MARCH 2021

VOL. 3 - N. 1/2021

SOCIETÀ ITALIANA DI FARMACOLOGIA

pharmadvances

THE OFFICIAL JOURNAL OF SOCIETÀ ITALIANA DI FARMACOLOGIA

1/2021

Abstracts of

40° CONGRESSO NAZIONALE DELLA SOCIETÀ ITALIANA DI FARMACOLOGIA – PROCEEDINGS

THE SCIENTIFIC VALUE AND APPROPRIATE USE OF DRUGS

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THE OFFICIAL JOURNAL OF SIF
Società Italiana di Farmacologia

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Volume **3**, issue **1**, **2021**
doi:10.36118/pharmadvances.03.2021.01

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EDITORIAL

THE 40TH CONGRESS OF THE ITALIAN PHARMACOLOGICAL SOCIETY (SIF)

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This issue of Pharmadvances features the presentations (as abstracts) of the 40th Congress of the Italian Pharmacological Society (SIF), which took place online due to the Covid-19 pandemic (1). As readers will realize, pharmacological research in Italy and globally is going strong, thanks to advances in basic research, their quick implementation by the pharmaceutical industry, and to the availability of new drugs and formulations. Notable examples include the pharmacology of non-coding RNA, the role of epigenetic modulations, or gene therapy, just to name a few. Most of these areas of research were unheard of only few years ago. The fact that such topics represent a notable proportion of the Congress testifies to the swift evolution of basic pharmacology and its clinical applications. Precision medicine is also growing rapidly thanks to the even-increasing availability of big data and of algorithms that are able to "crunch" them and extract valuable information.

The opening Plenary Lecture is, quite obviously, dedicated to SARS-CoV-2 and the dramatic consequences it is having on our lives. Yet, the key message pharmacologists worldwide will certainly extract is that the unique, interdisciplinary combination of basic research, informatics, clinical research, industrial progress, etc. is leading to a much better characterization of an otherwise elusive virus, to the ultra-hurried production of efficacious vaccines, and to the active search for antiviral agents. Even though time appears to flow very slowly, fighting Covid-19 at this pace is an incredible achievement of humankind and, particularly, of pharmacologists.

The most important outcome of the meeting will undoubtedly be the great need for future investments and strict collaborations between basic science pharmacology, industry, and politics (2). Indeed, the new therapeutic approaches discussed at the Congress are quite more expensive (though more successful) than the more traditional ones we are accustomed to. Only careful cost analyses and a new paradigm applied to the "willingness to pay" as far as research and development are concerned will eventually translate into a sustainable health care system that can provide its citizens with the most advanced and effective therapies.

In conclusion, the 40th Congress of the Italian Pharmacological Society represents a unique opportunity to look through the window of innovation and gauge successes and failures. Only dedicated researchers and enlightened entrepreneurs will grant humanity better treatments and improved quality of life.

Francesco Visioli, PhD
Executive Editor

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Volume **3**, issue **1**, **2021**
doi:10.36118/pharmadvances.03.2021.02

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CYTOTOXIN EFFECT OF TRAVECTEDIN IN HUMAN ADRENOCORTICAL CARCINOMA CELL

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BACKGROUND: Adrenocortical cancer (ACC) is a rare and aggressive disease with a severe prognosis. Mitotane is the only drug approved for the treatment of ACC, with or without chemotherapy that includes etoposide, doxorubicin, and cisplatin. This pharmacological approach, however, has a limited efficacy and significant toxicity. DNA alkylation appears to be a critical point for inducing cytotoxicity in the ACC; indeed, cisplatin is a fundamental component of the chemotherapic scheme. Results obtained both in vitro and in vivo support the antineoplastic activity of another alkylating agent such as temozolomide. Trabectedin is an anti-tumor drug that acts as an alkylating agent, with a complex mechanism of action. Here, we investigated whether trabectedin could exert a cytotoxic activity in in vitro cell models of ACC.
METHODS: NCI-H295R, MUC-1, HAC-15, SW13 cell lines and 5 ACC primary cell cultures were used. The local Ethical Committee approved the project and written informed consent was obtained from all enrolled patients, underwent surgery for primary or metastatic ACC. Patient-derived ACC cell cultures were established using the tumor isolation kit from Miltenyi Biotec. The adrenal origin of primary cells was assessed by the positivity to the SF-1 expression. The Chou-Talalay method was applied to study the effect of drug combination on cutotoxicity. β- catenin intracellular localization was evaluated by western blot and immunocytochemistry. The expression of Wnt/β-catenin pathway related genes was evaluated by qRT-PCR.
RESULTS: Trabectedin demonstrated high cytotoxicity at sub- nanomolar concentrations in ACC cells and the effect was maintained after drug withdrawn. Trabectedin/mitotane combination exerted a synergic cytotoxic effect in NCI-H295R cells. The drug was able to modify β-catenin intracellular localization: the exposure of NCI-H295R cells to trabectedin, at its IC50 value, for 72h induced a reduction of 44 ± 7.3% of the nuclear β-catenin. Interestingly, The co-localization of β-catenin with the proteasome subunit PSMB5 was increased, suggesting that trabectedin induced a β- catenin translocation from the nucleus to the cytoplasm, in particular to proteasome, leading to its degradation. Finally, trabectedin exposure significantly increased the expression of some genes that encode proteins involved in the reduction of Wnt activity, while the protoncogene MYCC and other genes activating the Wnt pathway were significantly reduced in NCI-H295R.

CONCLUSIONS: Our results indicate that trabectedinexerted a cytotoxic effect in different ACC cell models, and synergizes with mitotane in NCI-H295R cells, providing the rationale for a prospective clinical trial. Data on the inhibitory effect of trabectedin on Wnt/β-catenin pathway are interesting due to evidence indicating that its activation is a major tumor driver in pathogenesis of ACC and a mechanism of ACC resistance to modern immunotherapy.

EFFECT OF FUSARUBIN, A COMPOUND ISOLATED FROM THE ENDOPHYTIC FUNGI OF CLADOSPORIUM SPP, ON SENESCENCE OF ACUTE MYELOID LEUKEMIA CELLS

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BACKGROUND: Endophytic fungi interact with host plants and alter aspects of their physiology, conferring increased tolerance to stress, improving immune system function, bolstering defenses against disease, and aiding substance absorption. Thus, the possibility of exploiting endophytic fungi as biocontrol agents has received increasing attention. Cladosporium species are endophytic fungi that grow on organic matter and are considered food contaminants. The anti-microbial and anti-tumor naphthoquinones fusarubin (FUS) and anhydrofusarubin (AFU) were isolated from a Cladosporium species residing inside Rauwolfia leaves.
METHODS: The impact of FUS and AFU on cell growth was assessed in acute myeloid leukemia (OCI-AML3) and other hematologic tumor cell lines (HL-60, U937, and Jurkat). Cell viability and cell cycle progression were examined by flow cytometry to measure the DNA amounts in nuclei colored with PI. Proteins were extracted and analyzed by Western blotting. The gene expression analysis was performed by real time PCR. Additionally, we tested the possible effects of FUS on primary hematological cells by culturing mouse bone marrow cells from mice.
RESULTS: Treatment with FUS or AFU reduced the number of OCI-AML3 cells as evaluated by hemocytometer. Flow cytometry analysis showed that this effect was accompanied by diverse impairments in cell cycle progression. Specifically, FUS significantly decreased the percentage of cells in S and increased the percentage in G2/M phases, whereas AFU increased the percentage of cells in G0/G1 phase and decreased the percentage in S and G2/M phases. Both substances significantly increased apoptosis at higher concentrations. FUS, that was more potent than AFU, up-regulated p21 expression in a p53-dependent manner, as detected by Western blot analyses, likely the consequence of decreased ERK phosphorylation, increased p38 expression (both of which increase p21 stability) and decreased Akt phosphorylation. The up-regulation of p21 was possibly a mark of senescence since it was not accompanied by an increase of caspase-9-dependent apoptosis. However, FUS was also able to induce apoptosis in 8% of

cells by the production of FasL, thus promoting caspase-8/3-dependent apoptosis.
CONCLUSIONS: In conclusion, we showed that FUS and AFU derived from the endophytic fungi Cladosporium species isolated from Rauwolfia serpentina exert anti-proliferative and pro-apoptotic effects on tumor and primary cells, in part due to MAPK-dependent up-regulation of p21. In these circumstances, p21 transiently blocks cell cycle progression leading to senescence or apoptosis, thus protecting the organism from damaged or mutated cells. These results suggest that FUS induces a differential effect on the same cell line: senescence on the large majority of cells and apoptosis on a small fraction of them.

BIOASSAY-GUIDED ISOLATION OF ANTIPROLIFERATIVE COMPOUNDS FROM LIMBARDA CRITHMOIDES (L.) DUMORT

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BACKGROUND: The genus Limbarda (Asteraceae), formerly included in the genus Inula, comprises two accepted species: L. crithmoides (L.) Dumort and L. salsoloides (Turcz.) Ikonn. L. crithmoides is a halophyte plant, commonly known as Inula crithmoides (L.), that is widespread across the salt marshes and sea cliffs of the Mediterranean Sea, French Atlantic coasts, English Channel and western European seaboards. Extracts of L. crithmoides have been applied to crops and weeds to investigate their properties, which confirmed their herbicidal potency. The antioxidant activity of L. crithmoides has been widely investigated and seems to be directly correlated with the presence of phenolic metabolites such as quinic acid derivatives.
METHODS: Aerial parts of L. crithmoides were collected during the flowering period (August) in Fano, Urbino. NMR spectra were recorded using Avance DRX-400 and DPX-200 spectrometers and compounds were recovered from the stationary phase by column chromatography and fractions were monitored by TLC. The antiproliferative activity was tested on the acute myeloid leukaemia cell line OCI-AML3. Cell viability and cell cycle progression were examined by flow cytometry to measure the amount of DNA in nuclei stained with propidium iodide.
RESULTS: H and DCM extracts from L. crithmoides were able to significantly decrease the OCI-AML3 cell number compared to the vehicle (control) at concentrations of 15 or 10 µg/mL. An analysis of the cell cycle revealed a significant increase in cells in the G0/G1 phase for both fractions.The H extract was more potent than the DCM extract in increasing apoptotic cell death. Using NMR spectroscopic data from H extract, compound 1 was identified as 10-acetoxy-8,9-epoxythymol tiglate, and compound 2 was characterized as 10-acetoxy-9Z-chloro-8,9-dehydrothymol. The compounds 1 and 2 were very effective in decreasing the number of OCI-AML3 cells through

both increasing apoptosis and blocking cell proliferation. Compound 2 was more potent than compound 1 as it exerted inhibitory effects at a lower concentration. This prompted to examine the expression of both p21 and p53 in OCI-AML3 cells untreated or treated with the M, DCM, H extracts and the compounds 1 and 2 isolated; the result showed that all the tested extracts and compounds induced an upregulation of p21, but only with the H extract and with compound 2 the increase of p21 was significant compared to controls; it was also seen that p21 was regulated through p53-independent pathways, as there did not change after treatment with any of the extracts or compounds.
CONCLUSIONS: This study showed that the M extract of L. crithmoides has cell proliferation inhibitory activity against acute myeloid leukaemia cells (OCI-AML3) and solvent partition showed that the H fraction was more active than the DCM fraction. Chromatographic purification of fraction H led to the isolation of two active thymol derivatives, 1 and 2. Compound 2 was shown to be highly active.

PHARMACOLOGIC PPAR-GAMMA ACTIVATION REPROGRAMS BONE MARROW MACROPHAGES AND PARTIALLY RESCUES HSPC MOBILIZATION IN HUMAN AND MURINE DIABETES

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BACKGROUND: Mobilization of hematopoietic stem/progenitor cells (HSPC) from the bone marrow (BM) is impaired in diabetes. This defect precedes the reduction of circulating HSPC, which, in turn, is a driver of micro- and macrovascular complications. Excess oncostatin M (OSM) produced by M1 macrophages in the diabetic BM signals through p66Shc to induce Cxcl12 in stromal cells and retain HSPC. BM adipocytes are another source of CXCL12 that blunts mobilization. We tested a strategy of pharmacologic macrophage reprogramming to rescue HSPC mobilization.
METHODS: Diabetes was induced by a single intraperitoneal injection of streptozotocin at 175 mg/kg. HSPC mobilization was induced by injecting animals subcutaneously with 200 mg/kg with human recombinant granulocyte-colony stimulation factor (G-CSF) daily for 4 consecutive days. Pioglitazone was given to animals by daily gavage at 20 mg/kg for 4 weeks. Human peripheral blood mononuclear cells were isolated from buffy coat from healthy donors by Histopaque 1077 density gradient centrifugation followed by a high-density hyperosmotic Percoll gradient. Mouse BM-derived cells were isolated by flushing with sterile PBS both femurs and tibia. For both settings, cells were differentiated and polarized toward resting, M1 and M2 macrophages with or without pioglitazone (10 uM). Conditioned medium was obtained by adding serum-free medium without stimuli for 48 h to macrophages. Femurs were fixed in 4% paraformaldehyde, decalcified, and cut in longitudinal sections to perform histology or immunofluorescence. The human study

for G- CSF–induced mobilization was approved by the local ethics committee, was conducted in accordance with the Declaration of Helsinki as revised in 2000 and is registered at ClinicalTrials.gov (NCT01102699).
RESULTS: In vitro, PPAR-γ activation with pioglitazone switched both human and mice macrophages polarization from M1 to M2, reduced Osm expression, and prevented trans-cellular induction of Cxcl12 on stromal cells by macrophages-conditioned medium (Fig. 1A-E). In diabetic mice, pioglitazone treatment downregulated Osm, p66Shc, and Cxcl12 in the hematopoietic BM, restored the effects of G-CSF, and partially rescued HSPC mobilization, but it increased BM adipocytes (Fig. 1F-H). Using Osm-/- mice, we could show that Osm deletion recapitulated the effects of pioglitazone on adipogenesis, which was p66Shc independent, and double knockout of Osm and p66Shc completely rescued HSPC mobilization (Fig. 1J). In the absence of OSM, BM adipocytes produced less CXCL12, being arguably devoid of HSPC-retaining activity, whereas pioglitazone failed to downregulate Cxcl12 in BM adipocytes. In patients with diabetes on pioglitazone therapy, HSPC mobilization after G-CSF was partially rescued (Fig. 1H).
CONCLUSIONS: Pioglitazone reprogrammed BM macrophages and suppressed OSM signaling, but sustained Cxcl12 expression by BM adipocytes could limit full recovery of HSPC mobilization in diabetic animals and patients.

SEX DIFFERENCES ON MITOTANE CONCENTRATION AND TREATMENT OUTCOME IN PATIENTS WITH ADRENOCORTICAL CARCINOMA

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BACKGROUND: In clinical settings, data regarding sex are rarely investigated. In women, factors as body size and composition, hormonal variations, metabolism and access to care systems and therapy, could strongly influence the pharmacological management and the outcome of treatments. To underline this sex-related difference, we retrospectively collected data of adrenocortical carcinoma patients treated with mitotane, and then we evaluated sex-related pharmacokinetics parameters.
METHODS: A fully validated chromatographic method was used to quantify mitotane concentration in plasma collected from adult patients, also considering the active metabolite o,p'-DDE. Statistical analyses have been used to evaluate the sex influence on drugs pharmacokinetics.
RESULTS: We found that sex resulted as predictive factor of plasma mitotane and o,p'-DDE concentrations and significantly influenced the achievement of the therapeutic target of mitotane, implying that female sex could be a risk factor of treatment failure.
CONCLUSIONS: These results suggest that mitotane therapy should be modulated according to patient sex. Furthermore, the proposed approach could contribute to facilitating and disseminating sex-specific pharmacology.

ANTI-INFLAMMATORY ACTION OF A NOVEL CCR6 ANTAGONIST IN ZYMOSAN-INDUCED PERITONITIS

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BACKGROUND AND AIM: CC chemokine ligand 20 (CCL20), expressed by lymphoid tissues and epithelial/endothelial cells, and its cognate receptor CCR6, localized on innate/adaptive immune cells, are involved in the recruitment of leukocytes from the circulation to the inflamed tissues, a central process in the pathogenesis of inflammatory disorders, like inflammatory bowel diseases. Recently, our group had synthesized a small molecule targeting CCR6 (MR120) that was effective in mitigating inflammatory cells recruitment in a murine model of TNBS-induced colitis, characterized by the prominent activation of adaptive immune responses. The aim of the present work is now to evaluate the efficacy of MR120 against the acute inflammatory responses accompanying zymosan-induced peritonitis, a model of septic shock endowed with the massive recruitment of polymorphonuclear leukocytes in the peritoneal cavity.

METHODS: Peritonitis was induced in Swiss mice (n=8-10/group) by intraperitoneal administration (i.p.) of 1mg/mouse of zymosan A in PBS. MR120 1 mg/kg, 5mg/kg or 1mg/kg twice injected (1D - 1h before and 2h after zymosan), dexamethasone 3mg/kg (Dx) or vehicle (C) were subcutaneously (s.c.) applied 1h before zymosan. Sham mice (S) received PBS 200 µL i.p. and vehicle 10 mL/kg s.c.. Four hrs after zymosan A injection, the peritoneal fluid was collected and the myeloperoxidase (MPO) activity, index of leukocyte recruitment, the total proteins content and the number of neutrophils, identified as Ly6G+F4/80- cells, were determined by spectrophotometric or cytofluorimetric assays. All experiments were performed according to the guidelines for the Care and Use of Animals (DL26/2014).

RESULTS: Zymosan significantly increased total proteins amount and MPO activity and produced the massive recruitment of neutrophils in the peritoneal cavity ($P<0.05$ C vs. S). MR120 1D remarkably reduced peritoneal proteins and MPO content, showing comparable efficacy to Dx ($P<0.05$ vs. C). No protective effects were exerted by the CCR6 antagonist when administered in single dose at 1 or 5mg/kg.

DISCUSSION AND CONCLUSIONS: Our results suggest that MR120 is beneficial in attenuating the acute inflammation associated with strong native immune responses: these findings complement those obtained in TNBS-induced colitis, suggesting that not only does CCR6 blockade affect the modulation of lymphocytes responses but it also apparently interferes with neutrophil recruitment directly.

DEVELOPMENT OF A NEW LUBELUZOLE DERIVATIVE WITH REDUCED HERG CHANNEL ACTIVITY BUT INCREASED USE-DEPENDENT SODIUM CHANNEL BLOCK

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BACKGROUND: Lubeluzole, a neuroprotective drug previously developed for ischemic stroke, is a potent inhibitor of skeletal muscle voltage-gated sodium channels hNav1.4 (Desaphy et al., Mol Pharmacol 2013). The drug also showed potent antimyotonic activity in vivo in an animal model of myotonia congenita, a rare disease characterized by skeletal muscle hyper-excitability and stiffness (Desaphy et al., Exp Neurol 2014). One concern of lubeluzole is its ability to induce long QT, which precluded further use in humans. Here, we developed a new derivative of lubeluzole with the aim of reducing hERG channel inhibition, while maintaining sodium channel inhibition.
METHODS: Lubeluzole and its derivative were synthesized in our medicinal chemistry unit. The compounds were tested on potassium and sodium currents recorded with patch-clamp in HEK293 cells transfected with hERG or hNav1.4. Lubeluzole and its derivative were tested in vivo in the rat model of myotonia.
RESULTS: We synthesized LUB-1, a new derivative with reduced lipophilia, by introducing hydroxyl groups in the two outermost aromatic cycles. Indeed, an increased lipophilia is thought to favor inhibition of cardiac hERG channels. We previously showed that hydroxyl substitution in meta position of mexiletine aryl ring does not impair INa block (Desaphy et al., Front Pharmacol 2012), while reducing the potency on hERG channels (Gualdani et al, Pharmacol Res Perspect 2015). As expected, introduction of hydroxyl groups in lubeluzole reduced hERG channel inhibition by almost four times (Lubeluzole IC50 = 12 ± 2 nM; LUB-1 IC50 = 45 ± 3 nM). Compared to lubeluzole, LUB-1 was less than 2 times less potent but more use-dependent in blocking sodium currents. The IC50 of LUB-1 for sodium current inhibition was 80 μM at 0.1 Hz and 4 μM at 10 Hz stimulation frequency (holding potential was -120 mV). In the myotonic rat, lubeluzole exerted antimyotonic activity with an ED50 of ~0.1 mg/kg. Unfortunately, LUB-1, tested at 1 mg/kg, induced unexpected side effects on rat motor performance, leading to interruption of in vivo experiments.
CONCLUSIONS: We obtained a lubeluzole derivative with reduced hERG channel block and enhanced use-dependent behavior on hNav1.4 sodium channels. However, the derivative exerted unexpected side effects in vivo, due to either off-target activity or pharmacokinetic differences (grant #19027 supported by Association Française contre les Myopathies and Bari University Research Grant (2017-2018)).

FUNCTIONAL AND PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL CLCN1 MUTATION FOUND IN A RUSSIAN FAMILY SUFFERING FROM BECKER'S MYOTONIA

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BACKGROUND: Myotonia congenita (MC) is a rare skeletal muscle disease characterized by sarcolemma over- excitability inducing skeletal muscle stiffness and movement impairment. It can be inherited either as an autosomal dominant (Thomsen's disease) or an autosomal recessive (Becker's disease) trait. Both types are caused by loss-of-function mutations in the CLCN1 gene, encoding for ClC-1 chloride channel. In this study we identified a novel ClC-1 mutation, p.G411C, in a Russian family affected by a severe form of Becker's disease. We investigated the molecular defect of G411C chloride channels in order to define the mechanisms causing MC and to correlate it with the clinical manifestations of MC patients.Through a multidisciplinary approach, we showed that G411C can be classified as a trafficking defective mutant. A potential strategy to restore G411C channel surface expression is based on pharmacological chaperones, molecules able to bind ClC-1 and rescue misfolded protein.We verified whether cell incubation with niflumic acid (NFA), a reversible inhibitor of ClC-1, could restore G411C channel membrane expression and activity.
METHODS: Recombinant human wild-type (WT) and G411C ClC-1 channels were expressed in HEK293 cells and whole-cell chloride currents were recorded with patch-clamp technique, in control condition and after 16-24h incubation with MG132 or NFA. Biotinylation assays and fluorescent cell confocal imaging of YFP-tagged ClC-1 mutants were performed to detect and quantify ClC-1 membrane expression before and after NFA/MG132 incubation.
RESULTS: The G411C mutation dramatically abolished chloride currents in transfected HEK cells.Confocal imaging and biochemical experiments revealed that the majority of G411C mutant channels did not reach the plasma membrane but remained trapped in the cytoplasm. Cell incubation with NFA had no effect on G411C chloride current density.The lack of chloride current after NFA incubation could be attributed to the loss of NFA binding to G411C, the inability of NFA to exert chaperone effect on G411C,or the inability of G411C to conduct chloride currents.To gain major information, transfected cells were incubated with the proteasome inhibitor MG132 for 16 hours. MG132 was able to restore membrane expression of G411C mutant channels. However, despite an increase in cell surface expression, no significant chloride current was recorded in the G411C-transfected cell treated with MG132, suggesting that this mutation produces nonfunctional ClC-1 chloride channels,that are likely rapidly degraded.

CONCLUSIONS: This study expands the spectrum of CLCN1 mutations in MC and contributes to the understanding of genotype-phenotype correlation. The comprehension of the molecular mechanisms underlying MC could help the discovery of new drugs targeting specific mutant channels defects, allowing the development of a personalized treatment for MC patients (Telethon- Italy GGP14096 and Bari University Research Grant 2017-2018).

NRF2-PATHWAY, RPE, AND STRESS RESPONSE: IN SEARCH OF THE BIOLOGICAL SIGNATURE AND A NEW POTENTIAL PHARMACOLOGICAL STRATEGY FOR AGE-RELATED MACULAR DEGENERATION

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BACKGROUND: Age-related macular degeneration (AMD) represents a main cause of irreversible visual impairment in the elderly. An early event triggering AMD is the deterioration of the retinal pigment epithelium (RPE), fundamental for the retina health. RPE is physiologically exposed to high levels of oxidative stress during its lifespan; thus, the integrity and well-functioning of its antioxidant systems are essential to maintain RPE homeostasis. Among RPE defensive systems, the pathway of Nrf2 (nuclear factor E2-related factor 2) plays a primary role. Literature evidence suggests that, in aged and especially in AMD RPE, there is an imbalance between the increased pro-oxidant stress and the impaired endogenous detoxifying systems, finally reverberating on RPE functions and survival. We studied the effects of some AMD-related noxae on Nrf2-pathway in vitro in a human RPE cell line (ARPE-19), by evaluating Nrf2 activation and changes in the expression of selected downstream genes relevant for RPE homeostasis. Since an increase of Nrf2 activity seems to be protective against oxidative stress, with the aim to find new pharmacologically active compounds potentially useful for AMD, we tested some Nrf2 activators for their capability to promote RPE protection against AMD-related noxae.
METHODS: Cell viability and Nrf2-pathway activation in wild type (WT) and Nrf2-silenced (siNrf2) ARPE-19 cells exposed to AMD-related noxae (H2O2, 4-HNE, MG132+Bafilomycin), were evaluated. Four nature-inspired hybrids (NIH) were characterized as Nrf2 activators, and their pharmacological activity was investigated in ARPE-19 cells. The Nrf2 activator dimethyl-fumarate (DMF; 10 μM) was used as a positive control. In particular, Nrf2 activators were tested for Nrf2 activation (gene expression, nuclear translocation, up-regulation of an Nrf2-target gene), cell tolerability, ROS-scavenging effects, protection from noxae.
RESULTS: Nrf2-pathway activation is a physiological protective response in stressed ARPE-19 cells, leading downstream to an up-regulation of the Nrf2-targets HO1 and p62. Nrf2-deficit reverberates on the cell viability under stress. Three out of the four tested NIH (5 μM) display both direct and indirect antioxidant properties, in addition to cytoprotective effects in stressed ARPE-19 cells. The observed pro-survival effects require the presence of Nrf2, with the exception of NIH1, able to exert a still significant,

albeit lower, protection even in siNrf2 cells, supporting the concept of the existence of both Nrf2-dependent and independent pathways mediating pro-survival effects.
CONCLUSIONS: By using some pharmacological tools as well as a reference compound, we dissected the role of the Nrf2-pathway in ARPE-19 stress response, confirming that an Nrf2 deficit predisposes the cells to a higher vulnerability to stress, and suggesting that the Nrf2 induction represents an efficient defensive strategy to prevent the stress-induced damage.

ADVERSE DRUG REACTIONS TO CONTRAST MEDIA FOR MEDICAL IMAGING: A REPORT FROM SARDINIAN PHARMACOVIGILANCE CENTER

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BACKGROUND: Contrast media are commonly used to enhance the quality of imaging by improving the visibility of specific organs1. As the use of iodinated contrast media is rapidly growing, so is the occurrence of hypersensitivity reactions2. On 7th July 2020, the Italian Medicine Agency (AIFA) published a Safety Communication regarding the adverse drug reactions (ADRs) to contrast media used for medical imaging. The risk of hypersensitivity is higher in patients suffering from allergy or atopic diseases and in patients who experienced previous reactions. Nevertheless, anaphylactic events are mostly unpredictable3. The frequency of such events is classified as rare or not known in the summary of product characteristics (SmPC) of contrast media.
METHODS: We searched the Italian Pharmacovigilance database and included all the ADRs reported in Sardinia to any suspected drug used as a contrast media from 2001 to 14th of July 2020. We searched by ATC classification (V08) and by active ingredient. All the reactions were revised manually.
RESULTS: We found 102 ADRs. 1 was excluded because it was a duplicate. The ADRs are irregularly distributed during the period of observation. Among these, 82 cases (81,19%) involve iodinated contrast media (V08A), 16 cases (15,84%) paramagnetic gadolinium-based agents (V08C), 2 cases (1,98%) contrast media for ultrasound (V08D) and 1 case (0,99%) radiologic contrast media not-iodinated (V08B). The most reported drugs are IOMEPROL (34 cases), IOPROMIDE (27 cases), IODIXANOL (9 cases) among iodinated drugs, and GADOTERIDOL (7 cases) among gadolinium-based agents. 53 cases are classified as severe (52,48%), 37 cases as not-severe (36,63%), 9 cases as not-defined (8,91%) and 2 cases were fatal (1,98%). According to the Naranjo probability scale, the causality assessment is probable in 87 ADRs (86,14%) and possible in 14 ADRs (13,86%). The age groups are all well-represented: 63 patients aged 18-64 years (62,38%), 36 aged over 65 years (35,64%), and 2 cases not specified. In 66 cases (65,35%) the ADRs are evaluated as allergic reactions, in 31 cases (30,69%) as anaphylactic/anaphylactoid reactions and 4 cases (3,96%) involved other reactions.
CONCLUSIONS: The reporting rate is inconstant, and it may be influenced by contingent

factors. We argued that more awareness and involvement of health care professionals is recommended, for example by active pharmacovigilance projects.

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DRUG UTILIZATION OF ANTIDEPRESSANTS IN TWO LARGE ITALIAN AREAS: A TEMPORAL TRAJECTORY ANALYSIS

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BACKGROUND: Antidepressant drugs (ADs) are one of the most prescribed drug class in western countries. They have broad therapeutic indications, from major depression to anxiety but also enuresis, chronic pain or eating disorders. Recently, the consumption of ADs is slightly increased in Italy, from 39 Defined Daily Dose (DDD)/1000 inhabitants per day in 2013 to 42 in 2018. Tuscany is the Italian region with the highest consumption of AD (62 DDD/1000 in 2018). This is evident when the Tuscany consumption is compared with those registered in other regions with similar demographics and socio-economic conditions such as Emilia Romagna (52.1). This study aimed to describe the ADs use in Florence (FLO) and Bologna (BO) in terms of baseline characteristics and pattern of utilization.

METHODS: A retrospective cohort study, based on administrative healthcare databases from BO FLO, was performed. All individuals with ≥ 1 AD pharmacy claim (ATC code: N06A*) between 2009 and 2013 were selected. The consumption of ADs in the two areas was calculated (DDD/1000 inhabitants per day) in the observed period. The latter cohort included only those with no previous use of AD in the 5 years prior the index date (ID), first observed AD, and at least 5 years of follow-up. Demographic and clinical data (age, sex, type of AD) were collected at ID. Twenty binary variables were defined during follow-up each indicating presence of AD in a specific trimester. Trajectory model were assessed and clustered in the two areas.

RESULTS: In the observed period, 357,504 prevalent users were included in BO and 815,915 in FLO. FLO area showed higher ADs consumption than BO (71.5 DDD Vs 57.8). Incident cases were 149,285 in FLO (IR: 2.12/100 person-years) and 70,108 in BO (1.92/100 py), with IRR 1.11 (CI95%: 1.10-1.12). The latter cohort included 122,846 in FLO and 55,780 patients in BO. The majority of them with 5 years of follow-up were female (64%) aged 65 and 84 years (32%), but in FLO patients aged ≤45 were slightly more common (30% Vs 28%). The most prescribed class was SSRIs (75%). During the follow-up 7 clusters of trajectories were identified: (1) 38% were treated for 1 trimester (TRI)

only; (2) 12% had repeated short cycles; (3) 14% had short cycle with at least 2 TRIs after the first TRI; (4) 9% had longer cycles during the first 2 years and then discontinued; (5) 18% more than 2 years with interruption of treatment; (6) 5% had long cycle (between 8 and 19 TRI); and (7) 4% were continuously treated for 5 years. The clusters were similarly distributed across the two areas, but those with longer treatments were more frequent in FLO. Similar results were observed in the two areas for older individuals and those treated with SSRIs.

CONCLUSIONS: In FLO higher use of AD was observed, especially in younger individuals. This may indicate, in this area, a possible use of ADs also for non-psychiatric conditions. Findings may indicate higher reluctance in FLO to withdrawn the ADs therapy.

TREATMENT WITH LUTEOLIN IMPROVES LIPOPOLYSACCHARIDE-INDUCED PERIODONTITIS IN RATS

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BACKGROUND: Periodontitis is one of the most common and most serious dental diseases that causes the progressive destruction of the bone surrounding the tooth. In the onset of periapical inflammation lipopolysaccharide (LPS) is involved in the deterioration of the alveolar bone, in fact, the increase in its concentration determinates the release of a variety of pro-inflammatory mediators, including prostaglandins and cytokines, which cause periodontal tissues inflammation through the activation of multiple pathways. Luteolin (Lut) is a molecule of natural origin present in a large variety of fruits and vegetables, and also in medicinal herbs. It has been shown to possess beneficial properties for human health. Thus, on this basis, the purpose of this work was to investigate the anti-inflammatory properties of Lut on an animal model of periodontitis induced by LPS in rats.

METHODS: Periodontitis was induced by a single intragingival injection of LPS (10 μg/μl) derived from Salmonella typhimurium. The animals were treated with oral administration of Lut at different doses (10, 30, and 100 mg/kg), starting from 1 h after the injection of LPS. At the end of the experiment, 14 days after LPS injection, the animals were sacrificed, the gums removed by surgical procedure and processed for biochemical analysis and histological examinations.

RESULTS: The results obtained showed that the administration of Lut, only at the higher doses of 30 mg/kg and 100 mg/kg, was able to reduce alveolar bone loss, mitigate tissue damage, decrease the presence of neutrophilic infiltration and the concentration of collagen fibers in gingivomucosal tissues. Moreover, Lut treatment was able to reduce mast cells degranulation, NF-κB activation as well as decreased the presence of pro-inflammatory enzymes and cytokines.

CONCLUSIONS: In conclusion, the results obtained demonstrated that Lut has shown to have good anti-inflammatory activity in counteracting the inflammatory state caused by LPS-induced periodontitis. Therefore, assumed the potential of Lut, its implementation could represent valid support in the pharmacological strategy for periodontitis thus improving the well-being of the oral cavity.

ADVERSE EVENTS ASSOCIATED WITH DIABETES THERAPY IN CLINICAL PRACTICE IN CALABRIA

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BACKGROUND: Type 2 diabetes mellitus (DM2) is constantly increasing worldwide. Nowadays, numerous pharmacological treatments are available to control glycemia and reduce cardiovascular risk. However, the increase in the number of antidiabetic drugs, with several possible combinations, has led to an improved risk of treatment emergent adverse events (TEAEs) insurgence. The aim of the study was the identification of the TEAEs that appeared in relation to the characteristics of the population and the type of treatment used.
METHODS: We conducted a prospective observational study to identify TEAEs occurring with hypoglycaemic drugs in a real-world setting. From September 2018 until December 2019, consecutive outpatient afferent to "Mater Domini" University Hospital have been enrolled. Inclusion criteria were: ≥18 years, one or more hypoglycaemic drugs and a DM2 laboratory confirmed diagnosis. Reported TEAEs were coded using MedDRA.
RESULTS: The study involved 670 patients (47.5% female). Mean age was 66.2 years (range: 18-99 years) and mean age onset of the disease was 52.7±13.4 years. Overall, 553 (82.5%) patients had at least one comorbidity, mainly hypertension (64.9%) and hypercholesterolemia (55.1%). Monotreatment patients were 282 (42.1%) compared to patients in cotreatment 388 (57.9%). Metformin was the most prescribed drug (62.7%; 420), followed by insulin (39.4%; 264). A total of 284 TEAEs from 212 patients have been reported. The most frequently identified TEAEs were gastrointestinal disorders (148; 52.1%), followed by endocrine disorders (64; 22.5%) and general disorders (24; 8.5%). Twenty-one (7.4%) severe events of hypoglycaemia have been described.
Moreover, patients in polytherapy with 2 drugs experienced more TEAEs (40.6%), compared to monotherapy (34.9%) or polytherapy with ≥3 drugs (24.5%). Most of TEAEs were associated with metformin (140; 66%) and insulin (100; 47.2%) treatments. In the univariate regression, mean age (OR 0.97; CI: 0.96–0.98; p <0.001) and age of onset of the disease (OR 0.97; CI: 0.96– 0.99 p <0.001) seems to significantly predict TEAEs insurgence. Moreover, a statistically significant association was found between the use of insulin (OR 1.60; CI: 1.15–2.22; p = 0.005), GLP-1 analogues therapy (OR 2.65; CI: 1.44–4.89; p = 0.002) and TEAEs occurrence. In the multivariate regression, insulin seems to be a predictor of TEAEs onset OR 1.82; CI: 0.99–3.33; p = 0.05).
CONCLUSIONS: This study seems to confirm the importance of the management of complex pharmacological treatments to avoid TEAEs and improve effectiveness. According to literature, almost all adverse events are not serious (only severe hypoglycaemias have been reported). Finally, the type of hypoglycaemic drug treatments influences the risk of occurrence of TEAEs.

SIMILARITIES BETWEEN GHB (r-HYDROXYBUTYRATE) AND GVL (r-VALEROLACTONE) IN A COMMON SYMPTOM OF OVERDOSE: THE RESPIRATORY DEPRESSION

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BACKGROUND: The diffusion of NPS (New Psychoactive Substance) in the illicit drug market isworldwide. These are sedative-hypnotic compounds that induce the victims to be subjected to nonconsensual acts. Drug-facilitated sexual assault (DFSA) is a relatively new term by which it isdescribed a sexual abuse where the victim is unable to react due to drug intoxication, and thesame happens in case of robberies or other similar crimes. Among the most common sedative-hypnotic drugs associated with DFSA there are γ-hydroxybutyrate acid (GHB), its precursors, andits "legal substitute" γ-valerolactone (GVL). GHB is a short-chain fatty acid present in many humantissues, resulting from GABA metabolism. GHB is a central nervous system depressant with avariety of therapeutic uses. In fact, it has been first developed as an anesthetic drug and then as atreatment for narcolepsy and alcohol addiction. Recreational GHB use and related harms were firstdocumented from the mid-1990s as a part of the suite of drug use by nightclub patrons. GHB isoften sold in liquid forms under various street names such as "Juice", "Liquid G", or "Grievousbodily harm". GHB induces euphoria, relaxation and diminishes muscle tension but at higherdoses (50–70 mg/kg) can cause drowsiness, nausea, headache, vomiting, confusion, convulsion,anesthesia, apoplexy, respiratory depression, and coma.Within 30 min of ingestion, the individual may struggle to talk or move and may pass out,becoming vulnerable to assault. GVL is reported to be a substance that can be used as a "legalsubstitute" for GHB, and several anecdotal comparison of effects between GVL and GHB can befound on the web. Until a few years ago the GVL was marketed as a dietary supplement under thetrade name of Tranquilli-G. Unlike other GHB analogues, GVL is not metabolized to GHB but isprocessed to γ-hydroxyvaleric acid (GHV) by liver and plasma lactonase. GHV, that is the 4-methylsubstituted analog of GHB, probably exerts its pharmacological effect by mimicking the action ofGHB. Despite the lack of direct conversion from GVL to GHB, there is evidence that GHV inducessome similar effects compared with GHB. Since no data are reported on the possible cardio-respiratory adverse effects induced by GVL, the aim of this study was to investigate the cardio-respiratory (heart rate, pulse distention, breath rate, SpO2 saturation) impairments of GVL andGHB (100-3000 mg/kg) induced by gavage administration in mice.
METHODS: Male ICR mice weighing 25–30 g were group-housed (8–10 mice per cage), exposed to a12:12 hours light-dark cycle at a temperature of 20–22°C and humidity of 45–55%, and provided adlibitum access to food and water. All experiments were performed be-

tween 8:30 AM and 2:00 PM.Experiments were conducted blindly by trained observers working in pairs. GVL (99.5%) waspurchased from Sigma Aldrich, and GHB was purchased from LCG standards S.r.l. Both compoundswere dissolved in saline (0.9 % NaCl) solution that was also used as vehicle and administered orallyusing gavage needles at a volume of 4 ul/g. After a preliminary study, GHB and GVL wereadministered in the dose range of 100-3000 mg/kg. In order to evaluate the breath rate, theanimal was left free in a cage and the respiration rate of was videotaped by a cameraplaced above observation's cage. A blinded, trained operator performed the off-line analysis ofmovies. The analysis frame by frame allowed to evaluate the number of breath rates per min(brpm) of the mouse. Breath rates were measured at 0, 10, 35, 65, 85, 125, 185, 245 and 305 minpost gavage administration. Cardio-respiratory parameters in awake and freely moving mice weremeasured by the MouseOx Plus System. Briefly, the analysis was conducted by placing a collararound the neck of the animal which detected (with a frequency of 15 Hz) the heart rate (HR),breath rate, oxygen saturation (SpO2) and vessel distention (μM). In the first hour, a "false" collarwas placed on the animal's neck for simulate the real one used in the test, thus minimizing thepossible effects of stress during the experiment. After this adaptation period, the "real" collar (withsensor) was replaced and baseline parameters were monitored for 60 min. Subsequently, micewere administered with GHB and NMP (3000 mg/kg) by oral gavage administration and data wasrecorded for 5 hours.

RESULTS: Breath rate was unvaried in vehicle-treated mice over the 5 hours observation. Gavageadministration of GVL and GHB (100-3000 mg/kg) rapidly and dose-dependently reduced basalbreath rate (257 ± 11 brpm) in mice. The inhibitory effect induced by GVL was long-lasting andobserved only at higher doses (2000 and 3000 mg/kg), while GHB was already active at lowerdoses (100-200 mg/kg) even if the effect was transient. GHB at higher doses (400-3000 mg/kg)caused a marked and persistent respiratory depression. In particular, GHB (3000 mg/kg)induced a reduction of about 40% at 10 min after administration, and a respiratory depressionthat reached 80% of inhibition from 125 min to 305 min. The comparison of maximal effects caused by two compounds in the breath rate test revealed the lower potency of GVL respect to GHB. In order to better understand the adverse effects caused by GVL and GHB on cardiorespiratory functions, we have investigated the effects of the gavage administration of the highest dose (3000 mg/kg) of two compounds on heart rate, breath rate, pulse distention and SpO2, using the MouseOx instrument. The gavage administration of GVL (3000 mg/kg) decreased immediately the breath rate. A persistent bradypnea appeared at 180 min and the maximal effect was registered at 300 min (~70% of reduction) after the gavage administration and lasted until the end of the test. The SpO2 saturation was reduced to about 15%after 120 min of administration and the effect persisted up to 5 hours of measurements. Thebreath rate was also reduced to about 35% immediately after GHB treatment and the bradypneaepisodes increased after the second hour of the treatment. In particular, the breath rate wasinhibited to about 77% and the effect persisted until the end of the experiment. The SpO2 was alsoreduced to about 25% at 100 min after treatment and the effect persisted up to 5 hours afterregistration. Moreover, GVL induced a significant bradycardia at 185 min. The maximal effect wasregistered at 300 min (~35% of inhibition) and the effect persisted until the end of the test.Differently to GVL, the gavage administration of the highest dose of GHB (3000 mg/kg) reducedimmediately the heart rate after the treatment and the maximal reduction (~33%) was registeredat 100 min. The bradycardia persisted up to 5 hours of measurement. At the same time, the pulsedistention was slightly reduced during the first hour after GHB administration, but the effect wassignificant and persistent during the last three hours where the pulse distention was

reduced toabout 34% at 300 min of the registration. All the animals tested with GVL at the dose of 3000 mg/kg on the breath rate and MouseOx were found dead the next day after the experimentation.However, the 50% of the animals treated with 3000 mg/kg of GHB died at the end of the MouseOxtest, showing severe respiratory depression.

CONCLUSIONS: The present study reported the first in vivo comparison between the acute effectscaused by GVL and GHB on respiratory and cardio-respiratory parameters in mice. We havedemonstrated that GVL administration impaired respiratory and cardio-respiratory parameters inmice at higher doses (100-3000 mg/kg) and induced death of all the animals after 24 hours ofadministration of the highest dose (3000 mg/kg).The changes of the cardio-respiratory functioninduced by GVL were similar but delayed in respect to the ones induced by GHB. These datasuggest that the harmful effects, toxicity and the risk of inducing death of GVL abuse are similarto the well known sedative hypnotic GHB.

BIASED AGONISM AT NOCICEPTIN/ORPHANIN FQ RECEPTORS – A STRUCTURE ACTIVITY STUDY ON N/OFQ(1-13)-NH2

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BACKGROUND: Nociceptin/orphanin FQ (N/OFQ) controls different biological functions via selective stimulation of the N/OFQ receptor (NOP). The pleiotropic actions of N/OFQ may limit the development of NOP ligands as innovative drugs. The pharmacological concept of functional selectivity (aka biased agonism) might be useful for amplifying beneficial actions and/or counteracting side effects. To investigate the potential of biased agonism at the NOP receptor, molecules displaying a large bias factor toward G protein and/orarrestin are needed. This study investigated the effector specific (G protein vs arrestin) structure-activity relationship of the NOP receptor full agonist N/OFQ(1-13)-NH2 and its derivatives.

METHODS: The ability of N/OFQ(1-13)-NH2and its derivatives to promote NOP/G protein and NOP/arrestin interaction was investigated using the bioluminescence resonance energy transfer (BRET) assay described by Malfacini et al., 2015. The N/OFQ(1-13)-NH2derivatives were Ala-(Compounds 1-11) and D-scan analogues (compounds 12-21) as well as peptides modified either in the amino acid side chain or in the peptide bond (compounds 22-33). Compounds 34-38 were analogues modified in the address domain. Novel compounds were obtained by adding at the C terminus of [Cys14]N/OFQ(1-13)-NH2, via thiol-Michael reaction, groups with different chemical features i.e. lipophilic (compounds 39-41), hydrophilic (compound 42), positively charged (compounds 43, 44), negatively charged (compounds 45,46), and aromatic (compounds 47, 48) moieties.

RESULTS: N/OFQ(1-13)-NH2displayedhigh-potencyforbothNOP/G-proteinandNOP/β-arrestin2 interaction. Compared to the standard, compounds 1-38 displayed no significant changes in biased factor. Similar results were found with hydrophilicand aromatic compounds. On the contrary lipophilic and positively charged compounds displayed higher potency at NOP/G protein than at NOP/ß-ar-

restin2, thus behaving as G protein biased agonists. [Cys(palmitoyl)14]N/OFQ(1-14)-NH2 (compound 40) displayed abias factor of 2.01, the largest bias factor reported in literature for a NOPagonist.
CONCLUSIONS: This study showed thatN/OFQ(1-13)-NH2C-terminal modifications with positively charged peptide sequences or linear aliphatic chains can change agonist potency towards G protein thus generating NOP biased agonists. [Cys(palmitoyl)14]N/OFQ(1-14)-NH2 can be used in future studies to investigate functional selectivity in the NOPreceptorfield.

DRUG DELIVERY APPROACH BASED ON SELECTIVE INTEGRIN LIGANDS: TARGETED PRO-APOPTOTIC EFFECT OF 5-FLUORURACIL-INTEGRIN LIGAND CONJUGATES

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BACKGROUND: Targeted drug delivery can be an effective strategy to increase the bioavailability of therapeutics specifically to cancer tissue, and to decrease the heavy side effects from non-specific delivery to healthy tissue, therefore greatly improving clinical outcomes. It has been recognized that the molecular interactions between receptors and ligands that control cell-to-cell communications could be effectively targeted. In this context, integrins are peculiar receptors able to activate several intracellular signaling pathways to regulate cell growth, survival, migration, invasion, and angiogenesis. Integrins are overexpressed in many types of cancer cells and they have been implicated in mediating several hallmarks of cancer, including cancer cell proliferation, dormancy, survival, stemness, metabolic adaptation, and metastatic niche. Chemotherapy is considered the standard of care for several locally advanced cancers. Antineoplastic drugs have been largely employed in this setting, with 5-fluorouracil (5-FU) and cisplatin the most often used drugs. In this study, 5-FU was used as a model drug for developing more selective antitumor therapies by efficiently delivering the anticancer drug to its target cells through selective integrin ligands. New conjugates formed by 5-FU and integrin specific ligands (5FU-integrin ligand conjugate: 5-FUILC), connected by a linker, were evaluated as targeted delivery system for antineoplastic drugs.
METHODS: The effects of the new conjugates were studied to unravel their ability to modify integrin-mediated cell adhesion in cancer cells in vitro, performing cell adhesion assays on Jurkat and K562 cells. Moreover, we analyzed the pro-apoptotic effect of the new 5-FUILCs (in comparison to the unconjugated drug 5-FU, 10-50-100μM, 72h) in cancer cells (expressing α4β1 or α5β1 integrin) and in non cancer cells, by Annexin V assay. To study conjugates internalization, fluorescent conjugated analogs were synthesized and the extent of their internalization into cancer and non-cancer cells (expressing different types of integrin) was quantified by flow cytometry.
RESULTS: The new 5-FUILCs were able to modulate cell adhesion mediated by α4β1 or α5β1 integrin with a potency in the micromolar range. Moreover, fluorescent conjugated ana-

logs were internalized in a concentration-dependent manner in cancer cells expressing α4β1 or α5β1 integrin but not in non-cancer cells, expressing only β1 integrin subunit. As expected, the unconjugated parental drug 5-FU triggered the apoptotic process both in cancer and non-cancer cells. On the contrary, the 5-FUILCs were able to induce apoptosis in cancer cells whereas none of the 5-FU conjugates showed pro-apoptotic effects in non-cancer cells.

CONCLUSIONS: Conjugation of the anticancer drug 5-FU with integrin-specific ligands led to a higher selectivity in targeting cancer cells and to an increased accumulation of the payload in tumors probably through integrin trafficking.

EFFECTS OF THE PARTIAL DELETION OF MGLUR5 ON PRO- AND ANTI-INFLAMMATORY FEATURES OF MICROGLIA DURING ALS PROGRESSION IN SOD1G93A MICE

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the selective death of upper and lower motor neurons (MNs). The etiology of the disease is not completely understood, and among the several mechanisms that concur to the pathogenesis and progression of the disease, glutamate (Glu)-mediated excitotoxicity and neural inflammation play a pivotal role. In addition to MNs, other different cells types such as astrocytes and microglia are involved in ALS progression, acquiring a reactive phenotype during disease progression. Metabotropic Glu receptors 5 (mGluR5) are deeply involved in ALS, even if the importance in the different cellular populations has not been investigated. In our previous studies we generated double mutant mice carrying the SOD1G93A mutation and the mGluR5 partial deletion (SOD1G93AmGluR5+/-). These mice displayed a delay of the pathology onset and an amelioration on survival probability and an improvement in clinical signs.

AIM: The aim of this study was to investigate the effect of the partial deletion of mGluR5 in microglia cells acutely prepared from mutant mice. Several metabolic analysis on the different animal models used are carrying on.

METHODS: Microglia cells were acutely isolated through a discontinuos Percoll gradient from motor cortex and spinal cord of WT, SOD1G93A and SOD1G93AmGluR5+/- mice at three different time points during disease progression. TMEM119-positive cells were analyzed by flow cytometry. The pro-inflammatory M1 and the anti-inflammatory M2 phenotypes were detected and confocal analysis were performed to demonstrate the existence of mGluR5 on microglia. We performed oximetric and luminometric analysis to evaluate the oxygen consumption and ATP synthesis.

RESULTS: The M1/M2 ratio augmented in the spinal cord of SOD1G93A, but it is statistically significant in SOD1G93AmGluR5+/- mice at the late symptomatic phase of the disease only, while did not change in microglia derived from

motor cortex. Our results also highlight a bioenergetic impairment in microglia derived from 120 day-old SOD1G93A mice respect to age matched controls, that is partially restored in SOD1G93AmGluR5+/- mice.

CONCLUSIONS: The reduction of mGluR5 in SOD1G93AmGluR5+/- mice forces spinal cord isolated from microglia toward a more pro-inflammatory phenotype, at least at the late stage of disease progression.

GENDER-SPECIFIC CIRCULATING MICRORNA SIGNATURES AS BIOMARKERS OF ATHEROSCLEROSIS, ATHEROSCLEROSIS PROGRESSION AND VASCULAR EVENTS

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BACKGROUND: Tools currently used for cardiovascular (CV) risk assessment have non optimal predictive capacity and the accurate identification of "at-risk" individuals remains a major challenge. Thus, novel biomarkers for cardiovascular diseases are needed. In this study we have tested whether circulating microRNAs (miRs) and ultrasonographic imaging biomarkers of arterial wall composition and/or plaque vulnerability (echolucency) can serve as novel prognostic biomarkers for CV diseases, and whether the differences possibly observed may help in understanding the gender- and country-specific differences in CV risk.
METHODS: To achieve this goal we have investigated the associations between miR signatures assessed in plasma samples collected at baseline and: (1) echolucency of cIMT and/or carotid plaques measured at baseline, and (2) incidence of VEs. The IMPROVE study involved 7 centres in 5 European countries (Finland, France, Italy, Netherlands, and Sweden). Collected variables included clinical, biochemical, genetic, socioeconomic, psychological, nutritional, and educational data, personal and family history of diseases, drug intake, and physical activity. Ultrasonographic variables (cIMT, plaques size, their echolucency and ICCAD) have been measured on ultrasonographic scans stored in the IMPROVE imaging-bank. Plasma concentrations of 218 miR involved in metabolic, atherosclerosis or cardiovascular disease were measured by quantitative PCR (qPCR), using selected TaqMan probes, on a high throughput OpenArray platform (Life Technologies). For various reasons it was not possible to measure such set of miR in the whole cohort. Therefore, in order to maximize the probability of identifying a miR signature with prognostic power we selected patients in the following way: firstly, we selected all subjects who had developed a vascular event during the follow-up and a double number of subjects who had not developed events matched by gender, age and Framingham risk score (FRS). Secondly, we selected 371 subjects of the lowest decile of baseline subclinical atherosclerosis distribution and 371 subjects of the highest decile and finally 348 subjects of the lowest decile of the distribution of the progression of subclinical atherosclerosis and 348 subjects of the highest decile.
RESULTS: The echolucency of the most echolucent between left and right thickest plaque did not predict coronary events. Indeed, although

a slight trend can be glimpsed, the Hazard Ratio of subjects with echolucency below the median is not significantly different from that with echolucency above the median.
Conversely, considering cerebrovascular events the Hazard Ratio of subjects with an echolucency below the median is approximately double that of subjects with an echolucency above the median ($p<0.016$).
Volcano plot analysis of the relationship between standardized miR and ultrasonographic variable considered, adjusted for age, sex, FRS and latitude show that no one of the 218 miR considered were associated with ultrasonographic markers of plaque dimension such as IMTmax or plaque area. By contrast, 19 and 9 miR were significantly associated to echolucency of CC-IMT and echolucency of plaques, respectively, even after further adjustment for many other confounders. A ROC Association Statistics, shows that compared to vascular risk factors, the signature of 19 miRNA selected on the basis of their association with the echolucency of CC-IMT were significantly associated to both cerebrovascular and coronary events. Whereas the 9 miRNA selected on the basis of their association with the echolucency of plaques were significantly predictors of coronary events. Such miRNAs, combined into scores allow to improve the recognition of vulnerable patients.
CONCLUSIONS: Echolucency of the blackest one between left and right thickest carotid plaques is a predictor of cerebro-vascular events independent of FRS, statins and IMTmax thus corroborating the concept that echolucency is a good index of the presence of vulnerable plaques. In addition, arterial wall echolucency also allows to select specific signature of miR associated to vascular events. Such miRNAs, detected on plasma samples collected at baseline, can be combined into a score which may improve the recognition of more vulnerable patients i.e. those patients who have a higher probability to develop a new vascular events in a relatively short period (three years) independent of latitude, vascular risk factors and subclinical atherosclerotic burden.

ALTERATION IN GUT MICROBIOTA COMPOSITION AND FUNCTIONAL RELEVANCE IN SUBCLINICAL CAROTID ATHEROSCLEROSIS

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BACKGROUND: Alterations in Gut Microbiota (GM) composition and function associate with advanced cardiovascular diseases (CVDs); whether this holds true also at early stages of CVD is not known. We profiled GM in low risk primary prevention subjects from the PLIC survey, a population-based study, to investigate the association between GM profile and markers of Subclinical Carotid Atherosclerosis (SCA).
METHODS: GM composition was assessed through 16S rRNA gene amplicon sequencing from 345 fecal samples and a complete Gut Metagenome shotgun sequencing was performed on 23 subjects with advanced SCA vs 23 age- and gender-matched subjects without SCA.
RESULTS: In presence of SCA, the relative abundance of Bacteroides was reduced, while Escherichia, Coriobacteriaceae and Streptococcaceae were increased. Furthermore, gut metagenome sequencing demonstrated a significant increase of Escherichia coli, Dorea longicatena, Streptococcus parasanguinis, Streptococcus anginosus, Coprococcus comes in samples from subjects with advanced SCA vs those without SCA ($p<0.01$). In subjects with advanced and stenotic SCA, we also predicted i) an increase of bacterial species with higher expression of functional metabolic pathways related to phosphatidylethanolamine, palmitate biosynthesis and amino acids metabolism while ii) a rarefaction of bacterial species involved in starch degradation pathway and synthesis of anti-inflammatory short chain fatty acids. At the species level, while we observed reduced abundance of Fecalibacterium prausnitzii in metagenomes of subjects with SCA, we highlighted a uniquely increased abundance of E.coli, overexpressing two genes belonging to caiTABCDE operon and over representing the dietary LCarnitine degradation pathway. E.coli abundance also coincided with elevation in circulating innate immune cell fractions and with extent and echogenicity of SCA. We finally explored a possible relevance for GM composition by statin, the most prescribed pharmacologic treatment for the prevention of clinical manifestation of CVD and for slowing the preclinical progression of SCA. Of note, we did not find differences nor in alpha, beta diversities neither on the prevalence of predicted metagenomic pathways, implying that associations we found between GM signatures and SCA are to be considered beyond the effect of these treatments on liver and intestinal cholesterol metabolism. Longitudinal evaluations are warranted to better dissect these aspects.
CONCLUSIONS: We have identified a new, specific signature of GM dysbiosis in subjects with SCA among the general population, and the metagenomic analysis led to the identification of altered bacterial species related to specific metabolic pathways which may be new potential biomarkers for clinical diagnosis/prognosis of carotid atherosclerosis progression.

CLINICAL COMPARISON OF TWO DIFFERENT MONOCLONAL ANTIBODIES ACTING AGAINST CALCITONIN GENE RELATED PEPTIDE FOR THE PREVENTIVE TREATMENT OF CHRONIC MIGRAINE: A REAL-LIFE STUDY

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BACKGROUND: Migraine is a primary headache characterized by recurrent attacks of unilateral, pulsating pain, lasting from 4 to 72 hours and associated with autonomic symptoms such as nausea, vomiting, phono-phobia and photo-phobia. If the disorder recurs for more than 15 days per months for, at least, 3 months, a diagnosis of chronic migraine (CM) is made [1]. CM is a difficult-to-treat disease and the common oral preventive treatments usually fail, thus inducing treatment discontinuation within the first 6 months [2]. The recent discovery of the involvement of calcitonin gene-related peptide (CGRP) in migraine pathogenesis and the development of specific anti-CGRP monoclonal antibodies (mAbs) has changed CM history [3]. Erenumab and Galcanezumab are two out of the 4 mAbs approved for CM treatment, with the first one acting against CGRP receptor and the second on CGRP itself. The aim of this study is to compare the efficacy and safety profiles of the abovementioned antibodies in patients who were prescribed both of them for their CM, switching from one to the other.
METHODS: Nine CM sufferers treated at the Medical Toxicology-Headache and Drug Abuse Center of the University of Modena and Reggio Emilia were consecutively enrolled. Seven of them started with Erenumab and switched to Galcanezumab, whilst the other two did the opposite. The reasons for the switching was were a poor clinical benefit (5 patients) or adverse event (AE) in one case. For every patient the number of headache days per months (Headache Index-HI), the number of analgesics taken per months (Analgesic Consumption-AC) and the pain intensity measured trough the numeric rating scale for pain (NRS) were collected at the beginning of the treatment and after 2 months. The abovementioned variables were analyzed with the Student's t test for paired data. Adverse events (AEs) were also collected and descriptively analyzed.
RESULTS: Erenumab and Galcanezumab both relieved patients' headache in terms of frequency and consumption of painkillers. The NRS score decreased similarly after 2 months of treatment both for Erenumab and Galcanezumab groups. Among a general comparable effectiveness of two antibodies, Galcanezumab was associated with a lower number of AEs. In particular, Galcanezumab was less likely to induce constipation than Erenumab. Indeed, all nine patients developed constipation with Erenumab (100%), mild in 6 cases (66.7%) and moderate in the other 3 (33.3%), whilst with Galcanezumab only one patient developed constipation (11.1%). This may be explained by the different target of the two antibodies: since Erenumab targets the receptor of CGRP, it is arguable that it exerts a more pronounced inhibiting action on gastrointestinal motility that Galcanezumab. This may be due to the block of the action of other molecules such as amylin and VIP that act, in part, trought through CGRP

receptor and may account for a resting activation even during the block of CGRP.
CONCLUSIONS: Erenumab and Galcanezumab were comparable in terms of effectiveness, but the latter displayed a better safety profile.
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POSSIBLE DRUG-DRUG INTERACTIONS IN THE TREATMENT OF ALCOHOL USE DISORDER

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BACKGROUND: Alcohol use disorder (AUD) is a major source of mortality and morbility worldwide, as it is responsible for the 5.1% of the overall burden of disease, configurating as a global public health problem. It should be noted that AUD is underdiagnosed as well as undertreated, and only 15% of patients with a severe AUD receive an appropriate pharmacological treatment. Despite of such a clear medical need, currently there are only a few drugs approved for the treatment of AUD, such as: benzodiazepines, acamprosate, baclofen, disulfiram, nalmefene, naltrexone and sodium oxybate [2]. Managing a patient with an AUD may be very difficult because of the lack of adherence towards prescribed treatment, the possibility of therapeutic failures and the eventuality of drug-drug interactions. The aim of this study is to summarize and briefly review all the possible interactions among drugs approved for the treatment of AUD.

METHODS: A research was performed in Pubmed using the following terms: "interaction" OR "parmacological interaction" AND the following drugs: benzodiazepines (BDZ), acamprosate, baclofen, disulfiram, sodium oxybate, naltrexone and nalmefene. Only English-written articles were included, until the 31-MAY-2020. Pre-clinical articles were not considered, since authors have decided to focus only on clinical ones.
RESULTS: BDZ may increase the sedative effect of various drugs, such as opioids, antidepressants, anticonvulsants, antihistamines and neuroleptics. Moreover, azolic antimicotics, HIV protease inhibitors, macrolids and calcium antagonists may inhibit CYP3A4 and increase BDZ plasmatic concentration. On the other hand, barbiturates and anticonvulsants may induce CYP3A4 and decrease BDZ plasmatic levels. Baclofen shows pharmacodynamic interactions with opioids, anasthetics, analgesics like ziconotide and triciclic antidepressants, increasing their sedative effects. Sodium oxibate may increase the sedative effect of valproic acid. Disulfiram interferes with drugs containing alcohol, isoniazide, warfarin and psycostimulatns such as amphetamines, metilphenidate, buspirone, marijuana. Moreover, Disulfiram inhibits CYP2E1, increasing the sedative effects of various anesthetics, such as halothane. The inhibition of CYP2C9 by Disulfiram may be responsible of the in-

crease of sedative effect by amitriptyline. Nalmefene and naltrexone should not be administered in patients taking opioids for pain control, because they can elicit a withdrawal syndrome. No clinically significant interferences were found regarding acamprosate.

CONCLUSIONS: BDZ and Disulfiram may be involved in different pharmacological interactions, some of which could even be life-threating for Disulfiram. Naltrexone and Nalmefene should not be administered in patients taking opioids, whilst Acamprosate does not seem to have clinically significant interactions.

TARGETING THE CHLORIDE INTRACELLULAR CHANNEL 1 ACTIVITY PINPOINTS A MOLECULAR VULNERABILITY IN HUMAN GLIOBLASTOMA STEM CELLS: ANTITUMOR EFFECTS OF NOVEL LINEAR AND CYCLIC BIGUANIDES

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BACKGROUND: Among intracellular mechanisms contributing to the antitumor activity of metformin, a biguanide with hypoglycemic activity, the inhibition of the chloride intracellular channel 1 (CLIC1) ion current observed in human glioblastoma (GBM) stem cells (GSCs) is an intriguing target for its pivotal role in GBM cell proliferation. However, in vitro metformin concentration required to induce GSC growth arrest is difficult to translate in a clinical setting. We previously reported that other known linear or cyclic biguanides (i.e. phenformin and cycloguanil) acting via CLIC-1 inhibition are more potent than metformin, thus suggesting a pharmacological class effect. Based on selectivity towards CLIC1 inhibition, we design and investigate novel biguanide derivatives to enhance metformin pharmacological profile.
METHODS: Nine compounds characterized by a linear or cyclized biguanide moiety were synthetized and tested on a panel of human GSC cultures derived from GBMs of different molecular subgroups, as well as on non-stem GBM cells and normal umbilical cord mesenchymal stem cells (ucMSCs) as cell types whose proliferation is independent from CLIC1 activity. Cell viability, self-renewal and proliferation were assessed by MTT assay, spherogenesis and cell count; cell motility was evaluated by transwell migration assay. The selective effect on CLIC1-chloride current was measured by electrophysiology. To better recapitulate the original tumor heterogeneity, GBM 3D spheroids were obtained by mixing GSCs with Matrigel, EGF and bFGF, and evaluating proliferating cells by EdU incorporation.
RESULTS: In the 5 CLIC1-expressing GSC cultures tested, 7 compounds showed a lower IC50 as compared to metformin. We selected a linear biguanide-related drug, Q48, and a cyclic compound Q54, endowed with higher efficacy and potency than metformin and no or negligible toxicity on non-stem GBM cells and ucMSCs. Both derivatives significantly impaired cell migration, self- renewal ability and CLIC1-mediated ion conductance. The antitumor activity of Q54 was observed also in 3D spheroids, in which it drastically reduced cell proliferation rate. It is noteworthy that two cultures (GBM39 and GBM44) are insensitive to metformin, Q48 and Q54 although retaining

GSC properties, and displayed low content of CLIC1 protein as compared to responsive GSC cultures.
CONCLUSIONS: Q48 and Q54 could represent novel CLIC1-selective biguanides with a better pharmacological profile than metformin. The lack of antiproliferative effect of metformin, Q54 and Q48 related to a constitutive lower expression of CLIC1 in two GSCs confirms the central role of CLIC1 in GBM biology and biguanide-mediated antitumor activity. Moreover, primary GSC cultures emerge as a key model in GBM research, with the potential to uncover patient-specific differences in drug response, a step further towards precision medicine in which smart application of CLIC1- targeted agents might be more successful in eradicating GSCs.

EFFECTIVENESS AND SAFETY PROFILE OF BIOLOGICAL THERAPY IN INFLAMMATORY BOWEL DISEASE: REAL LIFE DATA FROM AN ACTIVE PHARMACOVIGILANCE PROJECT

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BACKGROUND: Inflammatory bowel disease (IBD), characterized by chronic inflammation of the gastro-intestinal (GI) tract, affects a growing number of people worldwide. Biological therapies are now the mainstay for the treatment of IBD. With their large use in real life, post-marketing activities become crucial for monitoring the long-term safety. Aim of this project was to evaluate the effectiveness and the safety profile of biologics for the treatment of IBD patients during a prospective pharmacovigilance study.
METHODS: From January 2017 to July 2020, all patients with Crohn's Disease (CD) and Ulcerative Colitis (UC) followed by the IBD unit of University Hospital of Messina and treated with at least one biologic agent at the start of the study (index date) or commenced a biologic during the study period were enrolled. Demographic, clinical, and disease-related data (e.g., age, sex, diagnosis, drugs used, switch/swap to another biologic drug) were collected. A descriptive analysis of patients' characteristics at the index date was performed. Moreover, an analysis of all adverse events (AEs) and all primary/secondary failures expressed as number of AEs or failures/10 treatment years was carried out taking into account the total years of treatment for each biologic including all patients treated with a biologic at least once during the follow-up period.
RESULTS: A total of 605 patients were enrolled, 58.5% with CD and 41.5% with UC. Mean age (±SD) was 44 ± 17 years and 59.3% were males. Mean age (±SD) at diagnosis was 34 ± 17 years and the mean disease duration (±SD) was 10.5 ± 9.1 years. At the index date, the following treatments were used: 41.0% adalimumab (ADA), 33.7% infliximab (IFX), 21.5% vedolizumab (VED), 2.1% golimumab (GOL), and 1.7% ustekinumab (UST). Patients naïve for biologic therapy were 82.2%. During the study period, 28.3% of patients had at least one switch/swap to another biologic agent. The total years of treatment were 854 years for ADA, 635 years for IFX, 294 years for VED, 48 years for GOL, and 67 years for UST. Data for

AEs and failures (both expressed per 10 treatment-years) were the following: IFX - 0.8 and 0.6, ADA - 0.6 and 0.8, VED - 0.6 and 1.5, GOL - 0.8 and 3.3, and UST - 1.1 AEs and 0.8, respectively. During follow-up, 133 AEs were reported. Infections mainly occurred in patients treated with GOL and ADA (6.1% and 6.0%, respectively), skin reactions in patients treated with ADA (5.6%), while infusion reactions with IFX (9.9%). A higher frequency of malignancies was observed in patients on treatment with VED (2.8%).

CONCLUSIONS: There were no major differences for AEs between the different treatments, but a higher frequency of failures with GOL and VED, both rarely used as first line therapies. Nevertheless, the acquisition of data from clinical practice should be endorsed to better define the safety and efficacy profile of new biologic agents in IBD.

ADVERSE DRUG REACTIONS WITH ORAL ANTICOAGULANTS: DATA FROM SICILIAN SPONTANEOUS REPORTING SYSTEM DATABASE

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BACKGROUND: The direct oral anticoagulants (DOACs) were synthetized in order to avoid the limitations of vitamin K antagonists (VKAs). DOACs are related to a greater number of gastrointestinal bleedings and to a smaller number of intracranial hemorrhages as compared with VKAs. Therefore, it is important to deepen information about their safety profile. In view of the above findings, the aim of this study was to analyze ADRs reports with DOACs and VKAs using the Italian Spontaneous Reporting System (SRS) database.

METHODS: All ADR reports having as suspected drugs DOACs and VKAs and coming from the Sicilian SRS database into the period 2001-2019 were selected. In detail, all reports with the following single active substances were included as cases: dabigatran etexilate, rivaroxaban, apixaban, and edoxaban; acenocoumarol and warfarin were included as comparator group. Descriptive statistical methodology was used to evaluate characteristics of reports; moreover, the reporting odds ratios (RORs) and the relative 95% confidence intervals (CIs) were performed at SOC level. Furthermore, the causality assessment evaluation was performed using the Naranjo algorithm according to the Italian Medicine Agency (AIFA).

RESULTS: Of 521 reports related to anticoagulants, 444 (85.2%) and 77 (14.8%) regarding DOACs and VKAs, respectively. VKAs were most significantly related to serious ADRs compared to DOACs ($p < 0.001$). A significant ROR for DOACs was found for gastrointestinal disorders (ROR 4.73, 95% CI 2.61-8.58). Alternatively, VKAs were mostly significant related to blood and lymphatic system disorders, injury, investigations, and vascular disorders. ADRs belonging to gastrointestinal disorders were more reported for dabigatran etexilate (n = 179; 73.7%) while ADRs of blood and lymphatic system disorders were mainly found for acenocoumarol (n = 27; 57.4%). In detail, the most commonly reported Preferred Terms (PTs) for DOACs were dyspepsia (n = 89; 17.1%), ab-

dominal pain upper (n = 41; 9.2%), and pruritus (n = 26; 5.8%), while for VKAs were anemia (n = 21; 27.3%) and hypocoagulable state (n = 18; 3.5%). Regarding the causality assessment, the majority of ADR reports were categorized as possible (65.1%) followed by probable (32.6%); furthermore, 8 cases were doubtful and only 4 cases were considered as definite.

CONCLUSIONS: Our analysis suggests that DOACs were related to a higher reporting of non-serious ADRs over VKAs. DOACs, especially dabigatran, were mostly related to gastrointestinal disorders, but also to gastrointestinal bleedings. On the other hand, VKAs were more responsible of serious ADRs, regarding anemia and hypocoagulable state. Nevertheless, the high frequency of gastrointestinal disorders and skin manifestations, especially for dabigatran, highlights the need of a careful focus on prescribing.

ACTIVATION OF A2A ADENOSINE RECEPTOR AS A PROMISING THERAPEUTIC APPROACH FOR THE TREATMENT OF OSTEOPOROSIS

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BACKGROUND: Osteoporosis is a common skeletal disease characterized by a dampening in bone formation, caused by a suppression in osteoblast activity and an increase in the resorption of bone due to an enhanced activity of osteoclasts, leading to a progressive reduction in bone density with a consequent increase in fragility and susceptibility to fractures.

The A2A adenosine receptor (A2A-R) is a purinergic receptor belonging to the family of G protein-coupled receptors, whose activity is mediated by a G protein that activates adenylate cyclase (Gs). Some studies suggest that A2A-R could stimulate osteoblast proliferation and differentiation through the activation of Wnt/b-Catenin pathway, the main pathway involved in cellular proliferation and differentiation.

The aim of this study is to evaluate how the activation of A2A-R, using a specific agonist like CGS-21680, could stimulate cell proliferation and differentiation in an in vitro model of dexamethasone-induced osteoporosis in human fetal osteoblasts.

METHODS: Human fetal osteoblasts hFOB 1.19 (ATCC® CRL-11372™) were cultured in a 1:1 mixture of Ham's F12 Medium and Dulbecco's Modified Eagle's Medium (DMEM) under standard conditions. Upon reaching confluence, cells were treated with CGS-21680 (1.0 uM) following Dexamethasone (1.0 uM) challenging for 24 hours. qPCR and Western Blot were performed to evaluate the expression of markers involved in osteoblast proliferation and differentiation (RUNX2, BMP6, COL1A1, Osterix and Osteocalcin) and in Wnt/b-Catenin signalling pathway (Wnt5a, Wnt10b, b-catenin, DKK1 and Sclerostin). In addition, nuclear translocation of b-Catenin was evaluated by immunofluorescence.

RESULTS: Activation of A2A receptor by CGS-21680 stimulated proliferation and differentiation of osteoblasts, increasing the expression of BMP6, RUNX2, COL1A1, Osteocalcin and Osterix compared to cells treated with dexamethasone; caused an activation of Wnt/b-Catenin pathway, enhancing the expression of Wnt5a, Wnt10b and b-catenin, and reducing the expression of DKK1 and Sclerostin, main inhibitors of Wnt signalling. Finally,

CGS-21680 promoted the nuclear translocation of b-Catenin, activating the transcription of gene involved in osteoblast proliferation and differentiation.

CONCLUSIONS: These preliminary data suggest that the use of A2A-R agonists could represent a promising therapeutic approach for the treatment of osteoporosis.

ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECT OF LYCOPENE AS A STRATEGY FOR THE TREATMENT OF OSTEOPOROSIS

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BACKGROUND: Osteoporosis is a systemic skeletal disease characterized by a progressive loss of bone mass which leads to an increase in fragility and, consequently, a greater risk of fractures. Bone loss is determined by an alteration of the dynamic balance of bone remodeling, in which bone resorption mediated by osteoclasts exceeds the osteoblastic activity of bone formation. Several studies have found a link between oxidative stress and the pathogenesis of osteoporosis, since the damage induced by oxidative stress could cause an alteration of cells involved in bone remodeling. ROS, whose production increase with aging or in an inflammatory state, suppress osteoblast differentiation while promote osteoclast differentiation and activity.
Lycopene is a hydrocarbon carotenoid found mainly in tomatoes. It is known as a potent antioxidant and anti-inflammatory compound since it shows a high free radical-scavenging activity and inhibits the activation of NF-κB and the release of pro-inflammatory cytokines. For these properties, lycopene is considered an important nutrient for human health, since several studies have shown that a diet rich in lycopene can prevent various pathologies, including cardiovascular disease, prostate cancer, and male infertility.
The aim of this study was to evaluate the anti-inflammatory and antioxidant effects of lycopene on the H2O2-induced production of inflammatory mediators in human fetal osteoblasts.
METHODS: Human fetal osteoblasts hFOB 1.19 (ATCC® CRL-11372™) were cultured in a 1:1 mixture of Ham's F12 Medium and Dulbecco's Modified Eagle's Medium (DMEM) under standard conditions. Upon reaching confluence, cells were treated for 24 hours with lycopene at different doses (0.5, 1 and 2 uM) following H2O2 (0.2 mM) challenging for 24 hours. At the end of the treatment period, cell viability was evaluated by MTT assay and Trypan Blue staining; qPCR and Western Blot were performed to evaluate the expression of pro-inflammatory (TNF-a, IL-1b and IL-6), anti-inflammatory mediators (TGF-b and IL-10) and markers involved in apoptosis (Pro-caspase-3, Cleaved-caspase-3, Bax and BcL-2).
RESULTS: The treatment with lycopene increased cell viability as compared to H2O2 alone. The challenge with H2O2 for 24 hours stimulated nitrite/nitrate production and the expression of Pro-caspase-3, Cleaved-caspase-3, Bax, TNF-a, IL-1b and IL-6. Lycopene inhibited the induced production of apoptotic and pro-inflammatory mediators, nitrite/nitrate production, while increased the expression of the anti-inflammatory cytokine IL-10 and the pro-resolution factor TGF-b.
CONCLUSIONS: These preliminary data suggest that lycopene, a component of Mediterranean diet, could be used to reduce the inflammation and the oxidative stress related to osteoporosis.

DEVELOPMENT AND CHARACTERIZATION OF A KAPPA OPIOID RECEPTOR (KOR) PARTIAL AGONIST DISPLAYING MORE FAVORABLE PHARMACOLOGICAL PROFILE IN VITRO AND IN VIVO

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BACKGROUND: Kappa opioid receptor (KOR) agonists induce antinociception and side effects via G protein-dependent signaling and arrestin 3-dependent p38MAPK activation, respectively. Endogenous KOR agonist, dynorphin, is released in distinct brain regions following the development of addiction, thus contributing to craving for substances of abuse. Hence, KOR agonists preferentially activating G protein signaling are emerging as promising analgesic candidates with a more favorable profile, whereas KOR antagonists/partial agonists are studied as potential therapies for addiction.
Recently we identified CL39, a novel KOR selective partial agonist; here we investigate its G protein- versus arrestin-dependent signaling in vitro, and its analgesic versus sedative/aversive effects and anti-addiction properties in vivo. Classic KOR agonist U50,488 was employed as reference compound.

METHODS: G protein activation and arrestin 3 recruitment at KOR were investigated by measuring adenylyl cyclase inhibition and by performing arrestin complementation assay in U2OS and BRET assay in HEK-293 cells. Activation of distinct MAPKs over others and the subsequent functional selectivity on related cellular responses were studied in HEK-293, U87-MG astrocytoma cells and human astrocytes. Antinociception was assessed in the warm-water tail withdrawal test and in oxaliplatin-induced neuropathy in mice; aversive effects were evaluated through conditioned-place-preference in rats.
RESULTS: Similarly to U50,488, CL39 inhibited adenylyl cyclase, albeit displaying partial agonism. Conversely to U50,488, CL39 weakly recruited arrestin 3 at KOR and induced early (5-15 min), G protein-dependent ERK1/2 phosphorylation but neither late (60 min), arrestin-dependent ERK1/2 nor p38MAPK phosphorylation. U50,488, but not CL39, significantly increased U87-MG and normal human astrocytes cell proliferation in arrestin 3, p38MAPK-dependent fashion.
In vivo both CL39 and U50,488 (0-30 mg/kg; 0-60 min; i.p.) induced a significant, KOR-mediated, dose-dependent antinociception in warm-water tail-withdrawal test, displaying CL39 partial agonism. Conversely to U50,488, CL39 counteracted oxaliplatin-induced hyperalgesia and allodynia but did not determine any significant motor incoordination or aversion. CL39 effects on cocaine-induced conditioned place preference are being currently investigated and will be discussed at the conference.
CONCLUSIONS: CL39 is a KOR selective partial agonist preferentially activating G protein-dependent rather than arrestin-mediated signaling; as a consequence, CL39 fails to activate p38MAPK, that in turn is responsible for

astrocyte activation and other adverse effects as sedation, motor incoordination, anhedonia. Consistently, CL39 determine a significant, aversion-free antinociception in animal model of acute and chronic pain; thus emerging as a promising candidate to be further investigated as innovative analgesic and anti-addiction therapeutic.

KYNURENINE NEGATIVELY EXACERBATES THE THC EFFECTS ON TETRAD AND SENSORIMOTOR RESPONSES IN ADULT MICE

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BACKGROUND: The main psychoactive component of marijuana (Δ-9-tetrahydrocannabinol, THC) and synthetic cannabinoids intake, is correlated with untoward physiological effects in vulnerable individuals (D'Souza et al., 2016). Thus, cannabinoids misuse could be considered as a relevant factor in precipitating and/or perpetuating psychosis in these subjects. It has been reported, in rats and monkey, that the reinforcing effects of THC can be reduced by increasing endogenous kynurenic acid (KYNA) levels (Justinova et al., 2013). KYNA, a neuroactive metabolite deriving from tryptophan degradation (Schwarcz et al., 2012). Several studies suggest a pathophysiologically relevant association between increased brain KYNA levels and cognitive dysfunctions in individuals with schizophrenia (Wonodi and Schwarcz, 2010; Sathyasaikumar et al., 2011).
METHODS: Male ICR (CD-1®) mice (25-30 g body weight) were treated with THC (30 mg/kg; i.p.) and kynurenine (20 mg/kg, i.p.), alone or in combination. Following the drug administration, body temperature, acute mechanical and thermal analgesia, motor activity sensorimotor responses (to visual, acoustic and tactile stimulation) were evaluated. Furthermore, brain levels of KYNA were measured 1 and 4 hours after kynurenine injection.
RESULTS: Brain KYNA levels were significantly increased 1 hour, but not 4 hours, after kynurenine administration. The administration of kynurenine, amplified the THC-induced impairment of sensorimotor responses. In particular, kynurenine increased the THC-induced reduction in the visual placing response, acoustic response and tactile response (vibrissae, corneal and pinna reflexes). Furthermore, by using the "tetrad paradigm for screening cannabinoid-like effects" it has been observed that kynurenine significantly increased THC-induced motor activity reduction (as evaluated by the bar test, drag test and rotarod test) and hypothermia (core and surface body temperature), but not THC-induced analgesia.
CONCLUSIONS: Overall, the present data indicate that increased brain KYNA levels exacerbate "tetrad" and sensorimotor responses induced by the acute administration of THC. This confirm the existence of a cross-talk between the KP and endocannabinoid system which could be involved either in the psychotropic properties of THC or in the ethiopatogenesis of schizophrenia.

GLYCANS SIGNATURE OF IMMUNE CELLS IN EXPERIMENTAL ATHEROSCLEROSIS AND IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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BACKGROUND: Leukocytes infiltration and cellular extravasation observed in atherosclerosis at early stages take advantage of the interaction with endothelial glycoproteins, which expose Sialic Acid (Sia) as terminal glycan residues. The latter have been shown to affect several cellular functions, including immune cells interactions and immune response. Our aim was to profile immune cell glycan signature during atherosclerosis.
METHODS: Sialic acid (Sia) and Galactose (Gal) expression was analysed by flow cytometry via MAL and SNA (α2,3-linked and α2,6-linked Sia respectively) and RCA (Gal) lectin binding on the surface of T cells and monocytes from lymph nodes and blood of LDLR KO mice fed with chow (standard-) or WTD (high cholesterol diet) for 8 weeks and from blood of patients with familial hypercholesterolemia (FH).
RESULTS: Hypercholesterolemic LDLR KO mice fed WTD compared to chow presented a significant decreased expression of α2,3-linked Sia on T cells (0,68±0,040 fold* for CD4+, 0,49±0,02 fold* for CD8+), compared to LDLR KO mice fed chow diet. By contrast, monocytes showed an increased expression of both α2,6-linked Sia and Gal (2,12±0,15 fold* and 1,27±0,05 fold* respectively). In lymph nodes, CD4+ and CD8+ T cells showed a decrease expression of α2,3-linked Sia (0,88±0,13 fold for CD4+ and 0,86±0,14 fold for CD8+), while neither α2,6-linked Sia nor Gal expression showed significant changes.
Similarly, FH patients presented decreased expression of α2,3-linked Sia on circulating T lymphocytes (0,75±0,03 fold** for CD4+, 0,87±0,04 fold* for CD8+) as well as on CD14+ and CD16+ monocyte subsets (0,85±0,07 fold and 0,68±0,11 fold* respectively) compared to age and sex- matched controls. (*$p<0.05$; **$p<0.01$).
CONCLUSIONS: Our data indicate that hypercholesterolaemia impacts glycans signature of immune cells both in humans and in experimental models. The presence of increased α2,6/α2,3-linked Sia and Gal/Sia ratios observed in hypercholesterolaemic conditions supports a different immune activation during atherosclerosis and might pave the road to pharmacological approaches directed at targeting terminal glycan residues as a strategy to dampen the inflammatory response associated to atherosclerosis.

HEPARAN SULFATE BINDS THE EXTRACELLULAR FORM OF ANNEXIN A1 BLOCKING ITS ONCOGENIC EFFECTS ON PANCREATIC CANCER CELLS

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BACKGROUND: In pancreatic cancer (PC) progression the protein Annexin A1 (ANXA1) has been described as oncogenic factor promoting tumor cell metastatization and pathological angiogenesis. Thus, the need to inhibit its action, mainly the extracellular form, has become an appealing cue for the anti-cancer research. Heparan sulfate (HS) is a glycosaminoglycan belonging to the extracellular matrix where it binds several molecules, as growth factors and cytokines, generating a kind of reservoir in the extracellular environment.
METHODS: We started our study by showing the physical calcium-dependent interaction between HS and ANXA1 as both full-length protein and N-terminal and biologically active portion, Ac2-26, by biophysical techniques as the differential scanning fluorimetry and the surface plasmon resonance.
RESULTS: Through the use of HS as free molecule, we show, for the first time, its ability to inhibit the migration and invasion processes of human PC cells MIA PaCa-2 cells and partially revert their mesenchymal phenotype as reported through the expression of specific protein markers, as cytokeratins 8/18 and vimentin, and the growth in colonies and in 3D-spheroids. Furthermore, HS blocks the effects of Ac2-26, which enhances the aggressive behavior of PC cells if added alone. These effects appear evident also on endothelial cells whose activation is promoted by Ac2-26 but not in presence of HS. Thus, the interference of the interaction ANXA1-HS on angiogenesis strongly emerges. Moreover, once sequestered by HS, ANXA1 is not more able to bind the formil-peptide receptors (FPRs) preventing the increase of calcium mobilization, peculiar for cell motility. The specificity of the interaction between HS and ANXA1 is further explained by the lack of interference that the glycosaminoglycan carries out of the positive action of the peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) on its receptor, about calcium release.
CONCLUSIONS: These findings introduce a new important tale in the knowledge about the inhibition of the ANXA1 action in PC development. It could be hypothesized to use HS as scaffold for future investigation about the creation of synthetic molecules functioning as ANXA1 inhibitor. However, further information will be useful to highlight the interaction of HS with the protein, focusing on the characterization of the glycosaminoglycan and on in vivo assays.

THE NOVEL FINDING OF THE MESOGLYCAN FIBRINOLYTIC ACTION: THE ANNEXIN A2 IS THE KEY PLAYER

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BACKGROUND: Mesoglycan is a drug based on a mixture of glycosaminoglycans mainly used for the treatment of blood vessel diseases acting as antithrombotic and profibrinolytic drug. Beside the numerous clinical studies, until this moment there were no information about its function on the fibrinolytic cascade.
METHODS: Here, we have elucidated the mechanism of action by which mesoglycan induces the activation of plasmin from endothelial cells.
RESULTS: Surprisingly, by an overall proteomic analysis, we found that, following mesoglycan treatment, these cells show a notable amount of annexin A2 (ANXA2) at plasma membrane. This protein has been widely associated to fibrinolysis and appears able to move to membrane when phosphorylated. In our model, this translocation has proven to enhance cell migration, invasion and angiogenesis. Furthermore, the interaction of mesoglycan with syndecan 4 (SDC4), a co-receptor belonging to the class of heparan sulfate proteoglycans, represents the upstream event of the ANXA2 behavior. Indeed, the activation of SDC4 triggers the motility processes of endothelial cells culminating in the angiogenesis. Interestingly, mesoglycan can induce the release of plasmin in endothelial cell supernatants only in presence of ANXA2.
CONCLUSIONS: This evaluation has led us to suggest that mesoglycan triggers the formation of a chain mechanism starting from the activation of SDC4 and the related cascade of events, promoting the phosphorylation of ANXA2 and its translocation to plasma membrane. Here, ANXA2 links the tissue plasminogen activator bringing it closer to plasminogen which is so cleaved to release the active plasmin and to degrade the fibrin sleeves.

ROLE OF G PROTEIN-COUPLED RECEPTOR 21 IN MACROPHAGES

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BACKGROUND: GPR21 is an orphan and constitutively active receptor belonging to the superfamily of G-Protein Coupled Receptors (GPCRs), the largest genome protein superfamily targeted by drug discovery. GPR21 couples to the Gq family of G proteins and is markedly expressed in the hypothalamus and in macrophages. In particular, studies of GPR21 knockout mice indicated that GPR21 may act by promoting macrophage migration thus suggesting that GPR21 may be a novel control point coordinating macrophage pro-inflammatory activity. The aim of this study was to evaluate the role of GPR21 in human macrophages, analyzing (i) its involvement in cell migration and in pro-inflammatory cytokines production and (ii) its pharmacological inhibition by using the inverse agonist GRA2.

METHODS: THP-1 monocytes were differentiated into macrophages and polarized in M1 (LPS + IFN-γ) or M2 (IL-4 + IL-13). The expression of the receptor was evaluated through Real Time-PCR and Western Blot. A MTT assay was performed to evaluate potential toxicity of the GRA2 inverse agonist at the doses of 10, 30 and 60 mM. The constitutive activity of the receptor was verified through the IP One HTRF assay. Cell migration was evaluated by a Boyden chamber assay. Cytokine release was measured by ELISA. A migration assay was also performed on primary human peripheral blood mononuclear cells differentiated in macrophages and polarized in M1 (LPS + IFN-γ) and M2 (IL-4) in the presence and absence of GRA2 (30-60 mM).

RESULTS: Our data confirms that GPR21 is a constitutively activated receptor in our experimental condition and that GRA2 acts as an inverse agonist reducing endogenous basal production of IP1. GRA2 does not affect cell viability at the tested concentrations. The analysis of the migratory ability highlighted an opposite effect of GRA2 on M1 and M2 macrophages. Specifically, GRA2 significantly reduced M1 macrophages migration, while it induced a marked increase of M2 cell migration. These results were confirmed when the migration assay was performed with human primary cells. Finally, our data showed that the inverse agonist significantly reduces the release of TNF-α from M1 macrophages.

CONCLUSIONS: These results indicate an active role of GPR21 in the inflammatory process mediated by macrophages, by modulating both migration and pro-inflammatory cyto-

kine production. Moreover, we show that the inverse agonist is able to significantly reduce these receptor effects. Our preliminary data suggests that pharmacological inhibition of GPR21 could be a new strategy to reduce inflammation meriting further studies.

EFFECTS OF LINAGLIPTIN ON DPP-4 RELEASE FROM RENAL CELLS

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BACKGROUND: Dipeptidyl-peptidase (DPP)-4 is the pharmacological target of gliptins, drugs approved for the treatment of Type-2 diabetes mellitus. It is a membrane-bound protein expressed in a wide range of different tissues, with the highest activity per mg of protein content detected in the kidneys. However, a soluble DPP-4 (sDPP-4) form is also detected in biological fluids, thus indicating the existence of at least two forms of this enzyme, possibly playing a distinct pathophysiological role. Importantly, a significant sDPP-4 increase has been detected in plasma and urine of diabetic patients, although its origin is still unknown. The aim of this study was to assess (I) the role of kidney cells as possible sources of the sDPP-4 detected in urine; (II) the effect of DPP-4 inhibitors on the release of the enzyme.
METHODS: Release of sDPP-4 was evaluated by ELISA in cell-conditioned media of human immortalized podocytes and Primary Renal Proximal Tubule Epithelial Cells (RPTEC) derived from healthy subjects (RPTEC) or diabetic subjects (D-RPTEC) in the absence or presence of linagliptin (48h; 0.1 nM, 1 nM, 10 nM, 100 nM and 1000 nM) or sitagliptin (48h; 0.01 mM, 0.1 mM, 1 mM, 10 mM and 100 mM). Enzyme expression was determined by western-blot analysis. DPP-4 enzymatic activity was determined in both cell extracts and conditioned media by measuring the cleavage of the substrate H-Ala-Pro-7-amido-4-trifluoromethylcoumarin.
RESULTS: DPP-4 was detected in both podocytes and tubular cells. DPP-4 level was significantly higher in the medium conditioned from tubular cells in comparison to podocytes one ($p<0.01$). Through a specific activity test performed on the conditioned medium, it has also been established that the enzyme released is still active, although the enzyme activity found in the medium is very reduced, about a 250 times lower for tubular cells and 100-fold for podocytes, in comparison to the relative cell extract. No significant difference in enzyme expression, activity or release was detected between primary tubular cells extracted from healthy subjects and those derived from diabetic subjects. The results showed that linagliptin and sitagliptin did not affect the release of the enzyme by kidney cells at any doses testes.
CONCLUSIONS: Renal cells likely have just a marginal role as source of the sDPP-4 detected in the urine, thus indicating that the main origin is extra-renal and remain to be clarified. In addition, our data show that the treatment with DPP-4 inhibitors does not affect the release of the enzyme.

INVESTIGATION ON THE MECHANISM OF ACTION AND TRANSLATIONAL POTENTIAL OF DESOGESTREL IN CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

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BACKGROUND: CCHS is a genetic disorder affecting the Autonomic Nervous System and central chemosensitivity, due to heterozygous mutations in the PHOX2B gene, a transcription factor that drives the development of the autonomic visceral circuits. CCHS patients display hypoventilation and severe impairment of the central chemoreflex, especially during sleep. No pharmacological intervention is currently available for treating the disease or its symptoms, and recently it has been proposed that hormonal treatment (progestins) may provide partial recovery of chemoreflex impairment, therefore opening the possibility for a relief of the respiratory symptoms and a reduction of risks of death during sleep in CCHS patients. Preliminary results indicate that systemic progestin treatment rescues the chemoreflex response in an animal model in which central chemosensitivity is impaired. We also showed, in vitro, that the progestin desogestrel down- regulates the expression of PHOX2B and some of its target genes. Ion channels, a key class of genes for the chemoreflex response, are putative PHOX2B target genes and their expression is affected by the presence of mutant PHOX2B proteins. Here, we show that the progestin desogestrel regulates the expression of ion channels that are targets of PHOX2B, thus increasing our knowledge on the cellular mechanisms associated with the expression of PHOX2B and its mutant form.
METHODS: ChIP-seq analysis in IMR32 neuroblastoma cell line identified many PHOX2B target gene candidates, among which ion channels. To study desogestrel effect on their expression we generated stably over-expressing human progesterone receptor (hPRG) SK-N-BE(2)C and IMR32 neuroblastoma cell lines. One clone from each cell line has been treated with 1 nM 3- ketodesogestrel, the active metabolite of desogestrel, for 24 hours, and the expression of ion channels compared by qPCR, using a custom panel of ion channels genes (RT² Profiler PCR Arrays, Qiagen), chosen according to the ChIP-seq analysis. The effect of mutant PHOX2B proteins on the expression of some ion channels genes has been measured in a CRISPR-CAS9 Knocked-down PHOX2B expressing IMR32 cells.
RESULTS: Consistent with the PHOX2B role as transcriptional regulator, it is reasonable to suppose that transcriptional dysregulation might be an important mechanism of CCHS pathogenesis. Indeed, PHOX2B mutant proteins increases the expression of potassium channel genes, leading to an altered electrical activity and therefore excitability of the cells. Desogestrel up-or down- regulate a group of ion channels genes in both cell lines.
CONCLUSIONS: Our results suggest that transcriptional dysregulation of ion channels genes plays a role in the onset of respiratory problems associated with CCHS. Restoring their expression and/ or activity to the normal physiological level may lead to amelioration of CCHS symptoms. The modulation of ion channels genes by desogestrel is indicative of the role of these genes in the recovery of chemosensitivity.

ANTAGONISM AT THE GLUCOCORTICOID RECEPTOR DIFFERENTLY AFFECTS ALCOHOL SELF-ADMINISTRATION IN MARCHIGIAN SARDINIAN ALCOHOL PREFERRING AND NON-PREFERRING WISTAR RATS

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BACKGROUND: Alcoholism is a chronically relapsing disorder characterized by high alcohol intake and a negative emotional state during abstinence, which contributes to excessive drinking and susceptibility to relapse. Stress and its related hormones of the hypothalamic-pituitary-adrenal (HPA) axis are implicated in alcohol dependence. For instance, alcohol intake activates the HPA axis, releasing cortisol in humans and corticosterone (CORT) in rodents. Dysregulation of the HPA axis and the consequent alterations in brain glucocorticoid receptors (GRs) expression accompany compulsive alcohol intake in rats. Considering the importance of stress in the transition to alcohol dependence and the role of GRs in escalated alcohol intake, we investigated GR system in Marchigian Sardinian alcohol preferring (msP) rats, a line of rats genetically selected for excessive alcohol drinking, potentially driven by the attempt to self-medicate from their innate negative affective state. These animals have an up-regulated corticotrophin releasing factor receptor 1 system making them highly sensitive to stress and relapse. We investigated the effect of pharmacological blockade of the GR system on alcohol self-administration (SA) in male and female msP rats compared to Wistar controls.

METHODS: Male and female msP and Wistar rats were trained to self-administer 10% (v/v) alcohol. When a stable self-administration baseline was achieved the effect of intraperitoneal pretreatment with the GR and progesterone receptor antagonist mifepristone (0.0, 10, 30 and 60 mg/kg) and the selective GR blocker CORT113176 (0.0, 10, 30 and 60 mg/kg) on alcohol SA was tested. The two drugs were tested in different groups of rats. To evaluate whether the effects of the two compounds were specific for alcohol, the drugs were tested on saccharin SA as well.

RESULTS: Systemic injection with mifepristone dose-dependently reduced alcohol self-administration in both male and female Wistar rats. Conversely, mifepristone treatment did not affect alcohol intake in male and female msP rats. The GR antagonist CORT113176 decreased alcohol SA in male and female Wistar rats and in female msP rats, confirming an involvement of GR in the observed effects. Mifepristone and CORT113176 showed no effects on saccharin intake.

CONCLUSIONS: Altogether these data suggest that msP rats are less sensitive to the effect of GR antagonists on alcohol drinking. Furthermore, drugs appear to be more efficacious in female than in male rats. In the attempt to evaluate if msP and Wistar are characterized by a different regulation of of glucocorticoid mechanisms experiments are ongoing to assess plasma CORT levels in these two rat lines. The effect of chronic treatment with CORT113176 on alcohol self-administration is also under investigation. This work is supported by grant NIH AA017447 (to MR and RC).

THE HEPATIC MICROENVIRONMENT PRIMES COLORECTAL CANCER CELL BEHAVIOUR MODULATING MIRNAS EXPRESSION

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BACKGROUND: Metastatic spread to the liver accounts for about one third of all distant metastasis locations in colorectal cancer (CRC). The refractoriness to the pharmacological treatment is determined in particular by the microenvironment in which the malignant cells coming from the primary tumour take root, develop and grow generating the metastases. miRNAs are considered to regulate metastatic potential of various cancer cells and could be prognostic indicators of disease and of pharmacological outcome. Here we investigated the potential of miRNAs as biomarkers aiding to forecast the metastatic spread and the outcome of the pharmacological treatment in an in vitro model of hepatic metastases from CRC.
METHODS: The model is based on the CRC cell line HCT-116 cultured in a hepatic microenvironment (HME) constituted by fibronectin as structural component of the liver extracellular matrix, and by soluble factors secreted by the IHH hepatic cell line (IHH-CM). To get insights on the interplay between tumour cells and the HME we have analysed the miRNAs expression profile through TaqMan Advanced miRNA Cards.
RESULTS: The results have been related with functional features and behaviour of CRC cells in the HME, and with the response to drugs usually used to treat CRC in clinical protocols such as 5-FU. Among the great deal of information derived from the analysis some findings seem to be particularly relevant. miR-29a-3p, miR-29b-3p and miR-29c-3p, which act as tumour suppressor genes and have anti-fibrotic activity, are down-regulated in HCT-116 cells exposed to the IHH-CM respect controls in standard culture medium (ST-M); their down-regulation was related to the increase of TGF-b production measured in the same cells by ELISA. Other tumour suppressor miRNAs modulating the TGF-b pathway, such as miR-15a-5p and miR-590-5p, are down-regulated in HCT-116 cells grown in the HME in comparison to controls in ST-M, suggesting this pathway as relevant to trace the metastatic molecular features of HCT-116 cells in our model. Connected with TGF-b is the NOTCH pathway that emerged as very significantly related to the alteration of the miRNAs expression detected in our analyses. NOTCH crosstalk with TGF-b is involved in fibrosis of many organs, including the liver where this process helps disseminated tumour cells to arrest and grow as a metastasis. In addition, NOTCH signalling can also modulate stemness and chemo-resistance to 5-FU through the involvement of miR-139-5p. Although this miRNA is only weakly modulated in HCT-116 cells exposed to IHH-CM, other miRNAs related to 5-FU sensitivity are found to be down-regulated (miR-17-5p, miR-149-5p, miR-375 and miR-450a-5p) and all together they could be related to the tendency for a lower 5-FU response recorded in HCT-116 in the HME.

SENSORIMOTOR AND CARDIORESPIRATORY ALTERATIONS INDUCED BY 1-CYCLOHEXYL-X-METHOXYBENZENE DERIVATIVES IN MICE: COMPARISON WITH TRAMADOL AND PCP

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BACKGROUND: 1-cyclohexyl-x-methoxybenzene is a novel psychoactive substance (NPS) first seized in Europe in 2012 as unknown racemic mixture of its three stereoisomers, 1- cyclohexyl-ortho-methoxybenzene (ortho), 1-cyclohexyl-meta-methoxybenzene (meta), and 1- cyclohexyl-x-methoxybenzene (para). Each of the stereoisomers has structural similarity with that of tramadol and the phencyclidine (PCP). The recent trend on the analgesic tramadol use led to the increase of its implication in overdose death in Europe. On the other hand, phencyclidine (PCP) is a potent hallucinogenic drug, representing a synthetic arylcyclohexylamine and has been involved in many cases of intoxications. The abuse of Tramadol and PCP can produce severe adverse effects, characterized by sensory changes with dissociative, out-of-body feelings and distorted visual and auditory perceptions. Cognitive changes, such as memory impairment, altered perception of time and slowness are common, as are affective changes, although quite labile, varying between euphoria, anxiety, apathy and irritability. In light of these structural analogies and based on the fact that both tramadol and PCP are substances of abuse that cause toxic adverse effects in humans, the aim of this study is to investigate the pharmaco-toxicological effects caused by each of these 1-cyclohexyl-x-methoxybenzene derivatives, sensorimotor (visual placing, acoustic responses) and cardiorespiratory functions in CD-1 male mice and to compare them with those caused by tramadol and PCP. Moreover, we used naloxone to investigate the involvement of the opioid system in the appearance of pharmaco-toxicological effect of 1-cyclohexyl-xmethoxybenzene compounds.
METHODS: Adult male CD-1 mice, weighing from 25 to 30 grams, were used for this experiment. The effect of 1-cyclohexyl-x-methoxybenzene derivatives and tramadol was investigated using a battery of behavioral tests widely used in studies of "safety-pharmacology" for the preclinical characterization of new molecules in rodents. All drugs were injected by intraperitoneal administration at a volume of 4 ul/g. To investigate possible similarities between 1-cyclohexyl-xmethoxybenzene derivatives and PCP, we reported data from PCP (10 mg/kg) in the comparative figures of maximal effects. We used for the sensorimotor tests (visual placing and acoustic response) the range dose (0.1, 1, 10 and 100 mg/kg) for the three different 1- cyclohexyl-xmethoxybenzene derivatives and tramadol. For cardiorespiratory studies, we used the highest dose of 1-cyclohexyl-x-methoxybenzene derivatives (100 mg/

kg), tramadol (100 mg/kg) and PCP (10 mg/kg). For the antagonism studies, we used naloxone (6 mg/kg) for each treatment including antagonism with PCP (10 mg/kg). All experiments were performed between 8:30 AM and 2:00 PM. Experiments were conducted in blind by trained observers working together in pairs. The behavior of mice was videotaped and analyzed off-line by a different trained operator that gave test scores. Visual Placing response test was performed using a tail suspension modified apparatus able to bring down the mouse towards the floor at a constant speed of 10 cm/sec. Briefly, CD-1 mice were suspended 20 cm above the floor by an adhesive tape that was placed approximately 1 cm from the tip of the tail. The downward movement of the mouse was videotaped by a camera placed at the base of the tail suspension apparatus. Acoustic reflex was performed using four acoustic stimuli of different intensities and frequencies. Each sound test was repeated three times. A score of 1 was given if there was a response and a score of 0 was given if there was no response, for a total score of 3 for each sound. Cardio-respiratory parameters (heart rate, breath rate, oxygen saturation and npulse distention) were measured through the software MouseOx Plus in freely moving mice, monitored by a sensor collar applied around their neck and data was recorded for 5 h.

RESULTS: Systemic administration (0.1-100 mg/kg) of ortho, meta, para and tramadol reduced in a dose dependent manner the visual placing response in mice and the effect persisted up to 5 h at highest doses. Comparison of the maximal effect among 1-cyclohexyl-x-methoxybenzene derivatives, tramadol and PCP revealed significant differences among the effects of these compounds. In particular, PCP at 10 mg/kg was the most powerful compound in inducing the inhibitory effect on the visual placing responses ($p < 0.05$). Moreover, tramadol at 100 mg/kg was more effective in respect to para on visual placing impairment ($p < 0.05$). Pre-treatment with naloxone (6 mg/kg) partially prevented the inhibitory effect induced by ortho, meta and tramadol, while the effect of PCP was naloxone-insensitive. In addition, ortho, meta, para and tramadol reduced in a dose-dependent manner the acoustic response in mice and the effect persisted up to 5-hours at highest doses. Ortho, meta and tramadol inhibited the acoustic responses already at the dose of 1 mg/kg, while the para was effective starting from 10 mg/kg. The inhibitory effect caused by ortho and meta at the 100 mg/kg dosage was significant already after 10 min from the administration of the compounds. Otherwise, para and tramadol at 100 mg/kg were effective 60 min after their administration. Comparison of the maximal effect among 1-cyclohexyl-xmethoxybenzene derivatives, tramadol and PCP revealed significant differences among the effects of these compounds. In particular, ortho, meta and tramadol at 1 mg/kg were more effective in inhibiting the acoustic responses ($p < 0.05$) in mice. Pretreatment with naloxone 6 mg/kg did not prevent the inhibitory effect induced by ortho, meta, para, tramadol and PCP. Finally, the administration of the 1-cyclohexyl-x-methoxybenzene derivatives (100 mg/kg), tramadol (100 mg/kg) and PCP (10 mg/kg) affected the basal Breath Rate (BR) in mice. In particular, BR was transiently increased by ortho (max effect ~ +38% of basal values at 30 min) and meta (max effect ~ +21% of basal values at 15 min), but not para administration, while BR was transiently reduced by tramadol (max effect ~ -40% of basal values at 60 min) and PCP (max effect ~ -30% of basal values at 30 min). Pre-treatment with naloxone 6 mg/kg completely prevented bradypnea induced by tramadol, but was ineffective in blocking the effects caused by ortho, meta and PCP administration. The administration of tramadol (100 mg/kg) and PCP (10 mg/kg), but not 1-cyclohexyl-x-methoxybenzene derivatives (100 mg/kg) affected the basal SpO2 in mice. In particular, SpO2 was transiently reduced by tramadol (max effect ~ -20 % of SpO2 saturation at 30 min) and PCP (max effect ~ -19% of SpO2 saturation at 30 min) and their inhibitory effects disappeared at 60 min from compound

administration. Pre-treatment with naloxone (6 mg/kg) completely prevented the reduction of SpO2 induced by tramadol, but was ineffective in blocking the effect caused by PCP administration. The administration of the 1-cyclohexyl-x-methoxybenzene derivatives (100 mg/kg), tramadol (100 mg/kg) and PCP (10 mg/kg) affected the basal Heart Rate (HR). In particular, HR was transiently but maximally increased at 30 min by ortho (max effect ~ +25% of basal values), meta (max effect ~ +37% of basal values), para (max effect ~ +15% of basal values) and tramadol (max effect ~ +35% of basal values), while HR was mild and transiently reduced by PCP (max effect ~ -30% of basal values at 30 min). The HR facilitation persisted for the 1-cyclohexyl-x-methoxybenzene derivatives up to 120 min, while for tramadol up to 180 min. Pretreatment with naloxone 6 mg/kg did not prevent the increase of HR in mice induced by 1- cyclohexyl-x-methoxybenzene derivatives, tramadol and PCP administration. The administration of the 1-cyclohexyl-x-methoxybenzene derivatives (100 mg/kg), tramadol (100 mg/kg) and PCP (10 mg/kg) reduced the basal Pulse distention (Pd) in mice. In particular, Pd was maximally reduced at 30 min by ortho (max effect ~ -35% of basal values at 60 min), meta (max effect ~ -33% of basal values at 60 min), para (max effect ~ -25% of basal values at 30 min), tramadol (max effect ~ -30% of basal values at 15 and 30 min) and PCP (max effect ~ -25% of basal values at 30 min). Thereduction of Pd persisted for the ortho and meta up to 180 min, for tramadol up to 120 min and for para and PCP up to 60 min. Pre-treatment with naloxone 6 mg/kg did not prevent the reduction of Pd in mice induced by 1-cyclohexyl-x-methoxybenzene derivatives, tramadol and PCP administration.

CONCLUSIONS: The present study demonstrates that acute administration of the ortho, meta and para stereoisomers (0.1-100 mg/kg) impaired visual placing and acoustic responses and altered cardiorespiratory responses in the mouse in some cases with a similar profile to that of tramadol (0.1-100 mg/kg) and PCP (0.01-10 mg/kg). Naloxone (6 mg/kg) administration partially prevented the visual sensorimotor impairments caused by ortho, meta and para stereoisomers. While, the effects of tramadol are prevented (respiratory depression), partially prevented (visual placing) or not prevented (acoustic response, hearth rate and pulse distension) by the administration of naloxone. All PCP effects were naloxone-insensitive. The present data show that 1-cyclohexyl-xmethoxybenzene derivatives caused pharmaco-toxicological effects by activating both opioid and non-opioid mechanisms and suggest that their use could potentially lead to abuse and bodily harm.

EFFECTS OF AMINO ACID SUPPLEMENTATION IN A MOUSE MODEL OF HIND LIMB UNLOADING-INDUCED MUSCLE ATROPHY

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BACKGROUND: Skeletal muscle atrophy is a major consequence of various pathophysiological conditions, including neuromuscular disorders, disuse, aging and other chronic wasting diseases. Basically, a disequilibrium between anabolic/catabolic pathways leads to progressive loss of muscle mass and functional impairment. Branched Chain Amino Acids supplements (BCAAs; leucine, isoleucine, valine) are potentially useful to contrast muscle atrophy, since they account for 35% of muscle essential AAs and exert anabolic actions on protein synthesis. Here, we investigated the possibility to support BCAAs' action in muscle atrophy, by combining them with DFL24415, a newly-synthesized dipeptide – under patent confidentiality – thought to be involved in BCAAs catabolism and muscle protein balance.
METHODS: To this aim, a 4-week-treatment with an oral formulation of BCAAs (2:1:1) plus 33% DFL24415 (mix final dose: 984 mg/kg in drinking water) was performed on mice subjected to hind limb unloading (HU), a model of disuse leading to severe atrophy, dysfunction and slow-to-fast fiber-type shift in postural soleus (SOL) muscle. 10-week-old male C57BL/6J wild type mice were treated for 4 weeks in total (T0–T4) and underwent HU during the last 2 weeks (T2–T4). The outcome of HU/treatment was assessed on relevant in vivo and ex vivo readouts in comparison to untreated HU and non-HU mice.
RESULTS: At T2 (pre-HU), all mice groups had comparable body weight (BW). At T4, both untreated and treated HU mice had significantly lower BW compared to T2 values (-9.7% and -8.2%, respectively) and to age-matched non-HU mice (-17.3% and -13.4%, respectively). Ex vivo, untreated HU mice (n=6) had severely impaired SOL muscle contractile function compared to non-HU mice (n=8), as shown by the significant decrease of isometric twitch time-to-peak kinetic parameter (TTP; 28.22±2.7 vs 38.6±1.9 ms, $p<0.007$ by Student's t test) and of twitch and tetanic forces (sPtw: 14.9±2 vs 24.4±1.1, $p<0.0004$; sP0: 72.9±9.8 vs 179.8±3.2 kN/m2, $p<6.1*10-8$). This was paralleled by an alteration of SOL muscle elastic properties, measured as stiffness during eccentric contraction. Treated HU mice (n=6) showed a remarkable slow-down of TTP compared to untreated ones (+24%) and a significant improvement in sPtw (+43%, 21.3±1.5 kN/m2, $p<0.02$), sP0 (+62%, 117.9±15.2 kN/m2, $p<0.002$) and in eccentric contraction stiffness. Untreated HU mice also showed a significant decrease in SOL mass/BW vs non-HU and a reduction in muscle total protein, which were partially counteracted by treatment. In HU mice, a notable decrease of salivary IgA levels suggested a HU-related negative immune response modulation. Treated HU mice showed a remarkable recovery of this index (recovery score: 96.6%).
CONCLUSIONS: Overall, we collected first evidence that combining BCAAs and dipeptide DFL24415 could be effective in protecting from skeletal muscle atrophy. Ongoing histological, biochemical and molecular biology analyses will clarify the underlying mechanism for the observed benefit. [Supported by FARMIDIAB Project - Dompé farmaceutici S.p.A.].
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THE LOW-DENSITY LIPOPROTEIN RECEPTOR (LDL-R) IS AN IMMUNE-METABOLIC CHECKPOINT DURING CD8 T LYMPHOCYTES ACTIVATION

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BACKGROUND: Activation of T lymphocytes combines functional to metabolic rewiring of cell machinery, including cholesterol homeostasis. Here we evaluated the role of LDLR, as a key regulator of cholesterol import from blood to cellular compartment, on T cell biology.
METHODS: Immunophenotypic characterization of T cells from WT and LDLR KO mice was performed in vitro (anti-CD3/CD28) and in vivo (vaccination, homeostatic proliferation in Rag2 KO mice adoptively transferred with Tcells from LDL-R KO and WT mice) coupled to proteomic and seahorse analysis on isolated T cells. In parallel, T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.
RESULTS: LDLR mRNA expression increased after in vitro activation of CD8+, but not CD4+ T cells, suggesting a different regulation of cholesterol homeostasis between T cell subsets. Functionally, deficiency of LDLR mainly dampened CD8+ proliferation (-35%, $p<0.01$) paralleled by a reduction in INFγ production (-39.6%, $p<0.01$). In vivo antigen-specific activation by ovalbumin vaccination, but not homeostatic proliferation, resulted in a decreased proliferation and cytokines production (↓IFNγ $p<0.001$, ↓IL13 $p<0.01$, ↓perforin $p<0.05$) in CD8+ of KO mice. In addition, markers of rapid activation, such as CD69 (-32%, $p<0.01$), and AKT phosphorylation, a downstream molecule of the TCR, were decreased in KO CD8+ compared to WT, an effect that could partially depend on altered lipid homeostasis in CD8+ T cells as suggested by reduction in neutral lipids and lipid rafts staining. Finally, proteomic and seahorse analysis pointed out metabolic defects with reduction of both glycolytic and OXPHOS metabolism, thus linking functional to metabolic alteration in CD8+ T cells from LDLR KO compared to WT mice.
When tested in humans, CD8+ T cells from FH patients proliferated less (-36%, $p>0.05$) compared to sex- and age-matched controls; in addition, when CD8+T cells from FH vaccinated for seasonal influenza were tested in vitro with virus-derived peptides, presented a decreased granzyme production (-60.3%, $p<0.01$) compared to CD8+T cells from vaccinated controls, indicating a reduced CD8 effector response to virus infection.
CONCLUSIONS: LDLR plays a critical role in regulating the immunometabolic responses in CD8+ Tcells, and thus might represent a checkpoint linking cellular cholesterol metabolism to adaptive immune response. Pharmacological increase of LDLR expression may therefore enhance CD8 effector activation and be beneficial under pathological conditions associated to a failure of cytotoxic response, such as virus infection.

AGE-ASSOCIATED MORPHOLOGICAL CHANGES IN ASTROCYTES OF THE DORSAL DENTATE GYRUS: POTENTIAL RELEVANCE IN COGNITIVE DECLINE?

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BACKGROUND: In the adult brain, astrocytes exert a plethora of key functions. Indeed, they provide trophic support to neurons, participate in synaptic function and plasticity, mediate uptake and release of neurotransmitters, compose and regulate the blood–brain barrier. Moreover, in the hippocampal Dentate Gyrus (DG), astrocytes actively modulate adult neurogenesis (AN), a peculiar form of neuroplasticity which is involved in cognition and is deregulated in aging and several neurodegenerative diseases.
Astrocytes are also extremely heterogeneous in terms of molecular, structural and functional features. As of today, very little is known on the possibility that their heterogeneity may account for selective vulnerability of specific brain areas to age-related cognitive decline and neurodegeneration.
We decided to evaluate aging effects on adult murine astrocytes within different subfields associated with hippocampal AN and age-related cognitive decline.
METHODS: Hence, We performed a semi- automated morphological analysis of GFAP+-astrocytes in the Granule Cell Layer (GCL), Molecular Layer (ML), Hilus and Stratum Lacunosum Moleculare (sLM) of young (6-mo old, n= 4) and middle- aged (14-mo old, n= 5) C57Bl/6 male mice. Additionally, we evaluated changes in astrocyte morphometry along the DG dorso-ventral axis.
RESULTS: In the dorsal GCL, ML and sLM we observed an increased morphological astrocytic complexity in middle-aged compared to younger mice. Interestingly, no difference was observed in the Hilus between the two experimental groups. Worth of note, unlike what observed in the dorsal hippocampus, in ventral GCL and ML astrocytes did not display any age-related morphological changes. Entorhinal Cortex (EC) was also included since it represents the major cortical input to dorsal DG. Interestingly, EC astrocytes underwent remarkable atrophy in aging. Since dorsal, and not ventral, hippocampus is involved in cognitive functions, these findings appear worth of further evaluation.
CONCLUSIONS: Herein we describe, for the first time, remarkable heterogeneity in the astrocytic response to aging in the dorsal, and not ventral, hippocampus and in different subfields within dorsal DG. Our findings provide an additional level of complexity in the structural changes associated with brain aging. Future work will attempt to address if these region-specific changes may also occur in relevant animal models of neurodegeneration, their correlation with cognitive performance and their response to drugs.

FUNDINGS: This work was partially supported by PRIN 2017 and Fondazione Generali to MG. HB held a research fellowship (Bando Fondazione CRT, ID 393) supported by University of Piemonte Orientale.

NEURONS VS. ASTROCYTES: BIOENERGETIC SIGNATURE OF THE PRE- AND PERI-SYNAPTIC COMPARTMENTS IN AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neurodegenerative disease due to a progressive degeneration of cortical, brainstem, and spinal motor neurons. The etiology is still largely obscure and several mechanisms have been proposed for neurodegeneration, including mitochondrial dysfunction. Based on our previous results indicating abnormal exocytosis in the SOD1G93A mouse model of ALS, in this work, we characterized the aerobic metabolism and the glucose catabolism in two specific compartments actively involved in neurotransmission, the pre-synaptic district and the peri-synaptic astrocyte processes.

METHODS: Synaptosomes and gliosomes were used as models of the presynaptic and perisynaptic compartments, respectively. Both were purified motor from cortex (MC) and spinal cord (SP) of presymptomatic (30 and 60 days) or symptomatic (90 and 120 days) SOD1G93A mice, by homogenization and centrifugation on Percoll gradient. ATP production, oxygen consumption, respiratory complex, glycolytic and the Krebs cycle enzymes activities, lactate fermentation, and lipid peroxidation were investigated by spectrophotometric, oximetric and enzymatic assays.
Protein expression level was determined by western blot and the morphological characteristics of mitochondria by electron microscopy.

RESULTS: ATP/AMP ratio was lower in synaptosomes of SOD1G93A mice vs. controls. The energy deficiency was linked to the impairment of the oxidative phosphorylation (OxPhos) machinery and to increment of lipid peroxidation. These metabolic dysfunctions were present already from the pre-symptomatic stages of the disease in SP and at the symptomatic stages in MC and did not depend on the number of mitochondria or OxPhos protein expression. To understand whether the mitochondrial impairment might be a consequence of upstream metabolic damages we analysed the pathways involved in glucose catabolism. Unexpectedly, SP and MC synaptosomes from SOD1G93A mice displayed higher activity of the glycolytic enzymes hexokinase and phosphofructokinase and of the Krebs cycle enzymes citrate synthase and malate dehydrogenase. Lactate dehydrogenase activity was not modified. Differently from nerve terminals, gliosomes showed a reduction of the ATP/AMP ratio only in SP of SOD1G93A mice and at the late stages of the disease. Gliosomes also showed a precocious increment of oxidative stress, even in the absence of impairment in OxPhos activity. The glycolysis and the lactate dehydrogenase activities, but not the Krebs cycle, were increased in SP and MC gliosomes of SOD1G93A mice.

CONCLUSIONS: Our results suggest that pre-synaptic neuronal district present profound energy metabolism dysfunctions in ALS. Changes in the PAPs compartment seem sub-

ordinated to neuronal damage. The metabolic modifications of the glycolytic pathways may represent an attempt to restore altered energetic balance and indicate mitochondria as the main site of bioenergetic impairment for possible therapeutic intervention.

EPTRI, A PAEDIATRIC-DEDICATED RESEARCH INFRASTRUCTURE TO PROMOTE THE AVAILABILITY OF SAFER AND EFFICACIOUS DRUG FOR CHILDREN

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BACKGROUND: Minors cannot be compared to adults as they are continuously developing and their metabolism is changing over time. While they comprise 20% of the European population, only 30% of medicines have paediatric authorization. As a consequence, medicines are often used off-label exposing children to the risk of receiving non-efficacious treatments or developing unforeseen adverse drug reactions. To overcome this severe gap, EPTRI consortium put together all the available competences and technologies useful to enhance research in paediatric medicines from early drug discovery up to clinical phase to develop a new Research Infrastructure (RI) dedicated to translational paediatric research.
METHODS: EPTRI has been funded as a Design Study in the Horizon2020 Program under the INFRADEV-01-2017 call for proposals and has been characterised by 3 main phases. A Context Analysis phase, based on a broad survey, was aimed to map and analyse the needs of the potential users and the potential service providers to be included in the future RI, as well as the possible gaps to be covered. An Operational phase to design the different components of the new RI, including the governance and business model, the strategies for interaction with national Authorities, the ways of collaboration with the existing RIs, the IT-architecture,the operational models and the services to be provided. Finally, a Feasibility phase has been runto test and evaluate the operations of the future Research Infrastructure.
RESULTS: A Conceptual Design Report has been prepared as main final outcome of the project by the members of the Consortium describing the structure and services of the developing RI (Figure attached). EPTRI has been designed as a distributed RI with a Hub and Spokes model to connect many different re-

search units (RUs) dislocated across Europe and presenting their services through a Single Access Point. 259 RUs, identified during the context analysis phase, candidate as EPTRI providers from 29 EU/non EU countries, were distributed in 4 Thematic Research Platforms: paediatric medicines discovery; paediatric biomarkers and biosamples; developmental pharmacology; paediatric medicines formulations. A catalogue of centralised, integrated, common services has been described on the basis of the survey results that have highlighted, on one hand the needs and gaps encountered more frequently by the research community, and on the other hand the available expertise, resources, facilities existing in Europe and beyond. The RUs have been also organised in EPTRI National Nodes which represent a stable association between all institutions at any national level. Currently the most advanced node is the EPTRI-IT composed of more than 20 Institutions.

CONCLUSIONS: EPTRI as a new establishing European wide Infrastructure will ensure the provision of safe and efficacious medicines to European patients and will implement in the paediatric field innovative methods and technologies which have not been already included in the routine process to develop new drugs and medical devices.

PROSOCIAL EFFECTS OF CANNABIDIOL IN MICE

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BACKGROUND: Cannabidiol (CBD) is the second most abundant phytocannabinoid in Cannabis sativa after trans-Δ9-tetrahydrocannabinol, and it is devoid of psychoactive effects. Interestingly, it represents a potential pharmacotherapy for treating symptoms of various neuropsychiatric disorders such as addiction, anxiety and psychosis, disorders of motility. Recently, CBD has been authorized by the FDA to treat some rare forms of epilepsy and many trials have begun for the treatment of social deficit in patients with autism spectrum disorders. However, despite the therapeutic utility of CBD, its specific pharmacological mechanism remains not entirely clear. Indeed, CBD in addition to interacting with the endocannabinoid system (ECS) can also act on serotonin, adenosine, dopamine and opioid receptors behaving as a multi-target drug. In this study, at first, we confirmed the prosocial effect of CBD in mice; then, we demonstrated that this effect is oxytocin dependent. Finally, we tried to define the mechanism of action of the prosocial effects of CBD, mediated by the oxytocin pathway.

METHODS: CBD effect on social behavior has been tested in vivo on wild-type adult mice. Mice were exposed to intragastric administration of cannabidiol oil (20 mg/kg) or vehicle (MCT oil) for 2 weeks. Oxytocin receptor antagonist, L-371,257 (Tocris Bioscience, Bristol, UK) was intranasally administered (300 μg/kg). L-371,257 were administered 30 min before behavioral testing. Subsequently, mice were sacrificed and brain areas were isolated for molecular and chemical analysis in order to measure endocannabinoid and oxytocinergic tone. In order to define the molecular mechanisms of CBD, in vitro tests have been performed. BT-20 (human mammary gland/breast carcinoma) and HOS T85 (human osteoblast-like cell line) were used. In particular, we investigated the crosstalk between oxytocin and endocannabinoids.

RESULTS: A sub-chronic treatment (14 days) with CBD oil increased social interactions in

wild type mice. Furthermore, it induced an increase in mice communication. Moreover, we found that cannabidiol treatment increases oxytocinergic and endocannabinoid tone in several mice brain areas and that this action is blocked by oxytocin antagonist. We also demonstrated that the prosocial effect of CBD is mediated by the TRPV2 receptor.

CONCLUSIONS: These data show that CBD treatment is able to increase social behavior in mice. This effect is mediated by oxytocin and depends on the activation of the TRPV2 receptor. Due to both the social behavior and the oxytocin pathway alterations present in autism spectrum disorders patients, the use of CBD could represent a new interesting therapeutic strategy.

ZINGIBER OFFICINALE ROSCOE RHIZOME EXTRACT ALLEVIATES NEUROPATHIC PAIN IN MICE BY REDUCING NEUROINFLAMMATION THROUGH THE INHIBITION OF CLASS I HDAC ISOFORMS

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BACKGROUND: Current therapies for neuropathic pain are generally symptomatic and possess several side effects, limiting their prolonged usage. Thus, it is urgent to develop novel and safe candidates for the management of this chronic condition. For this purpose, we investigated the analgesic effect of a Zingiber officinale Roscoe extract (ZOE), standardized in gingerols and terpenes, in a mice model of peripheral neuropathy. We also explored the mechanism of action of ZOE and its main constituents using an in vitro model of neuroinflammation. HDAC has a pivotal role in the development of neuropathic pain, thus, we also investigated the ability of ZOE and its constituents to inhibit HDAC isoforms expression and activity.

METHODS: Peripheral mono-neuropathy was induced in mice, by spared nerve injury (SNI). The analgesic effect of ZOE after oral administration was assessed by measuring mechanical and thermal allodynia in SNI mice. The mechanism of action of ZOE and its main constituents was investigated using spinal cord samples and an in vitro model of neuroinflammation by ELISA, western blotting and immunofluorescence techniques.

RESULTS: Oral administration of ZOE 200 mg kg-1 ameliorated mechanical and thermal allodynia in SNI mice, with a rapid and a long-lasting effect without altering locomotor activity. In BV2 cells and spinal cord samples, ZOE modulated MAPKs activation and HDAC expression. The activity on HDAC was found to be selective for class I isoforms and mainly mediated by the terpenes fraction. The anti-inflammatory effect of ZOE and its constituents led to a neuroprotective effect on inflammation-impaired SH-SY5Y cell viability.

CONCLUSIONS: The oral administration of ZOE attenuated SNI-induced neuropathic pain symptoms by reducing spinal neuroinflammation, suggesting ZOE as a novel and interesting candidate for the management of neuropathic pain.

CHRONIC ETHANOL EXPOSURE INDUCES NEUROPATHIC PAIN IN MICE BY PROMOTING NEUROINFLAMMATION

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BACKGROUND: Alcohol use disorder (AUD) is defined by the emergence of negative affective symptoms during withdrawal. In particular, chronic or excessive alcohol consumption results in a hypersensitivity condition, that is considered a negative reinforcement process, facilitating AUD progression. The mechanisms responsible for the observed increasing of chronic pain associated with chronic alcohol consumption are still unknown. Neuroinflammation is a key contributor to alcohol reinforcement and it is well known to play a key role also in the pathogenesis of neuropathic pain. Thus, the aim of this project was to evaluate the development of neuropathy in a mouse model of alcohol dependence, and to investigate the role of neuroinflammation in this chronic condition.
METHODS: We used the chronic-intermittent ethanol two-bottle choice (2BC-CIE) paradigm to generate ethanol-dependent (Dep) mice exhibiting escalations in alcohol drinking after CIE vapor exposure, non-dependent (Non-Dep) mice with the same voluntary alcohol drinking history and ethanol-naive (naive) control C57bl/6J mice. Von Frey filaments were used to measure the mechanical allodynia in naive, Non-Dep and Dep mice during the 72 h of withdrawal and immediately after 2-bottle choice test. In vitro evaluations of microglia activation in spinal cord tissue of mice were also conducted.
RESULTS: A significant drinking escalation in the Dep group was observed in both sexes, compared to Non-Dep mice. Both male and female Dep mice developed a strong mechanical allodynia during the 72 h of withdrawal, which was completely reverted immediately after voluntary 2BC test. Also, the Non-Dep group showed a long-lasting increase of sensitivity compared to the naive group, which was not reverted by self-alcohol administration. An increase of microglia activation markers in the spinal cord tissue of 2BC-CIE were observed.
CONCLUSIONS: These results suggested that chronic alcohol exposure induced abstinence-related hyperalgesia in Dep mice, compared to Non-Dep and naive mice. Moreover, in our model we observed that increased hypersensitivity occurs in 40% and 50% of Dep male and female mice, respectively, compared to the naive group, highlighting that the chronic consumption of alcohol may also induces a direct alcohol-related neuropathy. Neuroinflammation, and in particular the role of microglia activation, may represent an interesting target for the management of this chronic pain condition.

EVALUATION OF THE ANTIPYROPTOTIC ACTIVITY OF NEW INFLAMMASOME INHIBITORS

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BACKGROUND: The inflammasome, an intracellular multiprotein complex responsible for the coordination of the innate immune response, plays a fundamental role in defending the body from potential threats. The activity of the inflammasome relies on the activation of caspase 1, a proteolytic enzyme that induces the cleavage and the release of interleukin 1β (IL-1 β) and IL18 and causes the cell death through pyropotosis. Numerous inflammasome variants have been identified, among which the absent inflammasome in melanoma 2 (AIM2), the NLRC4, NLRP1 and NLPR12 inflammasome and the extensively studied NLPR3 inflammasome. The fine regulation of inflammasome makes it a central player in the pathophysiology of numerous autoimmune and inflammatory diseases such as type 2 diabetes, gout, obesity, atherosclerosis, cryopyrinopathies, chronic inflammatory bowel diseases but also Alzheimer's and Parkinson's disease [1]. The aim of the present study was to evaluate new inhibitors of inflammasome NLRP3, synthesized by the SynBioMed group of the Department of Drug Science and Technology; the novel series of compounds was designed by taking as a template the compound INF39, which has demonstrated good pharmacological and toxicological properties [2].

METHODS: THP-1 cell line, human monocytes derived from an acute monocytic leukemia patient, was used to study if these compounds, used at a concentration of 10μM, were able to reduce inflammasome activation induced by treating the cells with LPS first, and then with ATP (5mM) or MSU (200μg/ml). Levels of lactate dehydrogenase (LDH), a cytosolic protein released in the extracellular space during pyroptosis, were evaluated using the Non-Radioactive CytoTox96® Cytotoxicity assay, while IL-1β concentrations were quantified through an enzyme-linked immunosorbent assay. MCC950, an established NLPR3 inhibitor [3], was used as control. Finally, the cytotoxicity of these inhibitors was evaluated after 72h of treatment through the MTT assay.

RESULTS: Compounds tested are not cytotoxic at 10μM concentration used in pyroptosis assays. The maximum inhibition of LDH release following ATP stimulation is about 45%; the same compounds are able to reduce IL-1β release by about 20-30%.

CONCLUSIONS: Future studies are required in order to perform a more accurate characterization of the anti-pyropototic activity of the novel series of compounds and to modulate their structures to increase their ability to inhibit NLPR3 inflammasome.

1) Awad et al. Pharmacol Ther 2018, 187:133-49
2) Cocco et al. J Med Chem 2014, 57:10366-82
3) Coll et al. Nat Med 2015, 21(3):248-55

HYALURONAN AS POTENTIAL MODULATOR OF THE GUT NEURO-IMMUNE FUNCTION AFTER INTESTINAL ISCHEMIA/REPERFUSION INJURY

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BACKGROUND: Hyaluronan (HA), an extracellular matrix glycosaminoglycan component, appears to be involved in the pathogenesis of chronic inflammatory bowel disease (IBD) by modulating toll-like receptor (TLR) pathways. Recently, HA was shown to maintain enteric neuron homeostasis by forming a well-structured perineuronal net, which is altered during inflammation. Since IBD often includes episodes of ischemia, in this study we aimed to assess the role of HA in the morphology and activity of the rat small intestine neuromuscular compartment after ischemia reperfusion (I/R).
MATERIALS AND METHODS: In vivo I/R injury was induced by clamping the superior mesenteric artery for 60 min, followed by 24 hours of reperfusion in adult male Wistar rats (300-350g), after general anesthesia. In some experiments, the HA synthesis inhibitor, 4-methylumbelliferone (4-MU, 25mg/kg), was intraperitoneally administered to normal (CTR), sham-operated (SH) and I/R animals 24 h before euthanasia.
RESULTS: In the I/R group, treatment with 4-MU dampened the marked increase of HA levels and density index of fluorescent HA binding protein (HABP) staining as well as the higher mRNA levels of the functional HA synthase, HAS2, in the small intestine neuromuscular and submucosal compartments. The increased number of neutrophils infiltrating the muscularis propria, myenteric ganglia and submucosal layer in the I/R group was significantly reduced by 4-MU treatment. In longitudinal muscle myenteric plexus (LMMP) preparations and in the submucosal layer of I/R rats TLR2 mRNA levels significantly increased and were reduced by 4-MU treatment. In LMMP but not in submucosal preparations TLR4 mRNA levels increased and were reduced by 4MU. The efficiency of the GI transit, measured as geometric center of non-absorbable FITC-dextran, was significantly reduced in the I/R group and was further reduced by 4-MU. In the I/R group, carbachol- and electrical field- (EFS, 0.1-40 Hz) stimulated contractions and EFS-induced (10 Hz) non-cholinergic non-adrenergic (NANC) relaxations were reduced with respect to both CTR and SH groups. I/R-mediated inhibition of EFS contractions, but neither of CCh-induced contractions nor of NANC relaxations, was abolished by 4-MU treatment.
CONCLUSIONS: Our data suggest that I/R injury increases HA levels in the neuromuscular and submucosal compartments of the rat small intestine which may depend on HAS2 transcription modulation and involves HA-mediated changes in TLR2 and TLR4 expression. During I/R, HA influences the GI transit mainly by regulating excitatory enteric pathways. Changes in gut HA homeostasis may have a role in development of enteric motor dysfunction after I/R.

ACTIVITY-BASED ANOREXIA ALTERS REWARD-RELATED MECHANISMS IN THE RAT NUCLEUS ACCUMBENS: FOCUS ON THE GLUTAMATERGIC SYNAPSE

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BACKGROUND: Anorexia nervosa (AN) is a complex mental illness characterized by restricted eating, an intense fear of gaining weight, body weight below 85% of expected body mass index and strenuous exercise regimens. AN begins with a restrictive diet and weight loss and progresses to an out-of-control spiral. At the neurobiological level, the imbalance between cognitive and reward networks likely interferes with motivation for treatment and ability to learn from experience, unabling patients to stop the vicious cycle of the disease. However, little is known about its aetiology or predictive factors. In order to identify molecular signatures of AN history in the brain, we evaluated if the combination of food restriction and intense exercise, in the activity-based anorexia (ABA) rat model, drives weight loss seeking during adolescence through enduring changes in the developing brain, altering the functionality of the synapses in the nucleus accumbens (NAc).
METHODS: Female adolescent Sprague-Dawley rats at postnatal day (P) 35 were individually housed and divided in two groups: controls (CTRL, food ad libitum–sedentary) and ABA (food restricted and free access to an activity wheel). On P38, food access for the ABA group was limited to 2 h per day but unlimited in amount, at the beginning of the dark cycle, till P42, when all ABA rats reached the anorexic phenotype. At the scheduled time, i.e. on P42, during the acute phase of the pathology, and on P49, after 7 days of weight recovery, animals were sacrificed and the NAc was dissected. Western blot analyses were run on protein extracts.
RESULTS: After 24 hours of AN induction, ABA rats reduced body weight and constantly increased wheel activity over days, as expected. Notably, ABA rats show increased mean and maximum speed during wheel activity at P40 and P41, a readout of their motivation to engage in intense physical activity. At the molecular level, in the membrane fraction of the NAc of ABA rats we found an increased GluA1/GluA2 AMPA receptor ratio both in the acute phase and after seven days of weight recovery. NMDA receptor levels, measured as GluN2A/GluN2B ratio, were increased at P42, while reduced at P49. Moreover, the expression levels of both PSD95, index of synaptic integrity, and DAT, the transporter responsible for dopamine reuptake, are reduced only after weight recovery.
CONCLUSIONS: Taken together, these results reveal that the induction of the anorexic phenotype dysregulates the glutamatergic synapse in the NAc, suggesting that these molecular determinants of maladaptive plasticity could represent a signal of altered processing of food reward and that might be, in turn, the trigger for the motivational mechanisms underlying AN. Furthermore, the recovery of body weight is not necessarily an index of newfound well-being since, in the brain, glutamatergic homeostasis is still altered.

CYTOFLUORIMETRIC ASSAY TO INVESTIGATE INTERPATIENT VARIABILITY ON BLINATUMOMAB RESPONSE: AN IN VITRO PROOF-OF-CONCEPT

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BACKGROUND: Relapsed/refractory acute lymphoblastic leukemia (ALL) represents a remarkable challenge for physicians. However, promising rescue therapy comes from the T-cell engager antibody blinatumomab that forces mature CD3+ T-lymphocytes and leukemic CD19+ B-cells into close proximity creating a tight cell-specific granzyme B -mediated cytolytic synapsis. Aim of the study is to provide a "proof of concept" that the CD19 surface expression regulated by the genetic status of PAX5, one of the most often mutated genes in B-ALL, could affect the blinatumomab response.
METHODS: Two human B-ALL cell lines (NALM6 and REH) have different PAX5 and CD19 protein levels, as verified by western blot and flow cytometry respectively. Co-cultures of B-ALL cells and mature CD3+ T-lymphocytes from healthy donors are seeded 10:1 in the presence of blinatumomab (1 ng/ml) and investigated after 3 and 7 days of incubation for cell morphology, CD19+/CD3+ composition and viability in cytofluorimetry by a cocktail of fluorescent anti-human antibodies (CD45, CD19, CD3, 7AAD, Syto16). Similar in vitro analyses are performed on primary leukemic and mononuclear cells isolated from bone marrow aspirates of 5 Philadelphia-negative B-ALL patients (median (IQ) age: 10.7(7.9–11.3) years; males: 60%) after 7 days of drug exposure.
RESULTS: Compared to REH, NALM6 cells express the full-length protein PAX5 and show more CD19 on cell surface (median intensity of the fluorescence peak: 28895 in NALM6 vs 9155 in REH). A significant increase in mortality of both B-ALL lines is observed in the presence of blinatumomab in the co-cultures (CD19+ gated 7AAD+, untreated vs treated, day+3, $p<0.001$; day+7, $p<0.0001$, two-way ANOVA, Bonferroni post-test). B-ALL cells mortality is more pronounced in the lower-expressing CD19+ REH compared to NALM6 at day+3 (44.46±6.9% vs 25.54±12.9%) and is almost complete at day+7 (REH: 95.12±2.1%; NALM6: 97.17±1.8%). Changes in co-culture composition can be detected only at day +7, with a decrease in B-ALL cells percentages and an increase in T-lymphocytes activation in blinatumomab treated samples compared to controls (both effects, $p<0.0001$): these effects are higher in REH co-cultures compared to NALM6 ones ($p<0.05$)(Fig1A). Patient B-ALL lymphoblasts and T-lymphocytes respond differently to blinatumomab in vitro: only two primary co-cultures show the simultaneous increase in blast mortality and T-lymphocytes activation by the drug (untreated vs treated, $p<0.0001$ for both effects), although at different concentrations) (Fig1B). These in vitro results are unrelated to the percentages of immature CD19+ B-cells present in the diagnostic samples.
CONCLUSIONS: The cytofluorimetric in vitro assay can measure both the blinatumomab effects on B-ALL cells death and T-lymphocytes,

appreciating the difference induced by the drug among co-cultures. Whether this difference is affected by the PAX5-regulated CD19 expression is unclear and additional studies are required to investigate on this still unproven issue.

UNIQUE INVOLVEMENT OF THE P2Y12 RECEPTOR IN THE EXPOSURE OF TISSUE FACTOR ON THE PLATELET MEMBRANE

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BACKGROUND: ADP plays a crucial role in platelet (PLT) activation being a cofactor in the PLT response to physiological agonists including thromboxane A2, collagen and thrombin. Its action is initiated by P2Y1 and amplified by P2Y12 receptors. We have reported that platelet ADP stimulation results in the exposure, on the cell membrane, besides P-selectin (Psel), of a functionally active Tissue Factor (TF), the main initiator of coagulation. However, the role of the 2 ADP receptors in PLT-TF modulation is still unknown. The aim of the study was therefore to assess whether 1) P2Y1 and P2Y12 receptors equally contribute to ADP-induced TF expression; 2) clopidogrel treatment reduces TF exposure; 3) TF and Psel are stored in the same cell compartment.
METHODS: PLTs from healthy subjects were in vitro treated with the P2Y1 antagonist MRS-2250 (1pM–100nM), or the P2Y12 antagonist AR-C69931MX (AR-C; 1pM–100nM). PLT-TF expression was analyzed by flow cytometry in resting conditions and upon stimulation with ADP (10μM), TRAP-6 (10μM) or U46619 (1μM) and pA2 of the 2 antagonists were calculated. A P2Y12-deficient patient was also studied. The relationship between the antiplatelet effect of clopidogrel, assessed by the VASP assay, and TF expression was investigated in 88 NSTEMI patients. Cytochalasin D (10μM) was used to investigate the involvement of open canalicular system (OCS) externalization in TF exposure.
RESULTS: In vitro PLT pre-incubation with anti-P2Y12 AR-C concentration-dependently reduced ADP-stimulated TF exposure. An almost complete inhibition was observed also when PLTs were stimulated with TRAP-6 or U46619. Of interest, the amount of PLT-TF observed in NSTEMI patients with a sub-optimal clopidogrel response (VASP>50%) was significantly higher than that measured in good responders. Psel levels measured in the 2 groups of patients were similarly reduced. Of note, the pA2 for AR-C to inhibit TF is 150 times lower than that of Psel, indicating that clopidogrel concentration, able to inhibit Psel, are not enough to reduce TF exposure. The unique involvement of the P2Y12 signalling was highlighted by results indicating that PLTs from a P2Y12 deficient patient did not express TF upon ADP stimulation. Since P2Y1 inhibition did not affect TF expression while it significantly reduced Psel expression, i.e. the alpha-granule secretion, data suggest a different cell localization of the two proteins. Indeed, inhibition of OCS impaired ADP-induced TF expression but it did not affect Psel.
CONCLUSIONS: Data indicate that 1) the release of endogenous ADP is a key event in TF

expression induced by classical platelet agonists; 2) ADP effect is mediated only by the P2Y12 receptor; 3) TF is not in the α-granule; 4) the therapeutic benefits of clopidogrel treatment may also rely on the inhibition of platelet TF expression, although at higher concentrations compared to those needed to regulate alpha -granule secretion.

IN UTERO THC EXPOSURE INDUCES A DIMORPHIC BEHAVIOURAL PHENOTYPE IN THE OFFSPRING: FOCUS ON LIMBIC MEMORY AND ALCOHOL VULNERABILITY

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BACKGROUND: Cannabis use during pregnancy is on the rise, driven in large part by increasing social and medical acceptance of its use and legalisation in many parts of the world. Since the endocannabinoid system regulates emotional and cognitive processes from early stages of life, in utero exposure to THC might contribute to the occurrence of a vulnerable phenotype later in life. Thus, this research investigated the effects of gestational THC exposure on aversive limbic memory and alcohol vulnerability in adolescent offspring.
METHODS: Rat offspring of both sexes were exposed in utero to THC (2 mg/kg, s.c., gestational day 5-21) or vehicle, and assessed for behavioural reactivity in the open field test, neutral explicit memory in the novel object recognition test, and limbic memory in the emotional object recognition test, starting from preadolescence. Moreover, the offspring were tested for vulnerability to alcohol in the binge-like intermittent two-bottle choice paradigm along adolescence.
RESULTS: Prenatal exposure to THC exerted dimorphic effects in preadolescent rats, inducing increased locomotor activity and emotional object deficits only in male rats. However, both male and female THC-exposed offspring displayed increased binge-like alcohol consumption, with respect to control offspring.
CONCLUSIONS: Gestational THC exposure induced sex-related effects on behavioural reactivity and cognition, which emerged when the integration between environmental encoding and emotional/motivational processing was required, and promoted the development of an alcohol- prone phenotype in both sexes since preadolescence.

IDENTIFICATION OF NOVEL THERAPEUTIC APPROACHES TO SELECTIVELY TARGET SENESCENT CELLS IN PROSTATE CANCER

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BACKGROUND: Despite recent progresses, prostate cancer (PCa) is still the major leading cause of cancer-related death among men worldwide. Commonly, PCa treatment is based on chemotherapy and radiotherapy, with or without a castration-based strategy in which androgen deprivation is achieved by pharmacological or surgical castration. Efficacy of all these types of therapies also relies on their ability to induce cellular senescence, which is defined as the irreversible arrest of cell growth. Moreover, these tumors often harbor PTEN-loss, which results in poor prognosis, therapy resistance and, nevertheless, acute loss of PTEN is responsible for a senescence response too, termed Pten-loss induced cellular senescence (PICS). However, despite the proliferation block, senescent cells remain metabolically and synthetically active: indeed, they release many cytokines and chemokines known as senescence-associated secretory phenotype (SASP) able to affect nearby cells. In particular conditions, SASP may promote cellular proliferation and angiogenesis, thus inducing tumour progression and invasiveness. Hence, elimination of senescent cells from tumours can avoid cancer metastasis and relapse. In this study, since our goal is to have an impact on senescent subset, we first analysed prostate tumour samples from Pten-null mice in order to identify molecular markers on it.
METHODS: The separation of senescent cells from non-senescent ones was performed using the C12-FDG staining and FACS-sorted cells were analysed by mass spectrometry. Further data examination using a bioinformatic approach identified membrane proteins upregulated by the senescent population.
RESULTS: Targets were validated by quantitative real time PCR, by flow cytometry and by western blot in different human cell lines after senescence induction using chemotherapy treatment.
Starting from a list of membrane proteins, we selected only the one that was significantly upregulated.
CONCLUSIONS: Our preliminary results show interesting and promising membrane proteins that can be exploited for the development of innovative drug delivery system in order to improve prostate cancer senescent cell clearance to boost the outcome of current therapies.

NEUROPSYCHIATRIC DISORDERS IN THE EXPERIMENTAL TREATMENT FOR COVID-19 WITH HYDROXYCHLOROQUINE

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BACKGROUND: Chloroquine and hydroxychloroquine are both drugs authorized to treat malaria and certain autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis.They have been recently used to treat patients with COVID-19 based on in vitro studies results, but their beneficial effects in this patient population are not established yet. It is well known that these drugs are associated with an increased risk of heart problems and several recent observational studies confirmed it. A limited number of case reports suggest that they may also cause neuropsychiatric disorders, such as agitation, insomnia, confusion, psychosis and suicidal ideation.
METHODS: From the first stages of the pandemic the Toxicology Unit of Careggi University Hospital monitored the occurrence of adverse drug reactions in patients undergoing experimental treatment for COVID-19. We took in considerations concomitant treatments and eventual drug interactions, comorbidities, time to onset.
RESULTS: In 2 of the 23 cases we recorded, neuropsychiatric symptoms occurred. Case 1 is a 80 years old man treated with hydroxychloroquine 400 mg for 7 days. He experienced a major depressive episode 7 days after starting treatment. He was concomitantly treated with darunavir/cobicistat. Case 2 is a 65 years old man treated with hydroxychloroquine 400 mg for 9 days. He manifested psychomotor agitation 20 days after starting treatment, and later developed hyperkinetic delirium despite the suspension of the drug. He was concomitantly treated with darunavir/cobicistat and tocilizumab. Both had no personal or familiar history of neuropsychiatric disorders and the symptoms improved after the drug was suspended and olanzapine was administered. In both cases causality was assessed with the Naranjo algorithm, with a score of 6 points corresponding to "probable".
CONCLUSIONS: For both patients, in the absence of personal or familiar history and since symptoms improved after the suspension of treatment, hydroxychloroquine could not be ruled out as a cause of neuropsychiatric disorders. Patients receiving chloroquine and hydroxychloroquine as experimental treatment for COVID-19 should be closely monitored while further analyses of available data are being carried out. Furthermore, a careful pharmacovigilance is necessary to obtain more data regarding hydroxychloroquine and neuropsychiatric disorders in patients exposed.

AMYLOID β FIBRILS DISRUPTION BY OLEUROPEIN AGLYCONE: INVESTIGATION OF THE MECHANISM OF ACTION OF THIS POLYPHENOL FROM EXTRA VIRGIN OLIVE OIL

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BACKGROUND: In the central nervous system, extra virgin olive oil (EVOO) produces interesting effects against neurodegenerative disorders including Alzheimer's disease (AD). The valuable properties of EVOO are largely ascribable to oleuropein aglycone (OA), the most abundant phenolic constituent. In particular, it has been demonstrated that, in mouse models of AD, OA produces neuroprotective effects reducing amyloid β (Aβ) aggregates and diminishing related cytotoxicity and inflammation. Biological data highlighted that OA is not only able to prevent Aβ aggregation but also that is able to disaggregate Aβ preformed fibrils.
METHODS: A comprehensive computational protocol for investigating the mechanism of action of OA as Aβ fibrils disruptor at molecular level has been developed. Initially, for establishing a potential high-affinity binding site of OA within the Aβ fibrils, we performed a blind docking calculation using AutoDock software. The identified binding site was used for a more accurate docking study employing Glide software using Extra Precision (XP) as scoring function. The obtained complex OA/Aβ fibrils was used for performing large-scale molecular dynamics (MD) calculations (5 μs repeated independently two times; aggregation time of 10 μs). MD simulation was performed, using CUDA API technology on two NVIDIA GPUs, by Desmond software in explicit solvent (TIP3P), employing OPLS3 as force field at constant temperature (300 K) and pressure (1.01325 bar) with NPT (constant number of particles, pressure and temperature) as ensemble class.
RESULTS: Results showed that OA initially interacts with a key motif (LVFFAED) of Aβ peptide, known to be extremely relevant for fibrils assembly and stability. Afterwards, the long-time MD simulation revealed that OA moved in depth within the Aβ fibrils targeting the mentioned motif in each chain. This movement of OA seems to "cut" the preformed fibrils, causing a significant disruption of the ordered structure. The results demonstrated that OA leads to a structural instability due to its insertion within the Aβ preformed fibrils. This event is a fundamental step for determining an effective Aβ fibrils disaggregation.
CONCLUSIONS: In this work we have described, for the first time, a detailed computational protocol useful for elucidating the mechanism of action of OA as Aβ fibrils disruptor at molecular level, establishing OA as a potent anti-amyloidogenic drug. This study also highlights the possibility of further exploration of natural/nutraceutical products about their prospective application for controlling neurotoxicity, proposing food components as preventive therapeutics against AD. In fact, by using computational approaches it will be possible to evaluate potential disease-modifying anti-Alzheimer's drugs (DMADs) from natural products or food components. Moreover, this study possesses relevant implication for the rational design of DMADs for developing innovative anti-AD drugs.

PROTECTIVE EFFECT OF CARBONIC ANHYDRASE INHIBITORS: ACETAZOLAMIDE AND AN11-740 IN BRAIN ISCHEMIA

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BACKGROUND: Ischemic stroke is a leading cause of death and disability worldwide. Since the only pharmacological treatment available to date for cerebral ischemia is tissue plasminogen activator (t-PA), search for successful therapeutic strategies still remain a major challenge. The loss of cerebral blood flow leads to a condition of hypoxia and glucose deprivation (oxygen, glucose deprivation: OGD) and subsequent tissue damage in the affected region (Gibson, 2013). One of the major pathogenic mechanisms of ischemic stroke includes the switch to the glycolytic pathway, which leads to tissue acidification (Siesjö, 1981). Carbonic anhydrase is the enzyme responsible for converting carbon dioxide into a hydrogen ion and a bicarbonate ion, thus contributing to pH regulation. Since in vitro studies document a decrease in pH in neurons and glial cells in hypoxic/anoxic conditions (Obara et al., 2008), carbonic anhydrase inhibitors (CAIs) could contribute to pH homeostasis under brain ischemia.
METHODS: Aim of this study was to investigate if acetazolamide (ACTZ) and a new synthetized carbonic anhydrase inhibitor, AN11-740, provided protection in two models of brain ischemia. In an in vitro model of acute rat hippocampal slices that underwent to severe, 30 min long, oxygen-glucose deprivation (OGD) episodes and in an in vivo model of focal cerebral ischemia induced by permanent occlusion of the middle cerebral artery (pMCAo) in the rat. In vitro, AN11-740 (3 μM) or ACTZ (20 μM) were applied 20 min before and during OGD application, whereas in vivo AN11-740 or ACTZ were administered (i.p.) 5 min, 6 and 20 h after pMCAo, at a dose of 1 mg/kg and 4.4 mg/kg, respectively. Twenty four hours after pMCAo, brain infarct volume and the cytoarchitecture of the ischemic tissue were determined by cresyl violet and hematoxylin/eosin and immunohistochemical analysis were performed to characterize neuronal damage and gliosis.
RESULTS: In vitro, the application of the selective CAIs significantly delayed the appearance of anoxic depolarization induced by OGD ($P<0.05$). In vivo, subchronic treatment with AN11-740 and ACTZ reduced the neurological deficit 24 hours after pMCAo ($p<0.01$) and decreased the infarct volume within the cortex and striatum (at least $p<0.05$). Moreover, CAIs reestablished the cytoarchitecture of the ischemic cortex and striatum, reduced the number of heterochromatic nuclei belonging to glial cells in striatum and cortex (at least $p<0.05$), and counteracted neuronal loss in core and peri-infarct area of both ischemic areas ($p<0.05$). Furthermore, ACTZ and AN11-740 reduced microglia activation ($P<0.05$) and partially conteracted the loss of astrocytes in the cortical and striatal peri-infarct area.
CONCLUSIONS: The results demonstrate that ACTZ and AN11-740 exert a protective effect in brain ischemia, both in vitro and in vivo. CAIs could be a useful treatment to complement the t-PA application in the therapeutic time-window after cerebral ischemia.

DRUGGABLE TARGET OXOEICOSANOID RECEPTOR 1 (OXER1) AND ITS RACK1-ASSOCIATED PATHWAY IN BREAST CANCER PROGRESSION

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BACKGROUND: BC (Breast Cancer) is a heterogeneous disease due to variable histological subtypes and differences in response to therapy and clinical outcome. In human BC, scaffold protein RACK1 (Receptor for Activated C Kinase 1) was reported as putative prognostic marker and drug target in BC due to its critical role in cancer cell migration and invasion. RACK1 increased expression negatively correlates with overall survival by promoting BC progression. Important binding sites on RACK1 promoter were found through in silico analysis, including c-Rel sites and a Glucocorticoid Responsive Element (GRE). Hence, RACK1 expression is controlled by a complex glucocorticoids-androgens balance and due to the hormonal nature of most BC types, androgen signaling in BC and its role in regulating RACK1 transcription became of pivotal interest. In this regard, an important role is now emerging for membrane androgen receptors (mARs), particularly for OXER1 (Oxoeicosanoid Receptor 1), involved in activating PI3K/Akt/NF-κB and Focal Adhesion Kinase (FAK) signaling pathways to promote survival and induce cell adhesion and migration.
METHODS: MCF7 and MDA-MB-231 treated with testosterone, testosterone-BSA-FITC, or nandrolone were analyzed to assess whether RACK1 transcriptional regulation was AR- or mAR-dependent, involving PI3K/Akt/NF-κB pathway. To this purpose luciferase reporter assay, qPCR and Western Blotting were performed on wild type, transiently or stably silenced cells for RACK1, OXER1 and AR. To evaluate androgens functional effect on this signaling cascade, we assessed cell proliferation through MTT, colony formation assay and cytofluorometry. Cell migration was evaluated through scratch-wound healing assay. To confirm nandrolone-mediated, OXER1-initiated effects on RACK1 expression, we performed in silico molecular docking and immunofluorescence. Finally, we validated our panel with patient-based transcriptomic data.
RESULTS: Our data confirmed RACK1 involvement in BC progression and prompted us to investigate hormone-related RACK1 expression following androgens treatment. We provided evidence that nandrolone exerts negative effects on BC cell proliferation and migration by antagonizing PI3K/Akt/NF-κB signaling pathway, ultimately leading to RACK1 expression down-reg-

ulation. We established that nandrolone impairs this signaling pathway and RACK1 expression through its binding to OXER1, whose increased expression is higher in tumors tissue compared to non-cancerous ones and correlated with ER and PR status in patients.
CONCLUSIONS: OXER1 provides a novel link between androgens and their AR-independent mechanisms, highlighting how androgenic molecules lacking an AR-dependent profile and with predominantly mAR-mediated effects (e.g. nandrolone) could be exploited to reduce BC cell migration and proliferation, finding a potential use for BC treatment. Indeed, our transcriptomic data suggest OXER1 as a possible therapeutic target.

CARDIOTOXICITY IN THE EXPERIMENTAL TREATMENT FOR COVID-19

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BACKGROUND: No therapy for the management of Sars-Cov-2 has been approved up to this day. Multiple and different therapeutic approaches have been used in experimental clinical trials. Hydroxychloroquine and azithromycin have been the most tested drugs between March 2020 and May 2020. Both drugs can increase QT interval on the electrocardiogram (ECG) as a possible adverse drug reaction (ADR) with following arrhythmic risk (e.g. torsade de pointes, TdP). Based on safety evidence, the use of these drugs is not currently recommended out of clinical trials or emergency programs, implying that hospitalized patients need to be put under monitoring. The main aim of this study was to monitor ADRs, QTc interval trends and arrhythmic risk in patients receiving HCQ for Sars-Cov-2 whether it was associated with azithromycin or not.
METHODS: The Clinical Toxicology and Poison Centre Unit at Careggi University Hospital (Florence) selected a group of patients positive for Sars-Cov-2 at rhino-pharyngeal swabs that were later admitted to an Internal Medicine Unit. This group started COVID-19 treatment with HCQ in the period from 06/04/2020 to 31/05/2020. Patients underwent several ECG checks for arrhythmic risk, following the Italian Society of Cardiology flow chart (updated to 27/03/2020) which stated to consider QTc elongation if > 460 msec. We decided to monitor the followings parameters: therapy in progress, admission and control QTcs during treatment, appearance of electrolytes alterations and development of other ADRs (including TdP). In case of QTc > 500 msec the COVID-19 therapy was ceased and patients were checked for normal QTc return after electrolytes correction.
RESULTS: The total number of patients treated with HCQ was 123. 18 patients (15%) had a QTc elongation above 460 msec at the control ECG, with 8 of them reaching a QTc above 500 msec and requiring therapy suspension. None of the patients developed arrhythmic complications including TdP but 13 out of 18 needed electrolytes correction. 14 out of these 18 patients showed normal QTc pattern after discontinuation of therapy. The remaining 4 patients died for COVID-19 complications and not for drug-related causes.
CONCLUSIONS: In conclusion, the proposed experimental use of HCQ with or without azithromycin during the COVID-19 pandemic has

been associated with ADRs such as prolongation of QTc interval. In our group this was shown in 15% of the admitted patients. The absence of arrhythmias (TdP) in our group could be due to early suspension of therapy and prompt correction of electrolytes alteration, in order to preserve our patients' already fragile health. Hydroxychloroquine should therefore be used with extreme caution and only if ECG and electrolytes monitoring are possible.

A NOVEL APPROACH FOR THE TREATMENT OF PANCREATIC DUCTAL ADENOCARCINOMA ADOPTING ANTI-GLYPICAN 1 CHITOSAN-BASED POLYMERIC NANOPARTICLESLOADED WITH CHEMOTHERAPEUTIC AGENTS

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BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer of the pancreas and it causes more than 330,000 deaths every year worldwide (1,2). In the majority of the cases the diagnosis occurs in an advanced stage where the disease is already spreads in different organs. In this context, surgery is not applicable and chemotherapy is slightly efficient causing severe toxicity to healthy tissues, for these reasons there is an urgent need of new therapeutic strategies (3,4). Glypican 1 (GPC1) is a cell surface proteoglycan that can be an useful target for active drug-delivering in the PDAC context due to its high expression in PDAC cells and its low/absent expression in most other tissues (5,6). Chitosan (CS) drug-loaded nanoparticles (NPs) conjugated with antibodies targeting tumor associated antigens represent a biocompatible, biodegradable, and non-immunogenic nanotechnological device potentially capable of focusing the toxicity on the tumor site (7). The aim of this research is to set up a drug-delivery strategy employing drug-loaded NPs conjugated with an antibody targeting GPC1 for the treatment of PDAC.
METHODS: GPC1 expression was performed using western blot (WB), immunofluorescence (IF) and flow cytometry (FC). The killing capability of chemotherapeutics agents was performed using flow cytometry using annexin 5 and 7-aminoactinomycin D. The mice model was established by subcutaneous injection of 5 million of PDAC-like BXPC3 cells in the flank of athymic nude mice.
RESULTS: The preliminary obtained data were focused on the validation of GPC1 as a specific cell surface PDAC associated antigen. WB, IF and FC confirmed the high expression of GPC1 in PDAC cell line (BXPC3) and the necessity to employ a C-terminal anti-GPC1 monoclonal antibody in order to obtain a specific targeting (Figure A-B-C). Among the different pharmacological compounds screened, doxorubicin showed the highest killing capability with an IC50 of 0.32 μM. The PDAC cell line BXPC3 showed tumorigenicity if subcutaneously injected in the flank of athymic nude mice.

CONCLUSIONS: Given the lack of an efficient therapy for PDAC patients, the obtained preliminary results are consistent with the hypothesis that this NPs-based therapeutic approach could represent an alternative strategy for PDAC treatment. The next experiments will be focused mainly on nanotechnological aspects: characterization of the nanoparticles, in-vitro and in-vivo evaluation of toxicological profile, biodistribution and killing efficacy of the nanoparticles.

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THE ECTO-5'-NUCLEOTIDASE INHIBITOR, ADENOSINE 5'-O-(ALPHA, BETA-METHYLENE)DIPHOSPHATE, AMELIORATES FEATURES OF ALLERGIC AIRWAY DISEASE IN MICE

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BACKGROUND: Asthma is the most common respiratory disease with increasing morbidity and mortality worldwide. The ecto-5'nucleotidase, CD73, is the main enzyme producing extracellular adenosine. While it is known that adenosine is actively involved in the clinical manifestation of bronchial asthma, very little is known about the role of CD73 in asthma pathogenesis. Here, we sought to investigate the effect of the pharmacological blockade of CD73 in a murine model of allergen airways disease.
METHODS: Mice were sensitized to ovalbumin (100 µg of OVA emulsified with aluminium hydroxide (13 mg/mL) by subcutaneous injection on day 0 and day 7. The CD73 inhibitor, Adenosine 5'-O-(alpha, beta-methylene) diphosphate (20 mg/kg), APCP, or vehicle were administered intraperitoneally 20 min before each OVA administration. Groups of mice were then challenged with OVA aerosol, or saline,

twice, on day 21 and 22. Mice were then sacrificed 24 h after the last challenge and lungs were excised. CD73 expression was evaluated by Western blot analysis, while CD73 specific activity was measured by Malachite green assay. The percentage of FcεRII+ B cells, CD4+ IL-4+ T cells and FcεRI+ mast cells was evaluated by flow cytometry. Haematoxylin and eosin staining was performed to evaluate morphological analysis. On isolated bronchi, reactivity to carbachol (10-9– 3 × 10-6 M) was evaluated.
RESULTS: Sensitization and challenge with OVA increased pulmonary CD73 expression and its specific activity. Mice treatment with CD73 inhibitor, APCP, reduced the percentage of FcεRII+ B cells and of CD4+ IL-4+ T cells infiltrating the lung induced by OVA challenge. Also, the percentage of FcεRI+ mast cells and the percentage of degranulated mast cells infiltrating the lung following OVA challenge were significantly reduced by treatment with APCP. In addition, morphological analysis of lung tissue obtained from APCP-treated mice showed reduced peribronchial and alveolar inflammatory cell infiltration. Furthermore, treatment with APCP protected mice from bronchial hyperreactivity to carbachol induced by allergen challenge.
CONCLUSIONS: Our results show that pharmacological inhibition of CD73 during sensitization phase ameliorates inflammation and bronchial hyperreactivity caused by allergen challenge, suggesting CD73 as a potential candidate for novel anti-asthmatic treatments.

VULNERABILITY AND RESILIENCE TO CHRONIC STRESS ALTER THE RESPONSE TO AN ACUTE NOVEL STRESSOR: INVOLVEMENT OF THE HYPOTHALAMIC-PITUITARY ADRENAL AXIS

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BACKGROUND: Stress is one of the main precipitating factors for psychiatric disorders. However, there are differences in the individual susceptibility to stress, with some individuals displaying vulnerability following stress exposure and others showing resistance to its maladaptive effect. Moreover, the effect of stress may depend on its duration and intensity and exposure to stressful events may alter the response to a subsequent stressor. In this study, we investigated the effect of the exposure to an acute restraint stress (ARS) immediately after 2 weeks of chronic mild stress (CMS) in both vulnerable (anhedonic) and resilient (non-anhedonic) rats, to evaluate the responsiveness of the two subpopulations of rats to the ARS. Moreover, we aimed to establish how long the anhedonic phenotype induced by CMS takes to normalize and we studied the response to these rats to the ARS once recovered. At molecular level, we focused on the contribution of the hypothalamic-pituitary-adrenal (HPA) axis functionality, the primary system mediating the stress response, by evaluating the corticosterone levels and the expression of genes responsive to the glucocorticoid receptor in dorsal (dHip) and ventral (vHip) hippocampus, brain regions strictly implicated in mood disorders and related to the emotional behavior.
METHODS: Adult male Wistar rats were exposed for 14 days to CMS and subjected to

ARS (1h) after the last episode of CMS, whereas a subgroup of vulnerable animals was left undisturbed until the recovery of the behavioral phenotype and than acutely stressed with one hour of ARS. Sucrose consumption test was performed at weekly intervals to assess the development of anhedonia. dHip and vHip were dissected, frozen on dry ice and stored at -80°. Total RNA was isolated and the analyses of mRNA were carried out by real time-PCR, whereas corticosterone (CORT) plasma levels were measured with a commercial ELISA kit.
RESULTS: CMS induced anhedonia in a subpopulation of stressed rats (vulnerable), while the remaining were resilient since they consumed the same amount of sucrose as controls. The anhedonic phenotype in vulnerable rats normalized after 3 weeks of rest from stress procedure. We found that CORT levels were increased in vulnerable but not in resilient rats and that the ARS induced an upregulation of its plasma levels in controls as well as in resilient animals. In vHip the acute challenge upregulated Gadd45β, Sgk1 and Dusp mRNA levels in control, resilient and vulnerable+washout rats, effects completely blunted in vulnerable animals. By contrast in dHip all the genes were positively modulated by the restraint independently from CMS pre-exposure.
CONCLUSIONS: These data suggest that CMS altered the ability of the HPA axis to deal with a challenging condition and indicate a different implication of the two hippocampal sub-regions in modulating the effect of a novel acute challenge in vulnerable and resilient animals.

USE OF MAGHEMITE NANOPARTICLES TO IMPROVE THE REGENERATIVE PROPERTIES OF BIOMATERIALS FOR BONE REPAIR

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BACKGROUND: Nanotechnology plays a key role in the development of new scaffolds for bone repair allowing the incorporation of nanomaterials able to improve regenerative properties. Recent evidences demonstrated that the combination of nanoparticles with biomaterials is able to improve proliferation and differentiation of cells seeded on them. The aim of this study is the evaluation of osteo-conductive and -inductive properties of the biomateials treated with the Maghemite Nanoparticles (MAG NPs).
METHODS: Osteo-conductive and -inductive properties of the biomaterial treated with MAG NPs have been assessed in vitro after 24 days of osteogenic differentiation. Cell biocompatibility and proliferation were evaluated by histological analysis (H&E) and DAPI staining, respectively; osteogenic differentiation was evaluated by alizarin red stain and immunofluorescence analyses.
RESULTS: Our data clearly highlight that the biomaterials treated with MAG NPs increase both osteo-conductive and osteo-inductive ability of cells seeded on them, in terms of cell adhesion, penetration, proliferation and differentiation.
CONCLUSIONS: Our results suggest that the excellent improvement of both osteo-conductivity and osteo-inductivity of the biomaterials treated with MAG NPs could pave the way for the development of innovative scaffolds to be applied in the field of bone repair.

A REAL-WORLD STUDY OF THE PRESCRIPTION PATTERN IN CASE OF OBSTRUCTIVE AIRWAY DISEASES ON A LARGE ITALIAN ADMINISTRATIVE DATABASE

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BACKGROUND: Achieving a good obstructive airway disease (OAD) control should be the main driver for the therapeutic choice. This real-world study aimed to describe the prescription pattern of OADs in the Italian clinical practice.
METHODS: Through specific algorithms based on the record linkage of healthcare administrative data (drug prescription, hospitalization, outpatient specialist care) collected in the 2015 ReS database, patients affected by OADs were selected and divided into 4 groups according to the following diagnoses: asthma, chronic obstructive pulmonary disease (COPD), asthma-COPD, undefined. Asthma was searched among subjects aged ≥12, while other conditions among those aged ≥40. Starting from the patient identification, a 1-year follow-up was analysed to describe the prescription patterns, by considering corticosteroids for systemic use [CSs] (at least 2 prescriptions) and drugs for OADs (ATC R03). Analyses were performed both on active substances (e.g. omalizumab, montelukast) and on therapeutic categories (e.g. inhaled corticosteroids [ICSs], long-acting-β-agonists [LABAs] and long-acting muscarinic agonists [LAMAs]).
RESULTS: Out of >7 million inhabitants in the 2015 ReS database, patients with OADs were 424,100. Among them, 110,453 (26.0%) had asthma, 229,747 (54.2%) COPD, 8,828 (2.1%) asthma-COPD and the remaining 75,072 (17.7%) resulted undefined. Over 1-year follow-up, CSs were supplied at least twice to 26.2% of asthma-COPD patients, to 14.9% of COPD, to 10.9% of undefined and to 9.2% of asthmatic ones. Among drugs for OADs, the 3 most prescribed were: beclometasone (36.4%), montelukast (24.9%), salbutamol (23.5%) for asthma; beclometasone (42.1%), salbutamol/ipratropium-bromide (20.6%), salmeterol/fluticasone (24.2%) for COPD; montelukast (69.4%), salmeterol/fluticasone (31.8%), beclomethasone (29,0%) for asthma-COPD; beclometasone (47.3%), salbutamol/ipratropium bromide (20.2%), salbutamol (15.0%) for the undefined OAD. Omalizumab was prescribed at least once to 2.3% of asthmatic patients and 6.9% of those with asthma/COPD, while to ≤0.2% of other groups. The prescription patterns for COPD patients consisted of ICS alone (37.5%), ICS/LABA (14.6%), LAMA and ICS/LABA (7.1%), whereas for those with asthma-COPD of ICS/LABA (33.7%), ICS and ICS/LABA (19.7%), LAMA and ICS/LABA (11.1%).
CONCLUSIONS: This real-world data study described the Italian prescription behaviour for different OADs, including defined diagnoses, the mixed and the undefined conditions.

PRESCRIPTION PATTERN OF ATYPICAL ANTIPSYCHOTICS AND CARDIO-METABOLIC PROFILE: AN ITALIAN LARGE REAL-WORLD STUDY

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BACKGROUND: In the last decades, the use of atypical antipsychotic drugs (AAPs) has increased worldwide and in Italy, often in the elderly, in case of comorbidities and through inappropriate use. This real-world analysis aimed to describe AAPs' prescription pattern to adults with/without cardio metabolic events (CMEs) or predisposing conditions (PCs) for CMEs in the Italian clinical practice.
METHODS: From the ReS database, collecting regional healthcare administrative data, adults with at least a prescription of one AAP (index date) reimbursed in 2015 were selected. New users were defined by the absence of any antipsychotic prescription in two years before index date. These patients were categorised into three groups, according to the presence/absence in the previous year of CMEs (diabetes, cerebrovascular and ischemic heart disease) and PCs (hyperglycaemia, dyslipidaemia and severe obesity): (X) with CMEs but without PCs, (Y) with PCs but without CMEs, (Z) without CMEs/PCs. Over 1-year follow-up, the AAPs' consumption (the most prescribed drugs), persistence to the treatment (patients treated with the same AAP in each quarter) were described for the overall population and for each group.
RESULTS: Out of >4 million adults in the 2015 ReS database, 50,893 patients were prescribed at least one AAP. New users were 12,128 (incidence: 2.8 x1,000). A monotherapy was prescribed to 99.5% of new users. Quetiapine, olanzapine and risperidone were the most prescribed AAPs in the whole cohort, both as first AP therapy (46.8%; 22.1%; 12.1%) and throughout 1 year (51.2%; 25.9%; around 9%). The ranking of these drugs was the same by analysing each group: in group X (n: 2,732) quetiapine, olanzapine and risperidone were prescribed to 65.4%, 17.7% and 13.3% respectively, in Y (n: 1,492) to 60.6%, 23.3%, 13.2%, and in Z (n: 7,904) to 44.4%, 29.3%, 16.8%. The 1-year persistence to quetiapine, olanzapine and risperidone reduced by about 50%, 60% and 70%, respectively.
CONCLUSIONS: This real-world data study described the prescription pattern of the first AAP in the presence of different patients' cardio-metabolic profiles. These findings can be useful to define AAP prescription tailoring programs according to specific cardio-metabolic profiles.

CANNABIS USE IN THE MANAGEMENT OF PAIN: EFFECTIVENESS AND SAFETY

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BACKGROUND: Analgesic therapy with cannabinoid-type substances (delta-9-Tetrahydrocannabinol and Cannabidiol) is an algological pharmacology field of emerging interest, however, it is still insuffciently explored. Consequently, the risk/benefit ratio related to the use of Cannabis and cannabinoids is still poorly defined. The scientific literature reports numerous examples on the beneficial effects of cannabis and delta-9-THC synthetic derivatives in pain associated with multiple sclerosis, cancer, neuropathies and HIV infection.
In Italy, since 2006 it is possible to prescribe cannabis for therapeutic purposes, however until 2016, cannabis-based preparations were partially imported, in particular from the Office of Medicinal Cannabis (Dutch Cannabis Agency) of the Dutch Ministry of Health, Welfare and Sport, according to the import procedure foreseen by a decree of the Ministry of Health (DM 11/2/97). Furthermore, following an agreement between the Italian Ministry of Health and the Ministry of Defense, from December 2016 also in our country the active substance derived from Cannabis called FM2 and more recently the substance FM1, both produced by the Italian Istituto Chimico- Farmaceutico Militare (Florence) are available for the prescription of individual galenic preparations based on Cannabis. FM2 consists of unfertilized, dried and ground female inflorescences containing acid precursors of delta-9-tetrahydrocannabinol (THC) corresponding to a THC percentage between 5 and 8% and a percentage of cannabidiol (CBD) included between 7.5 and 12%. FM1 consists of female inflorescences containing acid precursors corresponding to a percentage of THC between 13 and 20% and a percentage of CBD <1%.
Management of pain and its chronicization represents a health challenge of considerable social impact. In this context, the recent availability of cannabis derivatives with analgesic activity is not accompanied by sufficient information relating to the identification of the truly effective doses and the risks connected to their use. These uncertainties lie in an algological and palliativist context that is still culturally too fragile and empirically inhomogeneous. For this reason, it is considered necessary and urgent to monitor the therapeutic use of cannabis in the medical field and in particular in the algological field.
We designed for the University Hospital Policlinico "G. Martino" of Messina an active pharmacovigilance project based on Cannabis prescriptions in collaboration with Hub and Spoke Centers of Pain Therapy active in the territory of the Sicily Region.
Objective of the study is to improve the knowledge on the risk-benefit profile of the use of Cannabis derivatives in the management of pain.
METHODS: The collection of data on the effectiveness and adverse reactions deriving from use of Cannabis in the algological field envisaged by the project proposal is aimed at collecting data that can be used to establish the appropriateness of analgesic cannabis prescriptions. A web application has been pre-

pared to allow the single regional pain centers to enter data of prescriptions together with clinical data of single patient. Data will be associated with clinical evaluation of pain before cannabis therapy and during treatment.
RESULTS: Data will be collected during two years starting from October 2020.

CONCLUSIONS: We believe that the evaluation of clinical data on Cannabis use in the management of pain can help in the identification of the risk/benefit profile of Cannabis or other future cannabis products possibly supplied to regional hospitals by the Ministry of Health for pain therapy.

THE EFFECT OF MELATONIN IN AN IN VITRO INFLAMMATORY MODEL INDUCED BY GLIADIN IN ADIPOSE CELLS

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BACKGROUND: Gliadin, a fraction of gluten, represents the main antigen able to trigger an inflammatory reaction in the intestine. Previous studies have shown that gliadin is able to induce a chronic inflammation in white adipose tissue, increasing the expression of proinflammatory cytokines (F. L. P. Soares et al. 2013). Altogether, it determines the alteration of lipid metabolism, insulin resistance and the increase of body weight, typical characteristics of the metabolic syndrome. Melatonin (N-acetyl-5-methoxytryptamine), a hormone mainly synthesized by the pineal gland, regulates circadian rhythm in mammals. Melatonin is involved in a wide range of other physiological functions, including antioxidant, anti-inflammatory, immunomodulatory, and regulates vascular contraction. Moreover, melatonin levels inversely correlate with obesity. Since the reduction in melatonin levels is associated with insulin resistance and metabolic syndrome, the aim of this study was to evaluate the effect of Melatonin on the gliadin-induced inflammation in white adipocytes.
MATERIALS AND METHODS: Cell culture and treatment: 3T3L1 preadipocyte cells cultured in specific medium have been maintained for 12-14 days in differentiation medium in order to obtain adipocytes. White adipocytes were treated with 0,5, 1 and 2 mg/ml of gliadin for 24 hours to induce the typical inflammatory phenotype of adipose tissue. After 24 hours, the cells have been treated with Melatonin (1 μM) at different time points: 24, 48, and 72 hours. At the end of the treatment, cells were collected, RNA extracted, and used for molecular analyses.
RESULTS: The treatment with gliadin has resulted in an increase of inflammatory cytokines such as TNF-α, IL-6, IL-15 and also increased the expression of markers involved in lipid metabolism such as Leptin, Resistin, Visfatin Adiponectin, Lipoprotein lipase, hormone-sensitive lipase, Acetyl CoA Carboxylase. Melatonin anti-inflammatory effect was demonstrated through, the activation of MT1 and MT2 receptors as demonstrated by protein kinase A (PKA) and C (PKC) and ERK1/2 expression, which resulted in TNF-α, IL-6, IL-15 reduction, and IL-4 and IL-10 increase. In addition, it has also been demonstrated that melatonin reduced the expression of inflammatory adipokines, while increased the expression of Adiponectin, LPL, HSL, ACC.
CONCLUSIONS: These results support a hypothetic role of melatonin to prevent the onset of symptoms typical of metabolic syndrome in gluten sensitive subject.

COVID-19 PATIENTS AND THROMBOSIS: THE ROLE OF PLATELET AND ENDOTHELIAL ACTIVATION

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BACKGROUND: Patients with severe COVID-19 pneumonia experience hypoxemia, endothelial dysfunction and a systemic cytokine storm, causing micro- and macro-thrombosis. We hypothesize that the cytokine storm may lead to consistent cell-based Tissue Factor (TF)- mediated activation of blood coagulation, procoagulant microvesicles (MVs) release and massive platelet activation. The aim of this study was to assess in 46 COVID-19 patients: 1) levels of TF+ circulating cells and Mvs; 2) residual plasma thrombin generation capacity despite heparin treatment; 3) extent of platelet and endothelial activation. Finally, through an in vitro approach, we verified whether: 4) plasma from COVID-19 patients was able to reproduce the platelet activation observed in vivo when added to blood cells from healthy subjects (HS); 5) treatment with tocilizumab or antiplatelet drugs was effective in reverting platelet activation.
METHODS: TF+-platelets, -monocytes, -granulocytes, and -platelet-leukocyte aggregates (PLA), platelet activation markers (P-selectin and percentage of PLA) and the MV profile were evaluated by whole blood flow cytometry. Thrombin generation (TG), analyzed by CAT, and Nitric oxide (NO) and PGI2 synthesis assessed by LC-MS/MS were also measured. HS and coronary artery disease (CAD) patients were used for comparison.
RESULTS: COVID-19 patients had higher levels of TF+-platelets, -granulocytes and -MVs (2-to-3- fold) than HS and CAD ($p<0.0001$). A residual MV-associated-TG was measured in plasma of patients treated with prophylactic anticoagulation. Furthermore, a sustained platelet activation, in terms of P-selectin expression and PLA formation (10-fold vs HS and CAD), was also observed. Of note, the synthesis of NO and PGI2 that control platelet activation, was deeply affected ($p<0.0001$). Through an in vitro approach, we observed that COVID-19 plasma, added to blood of HS, induced platelet activation similarly to what observed in vivo. This effect was blunted by pre- incubation with tocilizumab as well as by aspirin and AR-C69931MX.
CONCLUSIONS: All together our findings revealed how the cytokine storm present in COVID-19 patients induces, in concert with the imbalance of the endothelial functions, a massive cell activation with production of TF, mainly by platelets, granulocytes and MVs, these latter responsible for the residual thrombin generation capacity measured in plasma of all patients treated with prophylactic anticoagulation. Furthermore, COVID-19 patients are characterized by a sustained platelet activation with formation of platelet-leukocyte aggregates that may be involved in the microthrombi found in autoptic specimens. Finally, the results provide insights into the IL-6 driven pathophysiological mechanisms that trigger the hypercoagulable state in COVID-19 and pave the rationale for the effectiveness of antiplatelet drugs.

DOXORUBICIN-INDUCED IMMUNOGENIC CELL DEATH IS ENHANCED BY ULTRASOUND EXPOSURE IN OVARIAN CANCER CELLS

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BACKGROUND: Ultrasound (US) is a mechanical wave that can be employed in many different fields. In recent years, the safety and very good tissue penetrating ability of US has prompted to the possibility of employing US-based strategies, not just as diagnostic tool, but also for therapeutic purposes. Therefore, US has been used for various therapeutic approaches such as promoting tissue healing, decreasing chronic pain, tumor ablation and drug release from drug delivery systems but, one of the most attractive approach, remains the sonodynamic therapy (SDT). SDT is an anticancer and antibacterial approach based on the use of US to activate a chemical compound, called sonosensitizer, through acoustic cavitation enhancing the sonosensitizer cytotoxicity. A chemical compound that can act as sonosensitizer, is doxorubicin, a potent anticancer agent used for the treatment of a huge variety of cancers. In particular it has a key role in the second line treatment of ovarian cancer. However, the overall survival rates of ovarian cancer remain considerably worse than those for other gynecological malignancies mainly because the cancer comes back, or recurs, after treatment in more than 70% of women with the disease. For this reason, many efforts in pharmacological research have been made in order to increase doxorubicin efficacy against ovarian cancer such as inducing immunogenic cell death (ICD) for a long lasting protective antitumor activity. On this point, US-based strategies, as SDT, seems to be highly encouraging.
METHODS: In this work, we addressed the relapsing issue, proper of ovarian cancer, investigating the increase of doxorubicin-induced ICD through US. In this regard, experiments have been carried out on the human ovarian cancer cell line A2780 to test if the activation of low doxorubicin concentrations by US is effective in maximizing the doxorubicin-induced ICD. Moreover, hypericin, a natural well-known ICD inducer, has been used as yardstick, in comparison to doxorubicin.
RESULTS: The activity of doxorubicin and hypericin under US exposure was first investigated on A2780 cell proliferation, observing a statically significant decrease of cell proliferation over time. Along with this, specific biomarkers associated with ICD have been taken into consideration such as the expression of calreticulin (CRT) at the cell surface and the ATP production. US exposure of doxorubicin or hypericin was able to induce a statistically significant increase of ATP production and CRT expression. Finally, the expression of genes involved in the ICD pathway (such as CRT, LC3II and HMGB1) have been analyzed by real time RT-PCR after US exposure of doxorubicin or hypericin.
CONCLUSIONS: The data obtained showed, for the first time, that the use of US is an efficient strategy to significantly boost doxorubicin-induced ICD, opening new encouraging developments in the treatment of ovarian cancer.

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN 9 (PCSK9) ASSOCIATION TO SERUM LIPOPROTEINS. EFFECT OF ANTI PCSK9 MONOCLONAL ANTIBODIES

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BACKGROUND: PCSK9 is a protein involved in low-density lipoprotein receptor (LDLR) degradation and low-density lipoprotein cholesterol (LDL-C) metabolism. Monoclonal antibodies (mAbs) blocking PCSK9 reduce LDL-C levels through the prevention of PCSK9 binding to the LDLR. PCSK9 associates with lipoproteins (LPs) in serum, we aimed at confirming this finding, and to understand whether treatment of patients with anti PCSK9 monoclonal antibodies results in a shift of this association.
METHODS: Fresh sera were collected from 60 volunteers, including Evolocumab-treated and untreated subjects. Lipoprotein fractions were obtained using different methods, including precipitation with phosphotungstic acid, fast protein liquid chromatography (FPLC), ultracentrifugation using KBr or iodixanol gradient (IGr). The lipoprotein fractions obtained were analyzed to detect PCSK9 with ELISA, while Lp(a), ApoB, ApoA1 and cholesterol were quantified using clinical-grade turbidimetry or colorimetry-based assays.
RESULTS: In the precipitation-mediated assay, around 80% of PCSK9 was found in the ApoB fraction of sera. With FPLC, around 11% of recovered PCSK9 was in the LDL fraction. Negligible amount of PCSK9 was detectable in lipoprotein fractions isolated by KBr ultracentrifugation; more than 20% of PCSK9 was found in LDL fraction obtained with the IGr ultracentrifugation. In Evolocumab-treated patients, preliminary IGr ultracentrifugation data showed a PCSK9/LDL-C ratio higher than in control subjects.
CONCLUSIONS: Based on our observations, it appears that the association of PCSK9 and LDL changes with the LPs isolation method and is sensitive to salt concentrations. No binding to other lipoproteins was detected. Preliminary data on Evolocumab-treated patients suggest that mAbs do not interfere with PCSK9 association with LPs. Further studies are needed to define the type of interaction and its possible biological consequences on both PCSK9 and LPs metabolism.

INDIVIDUAL TRAJECTORY TO DEVELOPMENT OF SUBSTANCE USE DISORDERS: FOCUS ON OPIOIDS AND PSYCHOSTIMULANTS

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Drug addiction is a chronic psychiatric disorder in which affected individuals lose control over drugs of abuse. Therapies for addiction do exist, but their efficacy is limited to subsets of responsive patients. In addition, many promising preclinical targets failed to translate into clinic. This could derive from a mismatch between preclinical research and clinical practice. While a group-based approach represents the standard in preclinical research, in humans not every drug user develops addiction, but only a subset of vulnerable subjects. This individual heterogeneity has been largely overlooked in preclinical research. In earlier studies, we studied the neuroimaging underpinnings of individual vulnerability to cocaine addiction at preclinical level. We reported that addiction vulnerable and resilient rats did not differ in grey matter volume (GMV), however, vulnerable rats showed reduced cerebral activity, whereas resilient rats showed activity comparable to cocaine naïve controls. Interestingly, cocaine addicts show both reduced GMV and brain activity. Which, together with our data, indicate that reduction in bran activity, but not GMV, is a translatable marker of vulnerability to cocaine addiction. More generally, our data indicated that individual variability in preclinical research could be used to highlight translational biological traits.

Next we switched our focus toward opioid use disorders (OUD), investigating how GMV interacts with heroin dependence. To answer this question, we subjected NIH Heterogeneous Stock (HS) rats to a longitudinal MRI study in which GMV was measured before and after exposure to heroin. Heroin-naïve HS rats were subjected to T2-weighted MRI acquisitions. Rats were then divided into sex-balanced heroin-naive and heroin-exposed groups (N=10-15). The heroin group was initially trained to 1h short access (ShA) heroin (60µg/kg/infusion) self-administration and then switched to 12h long-access (LgA). Motivation for heroin and heroin-primed reinstatement were also tested. After heroin training, T2-weighted MRI signals were again acquired in heroin-experienced and heroin-naive rats.

Heroin experienced rats showed escalation of heroin intake during LgA training. Motivation for heroin increased between ShA and LgA. In the reinstatement test, heroin primed reinstatement of seeking. HS rats showed high variation in heroin seeking behaviors. GMV did not differ between groups before heroin training, but there was a GMV reduction in the orbitofrontal, medial prefrontal, and insular cortices, of the heroin group. A larger insular GMV at baseline corresponded to a higher escalation of intake. We also found that a higher heroin intake, escalation and reinstatement ratio corresponded

to a bigger reduction of GMV in insular and frontal cortices.
In conclusion, HS rats developed heroin dependence-like behavior. Insular GMV in naïve animals predicted escalation and motivation for heroin. Heroin induced, and a larger GMV reduction was associated with increased addictive-like behavior.

A MULTI-SITE PRECLINICAL APPROACH HIGHLIGHTS EVIDENCE OF OUD INDIVIDUAL VULNERABILITY PROFILES IN THE RAT

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BACKGROUND: The misuse of opioids-based pain therapies has caused a resurgence of opioid use disorder (OUD) in the US known as the opioid epidemic. Not every drug user develops OUD, but only a subset of vulnerable subjects. Therefore, we set-out with a multi-site project to develop a model of individual variability in OUD. This would allow studying the neurobiology and genetic of OUD vulnerability, permitting to tailor pain treatments for patients carrying innate vulnerability to OUD and to foster the development of efficacious OUD therapies.
METHODS: NIH Heterogeneous Stock rats were screened by two independent units (MUSC N=170; UCAM N=134) in three blocks of experiments. In the first and third blocks, rats' innate exploratory activity, anxiety and pain perception were measured by open-field, elevated plus maze and tail-flick test respectively, before and after heroin history. In the second block rats' individual response to heroin reinforcement was tested. Rats were trained to heroin (20μg/kg/inf.) self-administration in 12-hours sessions under fixed ratio 1 contingency. After twelve sessions, total heroin consumption and escalation of intake were extracted. Then, motivation for heroin was scored in a progressive ratio session. Finally, heroin seeking was tested in two models of relapse, cued and heroin primed reinstatement.
RESULTS: To remove batch-specific effects, data produced at MUSC and UCAM were z-score transformed within site. The batch correction was validated by UMAP dimension reduction algorithm. Correlations were then computed between each rat using all available experimental measures. A K-Nearest Neighbors (KNN) graph was constructed, in which each rat shared an edge with the K=3 most highly correlated other rats in the data. A Bayesian stochastic block model (SBM) was fit to the KNN graph to assess possible clustering among rats. The number of SBM clusters was specified a priori using a singular value threshold heuristic applied to the KNN graph, which suggested M=3 distinct clusters in the network. Posterior estimation of the SBM parameters revealed statistically significant separation among the three clusters. Cluster 1 included 63 subjects (31 MUSC, 32 UCAM), cluster 2

150 (83 MUSC, 67 UCAM), and cluster 3 91 (56 MUSC, 35 UCAM). Behavioral differences between clusters were analyzed by Kruskal-Wallis test. Cluster 1 showed higher motivation for heroin (p=3.3e-10), heroin intake (p=1.2e-10), escalation (p=8.7e-9) and primed reinstatement (p=1.9e-9) compared to clusters 2 and 3. Clusters 1 and 2 showed higher cued-reinstatement (p=2.1e-9) compared to cluster 3. In all measure cluster 2 scored between clusters 1 and 3.

CONCLUSIONS: Our data provide evidence of individual vulnerability to OUD in the rat. In line with human OUD epidemiology, cluster 1 had 20% prevalence, it scored higher in all measures of addiction and may represent OUD vulnerable subjects. Cluster-3 may represent subjects with an innate resilience to develop OUD.

PLATELET ACTIVATION IN PATENT FORAMEN OVALE (PFO) PATIENTS WITH MIGRAINE ON ASPIRIN TREATMENT

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BACKGROUND: Migraine is a chronic neurovascular disorder with a multifactorial aetiology. Association with patent foramen ovale (PFO) has been reported, with symptom regression after PFO closure. Increased platelet aggregation has been documented in subjects with migraine, who, of note, were not on antiplatelet treatment. Current guidelines suggest the use of aspirin or clopidogrel. To date, however, no further insights into platelet activation in migraineurs with PFO treated with antiplatelet drugs have been reported.

The aim of this study was to characterize platelet activation, microvesicle (MVs) profile and platelet thrombin generation capacity (TGC) in PFO patients with migraine on aspirin before and after PFO closure.

METHODS: 61 PFO patients with migraine on aspirin treatment were enrolled. Platelet activation markers (Pselectin, activated glycoprotein IIb/IIIa [aGpIIb/IIIa], Tissue Factor [TF]) and the MV profile, focusing on those derived from platelets, granulocytes, monocytes, erythrocytes and endothelium, were evaluated by whole blood flow cytometry; and TGC by CAT the day before (T0) and 6-months after (T1) PFO closure. 8 healthy subjects (HS), treated with aspirin for 7 days, were recruited as controls. Compliance to antiplatelet therapy was assessed by arachidonic acid (AA)-induced platelet aggregation by light transmission aggregometry (LTA).

RESULTS: The treatment with aspirin was effective in almost all the enrolled patients: no platelet aggregation to AA was observed. The expression of classical marker of platelet activation, Pselectin and activated aGpIIb/IIIa, was similar in PFO patients before and after PFO closure and HS on the same pharmacological treatment. Conversely, in PFO patients at T0, TF+ platelets were significantly higher than in HS (3.3%±1.9 vs 2.3%±1.1, respectively; p=0.01): the presence of PFO causes an increase in platelet TF expression that is not sufficiently inhibited by aspirin treatment. After PFO closure, TF expression significantly decreased (p<0.0001). At T0, platelets generated a greater amount of thrombin than at T1, as well as the lag time, the thrombin formation time directly correlated to the amount of TF, was significantly faster at T0 than at T1 (26±7

and 33±12 min, respectively; p=0.005). Addition of a neutralizing αTF antibody confirmed that TGC was TF-dependent. The total number of circulating MVs, the most abundant of them coming from platelets, decreased after PFO closure (108450±49099 at T0 vs 79777±32591 at T1 vs 65647±26506 MVs/μl in HS; p=0.01), as well as the number of TF+ and of procoagulant (AnnV+/TF+) MVs (p<0.0001).

CONCLUSIONS: These data indicate that aspirin treatment in PFO patients is not able to fully control platelet activation. It is only after PFO correction that TF expression, the number of circulating MVs and platelet prothrombotic potential revert to physiological levels. PFO closure becomes crucial in restoring vascular homeostasis, thus favouring migraine resolution.

SIMVASTATIN TREATMENT DOES NOT AMELIORATE MUSCLE PATHOPHYSIOLOGY IN A MOUSEMODEL FOR DUCHENNE MUSCULAR DYSTROPHY

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BACKGROUND: Duchenne muscular dystrophy is an X-linked, recessive muscular dystrophy in which the absenceof the dystrophin protein leads to fibrosis, inflammation and oxidative stress, resulting inprogressive loss of muscle tissue. Currently there is no cure, and most of the pre-clinical studies inmouse models aim to develop new therapeutic strategies. Up to now, the only available treatmentis corticosteroids, able to counteract the inflammatory response. One big effort of the scientificcommunity is drug repurposing, i.e. using drugs already approved for other disorders, aspharmacological approaches even if unable to restore the primary defect, are able to massivelyameliorate patients' life quality. Drug repurposing is attractive as it decreases drugs developmenttime and animal use, thus decreasing the overall cost of research.
METHODS: Recent studies1 suggested that Simvastatin, which is a cholesterol lowering drug used forcardiovascular diseases2, has beneficial effects on several parameters in mdx mice, the mousemodel for DMD. To properly validate the effectiveness of simvastatin, we tested the effects of 12-week simvastatin oral treatment in adult (starting at 12 weeks of age) mdx mice.
RESULTS: The dose administrated to the mice was similar to the one published previously, 7-10 mg/kg/day.The outcome of the treatment was evaluated on several parameters, such as muscle function,histology or expression of genes involved in fibrosis, regeneration, oxidative stress and autophagy,mostly on diaphragm since is the most severely affected muscle. Functional performance,assessed after 4 and 8 weeks of treatment, using a four limb hang-

ing test showed no differencesin hanging times between control and treated mice. At the end of the treatment, force frequencyrelationship, a measure of muscle strength, and response to eccentric, lengthening, contractions,a measure of membrane integrity, were determined in situ in the tibialis anterior by stimulation ofthe sciatic nerve and ex vivo in the diaphragm by direct muscle stimulation. Both measure didn'tshow any improvement by simvastatin treatment. Markers of fibrosis and inflammation assessedboth by qPCR and at histological level didn't show any amelioration in treated mice in respect tothe control.Then, although the treatment protocol was similar to the one suggested by previous publications,simvastatin plasma levels were found be much lower than the ones observed in a previous study.Overall, Simvastatin treatment did not ameliorate the muscle pathology.

CONCLUSIONS: Results showed that simvastatin did not ameliorate disease pathology in mdx mice, which couldeither be due to the ineffectiveness of simvastatin itself or to the low simvastatin plasma levelsfollowing oral administration. Further studies are needed to assess whether a differentadministration route could give a better result for this specific drug.1. Whitehead et al., PNAS 20152. Vuorio et al., Cochrane database system 2019.

DETRIMENTAL EFFECTS OF THE 'BATH SALT' METHYLENEDIOXYPYROVALERONE ON SOCIAL PLAY BEHAVIOR IN MALE RATS

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BACKGROUND: Methylenedioxypyrovalerone (MDPV) is the most popular synthetic cathinone found in products marketed as 'bath salts', widely abused among teenagers and young adults. Synthetic cathinones have pharmacological effects resembling those of psychostimulants, which are known to disrupt a variety of social behaviors. To investigate the impact of MDPV on social behavior at young age, and the underlying neurobehavioral mechanisms, we focused on social play behavior, which is the most characteristic social behavior displayed by young mammals and it is crucial for neurobehavioral development.

METHODS: Eight experiments were performed to assess the effects of MDPV on social play behavior. In experiment 1 juvenile rats were treated with a broad range of doses of MDPV (0.025, 0.05, 0.1, 0.25, or 0.5 mg/kg) or saline (control group) while, in experiment 2, we tested the effects of the same doses of MDPV on social behavior in young adult rats. In experiment 3 we investigated whether the effects of MDPV on social play in juvenile rats depended on the behavior of the test partner and/or if the social repertoire of saline-treated rats was influenced by MDPV- treated partners, so we treated none, one, or both members of a test pair with MDPV (0.5mg/kg). Next, we investigated whether tolerance or sensitization would occur to the effect of MDPV on social play behavior after repeated treatment with either MDPV (0.5 mg/kg) or saline for 5 consecutive days. The 6th day, before testing, animals were treated with either saline or MDPV at the effective dose of 0.5mg/kg to assess the tolerance (experiment 4), or at the sub-effective dose of 0.1mg/kg to assess the sensitization (experiment 5). Therefore, in experiment 6 and 7 respectively, we treated animals with the dopamine receptor antagonist flupenthixol (0.125 mg/kg) and

with the α2 adrenoceptor antagonist RX821002 (0.2 mg/kg) before MDPV (0.5 mg/kg), to clarify whether MDPV exerts its effects on social play through dopaminergic or noradrenergic neurotransmission. Last, in experiment 8 we investigated whether dopaminergic and noradrenergic neurotransmission are simultaneously involved in the effect of MDPV, by treating the animals with sub-effective doses of both RX821002 (0.1 mg/kg) and flupenthixol (0.06 mg/kg), or with saline before MDPV 0.5 mg/kg treatment.

RESULTS: Treatment with MDPV reduced social play behavior in both juvenile and young adult male rats, and its play-suppressant effect was subject to tolerance but not sensitization. The effects of MDPV were blocked by either RX821002 or flupenthixol, given alone or together at sub-effective doses. To sum up, MDPV selectively suppresses the most vigorous social behavior of developing rats through both noradrenergic and dopaminergic mechanisms.

CONCLUSIONS: This study provides important preclinical evidence of the deleterious effects of MDPV on social behavior, and as such increases our understanding of the neurobehavioral effects of this popular cathinone.

IDENTIFICATION OF TARGETS FOR INDUCING DEATH OF REGULATORY T CELLS (TREGS) IN THE TUMOR MICROENVIRONMENT OF CLEAR CELL RENAL CELL CARCINOMA (CCRCC)

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BACKGROUND: Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney cancer in adults, responsible for approximately 90–95% of kidney cancers, and it is notoriously resistant to radiation therapy and chemotherapy. Immunotherapy is one of the most promising approaches for the treatment of human cancers. Antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) are the mechanisms by which some therapeutic monoclonal antibodies (mAbs), like rituximab, and trastuzumab kill cells. ADCC/ADCP requires binding of mAbs and activation of activating Fcγ receptors (FcγR) expressed by myeloid and NK cells, which promotes their killing activity.
Killing of regulatory T cells (Tregs) that infiltrates cancers may represent a new way to favor tumor rejection by the host's immune system (1), as demonstrated, for example, with the anti-GITR mAbs promoting tumor rejection in some murine models (2). In this context, it is crucial to find surface proteins expressed at high levels by Tregs in the tumor microenvironment (TME). The project aims to find the best mAb favoring ADCC/ADCP of Tregs in the TME of ccRCC.

METHODS: The study was segmented into three phases: 1) 32 Treg targets were screened through in-silico analysis of human-derived data and the expression level of the genes in ccRCC TME and healthy control tissue (HT) was evaluated; 2) The most promising targets were evaluated directly in human tumor-infiltrating lymphocytes (TILs) obtained from fresh tumor specimens, trough RT-qPCR, and multicolor

flow-cytometry; 3) mAbs against the validated targets were tested in primary cultures of TILs from ccRCC tumor specimens, for their ability to induce preferential Treg killing.
RESULTS: Trough the bioinformatic approach, we found that seven Treg markers were over-expressed more than 1.3 fold in ccRCC TME compared to HT. RT-qPCR was used to validate the over-expression of markers in 16 ccRCC specimens; five Treg markers (LAP, ENTPD1, HLA-DRA, OX40, and TIGIT) were confirmed to be significantly over-expressed by ccRCC samples. Then, we identified and characterized a never described Treg cell population through multicolor flow-cytometry analysis. Following the preparation of primary cultures of TILs from ccRCC, we tested the in vitro effects of some mAbs against the identified targets; results allowed us to identify mAbs that decreases the percentage of Tregs in ccRCC TME, while increasing proliferation rate of conventional T cells, and CD8+ T cells.
CONCLUSIONS: We identified 1) a new Treg subpopulation in the ccRCC TME and 2) potential new therapeutic targets in the treatment of ccRCC. In particular, the study results are proof of principle that mAbs against these targets can potentiate the patient's immune response, favoring tumor rejection.
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EXPANSION OF GITR+ TREG CELLS THROUGH MICROENCAPSULATED G3C HYBRIDOMA CELL GRAFT DELAYS THE ONSET OF SPONTANEOUS DIABETES IN NOD MICE

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BACKGROUND: Type 1 diabetes is due to the autoimmune disruption of pancreatic islet β-cells. As an alternative to lifelong insulin supplementation, potentiation of immune tolerance in patients with type 1 diabetes could prevent the autoimmune destruction of pancreatic islet β-cells (1). Regulatory T cells (Tregs) are specialized cells that control immune responses to pathogens and mediate immunological self-tolerance and homeostasis. Glucocorticoid-induced TNFR-related (GITR or TNFRSF18) receptor plays a pivotal role in the maturation and expansion of Tregs, both thymus-derived (tTreg) and peripherally-derived (pTreg)(2). This study aims to assess whether the G3c monoclonal antibody (mAb), which triggers GITR, promotes the expansion of regulatory T cells (Tregs) in diabetic-prone NOD mice preventing diabetes onset.
METHODS: G3c mAb was delivered via G3C hybridoma cells enveloped in alginate-based microcapsules that were grafted intraperitoneally into NOD mice under general anesthesia. For each mouse, 1.5x10^6 G3C hybridoma cells in 1 mL of microcapsules (G3C/cps) or 1 mL of empty microcapsules (e/cps) as a control, were grafted. After the graft, all animals were monitored, as far as not fasting blood glucose and body weight,

twice a week for 21 days (short-term study) and 90 days (long-term study). The following morphological and immune molecular tests have been scheduled: 1) morphological evaluation of the pancreas; 2) immunophenotyping of immune cells from pancreas and spleen; 3) ELISA for plasmatic IgGs and G3C levels.

RESULTS: In the short-term study, after G3C/cps graft, we observed expansion of conventional CD4+CD25+GITRhighFoxP3+ Tregs and non-conventional CD4+CD25-/lowFoxP3low-GITRint/high (GITRsp) Tregs in the spleen. Expansion of both Treg subsets, including antigen-specific Tregs (IGRP, NRP, and InsB Tetramer+ Treg cells), was also observed in the pancreas of G3C/cps-treated as compared to e/cps-treated NOD mice. Moreover, the number of intact islets was higher in the pancreas of G3C/cps-treated than in the pancreas of e/cps-treated and untreated animals.

In the long-term study, all but two G3C/cps-treated mice (95%) did not develop diabetes, and all but one survived till the end of the study. In comparison, only 50% of untreated NOD mice survived at the end of the study. Interestingly, expansion of GITR+ Tregs in the spleen of G3C/cps grafted mice was still present and the level of FoxP3 mRNA in the pancreas of G3C/cps-treated mice was 4000 fold higher than in the pancreas of e/cps and untreated diabetic mice.

CONCLUSIONS: In conclusion, long-term GITR triggering induces Treg expansion, thereby delaying/preventing diabetes development in NOD mice (3). This therapeutic approach may have promising clinical potential for the treatment of inflammatory and autoimmune diseases.

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ANTI-TUMOR ACTIVITY OF THE EXTRA-VIRGIN OLIVE OIL POLYPHENOL OLEACEIN IN HUMAN MELANOMA CELLS THROUGH THE MODULATION OF MIRNA EXPRESSION

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BACKGROUND: Extra-virgin olive oil (EVOO) polyphenols contribute to Mediterranean diet health-promoting properties. One of the most abundant secoiridoid present in EVOO, Oleacein (OA), demonstrated anticancer activity against several tumors. Nevertheless, its role against melanoma has not still investigated. This study aimed at determining in vitro the anti-melanoma activity of OA and the relative mechanism of action.

METHODS: To this aim, 501Mel melanoma cells were treated with OA. In OA-treated cells, the apoptotic process and cell cycle were evaluated. Levels of mRNA gene expression as well as miRNAs were measured by real-time PCR. Specifically, we concentrated on genes involved in the mammalian target of rapamycin (mTOR) signaling pathway, which is a positive regulator of cell growth and proliferation, and of BCL2 family, which is overexpressed in many cancers including melanoma.
RESULTS: OA induced cell growth inhibition in 501Mel melanoma cells with an IC50 in the low micromolar range of concentrations. Moreover, an OA concentration approximating the IC50 induced G1/S phase arrest, DNA fragmentation, and down-regulation of genes encoding anti-apoptotic (BCL2 and MCL1) and pro-proliferative (c-KIT, K-RAS, PIK3R3, mTOR) proteins, while increased transcription levels of the pro-apoptotic protein BAX. Concordantly, OA increased the levels of miR-193a-3p (targeting MCL1, c-KIT and K-RAS), miR-193a-5p (targeting PIK3R3 and mTOR), miR-34a-5p (targeting BCL2 and c-KIT) and miR-16-5p (targeting BCL2, MCL1, K- RAS and mTOR), while decreased miR-214-3p (targeting BAX). These modulatory effects might contribute to the inhibition of 501Mel melanoma cell growth observed after treatment with an olive leaves-derived formulation rich in OA, with potential application against in situ cutaneous melanoma.
CONCLUSIONS: Altogether, these results demonstrate the ability of OA to contrast the proliferation of cutaneous melanoma cells through the transcriptional modulation of relevant genes and microRNAs, confirming the anticancer potential of EVOO and suggesting OA as a chemopreventive agent for cancer disease therapy.

CARNOSINE NEGATIVELY MODULATES PRO-OXIDANT AND PRO-INFLAMMATORY ACTIVITIES OF M1 MACROPHAGES

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BACKGROUND: Different types of cells are involved in the innate immune response, with macrophage cells representing those primarily activated, especially under different diseases characterized by oxidative stress and systemic inflammation such as depression and cardiovascular disorders. Carnosine is a natural endogenous dipeptide widely distributed in

mammalian tissues, existing at particularly high concentrations in muscles and brain and possessing well-characterized antioxidant and anti-inflammatory activities.

METHODS: In an in vitro model of macrophage activation (M1), represented by RAW 264.7 cells stimulated with lipopolysaccharide + interferon-γ (LPS + IFN-γ), we here report the ability of carnosine to modulate pro-oxidant and pro-inflammatory activities of macrophages. An ample set of parameters aimed to evaluate cytotoxicity (MTT assay), energy metabolism (HPLC), gene expressions (high-throughput real-time PCR (qRT-PCR)), protein expressions (western blot) and nitric oxide (NO) production (qRT-PCR, HPLC), was used to assess the effects of carnosine on activated macrophages challenged with a non-cytotoxic LPS (100 ng/mL) + IFN-γ (600 U/mL) concentration.

RESULTS: In our experimental model, therapeutic concentrations of carnosine exerted the following effects: 1) an increased degradation rate of NO into its non-toxic end-products nitrite and nitrate; 2) the amelioration of the macrophage energy state, by restoring nucleoside triphosphates and counterbalancing the changes in ATP/ADP, NAD+/NADH and NADP+/NADPH ratio obtained by LPS + IFN-γ induction; 3) a reduced expression of pro-oxidant enzymes (NADPH oxidase, Cyclooxygenase-2) and of the lipid peroxidation product malondialdehyde; 4) the rescue of antioxidant enzymes expression (Glutathione peroxidase 1, Superoxide dismutase 2, Catalase); 5) an increased synthesis of transforming growth factor-β1 (TGF-β1) combined with the negative modulation of interleukins 1β and 6 (IL-1β and IL-6), and 6) the induction of nuclear factor erythroid-derived 2-like 2 (Nrf2) and heme oxygenase-1 (HO-1).

CONCLUSIONS: In the present study we provided the first evidence that carnosine can negatively modulate the pro-oxidant and pro-inflammatory activities of activated (M1) macrophages. Our results suggest a dual antioxidant activity of carnosine as demonstrated by its ability to induce and potentiate the antioxidant machinery, simultaneously decreasing the expression of pro-oxidant enzymes and lipid peroxidation. All the aforementioned effects of carnosine suggest a therapeutic potential of this dipeptide in counteracting pro-oxidant and pro-inflammatory phenomena observed in different disorders characterized by elevated levels of oxidative stress and systemic inflammation such as depression and cardiovascular disorders.

TARGETING MICROGLIA TO MODULATE EARLY EFFECTS OF β-AMYLOID PEPTIDE

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BACKGROUND: Neuroinflammation is a feature common to several neurodegenerative conditions including Alzheimer's Disease (AD). Microglia, the resident immune cells of CNS, exert a crucial role during the development of neuroinflammation. Upon activation, microglia can polarize into two different phenotypes, the first, M1 phenotype, characterized by release of pro-inflammatory cytokines and molecules and the other, M2 phenotype, with an anti-inflammatory and repair-promoting role. This is characterized by secretion of neurotrophic factors and cytokines including brain-derived neurotrophic factor (BDNF). Based on our previous observation that low concentrations of β-amyloid (Aβ) rapidly increase BDNF expression in primary cultures of rat microglia, we decided to characterize this response to identify possi-

ble steps of intervention to maintain the early, beneficial response against Aβ.
METHODS: The human microglial cell line HMC3 cells was exposed to low concentrations of Aβ 1-42 (Aβ42) oligomers (200 nM-2 μM) and cellular extracts were analyzed by Real Time PCR, western blot, immunocytochemistry, ELISA and enzymatic activity assays.
RESULTS: Aβ42 induced the expression of BDNF in HMC3 cells as revealed by enhanced BDNF mRNA by Real Time-PCR. This effect was rapid (3 h) and sustained at 6 h and accompanied by an increase of BDNF content in the total protein extracts and in the cytosolic fraction. Aβ42 increased also released BDNF. In parallel, Aβ42 induced the expression of the deacetylase sirtuin-1 (SIRT-1), that increased in total protein extracts, as well as in cellular subfractions as shown by western blot and immunocytochemical analysis. Increased SIRT-1 levels were persistent after 24 h of exposure to Aβ42 and paralleled by a reduction of NFkB expression. Aβ42 induced an early induction of pAMPK and a subsequent stimulation of SIRT-1 enzymatic activity. Interestingly, the reported effects were sensitive to the SIRT-1 inhibitor EX527.
CONCLUSIONS: Our data confirm that in response to low concentrations of Aβ microglia polarize towards a BDNF-releasing phenotype, an effect mediated by the pAMPK/SIRT-1 pathway. The complete characterization of this pathway will serve as a basis for the study of microglial-neuronal crosstalk in response to Aβ challenge and can offer potential new targets for pharmacological intervention.

RETROSPECTIVE ANALYSIS OF ADVERSE DRUG REACTIONS REPORTED IN PEDIATRIC PATIENTS IN CALABRIA REGION

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BACKGROUND: Considering the shortage of clinical trials involving pediatric populations, several drugs are administered to children without authorized indications (off-label use), thus increasing the number of adverse drug reactions (ADRs) reported among these patients as long as the therapeutic risk. Monitoring the effects of the drugs, after their commercialization, is the primary endpoint of pharmacovigilance.
METHODS: In this retrospective analysis, ADR reports related to children and adolescents from Calabria (Italy) were retrieved from the data provided by Regional Center of Pharmacovigilance, considering the period between 2010 and 2019. After the exclusion of the data-missing reports, the characteristics of the remaining part were investigated in terms of related medications and ADR characteristics, across different age-groups. Vaccine-related reports and medications administered with off-label indications have been also evaluated.
RESULTS: Among 811 spontaneous reports provided, 806 have been analyzed. Of these, 47.6% were due to vaccines and 52.4% with other drugs, mainly with antibiotics (19.7%), Nonsteroidal anti-inflammatory drugs (11.2%), Central nervous system (CNS) drugs (9.4%) and gastrointestinal agents (2.2%). 50.4% of reports regarded patients younger than 2 years, most of which ADRs were not serious (73.2%). Only

15.4% of all ADRs were reported as serious, of which 76.6% led to hospitalization, mainly among patients <2 years old (42.1%). Almost all the reports were provided by hospital physicians (40.9%) and other health professionals (48.5%). Overall, 1008 ADRs were reported, most of which were not serious (70%) and resulted in skin reactions (83.3%), fever (83.1%) and agitation (71.5%). Whereas among serious events, CNS related (29.8%), allergy (27.6%) and skin reactions (12.8%) were the most frequent. Sorting the ADR reports for age-groups, 60.22% of them were related to patients <2 years old, with a higher prevalence of hyperpyrexia (98.8%), agitation (88.5), fever (86.6%) and local reactions (75.7%). Vaccine's ADRs were mostly reported in patients <2 years old (83.1%) whereas other drugs reports were almost equally distributed among the different age groups. Vaccine-related ADRs were hyperpyrexia (98.8%) fever (95.1%), local reactions (90%), agitation (87.3%), and CNS related (74.2%); on the other hand, skin reactions (90.6%) and erythema (83.5%) were the most common findings related to other drugs' ADRs. Finally, 26 reports were related to off-label administration, mainly for reference population (drugs not authorized for pediatrics) (73%), dose (15.3%) and indication (11.4%). Most of these reports indicated skin reactions (29%), erythema (12.9%), CNS-related (12.9%) and allergy (12.9%) as the most frequent ADRs, and antibiotics and antifungal as the most frequent drugs related to them.

CONCLUSIONS: This study underlines the safety concerns related to drug administration in children and reflects the different patterns of ADR distribution across different age-groups.

THE ANTI-INFLAMMATORY PROPERTIES OF KYP-2047 AS THERAPEUTIC POTENTIAL IN THE TREATMENT OF CHRONIC VENOUS INSUFFICIENCY

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BACKGROUND: Chronic venous insufficiency (CVI) is a common disorder related to functional and morphological abnormalities of the venous system and associated with a variety of symptoms in later disease stages; despite the high prevalence of this pathology, its exact etiology remains unclear and suitable pharmaceutical therapies have not been explored to date. Although venous hypertension and venous biomechanical stretch have been known to be crucial for development of the illness, recent findings indicate that inflammatory processes and angiogenesis alterations greatly concur to the onset of varicose vein. KYP-2047 is a very potent, selective inhibitor of Prolyl oligopeptidase (POP), a serine protease involved in the release of pro-angiogenic and anti- fibrogenic molecules. The aim of the present study was to evaluate the capacity of KYP-2047 to influence the angiogenic and inflammatory mechanisms involved in the pathophysiology of CVI, to restore normal vascular blood flow.

METHODS: The properties of KYP-2047 have been tested on in vivo model of CVI-induced by saphene vein ligation. Rats were subjected to CVI induction followed by KYP-2047 treatment for 24 hr. Then, some of saphene veins taken, were cultured to perform immunofluorescence evaluation for VEGF and CD34 markers expression. Histological analysis, Masson's

trichrome staining and mast cells evaluation were performed on saphene vein samples. Release of cytokines and nitric oxide synthase production were evaluated.
RESULTS: Decreased expression of VEGF and CD34 has been found in rats CVI-injured treated with KYP-2047, compared to the damage group, confirming KYP-2047 role in the management of angiogenesis. KYP-2047 treatment has reset the histological abnormalities of the venous wall, reducing cytokines and mast cells levels, bringing out its vasculoregulatory and anti-inflammatory properties.
CONCLUSIONS: For the first time, this research revealed the therapeutic potential of KYP-2047 as a helpful treatment for the management of CVI, suitable to reduce varicose vein pathophysiology and to regularize venous tone.

EFFECTS OF ULTRAMICRONIZED PALMITOYLETHANOLAMIDE TREATMENT ON THE GLUTAMATERGIC ALTERATIONS AND MITOCHONDRIAL IMPAIRMENT IN 3×TG-AD MICE

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BACKGROUND: Mitochondrial dysfunction plays a pivotal role in glutamate-induced excitotoxicity in Alzheimer Disease (AD) pathogenesis. Moreover, inflammation significantly contributes to disease progression and chronicity. In this regard, Palmitoylethanolamide (PEA), acting as a peroxisome proliferator activated receptors (PPAR)-alfa agonist, is an endogenous lipid that modulates inflammation and protects cells from glutamate toxicity. We exploited the availability of a triple transgenic model of AD (3×Tg-AD) to test the hypothesis that (i) the progressive accumulation of Abeta might affect mitochondrial functioning and glutamatergic transmission; (ii) chronic treatment with ultramicronized-PEA (um-PEA) might modulate the onset and the progression of AD.
METHODS: Both young ("presymptomatic", at 3 months of age) and old ("symptomatic", at 9 months of age) 3×Tg-AD mice were subcutaneously implanted with a 90-day-release pellet containing either 28 mg of um-PEA or placebo. At the end of 90-day treatment, we tested our hypothesis following an integrated approach, involving microdialysis/HPLC and mitochondrial experiments. In particular, the glutamate release was evaluated in the ventral hippocampus (vHIPP) of freely moving mice under basal and stimulated condition. Mitochondrial respiration and ATP homeostasis were evaluated in the frontal cortex (FC), vHIPP, brain regions manly affected by AD pathology.
RESULTS: The basal extracellular glutamate levels in the vHIPP of 6-month-old 3×Tg-AD mice were significantly higher compared to age-matched Non-Tg mice, whereas any genotype-related differences were observed in 12-month-old mice. Chronic um-PEA treatment did not affect the basal output of glutamate at both ages. Moreover, we observed the total lack of response to K+- stimulation in the

vHIPP of 3×Tg-AD mice compared to Non-Tg group, at both 6 and 12 months of age. Chronic treatment of um-PEA did not ameliorate the impaired K+-evoked output of glutamate both in 6- and 12-month-old 3×Tg-AD mice. As far as the mitochondrial oxygen consumption and ATP homeostasis, no difference was observed within the FC and vHIPP among 6-month-old 3×Tg- AD and Non-Tg mice, respectively treated with either um-PEA or placebo. In contrast, 12-month- old 3×Tg-AD mice exhibited high complex I respiration rate of hippocampal mitochondria, whilst a reduction was observed in the FC, which was attenuated by chronic treatment with um-PEA. Moreover, F0F1-ATPase activity and ATP content were reduced both in vHIPP and FC of transgenic mice and um-PEA treatment counteracted these effects only in the latter area.

CONCLUSIONS: Our data suggest that impairments of mitochondrial bioenergetics might sustain the failure in the energy-requiring glutamatergic transmission and that the chronic um-PEA treatment partially restores such alterations in old symptomatic mice, thus suggesting that um-PEA might represent a promising target for the development of novel anti-Alzheimer's therapies.

DEVELOPMENTAL FRAMEWORK OF ALCOHOL BINGE-DRINKING: THE IMPACT ON SOCIABILITY AND STRESS RESPONSIVITY, WHILE INVESTIGATING ON ECB SYSTEM MANIPULATION AS A POTENTIAL THERAPEUTIC STRATEGY

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BACKGROUND: Social environment challenges the behavioral homeostasis in adolescence, the ability to recover from adversities, and the appropriate social interactions, whose disruption leads to risky behaviors, including the engagement in binge alcohol drinking pattern – episodic excessive consumption followed by variable mini-withdrawals. Since adolescence-unique neuroadaptations make this epoch a crucial transition to adulthood, the relationship between binge-pattern and reactivity to stress, as well as sociability, needs to be addressed. Furthermore, the potential of cannabidiol (CBD) as a rescue strategy deserves a careful evaluation.

METHODS: We examined i) the effects of binge alcohol drinking (BAD) during adolescence and withdrawal (WDL) on behavioral correlates of social dimension ii) whether BAD in adolescence affects social stress (SS) response, exploring copying strategy along the WDL period iii) how the SS exposure alters the social responding of adult rats who underwent binge drinking during adolescence together with corticosterone serum concentration. At last, we assessed the effects of CBD on BAD vulnerability and induced impairments.

RESULTS: At protracted alcohol withdrawal from the last BAD session, rats displayed decreased social preference. Moreover, BAD during adolescence decreased the defensive behavior at both acute and late WDL. Finally, binging on alcohol during adolescence, SS exposure at adulthood, and their interaction de-

creased social preference in rats and altered coping with stress. In fact, we observed a persisting increase in corticosterone serum concentration in withdrawn rats, independently of SS exposure. CBD decreased alcohol consumption among adolescent rats and counteracted alcohol induced deficits on sociability of adult rats. However, a complex interaction between BAD, CBD and SS exposure emerged.

CONCLUSIONS: A significant impact on social interactions and stress reactivity results from binge- like alcohol consumption in adolescence, as well as to alcohol dependence onset later in life. Prevention campaign and rehabilitation programs for self-awareness and resilience abilities are needed, until the therapeutic potential of neuroactive compounds like CBD needs further estimation.

MONTELUKAST, AN AVAILABLE AND SAFE ANTI-ASTHMATIC DRUG, EXERTS CARDIAC PROTECTION AFTER MYOCARDIAL INFARCTION

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BACKGROUND: Preclinical and clinical data indicate that the 5-Lipoxygenase pathway becomes activated in cardiovascular diseases and suggests an important role of CysLTs in atherosclerosis and in its ischemic complications such as myocardial infarction (MI). Moreover, CysLT modifiers, which are generally safe and well tolerated, show significant cardiovascular protection in the experimental settings. Although several data indicate a protective role of CysLTs in MI, no data are available about the benefit of CysLTs receptor (CysLTR) antagonists treatment. This study aims to investigate the effects of montelukast, a CysLTR-1 antagonist, in mouse model of MI investigating the effects on ventricular function, hypertrophic remodelling of the remote non- infarcted region and inflammatory patterns.
METHODS: MI was induced in C57BL/6N female mice by left anterior descending (LAD) coronary artery ligation. 24 hours after LAD ligation mice were subjected to cardiac magnetic resonance imaging (cMRI) and randomized to receive montelukast 10 mg/kg/die (MI+MTK, n=9) or vehicle (MI, n=9). Sham-operated mice were used as controls (SHAM, n=8). Four weeks after surgery mice were subjected to cMRI, sacrificed, and hearts collected. The expression of the genes was measured by the use of qPCR 48 h after MI.
RESULTS: Our results demonstrated that, compared to SHAM, 24 hours after LAD ligation, the infarcted groups (MI and MI+MTK) exhibited severe LV dilatation, a concomitantly significant reduction of ejection fraction (EF%) ($p<0.001$) and regional contractility of remote non-infarcted region ($p<0.001$). At the end of experimental protocol, compared to MI group, MI+MTK mice showed a reduction in LV volumes, and a significant increase in EF% ($p<0.01$) indicating a protective effect of montelukast in preserving LV contractility. Moreover, montelukast treatment was able to reduce LV mass increase ($p<0.05$) and wall thickening, counteracting maladaptive remodelling. These results were supported by histological and molecular analyses performed on non-infarcted tissue. We showed that montelukast prevented cardiomyocytes hypertrophy significantly counteracting the increase in pGSK3ß/GSK3ß, a regulator of

hypertrophic pathway, in MI animals ($p<0.001$). Moreover, montelukast significantly decreased mRNA expression of Il1ß, Tgfß and CCl2 thus confirming its anti-inflammatory properties.
CONCLUSIONS: Our data strongly suggest the development of hypertrophic condition in remote non- infarcted tissue after MI and demonstrates the ability of montelukast to preserve this maladaptive condition sustaining contractility. The beneficial effects of MTK could be partially related to its anti- inflammatory properties exerted during the acute post-ischemic phase. These results providing the opportunity for montelukast "repurposing" in cardiovascular diseases and in particular in myocardial infarction.

MONTELUKAST PROTECTING OLIGODENDROCYTES IMPROVES FIBER CONNECTIVITY AND FUNCTIONAL RECOVERY IN A MOUSE MODEL OF STROKE

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BACKGROUND: Neuroprotective drugs have been proposed to improve long-term recovery after stroke, but in many cases fail to reach clinical effectiveness. Recent studies suggested that restorative therapies should combine neuroprotection and remyelination. Montelukast, an anti- asthmatic drug, was shown to exert neuroprotection in models of central nervous system injuries, but whether this could occur by influencing myelin-producing oligodendrocytes remains to be determined. Here, reporter inducible GPR17iCreERT2:CAG-eGFP mice were used to assess the effect of montelukast on oligodendrocyte precursor cells (OPCs) made fluorescent by GFP labeling upon tamoxifen administration in ischemic brain remodeling.
METHODS: 11 week-old GPR17-iCreERT2:-CAG-eGFP male mice were subjected to permanent middle cerebral artery occlusion (pMCAo) and treated with montelukast (10 mg/kg/day) or vehicle for 8 weeks. Sham operated mice were used as control. Electrophysiological analyses were performed weekly to evaluate functional outcomes. White matter integrity and oligodendrocyte dynamics were assessed by immunohistochemistry. Finally, the effects of montelukast on motor coordination were evaluated.
RESULTS: Electrophysiological analyses showed that, at all time points from week 3 to week 7 vs baseline ($p<0.05$), pMCAo induced a significant decrease in the amplitude of field potentials, indicating a significant reduction in fiber connectivity. Conversely, in montelukast treated mice, the significant reduction of the amplitude of field potentials observed at week 4 and week 5 vs baseline ($p<0.05$ for both) was completely recovered and returned to baseline by the 5th-7th week after stroke, suggesting improved fibers reorganization and connectivity. Moreover, immunofluorescence

analyses showed that montelukast treatment was able to induce an increase in OPCs recruitment and proliferation during the acute phase (2 weeks after pMCAo), and to promote their differentiation to mature oligodendrocytes at chronic phase of brain ischemia (8 weeks after pMCAo), suggesting a link between OPCs activation and maturation and fiber connectivity. Finally, montelukast treatment demonstrated its effectiveness in preventing ischemia-associated deficit in motor coordination performance.

CONCLUSIONS: We demonstrated that montelukast improves long-term functional recovery after brain ischemia by enhancing the recruitment and maturation of OPCs towards myelinating stage. These results raise the concrete possibility of repositioning a safe, already marketed drug, with immediate advantages for patients suffering from stroke.

ADOLESCENT LONG-TERM COCAINE EXPOSURE IMPACTS COGNITION AND NEUROPLASTIC MECHANISMS IN THE RAT PREFRONTAL CORTEX

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BACKGROUND: Adolescence is a critical period marked by a heightened vulnerability to substances of abuse. This higher sensitivity could be analogous to that of young humans who are more susceptible to the rewarding effects of drugs and less affected by their negative-collateral effects. Previously, it has been shown that cocaine impacts on the dopaminergic system by affecting dopamine homeostasis and altering mainly the Ventral Tegmental Area signaling to the rest of the mesocorticolimbic system, involving structures such as Nucleus Accumbens and Prefrontal Cortex (PFC). Previous studies suggest that the PFC and also the hippocampus play a role in representing information about temporal order. In addition, Brain-Derived Neurotrophic Factor (BDNF) plays a key role in modulating cocaine effects on the brain.

METHODS: In this study, adolescent rats were treated with a long-term cocaine protocol for 15 days: from postnatal day (PND) 28 to PND 42, that roughly parallels adolescence in humans (5 mg/Kg subcutaneous). After the treatment, animals underwent 14 days of drug-withdrawal and, subsequently, a Temporal order Object Recognition (TOR) cognitive test was performed. In an attempt to find a putative underlying molecular mechanism, we analyzed the expression of the neurotrophin BDNF in the whole homogenate of the Infralimbic cortex (ILCtx) and the Prelimbic Cortex (PLCtx), the brain subregions of the PFC that modulate recognition memory. BDNF, ERK I/II, TRKB and AKT proteins were analyzed by western-blot assay.

RESULTS: Behavioral results indicate that cocaine exposure during adolescence impaired the cognitive performance in the TOR test. Such behavioral observation was paralleled by alterations in BDNF and its intracellular signalling in cocaine-in withdrawn rats. In particular, we found changes in ERK, AKT and TRKB phosphorylation state and expression in the homogenate of the ILCtx between cocaine and control groups. Such changes indicate a dysregulation of the BDNF-ERK pathway which may implicate functional alterations since BDNF is known to modulate both functional and structural long-term potentiation.

CONCLUSIONS: Our results point to adolescence as a crucial developmental period of vulnerability for psychostimulant-induced cognitive impairment, here emphasized by long-term withdrawal, following repeated exposure during adolescence.

IDENTIFICATION OF SEX-SPECIFIC PHENOTYPES IN THE SECRETOME OF HUMAN ENDOTHELIAL CELLS

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BACKGROUND: Cellular sex has rarely been considered as a biological variable in preclinical research, even when the pathogenesis of diseases with predictable sex differences is studied. In this perspective, proteomics, and 'omics approaches in general, can provide powerful tools to achieve comprehensive cellular maps, thus favouring the discovery of still unknown sex-biased physio-pathological mechanisms.
METHODS: Proteomic and gene ontology analyses followed by ELISA validation were performed on secretome from human serum-deprived male and female endothelial cells (ECs). Apoptosis was detected by FACS and Western blot techniques, and efferocytosis through the ability of a macrophage cell line to engulf apoptotic ECs. PTX3 expression and silencing efficacy were assessed by RT-qPCR.
RESULTS: Proteomic analysis showed different secretory phenotypes, due the secretion of different sets of proteins, in serum-deprived male and female ECs. Gene ontology analysis revealed that secretome from male ECs was enriched in proteins related to cellular responses to stress and to the regulation of apoptosis. Accordingly, a higher proportion of serum-deprived male ECs underwent apoptosis in comparison to female ECs, suggesting that male and female ECs adopt different strategies in response to cellular stress. Among the secreted proteins, we consistently found higher levels of PTX3 in the male EC secretome. Searching for the biological significance of this difference, we silenced PTX3 expression and discovered a male-exclusive requirement for PTX3 in the resolution of the apoptotic process, specifically in efferocytosis.
CONCLUSIONS: Our results, showing different secretory phenotypes in stressed male and female ECs, propose the control of secretory pathways and secreted proteins as a possible new mechanism involved in the definition of sex-specific biological properties. Regarding PTX3, its pleiotropic functions in cardio-vascular biology, although still debated, are of growing interest, and the discovery of sex-specific difference in its secretion and activity may have important implications for the understanding of EC physiopathology. Overall, our study confirms that an upgrade in knowledge of endothelial sex-biased differences will be needed to identify novel sex-specific pathogenetic mechanisms and pharmacological targets for the prevention and treatment of endothelial dysfunction at the onset of atherosclerosis and cardiovascular disease.

CROSSTALK BETWEEN TOLL-LIKE RECEPTOR 4 AND DOPAMINE IN A MOUSE MODEL OF DEXTRAN SULFATE SODIUM-INDUCED COLITIS

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BACKGROUND: Changes in dopamine levels, deregulated dopaminergic machinery and altered Toll-like receptor 4 (TLR4) expression have been consistently associated with clinical and preclinical settings of IBD. In this study, we aimed to assess the crosstalk between the enteric dopaminergic system and TLR4 signaling in the enteric nervous system (ENS) of a mouse model of dextran sulfate sodium (DSS)-induced colitis.
METHODS: Male C57/Bl6 (WT) and TLR4-/- mice (8±1 weeks old; N=32 mice) received 1.5% DSS in drinking water for 5 days, switching thereafter to regular drinking water for 3 days. Small intestine inflammation was evaluated by measuring disease activity index and by histological analysis. Gastrointestinal transit was measured by nonabsorbable-FITC-labeled dextran distribution. Changes in ileal muscle tension were isometrically recorded following: i) cumulative addition of dopamine (0.1-300 μM); ii) electric field stimulation (EFS, 4 Hz) in presence of 30 μM dopamine with or without 10 μM SCH-23390 (D1R antagonist) or 10 μM sulpiride (D2R antagonist). Immunofluorescence distribution of the neuronal HuC/D or glial (GFAP and S100β) markers and dopamine β-hydroxylase (DBH) and dopamine transporter (DAT) were determined in longitudinal muscle myenteric plexus whole-mounts (LMMPs) preparations by confocal microscopy.
RESULTS: In WT mice, DSS treatment determined a delayed gastrointestinal transit, a reduction of dopamine-induced relaxation (-26%, N=5, P<0.05), reactive gliosis and 1.2-fold increase in DBH immunoreactivity. After DSS treatment TLR4-/- mice showed a significant increase in dopamine-induced relaxation (+30%, N=5, P<0.01) and a 2.3-fold increase in 4-Hz EFS-elicited contraction (N=5, P<0.001), which was sensitive to D1R and D2R activation. In DSS-treated TLR4-/- LMMPs, the ENS neurochemical coding was altered as evidenced by a reduced number of HuC/D+ neurons (-12%, N=5, P<0.05), a 1.4-fold increase of DBH immunoreactivity. No significant change was, however, observed in both GFAP and S100β staining.
CONCLUSIONS: In mice, TLR4 signaling influences the severity of small intestine inflammation as well as ENS activity and neurochemical coding, sustaining a dopaminergic-mediated control of the small intestine neuromuscular function.

SMALL INTESTINE NEUROMUSCULAR DYSFUNCTION IN A MOUSE MODEL OF DINITROBENZENE SULFONIC ACID-INDUCED COLITIS: INTERACTION BETWEEN TOLL-LIKE RECEPTOR-4 AND SEROTONIN

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BACKGROUND: Changes in serotonin (5-HT) levels, anomalies in serotonergic and cholinergic machinery and altered Toll-like receptor 4 (TLR4) expression have been shown in IBD in patients and related animal models. Thus, we aimed to assess the crosstalk between the enteric serotonergic system and TLR4 signaling in a mouse model of dinitrobenzene sulfonic acid (DNBS)-induced colitis.
METHODS: Male C57/Bl6 (WT) and TLR4-/- mice (9±2 weeks old; N=10 mice) were pre-sensitized with 1% DNBS, and after 1 week were intrarectally instilled with 2.5% DNBS. Small intestine inflammation was assessed by measuring the indices of disease activity and by performing histological analysis of ileal tissue samples. Changes in ileal muscle tension were isometrically recorded following: i) carbachol cumulative addition (CCh; 0.1-100 μM); ii) electric field stimulation (EFS, 0-40 Hz); iii) 60 mM KCl; iv) 30 μM 5-HT addition with or without 0.1 μM ondansetron (5-HT3R antagonist). Immunofluorescence distribution of the neuronal HuC/D and nNOS markers or the glial GFAP marker were determined in longitudinal muscle myenteric plexus whole-mounts (LMMPs) preparations by confocal microscopy.
RESULTS: In WT mice, DNBS treatment affected receptor and non-receptor mediated excitatory responses (+120% of Emax to CCh and +103% of contraction to KCl, respectively; $P<0.001$, N=5 mice/group) as well as the cholinergic neurotransmission (-50% at 10 Hz; $P<0.01$, N=5 mice/group) and 5-HT-mediated response (2-fold increase to 30 μM 5-HT,$P<0.001$, N=5 mice/group). After DNBS treatment TLR4-/- mice showed a significant increase in the excitatory response (+98% of Emax to CCh; +80% of contraction to KCl; +120% at 10 Hz; $P<0.001$, N=5 mice/group) and a significant reduction of 30 μM 5-HT-mediated response (-50%, $P<0.001$, N=5 mice/group). These changes were associated to a significant decrease of the total number of HuC/D+ neurons (-44% and -19% for WT DNBS and TLR4-/- DNBS mice, respectively) together with a 1.3-fold increase in S100- immunofluorescence in WT mice after DNBS treatment.
CONCLUSIONS: These findings suggest an important role of TLR4 in small intestine neuromuscular dysfunction during colitis and provide novel information on the potential benefits of targeting TLR4 in gut disorders that exhibit aberrant cholinergic and 5-HT signaling.

EXOSOMES: A SIGNAL FROM ASTROCYTE PROCESSES TO NEURONS

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BACKGROUND: Extracellular vesicles (EV) subserve non-classical signal transmission in the central nervous system (CNS). Exosomes - EVs of about 30 - 100nm diameter, released into the extracellular space upon fusion of multivesicular bodies with the plasma membrane - play multiple roles in both physiological and pathological conditions in CNS. Neurons, microglia and oligodendroglia, can release exosomes; while cultured astrocytes have been reported to secrete exosomes, less is known on the ability of astrocytes to release exosomes in neuron-astrocyte networks. There, perisynaptic astrocyte processes function as sensors of transmitters in the extracellular environment and modulate neural activity by clearing glutamate and by releasing gliotransmitters; they regulate extracellular space volume and coverage of synapses. Indeed, they represent the astrocyte compartment specially devoted to bidirectional neuron-astrocyte communication in the tetrapartite synapse and to regulation of synapse plasticity. Here we assess if the astrocyte processes could convey messages through EVs.
METHODS: Freshly isolated astrocyte processes (gliosomes) were prepared from adult rat cerebralcortex and superfused with standard medium. EVs were collected in 10 min superfusate sample. We assessed the purity of gliosomes with IF analysis and WB, the presence of multivesicular bodies in the astrocyte processes with EM. The presence of exosomal and glial markers in gliosomes and EVs was assessed by WB. The vesicle size was measured with DLS, while the exosome cell target was studied in primary astrocyte-neuron co-culture with IF and confocal microscopy.
RESULTS: The astrocyte processes are a purified preparation that presented multivesicular bodies and expressed Alix and TSG101, consistent with their ability to release exosomes. Notably, the astrocyte-released EVs have the typical cup-shaped appearance, the size, and markers for the exosomes and for their parental astrocytic origin. The astrocyte-released exosomes were proved positive for neuroglobin (NGB), a protein functioning as neuroprotectant against cell insult. The exosomes were able to selectively target neuronal cells and to be internalized by neurons.
CONCLUSIONS: The main finding is that the astrocyte processes acutely prepared from astrocytes matured in a neuron-astrocyte network in CNS might participate to signal transmission by releasing exosomes, which, in turn, might target near or long-distance targets by volume transmission. The possibility that exosomes

might transfer NGB to neurons would add a mechanism to the potential astrocytic neuroprotectant activity. Notably, the exosomes released from the processes of astrocytes maintained markers, which prove their parental astrocytic origin. This potentially allows the assessment of the cellular origin of exosomes that might be recovered from body fluids.

PERAMPANEL IN PARTIAL SEIZURES, THE IMPORTANCE OF A METHOD FOR MEASURING BLOOD LEVELS IN THE ADJUSTMENT OF MULTI-DRUG THERAPY

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BACKGROUND: Nowadays, several antiepileptic drugs are suitable for patients suffering of seizures, used in monotherapy or in association in case of therapeutic failure. Perampanel (PMP) is the first of a new class of antiepileptics, the non-competitive antagonists of α-amino-3-hydroxy-5-methyl-isoxazolonepropionate receptors (AMPA). Perampanel initial dose is 2 mg / die, increased usually to 8 mg/die, up to 12 mg/die, based on clinical response and tolerability. Perampanel is mainly metabolised via CYP3A-mediated primary oxidation. Several studies have shown that adverse reactions to PMP (such as dizziness, somnolence, anger, anxiety, confusional states, dizziness, etc.) are dose-dependent. Perampanel clearance can be significantly affected by CYP inducing antiepileptics, often used in co-administration, such as carbamazepine, phenytoin, oxcarbazepine, topiramate that may lead to a therapeutic failure. The possibility to quickly evaluate PMP blood levels is therefore crucial to maintain therapeutic levels. Here we present a simple, fast and cheap method for dosing PMP in plasma samples.
METHODS: We used a HPLC system equipped with C18 column and we compared two different detection techniques to simultaneously evaluate the presence of PMP and the internal standard (IS) Ketoprofen. More specifically a Fluorimeter (FL) was used to detect PMP and a Photo Diode Array (PDA) was used to measure Ketoprofen. Perampanel-PDA/ketoprofen-PDA area ratio and PMP-FL/ketoprofen-PDA area ratio were used to quantify drug in plasma samples.
RESULTS: The proposed method has been validated completely for both PDA/PDA and FL/PDA detection approaches according to EMA guidelines in terms of linearity (Figure 1), selectivity, precision, accuracy and carry-over parameters. Passing-Bablok regression and Bland–Altman plot have been used to evaluate the data obtained and to confirm the possibility to use a combination of both detectors to measure PMP and Ketoprofen in plasma samples. Analyses performed on samples withdrawn from 10 patients were compared with the data obtained using a LC-MS/MS commercially available CE-IVD kit. Results are comparable, showing that our method can be currently used in clinical routine where therapeutic monitoring of PMP is required.
CONCLUSIONS: Perampanel is an ideal candidate for therapeutic drug monitoring. The

possibility of having a fast and highly processive method for dosing blood PMP levels is essential to adjust the dose during antiepileptic multi-drug therapy. Although mass spectrometry (MS) coupled to liquid chromatography remains the gold-standard to quantify this kind of molecules in biological matrices, the availability of a HPLC-based method allows to obtain a reliable dosing of this drug in clinical settings where MS is not available.

DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR OROTIC ACID AND OROTIDINE 5'-MONOPHOSPHATE DETECTION IN HUMAN PLASMA AND URINE

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BACKGROUND: The orotic acid (2,4-dioxo-1H-pyrimidine-6-carboxylic acid; Vitamin B13) is an intermediate metabolite of pyrimidine nucleotides biosynthesis and represents a minor diet constituent. The precursors of orotic acid in human metabolism are the cytosolic carbamoyl phosphate and aspartate via dihydrorotate: this biosynthesis is catalyzed by CAD gene encoding multifunctional enzyme. The multimeric protein called uridine 5'-monophosphate synthase is constitued by two domains that catalyze uridine monophosphate synthesis: orotatephosphoribosyltransferase (OPRTase; EC 2.4.2.10) and orotidine 5'-phosphate decarboxylase (OMPdecase; EC:4.1.1.23). The complete pathways of orotic acid biosynthesis is reported in Fig. 1. The step (5) represented in Fig.1 is directly involved in metabolism of 5-Fluorouracil (5-FU), because this anticancer drug is competitive substrate of OPRTase. In particular, the transferase activity of OPRTase multicomplex enzyme is inhibited by 5-FU at 59% level of control. The other end OPRTase is involved in metabolic disorders as congenital orotic aciduria and consequently the urinary orotic acid is quantified in clinical routine analysis for differential diagnosis of hereditary metabolic disease. Therefore, we aimed to develop a high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) assay that allows the simultaneous and sensitive detection of an orotic acid and orotidine 5'- monophosphate as metabolic and toxicological biomarkers in plasma and urine.
METHODS: The implementation and validation of chromatography and spectrometric method are developed in accordance with UNI EN ISO/IEC 17025:2018 and Eurachem Guide Lines (Method validation in clinical chemistry follows the established standards and procedures accepted by all disciplines of chemical metrolo-

gy). The clinical aspects are tested by analyzing diagnostic proficiency testing (external quality assessment - EQA) and other samples from patients with metabolic and malignant disorders.
RESULTS: The analytes, orotic acid and orotidine-5'-monophosphate are identified and quantified with high performance parameters of repeatability, reproducibility, robustness, precision and accuracy. The quantification method is based on internal standard approach for signal and matrix effect suppression. Whatever analytes is identified and quantified by two MRM transition with S/N>50 in LOD range. The analytical method clearly distinguish between urine and plasma specimens from the normal and pathological patients at 97.5% of level of confidence.
CONCLUSIONS: The HPLC-MS/MS method to be suitable for differential diagnosis of hereditary metabolic disease and metabolic monitoring of toxicity induced by anticancer drug. The analytical protocol is rapid and ideal to be used in routine analysis of clinical chemistry laboratory.

EFFECT OF SEASONS ON FOUR ANTI-EPILEPTIC DRUGS PLASMA LEVELS

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BACKGROUND: Therapeutic drug monitoring of anti-epileptic drugs is widely use for the management of epilepsy and to avoid treatment failure or explain adverse events onset. In this study, weexploredthe role of months and seasons of withdrawal for plasma quantification of oxcarbazepine, lamotrigine, phenytoin and levetiracetam pharmacokinetics and outcome cutoffs prediction.
METHODS: One hundred and seventy-five adult patients were enrolled. Drugs plasma concentrationswere measured by HPLC-UV methods.
RESULTS: We reported that oxcarbamazepine levels were higher in autumn and winter than those reported in spring and summer. In logistic regression model, warm seasons have been retained as therapeutic range negative predictive factors.If we separately evaluate males and females, the influence of seasons on oxcarbamazepine concentration remained only in male patients, also considering the logistic regression analysis.No factors significantly influenced lamotrigine, phenytoin and levetiracetam levels or were retained in regression model as treatment outcomes predictive factors.
CONCLUSIONS: These results, for the first time, suggest the effect of seasons on oxcarbamazepine. Apply a seasonal and gender specific approach should be the key to optimize treatment in each patient, in each period of people life.

ADVERSE DRUG REACTIONS WITH NEWER LONG-ACTING INJECTABLE ANTIPSYCHOTICS IN ITALY: A SPONTANEOUS REPORTING ANALYSIS

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BACKGROUND: Antipsychotics (APs) are the gold standard for treatment of schizophrenia. The longacting injectable formulations (LAI) have been developed to improve adherence reducing the risk of relapses. Since the introduction of risperidone LAI in 2006, three additional second-generation antipsychotics (SGAs) have become available as LAI formulations: olanzapine pamoate, paliperidone palmitate, and aripiprazole LAI. Safety profiles of these new SGA-LAIs seem to be consistent with their oral parent formulations, even though unexpected safety signals occasionally emerged from clinical practice. Considering this, the aim of our work was to describe adverse drug reactions (ADR) associated with SGA-LAIs the Italian Spontaneous Reporting System (SRS) database.
METHODS: We performed an analysis of the Italian SRS database, selecting all the ADR reports having as a suspected drug one or more SGA-LAI from their market introduction in 2006 to December 2019. We described the frequency of drug specific ADRs by MedDRA System Organ Class (SOC) and Preferred Terms (PTs).
RESULTS: Over a 13-year time frame, 558 reports of ADRs related to second-generation LAI antipsychotics were identified in the Italian SRS database. The observed median age of LAI antipsychotic users was 42 years (IQR: 32,5-51). The reports concerned more commonly males (n=305; 54.7%) than females (n=244; 43.7%). Serious ADRs accounted for 40.7% (n=227) of total SGA-LAIs related reports. Paliperidone palmitate was associated with the highest number of reports (n=204; 36.4%), followed by aripiprazole LAI (n=142; 25.5%), olanzapine pamoate (n=133; 23.7%) and risperidone LAI (n=81; 14.5%), with two reports presenting a combination of two SGA-LAIs as suspected drugs. Reported ADRs were mainly nervous system disorders (n=238; 24.3%) such as akathisia (n=47), sedation (n=44) and somnolence (n=27) followed by psychiatric disorders (n= 154; 15,7%) like drowsiness (n=29). The rate of extrapyramidal effects on total ADR reports was 24.7% with aripiprazole LAI, 20.6% with paliperidone palmitate, 19.8% with risperidone LAI, and 11.2% with olanzapine pamoate. The ADR known as post-injection delirium/sedation syndrome (PDSS) was reported in 43 cases all related to olanzapine pamoate. However, only 5 of those cases presented the proper codification for this ADR, the others were identified thanks to a clinical picture compatible with PDSS (presence of excessive sedation, confusion, and disorientation, occurring within few hours after injection).
CONCLUSIONS: Our analysis shows that the tolerability and safety profiles of second-generation LAI antipsychotics, while being similar to those of the parent oral formulations, differ among the available agents. These results highlight the importance of the national SRS database as an evaluation tool for the safety of SGA LAIs. In addition to that, the relatively high number of cases of PDSS related to olanzapine pamoate remarks the necessity of a high attention level regarding this potentially serious ADR.

ANTI-INFLAMMATORY AND ANTIOXIDATIVE PROPERTIES OF FORTIFIED CITRUS OLIVE OILS ON AORTA VESSELS OF HIGH FAT DIET-TREATED RATS

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BACKGROUND: In the last years, the development of foods fortified with natural antioxidants have been proposed for their nutraceutical properties. In particular, Citrus peel containing bioactive compounds, such as carotenoids, flavonoids and limonoids, could be suitable to formulate enriched olive oils able to boost a healthy nutrition. Due to their phytochemical content, Citrus fruits including orange (Citrus x aurantium L.) and lemon (C. limon) exhibit interesting properties in health protection and disease prevention. Our aim was to evaluate the protective effect of extra-virgin olive oil (EVOO) enriched with fruit extracts on isolated aortic vessels from high fat diet-treated rats.
METHODS: In order to obtain a food enriched with nutraceutical value typical of Citrus fruits, peels or leaves of Citrus were cryomacerated and pressed with olive fruits. The anti-inflammatory and antioxidative properties of fortified Citrus Olive Oils were studied on isolated aorta from adult male Wistar rats (3-4 months old) treated for 21 days with: a standard diet (STD); a high fat diet (HFD); HFD + EVOO; HFD + CaOO (Citrus x arantium EVOO); HFD + ClOO (Citrus limon EVOO). The effect of HFD on the rat lipid profile was assessed on blood by using Cobas b101 instrument. To investigate the contribution of CaOO and ClOO on endothelial function, endothelial cell sprouting activity in 3D culture was assessed ex vivo, in aortic rings isolated from treated rats. The expression of inflammation/oxidative stress markers were evaluated in aorta tissues by qPCR and western blot. Finally, inflammatory/immune cell infiltration was investigated by assessment of CD40 in aortic histological sections.
RESULTS: HFD promotes an increase in total cholesterol, LDL cholesterol and triglycerides, and a marked reduction in HDL levels in rats. The addition of EVOO modestly contributed to the lowering of total cholesterol and LDL levels, while both the Citrus enriched Olive Oils were endowed with more evident hypo-cholesterolemic properties. Animals supplemented with EVOO as well as CaOO and ClOO markedly reduced plasma triglycerides. Aortic rings dissected from rats fed with HFD showed an impairment to form branching micro-vessels in response to the angiogenic factor VEGF. In aortic rings from EVOO, CaOO and ClOO, VEGF significantly promoted vessel sprouting. At molecular level, the HFD diet significantly declined eNOS and ALDH1A1 protein levels, while increased the expression of iNOS, COX-2 and mPGES-1 in aortic tissues. EVOO, CaOO and ClOO supplementation in the HFD reverted eNOS, ALDH1A1, iNOS and COX-2 expression levels. In addition, HFD increased protein levels with adducts of 4-HNE, a product of lipid peroxidation, while EVOO, CaOO and ClOO significantly reduced the 4-HNE-protein adducts, suggesting an antioxidant effect of the fortified diet at vascular

level. Photomicrographs of aortic wall sections from all experimental groups revealed that expression of CD40 was absent in STD, EVOO, CaOO and ClOO groups, while it was positive in HFD group.

CONCLUSIONS: These data suggest a protective activity of the three oils that might be associated with both their ability to reduce the expression of anti-inflammatory/anti-oxidative stress markers and to preserve endothelial function.

ANTIMUTAGENIC AND CHEMOPREVENTIVE PROPERTIES OF 6-(METHYLSULFINYL) HEXYL ISOTHIOCYANATE ON TK6 HUMAN CELLS BY FLOW CYTOMETRY

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BACKGROUND: 6-(methylsulfonyl) hexyl isothiocyanate (6-MITC) is the main bioactive compound present in *Wasabia japonica* rhizome. Several scientific studies have shown that 6-MITC possesses interesting antimicrobial, anti-inflammatory, antiplatelet and antioxidant properties which therefore suggested us it could have an interesting chemopreventive potential. In a recent publication, we demonstrated, in two different leukaemia cell lines, its ability to modulate several mechanisms supporting its antitumor activity. For this reason, we thought useful to continue the research, by investigating the potential antimutagenic activity of 6-MITC and thus better define its profile as a possible chemopreventive agent.

METHODS: 6-MITC antimutagenic effect against two known mutagenic agents: the clastogen Mitomycin C (MMC) and the aneuplodogen Vinblastine (VINB), was analyzed, in terms of micronuclei frequency decrease, after short- and long- time treatment on TK6 human cells, using a new automated protocol of the "In Vitro Mammalian Cell Micronucleous Test" by flow cytometry.
RESULTS: The results showed a different behavior of the isothiocyanate. In particular, 6-MITC was unable to counteract the MMC genotoxicity, but when it was associated with VINB, a statistically significant decrease in the micronuclei frequency was registered.
CONCLUSIONS: Overall, the results obtained suggest a potential antimutagenic activity of 6-MITC, in particular against the aneuploidogen agents. This ability to inhibit or counteract the mutations at the cellular level has a great therapeutic value, and it represents a mechanism through which a chemopreventive agent can express its activity.

DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE MONITORING OF NEW GLUTEN EXPOSURE BIOMARKERS

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BACKGROUND: Celiac disease (CD), an immune-mediated enteropathy of the small intestine, caused by the ingestion of gluten in genetically predisposed individuals. The only treatment available for CD is the gluten-free diet (GFD). A tight adherence to GFD is essential to reduce symptoms, avoid nutritional deficiencies and improve patient's quality of life.
In order to evaluate exposure to gluten, great interest has been devoted to the definition of biomarkers of gluten exposure and to development of methods allowing their detection and quantization. A "33 mer" peptide, alkylresorcinols (ARs) and their main urinary metabolites (3,5-dihydroxybenzoic (DHBA) acid, 3-(3,5-dihydroxyphenyl)-propanoic (DHPPA) acid) were suggested as potential molecular biomarkers for short-term monitoring of the compliance to GFD.
The aim of this research was to develop and validate analytical methods using ultra high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) for the monitoring of these biomarkers in human biological matrices.
METHODS: High-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) is considered a gold standard technique for the detection of traces of exogenous substances within complex matrices (such as stools and urine). UHPLC-MS/MS analyses were carried out by an Ultimate 300 UHPLC coupled with a TSQ-Endura ESI-Q-q-Q mass spectrometer (Thermo-Fisher). Multiple reaction monitoring transitions were set to detect and quantify the "33 mer" peptide, the main alkylresorcinols urinary metabolites DHBA and DHPPA.
RESULTS: To develop an analytical method suitable for the identification and quantization of traces of the selected biomarkers in different human samples, an accurate optimization of any pre-analytical and instrumental parameter was required. In that aim, we started with the optimization of mass spectrometric conditions: for each compound we identified the molecular ion and the specific ion products derived from its fragmentation. To prevent misidentifications two transitions were selected for each molecule. Chromatographic separation of the compounds was achieved using a reverse-phase C18 column and a mixture of 0.1% formic acid in water and acetonitrile as mobile phase. Specific gradients were developed for the three chosen biomarkers. Finally, we optimized the pre-analytical procedure to obtain suitable samples in urinary matrix, taking into account both the optimal recovery and the reproducibility of the method.
CONCLUSIONS: Monitoring the "33 mer" peptide, DHBA, and DHPPA by LC-MS/MS might represent a promising tool to help CD patients to keep their diets under control, and the phycsicians to understand the causes of any adverse events potentially occurring. In this framework, the proposed approach provides good efficiency and wide applicability, even if further validation has to be performed.

THERAPEUTIC DRUG MONITORING OF OPIOIDS FOR PAIN MANAGEMENT

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BACKGROUND: Opioids are a class of drugs used to treat different forms of pain. Starting 1986, the World Health Organization (WHO) has been proposing a three-step analgesic ladder that includes anti-inflammatory, weak and strong opioids, which may be associated with other classes of drugs.
Noteworthy, opioids are drugs that have a reduced therapeutic index; TDM allows establishing the most appropriate treatment in terms of clinical efficacy, dose optimization, thus minimizing possible adverse reactions and drug interactions. Opioid drugs can be dispensed through different routes of administration, which are chosen based on patient's condition, type, intensity of pain and chemical characteristics of the drugs themselves. The use of these drugs in clinical practice is managed by the clinician based on the observed reduction or absence of pain and possible onset of adverse drug reactions. Indeed, there is a fundamental lack of studies addressing the individual pharmacokinetic response to these drugs, which hampers a rationalized fine-tuning of the therapy personalized to the specific patient.
METHODS: We developed a selective and sensitive LC-MS/MS assay to monitor of a large panel of opioids and related metabolites in blood plasma including morphine, buprenorphine, fentanyl, meperidine, oxycodone, oxymorphone, dihydromorphine, norbuprenorphine, morphine 3- and 6-glucuronide and hydromorphone, hydrocodone. Molecules and internal standards were extracted from blood plasma using a single step protein precipitation in methanol. Moreover, the efficacy of this approach was also evaluated on whole blood microsampled using innovative systems and on saliva samples.
RESULTS: We developed, optimized and validated an LC-MS/MS-based assay to monitor a large panel of opioids and related metabolites in blood plasma. Molecules and internal standards were extracted from blood plasma using a single step protein precipitation in methanol. Moreover, the efficacy of this approach was also evaluated on whole blood microsampled using Volumetric Absorbitive Microsampling Systems (VAMS) and on saliva samples. The method was linear in range from 0,1 to 100 ng/mL.
CONCLUSIONS: The methodology developed may be easily implemented in clinical settings such as Palliative Care and Pain Medication Units where polytherapeutic treatments are often used and rapid and versatile methodologies such as those based on mass spectrometry can be informative and useful to help the clinician in decision making process.

BARICITINIB COUNTERACTS METAFLAMMATION THUS PROTECTING AGAINST DIET-INDUCED METABOLIC ABNORMALITIES IN MICE

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BACKGROUND: Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway exerts a pathologic effect exacerbating metaflammation, a chronic inflammatory state that characterizes diet-related metabolic disorders. We investigated the effects of the Jak1/2 inhibitor baricitinib, recently approved for the treatment of rheumatoid arthritis, in a murine model of high-fat-high-sugar diet (HD).
METHODS: 4-week old male C57BL/6 mice were fed with a control normal diet (ND) or a high-fathigh sugar diet (HD) for 22 weeks. A sub-group of HD fed mice was treated with baricitinib (10 mg/kg die, p.o.) for the last 16 weeks (HD + Bar).
RESULTS: HD group showed increase in: body weight, triglycerides, cholesterol, LDL, blood glucose levels ($P<0.05$) and an impairment in OGTT, compared to ND. HD resulted in increased leptin, resistin and insulin plasma levels ($P<0.05$), impaired insulin signaling transduction and reduced GIP, GLP-1 and ghrelin ($P<0.05$). HD also led to increased systemic proinflammatory markers IL-1β, INF-y; TNF-α ($P< 0.05$), and to reduced anti-inflammatory IL-10 and IL-6 ($P< 0.05$).
Despite HD+Bar did not change diet-induced microbiota imbalances, the metabolic abnormalities were reverted.
CONCLUSIONS: In summary, our data suggest that Jak2/Stat2 pathway may represent a novel candidate for the treatment of diet-related metabolic derangements, with potential for EMA- and FDA-approved Jak inhibitors to be repurposed for the treatment of type 2 diabetes and/or its complications.

INHIBITION OF BRUTON'S TYROSINE KINASE REGULATES MACROPHAGE NF-KB AND NLRP3 INFLAMMASOME ACTIVATION IN METABOLIC INFLAMMATION

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BACKGROUND: Currently there are limited medicines available for the treatment of metabolic inflammation in diseases such as obesity and type 2 diabetes (T2D). Bruton's tyrosine kinase (BTK) is present in a wide variety of cells not just B-cells, these include monocytes and macrophages and has been implicated in the regulation of the NF-kB and NLRP3 inflammasome activity.
METHODS: Using in vivo models of chronic inflammation [high-fat-diet (HFD) feeding] we investigated if ibrutinib, an FDA approved medicine that targets BTK, may represent a novel anti-inflammatory drug for the use in treating metabolic inflammation.
RESULTS: HFD feeding was associated with increased BTK expression and activation, which was significantly correlated with monocyte/macrophage accumulation in the liver, adipose tissue and kidney. Treatment of mice fed a HFD with ibrutinib inhibited the activation of BTK and reduced monocyte/macrophage recruitment in the liver, adipose tissue and kidney and reduced inflammatory gene expression; this was coupled with decreased activation of NF-kB and the NLRP3 inflammasome in vivo. As a result, ibrutinib treated mice fed on a HFD displayed improved glycaemic control as a result of restored signalling through the IRS-1/Akt/GSK-3b pathway; protecting mice against the development of hepatosteatosis and proteinuria.
CONCLUSIONS: In the present study we provide "proof of concept" evidence that BTK is a novel therapeutic target for the treatment of diet induced metabolic inflammation.

NEUTRALIZATION OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (ENAMPT) AMELIORATES EXPERIMENTAL COLITIS: POSSIBLE INTERVENTION ON MACROPHAGE PLASTICITY

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BACKGROUND: Inflammatory bowel disease (IBD) is a chronic idiopathic disorder. Although biologics have increased the probability to maintain remission of the disease, a considerable percentage of patients still do not respond to therapies. Therefore, there is an unmet medical need for novel targeted therapies to control these diseases. In this setting, Nicotinamide phosphoribosyltrasferase (NAMPT), a pleiotropic enzyme involved in cellular metabolism, has been postulated as a novel target. NAMPT is present in two different forms in cells: an intracellular form, called iNAMPT, involved in NAD synthesis (Chiarugi et al., 2012), and an extracellular form, eNAMPT that acts as cytokine on different cell types (i.e. immune cells), binding to a still unknown receptor (Camp et al., 2012). Importantly, eNAMPT levels are increased in IBD and its levels correlate with the stage of the pathology: in an active state of the disease its levels are extremely high, however they are partially reduced in a remission stage (Moschen et al., 2007). The aim of our work was to determine the if eNAMPT could be a novel target, thanks to a novel anti- eNAMPT antibody (C269) generated by our group.

MATERIALS AND METHODS: The murine DNBS-model was used to emulate IBD disease in vivo. Briefly, Balb/c mice were intrarectally administered with DNBS and sacrificed after 5 days. Every two days we injected 50µg of C269 (and respective IgG1) or recombinant eNAMPT (50µg/mice daily). After the sacrifice, we collected blood for ELISA analysis, colon for RT-PCR and IHC and we extracted cells from lamina propria for FACS analysis. At the cellular levels, peritoneal macrophages (PECs) were extracted from C57BL/6 mice peritoneum, after 5-days 3% thioglicollate induction. PECs were stimulated with 100 ng/ml IFNγ and/or 500 ng/ml recombinant eNAMPT. mRNAs were extracted for RNAseq, while the macrophage migration was evaluated with wound healing assay.

RESULTS: Exogenous administration of eNAMPT (i.p. 50ug/mice, endofree) in DNBS model determined a worsening of IBD symptoms (increased weight loss, colon shortening and tissue damage). These symptoms are reduced after the treatment with the anti-eNAMPT antibody, observable in a reduction in IBD symptoms and a reduction in mRNA proinflammatory IFNγ-associated genes, usually upregulated in IBD. Moreover, C269 reduced the frequency of myeloid and T cells in lamina propria.

Ex vivo data on PECs reveals that eNAMPT has pro-inflammatory and pro-migratory activity. We have demonstrated that eNAMPT priming of PECs enhances IFNγ-dependent response, through STAT and NF-κB-dependent mechanism, reverted with C269 pre-treatment. Furthermore, eNAMPT also induced macrophage migration, in the same manner of LPS.

CONCLUSIONS: Taken together, our data demonstrated that eNAMPT exacerbates DNBS-associated symptoms, in which its neutralization could ameliorate the pathogenesis of the disease, focusing on macrophages plasticity as a possible target. These data prompt anti-eNAMPT antibody as a possible treatment in IBD.

POST-TRAUMATIC STRESS DISORDER (PTSD): AN UPDATED ANIMAL MODEL PREDICTING SUSCEPTIBILITY AND RESILIENCE

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BACKGROUND: Post-traumatic stress disorder (PTSD) is a psychiatric disorder whose pathogenesis relies on a maladaptive expression of the memory for a life-threatening experience, characterized by over-consolidation, generalization and impaired extinction, which in turn are responsible of dramatic changes in arousal, mood, anxiety and social behavior. Even if human subjects experiencing a traumatic event during lifetime all show an acute response to the trauma, only a subset (susceptible) of them ultimately develops PTSD, meanwhile the others (resilient) fully recover after the first acute response. The majority of the available animal models of PTSD lacks of a dynamic dissection to better understand how this acute response can turn into PTSD-related maladaptive changes.
METHODS: Here we have implemented our experimental PTSD model previously developed, making it suitable to differentiate between susceptible (high responders, HR) and resilient (low responders, LR) rats in terms of over-consolidation, impaired extinction, and social impairment long after trauma. Rats were exposed to five footshocks paired with social isolation. One week after trauma but before extinction, animals were tested in the Open Field and Social Interaction tasks for the identification of a predictive variable to identify susceptible and resilient animals before the possible appearance of a PTSD-like phenotype.
RESULTS: A significant negative correlation was found between the total distance traveled, which is an index of the exploratory activity, in the Open Field test performed 5 days after trauma exposure and the freezing behavior shown by rats during the first extinction session (day 7) and the extinction retention test (day 16), indexes of the over-consolidation and impaired extinction of the trauma experience, respectively. Conversely, a significant positive correlation was found between the total distance traveled and the time spent in social interaction in the Social Interaction test performed 19 days after trauma exposure as an index of social alterations in the PTSD model. We then segregated animals in HR and LR, according to the 25th and 75th percentile of the experimental group's distribution for the exploratory activity in the Open Field test and we found that HR rats displayed increased freezing response compared to LR rats, across retrieval and extinction sessions. Moreover, we found that HR rats spent less time interacting with a conspecific after trauma with respect to the LR rats.
CONCLUSIONS: Our findings show that exploratory activity after trauma in a novel environment is a very robust variable to predict susceptibility towards a PTSD- like phenotype thus suggesting that this parameter is a reliable predictive variable able to predict individual differences in later developing a PTSD-like phenotype with a high translational value with respect to the cognitive and emotional clusters observed in the human pathology.

PERICYTE-LIKE DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS: AN IN VITRO STUDY

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BACKGROUND: Pericytes support endothelial cells and play an important role in stabilizing the vessel wall at the microcirculation level. The loss of pericytes, as occurs in diabetic retinopathy, results in a breakdown of the Blood-Retinal Barrier (BRB) and infiltration of inflammatory cells. Thus, pericyte-like differentiated human Adipose-derived mesenchymal Stem Cells (hASCs) may represent a therapeutic tool for restoring a damaged BRB.
METHODS: To induce a pericyte-like phenotype, hASCs were cultured in different conditions and compared to human Retinal Pericytes (hRPCs). Pericyte-like differentiation of hASCs was monitored by assessing the expression of neural/glial antigen 2 (NG2) and α-smooth muscle actin (α-SMA) through immunofluorescence and western blot analysis. Interactions between human Retinal Endothelial Cells (hRECs) and different groups of hASCs were investigated in co-culture experiments. Some typical junctional proteins, such as Vascular Endothelial (VE)-cadherin, Zonula Occludens-1 (ZO-1), Occludin and also α-SMA have been revealed by immunofluorescence. To evaluate interactions between hRECs and hRPCs or pretreated hASC, trans-endothelial electric resistance (TEER) has been measured in an in vitro model of BRB. The pericyte-like phenotype of PM-hASCs was also confirmed in three-dimensional co-cultures by in vitro tube formation assay in Matrigel Basement Membrane Matrix system.
RESULTS: The closest pericyte-like phenotype was observed when hASCs were cultured in Pericyte Medium (PM-hASCs). PM-hASCs and hRPCs showed a similar expression of α-SMA and NG2, that are acknowledged pericyte markers. Immunofluorescence results corroborated with western blot analysis, which reveals that α-SMA and NG2 levels of PM-hASCs were similar to HRPCs. The expression of VE-cadherin, ZO-1 and Occludin was considerably increased in hRECs, particularly when PM-hASCs were present in co-coltures, similar to hRPCs. After 1 days of co-colture with hRECs, α-SMA immunoreactivity was poorly detectable in hRPCs and PM-hASCs, whereas was clearly visible in these two conditions after 4 day of co-colture. This probably suggests a pericyte switching toward the contractile phenotype, characterized by a strong α-SMA expression. Values of TEER, that are an in vitro index of BRB integrity, significantly increased when HRECs has been co-coltured with hRPCs or PM-hASCs. Three-dimensional co-coltures in Matrigel, where vessel-like tubular structures are spontaneously formed by endothelial cells, showed that
PM-hASCs and hRPCs similarly localized around the tubular structures formed by hRECs. These findings suggest a comparable phenotype between hRPCs and PM-hASCs.
CONCLUSIONS: In conclusion, PM-ASCs seem able to strengthen the intercellular junctions between hRECs, and the integrity of the BRB; thus, hASC-based therapeutic approaches may be used to restore the integrity of retinal microcirculation.

ADHERENCE TRAJECTORIES OF BIOLOGIC DISEASE MODIFYING ANTI-RHEUMATIC DRUGS RECORDED IN TUSCAN ADMINISTRATIVE HEALTHCARE DATABASE: THE PATHFINDER STUDY

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BACKGROUND: Adherence to disease modifying anti-rheumatic drugs (DMARDs) is crucial for disease control in rheumatoid arthritis (RA) [1]. Adherence trajectory model may provide interesting information about drug utilization patterns in RA patients, but their clinical interpretation deserve caution. This study was aimed at identifying and describing trajectories of adherence to biologic (b) DMARDs.
METHODS: We conducted a drug-utilization study using Tuscan administrative healthcare databases. We included patients with a first dispensation of a bDMARD (index drug: infliximab, adalimumab, certolizumab, etanercept, golimumab, abatacept, tocilizumab) from 2010 to 2015 (index date) and a record of RA diagnosis in the five years before or one year after the index date, or with a rheumatologic visit within one year before or after the index date. Patients with rituximab as index drug were excluded because of the potential oncologic indication. Patients were observed for 3 years until the end of the study, death, or drop out from databases, and records of pregnancy and cancer were also used as censoring event. We evaluated adherence to bDMARDs every 3 months through the Medication Possession Ratio, by classifying the follow up period in 12 adherent values. We identified the longitudinal adherence trajectories to bDMARDs and clustered in groups [2,3]. Each cluster was labeled based on adherence trajectories and baseline characteristics of each trajectory were described.
RESULTS: We identified 11,110 new users of bDMARDs in the study period in the Tuscan healthcare databases. After excluding those living outside Tuscany, those using rituximab as index drug and those with short look-back period, the cohort included 6,323 patients. Among these, 3,347 meet the criteria for RA diagnosis and were included in the final cohort. The 67.3% were female; the mean age was 53.2 (standard deviation, SD, 16.6). The index bDMARDs were: etanercept (38.3%); adalimumab (32.3%); infliximab (8.8%); golimumab (7.4%); abatacept (5.0%); certolizumab (4.5%); tocilizumab (3.7%). Three trajectories of adherence to bDMARDs were identified: the medium-high (2,486 patients, 74.3%), the moderate (554, 16.5%), and the low (307, 9.2%). The moderate and the low adherence trajectories were characterized by discontinuation of bDMARDs after 18 and 6 months, respectively. Patients in the medium-high adherence trajectory were younger (mean 52.5, SD 16.7; $p<0.001$) and displayed a higher use of NSAIDs (1,672 patients, 67.3%; $p=0.04$) than

those in the other trajectories. Infliximab was the drug most frequently dispensed as index drug in the medium-high adherence trajectory, while golimumab in the moderate, and abatacept and tocilizumab in the low adherence trajectory.

CONCLUSIONS: This study identified three different trajectories of adherence to bDMARDs: low, moderate and medium-high. Index bDMARD, age and baseline concomitant therapies could be explored as predictor of the adherence trajectory.

PENTAMIDINE NIOSOMES THWART S100B EFFECTS IN HUMAN COLON CARCINOMA BIOPSIES FAVORING WTP53 RESCUE

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BACKGROUND: S100B protein bridges chronic mucosal inflammation and colorectal cancer, since its capability to activate NF-kappaB transcription via RAGE signaling and sequestrate pro-apoptotic wtp53. As S100B inhibitor, pentamidine disengages S100B-wtp53 restores wtp53 mediated-pro-apoptotic control in cancer cells in different tumors. The expression of S100B, pro-inflammatory molecules and wtp53 protein expression in human biopsies deriving from control, ulcerative colitis and colon cancer's patients was evaluated and S100B targeting with niosomal PENtamidine VEhiculation (PENVE), to maximize drug permeabilization in the tissue.
METHODS: Cultured biopsies underwent immunoblot, EMSA, ELISA and biochemical assays for S100B and related pro-inflammatory/pro-apoptotic proteins. Exogenous S100B (0.005-5μM) alone or in the presence of PENVE (0.005-5μM), was tested in control biopsies while PENVE (5μM) was evaluated on control, peritumoral, ulcerative colitis and colon cancer's biopsies.
RESULTS: Our data show that S100B level progressively increases in control, peritumoral, ulcerative colitis and colon cancer correlating to a pro-inflammatory/angiogenic and antiapoptotic environment, featured by iNOS, VEGF and IL-6 Up-regulation and wtp53 and Bax inhibition.
CONCLUSIONS: PENVE inhibited S100B activity, reducing its capability to activate RAGE/phosphor-p38MAPK/NF-kappaB, and favoring its disengagement with wtp53. PENVE blocks S100B activity, rescues wtp53 expression determining pro-apoptotic control in colon cancer suggesting pentamidine reposition as anticancer drug.

OVERWEIGHT/OBESE PATIENTS AFFECTED BY CHRONIC LOW BACK PAIN UNDERGONE TO SULPHUROUS MUD-BATH THERAPY

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BACKGROUND: Several factors, including overweight and obesity, contribute to the pathogenesis of Chronic Low Back Pain (CLBP). A recent study found a significant causal effect of Body Mass Index (BMI) in acute and chronic back pain with the BMI increasing 1.15 times the odds of back pain (1). Moreover, both CLBP and obesity affect the Quality of Life (QoL), often causing disability, anxiety, depression, and insomnia with deleterious social and economic consequences (2-4). Among the non-pharmacological treatments, the salus per aquam (spa) Medicine, which uses spa mineral waters (or medical mineral waters), has been suggested to have beneficial effects in treating several diseases. In particular, the mud-bath treatment with sulphurous mineral water, represents a therapeutic tool in CLBP. Our study evaluated the efficacy of spa treatment, in particular with sulphurous mud-bath cycle, on pain and disability in Italian patients with BMI within (group A1) or above (group A2) the range of normality, suffering from CLBP with lumbar spine osteoarthritis**.**
METHODS: Forty-three patients (mean age 63±8.8 years old) underwent a 2-week sulphurous mud-bath treatment at the Terme of Telese S.p.A. (Benevento-Italy). To determine whether there were differences between patients with different BMI, we evaluated: low back pain using the Numerical Rating Scale (NRS-score) and disability-function of the lumbar spine using the Oswestry Disability Index questionnaire (ODI-I), before and after the spa treatment. Moreover, the safety of the treatments used was presumed by monitoring the occurrence of adverse events.
RESULTS: At the end of the sulphurous mud-bath cycle, all enrolled patients showed a significant decrease ($p<0.05$) of the evaluated parameters. The reduction was more consistent in the patients with the normal BMI (A1 group: 60% reduction of the pain symptomatology and 38% reduction of the total ODI-I score) when compared with the overweight/obese patients (A2 group: 46% reduction of the pain symptomatology and 37% reduction of the total ODI-I score).
CONCLUSIONS: The study highlights the beneficial effects of the sulphurous mud-bath cycle on the painful symptomatology and the QoL in both normal weight and overweight/obese patients suffering from CLBP associated with lumbar spine osteoarthritis.

DIGITAL HEALTH AND OXIDATIVE STRESS

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BACKGROUND: The combination of digital and health environments recently has taken on an important role. Indeed, technologies, digital education, and even viruses force a paradigm shift in the health ecosystems. Nowadays, digital technologies can be considered a powerful weapon for designing new solutions and critical systems that can support data analysis for basic and clinical experimental research and diagnostics. In Medicine, oxidative stress is caused by excessive production of reactive oxygen species (ROS) in cells and tissue, which, when in excess, can induce cellular and tissue damage, which, if not promptly repaired, will favor the appearance or accelerate the progression of several diseases. Unfortunately, oxidative stress does not give rise to specific clinical manifestations, so it can only be identified if the biological samples collected are subjected to some simple laboratory tests. Therefore the aim of our pilot project was to design and develop software capable of interacting with laboratory machinery (also called equipment or device) that allows us to measure parameters (e.g., reactive oxygen metabolites) that allow us to evaluate oxidative stress. At the same time, a cloud platform was developed to store and analyze the data collected by the equipment.

METHODS: The proposed solution focuses on the Free System-Diacron International device (Grosseto-Italy), whose function is to perform photometric analyzes of biological samples to determine the redox state of an organism in human and veterinary fields, using suitable kits (such as d-ROMS test, BAP test, SHP Test, OXY-adsorbent Test). The device, which is the focus of our research, is controlled by an embedded display and keyboard, producing an analog output printed on paper.
THE PROPOSED SYSTEM: The proposed system's architecture consists of three entities: Free Device, Cloud, and Database. After the analysis of the test samples, the Free Device sends the acquired data to the Database. The data stored on the Database are made available in a secure way through a Cloud platform. The Medical Staff can retrieve the information from the Cloud platform. We remark that using the proposed system is more convenient than using the Free Device through its embedded display and keyboard. Again, the proposed system provides security facilities for the processing of sensitive data. More precisely, such data are managed securely, in compliance with the GDPR. Finally, saving data in digital format allows more efficient and fault-tolerant management of vast archives.
CONCLUSIONS: Since health is one of the top future topics, the proposed system implements some functionalities for operations that otherwise would involve a considerable waste of time. In particular, the proposed system includes searching for data according to the criteria established by the end-user (i.e., a doctor or nurse), such as searching based on the patient's date or name. Finally, any errors due to data transcription in a paper-based format and data loss are limited through our system. In conclusion, we can say that this pilot project highlights the importance of digital health in research and diagnostics.

RENIN ANGIOTENSIN ALDOSTERONE SYSTEM INHIBITORS ARE NOT ASSOCIATED WITH COVID-19 SEVERITY: A META-ANALYSIS OF RETROSPECTIVE STUDIES

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BACKGROUND: Angiotensin-converting enzyme 2 (ACE2) is a functional receptor for SARS-CoV-2, which is used by the virus to enter the cell. ACE2 is a key modulator of the renin–angiotensin–aldosterone system (RAAS), which is a signaling pathway involved in the regulation of vascular and heart function. Inhibition of RAAS by angiotensin-converting-enzyme inhibitors (ACE-I) or angiotensin-receptor blockers (ARB) may result in a compensatory increase in tissue levels of ACE2. The hypothesis that the use of RAAS inhibitors might be associated with COVID-19 severity has generated concern. We performed a set of meta-analyses aimed at evaluating the association of ACE-I and ARB use with severity of COVID-19.
METHODS: Articles were retrieved until July 13th, 2020 by searching in MEDLINE, EMBASE, Web of Science, and Cochrane library. Data were combined using the general variance-based method on the odds ratio estimate for each study. Heterogeneity was assessed by Higgin's I2 test. The main outcome was severity of COVID-19, including or not mortality. We performed different meta- analyses according to type of COVID-19 patients (restricted to hypertensive subjects or not), different RAAS inhibitors (ACE-I or ARB as unique or combined exposure) and outcomes. The exposure to either ACE-I or ARB was analyzed separately or in combination, and was tested for association with mortality or a combined outcome of severe illness and mortality. In all studies the control group consisted of COVID-19 patients without drug exposure.
RESULTS: A total of N=29,055 COVID-19 men and women adult patients (9,700 with hypertension) were included in the meta-analysis (19 studies). Use of ACE-I or ARB was not associated with COVID-19 severity, neither in 9 studies including both hypertensive and non-hypertensive patients (pooled odds ratio=1.18, 95%CI: 0.96 to 1.46; I2=78%) nor in 12 studies conducted in hypertensive patients (pooled odds ratio=0.90, 95%CI: 0.80 to 1.01; I2=5%). The lack of association was confirmed in several subgroups analyses according to type of outcome (severe COVID-19 only as the outcome; mortality only as the outcome) or exposure (ACE-I or ARB combined in a single group; ACE-I alone; or ARB alone). Selection bias was not revealed at visual inspection of funnel plots in all meta-analyses.
CONCLUSIONS: No association was found between ACE-I/ARB use and severity (including mortality) of COVID-19. These results should be considered with caution, because all the studies meta-analyzed were observational and retrospective, and the possibility of confounding could not be completely excluded. Randomized controlled clinical trials are still necessary to reach a firm conclusion regarding a potential beneficial or detrimental role of these drugs in patients with COVID-19. However, at present, this is the best available result that can help physicians in managing anti-hypertensive therapy with these drugs in COVID-19 patients.

DUAL CAR-T CELL THERAPY ANTI-CD138 AND CD19 COMBINED WITH A2AR/CD73 PATHWAY ANTAGONIST FOR THE TREATMENT OF MULTIPLE MYELOMA

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BACKGROUND: Many patients undergoing treatment for Multiple Myeloma (MM) eventually relapse and become resistant to therapy. CAR-T cell therapy combined with pharmacological drugs has shown great potential in terms of efficacy, safety and durable response (1). The interplay between MM cells and the immunosuppressive tumor microenvironment (TME) is crucial both for MM management and also for CAR-T cell therapy efficacy (2). CD138 is a member of the transmembrane heparan sulfate proteoglycan commonly expressed on MM cells' surface and CD19 is often present on the MM stem cells population, which is thought to play a role in relapsing (3). A2AR and CD73 have a role in the adenosine pathway, which protects MM cells by dampening the action of the immune system among the TME (4). To investigate the potential use of a dual CD138-CD19 CAR-T cell therapy, combined with A2AR and/or CD73 inhibitors for a novel cell-targeting system useful for the treatment of MM.

METHODS: CD138 and CD19 expression has been assessed both in MM cell lines (e.g. RPMI-8226, U266) and in other blood cancer lines (HL60, JURKAT). Western blot (WB) analysis was performed to assess CD138 expression. Flow cytometry (FC) was used to confirm the WB results. Eventually, immunofluorescence (IF) has been used to assess the antigens' localization. Patient-derived cells were obtained, separated and isolated with CD138+ immunomagnetic bead selection, then analyzed using FC in order to confirm CD138 expression among other B-cell lineage markers (e.g. CD27, CD38, CD78). The expression of CD73 and A2AR was also assessed.

RESULTS: Different clones of anti-CD138 antibody (Ab) have been analyzed (DL-101, B-A38, SP152) to evaluate the most suitable. These experiments have determined the region of the CD138 protein more useful to act as a specific target for the anti-CD138 Ab that will be manufactured for the CAR-T cell therapy.

The Ab clone DL-101 has better met the criteria of specificity and sensibility required. We have confirmed the protein expression of CD138 on various MM cell line models (e.g. RPMI-8226, U266) through WB, IF (A) and FC (B). We have also identified two negative controls, such as JURKAT and HL60. Analysis of the surface antigens (CD138, CD27, CD38, CD78) was performed using FC to characterize MM primary cells. The expression of CD73 and A2AR was also assessed.

CONCLUSIONS: According to the literature and in line with our results, we have identified U266 as the most suitable MM cell line model to use for further experiments. As a negative control, JURKAT cell line has shown a lack of CD138 expression. Those preliminary findings pave the way for the production of an anti-CD138 CAR-T by engineering T cells from MM patients and subsequent production of dual anti-CD138-CD19 dual CAR-T.

1. Kumar et al., Nat Rev Dis Prim 2017
2. Kawano et al., Immunol Rev 2015
3. Akl et al., Oncotarget 2015
4. Ohta et al., PNAS 2006

POSSIBLE PROTECTIVE EFFECT OF SODIUM BUTYRATE IN CHEMOTHERAPY-INDUCED NEUROPATHY AND DEPRESSIVE-LIKE BEHAVIOR

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BACKGROUND: Despite recent advances in chemotherapy drugs, their use is usually accompanied by side effects; a large proportion of cancer patients often show peripheral neuropathy, have experience in changes mood, including anxiety and depression, and gastrointestinal toxicity. Among new chemotherapeutic agents, paclitaxel emerged as one of the most powerful compounds although its known side effects. Gastrointestinal complications result in the disruption of the mucosal barrier leading to bacteria passing into the systemic circulation and causing serious central symptoms, related to gut-brain axis hypothesis. Dietary fiber fermentation by intestinal microflora results in the production of short-chain fatty acids and butyrate has been reported as a chemopreventive agent and a histone deacetylase inhibitor. The aim of the study is to examine whether sodium butyrate is able to attenuate chemotherapy-induced neuropathy, inflammation and behavioral changes.

METHODS: For the study mice received sodium butyrate at the dose of 30 mg/kg daily in drinking water for 44 days and from day 30 they also received the chemotherapic agent (paclitaxel-one cycle) intraperitoneally. Pain sensitivity and depressive-like behavior were performed 14 days from the first injection of paclitaxel and then central and peripheral tissues were collected for molecular analysis. Neuropathic pain was assessed by Randal-Sellitto and Von Frey tests, while depressive behavior was analyzed by tail suspension, forced swim test, and anxiety and locomotor behaviors were assessed by elevated plus maze and open field tests. We also performed biochemical analyses using western blots and RT-PCR to evaluate the expression of different markers and their possible modulation.

RESULTS: As expected, paclitaxel induced the development and maintenance of mechanical hyperalgesia and allodynia and also induced anxiety-like behavior, as assessed in the elevated plus maze test. In addition, paclitaxel-treated mice displayed depression-like behavior during the forced swim test and the tail suspension test. In vivo results showed that the sodium butyrate significantly reduced both neuropathy and depression in mice treated with paclitaxel. Moreover, preliminary molecular results also show that butyrate treatment improved central and peripheral inflammation and modulate the expression of neurotrophins and their receptors.

CONCLUSIONS: In conclusion for the first time we found the protective role of sodium butyrate in chemotherapy mouse model, through its pleiotropic mechanism of action, supporting analgesic, anti-inflammatory and neuroprotective effects.

EVALUATION OF NEUROPROTECTIVE EFFECTS OF QUERCETIN AGAINST AFLATOXIN B1-INTOXICATED MICE

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BACKGROUND: One of the main factors that can lead to the deterioration of poultry feed is fungal spoilage. Several factors can provide favorable conditions for the growth of fungi, like higher ambient temperatures, bad harvesting methods and inadequate drying of cereals in the field. All these factors can bring about an increase in the development of secondary fungal metabolites correlated with mycotoxin production. Certain strains of Aspergillus flavus and Aspergillus parasiticus produce secondary metabolites called aflatoxins that have been exhibited to be toxigenic, mutagenic, carcinogenic and teratogenic to several species of animals. Among the aflatoxins, aflatoxin B1 (AFB1) represents the most toxic and prevalent form. It was demonstrated that prenatal exposure to AFB1, in rat offspring, can delay the development of reflex response and learning ability, as well as motor activity, locomotor coordination and exploratory behavior. The ability of AFB1 to penetrate the brain may be partially attributed to its detrimental effects on the blood–brain barrier's integrity and therefore in the possible onset of cognitive deficits. Quercetin, an abundant flavonoid with antioxidant properties, which commonly presents in the diet, for example, in fruits like blueberries or vegetables such as onions, curly kale, broccoli and leeks, is a promising candidate for the prevention of adverse health effects. In the present study, we aimed to investigate the potential preventive effects of quercetin consumption in a mouse model ofAFB1-induced behavioral impairment.

METHODS: Balb/c mice were allocated into five groups. The administration of AFB1 and/or quercetin by oral gavage was carried out every three days for 45 days. Mice were subjected to behavioral test such as FST and EPM to study depressive and anxiety-like state; MWM to assess spatial learning and memory and NOR test to assess recognition memory function. Thiobarbituric acid-reactant substances evaluation, a suitable indicator of lipid peroxidation, was determined in whole brain; Serum TNF-α and IL1β levels were evaluated using ELISA kit and levels of reduced glutathione (GSH) superoxide dismutase (SOD) and catalase (CAT) activities were detected in whole brain tissues.

RESULTS: Oral administration of quercetin during chronic AFB1 exposure prevented memory impairment, such as the anxiety-like behavior induced by mycotoxin in adult mice. This protective effect of quercetin against AFB1 toxicity seemed to be related to antioxidant action in the brain, through decreased lipid peroxidation and preservation of detoxification enzymes like GSH, SOD and CAT.

CONCLUSIONS: We suppose that AFB1 is able to alter brain functions and highlight the capacity of quercetin as antioxidant to counteract these detrimental effects. Our results reinforce the idea that monitoring food preservation and mycotoxin levels are of fundamental importance when safeguarding food safety and the well-being of animals and humans.

CALCINEURIN REGULATES NEURO-IMMUNE NETWORK IN INFLAMMATORY BOWEL DISEASES

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BACKGROUND: Inflammatory bowel disease (IBD) pathogenesis remains unclear up to now but is thought to be a complex interaction of genetic, environmental and immunological factors. Increasing evidence indicates that also the ENS is involved in the pathogenesis of IBD. The ENS is an integrative neuronal network within the gut wall and is made up of two major cell populations: enteric neurons and enteric glial cells (EGCs). Abundant studies have demonstrated that the EGCs can exert their effects on immune system in the gastrointestinal tract. The disruption of the EGCs might represent one possible cause for neuro-immune dysfunction that may contribute to mucosal inflammation (Xie et al, 2020).
The Ca2+/calmodulin (CaM)-activated serine–threonine phosphatase, calcineurin (CaN), is abundantly expressed in neurons, but also in astrocytes (Lim et al., 2016). It induces transcription factors (NFATs) that are important in the transcription of IL-2 genes. When CaN is up-regulated plays a crucial role in astrogliosis and neuroinflammation (Pekny et al., 2016).
The aim of our work was to determine the role of CaN in ENS and how the deletion of this protein in EGCs could influence inflammation.
MATERIALS AND METHODS: Conditional CaN knockout in astrocytes and EGCs cells was generated by crossing CaNB1flox/flox mice (strain B6), in which third, fourth, and fifth exons in the CaNB1, a regulatory CaN subunit gene, were flanked by LoxP sequences, with the expression of bacterial Cre recombinase. The backcross generates two murine lines referred as ACN-Ctrl, as control, and ACN-KO.
Colitis was induced in mice with 2% of dextran sodium sulphate (DSS) in drinking water for 7 days. Mice were monitored daily in body weight and in disease activity index (DAI) that comprises of blood in faeces and stool consistency. After 7 days, the mice were sacrificed, colon length evaluated, and tissues harvested for RT-PCR, IHC and myeloperoxidase assay (MPO).
RESULTS: After the determination of deletion of CaN in EGCs in colonic tissue, through IHC, conditional EGCs ACN-KO mice were induced with DSS. Daily monitoring determined that DSS-induced mice resulted in a significant amelioration of IBD symptoms. We observed a decrease of body weight loss, a decrease of DAI as a result of less blood in faeces and more consistent stools, compared to CaN-Ctrl mice. At the sacrifice, we also determined less fibrosis in colon mucosa, observing colon length, because of attenuate inflammation.
mRNA was extracted from tissues, and gene expression evaluated for RT-PCR. We observed a significant reduction in Tnf, Il1 and Ifna. Moreover, we observed a significant reduction in MPO activity in CaN mice.
CONCLUSIONS: We demonstrated for the first time that CaN deletion in EGCs is responsible of the amelioration of DSS-induced symptoms, suggesting a pathogenic role of CaN in IBD.
This data confirmed the idea that CaN deletion may influence neuro-immune network in the colon, as a result of a possible lacking communication between EGCs and immune cells.

AN INTEGRATED RADIOGENOMIC APPROACH TO MONITOR CLONAL HETEROGENEITY OF EGFRMUTATED NSCLC

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BACKGROUND: Non-small cell lung cancer (NSCLC) is a dynamic disease. In the era of personalized medicine, the development of sensitive technologies able to monitor tumor heterogeneity is needed, in order to identify the most appropriate treatment, to improve the response rate and patient's quality of life, and to reduce healthcare-related costs. Radiomics and liquid biopsy have great potential, since are both minimally invasive, easy to perform, and can be repeated during patient follow-up, enabling the extraction of valuable information, such as tumor type, aggressiveness, and response to treatment.
METHODS: 7 patients with metastatic EGFR mutant NSCLC have been monitored during EGFR-TKIs treatment. Plasma-derived circulating free DNA (cfDNA) was analyzed by a digital droplet PCR (ddPCR), while radiomic analyses were performed using the validated LifeX® software by looking at multiple basal computed tomography (CT)-images. The dynamic of EGFR mutations in cfDNA was compared with that of radiomic features by Kendall's tau-b correlation coefficient. Then, for each EGFR mutation, a radiomic signature was the sum of the correlated most predictive features, weighted by their corresponding regression coefficients for the least absolute shrinkage and selection operator (LASSO) model, by performing 27-fold cross-validation. Receiver operating characteristic (ROC) curve analyses were computed to estimate their diagnostic performance.
RESULTS: Liquid biopsy monitoring during treatment was concordant with clinical and radiographical assessment. Direct and inverse correlations were found between radiomic features, activating EGFR (ex19del/L858R) copies/ml, T790M copies/ml and the total copies/ml ($p<0.05$).
The signatures achieved promising performance on predicting the presence of EGFR mutations ($R2 = 0.447$, $p<0.001$ for activating EGFR copies/ml; $R2 = 0.301$, $p=0.003$ for T790M copies/ml; and $R2 = 0.354$, $p=0.001$ for the total copies/ml), confirmed by ROC analysis.
CONCLUSIONS: Together, radiomics and liquid biopsy could provide a promising strategy to detect clonal heterogeneity and ultimately identify patients at risk of progression during treatment, due to the appearance of new mutations and therefore addressable by new therapeutic management.

GENDER, MGMT METHYLATION, AND LEVETIRACETAM PLASMA LEVELS CORRELATE WITH SURVIVAL BENEFIT IN GLIOBLASTOMA PATIENTS TREATED WITH ADJUVANT TEMOZOLOMIDE: RESULTS FROM A NON-INTERVENTIONAL RETROSPECTIVE CLINICAL STUDY

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BACKGROUND: Glioblastoma multiforme (GBM) is a highly aggressive brain tumor, often occurring with seizures. The current standard of care for cancer patients who present seizures includes the use of antiepileptic drugs (AEDs), such as levetiracetam (LEV). Some preclinical studies have shown that LEV may act as histone deacetylases inhibitor (HDACi) through the modulation of O6- methylguanine-DNA methyltransferase (MGMT) expression, thus enhancing temozolomide (TMZ) activity.
METHODS: In this retrospective, non-interventional clinical study, patients with glioblastoma underwent surgery and radiotherapy and received LEV during adjuvant TMZ chemotherapy. Therapeutic drug monitoring of LEV plasma concentrations was performed through a validated assay using high-performance liquid chromatography (HPLC) with UV-detection, after purification by protein precipitation and solid-phase extraction. The average concentration of the drug throughout follow-up has been related to both patient's clinical characteristics and outcomes. Differences were considered significant at $p<0.05$. Statistical analysis was performed using the open-source statistical language R (R Foundation for Statistical Computing, Vienna, Austria) through the free and open statistical software program JAMOVI® (Version 1.1.9; retrieved from https://www.jamovi.org).
RESULTS: Fourty patients (43% female; mean age = 54.73 ± 11.70 years) were enrolled, and GBM MGMT methylation status assessed. All patients were treated with adjuvant TMZ and LEV to seizures control. While age and TMZ dose (75.17 ± 0.35 mg/m2) did not seem to affect clinical outcomes, median progression-free survival (PFS) was significantly longer in patients harboring methylated MGMT (548 vs. 246 days, log-rank $p<0.001$). Such beneficial effect was more prominent in males (815 vs. 360 days of men without methylation, log-rank $p = 0.029$; 815 vs. 216 days of women without methylation, log-rank $p < 0.001$), and in patients whose LEV average concentration was ≤ 25.4 µg/mL (815 vs. 274.5 days, log-rank $p=0.003$). Overall, female patients showed a longer overall survival (OS) (1220 vs. 574 days, log-rank $p=0.03$), and LEV average concentration positively correlated with OS ($R = 0.35$, $p=0.029$), too. Survival analyses also revealed that men with methylated MGMT lived longer (699 vs. 392 days, log-rank $p=0.05$) and that men with methylated MGMT and treated with high plasma levels of LEV lived much longer than those with no methylation and proven low

LEV average concentration (937 vs. 365 days, log-rank p=0.05).
CONCLUSIONS: Female gender and MGMT methylation status may have an impact on PFS and OS of patients with GBM. Nonetheless, where these characteristics are lacking, high plasma concentrations of LEV may succeed with mechanisms not directly involved in controlling epileptic seizures, supporting TMZ effect, and extending patients' survival.

THE POTENTIAL ROLE OF CD69 AS MARKER OF INFLIXIMAB RESPONSE IN PEDIATRIC PATIENT WITH INFLAMMATORY BOWEL DISEASE

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BACKGROUND: Infliximab (IFX), a chimeric antibody to tumor necrosis factor α (TNF), is a highly effective treatment for induction and maintenance of remission in pediatric patients with inflammatory bowel disease (IBD). The molecular mechanisms by which IFX regulates inflammation have not been clarified yet. The proportions of circulating activated CD4(+) and CD8(+) T lymphocytes are increased in patients with IBD and are related to plasma concentration of pro-inflammatory cytokines. Recent studies have identified genes regulated by anti-TNF therapy such as CD69, a membrane receptor transiently expressed on activated lymphocytes at inflammatory sites. During inflammation, T cell activation induces several signalling cascades, including nuclear factor-kappa B (NF-κB) pathway, that enhance the release of proinflammatory cytokines, which maintain and upregulate CD69 expression on cell surface. The aim of this study is to evaluate CD69 as a marker of IFX response on an immortalized T cell line and on peripheral blood mononuclear cell (PBMC) of healthy donors.
METHODS: Jurkat cells and PBMCs were activated with PMA/ionomycin for 4 and 8 hours respectively and then treated with different concentrations of IFX overnight. The effect of IFX was evaluated both as decrease of CD69 mean fluorescence intensity (MFI) by flow cytometry and p65 (a subunit of NF-κB) and IKB protein expressions by western blot analysis. Statistical analyses were performed by two-way ANOVA. The data are reported as means ± standard error (SEM) of three independent experiments.
RESULTS: CD69 cell surface expression decreased from 100 to 91.7 ± 3.61, 87.4 ± 6 and 73.1 ± 0.98 % in activated Jurkat cells treated with IFX 0, 1, 10 and 100 μg/mL respectively ($P \leq 0.001$). Western blot analysis revealed a significant difference in IkB protein expression ($P \leq 0.0001$): activation reduced IkB protein concentration in Jurkat cells (fold change = 0.6 ± 0.03) while no significative change was observed for p65 (fold change = 1.06 ± 0.07) ($P = 0.3$). Treatment with IFX 100 μg/mL led to an increase of IκB protein expression in comparison to untreated cells (fold change = 1.24 ± 0.1). Preliminary data obtained on CD4+ and CD8+ T cells isolated from PBMCs of a healthy donor show that CD69 MFI decreased from 100 to 73.5, 30.9 and 23.3% and from 100 to 72.3, 31.3 and 22.4%

after treatment with IFX 0, 1, 10 and 100 μg/mL respectively. Interestingly, the percentage of CD69 positive CD8+ cells decreased after IFX treatment from 100 to 98.5, 86.4 and 73.1%. No change was observed after IFX treatment on the percentage of CD69 positive CD4+ cells.

CONCLUSIONS: When T-cells are treated with IFX, probably the inhibition of TNF and, consequently, the increase of IκB protein expression, inhibitor of NF-κB, leads to the diminished cell surface expression of CD69 making it a possible marker of IFX response.

VITAMIN D GENETICS AND INFLAMMATORY BOWEL DISEASE PATIENTS TREATED WITH ADALIMUMAB: A STUDY ON PREDICTORS OF EFFICACY AT 3 MONTHS OF THERAPY

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BACKGROUND: In the era of "individualized medicine", genetics and pharmacokinetics have to cooperate leading to tailored treatment, with maximal efficacy and reduced side effects.
Adalimumab (ADA) is a therapeutic human anti-tumor necrosis factor (TNF) α monoclonal antibody also used in inflammatory bowel diseases (IBD), such as Crohn's or ulcerative colitis.
Vitamin D (VD) is important for several biological functions, such as regulation of the immune response and modulation of expression of genes encoding enzymes and transporters involved in drug metabolism and transport. VD is activated by cytochrome (CYP) 27B1, inactivated by CYP24A1, transported in kidney by VD binding protein (VDBP, encoded by GC gene) and carries out its activities through its receptor (VDR).
IBD affected patients generally show reduced VD levels, but no data are available in literature concerning VD genetics and ADA clinical response, other than pharmacokinetics.
For these reasons, aim of this study was to describe the relationship between vitamin D pathway-related gene single nucleotide polymorphisms (SNPs) and ADA clinical outcome, other than its plasma concentrations, at 3 months of therapy, in a cohort of IBD affected patients.
METHODS: ADA treated individuals were included in the study after signing a written informed consent. SNPs in CYP27B1, CYP24A1, GC and VDR genes were analyzed through real-time PCR. ADA concentrations were determined using enzyme linked-immunosorbent assay (ELISA). Clinical outcome was considered as clinical response and remission of pathology at 3 months of therapy.
RESULTS: In this study, 69 patients were enrolled. Median age was 40 (IQR 31-56) years,

body mass index 22 (IQR 20-24) Kg/m2 and median basal calprotectin was 396 (IQR 188-851) mg/Kg. Males were 40 (58%), 3 (4.3%) subjects had ulcerative colitis and 36 (53.7%) had a PCR value over the laboratory referred limit.
We documented the following associations: CYP27B1+2838 CT/TT with perianal disease (p= 0.002), basal calprotectin (p= 0.018) and T3 calprotectin (p= 0.035), figure 1; CYP27B1-1260 GT/TT with perianal disease (p= 0.006), basal calprotectin (p= 0.036) and T3 calprotectin (p= 0.024); VDR ApaI CA/AA with basal calprotectin (p= 0.014) and T3 calprotectin (p= 0.036); VDR BsmI GA/AA with perianal disease (p= 0.036) and, finally, GC 1296 TG/GG with basal calprotectin (p= 0.014) and T3 calprotectin (p= 0.052), figure 2.
Logistic regression analyses were performed to understand which factors were able to predict the clinical response and remission at three months of treatment: systemic steroid use (p<0.001), GC TG/GG (p= 0.076, figure 3) and VDR Cdx2 GG were retained in the final model for the clinical response, whereas systemic steroid use (p<0.001), immunosuppressant drugs (p=0.098), GC 1296 TG/GG genotype polymorphism (p= 0.044, figure 4) and alcohol use (p<0.001) for the remission.
Finally, in a sub-population of 23 subjects, ADA plasma levels at 3 months of therapy were available: their median concentrations were 7.4 (IQR 5.5; 11.7) ug/mL. CYP24A1 3999 (p=0.025) and VDR TaqI (p=0.016) SNPs affected these levels.
CONCLUSIONS: This is the first study reporting the association between vitamin D pathway-related genetics and ADA treatment in a cohort of patients affected by IBD. Further studies in different and larger cohorts are needed to clarify these aspects.

HUMAN-IPSC DERIVED CARDIOMYOCYTE MITOCHONDRIAL FUNCTIONALITY AND METABOLISM ARE AFFECTED BY LIPOPROTEIN METABOLISM

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BACKGROUND: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is secreted into the circulation by the liver and controls the expression of several receptors of the LDL-R family and of CD36 guiding their degradation in the lysosome. Physiologically, this mechanism limits an exaggerated expression of lipoprotein receptors which viceversa might lead to peripheral lipid accumulation which in the case of the heart might result in cardiac lipotoxicity. Aim of this project is to test the hypothesis that PCSK9 might regulate lipoprotein and fatty acid metabolism in cardiomyocyte, thus, in turn, affecting mitochondria physiology and their metabolism.
METHODS: Cardiomyocyte have been differentiated from human-inducible Pluripotent Stem Cells (h-iPSCs) and after 30 days of culture have been have been treated for 24h with PCSK9 (2ng/ml) followed by incubation with

LPDS (10%), VLDL (50 µg/ml) or LDL(100µg/ml). At the end of the experiments cells were profiled by real time QPCR, FACS analysis, western blot and mass spectrometry.
RESULTS: After 30 days of differentiation, cells presented an elevated expression of Troponin T, confirming the acquisition of a mature cardiomyocyte phenotype (iPSC-CM) and genes involved in fatty acid metabolism (CD36, CPT1b, PPARg). Incubation of iPSC-CM with VLDL reduced the expression of mitochondrial fusion protein (MFN1) while mitochondrial fission (DRP1) protein were increased. This profile was associated with increased accumulation of neutral lipids and a decreased mitochondrial mass compared to control. This phenotype was reverted when cells were pre-incubated with PCSK9.
CONCLUSIONS: Our data suggest that elevated VLDL concentrations favor lipid accumulation in cardiomyocytes followed by reduced mitochondrial mass in cardiomyocyte. Incubation with PCSK9 limits cardiolipotoxicity by reducing lipid receptor expression and lipoprotein uptake.

INCREASED LEVELS OF TRACKABLE COPY NUMBER ABERRATIONS (CNAS) IN THE CELL-FREE DNA (CFDNA) OF LOCALLY ADVANCED RECTAL CANCER (LARC) PATIENTS RECEIVING NEOADJUVANT CHEMORADIOTHERAPY (NCRT) AND PHARMACOLOGICAL IMPLICATIONS ON TREATMENT OUTCOME

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BACKGROUND: The standard of care for the management of locally advanced rectal cancer (LARC) relies on 5-fluorouracil-based neoadjuvant chemoradiotherapy (nCRT), followed by total mesorectal excision (TME). The achievement of a pathological complete response (pCR) after nCRT is observed in up to 30% of patients and is positively associated with a lower risk of local and distant recurrence. The need to discriminate good from poor responder in the early steps of nCRT is urgently required to optimize the following therapeutic strategies and to refine current multimodal pharmacological schemes. The chance of interrogating circulating tumor DNA (ctDNA) represents an appealing tool for the real-time monitoring of treatment response in a low-invasive manner and to sustain the decision making for treatment's personalization. We monitored the presence and the fluctuation of copy number aberrations (CNAs) by means of low-coverage shallow whole genome sequencing (sWGS) in the cfDNA of 40 LARC patients receiving nCRT to

assess its potential role as an early biomarker of treatment efficacy.

METHODS: 100 blood samples were collected from 40 consent LARC patients with available clinical data and from 16 healthy donors at the Clinical Pharmacology Unit of IRCCS CRO Aviano (Italy). Plasma was separated in a two-steps centrifugation protocol and cfDNA was extracted using the Qiaseq ccfDNA MiniKit (Qiagen). Sequencing libraries for sWGS were prepared from 5-10 ng of cfDNA using the KAPA Hyper Prep Kit for Illumina platforms (Roche). Pooled libraries were sequenced on an Illumina HiSeq4000 with 100 bp paired-end reads. Sequencing reads were processed and aligned against reference genome by the automated pipeline OTP1. Genome-wide copy number profiles and tumor fraction (TFx) were estimated from sWGS data using ichorCNA2. The response to nCRT was assessed using the Mandard's tumor regression grade (TRG) scale3.

RESULTS: For all LARC patients the plasma sample collected at the time of diagnosis (T0, n=40) was available. Further longitudinal plasma samples were collected in the course of nCRT (T1, n=19) and after nCRT (T2, n=14). The presence of CNAs was detected in the cfDNA of 7/40 patients (17.5%) evaluated at the T0, with a median TFx of 10.41% (range 5.89–27.34). When comparing the variation of TFx between T1 and T0, 7/19 evaluable patients (36.8%) showed an increase of the TFx (median 14.28% range 5.65–28.81). In this first group 6/7 patients (85.7%) achieved a pCR. On the contrary, 12/19 patients (63.2%) showed no trackable CNAs or, when trackable, they had a reduction of calculated TFx at T1. In that second group, only 3/12 patients (25.0%) reported a pCR. Subjects with increased TFx at T1 (n=7, first group) presented an increased likelihood of achieving a pCR than those without detectable or reduced TFx (n=12, second group) (OR 3.43, 95%CI 1.23–9.56, P=0.0106). Presence of CNAs at the T2 was observed only in 3/14 evaluable patients (21.4%) with an average TFx of 20.02%, and none of them achieved a pCR. No CNAs were detected in the cfDNA of healthy donors.

CONCLUSIONS: The identification of CNAs and their quantification by means of TFx estimation is feasible in the cfDNA of LARC patients. Preliminary data suggests that an increased TFx in course of nCRT is associated with a higher probability of achieving a pCR, whereas the permanence of TFx after nCRT is a marker of poor outcome. These results sustain the need for a wider prospective evaluation of the predictive role of ctDNA for the early monitoring of nCRT sensitivity.

A GENOTYPING/PHENOTYPING APPROACH TO MANAGE THE FLUOROPYRIMIDINES-BASED THERAPY: CLINICAL CASES

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BACKGROUND: Fluoropyrimidines (FP) are chemotherapy drugs used for the treatment of solid tumors. DihydroPyrimidine Dehydrogenase (DPD), a key enzyme in the FP metabolism, is encoded by a polymorphic gene (i.e. DPYD). Four polymorphisms are associated to reduction or abrogation of the DPD activity, leading to severe Adverse Drug Reactions (ADRs). Recently, the European Medicines Agency stated that the DPYD Pharmacogenetics (DPYD-PGx) should be performed prior to start a treatment with FP and heterozygous for one of four polymorphisms should receive reduced starting doses of FP while homozygous have to use alternative drugs.
METHODS: We describe a case series of patients (Pts) enrolled at Oncology Unit of University Hospital of Salerno. The DPYD-PGx was performed by real-time PCR. A clinical monitoring was carried out and the ADRs were graded according to Common Terminology Criteria for Adverse Events. Plasmatic dihydrouracil/uracil (UH2/U) ratio was evaluated by high-performance liquid chromatography whereas plasmatic 5-FU clearance was determined by ultra-high performance liquid chromatography combined with tandem mass spectrometry.
RESULTS: Table 1 shows the main characteristics of 11 patients treated with FP and identified as carriers of DPYD variants. Precisely, 6/11 were DPYD*2A heterozygous and 5/11 were DPYD c.2846 heterozygous. A pre-therapeutic DPYD-PGx was performed in 8/11 cases while, in 3/11 (case 1,4 and 5), DPYD-PGx was required after the occurrence of grade 3 ADRs. Pt 1 reported grade 3 neutropenia and mucositis after three cycles of chemotherapy. He was then identified as DPYD*2A. Plasmatic UH2/U ratio was 3.52. Based on these results, 5-FU dose was reduced to 50%. At the fourth cycle of therapy, the pharmacokinetic analysis revealed a trough 5-FU plasma concentration of 950 ng/ml. At the sixth cycle of therapy, 5-FU plasma concentration was 400 ng/ml and Pt did not experience grade 3 ADRs. Pt 4 reported grade 3 vomit after the second cycle; he was then identified as DPYD*2A heterozygous so he continued to be treated only with oxaliplatin. Plasmatic UH2/U ratio was 7.09. Pt 5 showed grade 3 vomit after the eighth cycle of chemotherapy. Then he was identified as DPYD c.2846 heterozygous and 5-FU dosage was halved. Plasmatic UH2/U ratio was 3.88. Conversely, in the other patients a pre-therapeutic DPYD-PGx was performed, they were treated with a starting halved dose of 5-FU and no severe ADR were reported, except for Pt 3 who reported grade 3 and 4 diarrhea throughout the duration of therapy. Pt 3 was found hetero-

zygous for DPYD c.2846 A>T. Plasmatic UH2/U ratio was 1.77. 5-FU plasma concentration was high during all therapy cycles (table 1).
CONCLUSIONS: Routine use in the clinical practice of DPD-PGx is more than justified. Other factors may influence FP safety and researchers 'efforts are addressed to find phenotyping methods to complement the DPYD-PGx to better personalyze and optimize the FP-based chemotherapy.

IMPACT OF EARLY LIFE SOCIAL EXPERIENCES ON ALCOHOL DRINKING AND NEURODEVELOPMENT IN GENETICALLY SELECTED ALCOHOL PREFERRING RATS

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BACKGROUND: Several studies have shown that in mammals neuronal and behavioural development is strongly influenced by early negative or positive social experiences. For instance, in the rat, nesting conditions and early life stress may influence animal's neurodevelopment and affect various neurocircuitry functions including those associated with the brain reward system. Ultimately, this may contribute to exacerbate psychiatric-like conditions, such as individual propensity to develop substance abuse, anxiety or depression. Here we sought to explore the impact of nesting condition and early life maternal separation on alcohol drinking of genetically selected male and female marchigian sardinian alcohol preferring (msP) rats.
METHODS: Offspring from 36 female and 24 male msP rats were subjected to different nesting conditions. Specifically, female rats were divided into two groups: 18 were single-mated with a male rat (standard housing condition, SH) and 18 were housed in groups of three and each group were mated with a male rat (communal nesting condition, CN). Starting from postnatal day (PND) 14 until PND 21, half of the SH and CN offspring were separated and daily isolated for 30 minutes (early social isolation, ESI), whereas the other half of the offspring were maintained in standard condition and represented the control groups. At PND 21, the rats were weaned and housed in groups of three according to their experimental group (SH, SH+ESI, CN, CN+ESI).
RESULTS: From the offspring generated, 12 rats for each group (6/sex) were sacrificed at PND 35 or at PND 75. In these rats cerebral and blood tissues were sampled for biochemical analyses. The remaining littermates (10/group, sex balanced) have been exposed for one week to an intermittent two-bottle choice drinking (choice between 10% alcohol and water). Behavioural data revealed that all groups assumed high amounts of alcohol from this first

day of exposure. No significant difference were detected between groups ($F(3,16) = 2.71$, $p > 0.05$). Subsequently rats have been trained to alcohol operant self-administration under fixed ratio 1 (FR1) schedule of reinforcement. During the initial training no differences were detected between groups ($F(3,16) = 1.18$, $p > 0.05$). Experiments are ongoing to evaluate the propensity to develop alcohol seeking behaviour in response to the pharmacological stressor yohimbine and to evaluate the molecular and biochemical parameters associated with the different social experiences.

CONCLUSIONS: Preliminary findings demonstrate that different nesting condition associated with exposure to early life stress may not modify the propensity to consume alcohol in a rat line characterized by innate predisposition to excessive drinking. This work is supported by grant PRIN 2017 (2017SXEXT5).

EFFECTS OF SODIUM BUTYRATE (NAB), ALPHA-LACTOALBUMIN (ALAC) AND THEIR COMBINATION ON AUTISTIC-LIKE BEHAVIOR IN BTBR MICE

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BACKGROUND: Autism Spectrum Disorder (ASD) is one of the most common neurodevelopmental disorders worldwide, affecting approximately 1 in 54 children. The behavioral symptoms of ASD include dysregulation of social communication and the presence of repetitive behaviors. In addition to these symptoms, children with ASD are affect by gastrointestinal (GI) problems1. GI alterations are strongly correlated to irritability, anxiety, and modification of social behavior2. Moreover, it was reported that modification of gut microbiota and alterations in gut-brain axis communication, are associated with a development of different diseases ranging from inflammatory bowel disease (IBD) and obesity, to neurological diseases and neurodevelopmental disorders such as Parkinson's disease, epilepsy and ASD. Recently, it was reported that ALAC, a whey protein rich in tryptophan, is effective in some animal models of epilepsy and epileptogenesis, can inhibit gut inflammation and contribute to the development and maintenance of gastrointestinal physiological functions3. It was previously observed that NaB, a short chain fatty acid (SCFA), normally produced in the intestine by the gut microbiota, is important in maintaining gut health and in reducing gut inflammation and oxidative stress. Finally, changes in SCFAs levels in mouse models of ASD likely contribute to ASD symptoms4. Accordingly, we tested ALAC, NaB and their combination on autistic-like behavior in BTBR mice.

METHODS: Male BTBR mice were orally treated for 15 days with NaB (30, 100, 300 mg/kg), ALAC (30, 100, 300mg/kg) and with their co-administration (NaB 100 mg/kg +ALAC 30, 100, 300mg/kg) and compared with vehicle group (C57/BL6). At the end of treatments, animals were subjected at different behavioral tests such as three-chamber social interaction

test, marble burying test, elevated plus maze and passive avoidance test.
RESULTS: Our results indicate that NaB, ALAC and their co-administrations revert the altered behavior of BTBR mice. In particular, single treatment produced a significant effect on social, repetitive and perseverative behavior and also on learning and memory performance if compared with vehicle group. This effect becomes more marked using the co-administration. Moreover, in the forced swimming test, the co-administration only was effective in producing antidepressant-like activity.
CONCLUSIONS: Our results suggest that treatments with NaB and ALAC may modulate autistic-like behavior in BTBR mice and their combination has further advantages in comparison to single drug administration.

PHARMACOLOGICAL INHIBITION OF FAAH REDUCES DEPRESSIVE-LIKE AND ANXIETY-LIKE BEHAVIOURS INDUCED BY THE ABSTINENCE FROM A CAFETERIA-STYLE DIET

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BACKGROUND: Obesity has been linked to a higher risk of depression and anxiety. Diet restriction and abstinence from high-palatable food exacerbate these comorbidities, thus sustaining the vicious circle of "food addiction". Endogenous acylethanolamides (such as anandamide, oleoylethanolamide, palmitoylethanolamide) might play a key role in this scenario since they participate to the mechanisms regulating reward and mood tone. This study aimed to explore whether the abstinence from a palatable diet, after a long-term ad libitum consumption of high palatable food, might produce alterations of the emotional reactivity and mood tone and whether the pharmacological inhibition of fatty acid amide hydrolase (FAAH) by PF-3845 treatment (which causes an increase of acylethanolamide tone) could ameliorate such alterations.
METHODS: We used a rat model of diet-induced obesity based on a cafeteria-style diet. After the first 40 days of exposure to cafeteria diet, rats underwent an abstinence period of 28 days. During this period, animals were separated in two different groups: one group was chronically treated with PF-3845 (10 mg/kg, intraperitoneally) and the other group was treated with vehicle (ethanol/tween80/saline 5/5/90). At the end of the treatment rats were subjected to behavioral tests including the open field test, the elevated plus maze and the forced swimming test and then sacrificed. Brains were collected, microdissected in different areas and processed for both HPLC analysis of monoamines (dopamine, noradrenaline, serotonin and their main metabolites) and western blot analysis of different proteins involved in the synthesis

and degradation of acylethanolamides (monoacylglycerol lipase or MAGL; N-acyl phosphatidylethanolamine-specific phospholipase D or NAPE-PLD; diacylglycerol lipase alpha and beta or DAGL-α, DAGL-β; FAAH) and linked to inflammatory processes (cyclooxygenase 2 or COX2, allograft inflammatory factor 1or IBA-1, glial fibrillary acidic protein or GFAP).
RESULTS: Our results show that after the abstinence from the cafeteria-style diet, rats displayed an anxiety-like and depressive-like behaviour; interestingly the pharmacological treatment with PF-3845 was able to revert such behavioral effect, by exerting an anxiolytic-like and antidepressant-like effect. A variety of alterations of the monoaminergic transmission were observed in selected brain areas and some of those alterations were partially recovered by FAAH inhibition. Moreover, we observed that abstinence was able to alter the expression of proteins involved in neuroinflammation and affecting the endocannabinoid system in different brain areas; PF-3845 treatment was able to partially restore these alterations.
CONCLUSIONS: In conclusion, the results obtained from the present study support the role of the endocannabinoid system as a valid pharmacological target for the development of treatments for the neurofunctional alterations related to obesity.

THE ROLE OF LOCAL ETHICS COMMITTEES (EC) DURING THE COVID-19 PANDEMICS: THE EXPERIENCE OF ASL LECCE EC AS A MODEL FOR FUTURE RE-ORGANIZATION SCENARIOS

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BACKGROUND: During the Covid-19 pandemics, the urgent need for coordinating and analyzing scientific evidences has forced the government and AIFA to start up new regulatory procedures for clinical research. Before the pandemics, the reduction of local ECs from 90 to 40 was expected, based on Law n.3/2018 and the EU Reg. 536/2014. This transition may have complex consequences, as
reported by Petrini C, and is still far from being completed. However, the SARS-Cov2 pandemics has already led to a temporary re-organization. All phase I-II-III-IV clinical trials and observational studies on drugs/therapies for Covid-19 are evaluated by only one designated national EC. This also applies for compassionate use programs. In this context, the local ECs can only accept the decisions and evaluate Covid-19 non-pharmacological studies and nominal compassionate use requests. This report aims at evaluating if and how the pandemics impacted on the activities of a local EC.
METHODS: The ASL Lecce EC activities during the period March 2020-June 2020 have been analyzed and compared to the same time-frame in 2017, 2018 and 2019. The Covid-19-related activities have been analyzed in the same period, in terms of number, nature and main features of studies submitted.

RESULTS: In March-June 2020, in an unprecedented period of pandemics, the activities of ASL Lecce EC have not been reduced. In fact, 7 meetings have been held, compared to 5 in 2017 and 2018, and 4 in 2019. This is reflected in the increased amount of evaluations produced. Figure 2 shows the distribution of the 11 Covid-19 proposals. Only one clinical trial has been submitted, promoted by the regional government, testing the treatment with convalescent plasma. In the same period, to our knowledge, of the 35 Covid-19 clinical trials published on AIFA website, only one study has involved the hospitals of the ASL Lecce. Furthermore, no local center has been involved in Covid-19 compassionate use programs.
CONCLUSIONS: The global pandemics and lockdown have forced a sudden re-organization of procedures for clinical research. While one national EC has been identified, the local ECs have started to prepare for what might be the future: real-world studies on clinical practice, studies on biological samples, often locally-promoted studies, sometimes in partnership with the university. This is also one of the possible scenarios depicted by Petrini C in a recent editorial, as an option for giving value to the expertise accumulated by local ECs in decades of activities. As this report shows, for the ASL Lecce EC as a model of local Ecs, these changes have been immediately operational and have not caused a reduction or suspension of activities. However, the peripheral areas may be excluded from the pharmacological research, as it has been for Covid-19 trials, thus a stronger connection between "central" and local ECs need to be guaranteed in the process, for a feasible and equal access for all patients to experimental trials.

THE "RIGHT TO HOPE" ETHICAL DILEMMA: A REPORT OF ASL LECCE'S ETHICS COMMITTEE'S AUTHORIZED COMPASSIONATE USE TREATMENTS IN 2019

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BACKGROUND: Compassionate use programs (CUP) allow the therapeutic use of unauthorized medicines. In Italy, CUPs are regulated by the Ministerial Decree dated 7th September 2017: the use of investigational drugs can be authorized by Ethics Committees (EC) for the treatment of patients with serious diseases, rare diseases or rare tumors, if no clinical trials or treatment alternatives are available. The treating physician assumes the full responsibility for adopting the CUP. The ECs have to preliminarily evaluate the CUP risk/benefit profile in that patient, thus guaranteeing an external independent assessment of both the regulatory requirements and the clinical motivations. However, the decision has to be rapidly taken and the formal checking of documents' compliance may outweigh the actual "ethical" evalua-

tion of the case. This is even more accentuated in case of "emergency procedure" requests. The ASL Lecce EC has recently observed a rise in CUP requests, often via "emergency procedures" and, in order to examine the outcomes and the decision making process, decided to solicit detailed follow-up reports.

METHODS: All CUP procedures authorized in 2019 have been examined, including details about start and end of treatment, reasons for discontinuation, adverse events, survival. This preliminary report is intended to set the basis for internal discussion.

RESULTS: In 2019 the EC examined 27 CUP requests, of which 15 via "emergency procedure", and authorized all of them. The mean time for authorization was 5 days and, for nearly 50% of requests, 3 days. Of all requests, CUP for oncology and hematology malignancies were prevalent (14 and 10, respectively). Nearly 27% of patients never started the treatment, mostly because of death or steady worsening of disease. While for 6 patients the treatment is still ongoing, 13 patients discontinued the treatment. Of these, only one patient had to interrupt the treatment due to a serious adverse event (SAE). Surprisingly, of all patients, only 2 SAEs and 3 AEs (Grade 1, for the same subject) have been reported.

CONCLUSIONS: ASL Lecce's EC authorized all CUPs requested in 2019, based on the documents submitted and the clinical motivations declared. However, preliminary findings highlight the need to re-define the process of decision-making, for both clinicians and EC members. A large quote of patients never started the treatment, because already in poor clinical conditions. The procedure of requesting a CUP in these patients may have provided "the right to hope" but delayed (or denied) the implementation of a palliative care approach. This has also been reported in a recent work by De Panfilis L. for the EC of Reggio Emilia, whose conclusions encouraged a simultaneous right-to-hope/palliative-care approach, that has to be clearly communicated to the patient. Furthermore, the reduced number of AE/SAE reported in 2019 appears unrealistic and urges the EC to recommend a more accurate vigilance of drug-related events.

COLLECTING BIOLOGICAL SAMPLES FOR FUTURE RESEARCH IN CLINICAL TRIALS: TIME TO ALIGN THE CURRENT PROCEDURES FOR EVALUATING INFORMED CONSENT FORMS

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BACKGROUND: The EU Regulation n.536/2014 on clinical research clearly states that the use of biological samples collected during trials may be allowed "for future scientific research". It also sets the rules: the subject must give consent and has the right to withdraw it at any time; furthermore, research projects based on such data have to be reviewed on ethical aspects before being conducted. In Italy, the Law n.3 dated 11th January 2018 envisages simplified procedures for the use of biological or clinical residual material from previous diagnostic or therapeutic activities. In this scenario, the rules set by the General Data Protection Regulation (GDPR) provide specific indications to guarantee the correct and transparent treatment of data. The Ethical Committees (EC) are at the first lines called to judge the ethical and scientific value of such proposals and accurately scrutinize informed consent forms (ICF). This preliminary report aims at providing a picture of the present context from the point of view of a local EC.

METHODS: All clinical trials evaluated by the ASL Lecce EC from June 2018 to June 2020 have been examined, in terms of: presence of biological sample collection for further/future use; number and type of ICF/privacy consent forms; presence of sub-studies; clarity of information provided.

RESULTS: In the period examined, the ASL Lecce EC evaluated 77 clinical trials. Of these trials, 46 (59,7%) required a specific consent for further biological analyses within the study purposes and 32 (41,5%) also requested a consent for future research. Globally, 28 studies proposed one "broad" ICF, while for 18 a specific ICF for optional analyses was provided. Since 2018, the number of studies requiring the patient's consent for further/future research has steadily increased. The procedures for informing the patient and obtaining the consent, though respectful of GDPR prescriptions, may largely vary. According to the EU Reg. 536/2014, local ECs are specifically entitled to judge the nature, clarity and law compliance of ICF.

CONCLUSIONS: The legitimate need of researchers to collect biological samples and genomic data is encouraged by the legislation, and strictly regulated by the GDPR. The ASL Lecce EC has analyzed the changes brought out by the new regulations in the last two years and found an increase in the number of studies requiring a consent for further/future research, though with varying approaches, from one broad consent to 3 or more different ICF. The large diversity of approaches may confound local investigators and patients about their rights, thus local ECs are urged to provide recommendations for aligned procedures. The ASL Lecce

EC suggests the use of a specific optional ICF, allowing the patient to clearly understand that separate part of the study and what it entails. Furthermore, whenever possible, distinct biologic sub-studies should be designed and submitted as related though separate entities from the trial.

EFFECTS OF CHRONIC EXPOSURE TO LOW DOSES OF Δ-9- TETRAHYDROCANNABINOL IN ADOLESCENCE AND ADULTHOOD ON SEROTONIN/ NOREPINEPHRINE NEUROTRANSMISSION AND EMOTIONAL BEHAVIORS

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BACKGROUND: Chronic exposure to the δ9-tetrahydrocannabinol (THC), one of the main cannabis pharmacological component, during adolescence has been shown to be associated with an increased risk of depression and suicidality in humans. However, little is known about the impact of the long-term effects of chronic exposure to low doses of THC in adolescent compared to adult rodents.
METHODS: THC (1mg/kg i.p., once a day) or vehicle was administered for 20 days in both adolescent (post-natal day, PND 30-50) and young adult Sprague-Dawley rats (PND 50-70). After a long washout period (20 days), several behavioral paradigms were carried out. In particular, we performed behavioral paradigms to assess anxiety, the novelty suppressed feeding, the elevated plus maze and the open field test. Despair-like behavior was assessed employing the forced swim test, while anhedonia-like was assessed with the sucrose preference test. Finally, in vivo single unit electrophysiological recordings of serotonin (5-HT) and noradrenaline (NE) neurons were carried out in the dorsal raphe nucleus (DRN) and in the locus coeruleus (LC), respectively.
RESULTS: Adolescent THC exposure resulted in depressive like-behaviors: a significant decrease in latency to first immobility ($P<0.001$) in the forced swim test and in anhedonia-like effect in the sucrose preference test were detected ($P<0.0001$). Any effect was found in the open field test concerning the distance travelled ($P=0.3141$) and in the number of entries in the center ($P=0.4885$). Decrease entries in the open arm ($P<0.001$) were observed in the elevated plus maze after adolescent and adult exposure, indicating mild anxiety-like phenotype. Any significant effect was found in the novelty suppressed feeding test. A significant reduction in dorsal raphe 5-HT neural activity ($P=0.0003$) without changing locus coeruleus NE neural activity ($P=0.2191$) was found in THC adolescent and adult exposure. In addition, the adolescent exposure of THC produced an irregularity in the firing rate in both serotonergic ($P<0.0001$) and noradrenergic activity ($P<0.0001$).
CONCLUSIONS: Altogether, these findings suggest that low doses of chronic THC exposure during the developmental period and adulthood could result in increased vulnerability of the 5-HT system and anxiety-like symptoms;

however, depressive-like phenotypes occur only after adolescent, but not adult exposure, underscoring the higher vulnerability of young ages to the mental effects of cannabis.

LONG-TERM PHARMACOKINETICS OF DALBAVANCIN IN PLASMA FROM PATIENTS WITH GRAM-POSITIVE INFECTION

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BACKGROUND: Dalbavancin is a potent lipoglycopeptide active against Gram+ bacteria with a very long halflife and excellent tolerability, indicated for numerous difficult to treat conditions. Dalbavancin has been approved for Gram+ infections of soft tissues in a single intravenous infusion (30 minutes), but its range of application and the relative posology is still debated. Nowadays little is known about its pharmacokinetics (PK) and PK/pharmacodynamic (PK/PD) profile in the real-life.
MATERIALS/METHODS: Two groups of patients who received Dalbavancin have been enrolled, receiving respectively one or two doses (1500 mg vs 2 x 1500 mg doses within two weeks), respectively. Given informed consent, plasma Dalbavancin concentrations were measured through a UHPLC-MS/MS analytical Kit (CoQuaLab) at the end of infusion, after one hour and then weekly. AUCs and half-lives (t1/2) were calculated through Phoenix WinNonLin ® (Certara) software in the two groups. Terminal t1/2 and AUCs have been calculated for the patients who had at least 8 weeks (w) of PK follow-up after last intake. Then, AUC/MIC and the estimated T>MIC (considering a logarithmic model based on the terminal t1/2 and the concentrations at 2 weeks after the last infusion) were calculated according to the maximum reported MIC for susceptible microorganisms (0.125 mg/L).
RESULTS: Ten patients were enrolled: age 60 (IQR 52 - 74), 4 with soft-tissues and 6 with osteoarticular infections. Six patients received 1 single dose and 4 patients received 2 doses. After the first dose, the mean Cmax was 355.7 mg/L (SD 84.3 mg/L), while at the second dose it increased to 376.1mg/L (SD 26.1 mg/L). Dalbavancin concentrations showed multifasic PK, with a relatively fast distribution t1/2 in the first hour after the infusion (3.9 h, SD 1.5 h), an intermediate t1/2 within the first 2 w (117.4 h, SD 49.7 h) and, finally, an extremely long terminal t1/2 (554.3 h, SD 223.1 h). Mean observed AUC for the patients who had one single dose was 52670 mg/L*h (SD 20070 mg/L*h) while it was 134413 mg/L*h (SD 7454 mg/L*h) in the double dose group. Mean AUC/MIC were as high as 4.5x105 and 1.1x106 in the single and double dose groups, respectively. Estimated T>MIC was 30.2 w (SD 14.2 w) in the single dose group and 34.3 w (SD 11.2 w) in the double dose group. There were no adverse events nor discontinuation. Nine (90%) had favorable outcome.
CONCLUSIONS: This is among the first evaluations of Dalbavancin PK profile in a real-life clinical setting. By a theoretical PK/PD perspective, our data support a potential longer antibiotic activity than previously considered both for single or double 1500 mg dose administration, with estimated T>MIC ranging

from 4 to 11 months. Moreover, the evidence of an extremely long terminal halflife underlies a high penetration and permanence time within the tissues, increasing the interest for studying intracellular and intratissue disposition of this drug.

DONOR *CYP3A5* GENOTYPE AND GRAFT-TO-RECIPIENT WEIGHT RATIO AS PREDICTORS OF TACROLIMUS PHARMACOKINETICS IN PEDIATRIC LIVER TRANSPLANT PATIENTS

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BACKGROUND: To date, immunosuppressive treatment with tacrolimus (TAC) is widely used for adults as well as paediatric patients who received liver transplantation. TAC concentrations show a wide inter-patient variability, so the therapeutic drug monitoring (TDM) is considered pivotal for guiding posological adjustments, particularly within two weeks after transplantation. Nevertheless, these posological adjustments need strict daily monitoring and, most often, the optimal dose (and concentration) is reached only after several days. This problem is particularly important in the paediatric context, since patients and graft characteristics show a relatively wider variability, starting from the ratio between graft size and patients weight (Graft-to-Recipient Weight Ratio, GRWR). In this scenario, deepening the knowledge of the factors affecting TAC pharmacokinetics could help to optimize the starting dose, reducing the need for continuous dose adjustments on treatment and, potentially, further reducing the risk of acute rejection. Therefore, this work aimed at describing TAC concentrations in paediatric patients during the first two weeks after transplantation and studying their correlation with patients and donors characteristics.
METHODS: Paediatric patients treated with tacrolimus after liver transplantation have been prospectively enrolled in this study and underwent daily TDM of TAC concentrations in whole blood during the first 15 days of treatment, as required by the guidelines for immunosuppressive treatment, through a UPLC-MS/MS method. Patients polymorphisms on CYP3A4, CYP3A5 and ABCB1 genes were tested by real-time PCR. Patients and graft characteristics were then tested for correlation and predictivity for TAC concentrations by using SPSS software.
RESULTS: Tacrolimus concentrations normalized by dose resulted strictly associated with donor CYP3A5 genotype and, strikingly, with GRWR. By multivariate analysis, these variables resulted the best independent predictors of the final required dose of TAC and, moreover, a GRWR higher than 4% was associated with a higher risk of acute rejection.

CONCLUSIONS: The significant predictive effect of donor CYP3A5 genotype and GRWR on TAC concentrations in the first weeks of treatment indicates graft catabolic capability as the most important determinant of tacrolimus pharmacokinetics in the paediatric context. Anyway, further larger prospective studies should be conducted for the evaluation of the possible beneficial effect of a "graft-focused" starting dose in reducing the onset of acute graft rejection.

SAFETY PROFILE OF BIOLOGICS IN RHEUMATOLOGIC DISEASES IN A REAL WORLD SETTING IN CALABRIA REGION

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BACKGROUND: Biologic drugs have led to significant progresses in the treatment of several inflammatory pathologies. Despite their increasing use and number in clinical practice, the evidence on their long-term safety (adverse drug reactions events [ADRs]) is, however, relatively scarce. The following active pharmacovigilance study aims to evaluate biologic drugs use in naïve and nonresponder patients to previous treatments and therapeutic switch strategies as well as to report occurrence of ADRs .
METHODS: A retrospective analysis of patients records with rheumatological diseases naïve to biologics in the period 2013-2015 was carried out, to monitor therapy discontinuation/modification, ADRs and switches. The observation period began from the first day of administration until the therapeutic failure (dropout), or the date closest to the end of the first observation period (36 months). All patients of the first cohort who underwent a therapeutic switch to another biologic drug were further monitored for 12 months (second cohort). All ADRs have been coded according to the MedDRA dictionary version 20.
RESULTS: Overall, the sample consisted of 97 patients with an average age of 58.8±11.4 years, 66 (68.0%) women and most affected by psoriatic arthritis 40 (41.2%), with an average period from diagnosis of disease of 10.7±5.2 years. The most prescribed drug was etanercept (34%) followed by adalimumab (30.9%), infliximab (14.4%), abatacept (5.2%), belimumab (4.1%), certolizumab, golimumab and ustekinumab (3.1%), rituximab (1.1%) and finally tocilizumab (1%). By assessing long-term efficacy (36 months), 52 patients (48.5%) discontinued treatment with consequent switch. Switches were reported mostly for adalimumab (14; 46.7%), etanercept (20; 60.6%) and infliximab (7; 50.0%). Regarding safety, 23 ADRs have been reported by 14 patients, mostly due to etanercept treatment. The most frequent identified ADRs were infections and infestations. In the second cohort (followed 12 months), several patients also discontinued the second biologic drug, in particular: 7 (50%) with adalimumab, 7 (63.6%) with etanercept, 2 (20%) with golimumab, 2 (66.7%) with infliximab, 1 (50.0%) with rituximab and 1 (50.0%) with ustekinumab. Nine ADRs were reported by 6 patients treated with adalimumab (3) with rituximab (2) and one with secukinumab; in particular 1 case of alopecia by rituximab, 3 case of infections by adalimumab and 1 by rituximab, finally, 2 case of rhinitis by adalimumab and 1 by rituximab and 1 case of dyspnoea by rituximab.
CONCLUSIONS: Considering the low rate of ADRs reported and discontinuation from therapy, our data seems to confirm a good safety

profile of biologics. The reported ADRs have been generally mild to moderate and mostly related to infections. However, further studies are needed to assess longterm safety of biologics.

ADVANCED OR METASTATIC NON-SMALL-CELL LUNG CANCER (NSCLC): CLINICAL AUDIT ABOUT TREATMENTS OF FIRST-LINE AND SECOND-LINE THERAPIES

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BACKGROUND: NSCLC has been profoundly changed over the last few years with the development of new tyrosine kinase inhibitors and the introduction of immunotherapy. For the purpose of assessing appropriateness of first-line(1L)and second-line(2L) treatments of patients(pt)with NSCLC, with squamous or non-squamous histology, in 2019 a clinical audit was carried out in S. Orsola-Malpighi University Hospital-Bologna that has seen the cooperation of pharmacists, clinicians and Clinical Governance's representatives. The clinical audit's aim was to evaluate the accession in the medical practice to the standards(std) defined at the regional and national levels,and the achievement of the objectives of the company budgets expected from Management for 2019.
METHODS: Criteria, indicators (ind) and reference std were defined relying on AIFA's indications, GreFO's (Regional Group on Cancer drugs) recommendations and based on the relative algorithm with indications on the use of medications under evaluation: immunotherapy(nivolumab, pembrolizumab and atezolizumab),EGFR inhibitors(erlotinib, gefitinib, afatinib, osimertinib), ALK inhibitors(Alectinib),ROS1 inhibitors(Crizotinib), chemiotherapy based on platinum, pemetrexed, docetaxel and nintedanib. It was evaluated pt with NSCLC that started the treatment in 2019. For histology, lines, treatment setting,EGFR-ALK-ROS1 mutational status, PS and PD-L1 were consulted different sources: drug distribution program for oral drugs, Log80® program for the management of infusion therapies, AIFA's Register and patients'medical records.
RESULTS: Compared to the 11 established ind, 170 pt have been analyzed of which 130 pt treated in 1L and 58 pt treated in 2L. Non mutated pt candidates for 1L were treated with pembrolizumab n°31(100%,PS0-1 e PD-L1>=50%) while the pt EGFR mutated were treated with osimertinib n°12(57,1%),gefitinib n°8(38,1%), afatinib n°1(4,8%),the 3 pt ALK mutated with alectinib (100%) and 1 pt ROS1 rearranged with carboplatin-pemetrexed(100%) not treated with crizotinib for QT interval lengthening. For the 2L,non mutated (n°48)were treated with immunotherapy n°41(91,1%)and in a very small part with docetexel-nintedanib n°2(4,3%);7 pt EGFR-T790M mutated were treated with osimertinib n°7(100%).The opportunity cost was calculated for the 2L immunotherapy in which atezolizumab was the cheapest; in line with that, it was the most prescribed (61.9%).
CONCLUSIONS: The clinical audit highlighted a complete accession to the indications

offered by GReFO. For all the ind, the target was considered achieved, except for 2 (ind. 10 and ind. 11) that couldn't be determined due to the absence of pt. There is to report that until November 2019, Osimertinib used in the 1L was a therapeutic option on CNN (non-negotiated class C), so it couldn't be considered a totally available therapeutic alternative, this affected the final result, determining a percentage of use lower than the expected, consequently there is increased use of gefitinib and afatinib. It is essential to underline that, even though the population monitored was numerically important(170 pt),the indicators were calculated on particular subgroups of pt; for some ind this determines a small sample population. Overall, in this clinical audit, we confirm that in our hospital the accession to the reference std in the treatment of NSCLC has been appropriate.

PROTECTIVE EFFECT OF THE ADENOSINE A2B RECEPTOR AGONIST BAY60-6583 ON CEREBRAL DAMAGE INDUCED BY TRANSIENT FOCAL CEREBRAL ISCHEMIA IN THE RAT

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BACKGROUND: Cerebral ischemia is a multifactorial pathology characterized by different events evolving in time. The acute injury, characterized by a massive increase of extracellular glutamate levels, is followed by a secondary brain injury that develops from ischemia. During ischemia adenosine increases extracellularly and acting on its receptors (A1, A2A, A2B and A3) exerts an important role. Few studies have investigated the role of adenosine A2B receptor in brain ischemia because of the few selective ligands developed so far. A2B receptors are scarcely but widely distributed in the brain on neurons, glial and endothelial cells and on hematopoietic cells, lymphocytes and neutrophils, where they exert mainly anti-inflammatory effects, inhibiting vascular adhesion and inflammatory cells migration. Aim of our study was to assess the putative neuroprotective effects of the adenosine A2B receptor agonist, BAY60-6583 (BAY), chronically administered in a model of transient focal cerebral ischemia in the rat.
METHODS: Wistar rats weighting 270-290 g, were used. Transient cerebral ischemia was induced by 1 h occlusion of Middle Cerebral Artery (tMCAo) by the monofilament technique. BAY was administered starting 4 h after ischemia according to a chronic protocol (0.1 mg/kg, i.p., twice/day for 7 days). Neurological deficit was evaluated by the modified Neurological Severity Score (mNSS) test 1, 5 and 7 days after tMCAo. Seven days after tMCAo, the ischemic brain damage by cresyl violet and Hematoxylin and Eosin (H&E) staining was evaluated. Three confocal acquisitions of immunohistochemical analysis by using antibodies anti-NenN for neurons, anti-IBA1 for microglia and anti-GFAP for

astrocytes were performed and inflammatory cytokines were measured by ELISA kit in the plasma. Two days after tMCAo, the blood cell infiltration in ischemic areas was evaluated by using anti-HIS-48 antibodies for granulocytes.
RESULTS: Chronic treatment with BAY significantly improved neurological deficit up to 7 days after tMCAo (*P<0.02). Seven days after ischemia, BAY has significantly reduced the volume of ischemic brain damage in cortex and striatum (**P<0.001, *P<0.05), has reestablished the tissue cytoarchitecture, has counteracted ischemia-induced neuronal death (*P<0.05) and has reduced microglia and astrocyte activation. Moreover, 7 days after ischemia, BAY decreased the expression of the pro-inflammatory cytokine TNF-α and increased the IL-10, an anti-inflammatory cytokine in peripheral plasma (*P<0.05). Two days after ischemia, BAY60-6583 reduced blood cell infiltration in the ischemic cortex (**P<0.004).
CONCLUSIONS: Results demonstrate that stimulation of adenosine A2B receptor located on brain cells, on vascular endothelial cells and on blood cells attenuate the neuroinflammation that develops after ischemia.

USE OF HYDROXYCHLOROQUINE IN COVID-19 PATIENTS IS ASSOCIATED WITH REDUCED MORTALITY: FINDINGS FROM THE OBSERVATIONAL MULTICENTRE ITALIAN CORIST STUDY

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BACKGROUND: Hydroxychloroquine (HCQ) might inhibit the intracellular glycosylation of angiotensin converting enzyme 2, the receptor used by the SARS-CoV-2 virus to enter the cells, resulting in a reduced ligand recognition and internalization of the virus and exerting a possible protective role in SARS-CoV-2 infection. Moreover, due to its immunomodulatory, anti-inflammatory and anti- thrombotic effects, HCQ could also modulate the severity of the disease. HCQ was thus proposed as a potential treatment for COVID-19. We set-up a multicenter Italian collaboration to investigate the relationship between HCQ therapy and COVID-19 in-hospital mortality.
METHODS: This national retrospective observational study was conceived, coordinated and analysed within the CORIST Project (ClinicalTrials.gov ID: NCT04318418); 3,451 unselected patients hospitalized in 33 clinical centers distributed throughout Italy from February 19, 2020 to May 23, 2020, with laboratory-confirmed SARS-CoV-2 infection, were included. The primary end- point in a time-to event analysis was in-hospital death, comparing patients who received HCQ with patients who did not. We used multivariable Cox proportional-hazards regression models with inverse probability for treatment weighting by propensity scores, with the addition of subgroup analyses.
RESULTS: Out of 3,451 COVID-19 patients, 76.3% received HCQ. Death rates for patients

receiving or not HCQ were 8.9 and 15.7 per 1,000 person-days, respectively. After adjustment for propensity scores, we found 30% lower risk of death in patients receiving HCQ (HR=0.70; 95%CI: 0.59 to 0.84; E-value=1.67). Secondary analyses yielded similar results (Table). The inverse association of HCQ with inpatient mortality was particularly evident in patients having elevated C-reactive protein (CRP) at entry: HR=0.59, 95%CI: 0.47 to 0.73 in patients with CRP ≥10 mg/L and HR=1.23, 95%CI: 0.86 to 1.77 in patients with CRP <10 mg/L.

CONCLUSIONS: Our study, including a large real-life sample of patients hospitalized with COVID-19 all over Italy, shows that HCQ use (200 mg twice daily) was associated with a 30% reduction of overall in-hospital mortality. In the absence of clear-cut results from controlled, randomized clinical trials, our data do not discourage the use of HCQ in inpatients with COVID-19. Given the observational design of our study, however, these results should be transferred with caution to clinical practice.

POLYPHARMACY IN A PEDIATRIC AUTOIMMUNE HEPATITIS COHORT: THE FEDERICO II TEACHING HOSPITAL EXPERIENCE

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BACKGROUND: Autoimmune hepatitis (AIH) is a chronic condition that frequently requires a polypharmacological treatment in order to avoid liver damage. Pediatric polypharmacy is defined as the prescription or consumption of two or more distinct medications for at least one day. Patients with AIH are subjects at high risk of polypharmacy because liver disease requires immunosuppression, vitamin supplementation in case of cholestasis and treatment of immunosuppression- related side effects. Nonetheless, very few data are available on prevalence and severity of polypharmacy in pediatric patients with AIH.

METHODS: We reviewed the pharmacotherapy of AIH patients in regular follow-up (FU) at the Federico II teaching hospital till December 2019 in order to search for drug-drug interactions (DDIs). Polypharmacy was evaluated at the last observation. Vitamin supplements were given if a deficit was confirmed by blood tests and stopped when the deficit was corrected; as a consequence we considered them as drugs. We used both the checkers provided by IRCCS Mario Negri (Intercheckweb) and Medscape, which give different and additional information. These checkers classify DDIs in 4 classes based on their clinical relevance and on the strength of supporting clinical evidence: red (high risk with strong evidence), orange (moderate risk with good evidence), yellow (moderate risk with limited evidence) and green (low risk with poor evidence).

RESULTS: 19 (5 males) AIH patients were analyzed. Mean age (years) & standard deviation (SD) at diagnosis were 8.58 & 5.17; mean age (years) & SD at the last FU were 13.89 & 4.47. In the overall population, 17 patients

(89.47%) took 2 drugs, 16 (84.21%) 3 drugs, and 9 (47.37%) 5 drugs. Mean drug number & SD at the last FU were respectively 4.37 & 2.39. The most frequent drugs were: glucocorticoids (21.69%); ursodeoxycholic acid (18.07%); proton pump inhibitors (15.66%); azathioprine (13.25%) (in Table 1 the detailed list of the observed drugs). Mean DDIs according to Intercheckweb & Medscape are 0.05 & 0.26 respectively; SD of DDIs according to Intercheckweb & Medscape are 0.23 & 0.65 respectively. Almost all the observed DDIs were to monitor or of unknown significance (Table 2).

CONCLUSIONS: Although the small sample might limit the reliability of the analyses, the pharmacological therapy in AIH patients is per se significant with almost 90% of them reaching the pediatric polypharmacy cut-off. Despite the heavy drug burden, few DDIs were observed with the most part of unknown clinical meaning. Nevertheless, each drug has to be chosen wisely, because some of them such as azathioprine and proton pump inhibitors can create interactions with significant clinical implications.

PHYTOCHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF A COMMERCIAL *HARPAGOPHYTUM PROCUMBENS* (BURCH.) DC. EX MEISN. EXTRACT

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BACKGROUND: *Harpagophytum procumbens* (Burch.) DC. ex Meisn. is a medicinal plant used worldwide as a remedy for joint pain associated with osteoarthritis and rheumatic ailments. Iridoid glucosides are considered the bioactive constituents, although further unknown phytochemicals seem to be involved in its bioactivities. In the present study, a commercially available extract from *H. procumbens* root, characterized to contain 1% harpagoside, has been evaluated for its phytochemical composition and biological activities, in order to identify novel bioactive compounds; particularly, its ability to modulate different targets of endocannabinoid system, including CB2 receptor and FAAH enzyme, was studied.
METHODS: In order to better recover the bioactive phytochemicals, the crude extract was dissolved in different solvents, including dimethyl sulfoxide (DMSO), ethanol (EtOH) 100% v/v, EtOH 50% v/v and deionized water, and any insoluble residue was removed by centrifugation. Total polyphenols, tannins and flavonoids were spectrophotometrically determined, while the volatile compounds were identified by GC-MS analysis. A preliminary cytotoxicity assay in human primary synoviocytes was performed; then, nontoxic concen-

trations of the extracts were assessed for the ability to affect endocannabinoid system. The effects of the treatments on protein and gene expression of the cannabinoid receptors were determined by immunofluorescence and quantitative-real time-PCR. Furthermore, the ability of the extracts to inhibit the fatty acid amide hydrolase (FAAH) was evaluated using a fluorescence-based kit.

RESULTS: DMSO fully dissolved the crude extract, whereas insoluble residues were produced with the other solvents; moreover, the solvent allowed to recover the highest levels of total polyphenols, tannins and flavonoids, followed by EtOH 100% v/v, EtOH 50% v/v and H_2O. GC-MS highlighted the presence of different terpenoids, among which β-caryophyllene and eugenol. The extracts induced a significant increase in the CB2 mRNA expression and protein levels in synoviocytes. Moreover, they inhibited FAAH activity, although with a different potency. Under the same experimental conditions, harpagoside lacked FAAH inhibition, despite a strong effect of the positive control JZL 195. On the basis of this evidence, a modulation of the endocannabinoid system seems to be involved in the bioactivities of the tested extracts. Moreover, our results highlight the presence of different terpenoids along with polyphenols in *H. procumbens* samples. Among the identified constituents, β-caryophyllene is known to induce CB2-mediated antinflammatory effects, thus suggesting its possible contribution to phytocomplex bioactivity.

CONCLUSIONS: The present results highlight, for the first time, the involvement of an endocannabinoid system modulation in the antinflammatory and analgesic effects of *H. procumbens* and improve the mechanistic knowledge in support of its use in arthritic diseases.

IMPACT OF CHRONIC PSYCHOSOCIAL STRESS ON INSULIN PATHWAY AND HEPATIC REDOX STATE: AN IN VIVO STUDY

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BACKGROUND: Several lines of evidence have reported that chronic psychosocial stress plays a key role in the onset and progression of psychosis, and that stress-induced redox imbalance may contribute to the pathophysiology of glycemic and lipidic dysfunctions observed in this psychiatric condition. We previously demonstrated central and peripheral redox dysregulation, as well as metabolic dysfunctions, in the rat model of social isolation, a non-pharmacological model of inducing long-term alterations resembling human psychosis. Here, by using this animal model, we investigated the impact of chronic psychosocial stress on insulin pathway and redox state in the liver.

METHODS: Adult male and female Wistar rats were mated to obtain litters. After weaning, male pups were isolated or grouped for seven weeks. The glucose tolerance test (GTT) was used to assess the glucose tolerance of rats at the end of isolation period. Peripheral insulin sensitivity was assessed by the insulin tolerance test (ITT), 48h after the GTT. Hence rats were euthanized, livers and plasma were

collected. In the liver, the insulin receptor, glucose transporter 2, carnitine palmitoyltransferase 1 and superoxide dismutase proteins were quantified by Western Blotting analysis. The reactive oxygen species ROS were measured by using the fluorogenic dye 2', 7' dichlorofluorescein diacetate. The concentration of malondialdehyde (MDA) was evaluated by commercially available assay kit. Plasmatic insulin levels were assessed by commercially available ELISA kit. The homeostatic model assessment (HOMA) was used to quantify insulin resistance and beta-cell function The HOMA index was calculated according to the formula (glycemia x insulin)/22.5. Data were analyzed by using GraphPad 5.0 software for Windows. T-test and Two-away ANOVA for repeated measures were carried out, P-value was set at 0.05.

RESULTS: Whereas isolated rats were euglycemic compared to controls, enhanced insulin levels and HOMA index were detected following seven weeks of social isolation. This was accompanied by a decrease in the expression, at protein level, of insulin receptor, glucose transporter 2 and carnitine palmitoyltransferase 1 in the liver of isolated rats, where we also found elevations in hepatic reactive oxygen species production and lipid peroxidation, as well as decreased expression of antioxidant enzymes, including superoxide dismutase.

CONCLUSIONS: Our results provide new insights in the molecular mechanisms linking psychosocial stress, redox imbalance and metabolic dysfunctions, opening novel therapeutic perspectives for the treatment of peripheral alterations associated with stress-induced mental disorders.

PRO-CALCIFIC DIFFERENTIATION OF VALVE INTERSTITIAL CELLS IS PREVENTED BY ACETYLSALICYLIC ACID THROUGH GENERATION OF ANTI-INFLAMMATORY LIPOXINS

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BACKGROUND: Calcific aortic valve disease (CAVD) is the third cause of cardiovascular disease in the Western world and its prevalence is expected to increase due to ageing of population. To date, there is no effective pharmacological therapy to halt or delay CAVD progression and the only therapeutic option remains the replacement of the aortic valve. Our purpose is to study the effects of acetylsalicylic acid (ASA, aspirin) and anti-inflammatory lipoxins (aspirin-triggered lipoxins, ATL) on the process of pro-calcific differentiation of valve interstitial cells (VICs), in order to develop novel therapeutic strategies for CAVD treatment.

METHODS: A clone of VICs able to acquire a pro-calcific phenotype was treated with endotoxin (LPS, 500 ng/ml) to induce calcification, with ASA at two different concentrations (1 mM and 10 mM) and MMK1 (50 μM). After 12 days of treatment, alkaline phosphatase (ALP) activity and calcium deposition were determined through colorimetric assays. Proteins and RNA were extracted from VICs to perform western blotting (WB) and RT-PCR analyses. A RP-HPLC analysis was conducted on the culture medium to assess the production of arachidonic acid

metabolites and ATL (such as PGE2, 15-HETE and 15-epi-LXA4). Lastly, immunohistochemical analysis was performed on pathological and healthy valves to investigate the expression of FPR2, the main ATL receptor.
RESULTS: Treatment of VICs with ASA reduced expression of pro-inflammatory cytokines (IL-6, TNF-α and IL-1β , $p<0.001$), ALP activity ($p<0.001$) and calcium deposition ($p<0.05$) induced by LPS. Moreover, cells treated with ASA showed an increased production of ATL, such as 15-HETE and 15-epi-LXA4 ($p<0.001$). We also observed that FPR2 was expressed within human pathological valves and was increased in VICs treated with LPS. Finally, in vitro studies showed a significant reduction of ALP activity in VICs treated with LPS and concomitant MMK1, a selective agonist for FPR2.
CONCLUSIONS: Treatment with ASA prevents inflammatory activation and pro-calcific differentiation of VICs. The anti-calcific effect of ASA might be partly due to the production of ATL and the consequent activation of FPR2. These promising findings can offer the opportunity to develop novel pharmacological therapies for CAVD.

PPARγ DRIVES IL-33-DEPENDENT ILC2 PRO-TUMORAL FUNCTIONS

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BACKGROUND: Innate Lymphoid Cells (ILCs) are the most recently identified family of innate immune cells, that are emerging as potent orchestrators of immune response. In particular, ILC2s have been reported to play a critical role in disparate inflammatory diseases including asthma, chronic rhinosinusitis and allergic rhinitis. Moreover, we and others have recently reported dominant pro-tumoral functions of ILC2 in cancer. Nonetheless, regulatory factors that dictate ILC2 activation and function remain poorly studied. Peroxisome proliferator-activated receptor gamma (PPARγ) regulates the transcription of genes associated with lipid metabolism and is expressed in different immune cells, including lymphocytes, monocytes, dendritic cells and platelets where it mainly exerts anti-inflammatory effects. Here, we characterize the expression and functional role of PPARγ in human and mouse ILCs to assess whether PPARγ can be targeted in the context of an ILC-directed immunotherapy.
METHODS: mRNA-sequencing was performed on freshly-sorted ILCs from healthy donors (HDs) peripheral blood mononuclear cells (PBMCs). PPARγ expression was confirmed by qPCR and western blot analysis in both human and mouse ILCs. PBMCs and lymph nodes from colorectal cancer (CRC) patients were used to analyze ILC frequencies, phenotype and cytokine secretion by flow cytometry assay.
RESULTS: We found that PPARγ is selectively expressed in ILC2s in humans and in mice, acting as a central functional regulator. Pharmacologic inhibition or genetic deletion of PPARγ in ILC2s significantly impaired IL-33-induced Type-2 cytokine production and mitochondrial fitness. Further, PPARγ blockade in ILC2s disrupted their pro-tumoral effect induced by IL-33-secreting cancer cells. Lastly, genetic

ablation of PPARγ in ILC2s significantly suppressed tumor growth in vivo.
CONCLUSIONS: Our findings highlight a crucial role for PPARγ in supporting the IL-33 dependent pro-tumorigenic role of ILC2s and suggest that PPARγ can be considered as a new druggable pathway in ILC2s to inhibit their effector functions. Hence, PPARγ targeting might be exploited in cancer immunotherapy and in other ILC2-driven mediated disorders.

EFFECTIVENESS AND SAFETY BETWEEN RACEMIC METHADONE AND LEVOMETHADONE IN OPIOID USE DISORDER

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BACKGROUND: The prescription of opioids for the treatment of chronic non cancer pain (CNCP) is constantly increasing. Methadone (a mixture of levo and dextromethadone) is used as substitution therapy in heroin use disorder as well as in any opioid use disorder. Long-lasting therapy with this drug is often burdened by side effects, mostly caused by dextromethadone. Levomethadone should guarantee an important reduction of side effects and a better pain relief.
METHODS: We led an observational prospective monocentric study on patients attending the outpatient clinic of the Toxicology Unit of Careggi University Hospital who were in therapy with methadone (>5 mg/day) or other opioid drugs. Patients with CNCP were evaluated with pain assessment scales, meanwhile patients with heroin use disorder were evaluated with Clinical Opiate Withdrawal Scale (COWS). Side effects such as constipation, sedation and nausea were studied based on patients' reports. Statistical analysis was assessed by Excel software (mean, standard deviation, standard error, t-test, p-value< 0.05).
RESULTS: We recruited 17 patients (13 women, 76.5% and 4 men, 23.5%), with a mean age of 55.2±7.8 years. Twelve patients (70,6%) suffered from headache (75% migraine, 16.7% muscletension headache and 8.3% cluster headache), 17.6% presented chronic neuropathic pain and the rest had a previous history of heroin use disorder in chronic therapy with methadone. Psychiatric comorbidity was present in 14 patients (82.4%). These patients were treated with SSRI (35.7%), SNRI (35.7%), trazodone (14.3%), benzodiazepines (50%), tricyclic antidepressants (21.4%), NaSSa (7.1%) and atypical antipsychotics (7.1%). In patients with chronic pain, VAS decreased significantly from 4.8±0.4 to 3.3±0.6. Most of the items of the Unified Pain Measurement Scale showed a statistically significant reduction of the mean value after switch to levomethadone therapy. Patients with a previous diagnosis of heroin use disorder didn't show any signs or symptoms of opioid acute withdrawal. Weekly headache attacks reduced from 7.1±1.2 per week with methadone to 3.5±0.6 with levomethadone. Methadone adverse effects such as nausea and constipation disappeared with levomethadone. Finally, number of pills per month was

significantly reduced (54.4±11.7 on racemic methadone vs 26±7.7 on levomethadone).
CONCLUSIONS: Levomethadone showed to be equivalent compared to methadone in the management of CNCP. Moreover, levomethadone showed a lower incidence of adverse drug effects as well as decreasing number of symptomatic drugs per month. Therefore, levomethadone could be considered as a promising therapeutic option in patients affected by any opioid used disorder and/or dealing with methadone adverse drug effects.

INVESTIGATING GLIAL CELLS AFTER AN ACUTE STRESS: EVIDENCE FROM A PRECLINICAL MODEL

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BACKGROUND: Stress represents one of the major risk factors for the development of neuropsychiatric disorders. Studies so far have been focused much more on the behavioral and molecular consequences of chronic stress exposure than of acute stress. However, even a single traumatic event could have a dramatic impact on people's life. The different ability to cope with stress is determined by resilience and vulnerability factors, not yet fully understood. Recent evidence show that acute stress exposure could alter the brain's neuroarchitecture in specific areas, including the frontal regions. Rats exposed to an acute stress show reduced dendritic length in the prelimbic cortex (PrL), a subregion of the frontal cortex. Few studies have been carried looking at the possible impact of acute stress exposure on glial cells, despite growing evidence demonstrates their major role on brain homeostasis. We therefore focused our study on that, looking also at the possible beneficial effect of an acute ketamine treatment. Indeed, the non-competitive NMDA receptor antagonist ketamine induces a rapid and sustained antidepressant response at sub-anesthetic doses, thus representing a new approach for reducing post-trauma symptoms within hours after treatment.
METHODS: Male Sprague-Dawley rats were repeatedly exposed to a footshock stress for 40 minutes and divided into resilient and vulnerable animals based on their anhedonic behavior following the stress procedure, then sacrificed 24 h later. To mimic a pharmacological treatment following a traumatic event, a second cohort was challenged with ketamine (0, 10 mg/kg, i.p.) 48 h after the exposure to the footshock stress. Gene expression of markers of glial cell morphology and functions as well as of neuroinflammation was studied in the PrL of both cohorts of rats.
RESULTS: Data reveal microglial activation 24 h after stress in the PrL of vulnerable rats compared with resilient animals. Vulnerable rats show also an upregulated IL-1β mRNA and downregulated BDNF mRNA compared with control rats. Data from the brains collected 48

h after stress exposure show a difference between resilient and vulnerable rats in the gene expression of S100B, a neurotrophin released by astrocytes with neurotoxic effects at high concentration. Indeed, we detected higher level of S100B mRNA in vulnerable rats than resilient ones, that was not affected by the acute ketamine treatment.

CONCLUSIONS: Our results suggest that alteration of glial cells functions could be involved in the resilient or vulnerable response to acute stress. Interestingly, our data propose that microglia could be activated right after an acute stressful event, while astrocytes could have a role at later time points. However, further studies are needed to demonstrate it.

POLYMORPHISMS IN DOPAMINERGIC AND SEROTONINERGIC RECEPTOR GENES AND CARIPRAZINE CLINICAL EFFECTS: A PROSPECTIVE STUDY IN SCHIZOPHRENIC PATIENTS

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BACKGROUND: Cariprazine (CAR) is a novel neuroleptic recently approved for schizophrenia treatment in adults. CAR acts as partial agonist on dopamine receptors (DR) D2 and DRD 3, with a higher affinity for DRD3. In addition CAR has peculiar antagonist activities on serotonin receptor (5-HTR) 1A and 5-HTR2A and adrenergic receptor (AR) alpha 1B (*Mazza M, et al. CNS Neurol Disord Drug Targets. 2018; 17:723-727*). Both DR and 5-HTR genes are characterized by the presence of several genetic polymorphisms which may affect their expression and function and evidence exists about their relevance for clinical response to antipsychotic/neuroleptic medication in schizophrenic patients (*Meltzer HY. CNS Neurol Disord Drug Targets. 2017; 16:900-906; Naumovska Z, et al. Pril (Makedon Akad Nauk Umet Odd Med Nauki)*. 2015; 36:53-67). However, no information exists, so far, about their possible influence on the therapeutic efficacy and/or on the side effects of CAR. The aim of the present study is therefore to assess the role of genetic variants in DR and 5-HTR genes in the response to CAR.

METHODS: This is a genetic, prospective, pilot, research study in which 250 schizophrenic patients diagnosed according to DSM5 diagnostic criteria who start therapy with CAR are consecutively recruited over a period of 36 months. The psychometric scales used to evaluate CAR clinical efficacy and side effects are: Collected Clinical data included age at onset, Positive and Negative Symptoms of Schizophrenia (PANSS), Brief Assessment of Cognition in Schizophrenia (BACS), Brief Psychiatric Rating Scale (BPRS), Clinical Global Impression (CGI), Young Mania Rating Scale (YMRS), Beck Depression Inventory Scale. Single Nucleotide Polymorphism (SNPs) in DRD2, DRD3, HTR1A and HTR2A genes are evaluated by Real Time PCR using Taq-Man probe. CAR plasma levels are also assessed by means a liquid chromatography-mass spectrometry technique with a lower limit of quantification of 0.02 ng/mL.

RESULTS: So far, we have enrolled 32 patients (14 women, mean age at onset 26±7 years). At the time of this submission, patients recruit-

ment and genotyping are still ongoing. An interim analysis of study data, including correlations with CAR response and side effects will be performed in the near future.
CONCLUSIONS: Our study points at providing reliable markers for evaluation of the clinical response to CAR. This approach will eventually result in a more precise tailoring of pharmacological regimen, with a view towards personalized medicine, which, in addition to benefits for patient, would reduce the management costs of the therapy.

EFFECTS OF ACETYLCHOLINESTERASE INHIBITORS ON BRAIN ENERGY METABOLISM OF NON-SYNAPTIC AND SYNAPTIC MITOCHONDRIA

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BACKGROUND: Degeneration of cholinergic neurons is one of the physiopathological hallmarks of primary Dementias. Improvement of cholinergic neurotransmission through inhibition of Acetylcholine catabolism is a valid approach to slow down disease progression, and Acetylcholinesterase Inhibitors (AChEI) are commonly used symptomatic treatment. Physostigmine (Phy) is the prototype of these drugs and its effects on brain Energy Metabolism have not been studied on subcellular structures. Because mitochondrial dysfunction is a common feature of many neurodegenerative diseases, including Dementias, in this study we preliminary investigate the effects on neuronal energy-yielding mitochondrial pathways exerted by Phy and Neostigmine (Neo), the latter being an AChEI which does not cross the blood-brain barrier (BBB) in physiological conditions.
METHODS: The effects of acute Phy and Neo treatments (0.015 mg/kg i.m., 20 min) were studied on mitochondrial metabolism of temporal cerebral cortex of 3-month-old male Wistar rats. Because of brain mitochondria microheterogeneity, the following populations were purified: non- synaptic mitochondria (FM) of neuronal soma, located in vivo in post-synaptic compartment, and intra-synaptic "light" (LM) and "heavy" (HM) ones of pre-synaptic compartment. The catalytic activities of regulatory enzymes of energy-yielding metabolic pathways have been assayed: citrate synthase, succinate dehydrogenase, malate dehydrogenase for Krebs' cycle; Complex I-III, Complex II and Complex IV for Electron Transport Chain (ETC).
RESULTS: In FM, none of the assayed enzymes were modified by the tested drugs, which exerted their effects only on intra-synaptic mitochondria: (a) in LM both drugs increased citrate synthase, Complex I-III (NADH-cytochrome c reductase) and Complex IV (cytochrome oxidase) activities, while Complex II (succinate dehydrogenase) was enhanced only by Phy; (b) in HM both drugs increased citrate synthase, and Neo ETC Complexes. Malate dehydrogenase was not modified in any mitochondrial population.
CONCLUSIONS: Both drugs displayed similar changes for the assayed enzyme activities depending on subcellular localization of mitochondrial types: in post-synaptic compartment (FM), the Energy Metabolism was unaffected while, in pre-synaptic one (LM, HM) energy production pathways were increased. These results are coherent with the enhanced Acetylcholine turnover in pre-synaptic terminal, requiring higher energy availability, and provide new

insights about AChEI pharmacodynamics on brain Bioenergetics, independently from their ability to cross the BBB. Further studies are recommended on the same enzymatic systems in Dementia experimental animal models and after chronic treatments, in accordance with the hypothesis that mitochondrial Energy Metabolism is a potential physiopathological factor and thus target for therapeutic strategies.

ACKNOWLEDGEMENTS: This Study is supported by Conferenza Episcopale Italiana and bt the Project "Progressi in Biologia e Medicina" of Fondazione Ghislieri for Dr. Ferrari's fellowship.

DIAGNOSTIC DELAY AND DRUG UTILIZATION PATTERNS IN PATIENTS WITH CROHN'S DISEASE: ANALYSIS OF AN ITALIAN REGIONAL ADMINISTRATIVE HEALTHCARE DATABASE

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BACKGROUND: Diagnostic delay (DD) in Crohn's disease (CD) is an important issue for disease management, due to its high frequency often caused by the difficulty in recognizing non-specific abdominal symptoms. DD is documented mainly in studies based on disease registries. The aim of this study was to quantify putative DD in a real-world cohort of CD patients, and to assess whether DD affected adherence and related patterns for first line drug treatments.
METHODS: In this retrospective cohort study, data was retrieved from the administrative healthcare databases of Tuscany, an Italian region. Patients were included if they had a first record of ICD-9 diagnosis or disease exemption or a first record of dispensation of oral budesonide as CD patient from 6/1/2011 to 6/30/2016 (index date, ID). Patients <18 years old at ID or with look-back period <5 years or follow-up period <3 years were excluded. Patients were classified with DD if they had at least one access to emergency department (ED) or hospitalization for gastro-intestinal (GI) causes earlier than 6 months before ID and during the look-back period. Trajectories of adherence to drug treatment were computed with a three-step procedure for drug-utilization analysis: 1) computation of 24 statistical measures; 2) factor analysis; 3) cluster analysis. Patients with DD and trajectories were described.
RESULTS: 3347 patients were included in the CD cohort. Patients with suspected DD were 2073 (61.9%). Their mean age was 49.6 years (standard deviation [SD] 17.7) and 1073 (51.7%) of them were female. Over the 6 months preceding the ID, they were taking a mean of 5.0 (SD 4.5) concomitant drugs. A suspected

DD of 7-18 months was observed in 759 patients (37%), while the remaining 1314 (63%) displayed a suspected DD of 19-60 months. For the drug utilization analysis, we excluded 1266 patients from the initial CD cohort due to lack of drug dispensations during follow-up. Therefore, the trajectories' analysis was conducted on 2081 patients. Trajectories over DD defined three different clusters. Cluster 1 (n=931 patients) described the highest adherence pattern (40% after 5 months), which likely identified patients with a prolonged treatment with budesonide. Cluster 2 (n=604) and Cluster 3 (n=546) described low adherence patterns (almost 0% and 20% after 5 months, respectively), and might represent patients with acute treatment, likely identifying a proper utilization of budesonide. Cluster 1, 2 and 3 included 58.9%, 64.9% and 61.4% of patients with suspected DD, respectively. Among the three clusters, the distribution of DD was not significant (p=0.0598) at level α=0.05.

CONCLUSIONS: This study showed a considerable level of suspected DD in Tuscany CD patients. The majority of them displayed a potentially inappropriate drug utilization behaviour, while others showed a treatment profile consistent with clinical guidelines.

MPEP, A SELECTIVE MGLUR5 NEGATIVE ALLOSTERIC MODULATOR, ATTENUATES FAT ACCUMULATION IN AN IN VITRO MODEL OF BENIGN STEATOSIS

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BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is a liver disorder caused by fat overload, usually associated with obesity, insulin resistance, hypertension and dyslipidemia. In Western countries, NAFLD prevalence is about 20-40% in general population, rising up to 75% in obese or diabetic people. It has been previously found that hypoxic hepatocytes release more glutamate than normoxic cells and that the blockade of metabotropic glutamate receptor type 5 (mGluR5) protects against hepatic ischemic injury. Recently, it has been shown that an increase in glutamate secretion also occurs in alcohol-induced steatosis, resulting in mGluR5s hyperactivation on stellate cells and abnormal fat accumulation in hepatocytes1. No study is currently available on the role of mGluR5 in NAFLD development. Aim of this work was to elucidate the role of mGluR5 in an in vitro model of benign steatosis.

METHODS: HepG2 cells were pre-incubated for 12 hours with 100 μM (S)-3,5-Dihydroxyphenylglycine (DHPG), an mGluR5 agonist, 300 nM 2-Methyl-6-(phenylethynyl)pyridine (MPEP), a negative allosteric modulator, or vehicle; cells were then treated with 1.5 mM oleate/palmitate (O/P) for another 12 hours. Cell viability was evaluated with the MTT assay; fat accumulation was measured using the fluorescent dye nile red; SREBP-1, PPAR-α, iNOS, pNFkB, NFkB and Caspase-3 protein expression were evaluated by Western blot; NFkB activity was evaluated as pNFkB/NFkB ratio.

RESULTS: mGluR5 modulation did not alter cell viability in O/P-treated cells; MPEP prevented intracellular lipid accumulation in O/P treated cells; MPEP administration was also associated with a reversion of O/P-induced changes in SREBP-1 and PPAR-α expression, two proteins involved in free fatty acid (FFA) metabolism and

uptake. No changes were observed in iNOS and Caspase-3 expression, or in NFkB activity.
CONCLUSIONS: We found for the first time that the pharmacological blockade of mGluR5 reduces FFA accumulation in an in vitro model of benign steatosis. We also found that mGluR5 inhibition is associated with a reversion of FFA induced changes in SREBP-1 and PPAR-α protein expression, suggesting that mGluR5 hyperactivation may promote changes in the expression of enzymes involved in FFA metabolism and uptake. Recently, significantly high glutamate levels have been found in serum and liver of patients with NAFLD; the increase in glutamate was significantly correlated with the severity of NAFLD and fibrosis, so suggesting the possibility of mGluR5 hyperactivation in NAFLD. Moreover, considering that mTOR is the main SREBP-1 and PPAR-α regulator and that mGluR5 has been already found to control mTOR activation in the CNS, we also hypothesize that a similar mechanism may reasonably occur also in human hepatoma cells.

CARNOSINE EXERTS ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS IN MURINE MACROPHAGES TREATED WITH PHORBOL 12-MYRISTATE 13-ACETATE

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BACKGROUND: Carnosine is an endogenous dipeptide composed of β-alanine and L-histidine present at high concentrations in several mammalian excitable tissues such as muscles and brain. Carnosine has been shown to be involved in different cellular defense mechanisms including inhibition of protein cross-linking, reactive oxygen and nitrogen species detoxification as well as counteraction of inflammation. As a part of the immune response, macrophages are the primary cell type that are activated and play a crucial role in different diseases associated with oxidative stress and inflammation, including cardiovascular and neurodegenerative diseases.
METHODS: By employing microchip electrophoresis with laser-induced fluorescence and MitoSOX Red probe, we first investigated the production of superoxide anion (O2−•) induced by phorbol 12-myristate 13-acetate (PMA) and superoxide dismutase (SOD) inhibitors, in the

absence or in the presence of increasing concentrations of carnosine and anserine, in RAW 264.7 cells, representing an established in vitro experimental model of oxidative stress. We also studied the variation of parameters representative of cellular energy metabolism (HPLC analysis), the expression of oxidative stress related enzymes, and the expression of pro- and anti-inflammatory cytokines (quantitative real time PCR) in stimulated RAW 264.7 cells in the absence or in the presence of therapeutic concentrations of carnosine. Lastly, relying on the fact that Akt protein is overactivated in stressed macrophages, we also evaluated the effects of carnosine on PMA-induced activation of Akt in RAW 264.7 cells by Western blot.

RESULTS: Carnosine was able to decrease the intracellular concentration of O2−• and the expression of Nox1 and Nox2 enzyme genes. These antioxidant effects of carnosine were accompanied by the attenuation of the PMA-induced Akt phosphorylation, the downregulation of TNF-α and IL-6 mRNAs, and the upregulation of the expression of the anti-inflammatory mediators IL-4, IL-10, and TGF-β1. Additionally, carnosine at the highest dose (20 mM) significantly improved macrophage energy state, evaluated through the increase both in the total nucleoside triphosphate concentrations and in the sum of the pool of intracellular nicotinic coenzymes. Finally, carnosine was able to decrease the NADP+/NADPH ratio in a concentration-dependent manner.

CONCLUSIONS: We provided evidence that the effects of carnosine are not only connected to the modulation of canonical pathways and activities causing oxidative/nitrosative stress and inflammation, but also that they are strictly linked to the capacity of this molecule to interact positively with the cell bioenergetics, improving mitochondrial oxidative phosphorylation and electron transport chain. Our data suggest a multimodal mechanism of action of carnosine which exerts both antioxidant and anti-inflammatory effects in an experimental model of inflammation-related oxidative stress.

CARBONIC ANHYDRASE IX: A NOVEL PHARMACOLOGICAL TARGET TO IMPAIR TUMOR CELLS PROLIFERATION, INVASIVENESS AND MALIGNANCY

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BACKGROUND: Carbonic anhydrase IX (CA-IX) belongs to the CA family, ubiquitous zinc enzymes, implied in different pathological and physiological processes. CA-IX is expressed ectopically by almost all solid tumors, especially in the advanced stages under hypoxia control and plays a pivotal role in regulation of pH in tumor milieu catalyzing carbonic acid formation by hydrating CO2. An acidification of tumor microenvironment contributes to tumor progression via multiple processes, including reduced cell-cell adhesion, increased migration and matrix invasion. According to the role of CA-IX activity in several phases of cancer development (metabolic transformation, growth and progression, invasion and metastasis) CA-IX inhibition has been considered a potential target for novel anticancer therapeutic strate-

gy. We aimed to assess whether the pharmacological inhibition of CA-IX could impair tumor cell proliferation and invasion.
METHODS: Tumor epithelial cells from breast (MDA-MB-231) and lung (A549) cancer were used to evaluate the cytotoxic effect of sulfonamide CA-IX inhibitors. Two ureido-benzene-sulphonamide analogues were testes as CA-IX enzyme blockers, SLC-0111 and AA-06-05. Tumor cell viability was assessed by colorimetric MTT Assay, after treatment with increased doses of CA-IX inhibitors. From a molecular point of view the impairment of tumor cell viability was corroborated by Western blot, to quantify the principal apoptotic biomarkers. Boyden chamber assay, a migration test, was performed to assess the decrease of invasiveness of tumor cells, responding to CA-IX inhibition, following by Western blot analysis of principal epithelial mesenchymal transition (EMT) markers. Media conditioned by tumor cells, treated with CA-IX inhibitors, were used to perform gelatin zymography, a functional test based on the gelatinase activity of matrix metalloproteinase MMP-2 and MMP-9.
RESULTS: In both tumor cell models MDA-MB-231 and A549, SLC-0111 and AA-06-05 inhibited cell proliferation, migration and invasion through shifting of the mesenchymal phenotype toward an epithelial one and by impairing MMP-2 activity. The antitumor activity was elicited via apoptosis pathway activation. An upregulation of p53 was observed, which in turn regulated the activation of caspase-3. Inhibition of proteolytic activity was accompanied by upregulation of the endogenous tissue inhibitor TIMP-2.
CONCLUSIONS: Collectively, these data strengthen a close relationship between CA-IX and cancer malignancy. Therefore, is confirmed the potential use of CA-IX inhibitors, and in particular SLC-0111 and AA-06-05, as agents to be further developed, alone or in combination with other conventional anticancer drugs.

INHIBITION OF INFLAMMASOME ACTIVATION RESTORE THE PATHOLOGICAL SIGNS IN A RAT MODEL OF DIARRHEA- PREDOMINANT IRRITABLE BOWEL SYNDROME (D-IBS)

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BACKGROUND: Diarrhea-predominant irritable bowel syndrome (D-IBS) is classified as a functional gastrointestinal disorder characterized by multi-factorial alterations, including altered gastrointestinal (GI) motility, visceral hypersensitivity, and intestinal inflammation. The bowel inflammatory response led to induce innate immune response trough the inflammasome activation. Once activated, NLRP3, the main component of inflammosome, leads to caspase 1/ASC-inflammasome-mediated activation, and the production of inflammatory mediators including cytokines and chemokines. Pharmacological inhibition of NLRP3 activation results in powerful therapeutic effects in a wide variety of rodent models of inflammatory diseases. The aim of this study was to evaluate the

effect of the inflammasome blocking agents BAY 11-7082 (10 mg/kg and 30 mg/kg) in a rat model of diarrhea- predominant irritable bowel syndrome (D-IBS).
METHODS: D-IBS was induced by intracolonic instillation of 1mL 4% acetic acid at 8 cm proximal to the anus for 30s. BAY 11-7082 (30 mg/kg) was administered daily for 1 week by oral gavage. For tissue examination, colon-rectal portions were taken 2 weeks after D-IBS induction to perform the histological analysis, immunistochemical analysis of tight junctions such as zona occludens-1 (ZO-1) and occluding, and for Western blot analysis of NRLP3, caspase-1, IL-1b, TNF-a, IkBa, NF-kB, and iNOS.
RESULTS: The results obtained showed that the treatment with BAY 11-7082, following histopathological analysis of colon tissue, showed that BAY 11-7082 treatment significantly reduced tissue injury due to acetic acid instillation, characterized by edema, neutrophil infiltration and loss of tissue structure. We also demonstrated that BAY 11-7082 treatment led to inhibit the NLRP3 inflammasome activation and NF-kB translocation in the nucleus, reducing inflammatory mediators production. Moreover, treatment with BAY 11-7082 restored the altered expression of tight junction proteins following D-IBS induction.
CONCLUSIONS: Taken together, our data demonstrate that D-IBS induced NLRP3 inflammasome pathway activation, accompanied by the production of proinflammatory response. The modulation of inflammosome pathway with BAY 11-7082 inhibitor significantly reduced the signs of D-IBS therefore, can be considerate a valuable strategy to reduce the development of D-IBS.

GILZ (GLUCOCORTICOID-INDUCED LEUCINE ZIPPER) REGULATES LEUKOCYTE LIVER INFILTRATION VIA REGULATION OF CCL2 EXPRESSION DURING LIVER FIBROSIS DEVELOPMENT

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BACKGROUND: Glucocorticoids (GC) are the most powerful anti-inflammatory and immunosuppressive drugs available, used to treat chronic inflammatory and autoimmune diseases. Glucocorticoid-induced leucine zipper (GILZ or tsc22d3) is rapidly induced by GCs and acts as an effector of many anti-inflammatory effects of GC in several cell types, including the regulation of cell survival, proliferation, differentiation and migration. It inhibits MAPK/Erk, Akt pathways and promotes TGF-b signaling in T cells. In human monocytes GILZ suppresses NF-kB- regulated expression of chemokines including CCL2 and CCL3. Inflammation promotes many pathologic conditions associated with tissue regeneraion, including liver fibrosis (LF). Given GILZ's role in regulating TGF-b singling and leukocyte migration, we hypothesized that it may play a role in LF development.
METHODS: We used WT and GILZ KO mice in experimental model of carbone tetracloride (CCl4)- induced LF. We compared the inflammatory (72hr after CCL4 injection) and chronic/late (7 weeks after CCL4 injection) phases of LF development in WT and GILZ KO mice. We evaluated gene expression by quantitative

red real time PCR (qPCR) and Western Blot. Infiltration of cells into livers was evaluated by flow cytometry. Pharmacological rescue of leukocytes liver infiltration was performed by injecting siRNA in vivo in the form of lipid nanoparticles.
RESULTS: We found enhanced leukocytes infiltration in the livers of GILZ KO mice at the acute phase of LF development, associated with increased production of pro-inflammatory IL-6 and IL-4 cytokines. This increase in leukocytes was significant fo rthe population of NK and T cells. This increased presence of T and NK cells in GILZ KO compared to WT livers is likely due to the increased production of CCL2 and CCL3 chemokines as evidenced by qPCR analysis of mRNA expression. Pharmacological inhibition of CCR2 expression through lipid nanoparticles (LNPs) containing siRNA for CCR2 was capable of limiting leukocyte infiltration into the liver of GILZ KO mice. Consistently with enhanced inflammatory phase in GILZ KO livers, the absence of GILZ resulted in the enhanced LF development upon chronic exposure to CCl4 as evidenced by elevated collagen deposition in the livers of GILZ KO mice compared to similarly treated WT mice.
CONCLUSIONS: Altogether, these data evidence the increased inflammation and fibrosis in the livers of GILZ-KO compared to WT mice. Thus, GILZ exhibits a protective role in liver fibrosis, consistent with its anti-inflammatory function in different cell types. Therefore, modulation of GILZ expression represents a potential pharmacological tool in the treatment of liver inflammatory disorders.

MECHANOSENSITIVE AND PHARMACOLOGICAL PROPERTIES OF PIEZO1 CHANNEL IN EMBRYONIC MESENCEPHALIC IMMORTALIZED NEURONS

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BACKGROUND: Mechanical and physical cues are important components by which cells perceive environment changes. Mechano-transduction enables cells to sense and adapt to external forces and physical constraints. Nevertheless, the way by which these mechanical stimuli are transduced into biological signals is still largely unknown. Piezo1 is a recently discovered transmembrane protein, acting as mechanosensing and non-selective ion channel. It is ubiquitously expressed in cells where displays a tight interplay with plasma membrane, extracellular matrices and cytoskeleton elements. In vivo, cells are embedded in a tissue environment with lower elasticity (from 0.2 to 64 kPa) as compared to the common in vitro culture. Moreover, different pathologies contribute to create a different environment that lead to biomechanical abnormalities, such as in tumours or in neurodegenerative diseases. The purpose of this study is to understand how different mechanical cues interfere with Piezo1 functioning to modulate neuronal activity.
METHODS: We explored the role of Piezo1 channel in mouse immortalized embryonic mesencephalic neuron line (named A1), representing a good in vitro model combining indefinite proliferation and neuronal features. We transfected A1 cells with human Piezo1-GFP plasmid (A1-OV) and evaluate the consequent alterations in cellular shape, by digital holography microscopy (HoloMonitor), and in cell via-

bility. We also checked whether substrate stiffness influences Piezo1 expression and activity by the use of specific plastic ware coated with different polyacrylamide hydrogels (0.2kPA-50kPa). Moreover, we investigated the activity of the channel using live cell Ca++ imaging after treatment with YODA1, a specific Piezo1 agonist.

RESULTS: Compared to A1-WT, A1-OV cells are thicker but smaller as surface area and show lower viability. A1-OV viability was stiffness-dependent, since it increases with substrate rigidity. Piezo1 over-expression leads to an increased expression of different cytoskeleton elements (i.e. filamin-A, vinculin, GFAP), independently from substrate stiffness, while A1-WT showed a lower expression of these markers when grown on a softer substrate. Increased cytoskeletal protein expression caused an increased rigidity in A1-OV cells, measured by PIUMA-CHIARO nanoindenter. We also performed Yoda1 dose-response curves regarding intracellular Ca++ increase, in A1 and A1-OV cells. The curves show that the overexpression of the channel increases A1 response to Yoda1.

CONCLUSIONS: These data show that in an in vitro neuron-like system Piezo1 channel acts as a sensor of different stiffness settings, allowing cells to change their characteristics based on the type of substrate in which they are plated. Since neuron mechanotransduction might be affected by environment changes due to normal aging, neurodegeneration processes or other pathologies, Piezo1 could represent a novel pharmacological target to improve neuronal survival.

HYPERICYN AND ROSE BENGAL ARE EFFECTIVE SONOSENSITIZER AGENTS ACHIEVING SIGNIFICANT ANTICANCER EFFECTS UNDER US EXPOSURE ON A THREE-DIMENSIONAL COLON CANCER MODEL

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BACKGROUND: One of the main advantages of using ultrasound (US) in combination with a chemical agent (sonosensitizer) is the ability to maximize its anticancer activity in the so called sonodynamic therapy (SDT). SDT shares common features with the clinically approved photodynamic therapy (PDT), distinguishing itself by the use of US instead of light to achieve a better tissue penetration. The way in which SDT achieves cytotoxic effects remains still under debate, but one of the main activities involves the sonosensitizer-mediated reactive oxygen species (ROS) production. In this work the anticancer activity of hypercin (HYP) and rose bengal (RB), two well-known photosensitizers, was investigated under US stimulation on an in vitro three-dimensional (3D) colon cancer model, taking PDT as a reference. The focus on 3D model is important since cancer cells organized into 3D structure share similar aspects of solid tumour, serving as a more feasible tool before moving to the in vivo setting compared to two-dimensional (2D) cell culture models.

METHODS: The effects of US exposure of HYP and RB have been studied on a human colon cancer (HT-29) cell line grown into 2D mono-

layers or 3D spheroids by coating 96-well plate with agarose. The synergic activity of HYP or RB in combination with US was assessed by evaluating treatment effects on cell growth and cell death, by cytofluorimetric assays and confocal imaging.
RESULTS: In order to select the proper non-cytotoxic concentration of HYP and RB and the proper time to perform SDT, cytotoxicity assays and cytofluorimetric evaluations of cellular uptake were performed, resulting for 2D cell cultures 0.1 μM HYP and 10 μM RB for 24 h incubation, and for 3D spheroid cultures 0.2 μM HYP and 30 μM RB for 24 h of incubation. On 2D HT-29 cell cultures, SDT showed a strong effect 48 h after the treatment, and on 3D HT-29 spheroids, SDT induced a significant decrease in spheroid volumes also at 48 h after the treatment. Moreover, it was also investigated the effect of SDT with HYP or RB on HT-29 resistant cells, and the expression of P-gp was not able to interfere with cell responsiveness to SDT.
CONCLUSIONS: Data point out that HYP and RB display interesting anticancer effects under sonodynamic treatment and not only under photodynamic treatment.

MONITORING THE CONSUMPTION AND EXPENDITURE OF SYSTEMIC ANTIBIOTICS: ANALYSIS IN AN APULIAN AOU

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BACKGROUND: Antibiotic resistance is an emerging growing global public health problem. In 2017, the Italian National Antimicrobial Resistance Contrast Plan (PNCAR) was approved for monitoring systemic antibiotic usage and prescription. Antibiotic consumption indicators are specific parameters for standardized usage comparison between pharmaceutical forms, dosages and among hospitals. In this work, we report antibiotic consumption monitoring of the University Hospital Ospedali Riuniti of Foggia (OO.RR.) in 2019.

METHODS: The Pharmacy Unit of the OO.RR. performed antibiotic consumption monitoring by periodic multidisciplinary meetings. Data analysis was carried out using the Defined Daily Dose (DDD) per 100 bed days (DDD/100 bd) indicator, an estimator of therapeutic intensity. Healthcare costs of antibiotic usage were monitored as a weighted average value for days of hospitalization.
RESULTS: During 2019, the OO.RR. of Foggia showed an increase in systemic antibiotic consumption of 3% with a DDD value of 75.1 compared to a DDD of 72.6 in 2018. The PNCAR considers essential monitoring two classes of antibiotics: carbapenems and fluoroquinolones. In our Hospital, an increase in carbapenem consumption of 22% was documented, while a decrease in fluoroquinolones of 28% was observed. The three most prescribed classes of antibiotics in 2019 were: penicillins combined with beta-lactamase inhibitors (25.4 DDD); third generation cephalosporins (13.1 DDD); and fluoroquinolones (11.9 DDD). The ten most used active principles were: amoxicillin/clavulanic acid; ceftriaxone; levofloxacin;

teicoplanin; piperacillin/tazobactam; clarithromycin; ciprofloxacin; metronidazole; meropenem; and daptomycin. Antibiotics for treatment of infections caused by Multiple Drug Resistance (MDR) pathogens represented about 20% of total consumption in our hospital with a 2% decrease compared to 2018. In 2019, the healthcare costs decreased of 2.9% of cost per hospitalization days, 6.5 € compared to 6.7 € in 2018, still higher than the median national cost (4.4 € in 2018). In particular, the ATC class III level, "Other antibacterials" has the highest spending per day of hospitalization (2.77€), followed by "Other beta-lactams" (1.84€) and Penicillin (0.967€).The ten active principles with the greatest expense per day of hospitalization were teicoplanin, daptomycin, tigecycline, piperacillin/tazobactam, ceftazidime/avibactam, amoxicillin/clavulanic acid, meropenem, ceftobiprole/medocaril, cefepime and ceftolozano/tazobactam.

CONCLUSIONS: In our OO.RR. of Foggia in 2019, we registered an increase of total antibiotic and carbapenems consumption; however, fluoroquinolones and antibiotics for MDR infections were less used compared to 2018 suggesting that Italian regulations, just approved in 2017, are already applied for preventing antibiotic resistance. However, further improvements are required to better manage antibiotic consumption and decrease healthcare cost below the national

PRECISION THERAPY FOR PEDIATRIC *BCR-ABL1 LIKE* ACUTE LYMPHOBLASTIC LEUKEMIA WITH ACTIVATION OF THE ABL PATHWAY: SET UP OF AN IN VITRO SYSTEM FOR DIAGNOSIS AND CLINICAL MONITORING

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BACKGROUND: Pediatric BCR-ABL1 like acute lymphoblastic leukemias (ALL) have a heterogeneous genetic background, characterized by a potential over-activation of the ABL1 kinase pathway and by a high risk of relapse when treated with conventional chemotherapy. Aim of the study is to set up a biosensor in vitro assay to investigate the ABL1 activity in blasts at diagnosis, as an integrative information to cytogenetics, and to screen the efficacy of tyrosine kinase inhibitors (TKIs) on BCR-ABL1 like ALL cells.

METHODS: The biosensor (PABL) consists in a biotynilated peptide probe whose sequence comprises a reporter region with the tyrosine (Y) phosphorylation site for ABL1 and a targeting region to increase the specificity for the kinase. PABL phosphorylation is quantified by an ELISA assay, using a neutravidin-coated plate as solid support to bind the probe and an anti phospho-Y primary antibody conjugated to a fluorescent secondary antibody to reveal the PABL phosphorylation after cell lysates incuba-

tion in the presence or absence of TKI (imatinib 5 μM, ruxolitinib, 52 nM and 5 μM, Fig.1A). Four leukemic cell lines were used, including NALM6 and ALL-SILL (ALL models harboring the ETV6-PDGFRB and NUP214-ABL1 translocation respectively, both leading to ABL1 pathway activation), K562 (BCR-ABL1 positive control) and REH (negative control). Cell lines were first characterized in western blot to confirm the presence of candidate chimeric proteins, and screened for TKI sensitivity by MTT assay. Additional biosensors (Y-site-mutated (PABL-F) and fully-phosphorylated probe (PPHOSPHO-ABL)) were included in the ELISA assay.
RESULTS: K562 was the most sensitive cell line to imatinib (IC50 = 0.38±0.13 μM) followed by NALM6 (5.56±0.65 μM) and ALL-SIL (8.02±0.36 μM); REH were not sensitive to imatinib in the concentration range tested. As expected, none of the cell lines resulted to be sensitive to ruxolitinib, known to be a specific inhibitor for JAK1/2. ELISA analysis showed a significant PABL phosphorylation in all the lines tested (average basal phosphorylation of lysates vs PABL phosphorylation: 6.84 ± 1.46% vs 32.44 ± 3.25%, two-way ANOVA p-value < 0.0001, percentages relative to PPHOSPHO-ABL). K562 cells presented the highest kinase activity on PABL (41.43 ± 3.45%) not significantly different from that of ALL-SIL (31.76± 2.79%); a decreased signal was instead observed for NALM6 (28.16 ± 3.23%, $p<0.001$) and REH (28.4% ± 3.5, $p<0.05$). Phosphorylation was ABL1-mediated, as demonstrated by the specific inhibition of imatinib ($p< 0.0001$ for K562 and NALM6; $p=0.0002$ for ALL-SIL and $p=0.0068$ for REH, Fig 1B), and occurred on the Y phosphorylation site of the reporter region, as demonstrated by a site-mutated biosensor PABL-F ,whose phosphorylation is comparable to the basal level of cell lysates.
CONCLUSIONS: While requiring further optimization, the PABL-based ELISA assay paves the way to a point-of-care device that might improve the diagnosis and treatment of BCR-ABL1 like ALL children.

PEDIATRIC ADVERSE REACTIONS TO ANTISEIZURE MEDICATIONS - AN ANALYSIS OF DATA FROM THE ITALIAN SPONTANEOUS REPORTING SYSTEM (2001-2019)

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BACKGROUND: Spontaneous reports of adverse drug reactions (ADRs) are a valuable supplement to clinical studies in informing about the safety of medications. This is especially relevant for pediatric populations, which are not often included in large-scale clinical trials. The aim of this study was to evaluate patterns of pediatric ADRs to antiseizure medications (ASMs) reported to the Italian Spontaneous Reporting System (SRS) database.

METHODS: Suspected ADR reports ascribed to medications listed under ATC code N03, plus clobazam (code N05BA09), affecting individuals below age 18 years, and reported during the period November 1, 2001- May 31, 2019, were selected from the Italian SRS database. All ADRs were categorized based on a modification of the MedDRA® high-level term. Demographic characteristics and drug-related variables were evaluated using descriptive statistics.
RESULTS: A total of 956 reports listing a total of 1,806 ADRs ascribed to one or more ASMs were received for individuals in pediatric age. The most commonly reported ADRs were skin rashes (24.0% of all reports), epileptic seizures (12.6%), gastrointestinal disturbances (11.8%), and somnolence (10.6%). A more detailed analysis was conducted on 675 reports listing a single ASM as suspected drug and occurring in patients with a specified or presumed diagnosis of epilepsy. Of these, 75 (11.0%) were newborns and infants, 137 (20.3%) were children of pre-school age (2 to 5 years), 240 (35.6%) were children aged 6 to 11 years, and 223 (33.0%) were adolescents (12 to <18 years). The most common indication category was epilepsy not otherwise specified (n = 385, 57%). Mostly of reports concerned carbamazepine (161 reports for a total of 309 ADRs), valproic acid (112 for 198 ADRs), levetiracetam (80 for 153 ADRs), lamotrigine (61 for 113 ADRs), and topiramate (56 for 109 ADRs). ADR patterns differed widely across ASMs. Skin rashes were the most commonly reported ADR for lamotrigine (62.3%), carbamazepine (50.3%), phenobarbital (42.3%), and oxcarbazepine (33.0%). Of the 186 reports of skin rashes, 88 (40 ascribed to carbamazepine, 21 to lamotrigine, 9 to phenobarbital, 6 to oxcarbazepine, 6 to valproic acid, 2 to ethosuximide, and one each to levetiracetam, phenytoin, rufinamide, and zonisamide) were classified as serious: 17 cases of Stevens-Johnson syndrome and 3 cases of toxic epidermal necrolysis were observed. Other most commonly reported ADRs were gastrointestinal symptoms for ethosuximide (44%), irritability/aggression for levetiracetam (25.0%), epileptic seizures for valproic acid (16.1%), fever (often associated with hypohidrosis) for topiramate (17.9%), and utilization error (mostly accidental drug administration) for clonazepam (34.6%).
CONCLUSIONS: Patterns of spontaneous ADR reports are indicative of major differences in safety profile among individual ASMs. Most, but not all, frequently reported ADRs were in line with findings from clinical trials and observational studies.

ROLE OF *KLEBSIELLA PNEUMONIAE* AND CYTOTOXICITY OF THIOPURINES *IN VITRO*

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BACKGROUND: *Enterobacteriaceae* are increased at the expense of other bacterial species in the gut microbiota of inflammatory bowel disease (IBD) patients. Thiopurines are used in the maintenance of remission in IBD and, despite their proven efficacy, some patients do not achieve satisfying therapeutic effects. Since growing evidence validates the role of bacteria in influencing the efficacy of therapies, this project aims to investigate *in vitro* if *E. coli*, *S. enterica* and *K. pneumoniae*, belonging to *En-*

terobacteriaceae, could cause lack of response to thiopurines.
METHODS: Azathioprine (AZA), mercaptopurine (MP) and thioguanine (TG)(400 μM) were incubated in presence or not of bacteria and of their growth phase broths (GPB) for 4 h at 37 °C. Thiopurines were also exposed to a *K. pneumoniae* clinical isolate and to several concentrations of *K. pneumoniae* ATCC 13883 exopolysaccharide (EPS) (3.75-60 μg/mL). Viability of NALM6 B cells exposed to serial dilution of drugs (0.2-15 μM for AZA, 0.3-20 μM for MP and 0.08-5 μM for TG) in medium alone or exposed to bacteria, to GPB or to EPS was determined by MTT assay. To identify variations in the concentrations of thiopurines after exposure to the bacterial strains, absorbance peaks were analysed by UV spectrophotometry (280 nm for AZA, 320 nm for MP and 340 nm for TG). Statistical significance was assessed by two-way ANOVA for cytotoxicity tests and one-way ANOVA for UV spectra.
RESULTS: AZA, MP and TG (400 μM) did not alter the proliferation of all bacterial strains tested. Cytotoxic effects on NALM6 cells after treatment with AZA, MP and TG were significantly decreased after incubation with *K. pneumoniae* ATCC 13883 and its GPB (15 μM -12%; from MP and TG starting from 1.25 μM -31% and 22% respectively) ($P<0.001$). Absorbance peaks of MP and TG significantly decreased after incubation with *K. pneumoniae* ATCC 13883 (-32±0.02% and -30±0.03% respectively) ($P<0.001$ and $P<0.01$ respectively). The exposure to bacteria and GPB caused the reduction of the absorbance peak of AZA (respectively -25±0.01% and -17±0.03%) ($P<0.001$). Contrary to what was observed after the incubation with *K. pneumoniae* ATCC 13883, thiopurines exposed to *E. coli* and *S. enterica*, as well as their GPB, did not undergo evident variations in cytotoxicity and in absorbance. Preliminary data obtained treating cells with thiopurines exposed to several dilution of EPS showed no variation in cytotoxicity and in absorbance of AZA, MP and TG. Moreover, MP and TG, but not AZA, exposed to *K. pneumoniae* clinical isolate resulted to be decreased in cytotoxicity.
CONCLUSIONS: Among *Enterobacteriaceae*, only *K. pneumoniae* seems to be responsible for a reduction of thiopurines cytotoxicity. Since preliminary data did not highlight interference of bacterial EPS in thiopurines efficacy, the phenomenon could be mainly linked to a microbial biotransformation and for this reason quantification in *K. pneumoniae* ATCC 13883 of thiopurines and their metabolites is ongoing.

MESOLIMBIC DOPAMINE DYSREGULATION AS A SIGNATURE OF INFORMATION PROCESSING DEFICITS IMPOSED BY PRENATAL THC EXPOSURE

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BACKGROUND: Cannabis is the illicit drug most widely used by pregnant women worldwide. Its growing acceptance and legalization have markedly increased the risks of child psychopathology, including psychotic-like experiences, which lowers the age of onset for

a first psychotic episode. As the majority of patients with schizophrenia go through a premorbid condition long before this occurs, understanding neurobiological underpinnings of the prodromal stage of the disease is critical to improving illness trajectories and therapeutic outcomes. We have previously shown that male rat offspring prenatally exposed to Δ9-tetrahydrocannabinol (THC), a rat model of prenatal cannabinoid exposure (PCE), exhibit extensive molecular and synaptic changes in dopaminergic neurons of the ventral tegmental area (VTA), converging on a hyperdopaminergic state. This leads to a silent psychotic-like endophenotype that is unmasked by a single exposure to THC.
METHODS: Here, we further characterized the VTA dopamine neuron and sensorimotor gating functions of PCE rats exposed to an acute stress or a challenge of the D2 receptor agonist apomorphine, by using in vivo single-unit recordings correlated with Prepulse Inhibition (PPI) analyses.
RESULTS: At pre-puberty, PCE male rat offspring display a reduced population activity of VTA dopamine neurons in vivo, the majority of which are tonically active. PCE male progeny also exhibit enhanced sensitivity to dopamine D2 (DAD2) receptor activation by apomorphine and a vulnerability to acute stress, which is associated with compromised sensorimotor gating functions.
CONCLUSIONS: This data extends our knowledge of the multifaceted sequelae imposed by PCE in the mesolimbic dopamine system of male pre-adolescent rats, which renders a neural substrate highly susceptible to subsequent challenges that may trigger psychotic-like outcomes.

A NEW HUMAN BLOOD-RETINAL BARRIER MODEL BASED ON ENDOTHELIAL CELLS, PERICYTES, AND ASTROCYTES

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BACKGROUND: The blood–retinal barrier (BRB) is crucial for proper vision, and the loss of the integrity of this physical barrier greatly contributes to the vision loss in retinal diseases such as diabetic retinopathy. BRB dysfunction represents one of the most significant changes occurring during diabetic retinopathy.
METHODS: We set up a high-reproducible human-based in vitro BRB model using human primary retinal pericytes, retinal astrocytes, and retinal endothelial cells in order to replicate the human in vivo environment with the same numerical ratio and layer order. We first evaluated barrier tightness and paracellular permeability of our BRB system by measuring the trans-epithelial electrical resistance (TEER) and the flux of the water-soluble inert tracer, sodium fluorescein (Na-F). Next, we examined the functional integrity of the BRB by immunofluorescence staining. The effects of high glucose exposure were also assessed by measuring total reactive oxygen species production (fluorescence), and the expression levels of genes related to pro-in-

flammatory mediators and oxidative stress (quantitative real time PCR (qRT-PCR)). Lastly, we investigated the effect of high glucose exposure on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2), as well as on its downstream gene heme oxygenase 1 (HO-1), by Western blot analysis.
RESULTS: Our findings showed that high glucose exposure elicited BRB breakdown, enhanced permeability, and reduced the levels of junction proteins such as zonula occludens-1 (ZO-1) and vascular endothelial (VE)-cadherin. Furthermore, an increased expression of pro-inflammatory mediators (IL-1β, IL-6) and oxidative stress-related enzymes (inducible nitric oxide synthase, NADPH oxidase) along with an increased production of reactive oxygen species (ROS) were observed in our triple co-culture paradigm. Finally, we found an activation of immune response-regulating signaling pathways (Nrf2 and HO-1).
CONCLUSIONS: We developed the first high-reproducible in vitro BRB model mimicking the inner retinal barrier, entirely based on human cells (retinal pericytes, retinal astrocytes, and retinal endothelial cells). High glucose elicited remarkable changes in our triple co-culture system; first of all, the high glucose significantly reduced ZO-1 and VE-cadherin in endothelial cells decreasing BRB integrity; secondly, high glucose elicited genes expression related to inflammation and oxidative stress as well as antioxidant response. Taken together, the present findings show that our in vitro paradigm mimics the in vivo human inner BRB, suggesting that this model represents a useful tool for the drug discovery process in ocular pharmacology.

MALADAPTIVE RESPONSES TO STRESS ALTER GLUTAMATE RELEASE IN GLIOSOMES FROM RAT PRE-FRONTAL AND FRONTAL CORTEX

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BACKGROUND: Stress is a response retained throughout evolution and acts as an integral part of any biological system promoting an adaptive plasticity or maladaptive and harmful effects.
Exposure to acute or subacute stress can induce both rapid and sustained changes in synaptic function and neuroarchitecture. The aim of the present study was to investigate in the short term (24 hours after foot shock) effect of acute stress on the release of glutamate from gliosomes, representing the perisynaptic areas of astrocytes, of pre-frontal and frontal cortex (PFC/FC).
METHODS: Male adult Sprague Dawley rats (1 month old) were used. The sucrose preference was determined for 4-5 weeks and after acute foot shock (FS) stress. On the basis of sucrose consumption, animals were divided in vulnerable (VUL, subjects with a reduction in sucrose consumption > 25% after FS, compared to the basal consumption) or resilient (RES, subjects with a variation <10%). Rats were exposed to FS stress for 40 min during which random 0,8 mA shocks (length 2-8 s) were applied for 20 min. For release experiments, gliosomes were purified by homogenization and centrifugation on Percoll gradient. Gliosomes were incubated with [3H]D-Asp, to label the releasable glutamate (Glu) intra-terminal releasable pools,

and exposed in superfusion to a Ca2+-free solution, to TFB-TBOA (EAAT1 and EAAT2 glial Glu transporter inhibitor) and to KB- R7943 (inhibitor of the Na+/Ca2+ exchanger working in the reverse mode) under basal conditions or after depolarization with 15 mM KCl. Appropriate controls (without TFB-TBOA or KB-R7943) were always run in parallel.
RESULTS: About 50% of rats were VUL to FS stress, on the basis of sucrose test 24 hours after FS stress application, and about 50% were RES. The 15 mM KCl-evoked release of [3H]D-Asp was significantly increased after FS-stress in VUL rats only, while the basal release was unmodified. In VUL rats, the EAAT1 and EAAT2 inhibitor TFB-TBOA significantly reduced the KCl-evoked and the basal [3H] D-Asp release; while, Ca2+ omission or KB-R7943 produced a non-significant decrease of the stimulus-evoked [3H]D-Asp release.
CONCLUSIONS: These data suggest that acute FS stress produced an increase of [3H] D-Asp release from PFC/FC gliosomes, possibly increasing Glu availability in the synaptic cleft. This increase could be observed only in rat that were VUL to stress application. Excessive Glu release seems to be supported mainly by the inversion of glutamate transporters.

IMPULSE CONTROL DISORDERS BY THIRD GENERATION ANTIPSYCHOTICS

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BACKGROUND: In 2016 the FDA released a warning regarding the possible role of aripiprazole in inducing impulse control disorders (ICDs). Brexpiprazole and cariprazine, approved in 2015, share structure and molecular targets with aripiprazole and therefore potentially its serious sequelae. In absence of clinical studies investigating their relations with ICDs, we implemented a causality assessment using pharmacosurveillance data.
METHODS: The entire FDA Adverse Event Reporting System - Quarterly Data (from 2004 up to the first quarter of 2020) was downloaded and pre-processed for duplicates removal. Bradford Hill's criteria for causality assessment were then adapted accounting for both literature and multiple disproportionality approaches. We assessed the causal association between third generation antipsychotics (APS) and four groups of ICDs: pathological gambling, compulsive shopping, hypersexuality and binge eating. By the literature, we analyzed biological plausibility and analogy to drugs causing the event. By pharmacovigilance databases (FAERS), we evaluated strength (Reporting Odds Ratio-ROR), consistency across sensitivity analyses (doctor-reported, consumer- reported, different geographical areas, pre- and post-warning, psychotic patients), coherence with the pathogenetic impulsivity substrate ("impulsive behaviour"), specificity of the adverse event (by assessing association of ICDs with other APs, both on the whole population and among psychotic patients), temporal relationship (by checking for time to onset), biological gradient (relationship between the occurrence of ICDs and daily dose), reversibility of the adverse event (as dechallenge and rechallenge).
RESULTS: The cases of ICDs for aripiprazole, brexpiprazole and cariprazine were respectively 2435, 171 and 27. Strong associations were found for all third-generation APs with ICDs and with impulsivity, consistently across subpopulations. For aripiprazole, brexpiprazole and cariprazine, the ROR for gambling was respectively of 124 (95%CI=117-132), 26 (20-32)

and 21 (12-34); for compulsive shopping 208 (194-254), 35 (24-48), 32 (15-61); for hypersexuality 47 (43-51), 9 (6-14), 15 (7-29); for binge eating 9 (8-10), 24 (19-31), 12 (6-22). While disproportionality was found for many APs, for the psychotic subpopulation only third generation APs and lurasidone were possible causes. The median time to onset falls between days and months, doses involved are slightly higher than expected, and dechallenge and rechallenge rates are respectively around 20% and 3%. Analogy, plausibility, strength, consistency, coherence, temporal efficiency and reversibility were verified.

CONCLUSIONS: Unexpected signals of ICDs for third generation APs emerged as supported by likely causal association. This safety issue deserves clinical monitoring and implementation of cohort studies to investigate the association further. Our adjusted Bradford-Hill algorithm could be further used to standardize pharmacovigilance studies.

ANTIBIOTIC PRESCRIPTION AND CONSUMPTION AMONG ADULT OUTPATIENTS IN TWO ITALIAN REGIONS

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BACKGROUND: Antibiotics are among the most prescribed drugs in outpatient settings, but inappropriate consumption and over-prescription have been extensively reported, resulting in increased antimicrobial resistance. The Italian situation is critical, both with regard to the spread of antibiotic resistance and the consumption of systemic antibiotics. Our aim was to retrospectively evaluate the antibiotic prescribing patterns in two Italian Regions, using regional administrative pharmaceutical prescription databases, during a 3-year period (2014-2016).

METHODS: This analysis was based on baseline data from the EDU.RE.DRUG project (funded by the Italian Medicines Agency), regarding prescriptions from all general practitioners (GPs) (≈4,200) and their adult (≥40 years) patients (≈3,300,000) from four local health units (LHUs) in Lombardy and four in Campania. Annual antibiotic consumption (expressed as DID -number of Defined Daily Doses/1000 inhabitants per day) and prevalence (percentage of patients with at least one antibiotic prescription) rates were estimated at LHU level. ESAC (European Surveillance of Antimicrobial Consumption)-based quality indicators were also assessed to describe antibiotic use in primary care. Finally, a multivariate logistic regression analysis was performed to evaluate the association between the probability of receiving an antibiotic prescription and selected GP and patient's characteristics.

RESULTS: In 2016, the mean prevalence rates were 33.1% [range LHUs: 31.7-35.0] in Lombardy and 58.0% [57.0-59.3] in Campania, with a slight prevalence reduction compared to 2014 (-2.1% and -4.7%, respectively). A great regional variability was also found in mean antibiotic consumption in 2016 (13.1 DID [range LHUs: 12.3-14.5] in Lombardy and 28.0 DID [27.2-29.3] in Campania), with a decreasing trend compared to the mean values in 2014 (-4.4% and -3.5%, respectively). Higher consumption patterns in Campania LHUs were also showed by ESAC indicators. Over the 3-year period, penicillins were the most used (≈40% of total DID), followed by quinolones (≈25%) and macrolides (≈20%) in all the LHUs. The logistic regression analysis confirmed that living in

Campania increased the probability of receiving an antibacterial prescription (OR 2.28; 95% CI 2.27-2.29). Conversely, male and older (≥65 years) patients were 14% (OR 0.86; 95% CI 0.86-0.87) and 2% (OR 0.98; 95% CI 0.98-0.99) less likely to receive an antibiotic prescription compared to female and younger adults (40-64 years), respectively. Regarding GP's characteristics, being male or younger was a risk factor for antibiotic prescription.

CONCLUSIONS: Despite national and international guidelines aiming to optimize antibiotic prescription in community outpatients, and a slight decrease trend over time, we observed high antibiotic consumption rates, supporting a potential inappropriate prescription and use in Italy, with a significant variation between Regions. Therefore, both national and local interventions are highly recommended.

PHARMACOLOGICAL TREATMENT OF DIABETIC FOOT ULCER BASED ON QUERCETIN AND OLEIC ACID

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BACKGROUND: Wound healing, especially diabetic ones, is a relevant clinical problem, so it is not surprising that surgical procedures are often needed. To overcome invasive procedures, several strategies with drugs or natural compound are used. Recently, in an experimental study, we described an increase in keratinocyte proliferation after their exposition to quercetin (Qu) plus oleic acid (OA) used in equimolar concentration[1]. Pharmaceuticals use nanocrystal to increase the transfer of skin drugs. But the fluidity of nanocrystals suspension causes a short retention time for the direct drug delivery. So that nano-hydrogel are better carrier/ candidate to achieve this purpose.
METHODS: Herein, we reported both the clinical efficacy and the safety of nano-hydrogel embedded with quercetin and oleic acid in the treatment of lower limb skin wound in patients with diabetes mellitus (DM). Fifty-six DM patients (28 men and 28 women, mean age 61.7 ± 9.2 years) unsuccessfully treated with mechanical compression were enrolled and randomized to receive an add on treatment with hyaluronic acid (0.2%) or nano-hydrogel embedded with Qu and OA.
RESULTS: The treatment with nano-hydrogel embedded with quercetin and oleic acid significantly ($P < .01$) reduced the wound healing time, in comparison to hyaluronic acid (0.2%) without developing of adverse drug reactions, suggesting that this formulation could be used in the management of wound healing even if large clinical trials must be performed in order to validate this observation. Conclusions: Recently we documented both in vivo and in vitro Qu and OA as well as its ester obtained enzymatically is able to restore skin lesions acting as a GPR40 agonist[1].
CONCLUSIONS: in the present observational blinded study, we recognized that a topical formulation nano-hydrogel based and loaded with Qu and OA induced a rapid wound healing compared to topical administration of hyaluronic acid (2%).

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ANTIPROLIFERATIVE EFFECT OF CANNABINOIDS IN HUMAN CELL LINES

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BACKGROUND: The biological effects of cannabinoids, the main constituents of Cannabis sativa, are mediated by the activation of G protein coupled receptors, CB1 and CB2, mainly expressed in the central nervous system and in the peripheral nervous system respectively [1]. For a long time, the psychoactive effects of delta-9-tetrahydorcannabinol (THC) have limited the use of Cannabis in therapy, but in recent years the therapeutic potential of cannabinoids has become increasingly evident, particularly in neurodegenerative diseases [2]. We studied the antiproliferative effect of cannabidiol (CDB) and THC on two cell lines: SH-SY5Y, a neuroblastoma cell line, and THP-1, a human monocyte cell line. These two cell lines were chosen since in literature the neuroprotective effects of cannabinoids have been correlated not only to neuronal activity, but also to effects on immune system.
METHODS: The two phytocannabinoids have been tested alone or in association, in the same ratio in which they are present in Cannabis FM-2, for times ranging from 24 to 72h. Furthermore, the activity of the individual phytocannabinoids was compared with extracts produced in the laboratory of Organic Chemistry of Prof. Barge. The anti-proliferative effect was assessed using the Cell-Titer Glo (Promega) assay. In addition, the ability of CBD and THC, alone or in combination, to reduce the cytotoxicity induced by 6-hydroxydopamine (6-OHDA) in SH-SY5Y, Parkinson model in vitro, was evaluated [3].
RESULTS: CBD is more cytotoxic than THC in SH-SY5Y cell line, with an evident effect after 24 hours of treatment. The combination has a similar effect to CBD, which however tends to increase with the prolongation of the treatment. Nonetheless in THP-1 cell line the cytotoxicity induced by CDB and THC is comparable; the CDB + THC association is able to inhibit cell proliferation at a lower concentration. None of the tested compounds are shown to reduce 6-OHDA-induced cytotoxicity, when administered 30 minutes after 6-OHDA.
CONCLUSIONS: The effects of CBD and THC is cell type dependent, with a very similar effect in THP-1, while in SH-SY5Y CBD is more cytotoxic. In the future, times of treatment will be modulated and different ratios of CBD and THC will be tested, even in an in vitro Alzheimer model.

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SHOULD SKELETON INJURY BE ADDED TO THE EVOLVING LIST OF IMMUNE-RELATED ADVERSE EVENT WITH IMMUNE CHECKPOINT INHIBITORS? INSIGHTS FROM PHARMACOVIGILANCE

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BACKGROUND: The advent of immune checkpoint inhibitors (ICIs) has dramatically improved patients' life expectancy in various cancers. Although ICIs are associated with a variety of immune-related adverse events (irAEs), the potential effect on the skeleton is poorly defined albeit biologically plausible, considering that activated T-cells increased pro-inflammatory cytokines and up-regulated RANK-L, thus promoting bone loss.
METHODS: The US FDA Adverse Event Reporting System (FAERS) database was queried to retrieve ICI reports including anti-CTLA-4 (ipilimumab, tremelimumab), anti-PD-1 (nivolumab, pembrolizumab, cemiplimab), anti-PD-L1 (atezolizumab, avelumab, durvalumab) recorded as suspect between the first quarter (Q1) of 2004 and Q1 of 2020. We searched all the 112 preferred terms (PTs) listed in "Bone and joint injuries" High Level Group Term, and PTs "osteonecrosis", "osteonecrosis of jaw", and "osteonecrosis of external auditory canal". The event "fall" was searched as negative control. Disproportionality analysis was performed by calculating the Reporting Odds Ratio (ROR) with 95% confidence interval (CI), deemed statistically significant by a lower limit of the 95%CI>1, with at least five cases reported. All other drugs/events recorded in FAERS were used as comparator. Skeleton events emerging with significant disproportionality were characterized in terms of demographic information, latency, seriousness, fatality rate, therapeutic regimen, proportion of fall, overlap with other irAEs (myositis, endocrine and neurological events), confounders (including bone metastases, and agents acting on bone resorption or causing bone damage).
RESULTS: Overall, 95,787 reports mentioning at least one ICI as suspect were retrieved, and 650 patients (0.68%) with bone and joint injuries were found, accounting for 822 drug-event pairs. Median onset of skeleton events was 138 days. Statistically significant ROR was found for eight, two, and one AEs respectively with PD-1, PD-L1, and CTLA-4 inhibitors, being spinal compression fracture (N=47; ROR=2.51; 95%CI=1.91-3.40), pathological fracture (46; 3.17; 2.37-4.24), and femoral neck fracture (26; 2.38; 1.62-3.50) the most common. Concomitant bone metastases, endocrine and neurological irAEs were retrieved in 8.3%, 9.8%, and 11.6% of cases, respectively. Drugs acting on bone resorption or causing bone damage were concomitantly reported respectively in 12.1% and 15.8% of cases. PPIs were used in 16.3% of cases. Fall was reported in 13.0% of cases, with no significant ROR for any ICI class or combination. Myositis was not recorded. Overall proportion of deaths was 34.0%.
CONCLUSIONS: We characterized for the first time worldwide skeletal irAEs with ICIs. The increased reporting of serious spinal compression fracture in patients without concomitant irAEs and no apparent pre-existing risk factors calls for both close clinical monitoring and implementation of dedicated guidelines.

SERIOUS ADVERSE EVENTS WITH NOVEL BETA-LACTAM/BETA-LACTAMASE INHIBITOR COMBINATIONS: THE ROLE OF PHARMACOVIGILANCE IN PRIORITIZING MONITORING IN SEVERE MULTI-DRUG RESISTANT GRAM-NEGATIVE INFECTIONS

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BACKGROUND: Ceftolozane-tazobactam (C/T) and ceftazidime-avibactam (C/A) are novel beta-lactam/beta-lactamase inhibitors (BL/BLIs) characterized by activity against multi-drug resistant (MDR) gram-negative pathogens. Although the risk-benefit ratio is generally favourable in severe MDR infections, safety profile of these antibiotics is still poorly investigated.

METHODS: The US FDA Adverse Event Reporting System (FAERS) database was queried to retrieve C/T and C/A reports recorded as suspect between the first quarter (Q1) of 2015 and Q1 of 2020. In order to assign a clinical priority to emerging safety issues, the public list developed by the European Medicines Agency including 62 different reactions was used to select designated medical events (DMEs), namely rare, serious adverse events (AEs) with a recognized drug-attributable risk. DMEs showing significant disproportionality were further classified into three broad categories: 1. expected AEs (anticipated from pivotal trials); 2. disease-related AEs (underlying sepsis/septic shock represents per se a risk factor); 3. unexpected AEs. Furthermore, given the non-negligible risk of neurotoxicity reported with cephalosporins, the following preferred terms were also investigated: seizure, generalised tonic-clonic seizure, myoclonus, status epilepticus, neurotoxicity, and confusional state. Disproportionality analysis was performed by calculating the Reporting Odds Ratio (ROR) with 95% confidence interval (CI). Potential signals were defined by statistically significant ROR (lower limit of the 95%CI>1), with at least three cases reported. All other drugs/events recorded in FAERS and cephalosporins showing clinical evidence of neurological AEs (namely cefazolin, ceftriaxone, cefixime, cefotiam, ceftazidime, and cefepime) were respectively selected as comparator for analysis of DMEs and neurotoxicity.

RESULTS: Overall, 624 and 468 AEs mentioning respectively C/T and C/A were found, of which 65.4% and 86.3% were serious. DMEs were respectively reported in 67 (10.7%) and 46 cases (9.8%) with C/T and C/A. Increased reporting was found for acute kidney injury (N=21; ROR 5.04; 95%CI 3.26-7.79), agranulocytosis (12; 22.76; 12.85-40.31), and renal failure (25; 7.63; 5.11-11.39) with C/T. Acute kidney injury (15; 4.79; 2.87-8.02), anaphylactic shock (3; 7.35; 2.36-22.89), haemolytic anaemia (3; 12.34; 3.97-38.41), hepatic failure (4; 6.15; 2.30-16.46), and renal failure (11; 4.40; 2.42-8.00) emerged as over-reported DMEs with C/A. Compared to other cephalosporins, only generalised tonic-clonic seizure (11; 3.70; 2.01-6.83) with C/T exhibited significant ROR.

CONCLUSIONS: The post-marketing reporting pattern of life-threatening events with novel BL/BLIs is largely predictable based on pre-approval evidence and settings of use. The unexpected reporting of agranulocytosis coupled with over-reporting of generalised convulsive crisis exhibited by C/T calls for stringent clinical monitoring in high-risk patients.

IN VITRO BIOCOMPATIBILITY STUDIES OF NANOSTRUCTURES (NANOEMULSIONS AND NANOFIBERS) FOR EDIBLE APPLICATION

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BACKGROUND: Nanoencapsulation is a process designed to protect a bioactive compounds from physical conditions or physiological barriers, such as the gastrointestinal acidification, enhancing the stability, solubility and bioavailability of encapsulated matrix. Nanoencapsulation can be achieved through emulsification and electrospinning which allow to obtain nanoemulsions (NEs) and nanofibers (NFs), respectively. NEs are colloidal dispersions between oil and water with nano-scale droplet sizes, while NFs are elongated, and threadlike shaped. Nano-sized structures have particular properties, exploited in several applications, including foods. Gelatin-based nano fibers (NFs) and nanoemulsions (NEs) loaded with high oleic palm oil (HOPO) are promising materials for edible applications. In this study we investigated *in vitro* the biocompatibility and eventually toxic effects of these two nanostructures on human cells.

METHODS: We first evaluated the effect of NFs and NEs loaded with different HOPO concentrations, on fibroblast (CCD-18Co) and endothelial cells (HUVEC) viability by MTT assay. To this purpose, cells were stimulated for both short and long time, from 24 to 72 h, with a broad concentrations of test compounds (5, 10, 20 and 50 µg/ml), and cell metabolic activity was measured using MTT solution. Secondly, we analyzed markers of different cell processes, such as cell death (Caspase3), inflammation (COX-2), oxidative stress (Catalase) and HUVEC homeostasis (endothelial nitric oxide synthase eNOS) by Western blot. In the second analysis we stimulated cells for 24 h with nanostructures at the highest concentration of 50 µg/ml. Stimulation with hIL-1β (10 ng/ ml) was used as positive control for inflammation. Finally, CCD-18Co and HUVEC morphology was observed, using an optical microscope before and after stimulation with test compounds.

RESULTS: Our findings showed that NFs and NEs were not cytotoxic on both cell lines. Indeed, all the concentrations tested did not affect cells viability, which reached 90% at all time-points evaluated. In agreement, the expression of cleaved-caspase 3 did not vary after 24 h of CCD-18Co and HUVEC exposure to NEs and NFs. The expression of eNOS, a marker of endothelial function, was not altered. Similar results were obtained for cyclooxygenase and catalase, showing neither oxidative stress nor inflammatory response. Normal morphology was confirmed for both cell lines,with no toxic effect of NEs or NFs loaded with HOPO.

CONCLUSIONS: Considering the results obtained in this work, we conclude that NEs and NFs had not toxic effect on human cells in vitro. This work could contribute to the development of normative rules for nanostructures on foodstuffs. Although further in vivo studies must be carried out, the nanostructures analyzed may be considered for edible applications in food industry.

CHARACTERIZATION OF CARBONIC ANHYDRASE IX INHIBITORS ON HUMAN ENDOTHELIAL CELLS

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BACKGROUND: The vascular endothelium is one of the first barriers encountered by drugs and xenobiotics, which, once administered, enter the blood stream and diffuse to all organs through blood vessels. The continuous exposure of endothelial cells to drugs and chemical compounds turns out to be a huge risk for the cardiovascular system, as these substances could compromise endothelial vitality and function and create damages. For this reason, cardiovascular safety remains the major concern in drug development and it is fundamental for any medication to be screened for toxic effects on endothelial cells. In this study we focused our attention on carbonic anhydrase (CA)-IX inhibitors. CA-IX is an enzyme over-expressed in tumor cells as a response to hypoxia, which is involved in pH control of the neoplastic mass microenvironment and in tumor progression. We evaluated the safety on human umbilical vein endothelial cells (HUVEC) of CA-IX inhibitor AA-06-05, compared to its lead compound SLC-0111, for which the efficacy on tumor cells has already been proven.
METHODS: SLC-0111 is a ureido benzene-sulphonamide, currently in phase 2 clinical trial for the treatment of solid tumors associated to hypoxic microenvironment. AA-06-05 is a SLC-0111 congener obtained by a divalent isosteric replacement approach. First, we evaluated the presence of CA-IX and CA-I in HUVEC under normoxic conditions, in presence of high VEGF levels or 100 μM $CoCl_2$ to simulate hypoxia. Secondly, we exposed HUVEC to test compounds, with or without vascular endothelial growth factor (VEGF), for 48h. Metabolic activity was assessed by MTT assay and cell viability though proliferation assay. Pro-survival signals (AKT and ERK1/2) and CA isozyme expression, were evaluated by Western Blot.
RESULTS: In this analysis we detected an impairment in viability and mitochondrial metabolism of HUVECs treated with AA-06-05 (but not with SLC-0111) in the concentration range 1–10 μM. The inhibitor AA-06-05, compared to what observed for its lead compound SLC-0111, operates by interfering with CA-I and CA-IX functioning, reflecting negatively on HUVEC viability and metabolic capacity. These data were accompanied by an increase in the expression of the cell cycle negative regulator, p21, and a down-regulation of the pro-survival proteins ERK1/2 and AKT, both in their phosphorylated and total forms.
CONCLUSIONS: The data obtained document the likelihood for CA-IX inhibitor AA-06-05 to be developed as new anticancer drug, but a particular attention should be paid to its potential side effects on endothelial cells due to its targeting on other CA isoforms as CA-I, with ubiquitous expression and physiological significance.

TREATMENT WITH TAT-GILZ FUSION PROTEIN AMELIORATES DSS EXPERIMENTAL COLITIS IN MICE

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BACKGROUND: Glucocorticoids (GCs) are a class of hormones released by the adrenal cortex thatare used as powerful anti-inflammatory drugs in a variety of inflammatory, autoimmune and neoplastic diseases. Glucocorticoid-Induced Leucine Zipper (GILZ) is an early GC-induced gene that mimics several anti-inflammatory effects of GCs.Inparticular,GILZ-regulates the activity of cells of the immune system,including T lymphocytes, macrophages and B cells.Interestingly, GILZ has been found to be protective in some experimental models of inflammatory bowel diseases, but the exact mechanisms by which GILZ exerts its activity is still under investigation.In this work,we tested the potential anti-inflammatory effect of the trans-activator of transcription peptide-GILZ (TAT-GILZ) fusion protein in a colitis model of inflammatory bowel disease (IBD).
METHODS: The Dextran Sodium Sulphate (DSS) experimental colitis model was performed inthe C57BL/6 strain of 8-weeks old mice.Four days after colitis induction with 3% DSS in drinking water (acute colitis),4 separate groups of mice (n=5/group) were treated with-PBS (control), Dexamethasone (a synthetic GC), TAT (Control), and TAT-GILZ, respectively, for 5 days.Disease Activity Index (DAI) has been recordedthroughout the experiment.At the end of the experimental protocol, mice were sacrificed and colons collected to be analysed by real-time PCR, western blotting, histology and immunohistochemistry (IHC). A FITC-Dextran assaywas also performed to investigate variations on intestine permeability.
RESULTS: TAT-GILZ fusion protein ameliorated the symptoms of the disease as demonstrated by the reduction in weight loss, resulting in a lower DAI compared with colitic controls. Histological analysis showed a lower leucocyte infiltrate and a better reepithelizationin TAT-GILZ treated mice than in controls. Interestingly, TAT-GILZ treatment led to the decrease of intestinal permeability. Furthermore, in TAT-GILZ-treated mice, the analysis of RNA and protein expression of whole colons extracts showed high levels of tight junction proteins and ERK phosphorylation, similar to the healthy group. No differences were found in immune cells subsets and cytokine release across all groups.
CONCLUSIONS: TAT-GILZ fusion protein resulted to have beneficial effects on DSS experimental colitis symptoms after the onset of the disease.Interestingly, the treatment with TAT-GILZ ameliorated the gut permeability,which is altered in colitis and has a pathogenic role. At the molecular level, the better permeability was supported by an overexpression of tight junction proteinsand enhanced intracellular signalling, both contributing to reepithelization. In conclusion, the treatment with TAT-GILZ fusion protein could be a pharmacological tool to ameliorate the colitis symptoms.

A MULTIDISCIPLINARY APPROACH FOR THE IDENTIFICATION AND VALIDATION OF NOVEL PROSTATE CANCER TARGETS

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BACKGROUND: Prostate cancer has recently been classified as the second most frequently diagnosed malignancy among men and the fifth leading cause of cancer-associated death worldwide. Although the 5-year survival rate for patients diagnosed with localized and regional prostate cancer is almost 100%, metastatic and castration-resistant prostate cancers are currently difficult to treat. Therefore, there is an urgent need to develop new tools for prognostic patients' stratification. While pathological, surgical and biochemical prognostic factors are useful tools for risk stratification, novel biomarkers are required to improve the reliability of these conventional determinants. In this setting, next-generation sequencing revealed the existence of many genetic alterations and allowed the development of novel prognostic markers. For instance, the loss of the tumor suppressor gene Pten has been identified as crucial to the disease progression, while further genomic aberrations can accelerate carcinogenesis, increase the aggressiveness and promote the onset of resistant phenotypes. Accordingly, the aim of this project is the identification of overexpressed genes potentially compatible with the role of oncogene and therefore targetable with existing or innovative pharmacological strategies.
METHODS: In order to identify novel therapeutic targets in the treatment of prostate cancer, we performed RNA-sequencing and polysome profiling analysis on prostate specimens isolated from five different genetically engineered mouse models of prostate cancer. Next, in silico analyses have been carried out to select metabolic genes significantly and polysome differentially upregulated in all the mouse models tested. To validate the expression level of promising candidates both real-time PCR and western blot analysis have been performed. Lastly, the expression of gene candidates' cognate proteins has been immunohistochemically evaluated on human FFPE prostate cancer samples.
RESULTS: In this work we identified three promising gene candidates, which are significantly and polysome differentially upregulated in five different mouse models of prostate cancer. Interestingly, through in silico analysis these genes were found to harbor focal amplifications, while their expression pattern increases with tumor progression. Taken together, these analyses suggest a role of our candidates as potential oncogenes. Moreover, immunohistochemical analysis revealed a high heterogeneity of expression both intra and inter tumoral but a clear overexpression in high Gleason tumors.
CONCLUSIONS: This multidisciplinary and translational approach allowed the identification of three promising gene candidates

potentially compatible with the role of oncogenes. These promising, but still preliminary results, will be further validated in in vivo and in vitro models and in a large well-annotated human cohort paving the way for the discovery of novel therapeutic targets.

ROLE OF POLY(ADP-RIBOSE) POLYMERASE INHIBITORS IN COMBINATION WITH ASCORBATE AND HYPOMETHYLATING AGENTS FOR ACUTE PROMYELOCYTIC LEUKAEMIA RESISTANT TO ARSENIC TRIOXIDE

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BACKGROUND: Current treatment of acute promyelocytic leukaemia (APL) is based on the use of all-trans retinoic acid (ATRA) combined with anthracyclines or with arsenic trioxide (ATO). However, 5-10% of patients develop relapsed/refractory disease. Recent preclinical studies have shown that poly (ADP-ribose) polymerase inhibitors (PARPi) might be effective against myeloid malignancies. Besides possessing cytotoxic effects in tumour cells with DNA repair defects, PARPi modulate gene expression that is frequently altered in haematological malignancies. Herein, we have investigated PARPi activity in APL cells rendered resistant to ATO, as single agents and in combination with DNA demethylatig agents. In particular, we tested azacitidine (AZA) and decitabine (DAC), known to cause DNA damage involving PARP1 intervention, and ascorbate (ASC), which has recently been shown to promote DNA demethylation, mimicking the action of Ten-Eleven Translocation-2 (TET2), and to increase DNA oxidation that triggers a DNA damage response.
METHODS: In order to generate ATO-resistant cells, four different clones of the human APL NB4 cell line were exposed to increasing concentrations of ATO (0.1-1 μM). Sensitive (CL1–4) and resistant clones (CL1R-4R) were characterized for PML/RARA expression (pathognomonic of APL) and proliferation rate. Cells were treated with the PARPi olaparib (OLA, 1.25-10 μM), rucaparib (RUC, 1.25-10 μM), niraparib (NIR, 1.25-10 μM), veliparib (VEL, 5-20 μM) and talazoparib (TAL, 12.5-100 nM), as single agents or in combination with ASC (0.25-1 mM), AZA (1.25-1 μM) or DAC (25-500 nM). Drug concentrations tested included the Cmax values reached in cancer patients. Cytotoxicity was evaluated by MTS assay and count of viable cells, apoptosis by flow cytometry and detection of cleaved caspases, DNA damage by γH2AX immunofluorescence and immunoblot, reactive ox-

ygen species (ROS) by flow cytometry, and 5-hydroxymethylcytosine (5hmC) by dot blot analysis of genomic DNA.
RESULTS: ATO-resistant NB4 cells (ATO IC50 >1 μM) maintained the APL phenotype with PML/RARA expression. The PARPi IC50 values obtained in ATO-sensitive and -resistant clones were far below (OLA and NIR) or within (TAL) the plasma Cmax range, whereas for RUC and VEL IC50s were above Cmax. Treatment with ASC, AZA or DAC inhibited the growth of both ATO-sensitive and -resistant clones and exerted synergistic effects in combination with PARPi. The ASC and PARPi synergistic effect was observed at ASC concentrations that did not induce ROS production and was associated with a significant increase of 5hmC and γH2AX. Since active DNA demethylation involves DNA damage repair pathways, we speculate that PARPi can increase cell sensitivity to DNA damage induced by ascorbate.
CONCLUSIONS: Our data suggest the therapeutic potential of PARPi as monotherapy or in combination with AZA, DAC or ASC for refractory/recurrent APL through mechanisms involving modulation of DNA damage.

SEVERE MULTIPLE SCLEROSIS COURSE AFTER ALEMTUZUMAB TREATMENT: A CASE REPORT

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BACKGROUND: Alemtuzumab (AL) is an anti-CD52 monoclonal antibody approved for Relapsing Remitting Multiple Sclerosis (RRMS). Severe exacerbation of CNS inflammation has been reported in a minority of AL-treated MS patients, and in some associated with enhanced B-cell repopulation, suggesting a role for AL in triggering B-cell-driven intrathecal autoimmunity.
METHODS: We report clinical features, MRI and peripheral blood lymphocyte counts serially collected; cytokine profile was assessed on blood samples before each of the 3 AL cycles.
RESULTS: The patient was diagnosed with RRMS in 2007 after recurrent optic neuritis. She was previously treated with glatiramer acetate, interferons and dimethylfumarate, withdrawn for lack of efficacy. She experienced a relapse in November 2015 and was given the first AL cycle in February 2016 at the age of 36. Expanded Disability Status Scale (EDSS) was 1.5. CD19+ were normal in November 2015 (9.6%) and May 2016 (14.7%). The second AL cycle was given in March 2017. CD19+ were 31.1%. She was clinically and radiologically stable up to February 2018 when a relapse occurred (EDSS 2.5) with a new cervical enhancing lesion. Methylprednisolone (MP) prompted partial benefit. In March 2018 she worsened with gait ataxia, lower limbs weakness and paresthesias (EDSS 4.0). MRI showed new brain and spinal cord active lesions. CD19+ were normal in CSF and 22.3% in peripheral blood. MP and 5 plasma-exchanges were not effective. In May 2018 she received a third AL cycle. She relapsed in October 2018 (burning dysesthesias and hyperesthesia, CD19+ 33.8% and two new spinal enhancing lesions). She experienced further relapses with clinical worsening (EDSS 6.5) up to July 2019. Multiple MP courses were

not effective (CD19+ were 21.0%). Autologous blood stem cells transplantation was performed in August 2019. MRI performed in November 2019 did not show signs of disease activity (EDSS 6.5). The patient is currently treated with pregabalin, acyclovir and candidate to cannabinoids. Cytokine profile assessed before each AL course showed suppression of IL-10 and IL-1β and progressive reduction of IL-9, IFN-γ and TNF-α during follow up.
CONCLUSIONS. We report a case of recurrent CNS inflammation despite three AL courses, leading to severe symptoms and blood stem cells transplantation. Recurrent CNS reactivation was not paralleled by consistent peripheral B-cell increase as reported in the literature at least in some patients. The possibility of enhancement of autoreactivity against the CNS due to defective recapitulation after AL treatment should be further investigated with serial immunological and functional assessments to improve understanding of both AL loss of efficacy and/or AL-driven autoimmunity, hence guiding subsequent therapeutic decisions.

ATRIAL FIBRILLATION IN A 15-YEAR-OLD BOY AFTER ENERGY DRINK CONSUMPTION: A CASE REPORT

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BACKGROUND: Energy drinks (ED) are high-caffeine content beverages widely consumed especially by adolescents and young adults, also due to an aggressive marketing campaign. EDs contain a variable labeled caffeine amount, usually ranging 80 to 500 mg, as well as ingredients such as guarana extracts (also containing "masked" caffeine), taurine, ginseng, carnitine, maltodextrins, vitamins and amino acids. Caffeine is generally considered safe but its overconsumption has been associated with atrial fibrillation (AF), particularly in predisposed subjects, and some cases of AF and serious cardiovascular (CV) adverse events in adolescents consuming EDs were reported.
METHODS: We described a case of AF in an adolescent admitted to emergency room (ER) after ED consumption. Clinical history, concomitant medications, vital signs, instrumental exams, blood and toxicological tests results, and clinical course were retrieved from the involved subject and ER data.
RESULTS: A 15-year-old boy accessed the ER of ASST Grande Ospedale Metropolitano Niguarda, Milan, presenting with chest discomfort and palpitations. He had drunk one ED and two soft drinks, all containing caffeine in different amounts, approximately one hour before symptoms onset. Clinical history was negative except for occasional palpitations after exercise with spontaneous resolution. The patient was not taking any medication. At physical examination tachycardia (135 bpm) with irregular rhythm was present. Electrocardiogram confirmed the presence of AF. Blood tests were normal and toxicological tests were negative. Echocardiography was normal with preserved ejection fraction. Intravenous amiodarone 150 mg/3mL was administered in one hour, without resolution of AF. An electric cardioversion (70 J) was performed, with sinus rhythm restoration. No other arrhythmic episode was observed during hospital stay. The final diagnosis at discharge was

"AF induced by EDs in a subject with atrial hyperexcitability". He was recommended to avoid EDs and caffeine consumption. Follow-up visit at one month was negative.
CONCLUSIONS: Our patient caffeine total intake could be estimated around 220 mg (160 in ED; 34 in each soft drink). The sole presence of highly concentrated caffeine and other stimulants (e.g. taurine or guarana) in EDs does not justify AF occurrence in young and healthy individuals. However, EDs overconsumption, underlying CV abnormalities, or predispositions in users, may play a role in EDs toxicity. EDs consumption is also frequently associated with alcohol and other stimulants that may exacerbate their effects. Lack of clear toxicologic data and high-quality evidence on the topic associated with limited regulation for EDs formulation represents a potential health hazard for adolescents and young adults. Physicians should be aware of potential arrhythmogenic effects of EDs while counseling patients and should take into account EDs consumption during diagnostic procedures of acute CV events.

OVERALL EFFICACY AND SAFETY OF SAFINAMIDE IN PARKINSON'S DISEASE: A SYSTEMATIC REVIEW AND A META-ANALYSIS

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BACKGROUND: Safinamide (SAF) is a novel Parkinson's disease (PD) drug with possible anti-dyskinetic properties due to its action both on dopamine and glutamate systems. PD is a complex disease and multiple outcome assessment of SAF efficacy is needed.
METHODS: A systematic review with meta-analysis was performed. PubMed, EMBASE, Cochrane CENTRAL, LILACS, and trial databases were searched up to 31 October 2019 for phase 3 randomized controlled studies (RCTs) comparing SAF to placebo, alone or as add-on therapy in PD. Post hoc analyses were excluded. Data were extracted from literature and regulatory agencies. Primary outcomes were "on" time without troublesome dyskinesia, "off" time, and Unified PD Rating Scale (UPDRS)-III. Secondary outcomes were: any scales rating dyskinesia; UPDRS-II; and Parkinson's Disease Questionnaire 39. Assessed safety outcomes were patients experiencing any serious AE (SAE) and treatment discontinuation due to AEs. Meta-analysis was performed on each outcome with data from at least two included studies. Generic inverse variance on mean difference (MD) and Mantel-Haenszel method to

estimate odds ratios were used for continuous and dichotomous variables, respectively. Analyses were grouped based on 1) the presence of motor fluctuations and concomitant use of dopamine agonists (DA) or levodopa (LD), and 2) the administered dose of SAF. Evidence quality was assessed with the GRADE method.
RESULTS: Four studies with a total of 2166 participants were identified. Moderate quality of evidence based on 995 participants randomized in 2 RCTs showed an improvement in "on" time (MD=0.71 hours; 95% confidence interval, CI, 0.37 to 1.05), "off" time (MD=-0.81 hours; 95% CI -1.23 to -0.40), and UPDRS-III (MD=-2.04; 95% CI -3.04 to -1.05) in PD patients with motor fluctuations treated with LD and SAF 100 mg daily. Moderate quality of evidence based on 632 participants randomized in 2 RCTs showed little to no meaningful improvement in UPDRS-III (MD=-1.43; 95%CI -2.64 to -0.22) in PD patients without motor fluctuations treated with a single DA and SAF 100 mg daily. Moderate quality evidence showed UPDRS-II was improved in fluctuating PD patients taking LD (MD=-0.68; 95% CI -1.24 to -0.13; p=0.02; I2=33%) and slightly improved in non-fluctuating PD patients taking a DA (MD=-0.56; 95% CI -1.02 to -0.11; p=0.02; I2=0%) by SAF 100 mg. In fluctuating patients taking LD, SAF 100 mg was not effective in changing dyskinesias assessed with Dyskinesia Rating Scale (DRS), with low quality of evidence (Re-expressed standardized MD=0.42; 95%CI -4.34 to 5.04).
CONCLUSIONS: SAF is effective in fluctuating PD patients taking LD. Evidence for efficacy in non-fluctuating PD patients taking DA is limited. Inefficacy in DRS change with low quality may be due to a modified version of original DRS was used. DRS was afterward demonstrated to be not sensitive to changes in dyskinesia. Further trials are needed to better evaluate the anti-dyskinetic properties of SAF.

THERAPEUTIC DRUG MONITORING MIGHT IDENTIFY POOR RESPONDERS TO RUXOLITINIB TREATMENT IN MYELOPROLIFERATIVE NEOPLASMS

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BACKGROUND: Myeloproliferative neoplasms (MPNs) are a group of clonal hematologic disorders caused in more than 50% of cases by somatic mutations in Janus Kinase 2 (JAK2). In 2011, the first JAK1/2 inhibitor, ruxolitinib (ruxo), was approved for the treatment of myelofibrosis (MF); however, JAK inhibitors fail in 50-75% of cases over a 5-year follow-up, and discontinuation is mainly caused by treatment-related adverse events (ADRs). The pathophysiology of ADR and the lack of consistent molecular response to ruxo are still under investigation. Several mechanisms have been proposed, such as molecular processes that could skip JAK2 inhibition. Therefore, patients could be exposed to suboptimal or toxic drug doses leading to treatment inefficacy or ADR development. In this study, we correlated ruxo plasma levels with new biomarkers for therapeutic drug monitoring of MPN patients.

METHODS: Plasma samples from four MPN patients and 10 age- and sex-matched healthy controls (HC) were collected from EDTA whole blood. Patients were on ruxo treatment (median treatment length, 27 months; range, 18-44), and three of them were diagnosed with secondary myelofibrosis (number of patients JAK2+/JAK2-, 1/2) and one with JAK2+ polycythemia vera (PV) (clinical characteristics in Figure 1A). Ruxo plasma levels were measured by HPLC as previously optimized by Our Group, and proteomics analysis of pooled plasma samples was carried out by mass spectrometry. Statistical analysis was performed by RStudio and Prism, and protein pathway analysis by String and Reactome databases.
RESULTS: Ruxo-treated patients displayed a mean drug level of 127.8 ng/mL (range, 1-407 ng/mL). Plasma samples from healthy donors were used as negative controls. No correlations were described between ruxo concentrations and complete blood counts (CBCs); while a slight correlation between circulating ruxo levels and LDH was described ($r = 0.950$; $P = 0.0502$) (Figure 1B). The patient with the highest ruxo plasma levels and LDH had a diagnosis of JAK2+ post-PV MF and achieved only a partial response. Qualitative proteomics analysis of plasma samples from ruxo-treated patients revealed 12 candidate biomarkers of responsiveness to therapy, such as chemokine (C-X-C motif) ligand 2 (CXCL2) or ficolin-3. Protein pathway analysis showed that these proteins might be related to interferon signaling pathway, neutrophil degranulation or integrin signaling (Figure 1C).
CONCLUSIONS: Our preliminary data suggested that identification of a therapeutic ruxolitinib range by correlating circulating drug levels to biomarkers of disease activity and severity might help clinicians in early identification of poor responders and of ADR development.

GENDER DIFFERENCES IN PHARMACOKINETICS OF LEVODOPA IN PARKINSON'S DISEASE

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BACKGROUND: Levodopa (LD) is widely used for treatment of Parkinson's Disease (PD) because of significant improvements in motor symptoms; however, long-term treatments are complicated by drug-related motor fluctuations and dyskinesia. Evidence shows that women are more likely to experience those complications because of different pharmacokinetics (PK) of LD, such as higher area under the curve (AUC) values, increased peak concentrations (Cmax), and a slower clearance compared to men (Tomoaki K et al. 2014). Therefore, LD is more bioavailable in females (F) compared to males (M). In this prospective longitudinal study, we aim at identifying gender differences in PK of LD and gender-related candidate biomarkers of motor complications.

METHODS: A total of 23 PD patients (M/F, 15/8) were enrolled at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" of Salerno and at the Hermitage Center of Naples, and received 100 mg of LD and 25 mg of benserazide. Peripheral EDTA blood for PK analysis was drawn at the time of the first LD administration, and every 20 min for the next 2 hours, and then every 45 min for up to 210 min. After centrifugation, plasma was collected and stored at -80°C until use. Circulating LD levels were measured by liquid chromatography tandem mass spectrometry using an in-house method validated following the European guidelines for validation of analytical procedures. Drug concentrations were obtained by interpolating the peak area of LD/peak area of internal standard ratios with calibration curves, and results were then used for calculation of PK parameters using GraphPad Prism and RStudio software. Unpaired t-tests was performed for two group comparison. A P value < 0.05 was considered statistically significant.

RESULTS: Patients were divided in two groups based on sex, and PK data were compared between groups by unpaired t-test. AUC (mean+SD, 2055+1329 ng/mL vs 1015+649.8 ng/mL, F vs M; P = 0.0187), and half-life values (mean+SD, 2.38+1.1 h vs 1.66+0.5 h, F vs M; P = 0.0447) were significantly increased in women compared to men. Cmax values were also slightly higher in F compared to M (P = 0.0652). Similarly, the time to reach the Cmax (Tmax) was slightly higher in F compared to M (P = 0.0740) (Figure 1).

CONCLUSIONS: Our preliminary data confirmed the existence of gender differences in the PK of LD with increased bioavailability in females compared to males. Moreover, the 2-year follow-up results will likely add information on correlation between chronic exposure to higher drug concentrations, incidence of motor complications, and gender. Therefore, therapeutic drug monitoring of LD might be a useful clinical tool for tailoring dosages and reducing drug-related motor fluctuations and dyskinesia, especially in females.

OFF-LABEL USE OF MEDICINES: A CLINICAL AND REGULATORY OPPORTUNITY

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BACKGROUND: According to EMA, off-label use "relates to situations where the medicinal product is intentionally used for a medical purpose not in accordance with the authorised product information". In the absence of a defined risk-benefit ratio, off-label prescription could increase the risk of inappropriate use and medical error. However, a major advantage of off-label use is the satisfaction of medical needs, especially in cases where no other options are available. EU legislation does not regulate the way medicinal products are ultimately used in medical practice, on-label or off-label. Therefore, off-label prescribing is not currently regulated at EU level, but some countries adopted specific laws. In Italy, Law 94/1998 allows the physician to perform off-label prescriptions if supported by published clinical evidence, but National Health System (NHS) does not cover their cost. In a hospital setting, prescribers must request authorization for off-label treatment, and costs are covered by the hospital budget. At the University Hospital of Catania the Clinical Pharmacology Program supports the assessment, approval, management

and follow-up of prescriptions according to L. 94/1998 since 2012.
METHODS: We analyzed the off-label drug prescriptions assessed by the Clinical Pharmacology Program over the last 5 years.
RESULTS: From 2015 to 2019, 872 off-label prescriptions were approved for the treatment of 746 patients (1.2 prescription/patient). Almost 13% of subjects received 2 or more off-label drugs (in combination or as subsequent line of treatment). More than 44% of prescriptions were performed by onco-hematology Units, both pediatric (53.8%) and adult (46.2%). Rituximab (RTX) was the most prescribed drug (n=99; 11.4%), for different autoimmune diseases:

- neurological diseases (neuromyelitis optica, NMO, multiple sclerosis, encephalomyelitis) 49.5%;
- interstitial lung disease in systemic autoimmune disorders 21.2%;
- glomerulonephritis and other renal conditions 16.2%.

The appropriateness of RTX use in NMO has been assessed by the Clinical Pharmacology Program since 2012. In 2018 the risk-benefit profile of RTX in these patients has been recognized by the Italian regulatory authority and the drug has been included into the list of medicines reimbursed in Italy for unauthorized indication according to L. 648/1996.
CONCLUSIONS: Appropriateness of off-label drug prescriptions must be carefully assessed in order to ensure this use occurs only in presence of data supporting a favorable risk/benefit profile. The RTX example shows that a well-performed monitoring activity for off-label prescriptions in the hospital setting together with a continuous cooperation between pharmacologists and prescribers allow to detect unmet medical needs and to identify drugs with favorable risk/benefit profile. The off-label use represents an opportunity for patients but also a regulatory challenge to guarantee an equal and rapid access to effective and safe treatment.

IMPAIRMENT OF TESTICULAR FUNCTION IN ELECTRONIC CIGARETTE (E-CIG, E-CIGS) EXPOSED RATS UNDER LOW-VOLTAGE AND NICOTINE-FREE CONDITIONS

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BACKGROUND: To date, electronic cigarettes (e-cigarettes, e-cigs) have been used worldwide without people knowing the risks they were exposed. Recently, several scientific studies have pointed out how e-cigs can generate carcinogens and how the exposure can lead to genomic damage through inhibiting of DNA repair or disrupting the redox homeostasis. However, some research underlined how this phenomenon can be avoided by setting the device to a low-voltage regimen. To test this feasibility, we show the effects of e-cig vapour generated from a low-voltage device filled with a nicotine-free liquid on rat testicular functions.

METHODS: We investigated the chemical analysis of the e-cig vapour, the relative testis weight through the gonadosomatic index (GSI), the testicular androgenic enzymes activities, the testicular marker enzymes, the oxidative stress markers and the antioxidant and detoxifying enzymes. Moreover, we analyzed the xanthine oxidase and the lipoxygenase activity, the cytochrome P450-linked activities and we performed the alkaline DNA unwinding test.
RESULTS: The chemical analysis revealed the presence of carbonyls, such as formaldehyde, acetaldehyde and acrolein. Rats exposed reported a lower relative testis weight and higher levels of lactate dehydrogenase (LDH) as tissue damage marker, along with an impairment of 3 βhydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and glucose-6-phosphate dehydrogenase (G6P-DH) as key enzymes in the steroidogenesis pathway. The pro-oxidative environment was confirmed by the higher amount of reactive oxygen species (ROS), the development of lipid peroxidation and protein carbonylation, as well as from the disruption of antioxidant capability. Finally, we observed a higher rate of DNA unwinding in white blood cell line and boosted lipoxygenase-linked activity, a tumour promotion marker.
CONCLUSIONS: Our results show that even with the setting of the device in weak condition, exposure to e-cig vapours could alter the function of the gonads in male vapers.

THE MULTIMODAL MOR/DOR AGONIST LP2 EXERTS ANALGESIC EFFECTS IN AN ANIMAL MODEL OF NEUROPATHIC PAIN BY RESCUE OF TGF-β1 SIGNALING

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BACKGROUND: Neuropathic pain is one of the most disabling forms of chronic pain. Available therapies often provide incomplete pain relief and treatment-related side effects are common. Neuropathic pain is characterized by typical symptoms such as hyperalgesia and allodynia linked to an aberrant processing of pain transmission and to neuroinflammatory phenomena which include complex interactions between neurons, glia and immune system cells. Transforming growth factor-β1 (TGF-β1) is an anti-inflammatory cytokine, which protects against neuroinflammatory events underlying neuropathic pain. It has been hypothesized that a deficit of TGF-β1 signaling might contribute to the pathophysiology of chronic pain. We have recently synthesized a dual target MOR/DOR agonist with the (R/S)-2-methoxy-2-phenylethyl group as Nsubstituent, named LP2. In the present study, we analyzed the anti-allodinic effect of LP2 in a rat model of neuropathic pain induced by chronic constriction injury (CCI) of the left sciatic nerve and assessed whether LP2 exerts its analgesic effects by rescue of TGF-β1 signaling.
METHODS: The CCI model was performed in male Sprague-Dawley rats according to the original protocol of Bennett and Xie (1988), with

slightly modifications. LP2 was administered daily intraperitoneal at the dose of 0.9 mg/kg starting 11 days post-ligature until 21 days. The assessment of tactile allodynia was performed by measuring the withdrawal threshold of the hind paw in response to von Frey filaments at 0 (before surgery), 3, 5, 11, 16, and 21 days after CCI procedure. At 11, 16, and 21 days post-ligature, spinal cords were isolated and gene expressions of TGF-β1 and its receptor (TGFBR2) along with two well-known pro-inflammatory cytokines, IL-6 and IL-1β were analyzed by RT-PCR.

RESULTS: LP2 significantly ameliorated mechanical allodynia from the early phase of treatment up to 21 days post-ligature. The CCI condition significantly decreased the expression levels of TGF-β1 and of its receptor TGFBR2, while raised both IL-6 and IL-1β levels compared to sham condition ($p < 0.05$ for all cytokines). We then focused our attention on the protective effects exerted by LP2 in counteracting neuroinflammatory phenomena induced by CCI after 16 and 21 days. We found a significant decrease in mRNA expression levels of TGF-β1 and its receptor in CCI rats that persists even after 16 and 21 days ($p < 0.05$ vs. sham). Interestingly, the treatment with LP2 rescued both TGF-β1 and TGFBR2 levels in CCI rats ($p < 0.05$ vs. CCI) at both time points, whereas LP2 administration was not able to counteract CCI-induction of IL-6 and IL-1β.

CONCLUSIONS: We identified for the first time a selective deficit of TGF-β1 signaling in an experimental animal model of neuropathic pain which persists until 21 days post-ligature. Our dual target MOR/DOR agonist LP2 was able to restore TGF-β1 levels in the spinal cord of CCI rats suggesting that rescue of TGF-β1 pathway might contribute to the analgesic efficacy of MOR/DOR agonists in animal models of neuropathic pain.

SULFORAPHANE AS POTENTIAL INDUCER OF NON-CANONICAL CELL DEATH IN LEUKEMIA CELLS

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BACKGROUND: Apoptosis and autophagy are two forms of programmed cell death (PCD), involved in the antitumor activity of numerous drugs. Recently, other modes of PCD, defined as non-canonical, such as necroptosis and ferroptosis, have been identified and investigated. Since therapeutic approaches based on apoptosis induction led to a series of therapeutic failures, due to the activation of resistance mechanisms, the identification and characterization of further cell death pathways could be considered a promising strategy to improve current cancer therapies. Isothiocyanates (ITCs) are a class of molecules present in form of precursors in many cruciferous plants. ITCs showed a marked antitumor activity, validated through in vitro and in vivo studies, and clinical trials. The most characterized ITC is sulforaphane (SFN), widely studied for its ability to simultaneously modulate multiple molecular targets involved in carcinogenesis. The aim of this study was to deepen the pharmacological profile of SFN, and, in particular, its ability to induce non-canonical cell death, on two acute

myeloid leukemia (AML) cell lines (MV4-11 and U-937).
METHODS: The cytotoxic effect was analyzed with Trypan Blue exclusion method. Cell death mechanisms involved in SFN cytotoxicity were investigated through the analysis of nuclear morphology by fluorescence microscopy. To evaluate the induction of non-canonical cell death, AML cells were pre-treated with ferrostatin-1, necrostatin-1, and necrosulfonamide.
RESULTS: SFN exhibited cytotoxic effects on both AML cells. The analysis of nuclear morphology showed that, in both cell lines, SFN induced a dose-dependent increase in apoptotic cells fraction, while necrotic cells fraction never exceeded 10%; however, at the highest tested dose, the fraction of necrotic cells increased to about 70%. In addition, pre-treatment with pan-caspase inhibitor Z-VAD-FMK almost abrogated apoptosis induction by low dose of SFN, but it had no effect at the highest tested dose, thus suggesting the induction of different cell death mechanisms, depending on the tested concentration. Hence, to evaluate whether the cell death pathway activated by the highest dose of SFN belongs to PCD, AML cells were treated with SFN in presence of different pharmacologic inhibitors of non-canonical cell death. Pre-treatment of AML cells with ferrostatin-1, a ferroptosis inhibitor, switched the mode of PCD from necrotic to apoptotic. Similar results were observed after pre-treatment of AML cells with necrostatin-1 and necrosulfonamide, two inhibitors of necroptosis.
CONCLUSIONS: The obtained results suggest that SFN is capable of inducing apoptosis, but also ferroptosis and necroptosis on AML cells. Hence, SFN, showing to possess a pleiotropic antitumor activity, could be considered as a promising anticancer drug. Therefore, further studies will be conducted to define the molecular mechanisms involved in SFN-induced ferroptosis and necroptosis.

A SIMPLE METHOD TO ASSESS ALCOHOL WITHDRAWAL STATUS IN PATIENTS AWAITING A LIVER TRANSPLANTATION

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BACKGROUND: Alcohol use disorder (AUD) is a major cause of morbidity and mortality world-wide and its management deserves a multidisciplinary approach to stop alcohol consumption, preventing relapse and to treat alcohol damages. Alcohol exerts a negative effect on various organs and systems, but mostly on the liver. Alcohol consumption may lead to liver steatosis, hepatitis and, finally, cirrhosis, the last one often needing a liver transplant. Patients who need a liver transplantation and suffer for an AUD need to stop alcohol use in order to stay eligible. Here's why clinicians need

easy tool to monitor if patient has consumed alcohol and need a more aggressive treatment to stop it. Many methods have been used to follow patients' withdrawal toward time and one of these is the determination of ethylglucuronide concentration in the hair keratin matrix. Hair sample are easy to collect, the collection is not invasive and well-tolerated by patients and, moreover, hair analysis may explore alcohol consumption for many months in the past, according to the length of the hair. The aim of this study is to compare the results of hair analysis in a sample of patients waiting for a liver transplantation because of alcoholic cirrhosis.
METHODS: Patients awaiting for a liver transplantation at the University hospital of Modena and Reggio Emilia because of an alcoholic cirrhosis were consecutively enrolled between 1st January 2020 and the 31st May 2020. Patients were asked about their drinking behavior before sample collection and then a hair sample was picked. The hair sample collected was about 5 mm of width and a minimum of 4 cm in length. Hair were cut near the skin in the nuchal area, according to the International guidelines. This sample was subsequently analyzed with liquid chromatography tandem-mass spectrometry (LC-MS/MS) to reveal the presence of Ethylglucuronide.
RESULTS: Globally, 40 patients were analyzed. Their mean age was 52.44±7.22 years, 22 were male and 18 were female. All patients were diagnosed with an AUD, active or+ past. Globally, 16 out of 40 patients admitted the consumption of alcoholics when screened for the transplant, whilst 24 denied it. Ethylglucuronide determination in hair revealed that 11 patients had an active alcohol-drinking behavior despite the declared withdrawal at the time of the enrollment in the transplantation list. The adherence measured trough the Cohen's Kappa statistics between patients' declarations and hair analysis was low (Kappa=0.3398, P=0.045).
CONCLUSIONS: Ethylglucuronide measurement in hair trough LC-MS/MS may be a valuable tool to strictly monitor the alcohol withdrawal status of patients with an AUD. This may help clinicians to avoid a relapse of the alcohol-drinking behavior, thus implementing or changing patients' treatment.

GENDER DIFFERENCES IN PATTERN OF USE, BASELINE CARDIOVASCULAR RISK AND CONCOMITANT USE OF SEROTONINERGIC MEDICATIONS AMONG TRIPTAN USERS IN TUSCANY, ITALY

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BACKGROUND: Triptans, agonists for serotonine 5-HT1B/D receptors, are widely used in clinical practice as migraine-specific medications. Although they are considered safe when appropriately used, their vasoconstrictive properties together with and the possible occurrence of medication overuse headache (MOH) represent the main limitations to their use. Moreover, they should be used with caution in patients concomitantly taking serotonegic medications. Although triptans utilization

in clinical practice has been extensively investigated during the last decade, evidence on gender differences in pattern of use, concomitant cardiovascular diseases and serotoninergic comedication use is still limited.

METHODS: The population-based regional administrative database of Tuscany region, Italy, was used. Subjects with ≥1 prescription of any triptan (ATC: N02CC*) 2008 and 2018 were identified. Trends of annual prevalence and incidence of triptan use were observed by age groups and gender and reported as number of users per 1000 inhabitants. Prevalent users (PU) were patients with ≥1 triptan dispensing in the year of interest. New users (NU) were subjects with ≥1 triptan dispensing during the year of interest and none in the past. Patients already in treatment (AT) were those with 1≥ triptan dispensing during the year of interest excluding new users. Users with CV comorbidities representing an absolute or a possible CV contraindication to triptan use as well as those taking drugs potentially associated with SS were identified. Patterns of triptan use during one year follow-up were categorized as: sporadic, occasional, regular, and overuse. All analysis were stratified by gender.

RESULTS: A total of 86.109 triptan users were identified between 2008 and 2018, of which 64.672 were NU (men: 26.3%; women: 73.7%). About 10% of both male and female NU users were aged 65+. Absolute CV contraindication was found in 4.4% and 2.1% of male and female NU, respectively, while male and female AT with absolute CV contraindication were 2.4% and 1.5%, respectively. New triptan users for which triptans should be used with caution ranged between 25% and 30% among NU and between 28% and 36% among patients AT in both genders during the whole study period. Patients with serotoninergic comedication were 17.2% and 21.9% of male and female NU, respectively. Overusers were 0.1% among NU of both genders. Overusers were around 3% of male and 2% of female patients AT, respectively. Notably, about 60% of overusers concomitantly used serotonergic medications.

CONCLUSIONS: Based on the results from this study, further investigations are needed to address safety concerns related to the use of triptans in non-recommended age-groups, in patients with CV comorbidities and those concomitantly using serotonergic medications, also taking in consideration possible gender differences. In particular, a special attention should be paid to overusers concomitantly using serotonergic medications.

REAL-WORLD CHARACTERISTICS AND USE OF DISEASE-MODIFYING ANTI-RHEUMATIC DRUGS IN PATIENTS WITH RHEUMATOID ARTHRITIS: A CROSS-NATIONAL STUDY

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BACKGROUND: Rheumatoid arthritis (RA) is associated with significant morbidity and economic burden. Short-term glucocorticoids and conventional disease-modifying antirheumatic drugs (cDMARDs) are generally considered as the 1st line therapy, and biological (bDMARDs) or targeted synthetic (tsDMARDs) DMARDs are also available for more severe RA. The aim of the study was to evaluate and to compare the baseline characteristics and the pattern of DMARDs use in RA real- world settings from Southern Italy and United States.
METHODS: Using Caserta Local Health Unit (Italy) and Optum (United States) claims databases (DBs), covering 1.1 million and 53.3 million inhabitants respectively, all subjects aged ≥ 18 years with at least two RA diagnosis codes during the study period (Caserta DB: 2010-2018; Optum: 2010-2019) were identified. The second RA diagnosis date was defined as the index date (ID). RA patients with at least one cDMARD, bDMARD or tsDMARD dispensing any time prior to the first RA diagnosis date were excluded. The baseline patient characteristics (e.g. sex, age, comorbidities, number and type of concomitant drugs) were described. The frequency of treatment lines, that is glucocorticoids, cDMARDs, bDMARDs/tsDMARDs and the proportion of untreated RA patients during the first three years of follow-up were calculated.
RESULTS: Among 195,951 and 9,227 RA patients identified from Optum and Caserta DBs, respectively, two-thirds of them (68.1%) were females. RA patients from Optum DB were older than those from Caserta DB (mean age±-standard deviation: 66.8±14.2 years in Optum vs. 57.1±16.1 years in Caserta; P-value=0.012). In general, RA patients from Optum DB had more comorbidities than those from Caserta DB (P-value<0.001). Overall, hypertension (66.4%) and hyperlipidemia (57.1%) were the most common comorbidities. In both centers, less than 2% of RA patients had history of other autoimmune disorders for which bDMARDs are indicated (e.g. IBD, psoriasis, uveitis, spondyloarthritis or psoriatic arthritis). On average, RA patients had received 10 drugs within one year prior to the ID, and 40.2% of them had previously received more than 10 drugs, mostly NSAIDs and antihypertensive drugs. Half of RA patients from Optum DB had at least 1 opioid dispensing, compared to 14% of RA patients from Caserta DB, in line with literature. Overall, less than two-thirds (N=53,795; 65.2%) of RA patients were treated with glucocorticoids or any DMARDs during the first 3 years of follow-up. In particular, more than half (57.2%) of RA patients were exclusively treated with glucocorticoids, and 31.7% were treated with cDMARDs. Irrespective of the concomitant cDMARD/non DMARD use, only 11.0% of RA

patients received at least one bDMARD/tsDMARD (only 1.5% from Caserta DB) dispensing. Most frequently used bDMARD was adalimumab (54.2%), followed by etanercept originator (38.6%).

CONCLUSIONS: We noted substantial heterogeneity in baseline characteristics and access to bDMARDs among RA patients between the United States and Southern Italy. A significant proportion of them appeared to be untreated. With the increasing spectrum of therapeutic options and the new information on existing drugs, it is vital to ensure all RA patients are appropriately treated with DMARDs.

POST-AUTHORISATION STUDIES IN PAEDIATRIC POPULATION: DATA FROM THE EU-PAS REGISTRY

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BACKGROUND: In 2019, an analysis of the studies included in the European Union electronic Register of Post-Authorisation Studies (EU PAS Register®) from its establishment to 31st December 2018, with a focus on the Multi-database studies, was performed by the Working group 3 'Data Sources and Multi-source Studies' of the European Network of Centres for Pharmacoepidemiology and Phar-

macovigilance (ENCePP). In this framework, a subgroup, represented by TEDDY and University of Campania, has been set up with the aim to perform analyses on the PAS conducted in the paediatric population in order to:

1) describe the epidemiological research framework in this population, considering the different class of ages and identifying the still uncovered therapeutic areas for each of them;
2) evaluate the impact of the finalised PAS on the regulatory actions taken on the specific drug (in terms of marketing/SmPC changes); and
3) assess the possible impact of the recent regulatory initiatives in promoting the clinical research in this population.

METHODS: From the main project analysis, among PAS performed in paediatric patients (n= 465), only those including exclusively paediatric subjects (0-18 years) were selected for the analysis. The remaining PAS will be analysed on a later stage. Different sources were consulted to retrieve the relevant information, i.e. EU PAS register, European Medicines Agency (EMA) website, study protocols.

RESULTS: From a preliminary analysis, 94 studies were selected as performed exclusively in paediatric population: most of them were observational (n= 85; 90%) and 52 (55%) were imposed by a regulator. Risk assessment was the main purpose of the majority of studies (n= 69; 73%) which fall within the definition of Post-authorisation safety studies (PASS), followed by the effectiveness evaluation (n= 26; 28%) which fall within the definition of Post-authorisation efficacy studies (PAES). Among 87 studies focusing on drug evaluation, 48 (55%) included non-biologic drugs, 38 (44%) used biologic drugs and one study both type of medicines. Only 51 out of the 94 studies carried out in the paediatric population were finalised at the time of the data extraction. The final results of this analysis will be available by the end of the year.
CONCLUSIONS: Despite recent national and international initiatives taken to promote clinical research and observational studies in paediatrics, very few studies were performed in this frail population following the obtainment of the marketing authorisation of medicinal products. Accordingly, it is unlikely that these actions alone will fill the current knowledge gaps.

THE EFFECTS OF SULFORAPHANE ON INTERFERON-DRIVEN INFLAMMATION: IN VITRO AND IN VIVO STUDY

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BACKGROUND: Interferonopathies are characterized by an abnormal response to nucleic acid stimuli due to either deficiency of nucleases, or to defective regulation of downstream effector molecules, leading to excessive production of type I IFN. Currently, no drugs are authorized for a routine clinical use in these pathologies. In this work we studied the contribute of sulforaphane (SFN), a bioactive molecule contained in cruciferous vegetables, in the modulation of IFN inflammation in an immortalized human hepatocytes (IHH) line and in two healthy volunteers, focusing on STING, a key-component player in IFN pathway produc-

tion, and IFN signature (ISGs) modulation. SFN ability to induce the phase II enzyme GSTM1 expression, was also evaluated.
METHODS: IHH cells were treated with SFN 10 μM for 24 and 72 h in presence or absence of the pro-inflammatory stimulus cGAMP, added in the last 3 or 6 h of incubation. Two healthy volunteers were enrolled to take a SFN supplement. Peripheral blood samples were collected for RNA extraction before and after assumption. ISGs, STING and GSTM1 analyses were performed by real time PCR, GSTM1 genotype was assessed by TaqMan® CNV genotyping assay. Statistical analysis was done by ANOVA.
RESULTS: SFN exposure reduced significantly STING expression in comparison to untreated IHH cells ($p < 0.05$). The pro-inflammatory stimulus cGAMP, used to mimic the IFN cascade, prominently increased STING expression ($p < 0.001$) with a time dependent effect ($p = 0.033$, 3 vs 6 h cGAMP incubation). Interestingly, SFN pretreatment significantly downregulated STING ($p < 0.001$) even in presence of cGAMP ($p = 0.0037$). ISGs showed a similar behavior, stating a considerably higher IFN score in the presence of cGAMP with more than halved IFN score in pretreated cells. GSTM1 resulted upregulated ($p < 0.01$) after 24 h of exposure suggesting that SFN is a GSTM1 inducer. Afterwards, the SFN effect was evaluated in the two subjects enrolled. One volunteer presented the deletion of GSTM1, related to greater SFN excretion, while the other resulted wild type. A dose of 12.6 mg for the first 24 h, 25.2 mg for the next 48 h and, after 2 weeks of no assumption, 25.2 mg daily for 7 days were administered observing no differences in terms of STING, while, after an almost fourtimes-higher dose (90 mg) for 3 days, STING resulted downregulated only in the volunteer wild type for GSTM1. No appreciable variations in ISGs were identified after SFN assumption, likely because volunteers were healthy.
CONCLUSIONS: This study confirmed SFN inhibiting STING-mediated inflammation and ISGs expression in vitro. Moreover, a trend toward a downregulation of STING was recorded also in vivo. Results obtained have to be confirmed in a larger group of healthy individuals and in patients with type I interferonopathies to define if assuming SFN could be useful as support therapy.

STABILIZATION OF HIF-1β IN HUMAN RETINAL ENDOTHELIAL CELLS MODULATES EXPRESSION OF MIRNAS AND PROANGIOGENIC GROWTH FACTORS

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BACKGROUND: Retinal hypoxia is one of the causative factors of diabetic retinopathy and is also one of the triggers of vascular endothelial growth factor (VEGF) release. We hypothesized that specific dysregulated miRNAs in diabetic retinopathy could be linked to hypoxia-in-

duced damage in human retinal endothelial cells (HRECs).
METHODS: We investigated in HRECs the effects of chemical hypoxia (200 μM CoCl2) on the expression of HIF-1α, VEGF, placental growth factor (PlGF), and of a focused set of miRNAs. Moreover, in order to explore alternative factors and pathways regulated by those miRNAs, we predicted their combinatorial effect on biological pathways by means of the DIANA miRPath webserver.
RESULTS: We found that CoCl2 treatment, by inhibition of HIF-1α degradation, significantly ($p<0.05$) increased stabilization of HIF-1α protein in HRECs and modulates the expression of VEGF-A and PlGF mRNA. Furthermore, six miRNAs (miR-20a-5p, miR-20b-5p, miR-27a-3p, miR-27b-3p, miR-206-3p, miR-381-3p) were significantly ($p<0.05$) dysregulated in HRECs treated with CoCl2.
Through the bioinformatics approach we identified the TGFβ signaling pathway as the top-scored pathway dysregulated in HRECs challenged with chemical hypoxic stimuli.
CONCLUSIONS: This study suggests that ocular neovascularization, during hypoxia, would be promoted by the upregulation of PlGF and other factors induced by HIF-1α/miRNAs, i.e. VEGFA, and genes of the TGFβ1 signaling pathway. The present findings highlighted that proangiogenic factors are worthy to be further explored as potential targets for pharmacological modulation of local retinal hypoxic events, which are generally transient but detrimental in retinal degenerations.

CO-ADMINISTRATION EFFECTS OF ALPHA-LACTOALBUMIN WITH SODIUM BUTYRATE ON NEUROPSYCHIATRIC DISORDERS IN MICE EXPOSED TO CHRONIC UNPREDICTABLE MILD STRESS (CUMS)

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BACKGROUND: Chronic stress represents a considerable health concern for society, linked to several disease state including an augmented risk to contract neuropsychiatric disorders. Recently, the contribution of microbiota–gut–brain axis signaling in both the aetiology and treatment of stress-related disorders has been documented1. Interestingly, in silico metagenomics analysis predicted a shift in the functional profile of the microbiome: stressed mice showed decreased functional diversity and a lower prevalence of pathways implicated in the synthesis and metabolism of neurotransmitter precursors and short-chain fatty acids2. Accordingly, we have studied if an oral supplementation with alpha-lactoalbumin (ALAC), a whey protein rich in tryptophan, and/or sodium butyrate (NaB), a short-chain fatty acid (SCFA), could improve stress- induced neuropsychiatric deficits in mice.
METHODS: male BALB/c mice were subjected to CUMS, as previously described3. Oral supplementation of ALAC (125, 250 and 500 mg/kg/day), NaB (100 mg/kg/day) and their co- administration started at the end of CUMS protocol. After 15 days of treatment, mice were subjected to behavioral tests: forced swimming test (FST), sucrose preference test (SPT), open field test (OFT), elevated plus maze (EPM), passive avoidance (PA), novel object recognition test (nORT) and Morris water maze (MWM).

RESULTS: ALAC, at each dose used, and NaB were able to significantly ($p < 0.001$) reduce the immobility time, during the FST, in treated CUMS mice in comparison to untreated CUMS mice. Interestingly, the co-administration of ALAC, at each dose used, with NaB was significantly ($p < 0.001$) more efficacious than the respective mono-administration to reduce depressive-like behavior in CUMS mice. Similar results were observed in the SPT. Regarding anxiety-like behaviour, there were not significant differences in the parameters evaluated in the OFT among groups. At odds, in the EPM test, ALAC, at each dose used, and NaB were able to significantly ($p < 0.05$) increase the time spent in open arms in treated CUMS mice. Additionally, the co- administration of ALAC, at each dose used, with NaB was able to significantly ($p < 0.0001$) decrease anxiety-like behavior in co-treated CUMS mice, with the time spent in open arms being maintained similar to control levels. Interestingly, ALAC, at each dose used, and NaB were also able to significantly ($p < 0.05$) improve learning and memory in treated CUMS mice in comparison to untreated CUMS mice, however, only the co-administration was able to maintain cognitive performance similar to control levels.

CONCLUSIONS: these results show that ALAC and/or NaB treatment relieves enduring alterations induced by CUMS, emphasizing the importance of future research into microbiota-targeted therapies for stress-related disorders.

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LIMITED SAMPLE STRATEGY TO PREDICT THE AREA UNDER THE CURVE OF MYCOPHENOLIC ACID IN CARDIAC TRANSPLANT PATIENTS WITH DIFFERENT CLINIC CHARACTERISTICS: A SUB-VALIDATION STUDY

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BACKGROUND: The Therapeutic Drug Monitoring (TDM) of Mycofenolic Acid (MPA) is not yet performed in clinical practice, although the MPA Area Under the concentration time Curve (AUC_{0-12}) correlates with treatment outcome. The MPA AUC_{0-12} therapeutic range has been established (30 - 60 mg*h/L), but its evaluation is a cost and time-consuming procedure. In this context, Limited Sample Strategy (LSS) AUC_{0-12} represents a useful tool. Two LSSs were previously tested and validated in a cohort of cardiac transplant (HTx) recipients treated with Mycofenolate Mofetil (MMF) and Cyclosporine (CsA). The aim of this study is the validation of these LSSs in a heterogeneous cohort of HTx recipients, in terms of treatment (MMF combined with CsA or Tacrolimus), comedications, age, and organ function. The secondary objective of this study is the evaluation of MPA AUC_{0-12} values among non-rejected and rejected patients.

METHODS: The characteristic of the 29 patients enrolled were (mean ± SD): age (54.9 ± 13.1 years), post-transplantation time (3.2 ± 4.9 years), BMI (25.5 ± 4.9 kg/m^2), Creatinine Clearance (66.7 ± 27.7 mL/min), ALT (29.4 ± 21.8 IU/L), AST (24.4 ± 18.8 IU/L) and Bilirubin (1.0 ± 0.7 mg/dL).
The algorithms used for MPA $AUC_{0\text{-}12}$ evaluation were:
1) LSS3: $AUC_{0\text{-}12} = 5.568 + 0.902 * C_{1.25} + 2.022 * C_2 + 4.594 * C_6$
2) LSS4: $AUC_{0\text{-}12} = 3.800 + 1.015 * C_{1.25} + 1.819 * C_2 + 1.566 * C_4 + 3.479 * C_6$
Agreement between LSSs $AUC_{0\text{-}12}$ and the entire $AUC_{0\text{-}12}$ was tested by linear regression and Bland-Altman analysis, considering Pearson correlation coefficient (r) strong for value > 0.8. The Coefficient of determination (R^2) was tested to verify the goodness of fit of the regression line.
The evaluation of $AUC_{0\text{-}12}$ values between non-rejected and rejected patients was analyzed by Mann Withney test, p-values of a 2-sided test < 0.05 were considered as statistically significant.

RESULTS: The linear regression analysis state a strong correlation with the $AUC_{0\text{-}12}$ and a good fit of the regression for LSS3 and LSS4 (r = 0.92 and 0.94; R^2 = 0.84 and 0.88, respectively).
The Bland Altman Plot for LSS3 and LSS4 confirmed these results (p = 0.038 and p = 0.047, respectively). The visual inspection of the plot did not reveal any particular pattern, and the linear regression of plot dots proved the results (r = 0.47, R^2 = 0.22 for LSS3; r = 0.56, R^2 = 0.31 for LSS4).
The median value of $AUC_{0\text{-}12}$ (mg*h/L) was 47.9 (95% CI: 36.0 to 70.8) for non-rejected and 33.7 (95% CI: 23.6 to 48.2) for rejected patients (p = 0.03); both LSSs methods were able to replicate these results.
CONCLUSIONS: The study suggests the effectiveness of both LSSs to predict the MPA $AUC_{0\text{-}12}$ in a heterogeneous cohort of HTx recipients. It was evident also the distribution of non-rejected patients $AUC_{0\text{-}12s}$ near to the upper limit of the therapeutic range, while those of the rejected patients were closed to the lower limit. Further studies are needed to confirm the results and to prove the utility of MPA $AUC_{0\text{-}12}$ quantification.

GENDER MAY INFLUENCE THE IMMUNOSUPPRESSIVE ACTIONS OF GLUCOCORTICOIDS IN PAEDIATRIC PATIENTS WITH IBD

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BACKGROUND: Although the use of glucocorticoids (GC) is well established, the therapeutic response to these agents often shows important interindividual differences among paediatric patients with inflammatory bowel diseases (IBD). Steroid resistance is common with an incidence of 6-16% of subjects, in addition, around one third of patients who initially responded cannot discontinue therapy as disease activity recurs when the dosage is reduced (steroid dependent). Currently, GC resistance or dependence cannot be predicted by clinical or laboratory findings. The aim of this study was to investigate the association of gender and age with GC efficacy and the expression of GILZ, a gene involved in GC pharmacodynamics, in young children with IBD.
METHODS: One hundred thirty patients (mean age at enrolment 12.6 years, 53 Crohn's disease, 70 males) were enrolled in this retrospective study. IBD patients with active disease despite GC up to 2 mg/kg/day over a period of 4 weeks, were defined as steroid resistant. Patients unable to reduce steroids within 3 months of therapy start were considered steroid-dependent. Total RNA was extracted from biopsies of 14 patients (9 males) and levels of GILZ were evaluated by real-time PCR. Association between pharmacological phenotypes and the considered demographic variables was evaluated using linear mixed effects model.
RESULTS: After 4 weeks of treatment, 112 patients were sensitive to GCs and 18 were resistant; at this time-point, resistant patients were older than sensitive ones (mean age: 14.9 vs 12.1 years respectively, p=0.01). After tapering (12 weeks), 42, 71 and 12 patients were sensitive, dependent and resistant respectively; at this time-point, females were more prone than males to develop GC dependence vs a good response (p=0.028; OR: 2.44; CI: 1.10 - 5.26) while age had no effect.
Age was associated with response both at 4 and 12 weeks in the subgroups of females: resistant patients were older than sensitive ones at 4 weeks (mean age: 15.8 vs 11.6 years respectively; p=0.02). Likewise, at 12 weeks of therapy dependent patients resulted older than sensitive ones (mean age: 15.5 vs 12.6 respectively, p=0.05). No association of age with GC response was found in males.
In a subgroup of 14 patients (5 females, 3 CD), GILZ levels were higher in males in comparison to females (fold change: 2.5; p=0.031), highlighting a different activity of the GC receptor between genders. Patients with unfavourable response (7, dependent or resistant) presented lower GILZ expression at disease onset in comparison to the sensitive group (7, p=0.017), supporting the important role of GC receptor transcriptional activity in steroid ineffectiveness.
CONCLUSIONS: Older females with IBD have a higher incidence of GC unfavourable response. The study of the molecular basis underlying the sexually dimorphic actions of GCs may help us to design more personalized treatment strategies, accounting for gender.

DETERMINATION OF MITOTANE AND PRINCIPAL METABOLITE BY A SIMPLE HPLC-UV METHOD AND ITS VALIDATION IN HUMAN PLASMA SAMPLE

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BACKGROUND: Mitotane (dichlorodifenyldichloroethane, DDD) it is a polychlorinated compound derivative of dichlorodiphenyltrichloroethane (DDT) that is prescribed to in inoperable adrenocortical renal carcinoma and Cushing's syndrome. DDD and its principal metabolite, dichlorodiphenylethene (DDE), can accumulate in fat tissues and their plasma concentrations are more related with clinical improvement, than those of dichlorodiphenylacetate (DDA), another metabolite of DDD. Therapeutic monitoring of plasma concentrations is thus required to combine good therapeutic efficacy with acceptable toxicity. HPLC methods constitute a valid alternative to gas chromatography, and plasma concentrations of 14–20 mg/L are considered therapeutic concentrations.
METHODS: Chromatographic conditions: stationary phase was represented by a Higgins Analytical C18 5 µm column (250mmx4.6 mm), maintained at 35 °C. Mobile phase was made by water/acetonitrile (10/90, v/v) and pumped at flow of 1.0 ml/min. Peaks of interest were monitored at wavelength of 226 nm. Human plasma sample preparation: 200 µl of plasma sample was added with aldrin (as Internal Standard) and 200 µl of acetonitrile for protein precipitation. Samples were vortexed for 30 sec and centrifuged at 12000 rpm x 10 minutes. Clear supernatants (50 µl) were injected within the HPLC apparatus
RESULTS: The average recovery of analytes was 95%, and the method was linear (r2=0.9988 and 0.9964 for DDD and DDE, respectively) within the range 1-40 mg/L. The values of limit of quantitation and detection were 0.2 mg/L and 0.3 mg/L for DDD and 0.066 mg/L and 0.099 mg/L for DDE, respectively. It is worth noting that sample preparation and run time are short enough to allow the analysis of at least 4 samples per hour (15 min total run). Indeed, the retention time (RT) of DDD and DDE are 7.06 min and 9.42 min, respectively, while the RT of the internal standard is 12.67 min. Finally, the validation process returned inter- and intra- day mean accuracies (1.30% and 1.60%, respectively) and precision (6.45% and 7.70%, respectively) within the limit of FDA guidelines. Therefore, the method has been adopted at the TDM Lab of Clinical Pharmacology Unit, University Hospital, Pisa, for routine monitoring of mitotane plasma concentrations in patients affected by adrenocortical renal carcinoma.
CONCLUSIONS: In conclusion, a reliable and rapid HPLC-UV method was developed for the measurement of mitotane and DDE concentrations in plasma samples using aldrin as internal standard for better accuracy and precision over the range of drug concentrations expected after the administration of mitotane at standard doses. Moreover, the simple preanalytical preparation of samples and the reduced costs of HPLC platform certainly ensure a wide diffusion of the present method, optimal for routine in laboratory.

THERAPEUTIC MONITORING OF PIPERACILLIN IN ICU PATIENTS

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BACKGROUND: Infections remain one of the major causes of morbidity and mortality in patients admitted to intensive care units (ICUs). The rate of nosocomial infections among patients admitted to ICUs is 5-10 times higher than other general medicine and surgery wards. The most common nosocomial infections are pulmonary, urinary and blood stream infections that are often caused by P. aeruginosa, S. aureus, K. pneumoniae, S. marcescens, P. mirabilis and C. albicans. The infection determines a large number of victims each year. Indeed, it has been estimated that in Europe about 25,000 people annually risk death due to the infections by MDR organisms. Strong evidence in literature endorses the benefits of penicillin TDM and suggests the existence, in population of critically ill patients, of a correlation between the exposure of the target to the drug and the outcomes. Therefore, this study was aimed at developing and validating a simple HPLC method for monitoring plasma level of piperacillin in ICU patients.
METHODS: The HPLC-UV method was developed for piperacillin quantitation, starting from 200 µl of plasma samples treated with 200 µl of ACN+H3PO4 conc. 5 % (v/v) for protein precipitation. In plasma samples, ticarcillin was added as internal standard (IS). The stationary phase was HAISIL HL C18 250 mm x 4.6 mm x 5 µm (Higgins Analytical Inc., USA). Mobile phase was composed by 20 mM phosphate buffer (pH 2.5)/acetonitrile (73.5/26.5, v/v). The mobile phase was pumped within the HPLC system at a flow of 1 ml/min, while 30 µl of each sample were injected within the system. Absorbance of piperacillin and IS was measured at a wavelength of 220 nm. The method was validated according to the EMA 2015 guidelines, then it was applied to measure drug concentrations in 248 plasma samples from ICU patients (AbioKin project, Mario Negri, Bergamo, Italy).
RESULTS: The chromatographic run time was 20 minutes, with retention times of ticarcillin and piperacillin of 13.4 and 18.0 min, respectively. Results of validation were as follows: linearity range, from 10 up to 400 µg/ml (r2=0.9994), accuracy and precision (< 8%), reproducibility (< 9%), limit of detection (9.57 µg/ml) and limit of quantitation (3.15 µg/ml) and average recovery (>90%). The method was applied to 248 plasma samples, resulting in a mean±SD concentration of 86.88±79.40 µg/ml, with a median concentration of 65.10 µg/ml (MIN-MAX range, 0-399.56 µg/ml). Of note, no interfering peaks were detected within the chromatogram despite the number of co-administered drugs. The method allows the preparation and analysis of 3 samples/h, but the availability of chromatographic systems equipped with autoinjectors does extend the analyses overnight, thus increasing the output.
CONCLUSIONS: In conclusion, a new chromatographic method for selective quantitation of piperacillin was developed and validated in plasma samples, with optimal performance to be applied to drug monitoring for ICU patients. Indeed, the application of the present HPLC-UV method may result in a more appropriate prescription of piperacillin with a subsequent improvement in clinical outcomes while the risk for the selection of resistant clones will be reduced.

EFFECTS OF GENETIC DELETION OF NOCICEPTIN/ ORPHANIN FQ RECEPTOR ON HEROIN DEPENDENCE IN RATS

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BACKGROUD: Nociceptin/orphanin FQ(N/FQ) and its receptor (NOP) have been implicated in addiction-related behaviors. Previous studies demonstrated that the activation of NOP reduced opioid preference in operant behavior and conditioned place preference paradigms. However, recent studies using NOP KO rats suggested that NOP activation is necessary to maintain drug self-administration. Indeed, we recently have demonstrated that NOPKO rats self-administer less cocaine, ethanol and heroin under fixed ratio 1(FR1) and progressive ratio (PR) schedule of reinforcement compared to wild type (WT) counterpart. Of note, saccharin self-administration was not affected by NOP receptor deletion. Here we further investigated the role of NOP in the primary reinforcing properties of heroin, comparing heroin self-administration under FR and PR contingency between NOP KO rats, obtained by the constitutive inactivation of the NOP receptor function, and their wild type counterpart.
METHODS: W. Han and NOP KO male rats (N=16 each), bred at the University of Camerino, were initially trained to self-administer heroin (20 µg/inf.) under FR1 and PR schedule of reinforcement. To determine whether the 2 lines of rats show different sensitivity to heroin's rewarding effects and motivation to self-administer the drug, we studied the dose/response curve for heroin subjecting the rats to 4 different concentrations (1, 7, 20, 60 µg/inf.) under FR1 and PR schedule of reinforcement respectively , according to a counterbalanced latin-square design. Next, we wanted to confirm the functionality of NOP in the 2 lines by a pharmacological manipulation testing the effect of a selective NOP agonist (0.3 and 1 mg/kg by gavage) on heroin self-administration (20 µg/inf. under FR1 and PR contingencies), in W. Han and NOP KO rats.
RESULTS: W. Han and NOP KO rats showed similar acquisition pattern of heroin self administration. Analysis of the dose/response curve under FR1 found no statistically significant difference between lines. Although reaching any significance thresholds, under PR contingency a trend toward reduced motivation for heroin was observed in NOP KO rats compared to controls,. In both FR1 and PR experiments, statistical analyses revealed that the NOP agonist reduced heroin self-administration at the dose of 0.3 mg/kg in W. Han and at 1 mg/kg in NOP KO rats.
CONCLUSIONS: Results indicate that W. Han and NOP KO rats show similar sensitivity and motivation for the rewarding effects of heroin, and that both lines responded to the pharmacological effect of the NOP agonist. These data are in contrast with previous reports showing reduced self-administration by NOP KO rats suggesting that NOPr may have maintained a residual activity in the NOP KO line. To confirm these results we are currently repeating the experiment in W.Han and NOP KO rats purchased by a different breeder.

GLIAL CELL ACTIVATION AND ALTERED METABOLIC PROFILE IN THE SPINAL-TRIGEMINAL AXIS IN A MODEL OF MULTIPLE SCLEROSIS-ASSOCIATED TRIGEMINAL PAIN

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BACKGROUND: Trigeminal (TG) neuralgia is one of the worst multiple sclerosis (MS)-associated neuropathic pain syndromes, with a 20-fold higher risk in patients than in the general population (Husain et al. Curr Treat Options Neurol 2018). TG pain is often an early or even an onset symptom of MS, which does not generally correlate with the severity of the disease and with the presence of demyelinating plaques (O'Connor et al. Pain 2008), suggesting that pain and clinical signs in MS are triggered by parallel but independent yet-to-be identified mechanisms. Thus, our aims were to study the development of spontaneous TG pain in an animal model of Experimental Autoimmune Encephalomyelitis (EAE), and to analyze: i) the activation of glial cells (i.e. astrocytes and microglia in the CNS and satellite glial cells in the TG ganglion), and ii) metabolic changes in the spinal-TG system.

METHODS: EAE was induced in Dark Agouti male rats by intra-dermal injection of recombinant MOG1-125 protein fragment in Incomplete Freund's Adjuvant (IFA) and sodium acetate. Motor symptoms were monitored on a 1-7 points scale of ascending paralysis. The development of orofacial allodynia was evaluated by von Frey's hairs. At day post immunization (DPI) 21 or at the onset of EAE symptoms, animals were sacrificed, and the brainstem, TG ganglia and nerves were collected for analyses of glial cell activation and metabolomics/lipidomics analyses by mass spectrometry.

RESULTS: The subcutaneous injection of recombinant MOG1-125 protein fragment to Dark Agouti male rats led to the development of relapsing-remitting EAE, with a first peak after DPI 13, a remission stage from DPI 16 and a second peak from DPI 21. Interestingly, orofacial allodynia developed from DPI 1, i.e. well before the onset of EAE, and worsened over time, irrespective of the disease phase. Activation of glial cells both in the TG ganglia and in the brainstem, together with over-expression of glial purinergic receptors involved in pain transmission, was observed along with metabolic alterations in the TG ganglion, with no signs of demyelination in the brainstem. At EAE onset, brainstem glial cells were already activated and overexpressed the A3 adenosine receptor subtype (Magni et al. Brain Behav Immun 2020).

CONCLUSIONS: Our data show the spontaneous development of TG pain before the onset of relapsing-remitting EAE in rats, suggesting the existence of parallel mechanisms controlling motor symptoms and orofacial pain. The involvement of central and peripheral glial cell activation and metabolic alterations in the TG ganglia suggest their contribution to trigger the sensitization of sensory neurons. Thus, the reduction of glial cell activation and normalization of TG ganglia metabolism are interesting options to manage pain in MS.

Supported by FISM – Fondazione Italiana Sclerosi Multipla – grant cod.2016/R/7 to SC and financed or co-financed with the "5 per mille" public funding.

ACCIDENTAL INJECTION OF PSEUDORABIES PIGS VACCINE IN HUMANS

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BACKGROUND: Pseudorabies virus, a member of the herpes virus group, causes Aujeszky disease in pigs. The susceptibility of man to pseudorabies virus is controversial: single cases report are reported [1], but Aujeszky diseases is considered as non-transmissible to human. Suvaxyn Aujeszky 783+O/W® is a veterinary medicinal administered for active immunization of pigs to prevent Aujeszky's disease. The pharmaceutical form consist in: powder (live attenuated Aujeszky's virus), solvents (aluminium hydroxide, mineral oil, mannide mono-oleate and polysorbate-80), eccipient (thiomersal). We describe four cases of accidental injection in human.

CASE SERIES: Cases of accidental human injection of Suvaxyn Aujeszky 783 + O/W® referred to Pavia Poison Control Centre from 2014-2019 were retrospectively evaluated. Four patients (age 45 ± 11 y.o.; male-75%) were evaluated. In all cases, patients accidentally self-injected (3 at the fingers, 1 at the knee) a residual dose of vaccine during administration to pigs. All 4 patients developed a local reaction with pomphoid lesion at the injection site. In two cases, local clinical manifestations improved in 12-hours after symptomatic treatment. Two of them manifested also severe asthenia associated with abrupt appearance (1h after injection) of fever (37.8 and 39.7 C°). In both cases blood test were normal except for a rise of WBC with normal procalcitonin. Hyperthermia improved after 24-hours with antipiretics and without antibiotic administration.

CONCLUSIONS: In case of human injection, the EMA product's information reports, the risk of local inflammation due to mineral oil. A transient increase in body temperature, up to 40.5C° and lasting for up to 2-days, is described as acute adverse reaction in a small number of pigs after vaccination. In our experience hyperthermia could be appear even in human exposed and is probably due to a systemic inflammatory reaction related to mineral oil. No patients developed the zoonosis. Workers and health professionists should be aware of this specific risk. A revision of product characteristics including the possibility to have hypertermia also in human is suggested.

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BOWEL PERFORATION DUE TO METHOTREXATE THERAPEUTIC ERROR: A CASE REPORT

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BACKGROUND: Methotrexate (MTX) is widely used in the treatment of some autoimmune diseases. Adverse effects are often associated with therapeutic errors such as the daily intake rather than weekly intake, especially when the drug is self-administered. Among the adverse effects of MTX, the risk of bowel perforation is extremely rare (0.1%) 1,2. We describe a case of bowel perforation,occurred following daily intake of MTX.

CASE REPORT: A 68-year-old man was prescribed to take MTX 7.5 mg per os once a week, while waiting for switch to Abatacept for a recent reactivation of rheumatoid arthritis. After 10 days, he started having pharyngodynia, haematochezia and general malaise. At medical examination he presented oral and nasal mucositis and thrombocytopenia. The history revealed that he had been taken the prescribed dosage of MTX daily, instead weekly. MTX serum concentration measurement showed normal range values, and immediate therapy with folinic acid 1000 mg (mg/ m^2/die) and urine alkalinisation was started. After 7 days later, the patient worsened abruptly. An emergency CT scan revealed millimetric gas bubbles indicating bowel perforation. The patient underwent an emergency exploratory laparotomy that resulted in peritoneal toilette and sigma resections.
Anatomopathological findings were suggestive of MTX poisoning. The patient was discharged 17 days after admission in good clinical condition with planned rheumatologist controls for therapy management.

CONCLUSIONS: MTX-related bowel perforation, although potentially lethal, is rarely described in literature[1]. It is appropriate to consider that there are individual susceptibility and genetic predisposition for development of MTX adverse effects[3]. Furthermore, steroid therapy and/or a pre-existing diverticulitis disease, must be considered as risk factors for bowel perforation during MTX therapy[2]. All this should be taken into account during medical treatment with MTX.

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SEX-DEPENDENT BEHAVIORAL DEFICITS INDUCED AT EARLY LIFE BY IN UTERO CANNABINOID EXPOSURE DEPEND ON MGLU5 RECEPTOR SIGNALING

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BACKGROUND: Marijuana is the illicit drug most commonly used among pregnant and breastfeeding women. Different studies reported long-term adverse effects induced by in utero exposure to the main component of marijuana, Δ9 -tetrahydrocannabinol (THC), both in rodents and in humans. However, little is known about any potential sex-dependent effects of marijuana consumption during pregnancy on newborns at early developmental ages.
METHODS: We studied the effects of prenatal exposure to the cannabinoid receptor agonist WIN55,212-2 (WIN; 0.5 mg/kg from GD5 to GD20) on the emotional reactivity and cognitive performance of male and female rat offspring from infancy through adolescence and tested the role of mGlu5 receptor signaling in the observed effects.
RESULTS: When separated from the dam and siblings, prenatally WIN-exposed male infant pups emitted less isolation-induced ultrasonic vocalizations and showed increased locomotor activity compared with male control pups, while females were spared. These effects were normalized when male pups were treated with the positive allosteric modulator of mGlu5 receptor CDPPB. When tested at the prepubertal and pubertal periods, WIN-prenatally exposed rats of both sexes did not show any difference in social play behavior, anxiety and temporal order memory.
CONCLUSIONS: We reveal a previously undisclosed sexual divergence in the consequences of fetal cannabinoids on newborns at early developmental ages, which is dependent on mGlu5 receptor signaling. These results provide new impetus for the urgent need to investigate the functional and behavioral substrates of prenatal cannabinoid exposure in both the male offspring and the female offspring.

ACID-SENSING ION CHANNEL 1A IS INVOLVED IN HIPPOCAMPAL SYNAPTIC PLASTICITY ALTERATIONS INDUCED BY Aβ

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BACKGROUND: Acid-sensing ion channels 1a (ASIC1a) are widely distributed in the mammalian nervous system and in particular brain areas including hippocampus. ASIC1a is highly permeable to Ca2+ and its activation has a crucial importance in numerous physiological and pathological processes, including synaptic plasticity, learning and memory. To further understand the involvement of ASIC1a channels in the synaptic mechanisms underlying learning and memory, we carried out electrophysiological experiments investigating hippocampal long-term potentiation (LTP) and long-term de-

pression (LTD), using an in vitro model of Alzheimer's disease (AD).
METHODS: We performed whole cell patch clamp recordings of CA1 pyramidal neurons in acute slices obtained from C57BL6J mice. We investigated mGlu- and NMDA- receptor dependent forms of Long Term Depression (LTD) and Long Term Potentiation (LTP) elicited by electrical and chemical protocol. The in vitro model of AD was obtained applying Aβ (200 nM) on slice for 30 minutes before recording.
RESULTS: We observed a functional role of ASIC1a in the impairment of mGlu-receptor LTD induced by Aβ. Blocking ASIC1a prevented the LTD amplification induced by Aβ. We also observed that blocking ASIC1a restored the Aβ mediated alteration of NMDA-receptor LTP and LTD. Overall, these data demonstrate for the first time that ASIC1a is involved in the synaptic plasticity modifications triggered by Aβ.
CONCLUSIONS: These findings suggest a novel function of ASIC1a channels in the synaptic alterations associated with AD. Therefore, it is possible to hypothesize that ASIC1a represents a novel target for the development of drugs able to prevent and/ or treat memory decline in AD and related cognitive impairments.

BIOMARKERS OF PAIN IN WOMEN WITH BREAST CANCER

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BACKGROUND: Pain is a frequent symptom in cancer patients and its management is generally guided by pain subjectively perceived by patients and reported through self-reported scales. The utility of these tools is limited because strongly dependent on patients' opinions. For this reason, more objective instruments are desirable. Several studies have been carried out to identify potential pain biomarkers, however, without any specificity for the cancer typology (Wang et al., 2020). Pain is frequently perceived in women with breast cancer together sleep disorders, depression, fatigue and anxiety Moreover, exists a correlation between immune activation with release of proinflammatory cytokines and perception of pain (Starkweather et al., 2013) it has been suggested the existence of a negative correlation between pro-inflammatory cytokines levels and pain scores in breast cancer and that changes of the cytokines could be considered valid prognostic factors for the evaluation of the reduction of the intensity of breast cancer pain (Fazzari et al., 2020).
Pain is perceived with a high prevalence in women with breast cancer. Increased levels of reactive protein C (PRC) and IL-13 have been observed in these patients, suggesting a correlation between immune system activity and the release of proinflammatory cytokines and pain perception. Proinflammatory cytokines such as TNF-α and IL-6 have been indicated as possible markers for pain in cancer (Baamonde et al., 2007). Interleukin (IL)-33 has been shown to play a key role in many inflammatory conditions. Furthermore, it has been observed that it is able to modulate skin sensitivity in inflam-

matory pain in experimental animals (Verri et al., 2008). On the basis of this evidence we designed a study to evaluate the involvement of the axis of the cytokines IL-31/IL-33 cancer and the potential role of these cytokines as biomarkers in patients undergone to breast surgery for cancer pain.
METHODS: Brief Pain Inventory test and Numerical Rating Scale were performed in patients affected by bone metastasis for pain evaluation before and after breast surgery. At the same times a blood sample was withdrawn to evaluation of reactive C protein (CRP), IL-6, TNF-α, IL-31 and IL-33.
RESULTS: preliminary results show that level of substances investigated correlated with pain scales scores.
CONCLUSIONS: preliminary results suggest a positive relationship between reduction of inflammatory biomarkers and relief of pain after breast surgery in cancer patients. Moreover, results suggest that these substances can be used to guide and predict analgesic response in breast cancer.

EFFECTS OF BRANCHED-CHAIN AMINO ACIDS AND L-ALANINE SUPPLEMENTATION IN A MURINE MODEL OF PHYSIOLOGICAL EXERCISE

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BACKGROUND: Branched-chain amino acids (BCAAs: leucine, isoleucine, valine) account for 35% of skeletal muscle essential amino acids (AAs). As such, they must be provided by the diet to balance protein synthesis and proteolysis. Although substantial evidence has been collected about BCAAs as potentially helpful to support muscle anabolism and alleviate fatigue in subjects regularly practicing sports, food supplements containing BCAAs alone may not be effective in controlling muscle protein turnover, due to the rate-limiting bioavailability of AAs involved in BCAAs metabolism [1,2].

METHODS: We aimed to provide solid preclinical evidence about the real effectiveness and possible harmful effects of BCAAs supplementation in the context of a moderate, constant exercise in healthy individuals, and the potential usefulness of combining them with L-Alanine (ALA). This latter, controls BCAAs catabolism and reduces perceived fatigue during endurance performance when administered with BCAAs [3]. Therefore, we compared the effects of a 4-week-treatment with BCAAs alone (ratio 2:1:1) and three mixtures combining BCAAs with an increasing content of ALA (1ALA = *mix 1*, 2ALA = *mix 2*, or 3ALA = *mix 3*), dissolved in drinking water and administered to 10-week-old male C57BL/6J wild type (WT) mice subjected to an "amateur-like" running protocol. The outcome on muscle function, structure and metabolism was evaluated via multiple *in vivo* and *ex vivo* readouts, in comparison to age- and sex-matched mice treated with vehicle (water)[4].
RESULTS: A preliminary pharmacokinetics study confirmed that ALA boosts up BCAAs bioavailability, with *mix 2* showing the highest BCAAs muscle levels. After 4 weeks, all mixtures significantly improved exercised mice *in*

vivo force production and resistance to fatigue vs vehicle with the following order of potency: *mix 2* > *mix 3* > *mix 1* > BCAAs. *Ex vivo* data supported the best overall efficacy and muscle-specific effects of *mix 2*, with treated mice exhibiting significantly heavier hind limb muscles vs vehicle or BCAAs-treated ones, as well as a significant myofiber hypertrophy in tibialis anterior muscle, particularly for fast fibers, vs the BCAAs group. Also, *mix 2* controlled to a major extent the physiological increase of circulating CK and LDH induced by long-lasting exercise (-78% and -38% vs vehicle, respectively), suggesting a further protective action on muscle integrity and metabolism compared to other mixtures. Accordingly, a trend to increase of pAMPK/AMPK protein ratio and a significant reduction of IL-6 gene expression vs vehicle were found in the *mix 2* group [4].
CONCLUSIONS: Our findings corroborate the usefulness of BCAAs + ALA to preserve muscle health during exercise, likely due to an amelioration of BCAAs muscle distribution[4]. The results also increase the interest in the best combination for genetic and acquired muscle-wasting disorders [Supported by FARMI-DIAB Project - Dompé farmaceutici S.p.A. and PRIN-MIUR Prot. 2017FJSM9S_005].
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PD-1 POLYMORPHISM AS A PREDICTIVE BIOMARKER OF IMMUNE-RELATED ADVERSE EVENTS IN PATIENTS WITH SOLID TUMOURS TREATED WITH IMMUNE CHECKPOINT INHIBITORS

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BACKGROUND: Immune checkpoint inhibitors (ICIs), targeting programmed cell death-1 protein (PD-1) and its ligand (PD-L1), have changed the clinical course of malignant diseases, improving outcomes of cancer patients. However, these drugs are effective only in a subgroup of subjects and about 10% of patients develop severe immune-related adverse events (irAEs) causing severe and prolonged sequelae. Therefore, there is an urgent need to identify biomarkers to predict both the efficacy and safety of ICI-based immunotherapy. This study aimed to assess whether the presence of PD-1 single nucleotide polymorphisms (SNPs) correlated with progression-free survival (PFS) and overall survival (OS) and/or development of irAEs in cancer patients treated with ICIs.
METHODS: Caucasian patients with advanced cancer were prospectively enrolled from December 2016 to June 2020, after signing an informed consent. Patient's performance status was assessed according to ECOG (Eastern Cooperative Oncology Group) score, IrAEs were

graded according to Common Terminology Criteria for Adverse Events (version 5.0). PD-1 SNPs (rs10204525, rs2227981, rs7421861, rs11568821, rs36084323 and rs2227982) were selected based on data available in the literature, genotyping was performed by Taqman Real-Time PCR. A statistical analysis was done to evaluate the association between PD-1 SNPs and clinical outcomes, including development of irAEs. A P<0.05 was considered statistically significant.

RESULTS: Seventy-two patients (81.9% male; median age, 66.1 years old) were enrolled. ECOG performance status was 0 (36.1%), 1 (38.9%), 2 (19.4%) and 3 (5.6%). Non-small-cell lung carcinoma was the most common tumour (68.1%), followed by renal cell carcinoma (11.1%), head and neck squamous cell carcinoma (11.1%), melanoma (8.3%) and primary unknown tumour (1.4%). Most patients were treated with anti-PD-1 antibodies; specifically, 76.4% with nivolumab and 12.5% with pembrolizumab. Patients treated with anti-PD-L1 antibodies were 9.7% with atezolizumab and 1.4% with durvalumab. At a median follow up of 12.2 months, the median number of immunotherapy cycles was 15.9, while median PFS and OS were 4.15 and 10.7 months. At 20 months of follow-up, 30.5% of treated patients were still alive. No correlation between OS and PFS and PD-1 SNPs was found. In contrast, rs10204525 significantly correlated with the development of grade 1/2 and also 3/4 irAEs (P<0.002). Specifically, wild type patients developed more irAEs than those carrying heterozygous genotype.

CONCLUSIONS: PD-1 polymorphism (rs10204525) might be a useful biomarker to predict irAE development in cancer patients treated with ICI-based immunotherapy.

MULTICENTER PROSPECTIVE OBSERVATIONAL / EXPLORATORY STUDY ADDRESSED TO THE EVALUATION OF THE EFFECTIVENESS AND SAFETY OF PHARMACOLOGICAL THERAPY IN PATIENTS OPIOIDS-DEPENDENT IN MAINTENANCE THERAPY: FACTORS THAT INFLUENCE THE RESPONSE TO THERAPY IN THE SOUTH EAST AREA, PRELIMINARY RESULTS

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BACKGROUND: The study launched in May 2019 has the primary objective of assessing the efficacy and safety of rac-methadone, levomedone and buprenorphine in maintenance patients and naïve, in pharmacological polytherapy. The objective of the study is to evaluate whether there is a correlation of efficacy/safety of the drugs in subgroups of patients with comorbidity.

METHODS: The primary end point was the temporal reduction of urinary positivity to sub-

stance of abuse evaluated every three months, expressed as a percentage of responders out of the total number of recruits. Secondary endpoints were: the safety assessment with particular regard to those associated with drug interactions caused by poly therapies for patient cohorts. Psychopathology was assessed by ASI, Scl90 and a novel COVID-19 questioner.
RESULTS: To date, 134 patients have been recruited, made up of 78.3% males and 12.7% females with more than 98% of Italian Caucasian ethnicity. Comorbidity study showed that 20%+1.3, 12%+5.9, 7%+5, and 5%+0.9 had psychiatric, infectious, cardiological and metabolic comorbidities, respectively. At enrollment, 80% of patients were treated with rac-methadone with an average dosage of 54.4 mg+10 mg/day, 11% were treated with levomethadone with an average dosage of 41.11 mg+6.5 mg/day and the remainder were treated with buprenorphine/naloxone at an average dosage of 8 mg+2.1 mg/day. The starting Craving was equal to 36/100 according to VAS. Out of 50 patients who have completed the visits to date, after 180 days from recruitment showed a stability in urinary methadone positivity with a marked decrease in heroin positivity -53%+4%, cannabinols -48%+2% and cocaine -37%+6%. The following ADRs have been observed: constipation, muscle fatigue and one case of a Stevens-Johnson syndrome in the groups being treated. 10 patients at recruitment showed an average QTc of 430.2+12.4, average BPM of 75.23+5.3, systolic and diastolic pressure, respectively of 122.5+2.14 and 81.4+3.3 mmHg that was unchanged after 180 days. Adverse events of any nature were: decreased will, loss of work, increased use of alcohol, changes in personality and mood, including abulia, and depression.
CONCLUSIONS: Currently, the patients under monitoring were considered stabilized and safely treated after 180 days of observation in our SERDs with however an unexpected increased dosing of levomethadone. The collected data will allow the construction of therapeutic algorithms useful for improving the prescription of these drugs in clinical practice, particularly recommended in complex patients, abusers and / or in polytherapy.
The study was carried out with the unconditional contribution of Molteni Farmaceutici S.p.A.

AMYGDALA AND PREFRONTAL CORTEX EXCITATORY/ INHIBITORY BALANCE IN A RAT MODEL OF PRENATAL STRESS EXPOSURE

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BACKGROUND: Today more than 300 million individuals worldwide suffer from depression, making it an essential public health priority. Exposure to early life adversities has been identified as a critical risk factor for the lifelong susceptibility to depression. In particular, the intrauterine environment is sensitive to several factors, such as maternal stress and infection, affecting fetal development by interacting with the genetic machinery and determining long-lasting changes in brain function and psychological health. From a molecular standpoint, transcriptional alterations play a major

role in the behavioral phenotype. Though, the molecular underpinnings for enhanced vulnerability to psychopathology after gestational stress remain elusive. On these bases, the aim of this project was to identify brain region-specific transcriptional mechanisms relevant for depression vulnerability in adult male and female rats, previously exposed to a paradigm of prenatal stress (PS).

METHODS: Sprague Dawley male (n=18-20) and female (n=18-20) rats born to either dams exposed to restraint stress during the 3rd week of gestation or left undisturbed in their home cage (sham) were tested for anxiety- and depression-like at postnatal day 80. The behavioral battery included open field, novelty suppressed feeding (NSF), sucrose preference and social interaction tests. At sacrifice, one hemisphere was dissected for gene expression analysis, while the other hemisphere was used for immunohistochemistry. Two-way ANOVA followed by Fisher's LTD post- hoc analyses were used for statistical analysis and significance set at $p<0.05$.

RESULTS: We observed significantly heightened anxiety-like behavior in the NSF for adult PS group of male rats versus shams, as well as a trend towards decreased Z-emotionality in the composite Z-score grouping all behavioral tests assessed. We observed alterations at transcriptional levels of key markers of glutamatergic transmission (Gin2a, Grin2b, vGlut1) in the prefrontal cortex of PS males, as compared to the sham counterpart, along with a significantly heightened expression of Npas4 gene in Parvalbumin immunoreactive positive neurons of the PFC. In the amygdala, another brain area involved in anxiety and emotion regulation, PS males show robust alterations of mRNA levels of Vglut1 and Vgat, two key markers of glutamatergic and GABAergic transmission respectively, and a significant decreased Vglut1/ Vgat expression ratio, an index of excitatory- inhibitory (E/I) balance. Consistently, increased levels of Gad 1/67 gene, implicated in the biosynthesis of GABA, were found for both groups of male and female PS with respect to their relative counterparts. Interestingly, such levels correlated to NSF scores for the male rats.

CONCLUSIONS: These data suggest that altered GABAergic transmission from Parvalbumin cells in the PFC may contribute to E/I unbalance in amygdala and underlie impaired emotional phenotype in male rats exposed to stress in utero.

EFFECTS OF ENDOCRINE DISRUPTING CHEMICALS (EDCS) ON RACK1 EXPRESSION AND THEIR IMMUNOLOGICAL IMPLICATION

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BACKGROUND: Cancers, autoimmune diseases and allergies arose in most industrialized countries, and a role of endocrine disrupting chemicals (EDCs) have been hypothesized. EDCs have been linked with immune alterations due to inflammation-enhancing, immunosuppressive and immunotoxic properties. Elucidating how EDCs interfere with the immune response, ultimately contributing to immune-mediated disorders and immunotoxicity, is becoming of pivotal interest. Since we demonstrated a tight correlation between RACK1 expression and immune

cells activation through PKC activity, RACK1 was investigated as an important EDC target. Indeed, a hormone-related regulatory element for glucocorticoids and androgens was found in rack1 gene promoter region to mediate its transcriptional regulation, resulting in modulation of cytokine production.

METHODS: To investigate the ability of EDCs to modulate RACK1 expression, the human promyelocytic cell line THP-1 was treated with increasing concentrations of p,p'DDT (weak AR antagonist), p,p'DDE (strong AR antagonist), nandrolone (AR agonist), oestrogen-active compounds 17β-oestradiol, non-cell permeable 17β-oestradiol-BSA, 17β-ethynylestradiol (EE), diethylstilbestrol (DES) and zearalenone (ZEA), PFOS (GR agonist), flutamide and agonist G1. Luciferase reporter assay, qPCR, Western blot analysis and specific sandwich ELISA were performed to investigate rack1 promoter activity, RACK1 mRNA production, RACK1 protein expression and cytokines release respectively. Flow cytometric analysis was performed to evaluate CD86 expression.

RESULTS: Our results show that PFOS or p,p'DDT and p,p'DDE, in the opposite way of nandrolone, induced a significant decrease in RACK1 transcriptional activity, RACK1 expression, LPS-induced cytokine production (IL-8 and TNF-α) and CD86 expression. Consistent with its stronger AR antagonistic effect, p,p'DDE exerts a stronger repressor effect than p,p'DDT. On the other hand, 17β-oestradiol, DES, ZEA (through GPER activation) and EE increased RACK1 transcriptional activity and its expression, which paralleled an increase in LPS-induced IL-8, TNF-α production, and CD86 expression all dependent on RACK1/PKCβ activation. Flutamide completely prevented diethylstilbestrol-induced RACK1 transcriptional activity and protein expression, confirming a role for AR in RACK1 transcription regulation.

CONCLUSIONS: Complex effect results from the activity as agonist or antagonist of oestrogens, androgens or glucocorticoids indicating that RACK1 could be a relevant target of steroid-active compounds and EDCs. Hence, RACK1 represents a bridge between the endocrine system and the innate immune system, offering the opportunity to use RACK1 as a possible screening tool for immunotoxic potential of hormone-active substances.

ACKNOWLEDGEMENTS: Research has been supported by Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN2017, Project number 2017MLC3NF)

EVALUATION OF PHARMACOKINETICS AND PHARMACOKINETICS/PHARMACODYNAMICS OF DEFERASIROX IN PEDIATRIC PATIENTS: A MULTICENTER ITALIAN STUDY

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BACKGROUND: The chronic iron overload (IO) is a serious concern in pediatric patients because the severe damage to target organs, as well as liver, kidney and bone marrow. Among pediatric patients, deferasirox (DFX), an iron chelating agent, is used to treat IO in those children who undergo a hematopoietic stem cell transplantation (HSCT). However, the drug is characterized by a large interpatient variability and, more notably, its use is often associated with severe toxicities that require treatment discontinuation in approximately 10% of patients (Cappellini et al. Blood. 2011; Díaz-García et al. Nat Rev Nephrol. 2014). Therefore, the present study was aimed to investigate the pharmacokinetics of DFX in children who receive the drug after HSCT.
METHODS: The retrospective study was approved by the Ethics Committee of the IRCCS Burlo Garofalo, Trieste, Italy (protocol n. 1015/2015). The patient population included 39 children (26 males), aged 2-17 years who underwent to an allogeneic HSCT. IO was diagnosed by abdominal MRI so that DFX was started at a median daily dose of 500 mg. As per clinical routine, blood withdrawal were obtained at steady state according to a therapeutic drug monitoring (TDM) protocol (Maximova et al. Oncotarget. 2017). DFX plasma concentrations were measured by a validated HPLC-UV method (Rouan et al. J Chromatogr B. 2001) and findings were fitted by means of a population pharmacokinetic (POP-PK) analysis that adopted a nonlinear mixed effects modeling (NONMEM 7.3®). Appropriate statistical tests were used.
RESULTS: TDM results showed that minimum plasma concentrations (Cthrough) of DFX were 32.4±23.2 mg/L (mean±SD), hence suggesting a wide variability among patients. Of note, Cthrough values higher than a threshold of 7.5 mg/L were significantly correlated with hematological and hepatic/renal toxicities (p-value<0.0001 for both T-test and Fisher's exact tests). The POP-PK analysis resulted in a mathematical model that demonstrated how several factors had a significant influence on DFX pharmacokinetics. Indeed, lean body mass significantly influenced DFX bioavailability and absorption, body weight had an effect on volume of distribution, while liver and kidney functionality tests (i.e., ALT, AST, direct bilirubin and serum creatinine) did predict changes in drug clearance. When the mathematical model was used to predict drug pharmacokinetics in all patients, the association between high systemic exposure to DFX and the occurrence of hepatic and renal toxicities was demonstrated.
CONCLUSIONS: The POP-PK analysis of DFX in pediatric patients resulted in a model that enabled both the fitting of drug concentrations and the demonstration of a significant correlation between systemic exposure to DFX and toxicities. In future, the adoption of the present POP-PK model in clinical settings could be useful to personalize DFX dose on the basis of known laboratory parameters in children with IO.

ANTIBIOTIC USE IN A CHILDREN NEUROPSYCHIATRY UNIT OF THE UNIVERSITY HOSPITAL OF MESSINA

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BACKGROUND: Antibiotics are the most common medications prescribed to treat bacterial infections in children. This retrospective observational study was aimed to evaluate the prescription of antibiotics and their indications of use in the Child Neuropsychiatry Unit of the University Hospital of Messina (from January 2019 to December 2019).
METHODS: A cohort of paediatric patients aged 0-16 years was selected. Antibiotics class and active principles used were obtained from patients' medical records and analysed for each clinical case. Socio-demographic characteristics, active principles and admission diagnosis were recorded. Patients were stratified by age group into infants (1 month - 2 years), children (2-11 years) and adolescents (12-16 years). Active substances have been classified according to the anatomical therapeutic chemical classification (ATC). Indications of use of antibiotics were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) and grouped through the classification of the Organ Class System (SOC).
RESULTS: 38 children were treated with antibiotics and 71% of them were male. Children median age was 102.5 ± 20.5 months. Moreover, 68.4% of prescriptions referred to children, while 31.6% to adolescents, and no prescription was observed among infants. Antibiotics prescribed were: β-lactams, aminoglycosides, macrolides, tetracyclines, fluoroquinolones, glycopeptides, sulfametoxazole+trimethoprim, phosphomycin. The antibiotics were prescribed for the following diseases: bacterial infections, disorders of the nervous system, respiratory tract infections, general pathologies/administration site conditions, prophylaxis, ear disorders, musculoskeletal and connective tissue disorders and psychiatric disorders.
CONCLUSIONS: Our preliminary data confirm a wide use of different antibiotics, mainly in children, most of them generally considered not the first choice. The inappropriate use of these drugs will be investigated. Furthermore, this data should be compared with the results of other pediatric wards and for a longer observation period.

EARLY ALTERATION OF DIAPHRAGM ECHODENSITY AND FUNCTION IN *MDX* MICE BY NON-INVASIVE ULTRASONOGRAPHY: A CORRELATION WITH *IN VIVO* AND *EX VIVO* FUNCTIONAL READOUTS

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BACKGROUND: In the *mdx* mouse model of Duchenne muscular dystrophy (DMD), the structural and functional alterations of diaphragm occur at early age, with a progression closely resembling patients' condition. Ultrasonography has been recently proposed as a longitudinal, non-invasive technique in pre-clinical studies in mdx mice to assess diaphragm dysfunction and detect drug efficacy. However, the independent validation of the technique is still lacking and few data are available at the stages of the pathology at which most pharmacological studies are conducted.
METHODS: Ultrasound acquisition of diaphragm was performed on 3- and 6-month-old mdx mice, the preferred age-window for pharmacology studies, by using the ultra-high frequency ultrasound biomicroscopy system Vevo2100® (VisualSonics, Toronto, ON, Canada). The alteration of diaphragm function over time was measured as ultrasound movement amplitude. At the same time points, a first-time assessment of diaphragm echodensity was performed, as an experimental index of progressive loss of contractile tissue. A computational analysis was made to further validate echodensity measurements and to establish the role of the hyperechoic superficial abdominal wall (AW) thickness in ultrasonography signal penetration. A parallel evaluation of other in vivo and ex vivo dystrophy-relevant readouts was carried out.
RESULTS: Both 3- and 6-month-old mdx mice showed a significant decrease in diaphragm amplitude compared to wild type (wt) mice. This index was well-correlated either with in vivo running performance or ex vivo isometric tetanic force of isolated diaphragm. In addition, diaphragms from 6-month-old dystrophic mice were highly susceptible to eccentric contraction force loss ex vivo. Importantly, we disclosed an age-dependent increase in echodensity in mdx mice not observed in wt animals, which was independent from abdominal wall thickness. This was accompanied by a marked increase of pro-fibrotic TGF-β1 levels in the mdx diaphragm and of non-muscle tissue amount in diaphragm sections stained by hematoxylin-eosin.
CONCLUSIONS: Our findings corroborate the usefulness of diaphragm ultrasonography as a powerful tool to monitor mdx pathology progression. Importantly, our first-time observations on diaphragm echodensity support the use of ultrasonography for detecting fibrosis progression in the mdx mouse model (or in other murine models of DMD). This envisages the possibility to perform a longitudinal evaluation of the efficacy of pharmacological treatments in preventing or slowing this process with minimal invasiveness, which is a valuable approach for both clinically-oriented pre-clinical research and trials on DMD patients.
This work was supported by The Dutch Duchenne Parent Project (DPP NL; grant 2015) and by PRIN-MIUR (grant n. 2015MJBEM2_005 and 2017FJSM9S_005) to Annamaria De Luca

PREDICTIVE ROLE OF GENDER AND DPYD PHARMACOGENETICS IN LOCALLY ADVANCED RECTAL CANCER PATIENTS TREATED WITH FLUOROPYRIMIDINES

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BACKGROUND: Locally advanced rectal cancer (LARC) at clinical stages II to III treatment is based on neoadjuvant chemoradiotherapy (CTRT) based on administration of FP. Four single nucleotide polymorphisms (SNP) in DPYD were associated with a lower enzyme function and a higher frequency of developing severe and life-threatening toxicity to FP. FP have also substantial sex-related inter-individual variability in clearance/exposure with a 26% higher exposure in women potentially related to a differential outcome.
Aim of this study was to evaluate the impact of the genetics of DPYD and patients' gender on toxicity and response to treatment in a group of LARC patients treated with FP-based CTRT.
METHODS: A series of LARC patients enrolled between 2003 and 2019 in Padua and in CRO-Aviano hospital treated with FP-based CTRT and providing a blood sample were studied. The genotyping was performed on genomic DNA using TaqMan and KASP fluorescent probes. Four SNPs of DPYD were investigated rs3918290 (DPYD*2A), rs55886062 (DPYD*13), rs67376798 (DPYDc.2846A>T), rs75017182 (DPYD HapB3). Toxicity grade was assessed based on 4.0 Version of the Common Terminology Criteria of Adverse Events (CTCAE).
RESULTS: A total of 135 LARC patients were enrolled in the study. Fifty-one females (37.8%) and eighty-four males (62.2%), homogenous for treatment, were analyzed for all the DPYD variants. After genotyping analysis: none of the patients were mutated for DPYD*2A or DPYD*13 while 3/135 (2.2%) patients were heterozygous for DPYD c.2846A>T and 3/135 heterozygous for DPYD HapB3.
Female gender was associated with a higher probability of developing FP-related toxicity (OR:2.29, CI:1.11-4.76, P=0.031, by Fisher's Exact Test). The presence of at least one of the four DPYD variants increased the risk of hematological toxicity grade 3 (OR: 12.4, CI: 1.82-84.45, P=0.03) and the risk of hospitalization for therapy-related adverse reaction (OR:8.71, CI:1.36-56.01, P=0.052).
Focusing on the interaction between DPYD genetics and patients gender, we observed that 1/2 (50%) of female patients with at least one DPYD mutation developed a grade 3 non-hematological toxicity event against only 0/4 (0%) males. Regarding grade 3 hematological toxicity, 1/2 (50%) of female patients with at least one DPYD mutation developed a grade 3 hematological toxicity event and was hospitalized for toxicity, whereas only 1/4 (25%) had toxicity and related hospitalization.
CONCLUSIONS: We have herein demonstrated that beside the 4 DPYD SNPs commonly

analyzed and their important impact on toxicity risk, female gender demonstrated to increased grade 2-3 FP-related toxicity. Furthermore a significant interaction between gender and DPYD variants was highlighted in terms of severe toxicity (grade 3) and risk of hospitalization. These data could be useful to further stratify the population to prevent side effects and for clinical implications in the management of LARC patients' therapy.

ANTI-INFLAMMATORY AND PAIN RELIEVING ACTIVITY OF POSIDONIA OCEANICA (L.) DELILE: PRECLINICAL EVIDENCES

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BACKGROUND: Over the last years, the herbal market has rapidly increased and many surveys have been conducted that aim at finding natural ingredients with possible applications as food additives or medicine. In this area, special attention has been given to the sea environment as a rich source of new active compounds. The marine vascular plant *Posidonia oceanica (L.) Delile* is the only endemic species of the Mediterranean Sea belonging to Posidoniaceae family. It is a marine angiosperm that flowers underwater forming vast meadows covering tens of thousands of square kilometers considered an important marine habitat. The decoction of the leaves is traditionally used as a remedy for diabetes and hypertension by villagers living by the coast of Western Anatolia. However, recently some studies have shed light on other promising biological *in vitro* properties of the ethanolic extract obtained from *Posidonia oceanica* (POE) leaves, including anti-glycation effects, inhibition of tumor cell migration, and antioxidant and anti-inflammatory activities. In light of these considerations, the aim of the study was to investigate thoroughly the anti-inflammatory and analgesic role of POE following acute treatment in mice.
METHODS: CD-1 male mice were orally administered with POE (10 – 100 mg kg-1). The anti-inflammatory activity was evaluated in the formalin (1.25% intraplantarly, 30 ml) and carrageenan (1.5% intraplantarly, 80 ml) tests while, for the analgesic properties, mice were challenged with the hot-plate (49°C) and the writhing tests (acetic acid 0.6%).
RESULTS: POE administration, 2h after carrageenan injection, exerted a dose dependent anti-inflammatory effect that counteracted carrageenan-induced allodynia from 15 min up to 45 min after treatment. In line with these results, POE was also able to diminish the paw oedema evoked by carrageenan of about 30% confirming its anti-inflammatory properties. In the formalin test, pre-treatment with POE significantly reduced, in a dose dependent manner, the nociceptive behaviors evaluated as lifting, licking, shaking and flinching of the formalin-injected paws. The effect lasted up to 60 min after formalin injection demonstrating that POE was effective in both neurogenic

and inflammatory phases evoked by formalin. Moreover, in the hot-plate test, POE administration in naïve mice significantly increased the mice pain threshold 30 min after treatment highlighting analgesic properties. In the writhing test, a type of abdominal pain evoked by acetic acid injection, POE was not protective in reducing the abdominal contractions.
CONCLUSIONS: These results indicated Posidonia oceanica ethanolic extract (POE) as a new valid strategy against inflammation and inflammatory pain.

A NETWORK APPROACH FOR THE STUDY OF DRUG (CO)PRESCRIPTIONS: ANALYSIS OF DATA FROM THE ASL TO4 (REGIONE PIEMONTE, ITALY)

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BACKGROUND: With the impressive growth of accessible data, the problem of devising efficient quantitative methods for studying complex systems has emerged. The great power of networks in describing real-world systems has attracted a growing interest and this modelling approach has been applied in many research areas during the last decades. In the field of pharmacoepidemiology, the network approach has been applied to the analysis of data on drug prescription. In particular, in a Drug Prescription Network (DPN), each drug is represented as a node and two drugs co-prescribed to the same patient are represented as an edge linking the nodes. The aim of this study was to demonstrate the analytical power of the DPN-based approach when applied to the analysis of administrative pharmaceutical data.
METHODS: Data on drug prescription collected over a 12-month period (July 2018–June 2019) from the ASL TO4 (Regione Piemonte, Italy) were used to create several DPNs corresponding to the five levels of the Anatomical Therapeutic Chemical classification system. A total of 5,431,335 drugs prescribed to 361,574 patients (age 0–100 years; 54.7% females) were analysed. Statistical and network measures were used to characterize the structure of DPNs. These measures included, the density of the graph, the assortativity coefficient and the Pearson's correlation coefficient for binary variables. Network comparison was performed by computing the Euclidean distance between graphs.
RESULTS: DPNs were dense networks, with giant components, which contain all nodes. As graph density depends on the number of edges, with respect to the number of nodes in the network, this implies that co-prescription of drugs is a very common event, which can be quantified by specific network measures. In addition, DPNs showed disassortative mixing of node degrees, which implies a non-random connectivity into the networks. The structure of DPNs was strictly associated to the prevalence of drug (co)prescriptions. Finally, a number of clusters in the distance matrices were identified thus quantifying the relative contribution of each node in determining gender- and age-specific patterns in drug prescription.
CONCLUSIONS: Networks can be created easily from administrative data routinely col-

lected by health systems. Network-based methods have been proven to be a flexible and efficient approach to analyse administrative data on drug prescription. By complementing more traditional pharmacoepidemiology methods, this approach could provide an efficient strategy to study the complexity of drug prescription.

NATALIZUMAB DOSING IN MULTIPLE SCLEROSIS: *IN SILICO* ASSESSMENT OF DOSING STRATEGIES

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BACKGROUND: Natalizumab is an anti-alpha4-integrin cell adhesion molecule monoclonal antibody used in the treatment of multiple sclerosis (MS). Natalizumab treatment is associated with an increased risk of progressive multifocal leukoencephalopathy (PML), a rare albeit potentially lethal brain infection caused by the John Cunningham virus. In addition, although highly efficacious in decreasing disease activity, many patients report the so-called wearing-off symptoms at the end of the standard interval dosing (SID, 300 mg once every 4 weeks). Extended interval dosing (EID; 300 mg once every 5-8 weeks) of natalizumab has been demonstrated as an alternative dosing strategy to reduce PML risk. However, extending dosing intervals could increase the risk of disease activity. Therefore, there is a need for new natalizumab dosing strategies and tools to guide clinicians in making timely decisions for MS patients. However, owing to the complex relationship between natalizumab pharmacokinetics (PK) and individual patient factors (e.g., covariates such as body size), interpreting natalizumab concentrations to guide dosing decisions is not straightforward. Here we present a proof of concept where *in silico* methods can rapidly examine the relative benefits of selected maintenance dose protocols for natalizumab.
METHODS: Several dosing strategies (SID, EID and therapeutic drug monitoring-based with established algorithms) were simulated on a virtual population using the R statistical and programming language to assess their relative performance. Strategies were evaluated on their ability to maintain trough natalizumab serum concentrations above an established target, 2 mg/L, and antibody occupancy of alpha4-integrin binding sites above 50%. Natalizumab serum concentrations were predicted using a population 2-compartment model with both linear first-order and Michaelis-Menten elimination. The relationship between natalizumab serum concentration and alpha4 integrin saturation was described by a direct response model with a sigmoidal effect on alpha4 integrin saturation mediated by a maximum effect relationship with natalizumab concentrations.
RESULTS: A broad range of natalizumab serum concentrations and occupancy of alpha4-integrin binding sites was determined in both the SID and EID samples. Model-based dosing was superior in maintaining target trough concentrations and occupancy of alpha4-integrin binding sites. Moreover, model-based dosing results were consistent across a range of baseline covariate groups.
CONCLUSIONS: This *in silico* assessment of dosing strategies demonstrated that, when challenged with covariate and random effect changes occurring in individual pharmacokinetic parameters, model-based approaches were superior to other strategies. Model-based

dosing has not been tested clinically; however, the potential benefits of model-based dosing for natalizumab suggest that it should be investigated to optimize individual exposure.

PHARMACOLOGICAL ACTIVITIES OF PLANT-DERIVED OPIOID PEPTIDES RUBISCOLIN-6, SOYMORPHIN-6 AND THEIR C-TERMINAL AMIDE DERIVATIVES

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BACKGROUND: Rubiscolin-6 (amino acid sequence: YPLDLF) is an opioid peptide derived from the plant enzyme Rubisco, whilesoymorphin-6 (peptide sequence: YPFVVA) is an opioid peptide derived from the enzymatic digestion of soy proteins. In this work we investigated the pharmacological activities of rubiscolin-6, soymorphin-6 and their new C-terminal amides *in vitro* using cells expressing the human recombinant delta and mu opioid receptors and *in vivo* using the mouse tail flick and formalin tests.
METHODS: In *in vitro* experiments, we evaluated the capability of rubiscolin-6, soymorphin-6 and their new C-terminal amides to activate the mu- and delta-human recombinant receptors permanently transfected in CHO cells. In such cells, the expression of a chimeric G protein that forces the opioid receptors to couple with the calcium pathway allows measuring receptor activation with an automated calcium mobilization assay. In *in vivo* experiments, the tail flick test was used to determinate antinociceptive response induced by a thermal stimulus and compounds were injected at 10 µg/10 µL for intracerebroventricular (i.c.v.) administrations. In the formalin test, which measured the response to inflammatory pain, compounds were administered subcutaneously in the dorsal surface of the right hind paw of the mouse at the dose of 100 µg/20 µL.
RESULTS: In the calcium mobilization assay, rubiscolin-6 C-amide, soyamorphin-6 and soyamorphin-6 C-amide stimulated calcium mobilization in mu expressing cells only at micromolar concentrations while rubiscolin-6 was completely inactive. In cells expressing the delta receptor, all compounds showed an incomplete concentration response curve, being active only at micromolar concentrations. In the tail flick test, rubiscolin-6 induced a significant but transient antinociceptive effect whereas rubiscolin-6 C-amide induced a statistically significant, prolonged antinociceptive effect . Soymorphin-6 and soymorphin-6 C-amide exerted significant antinociceptive activity until 30 min after the administration. Rubiscolin-6 was not able to change the nociceptive effect of formalin whereas rubiscolin-6 C-amide reduced formalin-induced nociception in both test phases. Soymorphin-6 and soymorphin-6 C-amide did not change the nociceptive effects of formalin.
CONCLUSIONS: In conclusion, we found rubiscolin-6 derivative C-terminal amide able to exert strong antinociceptive effects after central and subcutaneous administration despite its low agonist potency on opioid receptors. These results push us to further investigate rubiscolin-6 amide mechanism of action in other experimental paradigms and to design other derivatives more active in animal models of pain and inflammation.

DISCOVERY OF OREXANT AND ANOREXANT AGENTS WITH INDAZOLE SCAFFOLD ENDOWED WITH PERIPHERAL ANTIEDEMA ACTIVITY

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BACKGROUND: In the last two decades, obesity has assumed the form of a pandemic, often related to the development of cardiovascular diseases (CVD) and strokes. There is a worldwide emergency that requires the development of novel and safer anti-obesity treatments. Rimonabant, a well- known inverse agonist of the type-1 cannabinoid receptor (CB1), associated with lifestyle modifications was successfully engaged in the control of obesity by reducing appetite and weight gain. Though this drug has shown to be effective to control body weight and to manage obesity, it has been withdrawn from the market due to its important side effects. Thus, a novel drug able to provide the same therapeutic efficacy without the dangerous side effects is an urgent need. We report a series of lonidamine joined Leu, tert-Leu and Val amino acids with different C-terminal functional groups (LONI1-4,11) designed as hybrids of Fubinaca family compounds and Rimonabant, as novel compounds endowed with orexant/anorexant activity.
METHODS: We evaluated the orexant/anorexant activity of LONI1-4,11 in the feeding test using CD-1 male mice after intraperitoneal (i.p.) administration (10 mg/kg). For a LONI11 formalin test and a tail flick test after an administration by the subcutaneous (s.c., 30–100 µg/20 µL) and intracerebroventricular (i.c.v., 10 µg/10 µL) routes, respectively, were also carried out in mice to investigate the antinociceptive property at the central and peripheral levels. Furthermore, Zymosan-induced edema (s.c., 100 µg/20 µL) and hyperalgesia (s.c., 100 µg/20 µL) assays were also performed to estimate the anti-inflammatory activity of LONI11 in mice model.
RESULTS: We observed a significant orexant effect for LONI11 and an intense anorexant effect for LONI2 and LONI4. LONI11 at the doses of 10 µg/10 µL and 100 µg/20 µL produced a slight antinociceptive effect after i.c.v. and s.c. injections, respectively, in tail flick test and in formalin test. In zymosan-induced edema and hyperalgesia, LONI11 reduced the percent of paw volume increase and paw latency after s.c. administration, also suggesting a possible peripheral anti- inflammatory activity.
CONCLUSIONS: These results could be useful as a starting point to optimize the pharmacological properties of rimonabant/Fubinaca hybrids and to better understand the molecular mechanism below their biological profile. An understanding of all the neuroendocrine networks and their roles in the hypothalamus to regulate the feeding behaviour could lead to the development of novel and safer approaches to manage the main nutritional disorders.

A NOVEL IDENTIFICATION SYSTEM FOR POTENTIALLY COUNTERFEIT MEDICAL DEVICES BASED ON THE VISUAL ANALYSIS THROUGH EVALUATION SHEET

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BACKGROUND: The counterfeiting of medical devices has been a growing problem for several years, especially since the extension of the internet. Regulatory authorities are responsible, in collaboration with relevant national and international stakeholders, for establishing mechanisms to detect falsified products circulating in their territories and for removing them from the market. Thus it might be useful develop a simple and affordable preliminary screening methods to be used by inspectors to decide in the field whether to collect a sample for further laboratory analysis or not. We here report a new method to compare the physical appearances of suspected samples with those of known genuine products based on the new regulation 2017/745 on medical devices.
METHODS: According to the new Regulation (EU) 2017/745 on medical devices, we have collected all the requirements regarding the information supplied with the device, including: qualitative composition of the device and quantitative information on the main constituent or constituents responsible for achieving the principal intended action, the labeling and packaging and the CE marking of conformity. Thanks to these pieces of information, along with those concerning the physical characteristics of the product, permit us to compare the potentially counterfeit medical devices with the known genuine products.
RESULTS: We developed a new and quick method to provide evidence that the sample comes from a falsified product. We have designed a table (Table 1) containing a list of questions to ask yourself to compare potentially counterfeit medical device with the known genuine products. The questions include the visual analysis of packaging and the information reported on the package (Does it reflect the appearance of the original form and colour? Is the packaging size the same as the original? Is this system homologous to the original? Do the information on the packaging reflect the appearance of the original colour and form?...), including qualitative and quantitative information (Since it is a substance-based medical device, is the overall qualitative composition of the device reported? Does this match that of its original counterpart?...), the presence and the conformity of CE marking (Is there an EC marking? Is the marking clear, legible and indelible?...) and the physical analysis of the characteristics of the product (Do they all have the same shape if they are tablets/capsules? Do all tablets/capsules have the same size among them and compared to the original? Do all the tablets have the same type of surface coating?...).The questions provide a simple yes or no answer.
CONCLUSIONS: Visual analysis is an important part of the inspection process in the pharmaceutical industry. Falsified medical devices may be identified from this simple and quick method.

SHEDDING LIGHT ON PERICYTES: A NEW INVOLVEMENT OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) IN TUMOUR ANGIOGENESIS

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BACKGROUND: Nicotinamide phosphoribosyltransferase (NAMPT) is a key enzyme for nicotinamide adenine dinucleotide (NAD) biosynthesis. NAMPT exists in two distinct forms: an intracellular form (iNAMPT) and an extracellular form (eNAMPT). Given the high energy demand of tumours, NAMPT is overexpressed in many cancer types, and drugs inhibiting its activity have entered in clinical trials for advanced haematological or solid malignancies.
eNAMPT is released by most of the cells, including tumoural cells. Its serum and plasma levels are increased in several cancer patients and in most of the cases they correlate with the stage of cancer progression.
Preliminary data obtained by an in vivo murine mammary carcinoma which is able to release an high amount of eNAMPT (in which NAMPT was fused to signal peptide of IgG (SP-NAMPT) and stably expressed in 4T1 cells) suggested that high levels of eNAMPT in the tumour microenvironment reduce tumour growth but increase microvessel density (CD31+) and pericyte coverage (NG2+) in tumours.
Several studies investigate the role of NAMPT on endothelial cells (ECs), but no data are available in literature on NAMPT and pericytes. The aim of this study was to shed light on the role of NAMPT on pericytes.

METHODS: To this end we evaluated: i) the characterization of iNAMPT and eNAMPT expression in these cells, ii) the expression of the main pericyte markers, iii) pericytes proliferation, iv) pericytes migration, v) pericytes ability to form microtubules under different stimuli (i.e. NAMPT inhibition or recombinant NAMPT stimulation).
RESULTS: The data obtained show that NAMPT is expressed at high levels in pericytes and it is also actively released by these cells. The eNAMPT secretion in pericytes is higher compared to ECs. I demonstrated that pericytes are highly sensitive to NAMPT inhibitor (FK866), with an IC50 of around 10 nM. Moreover, NAMPT inhibition reduces platelet-derived growth factor receptor (PDGFR)-β expression in a dose-dependent manner and affects their migration and the ability to form microtubules. Our results also suggest the existence of a synergism between eNAMPT and platelet-derived growth factor (PDGF)-B, a known important stimulus for pericytes recruitment during both normal and tumoural angiogenesis. Moreover, these in vitro data correlate with the in vivo murine model of mammary carcinoma, in which the expression of PDGFR-β was higher in SP-NAMPT tumours, which have been engineered to release high levels of eNAMPT in TME.
CONCLUSIONS: The results from this study show a strong involvement of NAMPT in pericytes and paved the way to assess future efforts to better characterize the involvement of NAMPT not only on pericytes, but also on the cross-talk between pericytes and ECs, in physiological and pathological conditions, such as cancer.

BERGAMOT ESSENTIAL OIL INHIBITS CHOLINERGICALLY-MEDIATED CONTRACTIONS IN RAT ISOLATED GUT

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BACKGROUND: Treatment options for management of irritable bowel syndrome (IBS) are scanty and include the combination of dietary and pharmacological interventions. These are aimed at managing lower abdominal pain, the predominant and most common symptom of IBS. However, none of the currently available treatments is able to abrogate the complex symptoms of IBS. In addition, chronic abuse of these remedies is endowed with side effects onset. These latter problems have directed patients to the complementary use of natural products including some essential oils, such as lavender and peppermint. A large body of evidence shows that bergamot essential oil (BEO) is endowed with reproducible analgesic and anxiolytic effects that may stem from the reported modulation of the excitatory and inhibitory neurotransmission in the central nervous system (CNS). Despite the diverse functional complexity of the enteric nervous system, it is conceivable that BEO may exhibit at the gastrointestinal level biological properties similar to those reported in the CNS. Accordingly, here we have investigated ex vivo the effect of BEO and its major components linalool, linalyl-acetate and limonene, on cholinergically-mediated contractions in gut preparations of rat.

METHODS: The experiments were performed using isolated jejunum, ileum and colon from male Wistar rats (250-300g). Each tissue was cut into full thickness strips along the longitudinal axis and was suspended in tissue baths containing Krebs solution (5% CO2 in O2; 37°C) under 1 g of tension for recording of isometric contractions in response to the application of acetylcholine (ACh). The experimental protocols were authorized by the Ministry of Health (Authorization Number 700A2.N.6TI; date of approval: 13/03/2018).

RESULTS: Administration of ACh (10-6M) elicited a contraction in isolated jejunum, ileum and colon of rat. Application of BEO (2.5x10-5 to 2.5x10-3%v/v) 5 min before Ach resulted in a concentration-dependent decrease in the height of the contraction elicited by ACh. The effects of linalool, linalyl acetate and limonene (2.5x10-6M, 2.5x10-5M, 2.5x10-4M) were also evaluated; the latter resulted in a concentration-dependent decrease of the height of ACh-contractions. Among the constituents of BEO, linalool showed the maximum inhibition in all types of tissues.

CONCLUSIONS: Our present data demonstrate that BEO inhibits cholinergically-mediated contractions in rat isolated gut. These original data suggest that BEO might be beneficial in the treatment of enteric spastic disorders where the predominant symptom is pain; incidentally, IBS is one such painful clinical condition in which a role for altered inhibitory and excitatory neurotransmissions has been envisaged. Further studies are needed for clinical translation of the present findings.

INHIBITORY EFFECT OF LINALOOL ON NON-CHOLINERGIC MEDIATED CONTRACTIONS IN ISOLATED COLON OF RAT

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BACKGROUND: Recent findings demonstrated that bergamot essential oil (BEO), largely through the actions of linalool, is able to influence neuromuscular contractions in enteric nervous system in rat and human, suggesting that BEO might be beneficial in the treatment of enteric spastic disorders such as irritable bowel syndrome (IBS). However, the mechanism underlying the latter effect of the phytocomplex remains unclear. Accordingly, to gain more insight in spasmolytic effect of BEO here we have investigated ex vivo the effect of linalool, the most active components of BEO, on non-cholinergic mediated contractions evoked by serotonin (5-HT) and substance P (SP) in colon preparations of rat.

METHODS: The experiments were performed using isolated colon from male Wistar rats (250-300g). Each tissue was cut into full thickness strips along the longitudinal axis and was suspended in tissue baths containing Krebs solution (5% CO2 in O2; 37°C) under 1 g of tension for recording of isometric contractions in response to the application of 5-HT and SP and acetylcholine (ACh). The experimental protocols were authorized by the Ministry of Health (Authorization Number 700A2.N.6TI; date of approval: 13/03/2018).

RESULTS: Administration of 5-HT (10-8M), SP (10-7M) or Ach (10-6M) elicited a reproducible contraction in isolated colon of rat. Application of linalool (2.5 x 10-6 M and 2.5 x 10-5 M, 2.5 x 10-4 M) 5 min before 5-HT, SP and ACh resulted in a concentration-dependent decrease in the height of the contraction elicited by all contracting agents.

CONCLUSIONS: Our present data demonstrate that linalool inhibits both cholinergic and non-cholinergic mediated contractions in rat isolated colon. The ability of linalool to interfere with the contractions induced by the different spasmogenic agents seems to suggest that its action may depend on the modulation of intracellular processes common to different stimuli rather than a receptor-type mechanism. Accordingly, in vitro studies demonstrated that linalool interferes with Na+, Ca2+ and/or K+ intracellular levels. These data confirm that spasmolytic effects of BEO underlie the involvement of complex mechanisms and deserve further investigation.

DRUG DELIVERY THERAPY STRATEGY THROUGH CHITOSAN-BASED POLYMERIC NANOPARTICLES COATED WITH ANTI-GLYPICAN-3 (GPC3) ANTIBODY AND LOADED WITH A CHEMOTHERAPEUTIC DRUG TO TREAT HEPATOCELLULAR CARCINOMA

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BACKGROUND: Hepatocellular carcinoma (HCC) is the most common liver cancer and the third leading cause of cancer-related death (1). Late diagnosis, therapies' side-effects and no curative treatments make necessary to find new markers for a target therapy (2). Glypican 3 (GPC3) is a cell-surface proteoglycan present in HCC and absent in healthy liver tissue and benign lesions (3,4). These features make it a promising target for a delivery approach.
The use of the biocompatible polymeric nanoparticles functionalized with antibodies targeting tumor-associated antigens (such as GPC3) and loaded with the most suitable chemotherapeutic drug is useful to overcome side-effects caused by the off-target action that is one of the major problems in the chemotherapeutic treatment (5). The aim of the project is to set up a nanoparticle-based drug delivery approach in order to carry a chemotherapeutic drug directly to the cells using chitosan-based polymeric nanoparticles coated with the anti-GPC3 antibody.
METHODS: GPC3 protein expression has been detected using three different techniques: western blot, immunofluorescence and flow cytometry.
Cytotoxicity tests have been made using flow cytometry and/or MTT assay.
In-vivo HCC xenograft murine model has been set up by subcutaneous injection of 5 millions of HUH-7 cells in the right flank of each mice. Once the tumours reached the dimension limit, the mice have been sacrificed to recover tumour and organs for subsequent immunohistochemistry analysis.
RESULTS: GPC3 protein studies using western blot (Figure A), immunofluorescence (Figure B) and flow cytometry (Figure C) confirmed its high expression in HCC cell lines (Hep3B2, HUH-7 and HepG2) and low or no signal in the negative control of breast cancer cell line MDA-MB-231. Further, results highlighted the C-terminal domain as the best region for an antibody interaction.
Drug cytotoxicity tests by MTT and flow cytometry confirmed that anthracyclines have a high cytotoxic action making them ideal nanoparticle-loading agents.
HCC xenograft nude mice model revealed that HUH-7 have a tumorigenic activity after subcutaneous injection.
In-vitro cytotoxicity of doxorobucin- or idarubicin-loaded chitosan-based polymeric nanoparticles has been evaluated in HUH-7 cell line.
CONCLUSIONS: Considering the urgent need of better strategies to overcome HCC in patients, this target therapy approach could be a new option for the treatment of early and advanced HCC. Further studies are scheduled to check GPC3 positivity in HCC tumour and healthy tissues from HCC xenograft murine model and to test nanoparticles killing capa-

bilities in-vitro, their biodistribution, targeting and killing capabilities in-vivo.

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BCL-XL OVEREXPRESSION DECREASES GILZ LEVELS AND INHIBITS GLUCOCORTICOID-INDUCED ACTIVATION OF CASPASE-8 AND CASPASE-3 IN MOUSE THYMOCYTES

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BACKGROUND: Apoptosis is a central process in thymus physiology since about 90% of thymocytes die, leaving only 10% to complete their maturation and migrate out towards peripheral lymphoid organs to mount a functional immune response.
Classically, apoptosis is described as occurring through two different energy-dependent molecular pathways — the extrinsic and the intrinsic pathways. Moreover, Bcl-2 family members play a key role in regulating thymocyte apoptosis via the intrinsic pathway by promoting a balance between pro-apoptotic, such as Bax, and anti-apoptotic members, such as Bcl-xL.
In the thymus, glucocorticoids (GCs) decrease Bcl-xL expression by activating signal transducer and activator of transcription 5B (STAT-5B) and recruiting it to the Bcl-xL promoter. GCs decrease Bcl-xL expression also through the transcription of glucocorticoid-induced leucine zipper (GILZ), a protein upregulated by GCs in the thymus.
METHODS: Lckpr-bcl-xL transgenic mice bred on the C57BL6/J background constitutively overexpressing Bcl-xL within all thymocyte subsets were a generous gift of Dr. Craig Thompson. Female wild type (WT) or lckpr-bcl-xL transgenic (TG) mice of 4–8 weeks of age were sacrificed by cervical dislocation, thymi were excided, thymocytes were reduced to single cell suspencion and counted with a hemocytometer. Single cell suspensions were cultured at a concentration of 3 x 106 with or without 10-7 M dexamethasone (DEX). Total proteins were exctrated and probed in Western blot experiments.

RESULTS: DEX increased the expression of GILZ in WT thymocytes but, surprisingly, the expression of GILZ in TG transgenic thymocytes was decreased irrespective of their culturing with or without DEX. Thus, Bcl-xL overexpression decreased the expression of the GC-induced protein GILZ.
Moreover, caspase-3 was not activated in untreated WT and TG thymocytes. The addition of DEX induced the activation of caspase-3 in control thymocytes; however, DEX treatment did not induce activation of caspase-3 in Bcl-xL TG thymocytes. DEX-dependent activation of caspase-8 was completely abolished in Bcl-xL TG thymocytes. Surprisingly, this was not the case when caspase-9 activation was probed: caspase-9 activation was not inhibited by DEX in Bcl-xL transgenic thymocytes, suggesting that in thymocytes, Bcl-xL inhibits caspase-3 activation through inhibition of caspase-8 but not caspase-9 activation.
CONCLUSIONS: Although the exact mechanistic role of GCs in death by neglect is currently unclear, their repression of Bcl-xL expression, a signature of death by neglect, indicates a high probability of their involvement in the process.
Our results indicated that increase of Bcl-xL inhibited activation of caspase-8 and caspase-3, but not of caspase-9, suggesting the presence of a new GC-dependent apoptotic pathway that links Bcl-xL with caspase-8, and is distinct from the well-known intrinsic (Bcl-xL-caspase-9) apoptotic pathway.

IDENTIFICATION OF A NEW TREG SUBSET WITH ANTI-PROLIFERATIVE ACTIVITY IN PATIENTS WITH B-ACUTE LYMPHOBLASTIC LEUKEMIA THROUGH BIOINFORMATICS

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BACKGROUND: B-acute lymphoblastic leukemia (B-ALL) is one of the most common pediatric cancer and accounts for 85% of all ALL cases (1). Aside from genetic alterations, previous studies suggest the immune system as a key player in the development and maintenance of B-ALL. Regulatory T cells (Tregs) have been implicated in tumor development and immune suppression. In this study, the expression, and the coordinate expression of 22 Treg markers were evaluated to investigate the increased/decreased number of Treg subsets in the peripheral blood mononuclear cells (PBMCs) of patients with B-ALL.
METHODS: Genevestigator software was used to obtain PBMC mRNA expression from 25 patients with B-ALL and 93 healthy donors (HDs) with matched age and sex. The amount of T cells in patients and HDs was evaluated by using T cell signature (TCS) genes (2). Then, in each patient and HDs, the Treg marker mRNA levels were normalized with those of the TCS. To find the Treg markers expressed in a coordinated fashion, the correlation of each Treg marker expression level with that of the other markers was investigated using the Spearman and Pearson correlation tests. Then, for the selected markers, the correlation of each marker with regulatory transcription factors (FoxP3, Helios), RORγt transcription factor, cytokines, IDO and the markers of cell proliferation (Ki-67 and PCNA) were also analyzed.

RESULTS: The normalized mean expression of 21 out of 22 Treg markers was higher in PBMC of patients as compared to HDs. The increase pattern of Treg markers was not homogeneous (ranging between 1.5 and 20 folds) and the expression of 11 markers was more than 8 folds in B-ALL patients than in HDs. In each patient the expression of the 11 markers was higher than the mean+2SD expression of HD, with the only exception of 4-1BB. Interestingly, 8 out of 11 Treg markers (CD25, LAG3, GITR, 4-1BB, OX40, PD-L2, CCR8, and CCR4) correlated each other significantly and with a correlation factor higher than 0.4, possibly indicating that they are expressed by the same cells, expanded in B-ALL. Each of the above-mentioned marker correlated with FoxP3 and IDO, confirming the Treg markers are expressed by a cell subset with regulatory activity, but correlated inversely with Ki-67 and PCNA, suggesting that the presence of this cell subset inhibited the growth of leukemic cells. The correlation of the markers with RORγt and IL-17 suggested that the subset is formed by FoxP3^{+}RORγt^{+} Tregs.
CONCLUSIONS: Bioinformatics analysis suggests that in PBMC of patients with B-ALL there is a FoxP3^{+}RORγt^{+} cell subset previously described as activated Tregs with immunosuppressive and pro-inflammatory activity (3). Our study suggests that future targeted immunotherapy of Treg cells could be a promising approach in B-ALL treatment.

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EFFECTS OF HIGH FAT DIET IN THE WAG/RIJ RAT MODEL OF EPILEPTOGENESIS, ABSENCE EPILEPSY AND NEUROPSYCHIATRIC COMORBIDITIES

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BACKGROUND: Recent studies reported that hyperglycemia and/or obesity, also characterizing metabolic syndrome, may precipitate and/or worsen seizures1. In this light, we decided to study the effects of metabolic syndrome on the development of absence seizures and neuropsychiatric comorbidities in WAG/Rij rats, a well-established genetic model of absence epilepsy, epileptogenesis, and neuropsychiatric comorbidities, hypothesizing that inducing metabolic syndrome would negatively impact on seizures and animal behavior.
METHODS: 4-months-old male WAG/Rij rats were fed with normo-caloric-diet (NCD) or high-fat-diet (HFD) up to 16 weeks, in order to develop metabolic syndrome as previously reported3. During this period, WAG/Rij rats were weekly weighed and, at the same time, food and drink intake were measured for all animals. After 12 and 16 weeks of dietary manipulation, an intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were performed in both NCD and HFD groups. At the end of 16th week, NCD and HFD rat groups were randomly divided into subgroups. Two subgroups of rats, for type of diet, were respectively subjected to EEG recordings and behavioral tests: forced swimming test (FST),

passive avoidance (PA) and novel object recognition test (nORT). Simultaneously, a subgroup of HFD fed rats was switched to NCD for 12 weeks (switch group). During this period EEG recordings were assessed every two weeks. At the end of the switch period the above reported behavioral tests were performed also in this group.

RESULTS: Surprisingly, HFD fed rats showed a non-significant weight gain in comparison to their respective controls. Likewise, IPGTT and ITT analysis, performed in both groups, did not show any significant difference. At odds, HFD diet significantly increases both the number (nSWDs) and total duration (dSWDs) of spike-wave discharges (SWDs) in comparison to NCD fed rats. Moreover, HFD fed rats showed an increased depressive-like behavior in comparison to their respective controls. Likewise, HFD fed rats in comparison to NCD fed rats showed an altered working memory. Conversely, cognitive performance did not differ in the PA test in both groups. Regarding switch group, we only noticed a reduction of dSWDs, whereas the nSWDs did not significantly change between groups. Likewise, switch group has shown a significant depressive-like behavior, whereas no significant difference was observed on memory performance, in nORT and PA, between groups.

CONCLUSIONS: Overall, our results indicate that HFD diet can worsen both epilepsy and their related neuropsychiatric comorbidities, although, in this strain, we did not notice any altered glycometabolic profile after a dietary manipulation. Therefore, the causes underpinning this effect might be many and various, warranting for further studies.

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ASSOCIATIONS OF CANDIDATE GENE POLYMORPHISMS WITH R-CHOP EFFICACY AND TOXICITY IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: A MULTICENTER PROSPECTIVE PHARMACOGENETIC STUDY

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BACKGROUND: R-CHOP standard chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) is successful in about 60% of patients (pts) with diffuse large B-cell lymphoma (DLBCL). Pts who do not benefit from it, due to tumor drug resistance, have a poor prognosis. Currently, knowledge of reasons of treatment related failures are scanty and predictive biomarkers of response are largely unknown. We hypothesized that germline polymorphisms of gene involved in pharmacokinetics/pharmacodynamics (PK/PD) of R-CHOP drugs may play a role in predicting DLBCL outcome. This multicentre prospective pharmacogenetic trial aimed at verifying this hypothesis.
METHODS: The study included chemonaive DLBCL pts (Ann Arbour I-IV stages) candidate to R-CHOP.
R-CHOP efficacy was evaluated by objective response (OR) rate (Cheson criteria), progression-free survival (PFS), overall survival (OS). R-CHOP toxicity was dichotomized in 0 vs 1-4 grade (CTCAE-NCI v.4.03).
Genomic DNA was extracted from blood of 187 pts. 56 SNPs from 41 candidate genes involved in R-CHOP PK/PD (www.pharmgkb.org) were analysed by a genotyping array (Affimetrix). By logistic regression analysis associations between SNPs and clinical/pathological characteristics, efficacy or toxicity were evaluated.
RESULTS: Median age was 62 years. Men were 93 and women 94. 45.5% of pts were in stage I-II, 54.5% in stage III-IV. 18.1% of pts had bulky disease. According to the revised IPI, 13.9%, 57.2% and 28.9% were in the low, intermediate and high-risk groups, respectively.
86.1% of pts had CR to R-CHOP whereas the remaining showed PR (10.2%) or PD (3.7%).
Statistically significant correlations between survival parameters and sex, stage and R-IPI were observed. Men showed worst OS and PFS than female (p=.001, p=.025, respectively). OS and

PFS decreased in a linear manner from stage I to IV (p=.016, p=.019, respectively) and from R-IPI 0 to R-IPI 3-5 (p=.002, p=.009, respectively).
MLH1rs1800734 SNP was shown to predict PFS (p=.043) and OS (p=.045). No statistically significant correlation was found between SNPs and OR.
Statistically significant correlations between maximum toxicity grade and three SNPs, NCF4rs11883112 (p=.031), CYP2C9rs1057910 (p=.017) and ACP5rs2305799 (p=.038) were observed. Specific toxicities, i.e. hematological toxicity and infection, were associated with CYP3A4rs776746 (p=.028) and PNPLA3rs738409 (p=.007) SNPs. With the exception of PNPLA3rs738409, pts with mutant genotypes showed a decreased incidence of toxicity than those with the respective wild-type genotypes.
CONCLUSIONS: Our data show that SNPs affecting one of the major components of DNA mismatch repair, MLH1, may predict response in DLBCL pts treated with R-CHOP. Also, the presence of mutant genotypes in genes involved in oxidative stress (NCF4, ACP5) or in some of the most relevant CYP450 isoforms (CYP2C9, CYP3A5) seem to protect pts from R-CHOP toxicity. These results are promising and warrant further investigation.

ADOPTIVE CELL TRANSFER OF ENGINEERED REGULATORY T CELLS IMPROVES EXPERIMENTAL ATHEROSCLEROSIS

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BACKGROUND: Immunosuppressive regulatory T cells exert a key role in controlling the inflammatory response that contributes to atherosclerosis. We have previously shown that Tregs can be manipulated to improve atherosclerotic plaque homing in experimental models, and here we present data on the impact of adoptive cell transfer with engineered Tregs on atherosclerosis progression.

METHODS: LDLR-KO mice fed with a cholesterol enriched diet (WTD) for 8 weeks received Tregs retrovirally transfected to express CX3CR1 or with an empty vector. Four weeks later we investigated atherosclerotic plaque histology and performed a detailed immunophenotyping followed by aortic proteomics analysis.
RESULTS: Regulatory T cells overexpressing CX3CR1 localized preferentially in the aorta of LDLR-KO mice fed with WTD for 8 weeks. The treatment with CX3CR1+-Tregs resulted in reduced atherosclerosis with a significantly decreased plaque area (99658±11782 vs 65016±9446 μm2) in the aortic sinus. Furthermore, the lesion presented increased fibrosis (+25%) and α-SMA+ area (+29%), and a lower lipid content (-12%) compared to mice treated with non-engineered Treg, highlighting an increased plaque stability. These results are in accordance with the activation of specific biological pathways, including fibroblasts mobility (z-score=2,727) and integrin signaling (z-score=1,732), that were increased in the aorta of CX3CR1+-Tregs treated mice models

compared to controls. In addition, apoptosis (z-score=-0,888) and necrosis (z-score=-1,498) were reduced, as well as cell infiltration (z-score=-2,206), whereas pathways involved in positive response to phagocytosis (as endocytosis, organization of actin cytoskeleton and LXR activation) were upregulated. Alongside, several factors involved in efferocytosis resulted increased (such as MFGE8, LRP1 and calreticulin), while markers of decreased phagocytic ability and subsequent necrosis, such as high mobility group box 1 (HMGB1), were reduced. Increased efferocytosis in LDLR-KO mice treated with CX3CR1+-Treg was also confirmed by detection of apoptotic cells surrounded by macrophages supporting an improved atherosclerotic plaque phenotype.
CONCLUSIONS: The administration of regulatory T cells with improved homing to the atherosclerotic plaque reduces plaque size and inflammatory burden and improves plaque stability. This study thus confirms the potential use of a Treg-based adoptive cell transfer as therapeutic tool able to dampen the immune response selectively in the atherosclerotic plaque.

BERGAMOT POLYPHENOLIC FRACTION EFFECTS ON EXPERIMENTAL METABOLIC SYNDROME

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BACKGROUND: The National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) identified the metabolic syndrome (MetS) as a multiplex risk factor for cardiovascular disease (CVD) that is deserving of more clinical attention. ATP III considered the "obesity epidemic" as mainly responsible for the rising prevalence of MetS and obesity and disorders of adipose tissue, the driving cause of insulin resistance and type 2 diabetes onset. Overwhelming evidence suggests that many of the features of MetS can be efficiently treated with natural approaches, such as plant-derived polyphenols. Among them, bergamot polyphenols present several beneficial effects, although the role of this phytocomplex in the onset of the syndrome was not fully elucidated. To this purpose, we investigated the effect of bergamot polyphenolic fraction (BPF) on dysfunctional adipose tissue, which represents the main initiator of MetS triggering systemic inflammation, oxidative stress, NAFLD and insulin resistance.
METHODS: MetS was induced in a subgroup of mice fed with a high fat diet plus high fructose-glucose water solution (WD SW) for up to 28 weeks. Body weight was assessed at baseline and once per week up to 28 weeks. Total body fat, lean mass, and body fluids were measured using the nuclear magnetic resonance spectroscopy device EchoMRI-700TM. Brown and fat adipose tissues (BAT and WAT) were weighed at sacrifice. PPAR-γ was assessed in WAT and BAT, UCP-1 was measured in BAT. Plasma malondialdehyde and total antioxidant status were measured spectrophotometrically. Liver steatosis, inflammation and oxidative damage were evaluated using hematoxylin/eosin staining, and immunohistochemistry.
RESULTS: Fat mass, BAT and WAT weight of MetS mice increased significantly, compared to

controls. BPF treatment was only associated to an increase in BAT weight. BPF induced PPAR-γ expression in WAT and prevented its overexpression in BAT. UCP-1 levels were down-regulated in BAT as compared to control and were not restored by BPF. BPF prevented adipose tissue inflammation down-regulating NF-kB expression and improved systemic and liver oxidative stress in MetS animals. This was accompanied by a normalization of plasma lipid profile and by an improvement of glucose tolerance and insulin resistance.

CONCLUSIONS: Overall, the improved glucose metabolism in adipose tissue, the amelioration of plasma lipid profile, the prevention of systemic inflammation and oxidative stress, showed by improved NAFLD, suggest the potential use of BPF in MetS patients suffering from T2DM.

EFFECT OF STATINS ON ALZHEIMER'S DISEASE AND DEMENTIA RISK: A META-ANALYSIS OF OBSERVATIONAL STUDIES

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BACKGROUND: Previous studies have suggested an impact of statins use on cognitive decline and dementia, but the relationship between statins and cognitive impairment remains elusive. To gain further insight into this possible effect, we conducted a meta-analysis of observational studies to investigate the impact of statin use on Alzheimer's disease and dementia incidence.

METHODS: This meta-analysis was conducted according to the PRISMA reporting guidelines. PubMed, Cochrane, and EMBASE were searched since inception to 2019/12/31. Inclusion criteria were: (1) observational studies (cohort or case-control studies); (2) adult subjects; (3) statin use compared to non-use; (4) reporting an adjusted estimate (such as odds ratio/risk ratio/hazard ratio) and 95% confidence intervals (CI) for Alzheimer's disease (AD) and/or dementia risk as outcomes. Estimates from original studies were pooled and compared using restricted maximum-likelihood random-effect model. Measures of effects were reported as odds ratio (OR) and 95% CI. We also performed several stratified analyses according to study design, sex, and statin lipophilicity.

RESULTS: A total of 38 studies (30 cohort studies and 8 case-control studies) met the criteria for the analysis. We found that statin use was associated with a decreased risk of dementia (28 eligible studies, OR 0.83 [CI, 0.79 to 0.87]) and a lower incidence of AD (20 eligible studies, OR 0.73 [CI, 0.65 to 0.83]). Stratified analyses according to the study design confirmed the risk reduction associated with statin use in cohort studies (OR 0.77 [CI, 0.67 to 0.89] for AD and OR 0.84 [CI, 0.80 to 0.89] for dementia), with an even greater effect estimated when only case-controls studies were included (OR 0.65 [CI, 0.54 to 0.77] for AD and, OR 0.78 [CI, 0.69 to 0.89] for dementia). Dementia risk reduction was not different between men (OR 0.87 [CI, 0.81 to 0.93]) and women (OR 0.86 [CI, 0.83 to 0.89]); the pooled sex-stratified analyses for AD risk (including only two studies for each subgroup) yielded non-significant results. Lipophilic statins showed a slightly greater risk reduction of Alzheimer's disease (OR 0.76 [CI, 0.61 to 0.95]) and dementia (OR 0.88 [CI, 0.80

to 0.96]) compared with hydrophilic statins, for which no significant results were observed for both the outcomes.
CONCLUSIONS: Observational studies on statin use and development of cognition symptoms suggest a preventive effect of statin use on Alzheimer's disease and dementia risk. Better designed studies are needed to draw unequivocal conclusions about the effect of statins on cognitive function, minimizing the potential for bias, and to confirm the underlying mechanism of action.

PHARMACOLOGICAL AND GENETIC MODULATION OF TRPM8 SIGNIFICANTLY EFFECTS COLON CARCINOGENESIS

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BACKGROUND: Transient receptor potential (TRP) melastatin 8 (TRPM8) is a cold-sensitive Ca2+ channel protein belonging to the TRP superfamily of ion channels [1]. TRPM8 is dysregulated in different primary tumors and, recently, it has been proposed as biomarker and target in prostatic cancer [2]. However, TRPM8 function in colorectal cancer (CRC), one of leading cause of cancer-related deaths, is largely unexplored to date. Here, we investigated TRPM8 involvement in colon carcinogenesis.
METHODS: TRPM8 mRNA expression was quantified in tumoral biopsies collected from patients at different stages of CRC. The functional role of TRPM8 in vivo was assessed either by using selective a TRPM8 ligand (i.e. WS12) or by its genetic deletion (i.e. TRPM8-/- mice) in experimental models of colon cancer [induced by azoxymethane (AOM) or generated by inoculation of CRC cells in immunodeficient mice]. Microbiota composition in stool and colon samples are being analyzed by 16S rRNA gene sequencing.
RESULTS: In clinically-diagnosed CRC patients, a trend toward an increase in TRPM8 expression in tumour biopsies compared to healthy tissues was observed. Interestingly, the genetic deletion of Trpm8 in mice resulted in a significant reduction in AOM-induced colonic tumours compared to wild-type mice. Additionally, a TRPM8 selective agonist WS12 (20 mg/kg, intraperitoneally), significantly reduced both numbers of AOM-induced tumors and tumor growth in xenograft model. Microbiota analysis in samples of mice (AOM model) treated with WS12 as well as from TRPM8-/- mice is ongoing.

CONCLUSIONS: Adaptive changes in TRPM8 expression in colonic tumors of CRC patients are observed. Moreover, TRPM8 modulation either through a genetic or pharmacological approach reduces tumor formation and growth in murine colon cancer models. These findings suggest a therapeutic potential of targeting TRPM8 in CRC.

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DEVELOPMENT OF ANALYTICAL PROTOCOLS FOR THE STUDY OF THIOPURINE PHARMACOKINETICS IN BIOLOGICAL MATRICES BY SERS SPECTROSCOPY

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BACKGROUND: Thiopurine drugs are widely used as anticancer and immunosuppressants. Mercaptopurine (MP) and its prodrug azathioprine (AZA) are characterized by dose-dependent adverse effects such as myelosuppression and hepatotoxicity often related to inter-individual differences involving the activity of important enzymes at the basis of their biotransformation. Surface-Enhanced Raman Scattering (SERS) spectroscopy is emerging in the biomedical field and represents a valid alternative as a non-invasive and non-destructive analysis method with affordable cost, shorter analysis timing and easier sample preparation. The aim of this study is to investigate thiopurines pharmacokinetics by means of SERS in a nontumoral human hepatocytes cell line (IHH) as *in vitro* model of the hepatic tissue.
METHODS: IHH cells were exposed for 12, 24 and 48 h to AZA and MP at a concentration giving the 50% of the effect (EC50) at 96 h. After treatment, cells were lysed and protein content over 10 kDa was removed by ultrafiltration and samples were maintained at -80 °C. SERS analysis was carried out using SERS substrates constituted by Ag nanoparticles deposited on paper, following a method described by Wu-Li-Ji Hasi (Anal. Chem., 2014). SERS spectra were measured at room temperature with i-RamanPlus, a portable Raman spectrometer. The excitation source consisted in a high-power laser (500 mW) with an emission at 785 nm. All spectra were recorded using an accumulation of one scan (10 sec exposure) at 4 random locations on the substrate surface, with 10 mW of laser power focused onto the sample. All data analysis was performed with the R software. Standard solutions of phosphorylated thiopurine metabolites thioguanosine mono-di-triphosphate (TGMP, TGDP, TGTP), thioinosine triphosphate (TITP) and non-phosphorylated thiopurine metabolites thioguanine riboside (TGR) and mercaptopurine riboside (MPR) were used to collect SERS spectra to identify the characteristic bands of each molecule. The band at 917 cm-2 was attributable to TGMP, TGDP, TGTP and TGR; the band at 1000 cm-2 to MP and the band at 1340 cm-2 to MPR and TITP.
RESULTS: The comparison between SERS spectra acquired for each treatment allows to associate the intensity variation of the characteristic bands of each metabolite with its change in appearance over time and enables

to evaluate changes concerning the drug uptake into cells. Thiopurine metabolites increase over time and some metabolites increase differently than others. In fact, thiopurine metabolites MPR and TITP together with MP have been seen to increase, over time, much faster than TGMP, TGDP, TGTP and TGR which instead tend to increase more slowly.

CONCLUSIONS: The results indicate that SERS could be a promising tool for the study of pharmacokinetics of thiopurines. Further studies could consider influences of metabolic enzymes affected by genetic polymorphisms, by forced expression of the candidate enzyme in the *in vitro* cellular model.

CORRELATION BETWEEN TRYPTOPHAN CATABOLISM AND THE INTRAUTERINE RENIN-ANGIOTENSIN SYSTEM IN HUMAN PLACENTA

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BACKGROUND: Pregnancy requires a refined regulation of the maternal and fetal immune system. IDO 1 and 2 (indoleamine-2,3-dioxygenase) and TDO (tryptophan-2,3-dioxygenase) are enzymes of tryptophan (Trp) metabolism, expressed in human placenta [1]. Trp is degraded into various metabolites by of enzymatic reactions, called the kynurenine pathway. The activity of this pathway increases under inflammation and can exert potent immune-regulatory functions [2]. Intrauterine renin-angiotensin system (RAS) is important for normal pregnancy progression, it is involved in placental development. Ang(1-7), acting on the Mas receptor, inhibits the pro-inflammatory actions of the AngII/AT1R interaction, maintaining the integrity of the fetal membranes during pregnancy [3].
AIMS: The correlation between Trp catabolism and the intrauterine Ang(1-7), whether there is any difference in their expression between maternal vs fetus, in placenta of term vaginal delivery; to assess how immune and inflammatory reactions cooperate in maintaining pregnancy.
METHODS: Samples of blood and human placentae, (maternal side placental tissue and fetal side placental tissue), were taken after birth, from pregnancies following normal vaginal delivery. The plasma was collected from peripheral blood for the measure of circulating Trp and cytokines. Expression of Ang(1-7), IDO and TDO was assessed by immunofluorescence. MRNA were measured by means of real-time polymerase chain reaction and data analysis was accomplished with 2'-ΔCT method.
RESULTS: The immunofluorescence allowed to evaluate the location in human placenta of IDO and TDO with subsequent count percentage of IDO and TDO positive cells and colorimetric analysis. IDO and TDO were localized both in the chorion and in the basal decidua; in these areas IDO and TDO are mainly expressed compared to the expression in placental villi. IDO/TDO positive cells are 34% in basal deciduas, in chorion are 50%. Preliminary data show a higher expression of both TDO and IDO1 RNAm in the fetal side of the placenta than in the maternal side and Ang(1-7) expression. These data will be correlated with circulating inflammatory cytokines.

CONCLUSIONS: The study of IDO, TDO and Ang(1-7) is fundamental to understand their role in physiological pregnancy. The focus on possible alterations in their expression and functional aspects will be useful to target their pathways in pathologic pregnancy.

DIFFERENT EFFECTS OF TRYPTOPHAN 2,3-DIOXYGENASE INHIBITION ON SK-MEL-28 AND HCT-8 CANCER CELL LINES

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BACKGROUND: Tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO1) catalyze tryptophan (trp) degradation. IDO1 expression is upregulated in cancer contributing to immunologic evasion. Despite promising results of some IDO1 inhibitors alone or in combination with PD1 inhibitors, their benefits in melanoma patients have not been completely demonstrated. The expression and function of TDO in melanoma and in colon cancer is not clear yet, and a further investigation is necessary. Moreover, it has been reported that miR-200c targeted TDO directly resulting in reduced production of the immunosuppressive metabolite kynurenine. We aim to characterize TDO expression in human malignant melanoma cells SK-Mel-28 and human colon cancer cell line HCT-8. Moreover, since TDO possess Glucocorticoid Responsive Elements, we aim to evaluate whether they can modulate TDO expression.
METHODS: TDO and miR-200 expression were assessed as real time-PCR. While cell proliferation was assessed as cell duplication, cell apoptosis and cell cycle were analyzed by means of flow cytometry.
RESULTS: SK-Mel-28 cells showed higher TDO levels compared to HCT-8 cells. A selective TDO inhibitor, 680C91, significantly impaired cell proliferation in a concentration-dependent manner, by inducing cell arrest during the G2 phase for SK-Mel-28 cells, while an early apoptosis was increasing in HCT-8 cells. No toxic effects were observed. These data demonstrated that TDO is highly expressed in SK-Mel-28 cells and may be involved in the regulation of their proliferation.
CONCLUSIONS: TDO may directly modulate cancer cell function rather than immune suppression and can be considered as a target for melanoma progression together with IDO1.

ADVERSE DRUG REACTIONS TO CONTRAST MEDIA FOR MEDICAL IMAGING: A REPORT FROM SARDINIAN PHARMACOVIGILANCE CENTER

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BACKGROUND: Contrast media are commonly used to enhance the quality of imaging by improving the visibility of specific organs[1]. As the use of iodinated contrast media is rapidly growing, so is the occurrence of hypersensitivity reactions[2]. On 7th July 2020, the Italian Medicine Agency (AIFA) published a Safety Communication regarding the adverse drug reactions (ADRs) to contrast media used for medical imaging. The risk of hypersensitivity is higher in patients suffering from allergy or atopic diseases and in patients who experienced previous reactions. Nevertheless, anaphylactic events are mostly unpredictable[3]. The frequency of such events is classified as rare or not known in the summary of product characteristics (SmPC) of contrast media.
METHODS: We searched the Italian Pharmacovigilance database and included all the ADRs reported in Sardinia to any suspected drug used as a contrast media from 2001 to 14th of July 2020. We searched by ATC classification (V08) and by active ingredient. All the reactions were revised manually.
RESULTS: We found 102 ADRs. 1 was excluded because it was a duplicate. The ADRs are irregularly distributed during the period of observation. Among these, 82 cases (81,19%) involve iodinated contrast media (V08A), 16 cases (15,84%) paramagnetic gadolinium-based agents (V08C), 2 cases (1,98%) contrast media for ultrasound (V08D) and 1 case (0,99%) radiologic contrast media not-iodinated (V08B). The most reported drugs are IOMEPROL (34 cases), IOPROMIDE (27 cases), IODIXANOL (9 cases) among iodinated drugs, and GADOTERIDOL (7 cases) among gadolinium-based agents. 53 cases are classified as severe (52,48%), 37 cases as not-severe (36,63%), 9 cases as not-defined (8,91%) and 2 cases were fatal (1,98%). According to the Naranjo probability scale, the causality assessment is probable in 87 ADRs (86,14%) and possible in 14 ADRs (13,86%). The age groups are all well-represented: 63 patients aged 18-64 years (62,38%), 36 aged over 65 years (35,64%), and 2 cases not specified. In 66 cases (65,35%) the ADRs are evaluated as allergic reactions, in 31 cases (30,69%) as anaphylactic/anaphylactoid reactions and 4 cases (3,96%) involved other reactions.
CONCLUSIONS: The reporting rate is inconstant, and it may be influenced by contingent factors. We argued that more awareness and involvement of health care professionals is recommended, for example by active pharmacovigilance projects.

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GLUCOCORTICOID INDUCED LEUCINE ZIPPER (GILZ) PEPTIDES AS NEW DRUGS FOR TREATMENT OF INFLAMMATORY BOWEL DISEASES (IBD)

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BACKGROUND: Glucocorticoids (GCs) are among the most commonly used drugs for treatment of inflammatory and autoimmune diseases, including inflammatory bowel diseases (IBDs). Their efficacy is mainly due to the regulation of intracellular signaling pathways and gene expression, including the inhibition of NF-kB activation and its nuclear translocation. However, the GCs clinical effects are transitory, disease recurs on tapering the drug, and chronic use of GCs often leads to serious adverse effects. For these reasons new drugs substituting GCs are needed. Our previous studies showed that GILZ, a human 135 amino acid protein, is potently, rapidly and invariably induced by GCs. GILZ mediates many of the GC anti-inflammatory effects. As GCs, GILZ suppresses activation of T lymphocytes and macrophages and inhibits experimental colitis in mice. GILZ mimics the GCs effect, such as NF-κB inhibition and augmented GILZ expression is sufficient to suppress NF-κB-induced inflammation; indeed, GILZ over-expression in TG-GILZ transgenic mice and *in vivo* delivery of GILZ recombinant protein cured experimental colitis in mice without apparent adverse effects.
METHODS: We have previously demonstrated that the region responsible of interaction of GILZ with NF-kB, and its consequent inhibition, is the C-terminal region of GILZ protein. Based on this knowledge, we have designed and prepared new short peptides (spanning the amino acid sequence from 96 to 137 of GILZ protein and containing the NF-kB binding domain) and tested them for their capability to inhibit NF-kB in human T lymphocytes and macrophages in vitro, and to cure colitis in vivo by using murine IBD models.
RESULTS: Data obtained indicate that the administration of one of the peptides tested reduces the inflammation and symptoms of colitis in our in vivo experimental model. Same peptide inhibits NF-kB activation and nuclear translocation in human T lymphocytes in vitro. Similar experiments testing other peptides based on GILZ protein are ongoing.
CONCLUSIONS: This study will provide new agents with anti-inflammatory activity based on the GILZ structure and will indicate the basis for new therapeutic approaches for human IBDs.

NEUROPROTECTIVE ROLE OF VEGF-A IN NON-VASCULARIZED RAT ORGANOTYPIC SPINAL CORD SLICES

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BACKGROUND: Organotypic spinal cord slices preserve the normal cytoarchitecture and local neuronal circuits of the spinal cord, offering a compromise between dissociated cell cultures and complete animal studies. In the last decades, these systems have been extensively used to study various physiological and pathological aspects, reaching from neuronal development to neurodegeneration, as well as drug evaluation and discovery of several compounds. Considering that the organotypic slices have a reduction in the normal vascular network, this system is particularly interesting to study the non-vascular role of the Vascular Endothelial Growth Factor-A (VEGF-A), recently emerged as able to directly affect neuronal and glial biology, exerting trophic and signaling properties in the nervous tissue. Therefore, we used the organotypic spinal cord culture model to evaluate the properties of VEGF-A on oxaliplatin-induced neurotoxicity regardless of the vascular component.
METHODS: Spinal cord slices were prepared from 4 PND rat pups, kept in culture for two weeks and then morphologically analyzed. The slices were incubated with increasing concentrations of oxaliplatin (1-100 μM) for 1, 3, 6 and 24 hours and then, the toxicity has been evaluated using the Propidium Iodide (PI, 5μg/mL) fluorescence and immunofluorescent staining. The effect of co-treatment with VEGF165b and specific antibodies to VEGFR-1 and VEGFR-2 was also evaluated.
RESULTS: First, the spinal cord slices were analyzed with GFAP (astrocytic cell marker) and NeuN (neuronal cell marker) antibodies by immunofluorescence analysis, in order to characterize their structural organization. To confirm our initial hypothesis, the slices were stained with RECA-1 antibody (vascular marker of the endothelium) and we find a reduced vascularization of the tissue.
From the toxicity studies, we observed that oxaliplatin causes a dose-dependent neurotoxicity and alteration of neurons morphology, in particular after 24 hours incubation. Moreover, activation of astrocytes (evaluated by immunofluorescence staining) was observed. The co-treatment with VEGF165b (the most expressed VEGF-A isoform, 100 ng/mL) showed neuroprotection of the nervous tissue, assessed by PI fluorescence, and reduction of astrogliosis (as a reduction of morphological alterations observed with GFAP marker). In order to investigate the molecular mechanism underlying this neuroprotective effect, we analyzed the role of the two main receptors of VEGF-A (VEGFR-1 and VEGFR-2) by using their specific antibodies.
The slices were incubated with D16F7 (anti-VEGFR-1 mAb) and DC101 (anti-VEGFR-2 mAb) alone, in co-treatment with oxaliplatin, and then in co-treatment with oxaliplatin plus VEGF165b.
CONCLUSIONS: Our results show a reduction in the expression of the vascular network in organotypic spinal cord cultures, thus allowing

to study various physiological and pathological aspects independently of the vessel component. We demonstrated the neuroprotective effect of VEGF165b against neurotoxicity evoked by oxaliplatin and clarified the role of its receptors in mediating this effect.

A SYSTEMATIC REVIEW, META-ANALYSIS AND META-REGRESSION EVALUATING THE ADVERSE REACTIONS TO ERENUMAB IN THE PREVENTIVE TREATMENT OF MIGRAINE

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BACKGROUND: Erenumab has recently been approved as a pharmacological treatment for the prevention of migraine. However, the incidence estimates of adverse drug reactions (ADRs) were not consistent among studies. Consequently, pooled measures of the incidences of ADRs that accounts for inter-study heterogeneity are desirable. In addition, little is known on the factors leading to such heterogeneity.
METHODS: Clinical trials evaluating the occurrence of ADRs related to erenumab in migraine patients were searched with Ovid MEDLINE until April 2020. Random intercept models were used to estimate the pooled incidence of the ADRs reported at least in 5 different study populations. To examine whether specific factors correlated with the pooled incidence, we performed random-effects meta-regression.
RESULTS: Of 138 retrieved references, 8 clinical trials were included in the meta-analysis. We observed a significant heterogeneity of the incidence estimates of back pain, influenza, nasopharyngitis, and upper respiratory tract infection (URTI). Most of the observed heterogeneity is ascribed to treatment duration for back pain ($p = 0.045$), influenza ($p < 0.001$) and URTI ($p < 0.001$), and significantly attributed to Body Mass Index (BMI) for nasopharyngitis ($p < 0.001$).
CONCLUSIONS: Back pain, influenza, nasopharyngitis and URTI showed a significant heterogeneity of incidence estimates.

THE ROLE OF ANTIOXIDANTS AS A POSSIBLE THERAPEUTIC STRATEGY AGAINST RISKS OF EXPOSURE TO ENVIRONMENTAL CONTAMINANTS

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BACKGROUND: Ochratoxin-A (OTA) is a mycotoxin that is a common contaminant of food products for both humans and animals. This mycotoxin has several toxic effects. In particular, ochratoxin has significant nephrotoxic potential. In fact, OTA has been described as being responsible for naturally occurring animal and human kidney disorders.
METHODS: The toxicity of this mycotoxin involves the induction of the oxidative stress pathways. Therefore, in the present study, we wanted to evaluate the potential protective effects of hydroxytyrosol (HT), a phenolic constituent with potent antioxidant activity, of extra virgin olive oil in three different renal cell lines, the Madin-Darby canine kidney cell line (MDCK), a pig kidney cell line (LLC-PK1), and a rabbit kidney cell line (RK 13), and in rats.
RESULTS: Our results clearly showed that renal cells respond to OTA exposure by reducing cell proliferation and the induction of oxidative stress. Pre-incubation of the cells with HT prevented the cellular cytotoxicity and increased reactive oxygen species (ROS) levels induced by OTA. In addition, the antioxidative activity of HT was studied by measuring malondialdehyde (MDA) and lactate dehydrogenase (LDH) levels and nitrosative stress. Finally, we investigated the capability of HT (20 mg/kg, intraperitoneally) to act in vivo. In rats, HT reduced oxidative stress and collagen accumulation in the kidney and counteracted the augmentations in AST, ALT, and creatinine levels following OTA induction (250 µg/kg for 90 days orally).
CONCLUSIONS: In conclusion, our findings demonstrate that HT is able to protect three renal cell lines from the damage induced by OTA and protect the kidneys of rats. Therefore, the use of this compound could be an important strategy for the treatment and prevention of this type of kidney dysfunction.

PUBLIC PERCEPTION OF LABORATORY ANIMAL TESTING: WILL IT CHANGE AFTER SARS-COV2?

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BACKGROUND: In recent years, the use of laboratory animals in biomedical research has been a matter of intense public debate. The most recent statistics suggest that about half of the Western population, who generally are sensitive to this discussion, are in favor of animal testing, but the other half oppose it. Over the years, the European Union (EU), Canada, the United States, and several other countries have introduced laws to regulate the use of laboratory animal testing. These laws are generally well balanced and have been promulgated after consulting the main stakeholders (i.e., researchers, patient associations, associations for the protection of animals, etc.) who are sensitive to this matter. Unfortunately, despite these efforts, the public debate has often suffered from fallacious information that is disseminated by individuals or groups who oppose animal testing. Researchers have neglected to respond to such aggressive media campaigns with adequately effective communication. A prototypical example is the widespread use of the term "vivisection" that is used in an effort to stigmatize laboratory animal testing, notwithstanding the fact that science abhors vivisection, which is an illegal behavior that was banned by law and abandoned decades ago. Something similar is also happening in the case of vaccination, against which false information campaigns have been launched by groups of people who are generically identified as "Anti-Vaxers". These groups advocate alleged reasons and deny the success of vaccination strategies to eradicate several serious infectious diseases, such as smallpox and poliomyelitis, although such opposition to vaccination carries an incalculable risk of severe public health damage.

METHODS: The presentation makes an analytic evaluation of the historical, philosophical and scientific literature on laboratory animal experimentation. Different ethical positions are analyzed and compared. The concepts of "free cruelty", "good cause" or "responsibility" are also discussed.

RESULTS: Without taking a rigid position, the presentation intends to analyze all the implications, both in the public perception and in the biomedical research, of using laboratory animals.

CONCLUSIONS: The recent SARS-CoV2 pandemic and its social and political impact and dramatic consequences on public health systems are bringing new attention to biomedical research. This situation provides an opportunity to replace popular demagogic arguments with a constructive debate on the importance of animal testing and vaccination. In recent decades, much has been done to protect the rights of laboratory animals, but it is also clear that, based on present knowledge and available technologies, in specific research fields it is not possible to completely abandon in vivo animal testing by replacing it with alternative methods.

WHEN THE GLUCOCORTICOID RECEPTOR MEETS THE MINERALOCORTICOID RECEPTOR IN THE NUCLEUS OF HUMAN CELLS

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BACKGROUND: Adrenal corticosteroids, such as glucocorticoids and mineralocorticoids, are indispensable for mediating response to stress, development, limiting inflammation, and maintaining energy and fluid homeostasis. These hormones exert their actions via binding to two closely related nuclear receptors, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). The GR has low affinity for corticosteroids, but is expressed in nearly every cell. In contrast, the MR shows a higher affinity for corticosteroids and its expression is largely confined to those tissues where electrolyte exchange and fluid balance are required. GR and MR act as ligand-activated transcription factors which, following interaction with co-regulators and DNA responsive elements, either promote or repress gene transcription. The affinity for the same ligands, structural homology, and binding to the same DNA regions suggest GR and MR can compensate for each other's actions. Yet, there are specific glucocorticoid and mineralocorticoid-mediated responses indicating GR-MR functional diversity.

METHODS: To investigate this interplay, we developed U-2 OS (human osteosarcoma) cell lines stably expressing GR, MR, and both GR and MR (GRMR).

RESULTS: Immunofluorescence analysis showed that treatment of these cell lines with 1 nM of the synthetic glucocorticoid dexamethasone (Dex) induced nuclear traslocation of GR and MR. Conversely, treatment with 1 nM of aldosterone (Aldo) promoted nuclear translocation of the MR only. Moreover, Proximity Ligation Assay revealed that, in the absence of ligand, GR associated with MR in the cytoplasm and, upon 1 nM Dex exposure, GR-MR dimers were detected in the nucleus of GRMR cells. Surprisingly, nuclear GR-MR dimers were also detected in the presence of Aldo, suggesting that it is necessary to activate at least one receptor to induce nuclear traslocation of the heterocomplex. To decipher the functional contribution of GR-MR dimers in the transcriptional response of GR to Dex and MR to Aldo, we performed RNA-seq in GR, MR, and GRMR cells treated with 1 nM of Dex or Aldo. Transcriptome analysis revealed that Dex-activated GR regulated the transcription of 6180 genes. Co-expression of MR resulted in a blunted Dex-mediated gene response which affected only 1608 genes, suggesting a functional antagonism of MR. Aldo-activated MR regulated the transcription of 1660 genes. However, co-expression of GR expanded the Aldo-mediated gene response to 3150 genes. Strikingly, 74% of these genes were also regulated by Dex via GR, suggesting that GR-MR dimers in the presence of aldosterone are able to mimic the glucocorticod transcriptional response.

CONCLUSIONS: Our data suggest that the role of distinct GR and MR homo- and hetero-dimers is relevant for regulating gene expression. Dissecting the mechanism and investigating the cross-talk between GR and MR may be useful to understanding these two receptors in health and disease.

THE LOSS OF EPHB1 RECEPTOR PROMOTES PRO-TUMORAL EVENTS IN GLIOBLASTOMA CELLS AND ITS OVEREXPRESSION IS CORRELATED WITH A REDUCTION OF CANCER CELL AGGRESSIVENESS

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BACKGROUND: The Eph receptor family of tyrosine kinases and its ligands, ephrins, are involved in regulating different cancer cell functions as Eph/ephrin signaling is implicated in tumorigenesis, metastasis, and angiogenesis. Every molecule of this system may act as tumor promoter or suppressor, dependent on cellular context and type of cancer. EphB1 receptor expression is altered in different brain tumors. Regarding glioblastoma, EphB1 downregulation may be correlated with aggressive cancer phenotypes, as this receptor may act as tumor suppressor. Starting from these evidences, we have investigated the role played by EphB1 receptor in human glioblastoma U87 cells.
METHODS: EphB1 mRNA and protein levels were measured in U87 cells via real-time PCR and western blotting, respectively. [3H] thymidine incorporation and wound healing assays were employed to evaluate cell proliferation and migration, respectively. U87-MG cells were exposed to EphrinB1-Fc (1 µg/ml), an EphB1 receptor soluble agonist, or to different EphB1 receptor peptide antagonists, that we have identified. Alternatively, U87-MG cells were transfected with p-CMV6- EphB1 plasmid, to overexpress EphB1 receptor, and then exposed to EphrinB1-Fc or peptides. Different EphB1 3'UTR fragments were cloned into a luciferase reporter vector.
RESULTS: In U87 cells we measured low levels of EphB1 whereas EphrinB1 mRNA were higher than those of EphB1. After transfection with EphB1 plasmid, EphB1 protein and mRNA were increased in transfected U87 cells. Interestingly, EphB1 upregulation and/or its activation by EphrinB1-Fc decreased cell migration and proliferation. The administration of EphB1 peptide antagonists to native or transfected U87 cells increased cell migration. Consistently, EphrinB1-Fc administration increased EphB1 phosphorylation in contrast to the peptide antagonists that decreased it. On the other hand, EphB1 protein levels are lower in U87 cells while mRNA levels are higher compared to SH-SY5Y cells. EphB1 expression levels are not affected by MG132, a proteasoma inhibitor; thus, highlighting the role played by post-transcriptional processes in regulating EphB1 receptor expression in U87 cells.
CONCLUSIONS: Results presented here demonstrate that the loss of EphB1 receptor expression and the subsequent reduced activity are relevant pro-tumoral events in U87 cells. Consistently, treating U87 cells with the EphB1 receptor agonist or antagonists further reduces or increases cancer cell aggressiveness respectively. Other different peptides, designed starting from antagonists already tested in vitro, are being studied to evaluate how their binding to EphB1 affects the receptor function. Furthermore, the different levels of EphB1 mRNA and protein seems to correlate the loss of EphB1 receptor expression in glioblastoma cells with some post-transciptional events involving miRNAs. For this purpose we cloned EphB1 3'UTR to perform gene reporter assay aimed at unraveling the molecular processes responsible for EphB1 receptor downregulation in glioblastoma cells, elucidating those, we could develop innovative pharmacological approaches to treat this aggressive malignant brain tumor.

ROLE OF THE ACTIVATED MICROGLIA IN HIPPOCAMPAL SYNAPTIC PLASTICITY ALTERATION INDUCED BY Aβ

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BACKGROUND: Microglia cells, the main category of macrophages present in the CNS parenchyma, play a central role in brain development and homeostasis. Neuroinflammation induces the switching of the activated microglia phenotype leading to the production of pro-inflammatory mediators, such as IL-1β, IL-6, TNF-α, chemokines, matrix metalloproteinase 2 (MMP2), nitric oxide and nuclear factor kappa-B (Nf-κB). Activated microglia contributes to synaptic degeneration and myelin damage that occurs in neurodegenerative diseases, including Alzheimer's disease (AD). Different evidences show that in AD there is a consistent activation of CSF1 receptor (CSF1R) in microglia which is responsible for pathological microglia activation. In this study, we first investigated hippocampal synaptic transmission and plasticity in models of neuroinflammation; we then evaluated whether pharmacological manipulation of microglia activation is able to restore synaptic dysfunction associated with these models.
METHODS: To clarify the role of CSF1R pathway in synaptic plasticity, we performed extracellular field potential recordings in acute hippocampal slices. *In vitro* inflammatory models were obtained applying Lipopolysaccharide (LPS) (10 μg/ml) or Aβ 1-42 oligomers (200 nM). To reduce the activation of microglia we used a specific inhibitor, Pexidartinib (PLX-3397) (10 μM) and a no-specific blocker, Minocycline hydrochloride (500 nM).
RESULTS: In the slices treated with LPS or Aβ we found an impairment of LTP. A restoration of LTP was observed in minocycline group. Next, we studied the specific microglial activation pathway mediated by CSF1R using a novel pharmacological tool, PLX-3397.
CONCLUSIONS: Preliminary results suggest a functional role of activated microglia in the synaptic plasticity alteration induced by inflammation. Overall, we show that the pharmacological modulation of microglia activation potentially restores synaptic dysfunction in experimental AD and highlight CSF1R as a promising target for AD treatment.

INVESTIGATION OF GENES AND MIRNAS ASSOCIATED WITH RISK-TAKING PROPENSITY: MARKERS SHARED WITH BIPOLAR DISORDER AND POTENTIAL ROLE AS BIOMARKERS OF LITHIUM RESPONSE

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BACKGROUND: In patients with bipolar disorder (BD), risk-taking propensity may contribute to poor clinical outcome, while the mood stabilizer lithium has been suggested to decrease impulsivity. Little is known on the casual genes underlying risk-taking, as well as the potential shared factors between BD, risk-taking and lithium response. We used different computational methods to identify genes relevant to BD and risk-taking propensity based on tissue-specific eQTLs across human brain tissues and whole blood. Additionally, we investigated enrichment of miRNAs among risk-taking associated variants and the expression of those miRNAs in lymphoblastoid cells lines (LCL) from patients with BD characterized for lithium response.
METHODS: eQTL informed gene-based analyses were conducted with eMAGMA. For BD, we used latest data from the Psychiatric Genomics Consortium, including 20,352 cases and 31,358 controls of European descent [PMID: 31043756]. For risk-taking we used data from a large GWAS meta-analysis on risk-tolerance including 466,571 UK Biobank participants and replication cohorts [PMID: 30643258]. 8,701,749 variants common to the two datasets were assigned to genes based on their association with gene expression in 13 brain tissues and whole blood in GTEx v7. Enrichment for miRNAs among risk-taking and BD associated variants was evaluated with MIGWAS. Significant miRNAs were further investigated in a dataset in which genome-wide expression levels were measured using next-generation sequencing in LCLs from 24 patients with BD characterized for lithium response with the Alda scale [PMID: 31801218].
RESULTS: In brain tissues, two genes were associated with BD and risk-taking propensity: CACNA1C in the cerebellar hemisphere (BD: Z=5.23, p=8.5E-08, adj p=0.0004; risk-taking: Z=4.60, p=2.10E-06, adj p=0.009) and ZSCAN9 in the hippocampus (BD: Z = 4.11, p = 2.0E-05 adj p = 0.04; risk-taking: Z = 5.24, p = 8.2E-08, adj p = 0.0002). CACNA1C is part of the druggable genome according to the DGIdb database. Variants associated with risk-taking or BD were significantly enriched for 22 and 19 miRNAs, respectively. While no miRNA was shared between the two traits, three of the risk-taking associated miRNAs (let-7b, mir-146b and mir-598) were part of the same families found to be differentially expressed according to lithium response in LCLs from patients with BD (let-7e-5p, let-7d-3p and hsa-miR-598-3p were downregulated, let-7f-5p, let-7a-5p and miR-146a-5p were upregulated in responders).
CONCLUSIONS: Genetic variants associated with risk-taking were enriched for genes associated with BD and with miRNAs differentially expressed in LCLs derived from patients with BD responders to lithium. These findings support the hypothesis of shared genetic markers between risk-taking propensity, BD and lithium response.

BINGING ON ALCOHOL AND SOCIAL STRESS IN ADOLESCENCE: A TRANSLATIONAL RESEARCH IN SICILY

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BACKGROUND: The adolescence is a time of transition from childhood to adulthood scarred by emotional and behavioral changes, due to the development of brain areas that shape the maturation of cognitive-emotional functions useful for juggling in a complex psychosocial environment. Besides, they modulate the propensity towards risky behaviors and decisions and the search for rewarding sensations often associated with the use of substances of abuse like alcohol. Indeed, alcohol use involves multiple health risks in early adolescence, both in the case of moderate or excessive intake. The ability of alcohol to affect the neurobiological adaptive responses that underlie the ability to counteract social stress during the adolescent period is still poorly studied.
METHODS: 750 male and female students, aged between 18 and 20, were recruited from High Schools in Palermo. During the 1st phase of the research, students performed an evaluation of alcohol drinking pattern - social (1-2 drinks/occasion) and binge (> 4-5 drinks/occasion) - and motives, using the Alcohol Use Disorder Identification Test (AUDIT) and the Drinking Motives Questionnaire-Revised Short Form. Furthermore, they underwent a psycho-diagnostic assessment based on the Millon Clinical Multiaxial Inventory score. During the 2nd phase of the research, 40 male and female students were assessed for stress responses trough the Trier Social Stress Test (TSST), cardiac interbeat intervals, and salivary cortisol. Furthermore, students were tested for anxiety, resilience and self-esteem using State-Trait Anxiety Inventory (STAI), Connor-Davidson Resilience Scale, and Basic Self-Esteem Scale.
RESULTS: 81% of students drunk alcohol. 75% of them were social drinkers (SDs), 20% of them engaged in binge drinking (BD) and, among them, 69% were female students. BDs displayed a high rate of anxiety and depression whereas SDs displayed low depression as BDs. Moreover, there was a prevalence of clinical and personality disorders according to the drinking pattern, and a main effect of impulsivity across groups. Furthermore, a positive correlation was found between AUDIT score and salivary cortisol, and an increase of the salivary cortisol levels in BDs and SDs compared to controls. Correlation test showed a positive relationship between AUDIT and STAI-S prior and after the TSST. Notably, we highlighted an increase in STAI-S score of BDs and SDs with respect to controls. BDs had the highest STAI-S score. Lastly, BDs and SDsn resilience scores were lower than controls.
CONCLUSIONS: These results highlight the detrimental impact of binge consumption patterns on the stress response, as well as on the emotional and affective sphere of adolescence. This research also suggests that the assessment of the psycho-pathological status of students should be considered for the increased likelihood of developing a dysfunctional habit of alcohol consumption.

THERAPEUTIC DRUG MONITORING OF PALBOCICLIB, RIBOCICLIB AND LETROZOLE IN WOMEN WITH BREAST CANCER BY LC-MS

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BACKGROUND: Breast cancer (BC) is the most common female tumor worldwide. Life expectancy and choice of therapy vary depending on stage and receptor status. Endocrine therapy (ET) is an effective treatment for most hormone receptor positive (HR+) BCs but unfortunately, one third of HR+ BCs lose clinical benefit over time because of drug resistance development. The cyclin-dependent kinase inhibitors (CDKis) palbociclib and ribociclib have been recently introduced in the treatment of women with HR+, human epidermal growth factor receptor 2 negative (HER2-) locally advanced or mBC, in combination with ET. Even though adverse reactions (AR) to these new drugs are usually low and easy to manage, in some cases, based on individual safety and tolerability, dose reductions or discontinuation of therapy are necessary. Therefor patients treated with CDKis would definitely benefit from therapeutic drug monitoring (TDM). This clinical practice would in fact allow the clinician to identify for each patient the individualized dosage associated to a positive risk-benefit ratio, avoiding severe AR. To date PK-PD relationship of CDKi is not definitively fixed and has to be further investigated. A limiting factor in this context is the paucity of dedicated bioanalytical methods.
METHODS: A novel bioanalytical method for the simultaneous quantification of palbociclib, ribociclib and letrozole (the oral aromatase inhibitor most commonly combined with palbociclib) has been developed. Being based upon the use of LC-MS/MS technology, the method is highly sensitive and specific hence enabling the quantification of a wide concentration range, ideally including all the plausible in vivo plasma values and would make possible to perform TDM with a low turn-around time. This is also due to the simple and fast sample preparation protocol based on plasma protein precipitation. During the validation, performed according to FDA/EMA guidelines, a remarkable carry-over effect was observed for palbociclib and ribociclib, requiring the development of a strategy to overcome this pitfall.
RESULTS: The validation process revealed high recovery and selectivity and the absence of matrix effect for the three analytes. The assay is linear for the three analytes in the tested concentration ranges, which were 0.3-250 ng/mL for palbociclib, 10-10000 ng/mL for ribociclib and 0.5-500 ng/mL for letrozole. The carry over phenomenon was notably reduced by setting up a dedicated washing method. Applying the method for the analysis of incurred samples, it was confirmed to be reliable and reproducible.
CONCLUSIONS: The first LC-MS/MS method for the simultaneous quantification of palboci-

clib, ribociclib and letrozole in human plasma was developed and fully validated. It could be applied to deepen the correlation between PK and PD and to personalize anticancer therapy in this clinical setting.

TRANSIENT MATERNAL IL-6 BOOSTS GLUTAMATERGIC SYNAPSES AND DISRUPTS HIPPOCAMPAL CONNECTIVITY IN THE OFFSPRING

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BACKGROUND: Early prenatal inflammatory conditions are now considered as a risk factor for different neurodevelopmental disorders, with long-term consequences on adult brain connectivity. Here we show that a transient IL-6 elevation, occurring at vulnerable stages of early neurodevelopment, impacts brain developmental trajectories through the aberrant enhancement of glutamatergic synapses and overall brain hyper-connectivity.
METHODS: We used a combination of functional and morphological approaches using both in vivo and in vitro models.
RESULTS: The IL6-mediated boost of excitatory synapse density results from the neuron-autonomous, genomic effect of the transcription factor STAT3 and causally involves the activation of RGS4 gene as a candidate downstream target. The STAT3/RGS4 pathway is also activated in neonatal brains as a consequence of maternal immune activation protocols mimicking a viral infection during pregnancy.
CONCLUSIONS: By demonstrating that prenatal IL-6 elevations result in aberrant synaptic and brain connectivity through the molecular players identified, we provide a mechanistic framework for the association between prenatal inflammatory events and brain neurodevelopmental disorders.

CANNABIDIOL TREATMENT INFLUENCES ULTRASONIC COMMUNICATION IN MICE

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BACKGROUND: Mice emit ultrasonic vocalizations (USVs) under different social conditions to convey information related to emotional states and mediate social interactions. USVs study has become a valid assay in behavioural phenotyping in the field of psychiatric and neurological disorders, starting from those characterized by communication and social interaction deficits such as neurodevelopmental disorders (NDDs) and in particular autism spectrum disorders (ASD). Alterations in USVs pattern are found in mice after pharmacological treatments in NDDs context. Recently, Cannabidiol (CBD) is used in some clinical trials to treat symptoms of NDDs.
In this study, we wanted to investigate if treatment with CBD was able to influence ultrasonic communication of mice.

METHODS: Adult mice were treated with intragastric administration of cannabidiol oil (20 mg/kg) or vehicle (MTC oil) for 2 weeks. Another group of mice was intranasally treated with oxytocin receptor antagonist (L-371,257) 30 min before behavioural testing. USVs of mice were recorded during social interaction test using an ultrasound sensitive microphone and quantitatively analyzed by Avisoft software. Each syllable was categorized manually using Scattoni classification.
RESULTS: CBD treatment increased ultrasonic vocalizations in adult mice. In addition, an altered qualitative ultrasonic communication in mice after CBD treatment has been found. In particular, only few categories of calls were modified by CBD treatment. Social communication was blocked by the action of oxytocin antagonist.
CONCLUSIONS: These data suggest that CBD treatment influences ultrasonic communication in mice and this is mediated by oxytocin pathway. Finally, CBD could be a promising pharmacological molecule to treat social alterations typical of NDDs.

METHYLGLYOXAL-ENRICHED DIET SELECTIVELY MODULATE HIPPOCAMPUS IN AGED-MICE

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BACKGROUND: Advanced glycation end products (AGEs) are produced by the non-enzymatic glycation of proteins, lipids and nucleic acids with sugars. Diet is one of the main sources of AGEs and, among them, methylglyoxal (MG) is one of the most prevalent form. AGEs mediate their pathological effects by activating signaling cascades via the receptor for advanced glycation end products (RAGE), which role has not been fully characterized yet. Interestingly, b-amyloid is also a ligand of RAGE. The accumulation of AGEs is implicated in many health disorders including neurodegenerative diseases such as Alzheimer's disease (AD). AD is the most common cause of dementia and by the time it is typically diagnosed, different brain regions are already irreversibly damaged, making vain any pharmacological intervention. Nowadays, it is well known that a prodromal phase occurs almost 15-20 years before the symptomatic state; therefore, preventive strategies, like dietary modification, could prevent brain deterioration. The aim of this study was to evaluate in vivo the effects of a MG-enriched diet and to study if MG can induce modifications directly in the brain, focusing on specific brain areas.

METHODS: Twenty aged mice (18-20 months) received orally 100 mg/kg of MG or vehicle (VH) once a day for 28 days. Mice were then subjected to sequentially behavioral battery tests: rotarod for locomotor activity, open-field for exploratory behavior and Y-maze for spatial memory. Mice were then sacrificed, blood was collected, and hippocampus, cortex and hypothalamus were isolated. The expression of RAGE, neuroinflammation and redox homeostasis markers as well as AD-related APP processing ones was evaluated by RT-PCR and Western Blot.

RESULTS: From behavioral tests, no differences were observed between the two groups. Nevertheless, the MG administration was effective since MG blood concentration was significantly higher in MG- treated mice. RAGE gene expression was found remarkably increased in MG treated mice and, most interestingly, this was seen only in the hippocampus. At this level, MG treated mice showed also low-grade inflammation (IL-1b) and increased expression of phase II antioxidant enzymes such as HO-1. No differences were found neither for microglia or astrocyte associated genes, nor for inflammatory mediators' genes such as iNOS and COX-2. Regarding the APP-amyloidogenic-pathway, we found that the MG treatment increased the gene expression of PS1 in the hippocampus. BACE1 showed only an increasing trend in MG treated mice, that became anyway significant if we consider only the male mice group.

CONCLUSIONS: These findings suggest a possible connection between dietary AGEs, hippocampus-selective increased expression of RAGE and APP-pro-amyloidogenic pathway. Hence, we propose that a MG-enriched diet might favor the establishment of an environment unable to properly respond to additional insults, thus reducing the ability of adaptive responses needed to preserve brain integrity.

POST-MARKETING SURVEILLANCE OF APPROVED CAR-T CELL THERAPIES: ANALYSIS OF US FDA ADVERSE EVENT REPORTING SYSTEM (FAERS) DATABASE

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BACKGROUND: Chimeric antigen receptor T (CAR-T) cells, approved by Food and Drug Administration (FDA) since 2017, represent a new approach to treat specific type of B-cell leukemia and lymphoma. Pivotal clinical studies of CAR-T cells reported several adverse reactions, such as cytokine release syndrome (CRS), neurological toxicity and B-cell aplasia and hypogammaglobulinemia. However, data from post-marketing setting are scarce. The aim of this study is to carry out a post-marketing evaluation of CAR-T cell treatment safety profile using the FDA Adverse Event Reporting System (FAERS).
METHODS: We have selected all reports of adverse drug reactions (ADR) related to Tisagenlecleucel and Axicabtagene ciloleucel from FAERS in the period October 2017-March 2020, after excluding reports from experimental studies. Descriptive analyses of reports for the two CAR-Ts have been conducted. Reporting odds ratio (ROR) was calculated as a measure of the ADR reporting disproportionality. CAR-T-related ADR reports were compared with two reference groups, i.e. "all other drugs" and "antineoplastic agents" (Anatomical Therapeutic Chemical L01). Time to Onset (days) was calculated for the most frequently preferred terms (PT). PT most frequently co-reported with CAR-T induced CRS were searched to identify cluster of signs and symptoms potentially correlated to CRS.
RESULTS: Overall, 2,661 reports of CAR-Ts (1,228 for Tisagenlecleucel and 1,433 for Axicabtagene ciloleucel) were identified. CAR-T therapies-related ADR reports mostly involved males (M/F ratio= 1.5). Median age was 20 [11-58] years and 61 [51-67] years for patients treated with Tisagenlecleucel, also indicated in paediatric B-cell acute lymphoblastic leukemia, and Axicabtagene ciloleucel, respectively. The most frequently reported PTs were CRS (47% vs 60%), pyrexia (36% vs 22%) and neurotoxicity (16% vs 38 %) for Tisagenlecleucel and Axicabtagene, respectively. The median time to onset of CRS was 3 [2-5] days, 2 [1-5] days for pyrexia, 3 [2-7] days for neurotoxicity. Pyrexia, neurotoxicity, hypotension and hypoxia were frequently co-reported with CAR-T-induced CRS. CAR- T cell therapies where disproportionally associated with neurotoxicity, CRS, cytopenia and hypogammaglobulinemia, coagulopathy, bacterial and viral infections, respiratory failure and cardiac arrhythmias, as compared to "all other drugs" and "antineoplastic agents".
CONCLUSIONS: The most frequently reported events associated with CAR-T therapies generally concerned neurologic and immune-related disorders, in line with pre-marketing trials. Potentially emerging safety issues, such as coagulopathy, respiratory failure and cardiac arrhythmias, require further investigation. CRS, pyrexia and neurotoxicity occurred in the first week consistently with evidence derived from experimental studies.

INVESTIGATION ON THE MOLECULAR MECHANISMS UNDERLYING THALIDOMIDE-INDUCE PERIPHERAL NEUROPATHY IN IBD PEDIATRIC PATIENTS BASED ON HIGH-THROUGHPUT TRANSCRIPTOME PROFILES

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BACKGROUND: Thalidomide is used in the treatment of many autoimmune, neoplastic and chronic diseases, including inflammatory bowel disease (IBD); in particular, it appears to be a valuable treatment option for IBD patients refractory to standard therapies. Peripheral neuropathy is one of the most frequent adverse events of the drug. It is an axonal, length-dependent, sensory neuropathy and is reported to be reversible after drug suspension. In a recent study on IBD pediatric patients in treatment with thalidomide, it was found in 72.5% of patients with length-dependent prevalence. To date the molecular mechanisms underlying this adverse effect are mostly unknown. In this context, the aim of this study is to widen the knowledge about the mechanisms of peripheral neuropathy, by evaluating mRNA profiles during thalidomide treatment of IBD patients.
METHODS: Ten IBD pediatric patients refractory to previous therapies and responsive to thalidomide were enrolled. Peripheral blood was obtained before and after twelve weeks of thalidomide treatment. mRNA profiles were sequenced using next generation sequencing Illumina platform. The differential mRNA expression analysis between the samples was conducted using the Differential Expression for RNA-Seq tool of CLC Genomics Workbenc v12.0. The differentially expressed genes were selected considering a p-value <0.01 and a fold change threshold of |2|. In order to investigate on the critical biologic functions, a hypergeometric test on gene ontology (GO) annotations was performed. Data were further analyzed using Ingenuity Pathway Analysis (IPA) database.
RESULTS: RNA-seq analysis identified 252 genes differentially expressed before and after treatment. The hypergeometric test revealed that some genes were found to belong to GO categories linked to functions and components of the nervous system. In particular, the most interesting GO categories emerged are: "synapse organization", "synaptic membrane", "postsynaptic membrane", "glutamatergic synapse". From IPA analyses, "Axonal Guidance Signaling pathway" resulted as one of the most altered pathways. The most relevant peripheral neuropathy-related genes are: ADAMTS2, UNC13B, CACNG6, SEMA3F, GABRE, CABP1. All those genes belonging to these categories have been selected and after that the main mechanisms that seem to emerge are changes in intracellular calcium levels, alteration in glutamate signaling, impaired activation of glial cells, and alteration in axonal guidance signaling-related genes.
CONCLUSIONS: In this study, we identified several mRNAs differently expressed which could help to elucidate the mechanism underlying peripheral neuropathy in young IBD patients in treatment with thalidomide, suggesting also new molecular targets involved in this adverse effect. Moreover, based on transcriptomics data, genes selected by this research could be validated in a cellular model in order to better define a pathophysiological mechanism of this complication.

A PROTEIN-RESTRICTED, AMINO-ACID DEFINED DIET IMPAIRS TUMOR GROWTH IN MICE BY THE SIMULTANEOUS INDUCTION OF ENDOPLASMIC RETICULUM STRESS AND MTOR INHIBITION

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BACKGROUND: Besides their role in protein synthesis, amino acids (AA) play a fundamental role as metabolic intermediates and signaling molecules for cell growth regulation through the mammalian target of rapamycin (mTOR) pathway. AA are involved in several pathways supporting tumor growth and AA addiction is a hallmark of the altered cancer metabolism. However, although targeting AA biosynthetic pathways is widely accepted as an anti-cancer approach, dietary AA supplementation as a therapeutic intervention is uncertain. We have previously shown that, in mice, essential amino acids (EAA) supplementation and feeding with a protein restricted-AA-defined diet extend life span and combat obesity, respectively. Since aging and obesity are frequently associated with cancer, we investigated the effect of an AA-defined diet and EAA supplementation on cancer growth in vivo and in vitro.
METHODS: Mice were fed with a diet where casein, the main component of rodent diet, was substituted with a specific AA formulation (SFA-EAA), or, as a control, with casein amino acids (SFA-CAA). After xenograft implantation, tumor development was measured into the two groups. For in vitro experiments, both cancer and non-cancer cell lines were supplemented with AA mixtures that reproduced the AA levels found in the plasma of mice fed with SFA-EAA or SFA-CAA, as treated or control groups, respectively.
RESULTS: In vivo tumor growth was reduced by the SFA-EAA diet, unlike the SFA-CAA diet. Mechanistically, SFA-EAA led to endoplasmic reticulum (ER) stress activation and mTOR inhibition both in vivo and in vitro, as measured by the induction of ATF4 and other ER stress markers, as well as by the reduction of phospho-p70S6K levels. Furthermore, in vitro SFA-EAA supplementation induced apoptotic cell death of several cancer cell lines, with no effect on non-tumor cells viability. Metabolomic and Seahorse analysis on cancer cells showed a decrease in glycolytic intermediates, ECAR inhibition, and a fall in intracellular levels of glutamate, glycine, and aspartate, after SFA-EAA treatment. Re-addition of glutamate to supplemented cells, as well as supplementation of galactose-adapted non-glycolytic cells, blocked the effects of the SFA-EAA mixture. Furthermore, SFA-EAA diet activated branched chain amino acids (BCAA) oxidation, and treatment of cancer cells with BT-2, an activator of BCAA catabolism, recapitulated the effects of the EAA mixture on cell viability and glycolysis inhibition.
CONCLUSIONS: Our data indicate that a protein-restricted, AA-defined diet feeding could be a useful anti-cancer strategy to combine with already developed antineoplastic drugs, such as mTOR inhibitors. Furthermore, our data also highlight a role for BT2 as a potential anti-cancer molecule.

TOXIC EPIDERMAL NECROLYSIS: ECONOMIC AND PHARMACOGENETIC CONSIDERATIONS FROM THE CARDARELLI HOSPITAL EXPERIENCE

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BACKGROUND: Toxic epidermal necrolysis (TEN) is a severe mucocutaneous reaction, mainly drug-triggered, characterized by extensive epidermal necrosis and detachment. Mucous membranes are affected in >90% of cases, usually at ≥2 distinct sites. Based upon the percentage of detached body surface area (BSA), we identify 3 conditions: Stevens-Johnson Syndrome (SJS), <10% BSA; Lyell Syndrome (LS) >30% BSA; SJS/LS overlap, 10-30% BSA. TEN estimated incidence is 2-7 cases/million people/year and, subsequently, it belongs to rare diseases. Drugs more frequently involved are: allopurinol; antibiotics, especially sulfonamides; aromatic anticonvulsants; Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) and Analgesic Drugs.
METHODS: We collected, from the reimbursement office, data of patients hospitalized at Cardarelli Hospital (Naples, Italy) and discharged with a diagnosis of TEN from January 2015 to December 2019. We also reviewed the literature searching for pharmacogenetics (PGs) determinants of drug induced TEN.
RESULTS: During the observed period, 45 people were admitted due to TEN. Overall mean & median hospitalization length (days) were 9.39 & 8.67 [7.6-13.13]. Overall mean & median hospitalization reimbursement were 2493.8980 & 2472.75 [2295.3-2703] [Table 1]. To note, this kind of hospitalizations are frequently poorly reimbursed. Almost all LSs and some SJSs are hospitalized for a period in an Intensive Care Unit (ICU), hospitalization in ICU can cost up to thousands of euros/day. Different studies validated the relationship between some drugs and TEN (Table 2). Just the relationship between HLA-B*57:01 and abacavir has found a clinical applicability: the test is mandatory required by regulatory United States of America and European agencies before its introduction in therapy.
CONCLUSIONS: Considering the wide use of some of the most frequent drugs causing TEN, the identification of PGs relating a drug to TEN with a strong clinical evidence could be important in order to replicate the abacavir experience. If PG isn't frequent enough in the overall population to justify a pre-emptive test, the known role of a PG in the etiology of TEN can be used to investigate the drug-induced TEN case first and, if positive, the patient's close relatives. In both the cases the PG test will allow an improving of quality of pharmacological therapies and the reduction of costs for health sistems. The characteristics of Cardarelli Hospital may lead to a selection bias: the availability of a Burn ICU can conduct to a relative increase in the observed cases of drug-induced TEN. On the other hand and thanks to this specificity, we are implementing a protocol in order to investigate the PG correlates of drug-induced TEN with also the aim of preventing drug-induced TEN in drug-induced TEN-relatives.

POLYPHARMACY IN A PEDIATRIC LIVER TRANSPLANTED COHORT: THE FEDERICO II TEACHING HOSPITAL EXPERIENCE

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BACKGROUND: Orthotopic liver transplant (OLT) is a lifesaving procedure in children with a heterogeneous group of liver diseases. Pediatric polypharmacy is defined as the prescription/consumption of ≥2 distinct medications for at least one day. OLT patients are at high risk of polypharmacy: they have to chronically take anti-reject drugs and very often they also need additional drugs (antimicrobials, vitamin supplements). Nonetheless, very few data are available on prevalence and severity of polypharmacy in pediatric OLT recipients.
METHODS: We reviewed the therapy of OLT patients in regular follow-up (FU) at Federico II teaching hospital till December 2019 searching for drug-drug interactions (DDIs). Polypharmacy was evaluated at the last follow-up (FU). Vitamin supplements were given if a deficit was confirmed by blood tests and stopped when corrected; as a consequence we considered them as drugs. We used both the checkers provided by IRCCS Mario Negri (Intercheckweb) and Medscape, which give different and additional information. These checkers classify DDIs in 4 classes based on their clinical relevance and on the strength of supporting clinical evidence: red (high risk with strong evidence), orange (moderate risk with good evidence), yellow (moderate risk with limited evidence) and green (low risk with poor evidence).
RESULTS: 43 (21 males) OLT patients were analyzed. The most frequent cause of OLT was biliary atresia (30 patients, 11 males). Mean & median age (months) at OLT were 18.28 & 12 [1-84], standard deviation (SD) and 95% confidence interval (CI) were 19.33 & 12.33-24.23; mean & median age (years) at the last FU were 11.28 & 12 [1-18], SD & 95%CI were 5.17& 9.69-12.87. In OLT population, 29 patients (67.44%) took 2 drugs, 17 (39.53%) 3 drugs and 5 (11.62%) 5 drugs. Mean & median drugs number at the last follow up were respectively 2.37 & 2 [1-7] and 0.14 & 0 [0-2]. The most frequent drugs were: tacrolimus (29.37%), ursodeoxycholic acid (11.89%), mycophenolate (6.29%), proton pump inhibitors (3.50%) and glucocorticoids (2.80%) (Table 1). Mean and median DDIs according to Intercheckweb and Medscape are respectively 0.56 & 0 [0-5] and 0.98.56 & 0 [0-9]. According to the checkers, a variable percentage (8-56%) of the observed DDIs required therapeutic changes; in 43-92% of cases the observed DDIs were to monitor or of unknown significance (Table 2).
CONCLUSIONS: Although the small sample might limit the reliability of the analyses, the pharmacotherapy in OLT patients is per se significant with almost 70% of them reaching the pediatric polypharmacy cut-off. Following the unavoidable heavy drug burden, clinically significant DDIs were observed. The interaction risk with immunosuppressant drug of acyclovir is worth noting, it is frequently used for Herpes Simplex & Varicella Zoster infections. Careful monitoring of OLT patients for life threatening complications related not only to OLT but also to the danger of DDIs has to be carried on.

AN INNOVATIVE APPROACH FOR SCREENING ANTIVIRAL AND ANTIPROLIFERATIVE DRUGS TARGETING G-QUADRUPLEX NUCLEIC ACIDS

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BACKGROUND: G-quadruplex structures, consisting in stacked guanine nucleobases, are formed by guanine-rich sequences such as those that are present in telomeres, promoter genes and viral genomes. Small molecules with peculiar structural features can interact with G-quadrupexes: in medicinal chemistry, this strategy is pursued for selectively interfering with gene expression, uncontrolled cell proliferation and viral replication. Synthetic ligands are currently studied as non-covalent G-quadruplex binders, along with some natural compounds [1]. In this contribution, we will present the practical application of an experimental approach, consisting in a cell free, mass spectrometry-based test for screening small molecules interacting with biologically relevant G- quadruplex structures and for the evaluation of their sequence selectivity.
METHODS: Oligonucleotides were heat-denatured and folded in 150 mM ammonium acetate before incubation with ligands. Samples were acquired after equilibration by direct infusion electrospray (ESI). Collision-induced dissociation (CID) experiments were performed on the DNA/ligand complexes. Binding affinity (BA) was calculated according to the formula: BA = (ΣIbound DNA/(ΣIfree DNA + ΣIbound DNA)) x 100. In CID studies, relative intensity (I %) of the DNA/ligand signal was plotted against the applied energy. I (%) = (Icomplex/(Icomplex + Idissociation products)) x 100 [2-4].
RESULTS: A pool of 50 natural, semi-synthetic and synthetic compounds bearing a planar scaffold, such as anthracenes, anthraquinones and flavonoids, were tested against a G-quadruplex sequence containing the human telomeric TTAGGG repeat, introducing a double stranded DNA (dsDNA) sequence as control. While tested anthracenes did not discriminate between different DNA structures, 2,6-disubstituted anthraquinones and hydroxy isoflavones were proved to be selective for G-quadruplex. In particular, the natural flavonoid osajin showed a BA selectivity ratio of 3.2 for G-quadruplex over dsDNA. CID fragmentation, combined with molecular docking, demonstrate that interaction mainly occurs via external stacking [5-6].
CONCLUSIONS: Currently, ESI-MS studies are being carried out on synthetic derivatives of the most promising molecules with the aim of improving affinity and selectivity. Preliminary results encourage further investigation on the potential application in the field of antiproliferative and antiviral agents.

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THE APPETITE-SUPPRESSANT AND GLP-1-STIMULATING EFFECTS OF WHEY PROTEINS IN OBESE SUBJECTS ARE ASSOCIATED WITH INCREASED CIRCULATING LEVELS OF SPECIFIC AMINO ACIDS

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BACKGROUND: The satiating effect of whey proteins depends upon their unique amino acid composition because there is no difference when comparing whey proteins to a mix of amino acids mimicking the amino acid composition of whey proteins. The specific amino acids underlying the satiating effect of whey proteins have been not yet investigated to date. The aim of the present study was to evaluate the appetite-suppressant effect of an isocaloric drink containing whey proteins or maltodextrins.
METHODS: The effects of an isocaloric drink containing whey proteins or maltodextrins on appetite (satiety/hunger measured by a visual analogue scale or VAS), anorexigenic gastrointestinal peptides (circulating levels of glucagon-like peptide 1 [GLP-1] and peptide YY [PYY]) and amino acids (circulating levels of single, total [TAA] and branched-chain amino acids [BCAA]) were evaluated in a cohort of obese female subjects (n = 8; age: 18.4±3.1 years; body mass index, BMI: 39.2±4.6 kg/m2).
RESULTS: Each drink significantly increased satiety and decreased hunger, the effects being more evident with whey proteins than maltodextrins. Similarly, circulating levels of GLP-1, PYY and amino acids (TAA, BCAA and alanine, arginine, asparagine, citrulline, glutamine, hydroxyproline, isoleucine, histidine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine, valine) were significantly higher with whey proteins than maltodextrins. In subjects administered with whey proteins (but not maltodextrins), isoleucine, leucine, lysine, methionine, phenylalanine, proline, tyrosine and valine were significantly correlated with hunger (negatively), satiety and GLP-1 (positively).
CONCLUSIONS: Eight specific amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, proline, tyrosine and valine) are implied in the appetite-suppressant and GLP-1-stimulating effects of whey proteins, which might be mediated by their binding with nutrient-sensing receptors expressed by L cells within the gastrointestinal wall. The long-term satiating effect of whey proteins and the effectiveness of a supplementation with these amino acids (i.e., as a nutraceutical intervention) administered during body weight reduction programs need to be further investigated.

EFFECTS OF AN ACUTE BOUT OF EXERCISE ON CIRCULATING EXTRACELLULAR VESICLES: TISSUE-, SEX- AND BMI-RELATED DIFFERENCES

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BACKGROUND: Exercise is recognized to evoke multisystemic adaptations that, particularly in obese subjects, reduce body weight, improve gluco-metabolic control, counteract sarcopenia and lower the risk of cardiometabolic diseases. Understanding the molecular and cellular mechanisms of exercise-induced benefits is of great interest due to the therapeutic implications against obesity.
METHODS: The aim of the present study was to evaluate time-related changes in size distribution and cell origin of extracellular vesicles (EVs) in obese and normal-weight subjects who underwent a moderate-intensity exercise on a treadmill (at 60% of their VO2max). Blood samples were drawn before, immediately at the end of the exercise and during the post-exercise recovery period (3h and 24h). Circulating EVs were analyzed by a nanoparticle tracking analysis and flow cytometry after labeling with the following cell-specific markers: CD14 (monocyte/macrophage), CD61 (platelet), CD62E (activated endothelium), CD105 (total endothelium), SCGA (skeletal muscle) and FABP (adipose tissue).
RESULTS: In all subjects, acute exercise reduced the release of total (i.e., 30-700 nm) EVs in circulation, predominantly EVs in the microvesicle size range (i.e., 130-700 nm EVs). The post-exercise release of microvesicles was higher in normal-weight than obese subjects; after exercise, circulating levels of exosomes (i.e., 30-130 nm EVs) and microvesicles were, respectively, lower and higher in females than males. In all experimental subgroups (males vs. females and obese vs. normal-weight subjects), acute exercise reduced and increased, respectively, CD61+ and SCGA+ EVs, being the effect on CD61+ EVs prolonged up to 24h after the end of the test with subjects in resting conditions. Total EVs, exosomes and CD61+ EVs were associated with HOMA-IR.
CONCLUSIONS: Though preliminary, the results of the present study show that a single bout of acute exercise modulates the release of EVs in circulation, which are tissue-, sex- and BMI specific, suggesting that the exercise-related benefits might depend upon a complex interaction of tissue, endocrine, and metabolic factors. EVs, native or pharmacologically modified, endowed with antidiabetogenic, antisarcopenic, and antiobesogenic properties, might represent, in future, a new (nano-)therapeutic option for obesity-related comorbidities.

REAL-WORLD SAFETY AND EFFICACY OF NEWER ANTISEIZURE MEDICATIONS: A RETROSPECTIVE STUDY

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BACKGROUND: Several new antiseizure medications (ASMs) have been recently introduced in clinical practice offering new possibilities for seizure control in treatment-resistant epilepsy (TRE)1. Over the past decade, four "third-generation" ASMs, lacosamide (LCM), eslicarbazepine acetate (ESL), perampanel (PER), and brivaracetam (BRV), have been approved as adjunctive treatments in focal TRE, which still represents a significant burden for patients. Despite the similar effectiveness of the new ASMs, no head-to-head studies have been performed so far2. Thus, in a clinical practice context, a lack of information on their comparative efficacy, as well as short and long-term safety profile, need to be filled up. Aim of this retrospective study was to assess the effectiveness and tolerability of newer ASMs in a real-world setting.
METHODS: This retrospective study collected clinical records data from the Neurology unit of the "Mater Domini" University Hospital between January 2019 and January 2020. All outpatients with focal TRE who were followed at least 1 month for the ongoing therapy with one of the newer ASMs during the study period, have been included. For each ASM, responder rate (≥50% reduction in seizure frequency), seizure-free rate, dropout rate and patients with treatment emergent adverse events (TEAEs) were assessed.
RESULTS: Data on 123 patients have been collected, 70 (56.9%) were females, mean age 39.8 years (±15.5 SD), follow-up mean duration 32.8 months (±24.7 SD). Most patients were treated with LCM n=50 (41%), followed by ESL n=32 (26%), PER n=31 (25%), BRV n=10 (8%). At baseline, 10% of patients was previously treated with ≥10 ASMs, 59% were taking ≥2 other ASMs, and seizure frequency was mainly monthly (49.6%). At last follow-up available, responder rates were 50% (BRV), 45% (PER), 37.5% (ESL) 34% (LCM); seizure free rates were 31% (ESL), 30% (BRV), 22.6% (PER), 10% (LCM). Overall, 18/123 (14.6%) patients discontinued treatment (10/18 due to lack of efficacy, 8/10 due to TEAEs). Dropout percentages were 24% (66.7% of all dropouts), 13% and 6.3% with LCM, PER and ESL, respectively, with statistical significance only for LCM (p=0.01); no discontinuation was observed in patients taking BRV. Finally, 35/123 subjects had ≥1 TEAEs, mostly dizziness (n=11); TEAEs rates were 35.5% (PER), 32% (LCM), 19% (ESL), 10% (BRV).
CONCLUSIONS: We report real-world data from patients with TRE treated with third-generation ASMs which overall seem to confirm the effectiveness and tolerability of these medications. However, the small sample size and the large disproportion among the treatment groups did not allow to make statistically significant comparisons. Therefore, results from larger cohorts and real-life prospective studies are needed to provide further information in order to make the best treatment choice in a specific patient.

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REAL-TIME TELEMETRY MONITORING OF ENERGY METABOLISM AND GLUTAMATE IN THE CENTRAL COMPLEX OF FREELY-WALKING GROMPHADORHINA PORTENTOSA

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BACKGROUND: Recently, the profound homology between the basal ganglia of vertebrates and the central complex (CX) of insects have been highlighted [1-3]. The CX is a fundamental structure of insect brain regulating functions such as sleep, memory or motion control. Lately, new animal models, alternative to mammalians, have been used to study neurochemistry and expand the knowledge of the basal ganglia neurophysiology and the related disorders. Here, energy metabolism has been studied in the CX of Gromphadorhina portentosa by means of amperometric microsensors and biosensors.
METHODS: Oxygen microsensors and glucose, lactate and glutamate biosensors have been implanted in the CX of cockroaches [1] and connected to a telemetric unit for the wireless transmission of electrochemical data. The oxygen microsensors were carbon-based discs further modified by means of a layer of nitrocellulose, in order to make the transducer selective only for oxygen, thanks also to the application of a cathodic potential of -400 mV vs Ag/AgCl. The biosensors, however, were platinum-based and suitably modified in order to make them selective for glucose, lactate and glutamate, by loading glucose, lactate and glutamate oxidase enzymes. Biosensors were further modified in order to shield them against interfering compounds. The hydrogen peroxide (HP) produced during enzymatic reaction is easily monitored on the platinum surface by applying an anodic potential of +700 mV vs Ag/AgCl. The amount of HP is proportional to the analyte present in the matrix.
RESULTS: The functionality of the biosensors was initially verified by the injection in the hemolymph or the neck of the insect of the various analytes. The increase in the currents following these injections, demonstrated the capability of the devices to monitor the analyte variations in the cockroach's CX. The functionality of oxygen microsensors was assessed by varying the concentration of oxygen in the air inspired by the animal. Physiological stimulations, as handling or immobilization, determined a decrease in oxygen, an increase in lactate and glutamate, while glucose underwent no significant modification. Interestingly, the exposition to anesthetics, as CO2 , chloroform or triethylamine produced a decrease in oxygen and lactate, a small increase in glucose, while glutamate remained practically unchanged.
CONCLUSIONS: By means of this model it was possible to study the central complex oxygen, glucose, lactate and glutamate dynamics, resulting as an alternative in- vivo model.

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EFFECT OF LMWH THERAPY ON THIRTY DAYS-MORTALITY IN COVID-19 PATIENTS

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INTRODUCTION: COVID-19 induced coagulopathy has been raising concerns and questions on the pathological mechanism of SARS-CoV-2. This complication has been reported as frequent in these severely ill patients, and disseminated intravascular coagulation may be present in the majority of deaths.
Moreover, autopsies performed on COVID-19 patients have indicated that the lungs are oedematous with patchy bleedings, with diffuse alveolar damage and extensive fibrin thrombi in distended small vessels and capillaries.
For these reasons, the administration of anticoagulants to hospitalised patients with severe COVID-19 has been widely recommended, though its efficacy is yet to be assessed.
METHODS: In this retrospective observational study, clinical, laboratory and therapy data of COVID-19 patients who were consecutively hospitalised in Internal Medicine Units of Niguarda Hospital from March 9th to May 15th 2020 were collected through electronical medical record research.
The primary endpoints considered for this analysis were thirty days-mortality and admission to Intensive Care Unit.
RESULTS: COVID-19 patients hospitalised from March 9th to May 15th 2020 in Internal Medicine Units of Niguarda Hospital were 217. Of these, medical records of a total of 165 consecutive patients were analysed: of these, 142 were administered low molecular weight heparin (LMWH), while 23 were not.
No statistically significant age difference was found between the two groups: considering all patients, the mean age was 64.9 (SD ±17.4).
There were no statistically significant differences between the two groups of patients in terms of frequency of hypertension, prior stroke, BMI, atrial fibrillation, peripheral vascular disease and coronary disease. Females were 45.8% of untreated patients, and 39.6% of treated patients, and represented 40.5% of total patients.
Patients were treated with LMWH according to clinical judgement of the physician.
Survival rate at 30 days was of 93% in LMWH-treated patients, while it was 37% in patients not receiving anticoagulant. At multivariable Cox proportional hazard regression analysis age (hazard ratio [HR] 1.063, 95% confidence interval [CI] 1.035-1.079, p<0.001), BCRSS algorithm (HR 3.039, 95%CI 1.823-4.074, p<0.001), were directly associated with death, whilst use of EBPM (vs. no EBPM) (HR 0.325, 95%CI 0.171-0.412, p<0.001) was inversely associated.
DISCUSSION AND CONCLUSIONS: The endothelial cells dysfunction induced by SARS-CoV-2 infection causes thrombin generation and a faulty fibrinolysis, which results in a diffuse hypercoagulable state. Moreover, the hypoxia state of COVID-19 patients with respiratory distress can stimulate thrombogenesis through increased blood viscosity and a hypoxia-triggered signaling pathway.

MATURITY ONSET DIABETES OF THE YOUNG IN PREGNANCY: MANAGEMENT, TREATMENT AND NEONATAL OUTCOMES

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BACKGROUND: Maturity onset diabetes of the young (MODY) is a subtype of genetically inherited diabetes, accounting for 1 to 5% of all diabetes diagnoses. As it generally arises in young adulthood, it can affect pregnant women with significant neonatal outcomes.
METHODS: The aim of our study was to describe the pregnancy management and outcomes of a woman affected with the rare MODY 7, caused by a *KLF11* gene mutation. We retrospectively collected data of her two pregnancies, cared for in our Interdisciplinary Diabetes and Pregnancy Center. Furthermore, we systematically reviewed pregnancy management and neonatal outcomes of all subtypes of MODY. Our strategy aimed to search the MEDLINE and Central database through PubMed and Cochrane to find articles about management and outcomes of MODY-affected pregnancies. The identification of relevant abstracts, their selection and extraction were performed independently by two authors and conflicts resolved by a third investigator.
RESULTS: The woman affected with MODY 7 received basal-bolus insulin with glycemia optimization during both pregnancies. Her newborns were not macrosomic nor did they have neonatal hypo- or hyperglycemia.
Our systematic review found specific guidelines for the management of pregnant women affected with MODY 2 and 5, but no recommendations on management or outcomes of rarer subtypes.
CONCLUSIONS: Our review is limited by the lack of data on rarer subtypes of MODY, and by the specific keywords and databases selected. However, our work lays an important first step in the direction of much-needed clinical data and recommendations in treating rare MODY subtypes, especially during pregnancy.

MODULATION OF N-ACYLETHANOLAMINE ACID AMIDASE (NAAA) IMPACTS COLORECTAL CANCER GROWTH

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BACKGROUND: Colorectal cancer (CRC) is a predominant healthcare problem in Europe (1) and accounts to become the third leading cause of predicted cancer deaths in the USA (2) for the year 2020. N-acylethanolamine acid amidase (NAAA) is a lysosomal enzyme responsible of the breakdown of N-acylethanolamides (NAEs) (i.e. palmitoylethanolamide, anandamide and oleoylethanolamide) (3). NAAA inhibition is reported to be effective in several inflammatory disorders including those affecting the intestine (4). However, the role of NAAA in cancer is greatly fragmentary with only a few reports published (5-6). We covered this gap by investigating NAAA effects in CRC.
METHODS: Primary human biopsies were obtained from surgically resected colorectal patients; NAAA, NAE targets, cyclins and CDKs expression were analyzed by RT-PCR; NAEs were measured by LC-MS; CRC xenograft and azoxymethane models were used to assess the in vivo pharmacological effect of NAAA inhibition; tumor secretome was analyzed by an oncogenic array; CRC cell lines were used for in vitro studies; cell cycle was evaluated by cytofluorimetry; NAAA was knocked down with siRNA.
RESULTS: NAAA expression was downregulated in human CRC tissues, with a consequential augmentation of NAEs levels and dysregulation of some of their targets (i.e. PPAR-α and CB1 receptor). The selective NAAA inhibitor AM9053 reduced CRC xenograft tumor growth by reducing the proliferative marker Ki67 and counteracted sporadic tumor development (azoxymethane model). Moreover, NAAA inhibition impacted the composition of the tumor secretome that negatively affected the expression of epidermal growth factor family members. In CRC cells, AM9053 reduced, via PPAR-α and TRPV1, cell proliferation and induced cell cycle arrest in the S phase (an effect associated to cyclin A2/CDK2 downregulation). NAAA knock-down mirrored the effects of NAAA pharmacological inhibition.

CONCLUSIONS: Our results provide novel insights into the functional role of NAAA in CRC progression.

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P2X7 RECEPTOR ANTAGONISM PRESERVES RETINAL GANGLION CELLS IN GLAUCOMATOUS MICE

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BACKGROUND: The investigation about the role of topical treatment with a selective P2X7 receptor antagonist is crucial to preserve retinal ganglion cells (RGCs) structure and function in a genetic model of age-related glaucomatous neurodegeneration: DBA/2J mice.

METHODS: Chronic treatment with P2X7 receptor antagonist, RGCs function was assessed with pattern electroretinogram (PERG), and RGCs density along with microglia activation were assessed in flat-mounted retina immunostained with RBPMS and Iba-1, respectively.

RESULTS: Untreated glaucomatous eyes displayed significant microglia activation, loss of PERG signal, and RGCs loss. In P2X7 receptor antagonist-treated eyes, PERG signal was significantly ($P<0.05$) improved compared to controls, along with a significant ($p<0.05$) reduction in terms of retinal microglial activation, and remarkable preservation of RGCs density.

CONCLUSIONS: These findings demonstrated that topical treatment with a P2X7 receptor antagonist has a neuroprotective effect on RGCs in glaucomatous mice, suggesting an appealing pharmacological approach to prevent retinal degenerative damage in optic neuropathy.

CLINICAL UTILITY OF PANEL OF PHARMACOGENETIC MARKERS ASSOCIATED WITH ADVERSE DRUG REACTIONS IN FLUOROPYRIMIDINE AND IRINOTECAN-TREATED CANCER PATIENTS ENROLLED IN THE PREPARE STUDY- RESULTS FROM A PRELIMINARY ANALYSIS

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BACKGROUND: Despite the evidence available of the clinical validity of pharmacogenetic (PGx) toxicity biomarkers linked to Fluoropyrimidines (FPs) and/or Irinotecan (IRI) based treatments, a consensus on the level of evidence required to support their translation into the clinics has not been reached yet. Ubiquitous Pharmacogenomics (U-PGx) consortium set up in 2016 the PREemptive Pharmacogenomic testing for prevention of Adverse drug Reactions (PREPARE) study. PREPARE is a prospective, controlled, block-randomized clinical study with the aim of assessing the clinical utility of implementing a panel of PGx markers into routine care on the prevention of toxic events, on the improvement of patients' quality of life and on the reduction of toxicity-related costs. The aim of this preliminary analysis was to compare the incidence of clinically relevant toxicities in oncologic patients, enrolled at CRO-Aviano Hospital, blinded and notblinded for their DPYD and UGT1A1 genotype at the beginning of FPs/IRI treatment.
METHODS: The 40-months enrollment was split into two (Standard of care and PGx testing arm) time-blocks and ended in July 2020. Enrolled patients were genotyped within a 2-days turnaround time using an allelic discrimination-based method. In the standard of care arm, patients and clinicians were blinded for their PGx results. In the PGx testing arm, patients' PGx results were incorporated in the (electronic) medical record along with clinical data and combined with a clinical decision support system. Physicians chose whether to use these results to preemptively guide drug and dose selection. All patients were followed from 3 to a maximum of 18 months.
RESULTS: A homogeneous group of 402 FPs/IRI treated patients (236 from the Standard of care arm and 166 from the PGx testing arm) was selected for a preliminary analysis. The distribution of patients' with DPYD and UGT1A1 actionable genotype was similar in both arms. A preliminary analysis on patients with actionable genotype showed a trend toward a reduced incidence of clinically relevant toxicities (defined as G≥4 for hematological toxicities and G≥3 for non-hematological toxicities according to CTCAE v.5.0): 38.5% of patients in Standard of care arm developed clinically relevant toxicities versus only 9.0% in PGx testing arm. Patients with actionable genotype in

the PGx testing arm received also less clinically relevant dose modification (>20%) following a toxic event compared to patients in Standard of care arm (53.8% vs 9.0%).
CONCLUSIONS: From a preliminary analysis on a homogenously group of 402 FPs/IRI treated patients enrolled in the PREPARE study it could be hypothesized that implementing PGx-guided drug and dose selection may decrease incidence of clinically relevant adverse drug reactions over 70% (among those with actionable drug-gene interactions). A huge international effort is about to demonstrate whether a pre-treatment PGx approach will positively impact health outcomes, decision making and costs.

TAMOXIFEN INDUCED CYTOTOXICITY IN THE ADRENOCORTICAL CARCINOMA MODEL OF NCI-H295R CELL LINE: INVOLVEMENT OF β ESTROGEN RECEPTORS

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BACKGROUND: Estrogen Receptors (ER) α and β are expressed at different intensity in both normal and neoplastic adrenal cortex [1], however, the physio-pathological relevance of their expression in the physiological regulation of adrenal cell proliferation is not yet fully understood. During neoplastic degeneration, there is a rearrangement of ER expression, but data are controversial. In the experimental cell model of ACC, namely the NCI-H295R cells, it has been suggested that the selective ER modulator tamoxifen inhibits proliferation [2]. Here, we investigated the ER-mediated mechanisms in the regulation of cell viability in NCI-H295R cells.
METHODS: ER expression was studied by q-RT-PCR and immunofluorescence. Estradiol measurement was done by ELISA. Cell viability was evaluated by MTT assay following exposure of cells for 4 days to increasing concentrations of tamoxifen or erteberel, a selective ERβ agonist. Steroidogenic Factor 1 (SF-1) expression was investigated by qRT-PCR and western blot. Total miRNA extraction was conducted following the kit manufacturer instructions and analyzed by q-RT-PCR.
RESULTS: NCI-H295R cells expressed low levels of mRNA encoding both ER subtypes, with a prevalence of ERβ mRNA. NCI-H295R cells produced and secreted β-estradiol, that was 10.01 ± 0.77 ng/ml. Interestingly, exposure of ACC cells to exogenous β-estradiol slightly stimulated cell viability at low concentrations, while the effect was reduced as concentrations increased. Exposure of cells to increasing concentrations of tamoxifen induced cytotoxicity, with an IC_{50} that was 5.4 μM (95%CI: 5.1-5.7 μM). The cytotoxic effect could be mediated by the reduction of SF-1 protein expression (- 51.56% ± 0.02%), with no change in the mRNA expression. This phenomenon could be explain by the involve-

ment of miRNA23a/b activity, as we observed an increase in miRNA23a expression ($2^{-\Delta\Delta Ct}$: +73 ± 0.04 fold increase in tamoxifen-treated cells, $p < 0.005$) and in miRNA23b levels ($2^{-\Delta\Delta Ct}$: +51 ± 0.02 fold increase in tamoxifen-treated cells, $p < 0.05$). Finally, exposure of NCI-H295R cells to the ERβ selective agonist erteberel induced cytotoxicity in a concentration dependent manner, with the IC_{50} of 5.14 (95%CI: 4.80-5.49 μM). **CONCLUSIONS:** ERβ stimulation mediated a cytotoxic effect in ACC cells. Indeed, both erteberel and tamoxifen exposure exerted a cytotoxic effect on NCI-H295R cells, to which contributed the reduction of SF-1 expression, an adrenal key-protein involved in cell proliferation and migration [3]. The mechanism by which tamoxifen exert the cytotoxic effect is still under investigation. It could be hypothesized that it could exert this effect as ERα antagonist, leaving β-estradiol able to selective bind ERβ, that act as a tumor suppressor in a variety of tissues [4]. Furthermore, a non-genomic effect of tamoxifen cannot be excluded.

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YOSHIDA AH-130 ASCITES HEPATOMA-INDUCED CACHEXIA REPRESENTS A VALID MODEL TO STUDY THE ANTI-CACHECTIC EFFECT OF NATURAL COMPOUNDS

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BACKGROUND: Cachexia is a complex metabolic disorder which occurs in late stages of chronic disease including cancer and characterized by involuntary weight loss, caused by an ongoing wasting of skeletal muscle and loss of adipose tissue. Cachexia also affects cardiac muscle inducing an enhanced mortality.
METHODS: To better defined a possible strategy to counteract detrimental effects of cachexia, the Yoshida hepatoma model could be used as a model of skeletal muscle and cardiac atrophy. Rats are i.p. injected with Yoshida 108 AH-130 hepatoma cells. Body weight and body composition are assessed at baseline and at the end of the study (16 days). Cardiac function is analyzed by echocardiography at baseline and at day 11. At the end of the study, organs are removed and weighed.
RESULTS: Yoshida hepatoma-induced cachexia caused wasting and weight loss. This metabolic alteration is associated with a cardiac dysfunction (reduced LV EF, SV, CO) suggesting a close relationship between cardiac and skeletal muscle atrophy and cardiac function as also demonstrated by catabolic marker modulation.
CONCLUSIONS: Our results suggest that this model might be used to test the activity of natural compounds on cancer-induced cachexia in order to find a valid adjuvant support to conventional pharmacological therapy.

MANIPULATION OF DIETARY AMINO ACIDS PREVENTS AND REVERSES OBESITY IN MALE MICE

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BACKGROUND: The consumption of diets with a high ratio of saturated to unsaturated fatty acids (SFA diet) reduces the activation of energy metabolism, increasing adiposity and leading to defects in glucose homeostasis with the development of obesity and type 2-diabetes. When SFA diets are consumed, chronically raise cardiovascular risk and reduce the healthy disease-free lifespan. Modify the feeding habits may affect energy balance and represents a cornerstone of therapy in metabolic diseases. A growing body of evidence suggests that food can be considered as a cocktail of "hormones", a collection of molecules (e.g., free fatty acids, amino acids) with specific direct and indirect actions on receptors and signaling pathways. Several studies have shown the efficacy of central or peripheral administration of a single amino acid (e.g., glycine, leucine, or tryptophan) in modulating energy metabolism and body weight. Moreover, a sex-based difference in response to diets has been proved. For example, female mice are relatively less sensitive to the metabolic improvements observed following dietary protein dilution. Sexual dimorphism of mitochondria could take part in the sex specificity in response to diets.
METHODS: To find new nutritional approaches to fight metabolic diseases, we investigated the ability of a designer diet to promote energy expenditure. Male and female C57BL6/N mice (8 weeks old) were fed ad libitum for 6 weeks with 1) SFA diet; 2) SFA-EAA diet, isocaloric, isolipidic, isonitrogenous, in which protein (casein) was almost completely replaced by defined free essential amino acid formula (EAA); 3) SFA-CAA diet, as additional control diet, isocaloric, isolipidic, and isonitrogenous, in which casein was substituted with the amino acids designed on casein profile, and 4) normal chow diet.
RESULTS: Our data indicate that the SFA-EAA diet in male mice can: i) promote the brown fat thermogenic program and fatty acid oxidation, ii) stimulate uncoupling protein 1-independent respiration in subcutaneous white fat, iii) change the gut microbiota composition, and iv) prevent and reverse obesity and dysregulated glucose homeostasis in multiple mouse models, prolonging the healthy lifespan. These effects are independent of unbalanced amino acid ratio, energy consumption, and intestinal calorie absorption. A brown fat-specific activation of the mechanistic target of rapamycin complex 1 seems involved in the diet-induced beneficial effects. These effects seem to be sex-related and are blunted in female mice.

CONCLUSIONS: Hence, our results suggest that brown and white fat may be targets of specific amino acids to control thermogenesis, thereby contributing to the improvement of metabolic health. The apparent controversies about the effect of SFA-EAA diet in males and females suggest the importance of considering sex as a biological variable, which must be further investigated to understand how nutrients and estrogens could interact.

AUTOPHAGY PROMOTES SURVIVAL OF RETINAL GANGLION CELLS IN RODENT MODELS OF ACUTE GLAUCOMA

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BACKGROUND: Autophagy is a conserved lysosomal-dependent pathway responsible for the degradation of proteins and organelles. The three types of autophagy occurring in mammals, micro, macro and chaperone-mediated autophagy, differ for the mechanisms of cargo delivery to lysosomes. Beside the physiological turnover of cytoplasmic components, autophagy regulates cellular adaptation to different metabolic states and stressful conditions allowing cellular survival or, when overactivated, participating to cell death. Here we describe the role played by macroautophagy in the loss of retinal ganglion cells (RGCs) occurring in glaucoma, the most common neurodegenerative cause of irreversible blindness, and explore the pharmacological modulation of the process as therapeutic intervention for preventing ocular neurodegenerative diseases.
METHODS: Acute glaucoma was induced in adult rats, wild type C57BL/6J or GFP-LC3 transgenic mice by transient elevation of intraocular pressure (IOP). Expression of autophagy related proteins (Atg) was studied by western blotting and immunofluorescence. RGCs were retrogradely labeled by a fluorescent tracer and survival was assessed in autophagy-deficient mice (AMBRA1+/gt) and upon pro-autophagic treatments.
RESULTS: Transient IOP elevation induced in the retina a time-dependent modulation of autophagy, characterized by an acute upregulation of the autophagic flux followed by a reduction of autophagosomal turnover. The latter was associated with build-up of the autophagic

substrate SQSTM1/p62, decreased of ATG12-ATG5 conjugate, ATG4 and BECN1/Beclin1 expression. Induction of autophagy by fasting and rapamycin treatment prolonged the endogenous autophagy response and increased RGC survival, whilst genetic deficiency of basal autophagy was associated with increased RGC death.

CONCLUSIONS: Altogether, our data add knowledge to the dynamic of the autophagy process in the retina subjected to glaucoma-related insults, define autophagy as a determinant for RGC survival and identify this pathway as a relevant endogenous neuroprotective mechanism that can be targeted for retinal neuroprotection.

DETAILED IN VITRO PHARMACOLOGICAL CHARACTERIZATION OF THE CLINICALLY VIABLE NOP RECEPTOR ANTAGONIST BTRX-246040

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BACKGROUND: The peptide nociceptin/orphanin FQ (N/OFQ) is the endogenous ligand of the N/OFQ receptor (NOP) which is widely expressed in the central and peripheral nervous system. Selective NOP antagonists are worthy of testing as innovative drugs to treat depression, Parkinson's disease, and drug abuse. The aim of this study was to perform a detailed in vitro characterization of BTRX-246040 (also known as LY2940094, [2-[4-[(2-chloro-4,4-difluoro-spiro[5H-thieno[2,3- c]pyran-7,4'-piperidine]-1'-yl)methyl]-3-methyl-pyrazol-1-yl]-3-pyridyl]methanol) a novel NOP antagonist that has been already studied in humans.

METHODS: BTRX-246040 has been tested in vitro in the following assays: calcium mobilization in cells expressing NOP and classical opioid receptors and chimeric G proteins, BRET assay measuring NOP interaction with G proteins and b-arrestins, the label free dynamic mass redistribution assay, and the electrically stimulated mouse vas deferens. BTRX-246040 was systematically compared to the standard NOP antagonist SB-612111.

RESULTS: In all assays BTRX-246040 behaves as a pure and selective antagonist at human recombinant and murine native NOP receptors displaying 3 - 10 fold higher potency than the standard antagonist SB-612111.

CONCLUSIONS: BTRX-246040 is as an essential pharmacological tool to further investigate the therapeutic potential of NOP antagonists in preclinical and clinical studies.

SRC-TYROSINE KINASE INHIBITORS IN DUCHENNE MUSCULAR DYSTROPHY: EFFECT OF A NOVEL ORAL DASATINIB-CYCLODEXTRIN FORMULATION IN DYSTROPHIC MDX MOUSE

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BACKGROUND: Src Tyrosine Kinase (TK) protein is both overexpressed and overactivated in Duchenne Muscular Dystrophy (DMD), a rare X-linked disorder due to mutations in dystrophin gene, causing the absence of dystrophin protein in myofibers. In dystrophic muscles, Src-TK contributes to degradation of β-dystroglycan (β-DG), a component of dystrophin-associated glycoprotein complex, reinforcing damaging signals. Thus, Src-TK seems a promising therapeutic target for DMD. Recently, we performed in the mdx mouse, a 4-week subcutaneous treatment with the Src-TK's competitive inhibitor dasatinib, already in use as antitumor drug (dose: 5mg/kg; 3 times/week)[1]. Although well tolerated, dasatinib had no significant efficacy on pathology-related functional indices; however, an increased expression of non-phosphorylated β-DG was found. To overcome possible pharmacokinetic issues, a pilot study in wild type (WT) animals showed that the drug, complexed in hydroxypropyl-β-cyclodextrin (HPβCD) could be administered in drinking water (dose: 15mg/kg), was well tolerated and reached target tissues [2].
METHODS: We tested the effect of this new formulated dasatinib in male mdx mice (doses: 10 and 20mg/kg; 12 weeks in drinking water) starting at 4 weeks of age. Treatment outcome was evaluated by means of a multidisciplinary approach including in vivo and ex vivo readouts, in comparison to age- and sex- matched untreated mdx and WT mice.
RESULTS: Pharmacokinetic analysis confirmed that dasatinib reached both target tissues and plasma in a dose-related manner. In vivo, dasatinib at both doses (10 and 20 mg/kg) counteracted the exercise performance decrease observed in vehicle-treated mdx mice during an exhaustion test on treadmill, with a recovery score (R.S.) toward WT value of 28% and 17%, respectively. This was paralleled by an improvement in torque of hind limb plantar flexor muscles, mostly detectable in mdx mice treated with dasatinib 10 mg/kg vs untreated ones, at high stimulation frequencies (100 – 200 Hz). Ex vivo, dasatinib 10 and 20 mg/kg induced a trend of increase in tetanic isometric force of fast-twitch extensor digitorum longus muscle, with a R.S. of 31% for both doses. Dasatinib at 10mg/kg also increased tetanic isometric force of diaphragm (R.S. 45%), and partially recovered diaphragm elastic properties, measured as muscle stiffness during eccentric contraction. Moreover dasatinib, either 10 or 20mg/kg, notably decreased plasma levels of creatine kinase (R.S. 28% and 47%) and lactate dehydrogenase (R.S. 36% and 46%), biomarkers of damage in dystrophic muscles.
CONCLUSIONS: Our preliminary data support the interest of this new oral dasatinib formulation for DMD treatment and need to be supported by further experiments. Analysis of data from ultrasonography assessment of diaphragm function along with histological evaluation of diaphragm and gastrocnemius muscles, is currently ongoing [Supported by Duchenne Parent Project NL 2015].

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ETHNOPHARMACOLOGICAL EVALUATION OF CAMEROONIAN SPICES IN GASTRIC INFLAMMATORY MODELS

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BACKGROUND: Chronic gastritis is an inflammatory-based disease mainly due to *Helicobacter pylori* (*H. pylori*) infection. This bacterium is largely diffused in underdeveloped countries, where the economic situation limits the access to current drug therapies. In Cameroon, the traditional use of natural products is largely diffused and constitutes the first health resource among the population; unfortunately, the efficacy of these traditional preparations is generally not supported by scientific evidence. Nkui and Nahpoh are two Cameroonian traditional soups used for the treatment of various ailments, including stomach disorders.
This work investigated the antioxidant and anti-inflammatory activity of eleven spices included in Cameroonian traditional gastric remedies, focusing on NF-κB-dependent mediators at gastric level.
METHODS: The hydroalcoholic extracts were prepared from the following plants: *Xylopia aethiopica* (Dunal) A.Rich. (XA), *Xylopia parviflora* Spruce (XP), *Scorodophloeus zenkeri* Harms (SZ), *Monodora myristica* (Gaertn.) Dunal (MM), *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (TT), *Echinops giganteus* A.Rich. (EG), *Dichrostachys cinerea* (Forssk.) Chiov. (DC), *Afrostyrax lepidophyllus* Mildbr. (AL), *Aframomum melegueta* K.Schum. (AM), *Aframomum citratum* (C. Pereira) K.Schum. (AC), and *Zanthoxylum leprieurii* (Guill. & Perr.) (ZL). Two gastric epithelial cell lines were used to investigate antioxidant and anti-inflammatory activity, as a model of tumor (AGS) and non-tumor cells (GES-1).
RESULTS: All extracts inhibited intracellular ROS formation in at least one cell line at 10 μg/mL, but TT and AM gave the most interesting results. These two extracts belong to the group of six (XA, XP, TT, DC, AM, and AC) capable of reducing the NF-κB driven transcription, induced by TNFα in AGS (see figure attached) and in GES-1 cell lines. In addition, they also inhibited TNFα-induced IL-8 release and IL-8 promoter activity, with a lesser effect for XA. The measurement of IL-8 mRNA confirmed the effects at transcriptional level. Only XP, DC, TT, and AC reduced IL-6 secretion and mRNA levels in GES-1 cells. Interestingly, TT was the only extract that reduced TNFα-induced COX-2 mRNA levels, without affecting the gene expression of the COX-1 isoform. TT was also inactive on COX-2 and COX-1 mRNA levels in basal condition.
CONCLUSIONS: This study firstly demonstrates the antioxidant and anti-inflammatory activity, at gastric level, of different Cameroonian spices, largely used and diffused in some African countries. The anti-inflammatory activity of TT is of particular interest at gastric level, laying the foundations for a possible natural remedy widely accessible for Cameroonian people.

METALLOTHIONEIN-3 IS A POTENTIAL DRUG TARGET TO PREVENT TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA CELLS

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BACKGROUND: Metallothioneins (MTs) are involved in the regulation of zinc tracking, protection against reactive oxygen species, and adaptation to stress. Because of their broad range of properties, MTs have been suggested to promote angiogenesis, microenvironment remodeling, and immune escape in carcinogenesis (1). Among MTs, MT3 is significantly expressed in human glioblastoma (GBM) samples, where it correlates with poor patient survival (2,3). Nonetheless, the knowledge on MT3 expression, regulation and function in human gliomas is limited.
METHODS: Experiments were carried out in human cell lines obtained from the ECACC, including grade II 1321N1 astrocytoma cells, and A172 and U87 GBM cells. All cell lines were maintained under identical growth conditions, and initially investigated for their repertoire of MTs and zinc transporters by real-time PCR analysis. Based on the gene expression profiles, U87 cells appeared as the most appropriate GBM cell line to use in comparison with the grade II 1321N1 cells
RESULTS: U87 GBM cells expressed high levels of MT3 mRNA as compared to grade II 1321N1 astrocytoma cells. Different from 1321N1, U87 cells were resistant to the alkylating drug, temozolomide (TMZ, 100 M for 96 hours), which induced a massive accumulation of U87 into the S and G2 fractions of the cell cycle, but not apoptotic death. MT3 silencing did not affect U87 cell proliferation and survival, but it delayed G1/S transition and favored the occurrence of apoptosis in TMZ-treated U87 cells. MT3 silencing and TMZ converged on the activation of a checkpoint controlling the cell entrance into the S phase, namely the chk-1 protein (Sorensen et al., Cancer Cell 2003). Blockade of chk-1 by isogranulatimide (1 M) protected GBM U87 cells against the combined effects of MT3 silencing and TMZ treatment.
CONCLUSIONS: MT3 contributes to the intrinsic resistance of GBM cells to TMZ through the control of chk-1 activity.

THE ABSENCE OF SEROTONIN IN THE BRAIN ALTERS ACUTE STRESS RESPONSIVENESS BY INTERFERING WITH THE GENOMIC FUNCTION OF THE GLUCOCORTICOID RECEPTOR

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BACKGROUND: Serotonin is a neurotrophin strictly involved in the control of different behavioral processes such as mood and sleep. Alterations in serotonergic transmission have been related to a major predisposition to develop psychiatric pathologies, including depression. Here, we took advantage of tryptophan hydroxylase (TPH) 2 deficient rats, characterized by a complete absence of serotonin in the brain, to evaluate whether a vulnerable genotype may influence the reaction to an acute stressor. In this context, we investigated whether the activity of the hypothalamic-pituitary-adrenal (HPA) axis was altered by the absence of serotonin focusing on the genomic pathway of the glucocorticoid receptors (GRs). Moreover, we analyzed the transcription pattern of the clock genes that can be affected by acute stressors.
METHODS: Adult wildtype (TPH2+/+) and TPH2-deficient (TPH2-/-) male rats were exposed to one hour of acute restraint stress as an acute challenge. Rats were sacrificed immediately after the end of the stress and the prefrontal cortex was dissected, frozen on dry ice and conserved at -80°C. Protein and gene expression analyses were analyzed through real time PCR and western blot analyses.
RESULTS: The activation of the genomic pathway of the GRs depends on the translocation of the receptors into the nucleus. Here, we observed that the acute stress enhanced the translocation of glucocorticoid receptor in the nucleus specifically in TPH2+/+ animals. This effect was completely blunted in TPH2-/- rats, suggesting an impairment of the GR genomic mechanism due to the absence of the neurotransmitter in the brain. This alteration was mirrored in the expression of the GR-responsive genes: acute stress led to the upregulation of GR-target gene expression in TPH2+/+, while this increase in responsive genes transcription was reduced in TPH2-/- animals. Finally, clock genes were differently modulated in the two genotypes after the acute restraint stress.
CONCLUSIONS: Overall our findings suggest that the absence of serotonin within the brain in TPH2 deficient rats interferes with the ability of the HPA axis to correctly modulate the response to an acute stress, by altering the nuclear mechanisms of the GR and modifying clock gene expression.

PHARMACOLOGICAL PERSPECTIVES OF ZOLEDRONIC ACID AS AN AGONIST OF TRPV1 ION CHANNEL

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BACKGROUND: Zoledronic acid (ZOL) belongs to the family of bisphosphonates (BPs), drugs commonly used to treat several bone diseases, like osteoporosis and Paget's disease. Despite its belonging to WHO's List of Essential Medicines, many aspects linked to its pharmacological effects are still not clear. For example, empirical evidence has showed that ZOL may exert pain-relieving effects in the presence of various different noxious stimuli. At the same time, a recent FDA alert has reported an increased risk of bone pain and muscle aches in patients taking BPs, showing disabling pain condition. In both cases, the molecular pathways involved in ZOL-mediated pain are mostly unknown. Among the proteins involved in pain sensation, TRPV1 ion channel is universally recognized to play a key role. The selective channel agonist capsaicin is commonly used in fibromyalgia and joint conditions, as osteoarthritis. We have recently demonstrated that ZOL is an agonist of TRPV1 ion channel in bone cells, being able to activate strong outward capsazepinesensitive currents on pre-osteoblast like cells MC3T3-E1 and on native murine/rat mesenchymal stem cells. Effects of ZOL on ion channels in other tissues have never been reported. We examined ZOL effects on TRPV1 ion channel by performing patch-clamp experiments in neuronal SH-SY5Y cell line, TRPV1-transfected Xenopus laevis oocytes and HEK293 cells transfected with cDNA coding for wild-type and PI(4,5)P_2-binding site mutant TRPV1 ion channels.
METHODS: Patch-clamp experiments in cells were performed in whole cell configuration under physiological ionic conditions, while in Xenopus oocytes in inside-out configuration. Transient expression in HEK293 cells was achieved using the calcium phosphate co-precipitation method.
RESULTS: On neuronal SH-SY5Y cells, ZOL enhanced ion channel currents both at negative and positive voltages with a maximal activation of +351.9% at +100 mV with respect to the maximal current in the control condition. At positive membrane voltages ZOL-evoked currents were almost completely closed after the application of the selective TRPV1 antagonist capsazepine. In excised inside-out macro-patch in Xenopus laevis oocytes and in whole cell experiments on HEK293 cells, we found that ZOL caused a concentration-dependent enhancement of TRPV1 currents, with a maximal activation at high membrane potential (+180 mV). On K694A TRPV1 mutant, ZOL was still able to activate currents by+10%, +63% and +76% at -60 mV, +60 mV and +180 mV, respectively. No activation of currents was observed on the R579A mutant.
CONCLUSIONS: ZOL is an agonist of TRPV1 ion channel at micromolar concentration. The arginine in the S4-S5 linker of the channel is essential for drug interaction with the channel. TRPV1 ion channel appears now as an interesting target not only for explaining ZOL-mediated promineralizing properties in osteoblasts but also for ZOL effects in pain modulation.

BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF ATP-SENSITIVE POTASSIUM CHANNELS IN SKELETAL MUSCLE OF KIR6. 1WT/ V65M MICE RESEMBLING THE RARE CANTÙ SYNDROME

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BACKGROUND: Cantú syndrome (CS) is a rare condition primarily characterized by excess hair growth, heart defects and muscle symptoms. It arises from gain-of-function (GOF) mutations in *KCNJ8* or *ABCC9* genes, encoding Kir6.1 and SUR2 subunits, respectively, of ATP-sensitive potassium (KATP) channels. KATP over-activity has already been reported for Cantú cardiac and smooth muscle cells, causing low systemic blood pressure and cardiac enlargement; so far consequences in skeletal muscles are uninvestigated. We analyzed skeletal muscle properties of mutant Kir6.1[V65M] CS mice, by measurements of forelimb strength and ultrasonography of hind-limb muscles. Then, we investigated on the biophysical and pharmacological responses of KATP channel in native *Flexor digitorum brevis* (FDB) and *Soleus* (SOL) fibers by patch-clamp technique in parallel with histopathological, immunohistochemical and PCR analysis.

METHODS: For our blind investigations, we used 33-week-old transgenic heterozygotes mice Kir6.1[V65M] resembling the human CS generated through CRISPR/Cas9 technique. Forelimb strength was measured using a grip strength meter in parallel with ultrasonography evaluation of skeletal muscles (Vevo® 2100 system). Isolated fibers were obtained by enzymatic digestion from FDB/SOL muscles. KATP channel currents were recorded using inside-out macro-patch during pulses of -60 mV (Vm), in the presence of KCl on both sides of the membrane patches. Tissue samples for histological, immunohistochemical and PCR analysis were collected.

RESULTS: Forelimb strength was lower in Kir6.1$^{wt/VM}$ mice than in WT mice. The ultrasonography evaluation of the hind limb showed a significant enhancement of +21% of the mean echodensity in Kir6.1wt/V65M mice respect to the WT, suggesting the presence of fibrous tissue. Increased organ weights were observed in Kir6.1wt/VM mice with respect to the WT mice as regards heart, Tibial and Extensor digitorum longus muscles. Higher KATP channel current amplitude was recorded in FDB Kir6.1wt/VM fibers with respect to WT (mean current: -786.9±98.3 for WT vs -1092.6±130.9 for Kir6.1wt/VM, Student t test, $p<0.05$). A markedly reduced sensitivity to inhibitory glibenclamide was observed in both Kir6.1wt/VM FDB and SOL muscle fibers with respect to WT. Kir6.1 mRNA was found in all muscles by PCR.

Histopathological analysis revealed that the most affected muscle was Kir6.1wt/VM slow-twitch SOL muscle, showing degeneration, regressive-necrotic lesions with regeneration zone and replacement of myofibrils with connective tissues. PCR and immunohistochemical analysis revealed high expression of Atrogin-1, MuRF1 and BNIP3 mRNA/ proteins in Kir6.1wt/VM SOL.

CONCLUSIONS: Kir6.1wt/VM mutation in KATP channel leads to significant histopathological and gene expression changes in skeletal muscle, particularly slow-twitch SOL muscle, suggesting marked atrophy and autophagy.

REWARD-RELATED BEHAVIORAL AND NEUROCHEMICAL CHANGES IN A RAT MODEL OF AUTISM BASED ON PRENATAL EXPOSURE TO VALPROIC ACID

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BACKGROUND: Prenatal exposure to the antiepileptic drug valproic acid (VPA) induces autistic-like core behavioral symptoms in both humans and rodents, which makes it a good model to study the neural underpinnings of autism spectrum disorder (ASD). Rats prenatally exposed to VPA show profound deficits in the social domain. The altered social behavior displayed by VPA-exposed rats may be due to either a deficit in social reward processing or to a more general inability to properly understand and respond to social signals.

METHODS: To address this issue, we performed behavioral and neurochemical experiments and tested the involvement of the brain reward system in the social dysfunctions displayed by rats prenatally exposed to VPA (500 mg/kg).

RESULTS: We found that, compared to control animals, VPA-exposed rats showed reduced play responsiveness, impaired sociability in the three-chamber test and altered social discrimination abilities. However, when tested for socially-induced conditioned place preference, locomotor response to amphetamine and sucrose preference, VPA-exposed rats responded normally to social, drug and food rewards. At the neurochemical level, VPA-exposed rats showed altered expression of dopamine receptors in the nucleus accumbens (NAc).

CONCLUSIONS: These data suggest that social dysfunctions displayed by VPA-exposed rats are more likely caused by alterations in cognitive aspects of the social interaction, rather than to inability to enjoy the pleasurable aspects of the social interaction. The observed neurochemical alterations in the NAc may contribute to the inability of VPA-exposed rats to process and respond to social cues, or, alternatively, represent a compensatory mechanism towards VPA-induced neurodevelopmental insults.

TOXICOLOGICAL EVALUATION ON A CLUSTER OF INCREASED REPORTS OF HEPATITIS RELATED TO TURMERIC DIETARY SUPPLEMENTS IN ITALY

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BACKGROUND: Hepatitis related to dietary supplements consumption is a well know problem in medical literature. Few reports are published on turmeric related hepatitis. During 2019 in Italy there was an apparent increase in reports of cases of hepatitis related to intake of turmeric supplements. Together with the Ministry of Health and Istituto Superiore di Sanità, the risk on public health of these supplements was assessed.

CASE SERIES: 23 cases (22F; age 53 (29-71 y.o.)) of suspected curcumin-related hepatitis referred to the specific Italian System from October 2018 to September 2019 were evaluated. History, clinical, biochemical and instrumental data were collected and evaluated for each case. The causality probability was evaluated using the RUCAM score and the WHO criteria. All patients assumed different supplements containing curcumin for an average period of 98 days (8-730). No patient took the supplement in a higher dose than that suggested daily dose. In 10/23 cases (43.5%)the hepatitis was cholestatic, in 8 cases (35%) was non specific, but not cholestatic, and in 5 patients (21.5%) was acute non-specific with mild cholestasis. The causality probability evaluated through the RUCAM score resulted probable in 11 cases (48%), highly probable in 8 (35%) and possible in 4 (17%). The causality probability assessed with WHO criteria considered 13 cases (56.5%) as probable, 9 cases (39%) as possible and 1 (4.5%) certain because of the rechallenge of hepatitis after a second re-exposure to the same supplement.

CONCLUSIONS: From October 2018 until July 2019 a significant increase in the incidence of reports of liver damage related to the use of curcumin in Italy was registered. There is no a conclusive and well-accepted score to determine with certainty the causal link in these cases. The RUCAM score and the WHO criteria are to be considered useful and have given similar results. The Italian reporting system has proved its effectiveness. Considering the results of the causality assessment, curcumin might be considered as an infrequent cause of idiosyncratic liver damage. Consequently, further evaluations are needed to assess its safety, at least when administered in relevant doses for a prolonged period.

ANTIDOTE TREATMENT IN VIPER ENVENOMATION IN ITALY: A COMPARISON BETWEEN 4 ANTIVENOMS DURING A 6 YEARS STUDY

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BACKGROUND: Different products of viper antivenom are available in Italian hospitals. Clinical response to treatment with different antivenoms was prospectively evaluated in patients presenting with Grading-Severity-Score (GSS) ≥ 2 during 6 years' study period.

METHODS: All consecutive viper bitten patients referred to our Poison Control Centre (PCC) from 2013-2019 were investigated for sex, age, site of bite, time between bite and emergency department admission/antivenom administration (intravenous), antivenom and number of vials, GSS and clinical response (improvement/worsening after 6 hours), need of adjunctive doses, and adverse effects. Clinical manifestations were evaluated according to the GSS. Continuous variables' distribution was expressed with median and interquartile range. Univariate and multivariate mixed effects logistic regression was applied to estimate the probability of improvement based on variables values.

RESULTS: 114 patients, mean age 51 years old (range 35-67 years), males 72.8%, were included in the study. 19 were pediatric (range 1-16 years). Vipera aspis spp. was mainly involved. Upper and lower limbs were involved in 83 and 17% of cases, respectively. Time between bite and emergency department admission was 2 h (range 1-4 h) and showed: GSS-0 in 2/114 (1.75%), GSS-1 in 57 (50%), GSS-2 in 45 (39%) and GSS-3 in 10 (9%). Time elapsed between bite and antivenom administration was 10 h (range 5-20 h) in GSS-2 and GSS-3 patients, respectively. The 4 available antidotes were administered with a different frequency. Viper-Venom-Antitoxin® was administered in 61/114 cases (54%), European-Viper-Venom-Antiserum® in 31 (26%), and both ViperaTab® and Viekvin® in 11 cases each (10%). For all antiserum, a first dose of 1 or 2 vials was administered in 43 and 74/114 cases, respectively. A clinical improvement was registered after 6 h in 79/117 patients (69%). Seventeen patients needed a second dose (1 vial) of antivenom: 10/17 Viper-Venom-Antitoxin®, 4/17 European-Viper-Venom-Antiserum®, 2/17 ViperaTab®, and 1/17 Viekvin®. Nine (9/17, 53%) re-treated patients received Viper-Venom-Antitoxin® as first dose. Multivariate analysis identified the use of European-Viper-Venom-Antiserum® and Viekvin® ($p = 0.001$ and 0.041, respectively) compared to Viper- Venom-Antitoxin® as factors increasing the probability of improvement. The length of hospitalization was 5 days (range 4-7 days). Acute adverse reactions were registered in 3 patients (2 mild hypotension and 1 urticaria, all treated with European-Viper-Venom-Antiserum®) and mild serum sickness in 1 patient (treated with Viper-Venom-Antitoxin®). No lethal cases were registered.

CONCLUSIONS: Our experience evidenced that European-Viper-Venom-Antiserum® and Viekvin® were more effective in counteract the venom toxicity of Italian vipers. In general, 2 vials regimen as first dose offered better probability of clinical improvement also in pediatric population.

BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS OF DEMENTIA (BPSD): EVIDENCE ON PHARMACOUTILIZATION FROM A LARGE SAMPLE IN THE REAL-WORLD SETTING

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BACKGROUND: Dementia affects 50 million people worldwide and the most common form is Alzheimer's disease (AD). The 97% of demented patients develops symptoms known as behavioral and psychological symptoms of dementia (BPSD). Treatment of BPSD is a hard challenge, mainly for agitation. Data concerning the diagnosis and the pattern BPSD treatment in these patients are still scarce. This original study aims at testing the following hypotheses: 1) the number of patients receiving prescription of symptomatic drugs against AD, i.e. acetylcholinesterase inhibitors (AChEI) and/or memantine, may approximate the number of demented patients in Italy; 2) adherence to this treatment influences the prescription of drugs for BPSD, i.e. antipsychotics and antidepressants.

METHODS: This retrospective observational study includes a population of 298000 inhabitants, 84235 older than 60 years. The prescribing pattern of antipsychotics and antidepressants has been investigated in patients receiving concurrent prescriptions of anti-dementia drugs for 2 years. The stage of dementia at diagnosis was studied through data from a single center for cognitive disturbances and dementia (CDCD). The study was approved by Calabria Region Ethical Committee n. 31/2017. Data have been extracted from the provincial prescriptions database of Cosenza and the results have been evaluated statistically for difference using $\chi 2$ test for categorical variables considering $p < 0.05$ significant.

RESULTS: according to our results, 859 patients are treated with AChEI and/or memantine and 420 patients (48.89%) are adherent, receiving at least 80% of the recommended medications, and data from CDCD indicate a delay in dementia diagnosis. In fact, 62.14% of patients were referred to CDCD with a MMSE ≤ 20 underscoring a clinical condition of moderate-to-severe dementia. Antipsychotics and antidepressants are frequently used (20.61-20.71% and 42.37-51.43%, respectively) and the 16.43% of patients receive antipsychotics for longer than 6-12 weeks. Adherence did not influence prescription of antipsychotics and antidepressants. In fact, the use of antipsychotics is almost superimposable in the adherent (20.71%) and nonadherent (20.61%) patients and antidepressants are more extensively used

in both populations (nonadherent patients: 42.37%; adherent patients: 51.43%). Patients referred to CDCD receive fewer prescriptions.
CONCLUSIONS: This study, including a wide cohort of community demented patients, shows that: 1) delayed and reduced diagnosis of dementia; the rate of prescription of potentially harmful antipsychotics and antidepressants appears to be high and not impacted by adherence to anti-dementia drugs. These results support the development of strategies to improve the management of BPSD.

EVIDENCE FROM THE REAL-WORLD OF LIMITED ACCESS TO PAIN TREATMENT IN A LARGE SAMPLE OF PATIENTS SUFFERING FROM DEMENTIA

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BACKGROUND: Alzheimer's disease (AD), the most common form dementia, has a remarkable social burden on the global population. In this popolution, data concerned with the pattern of treatment of pain from the real-world settings of community are still very scarce. Chronic pain afflicts 72% of the oldest old (i.e. over 85 years old) patients and 60%–80% of demented patients living in nursing homes. The impaired communication skills of severely demented patients limit their self-report of pain, thus causing a phenomenon of under-detection and under-treatment, often contributing to the development of behavioral and psychological symptoms of dementia (BPSD) like agitation. Here we test whether or not adherence to the treatment with symptomatic drugs against AD, i.e. acetylcholinesterase inhibitors (AChEI) and/or memantine affects the pattern of prescription of analgesics, previously reported to be limited in this fragile population.
METHODS: This retrospective observational study concerns a population of 298000 inhabitants, among whom 84235 subjects older than 60 years, registered in the prescriptions database of the provincial health district of Cosenza. The prescribing pattern of analgesics has been investigated in patients receiving concurrent prescriptions of anti-dementia drugs for a two-year period. The study was approved by Calabria Region Ethical Committee n. 31/2017. Anonymized data have been extracted and results have been evaluated statistically for difference using $\chi 2$ test for categorical variables considering $p < 0.05$ significant.
RESULTS: The data show that 859 patients are treated with AChEI and/or memantine and 420 patients (48.89%) receive at least 80% of the recommended medications, thus resulting

adherent to the treatment. Adherence did not influence prescription of most of the analgesic drugs explored, but use of non-steroidal anti-inflammatory drugs was higher in non-adherent patients. An under-treatment of chronic pain is observed with opioids prescribed in the 4.76% and 12.46% of adherent and nonadherent patients and gabapentin and pregabalin used in the 4.29% and 4.07% of adherent and nonadherent patients, respectively. Moreover, the 0.48% of adherent and the 0.7% of non-adherent patients are treated with lamotrigine and the 6.43% of adherent and the 6.75% of nonadherent patients receive treatment with antidepressants, which can be used for neuropathic pain treatment.

CONCLUSIONS: This two-year period study, including a wide cohort of community demented patients, shows a limited prescription of analgesics that might represent a contributing factor for the development of BPSD in need for further investigation. To the best of our knowledge, this is the first study performed on a very large sample including community patients that has highlighted the prescribing attitude towards drugs for neuropathic pain treatment in patients suffering from dementia. Our data support the development of strategies to improve diagnosis, assessment and management of pain in non communicative patients suffering from dementia, reducing the off label use of unnecessary and harmful psychotropic drugs.

ENTERIC GLIA ACTIVATION IS ASSOCIATED WITH A DECREASE IN NEUROGENESIS AND NEURONAL DENDRITIC SPINES BOTH IN THE ENTERIC AND CENTRAL NERVOUS SYSTEMS, AND DEPRESSIVE AND ANXIETY-LIKE BEHAVIORS IN HFD MICE

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BACKGROUND: Mood and metabolic disorders are closely related, suggesting that a common pathophysiological process underlies these diseases. Indeed, a high-fat diet (HFD) induces impairment in energetic metabolism which is highly associated with the development of depressive-like behaviors and an intestinal "low-grade" inflammation called "leaky gut" syndrome. The enteric nervous system (ENS) is implicated in the maintenance of glycemia by regulating intestinal contractions and afferent nervous message to the hypothalamus that decreases the fed hyperglycemia. During metabolic disorders, intestinal inflammation leads to a dysfunction of ENS neurons, which favors duodenal hyper-contractility and an aberrant nervous message that fails to correctly control the glucose intake. Enteric glia are peripheral neuroglia surrounding the enteric neurons in the ENS that fulfill homeostatic functions in the digestive tract. However, reactive enteric glia mirror the astrocytes' ability to support a neuroinflammatory response and neuronal dysfunctions in certain circumstances. Here, we tested whether a long-term HFD induces (i) an impairment in intestinal permeability and a low-grade inflammatory state in the duodenum, (ii) enteric glia activation and neuropathological changes in the duodenal myenteric plexus of

the ENS; (iii) whether these changes propagate to the CNS, mirroring the neuroinflammatory scenario observed in the ENS, and eliciting (iii) anxiogenic/ depressive-like symptoms in HFD mice; (iv) we also investigated the enteric glia involvement in the HFD-mediated effects.

METHODS: C57Bl/6 male mice were exposed to a standard diet (SD, 6.2% fat) or high-fat diet (HFD, 72% fat) for 20 weeks. Overweight onset and progression were monitored by a weekly body weight assessment and correlated with mucosa histological damage and duodenal expression of tight junctions ZO-1 and occluding at 0, 6, and 20 weeks. The expression of specific markers of glia (GFAP, TLR-4, S100B), neurons (HuC/D, MAP-2), and neurogenesis (BDNF) were investigated in the myenteric plexus, nodose ganglia, and dentate gyrus of the hippocampus by immunohistochemistry analysis at the same times periods. The number of dendritic spines was evaluated in cultured neurons isolated from myenteric plexuses and hippocampi by PDS9.5 immunostaining. To evaluate whether peripheral ablation of glia functions inhibits peripheral and central neuropathological changes in HFD mice, one experimental group under HFD received a daily intraperitoneal (ip) administration of 1 µmol/kg of gliotoxin fluorocitrate (FC) from week 0. Depressive and anxiety behaviors were assessed by the recording of immobility time of mice undergo to tail suspension test and forced swimming test, and quantification of the percentage of time spent in the center and the number of entries in the center during the open field test, respectively.

RESULTS: HFD mice experienced a statistically higher value of gained body weight after 6 weeks and the difference increased along 20 weeks. At weeks 6 and 20, HFD mice showed depressive and anxiety-like behaviors with a significant increase of immobility time and decreased the time spent in the center and the number of entries in the center, in comparison to SD and FC-treated mice. Histopathological analysis revealed a time-dependent HFD-induced mucosal damage which was accompanied by marked infiltration of immune cells in the mucosa and decrease in ZO-1 and occludin expression. Also, an increase in GFAP, S100B, and TLR4 in the myenteric plexuses, nodose ganglia and hippocampi occurred at week 6 and 20. Enteric gliosis was associated with a progressive decrease in BDNF and the number of dendritic spines in myenteric plexus, nodose ganglia, and dentate gyrus, mirroring to the development of anxiogenic/depressive symptoms in HFD-treated mice. Gliotoxin fluorocitrate (FC) abolished these effects both in the ENS and CNS, suggesting that chronic glial activation triggers an impairment in peripheral neurogenesis that subsequently ascends to hippocampal circuits eliciting mood disorders development.

CONCLUSIONS: Our results confirm that a long-term HFD elicits an intestinal inflammatory condition associated with neurobehavioral alterations. Ascending neuropathological signals via the "gut- brain axis" seems to involve enteric glia reaction, which correlates with impaired neurogenesis and decrease in neuronal dendritic spines both in the enteric and central nervous systems.

A PATIENT-CENTERED APPROACH TO PREVENT ADVERSE CLINICAL EVENTS IN PATIENTS UNDERGOING VASCULAR SURGERY

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BACKGROUND: Antiplatelet treatment (AT) with P2Y12 inhibitors and salicylates shows patients' variable response due to, drug-drug interactions (DDI), body mass index (BMI), therapeutic adherence, gender and genetic background. The presence of CYP2C19-*2 and -*3 SNPs, associated to a phenotype of intermediate and poor metabolizers, impair clopidogrel (CLO) efficacy. The CYP2C19-*17 SNP identifies the rapid and ultra-rapid metabolizers. Patients with ABCB13435TT genotype have a higher rate of cardiovascular (CV) events than those with CC genotype, because of an influence on ADP dependent platelet reactivity. Polypharmacy and DDI increased the risk of adverse drug reactions (ADRs). Proton Pump Inhibitors (PPIs), primarily omeprazole and esomeprazole, might attenuated antiplatelet effect when administered with CLO. Female gender was associated with high residual on-treatment platelet reactivity (HTPR) and men are more responsive to aspirin than women in primary CV prevention. BMI impacts strongly on CLO and prasugrel response with higher HTPR incidence and lower bleeding in obese patients. At Clinical Pharmacology Unit of University Hospital of Salerno an observational study is ongoing aiming to associate factors influencing AT with platelet aggregation values and clinical outcomes in patients eligible for vascular surgery.
METHODS: Data of the enrolled patients were recorded in a Case Report Form. Blood samples were collected during the pre-hospitalization and the post-surgery visit. CYP2C19 genotyping was performed by RealTime PCR with allelic discrimination, while ABCB1 SNP by pyrosequencing. Multiplate® analyzer was used to measure platelet function (ADPtest, ASPItest). DDI were identified using Medscape® drug interaction checker.
RESULTS: A total of 138 patients (106 male/32 female, average age of 71.5 years) were enrolled. 55% of them had a carotid stenosis, 32% suffered from occlusive peripheral arterial disease and 13% had an abdominal aortic aneurysm. 28 patients were on AT with CLO, 84 with salicylates, 20 were in dual AT (ASA plus CLO), 3 in ticagrelor, 2 in ticlopidine and 1 in prasugrel. Twenty-one CLO users (15 male and 6 female) showed a residual platelet aggregation (RPA) (ADPtest>470 AU*min); 16/21 were also on PPIs. All the patients showing RPA, except for 1, were carriers of the described SNPs: 7 wt for CYP2C19 but ABCB13435CT, 3 CYP2C19*1/*2 and ABCB13435CT, 3 CYP2C19*1/*2 but ABCB13435CC, 2 wt for CYP2C19 but ABCB13435TT. 5 were CYP2C19*1/*17 (3 were also ABCB13435CC and 2 also ABCB13435TT). Only one subject was wt for both CYP2C19 and ABCB1. 14 patients on salicylates showed RPA (>500 AU*min)

(only one was female). All patients took at least 5 drugs and showed at least 4 DDI classified as serious/monitor closely. Among CLO users with RPA, 9 patients were in the range of normal weight (BMI 18.5-24.9), 9 were pre-obese (BMI 25-29.9) and 3 were in first class obesity. Among salicylates users with RPA, 5 were normal weight subjects, 7 were pre-obese and 2 were in first class obesity.

CONCLUSIONS: It seems appropriate to carefully monitor patients on AT with particular attention for the high risk subjects. To investigate the occurrence of any ADRs and adverse clinical outcomes (thrombosis, bleeding, hospitalization for CV and extra-CV causes and death) and to take into account all factors influencing AT are the only way to assure a personalized patients' global health.

CASE CONTROL STUDY UNDERLINES DIFFERENCES IN AZATHIOPRINE METABOLISM IN EARLY ONSET PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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BACKGROUND: Inflammatory bowel disease (IBD) is a chronic inflammation of the intestinal tract, that comprises two disorders, Crohn's disease (CD) and ulcerative colitis (UC), that may develop at relatively young ages. In early-onset (EO) IBD patients (age < 6 years old) generally the disease is more aggressive. At present, a curative therapy for IBD does not exist, but many drugs, such as the immunosuppressant azathioprine (AZA), are employed to induce and maintain disease remission.The response to the pro-drug AZA is highly variable and can be influenced by various factors such as the activity of the enzyme thiopurine methyltransferase (TPMT). Recently, age has also been demonstrated to affect the efficacy of thiopurines in pediatric IBD patients, with effects particularly strong in EO patients, who may need increased AZA doses. In this 1 : 3 case-control study we have analyzed the differences in AZA metabolites levels (TGN), AZA dose, TPMT activity and expression between EO and non-EO (NEO) pediatric IBD patients in order to identify pharmacokinetics differences between the two groups.
METHODS: 24 EO (age, 6 years, cases) and 72 NEO (aged>12 and<18 years, controls) IBD patients under AZA therapy for at least 3 months were enrolled. TGN and TPMT activity were assessed in patients' erythrocytes by high-performance liquid chromatography with UV detection method. TPMT gene expression analysis was performed in whole blood using TaqMan Gene Expression Assays in a small subset of patients composed by 11 EO and 16 NEO IBD patients. Statistical analyses were conducted by Wilcoxon's test.
RESULTS: 24 EO (mean age: 4.3 years, 14 females, 13 UC, 5 CD and 6 undetermined colitis) and 72 NEO (average age: 14.7 years, 33 females, 37 UC, 35 CD) pediatric IBD patients were enrolled. TGN levels were different between the two groups of patients (EO median: 267.5 pmol/8x108 erythrocytes, IQR: 155,

NEO median: 345.5 pmol/8x108 erythrocytes IQR: 193, p: 0.01), together with the ratio between TGN and dose in mg/kg (EO median: 97.75 IQR: 108 , NEO median: 169.4 IQR: 78, p: 0.01) while only a trend was observed in AZA pro-kg dose (EO median: 2.29 mg IQR: 0.83, NEO median: 2.03 mg IQR: 0.53 ,p: 0.06). Preliminary results showed no differences in TPMT expression (2-[dCt(EO – dCt(NEO)] = 0.88 , p: 0.7). Analysis on TPMT activity is still ongoing.

CONCLUSIONS: EO patients show lower levels of both TGN and TGN normalized to the pro-kg AZA dose, while no difference emerges from the other parameters analyzed. These results show a decreased bioavailability of AZA in EO patients, possibly linked to increased biotransformation. We will further analyze TPMT promoter methylation in order to clarify if an epigenetic regulation could explain the age-dependent differences in AZA metabolism between EO and NEO IBD patients.

HISTAMINE H3 RECEPTOR (H3R) LIGANDS-NITRIC OXIDE (NO) DONOR HYBRID COMPOUNDS AS A NEW THERAPEUTIC STRATEGY FOR THE TREATMENT OF GLAUCOMA

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BACKGROUND: Glaucoma is the leading cause of irreversible blindness worldwide, affecting 64.3 million people. The elevated intraocular pressure (IOP) is considered the prime factor responsible for the glaucomatous optic neuropathy involving death of retinal ganglion cells (RGCs) and their axons.
Several studies demonstrate that nitric oxide (NO) is involved in vasodilation, IOP homeostasis, modulation of ocular blood flow and apoptosis of RGCs. Further evidence shows that dysregulation in the production of NO at the endothelial level increases IOP by promoting the development or progression of glaucoma2. Histaminergic system has been implicated in the regulation of IOP and recent studies demonstrate that Histamine H3 receptor (H3R) antagonists are effective in reducing ocular hypertension and oxidative stress, and in preventing RGCs loss3. Based on this evidence, our project evaluates the capability of newly synthesized H3R ligands-NO donor hybrid compounds to reduce IOP and to prevent RGCs loss in a transient and a stable ocular hypertensive (OHT) model of glaucoma.
METHODS: The transient OHT model was induced by injection of 50 µL of 5% hypertonic saline into the vitreous and the stable one was induced by injecting 100 µL 0.1% carbomer in the anterior chamber. IOP measurements were performed with a Pneumotonometer prior to saline or carbomer injection (baseline), 30, 60, 120 and 240 minutes after transient OHT induction and, every day for 12 days, in stable OHT model before drug dosing. We measured cGMP with a specific kit and NO release was measured by Griess reaction .
RESULTS: Hybrid compound ST-1989 has proven to be effective in reducing IOP with a long-lasting effect in a transient model of ocular hypertension. These compounds increased

the availability of nitric oxide and stimulated GC increasing cGMP levels in aqueous humor. **CONCLUSIONS:** In conclusion these hybrid compounds are promising therapeutic strategy for the management of glaucoma, even if several aspects need to be deeply investigated.

DEVELOPMENT OF SENOLYTIC THERAPIES FOR TREATMENT OF AGEING- RELATED DISORDERS

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Cellular senescence is a phenomenon in which cells enter into an irreversible cell cycle arrest. It can be triggered by various stimuli such as shortening of telomeres, DNA damage, radiation, chemotherapeutic agents or loss of tumour-suppressor genes (PTEN). With ageing, senescent cells accumulate in different tissues and can cause a damage due to their secretome components known as senescence- associated secretory phenotype (SASP). In previous studies it was shown that selective clearance of senescent cells (senolytic therapy) can delay several age-related disorders and rejuvenate damaged tissues. We aimed to discover novel senolytic agents via high throughput screening of a library of 2500 natural compounds. The screening was performed by an automated high content imaging system (operetta) using engineered human fibroblasts (IMR-90) expressing green fluorescent protein (GFP) under the control of a senescence induced promoter. After two screening phases, we selected 4 compounds showing promising senolytic effect. By testing their efficacy at different concentrations on different senescent cell models, we selected one triterpenoid molecule (named NP12), which showed the strongest senolytic efficacy. We are currently performing in vitro studies, with the goal of understanding NP12 mechanism of action.

THE BLOOD-BRAIN BARRIER AS A NEW PHARMACOLOGICAL TARGET FOR THE S1P RECEPTOR 1 MODULATOR SIPONIMOD

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BACKGROUND: Sphingosine 1 phosphate (S1P), is a bioactive sphingolipid interacting with a class of 5 metabotropic receptors (S1P1-S1P5), largely distributed in several tissues. S1P, acting through S1P1, drives the egress of mature leukocytes from lymph nodes and functional S1P1 antagonists, such as fingolimod, are used in the treatment of multiple sclerosis (MS) reducing the rate of relapses. Fingolimod acts on 4 out of the 5 S1P receptors and more selective compounds are at present under study. Siponimod (BAF-132) is selective for S1P1 and S1P5 and has a shorter half- life, features that make it a good candidate for clinical practice. Similar to its analog fingolimod, siponimod, through S1P receptors, acts on endothelial cells and astrocytes, the two main principal cellular components of the blood-brain barrier (BBB), that plays an essential role in protecting the CNS and is affected in pathological conditions, like MS. Here, we investigated whether siponimod acting through S1P receptors modifies BBB properties.

METHODS: We used an in vitro BBB model, where human-derived cell lines, endothelial cells (TY-10) and astrocytes (hAST), were co-cultured. Cultures were exposed to an inflammatory insult (TNFα, 10UI + IFNγ, 5UI, T&I) in the presence of siponimod (BAF-132; 100 nM) and the S1P1 antagonist NIBR-0213 (1 μM). To investigate BBB properties, the tightness of endothelial junctions was tested by measuring both transendothelial electrical resistance (TEER) and barrier permeability to a dye-conjugated sugar (FITC-dextran). Further, the expression of the junctional proteins claudin-5 and Zo-1 was evaluated using western blot analysis and immunocytochemistry. Gene expression of inflammatory cytokines and chemokines was evaluated on astrocytes.

RESULTS: BBB exposure to inflammatory cytokines caused impairment of barrier properties, with increased permeability to dextran and reduced values of TEER. The expression of both claudin-5 and Zo-1 was reduced with a prevalent distribution in the cytosolic compartment. When cultures were pre-exposed to BAF-132 and challenged with T&I, the reduction of TEER values was partially prevented as well as the increase of dextran permeability. In addition, expression of Zo-1 and claudin-5 was preserved and both junctional proteins were visible at the cell boundaries. These effects were all detectable after a long-term exposure to BAF-132 (48 h), but not consistent at earlier time points (8-24 h). They were mediated by S1P1 as they were prevented by the S1P1 receptor antagonist NIBR-0213 and appeared to involve the PI3k/Akt pathway. On astrocytes, treatment with BAF-132 reduced the expression of inflammatory cytokines and chemokines induced by T&I, again an effect counteracted by NIBR-0213.

CONCLUSIONS: BAF-132 modulates BBB properties reverting its impaired function during inflammation. This suggests an additional effect for this compound in the treatment of MS.

A GENOTYPING/PHENOTYPING APPROACH WITH CAREFUL CLINICAL MONITORING TO MANAGE THE FLUOROPYRIMIDINES-BASED THERAPY: SYSTEMATIC REVIEW OF THE LITERATURE

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BACKGROUND: Fluoropyrimidines (FP) are used to treat solid tumors.The rate-limiting step of FP catabolism is catalysed by dihydropyrimidine dehydrogenase (DPD), encoded by DPYD gene.Patients carrying certain polymorphisms of DPYD gene have an increased risk of severe FP-related toxicity. In July 2020, the European Medicines Agency (EMA) stated that the FP pharmacogenetics(DPYD-PGx) including four DPYD polymorphisms (DPYD*2A, DPYD*13, c.2846A>T, c.1129-5923C>G or 1236G>A [HapB3]) is recommended before starting treatment with FP.Besides genetic variants, other factors are involved in the response variability to FP.A systematic review was performed to examine the studies investigating DPYD genotyping combined with phenotyping methods and/or clinical monitoring as predictive factors for FP-related toxicity.

METHODS: The systematic review was conducted using PubMed, Scopus and Cochrane databases until 3 July 2020.Only the clinical studies enrolling patients treated with FP and analyzing at least one of the four recommended SNPs of DPYD-PGx, have been included in the analysis.The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) was applied.

RESULTS: A total of 1932 studies were screened from the databases and after considering inclusion and exclusion criteria,22 articles were included in the analysis (Figure 1).(Table 1)Among the eleven studies with a DPYD-PGx/clinical monitoring combination,Lee et al., by testing 2886 patients, reported a significant association between DPYD*2A and DPYD c.2846A>T and grade ≥3 FP ADR[26].Among the three studies that performed a combined DPYD-PGx/phenotyping approach, Gentile et al.,by measuring the 5-FUDR (degradation rate) in peripheral blood mononuclear cells (PBMCs) utilising HPLC-MS/MS, found a significant correlation between several polymorphisms, including DPYD*2A and DPYD c.2846A>T, and this phenotyping marker[27].Eight studies have combined DPYD-PGx with phenotyping methods and clinical monitoring.Notably, Henricks et al. analysed all the four recommended DPYD SNPs and performed two phenotyping tests by measuring the UH2/U ratio in PBMCs and plasmatic 5-FU pharmacokinetics (PK) by UHPLC-MS/MS.The patients carrying DPYD c.1236G>A and DPYD c.2846A>T were more likely to manifest FP-related severe toxicity. The mean DPD enzyme activity was significantly lower in patients bearing these two genetic variants, as well as DPYD*2A, as compared to other patients.Only one patient carrying DPYD*13 showed a 60% DPD activity reduc-

tion.This patient was treated with a reduced 5-FU dosage for three treatment cycles, and no severe ADR occurred[35].
CONCLUSIONS: A combined genotyping/phenotyping approach, together with careful clinical monitoring is the best method to optimize the therapy with the fluoropyrimidines and minimize their toxicity. However, there is a need for large well-designed clinical trials to assess the utility of other phenotyping methods as well as genetic factors to complement the DPYD-PGx.

DEVELOPMENT OF A NOVEL NANOPLATFORM FOR THERANOSTIC APPLICATIONS IN HEPATOCELLULAR CARCINOMA (HCC)

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BACKGROUND: Hepatocellular carcinoma (HCC) is the most common type of liver cancer and it accounts for almost 90% of all cases. The staging and prognostic assessment of HCC is crucial for patients' management and HCC is divided into five stages ranging from very early to terminal, each of them related to different treatment options (e.g. ablation, resection, transplantation, chemoembolization, systemic therapy). To date, HCC early diagnosis is possible through radiological and biopsy approaches. Despite establishing screenings, a large number of patients is diagnosed outside surveillance showing symptoms, which often relate to an advanced disease stage and dramatically limited options (1). Discovering new markers for precision medicine and theranostic tools is needed to combine diagnosis and effective treatment of HCC patients. Glypican-3 (GPC-3) belongs to the glypican family of heparan-sulphate proteoglycans. While not expressed on healthy liver, its expression on HCC cells is high and related to tumor aggressiveness (2). Nanomedicine is emerged and nanoparticles (NPs) are used to build multifunctional nanosystems with therapeutic and diagnostic abilities in several cancers including HCC. By taking advantage of abnormal expression of GPC3 and using antibodies (Abs), active cancer targeting could be achieved for the selective accumulation of NPs in the tumor site (3). The development of a theranostic nanoplatform composed of gold NPs and capable of: i) active cancer targeting using anti-GPC3 Abs, ii) therapy through light-based techniques (e.g. photo-thermal therapy), iii) diagnosis by means of imaging (e.g. magnetic resonance).
METHODS: GPC3 expression and localization have been evaluated through western blot (WB), flow cytometry (FC) and immunofluorescence (IF) on different HCC cell line models found in literature, such as HUH7, HEPG2, HEP3B2 (as GPC3-positive), TIB75 and MDA-MB231 (as GPC3-negative).
RESULTS: Several antibodies against GPC3 (e.g. GC33, 1G12, 9C2) have been tested to determine GPC3 protein region more suitable to be recognized with high specificity and affinity. These experiments were also useful for establishing the fittest model to be used for further *in vitro* and *in vivo* studies. We confirmed high GPC3 protein expression (~ 96% positivity in FC) in HCC cell line models (e.g. HEP3B2, HUH7) and its virtual absence in negative controls (TIB75). In WB (fig. A), FC (fig. B) and IF (fig. C) experiments, the GC33 antibody showed the greatest ability to discrimi-

nate GPC3 protein among other glypican family members, making it our antibody of choice.
CONCLUSIONS: In line with literature and our results, we have identified GPC3 as a specific HCC antigen showing high expression in HCC cells and very low expression in negative controls. GC33 is the selected antibody for further studies of our project.

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IN VITRO AND IN VIVO PHARMACOLOGICAL CHARACTERIZATION OF NOVEL NEUROPEPTIDE S RECEPTOR ANTAGONIST WITH OXAZOLO[3,4-A] PYRAZINE STRUCTURE

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BACKGROUND: Neuropeptide S (NPS) is the endogenous ligand of a previously orphan G protein-coupled receptor (GPCR), now named NPSR. NPS has been identified in 2002 by reverse pharmacology approach. NPSR can signal via both Gq and Gs pathways to increase cellular excitability. In vivo, NPS has been shown to control several biological functions in rodents including stress, anxiety, social behaviour, locomotor activity, wakefulness, food intake and gastrointestinal functions, memory processes, pain, and drug abuse. As far as the therapeutic potential of selective NPSR ligands is concerned, NPSR agonists may be useful as innovative anxiolytics with no sedative effects, analgesics and memory enhancers. On the other hand, NPSR antagonists maybe useful to treat drug abuse disorders. Several NPSR antagonists with potent *in vitro* activity have been developed in the last years and few compounds are currently in use as pharmacological tools. Among these, SHA 68 is largely recognized as the most representative member of this class. The aim of this study was the *in vitro* and *in vivo* pharmacological characterization of a series of novel NPSR antagonists, which are analogues of SHA 68.
METHODS: The in vitro activity of the new compounds has been investigated in HEK293 cells stably transfected with the murine NPSR (HEK293mNPSR), using the calcium mobilization assay. The best compounds identified from the in vitro screening have been tested in vivo, in mice, in the locomotor activity test.
RESULTS: In the calcium mobilization assay NPS increased intracellular calcium levels with pEC50 and Emax values of 8.95 and 287 ± 26 % over the basal values, respectively. Inhibition

response curves to SHA 68 (0.1 nM – 10 μM), used as internal reference, were performed against the stimulatory effect of 10 nM NPS. SHA 68 concentration-dependently inhibited 10 nM NPS stimulatory effects with a pKB value of 8.12. 19 SHA 68 analogues have been tested under the same experimental conditions. Among these, DN10 and DN21 have been identified as the most interesting compounds, with pKB value of 7.38 and 7.82, respectively. These molecules were selected for in vivo testing. In vivo, NPS (0.1 nmol, i.c.v.) stimulated mouse locomotor activity. SHA 68 (50 mg/kg i.p.) only partially counteracted NPS-induced stimulant effects. DN10 (10 mg/kg) and DN21 (50 mg/kg) completely blocked NPS-induced stimulant effects. The in vivo action of DN21 reflects the in vitro potency of the compound that was very similar to that of the reference tool SHA 68. In contrast, DN10 displayed 5-fold higher in vivo potency than SHA 68, despite a 3-fold lower in vitro potency.

CONCLUSIONS: In this study, two potent and in vivo-effective NPSR antagonists have been identified. These may open new perspectives for addressing drug addiction. Moreover, they can be important research tools to investigate the neurobiology of the NPS / NPSR system.

ER-CHAPERONS IMPACT ON PROTEIN QUALITY CONTROL IN AORTA OF LDL-R KO MICE

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BACKGROUND: Vascular wall proteins undergo several post-translation modifications, including N glycosylation. Our aim was to investigate changes in N-glycosylation pathways during atherosclerotic plaque development in animal models.

METHODS: Shotgun proteomics was performed in aorta from LDL-R KO mice, fed in chow or WTD diet using orbitrap Fusion™ Tribrid ™ Mass Spectrometer followed by protein inference, label free quantification and pathway enrichment analysis.

RESULTS: Apoptosis, fibrosis, and ROS production were upregulated (z-score 2,9 to 2.2) while cholesterol efflux, phagocytosis and ATP production resulted decreased (z-score -1.6 to -2.7) in the aorta of WTD fed mice compared to chow. When the analysis was focused on enzymes for N-glycosylation cascade, OST complex, which controls glycans transfer from Dolicol-P-P to asparagine in ER, was up-regulated ($p=0.02$), glucosidases (Ganab, Prkcsh) which favor proper protein folding in concert with the lectin chaperon Calnexin/Calreticulin and ERp57 were also up-regulated. In parallel ERGIC-53 that operates the transport of glycoproteins from ER to Golgi was significantly up-regulated ($p<0.01$). These data suggest an increased production of glycosylated proteins during atherosclerosis coupled to a decreased protein response in the plaque. Indeed, the abundance and side-specific N-glycosylation of integrin β-1, laminin subunit γ -1, integrin α-8 were reduced in the plaque of WTD fed mice.

CONCLUSIONS: Our data suggest that altered protein glycosylation takes place in the aorta of WTD fed LDL-R KO mice. This can further affect the synthesis of atheroprotective glycoproteins.

EFFECTS OF N-ACETYLCYSTEINE ON SPONTANEOUS NON-CONVULSIVE EPILEPSY AND BEHAVIOUR, IN THE WAG/RIJ INBRED STRAIN OF RATS

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BACKGROUND: Increasing lines of evidence suggest a key role of oxidative stress in epilepsy N-acetylcysteine (NAC) is an antioxidant and a free radical-scavenging agent that acts as a precursor for glutathione synthesis as well as a stimulator of the cytosolic enzymes involved in glutathione regeneration. NAC has shown effectiveness against experimental seizures and chronic treatment with NAC has shown higher efficacy in preventing seizure than acute treatment. NAC appears to be also promising in the treatment of several psychiatric conditions. Based on this background, we studied the potential effects of a chronic oral treatment (30 day) with NAC (500 mg/kg/day) on absence seizures (electroencephalographic [EEG] recordings), depressive-like behavior (forced swimming test [FST]) and working memory (Novel object recognition test [NORT]), in WAG/Rij rats, a well-established genetic model of absence epilepsy, epileptogenesis, and neuropsychiatric comorbidity.

METHODS: All these experiments were run on ~6-month-old WAG/Rij rats with already established seizures. In details, treated rats received the drug orally for 30 days. At the end of treatment, EEG recordings and the above reported behavioral tests were performed in different groups of treated and untreated rats.

RESULTS: Our results have shown that in WAG/Rij rats, chronic NAC treatment significantly increased spike-wave discharges parameters (both numbers SWDs and duration of SWDs; $p = 0.03$) aggravating absence epilepsy while ameliorated depressive like-behavior in FST ($p = 0.037$) and the cognitive performance in NORT ($p = 0.023$). Data obtained from experiment were analyzed and compared by Student's t test.

CONCLUSIONS: These findings suggest that, although NAC improves depressive-like behavior and memory deficits, the use of NAC should be limited in patients with absence seizures due to the risk of aggravation of this epileptic condition.

ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN ADRENOCORTICAL CARCINOMA: DEFINING A ROLE ON CELL VIABILITY IN THE EXPERIMENTAL MODEL OF NCI-H295R AND MUC-1 CELL LINES

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BACKGROUND: Progesterone (Pg) and Estrogen (E) receptors are expressed in both normal and neoplastic adrenal cortex. During the neoplastic degeneration, an unpredictable rearrangement of their expression is observed, in particular for E receptors (ER); however, the role of both Pg receptors (PgR) and ER is not yet fully understood [1]. Previous results obtained by our research group demonstrated that Pg exerts a cytotoxic effect on AdrenoCortical Carcinoma (ACC) cell viability [2]; while the selective ER modulator tamoxifen inhibits ACC cells proliferation [3], although the role of each ER subtypes remains to be elucidated. Here, we performed an immunohistochemistry (IHC) analysis in a set of paraffine-embedded ACC tissues, to evaluate the amount of ER and PgR expression, then we characterized the cytotoxic effect of tamoxifen, Pg and mitotane, alone or in combination in the ACC experimental model of NCI-H295R and MUC-1 cell lines. Mitotane, indeed, is the only approved drug for the first-line therapy of ACC.
METHODS: Thirty-five paraffin embedded tumor samples belonging to ACC diagnosed patients were analyzed for ER and PgR expression by IHC, while their expression on ACC cell lines, namely NCI-H295R and MUC-1 cells was studied by qRT-PCR. Combination experiments were performed in ACC cell lines to evaluate the interaction of tamoxifen with mitotane or Pg on cell viability, according to the Chou and Talalay method. Cell viability was evaluated by MTT assay, while cell proliferation rate was assessed by direct cell counting.
RESULTS: IHC detected the absence or very weak expression of ER in ACC samples, while PgR proteins were expressed, although with a variability within the different samples. The same trend of ER and PgR expression at mRNA was observed in ACC cell lines. Tamoxifen induced a concentration-dependent reduction of NCI-H295R cell viability, with an IC50 that was 5.4 μM (95%CI: 5.1-5.7 μM). Mitotane and progesterone exerted a as well a concentration-dependent cytotoxicity in NCI-H295R cells with an IC50 that was, respectively, 5.15 μM (95% CI: 4.66-5.7 μM) and 25.6 μM (95%CI: 23.1-28.4 μM). MUC-1 cells, belonging from a heavily-treated patient, displayed a lower sen-

sitivity to the cytotoxic effect of each drug studied. Cytotoxicity was increased in an additive/synergic manner when cells were treated with a combination of tamoxifen and Pg or mitotane.
CONCLUSIONS: Tamoxifen and Pg exerted an additive/synergic marked cytotoxic effect in NCI-H295R cell line, suggesting that ACC could be considered as a steroid hormone-sensitive cancer, stratified on the intensity of ER/PgR expression. Evaluation of ER and PgR expression in ACC tissue samples could thus become a routine analysis in the histopathological assessment of ACC, with the aim to customize the therapy in accordance with patient-specific markers, in light of the personalized medicine era.

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EVALUATING THE THERAPEUTIC POTENTIAL OF ACETAZOLAMIDE IN THE RESTORATION OF ION CHANNEL FUNCTION IN SKELETAL MUSCLE OF AMYOTROPHIC LATERAL SCLEROSIS RODENT MODEL

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease. The causes are not fully understood and the mechanisms involved in motor neuron degeneration are multi-factorial and complex. The discovery of the pathological mechanisms leading to the disease is essential for therapeutic development. Skeletal muscle is severely compromised and its function was investigated in SOD1 G93A and MLC/SOD1 G93A murine models of ALS (Dobrowolny et al. J Cell Biol. 168, 193, 2005), which express the mutated SOD1 ubiquitously and selectively in skeletal muscle, respectively. We found a modification of ion channel function in both models. Indeed, a reduction of ClC-1 chloride channel activity, the typical muscle channel important for the repolarization of sarcolemma, was found in both animal models together with an abnormal hyperexcitability (Camerino et al., Sci Rep. 9, 3185, 2019). However, a restoration of the resting chloride conductance (gCl), sustained by the ClC-1 channel, was observed when acetazolamide (ACTZ) was applied in vitro in skeletal muscle of MLC/SOD1 G93A mice. Acetazolamide, a inhibitor of carbonic anhydrase, was already found to be useful for membrane excitability disorders. The mechanism of action of this drug may include the activation of the skeletal muscle ClC-1 (Desaphy et al. Exp. Neurol. 248, 530, 2013) or of the Ca2+-activated-K+ (BK) channels (Tricarico et al. Proc Natl Acad Sci USA 103, 1118, 2006). Based on these results we performed an in vivo treatment in a new generated animal model of ALS, the SOD1 G93A Knock-In (KI) in which the endogenous SOD1 has been substituted with the mutated one.
METHODS: Mice were divided into three groups: Wild-type (WT), SOD1 G93A KI injected with the vehicle (sterile water) and SOD1

G93A KI injected with acetazolamide 5mg/Kg (i.p.). Electrophysiological analysis in current clamp mode was applied to measure the resting chloride conductance (gCl) and action potentials parameters from single myofibers using two intracellular microelectrodes.
RESULTS: The functional characterization showed the modification of the membrane-stabilizing gCl leading to hyperexcitability in SOD1 G93A KI mice. A significant reduction of gCl (27% reduction) was found in the SOD1 G93A KI model with respect to WT. The excitability parameters were accordingly modified, since the threshold current needed to obtain the first action potential was reduced and the maximum number of spikes was incresed. Acetazolamide treatment was able to completely restore the gCl toward the WT value. Also the excitability parameters were ameliorated. Indeed, the threshold current and the maximum number of spikes were shifted toward the WT value.
CONCLUSIONS: These results show that ClC-1 channel is an important parameter involved in ALS pathogenesis and can be a pharmacological target. At this aim, acetazolamide deserves to be better investigated as a promising, prompt-to-use drug, to possibly ameliorate muscle function in ALS.

ALTERED NEUROMETABOLIC MECHANISMS IN A MUSCLE-TO-BRAIN CROSSTALK IN AN EXPERIMENTAL MODEL OF ANOREXIA NERVOSA

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INTRODUCTION: Anorexia nervosa (AN) is a serious mental illness characterized by high morbidity and mortality that prevalently affects adolescent females over males. The main symptoms are intense physical exercise combined to strict measures to reduce caloric intake, a distorted perception of body image and a constant fear of gaining weight. AN begins with a restrictive diet to lose weight and progresses towards an out-of-control spiral in which the complete control over the food intake and the body shape became extremely rewarding further reinforcing the dieting behavior. AN is a multifactorial disorder with unknown etiology, indeed all the current pharmacological treatments are only symptomatic.
Using the activity-based anorexia (ABA) rat model, the major aim of our work was to evaluate the impact of the combination of intense physical exercise and caloric restriction, on metabolic processes in the muscle and how this metabolic switch may affect brain functionality focusing our attention on the neurotrophin BDNF.
METHODS: To this end, female adolescent Sprague-Dawley rats at postnatal day (P) 35 were individually housed and divided in four groups: controls (CTRL, food ad libitum–sedentary), FR (food restricted–sedentary), EXE (food ad libitum-exercise) and ABA (food restricted-exercise). On P38, the ABA group had free access to running wheels and access to food (2h/day) till P42, corresponding to the acute phase of the pathology. On P42 and on P49, after seven days of weight recovery, animals were sacrificed and trunk blood, soleus muscle and hippocampus (Hip) were collected. Total RNA and proteins were extracted and analyzed via Real- Time PCR and western blots, respectively.
RESULTS: After three days of AN induction, ABA rats lost more weight than FR rats. EXE group maintained a stable activity on the

wheel, on the contrary physical activity of ABA rats constantly increased over days. In the acute phase of the pathology ABA rats showed a marked reduction of triglycerides and cholesterol plasma levels, while the levels were restored during the recovery phase. At the molecular level, the altered expression of metabolic markers (FASN, CTP1 and Glut4), observed in the acute phase of the pathology in the soleus of ABA rats, were restored after recovery of body weight. Despite these data suggest that the metabolic state of the muscle is restored with body weight recovery, we observed increased levels of PGC1a and FNDC5 protein expression, that may, in turn, via a muscle-to-brain crosstalk alter BDNF and its signaling in Hip.

CONCLUSIONS: These data suggest that the combination between hyperactivity and reduced food intake may determine a series of events that can alter metabolic homeostasis and muscle to brain crosstalk even when body weight is restored. This maladaptive plasticity could represent a signal of altered processing of food reward, and a vulnerability trait for relapse.

AMPHETAMINE SELF-ADMINISTRATION REORGANIZES THE GLUTAMATE SYNAPSE IN THE NUCLEUS ACCUMBENS: THE ROLE OF SEROTONIN

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BACKGROUND: Amphetamine (AMPH) abuse represents a severe health issue; despite that, little is known about the molecular mechanisms that underlie the transition from hedonic to compulsive use. Previous studies have shown that the serotoninergic system is involved in this transition. In particular, the lack of the serotonin transporter (SERT) is linked to an increased susceptibility and enhanced motivation to take drugs, as we already demonstrated for cocaine. Thus, the major aim of our work was to evaluate the influence of serotonin in the transition to a compulsive AMPH intake and its impact on specific determinants of the glutamatergic synapse in the nucleus accumbens, a brain region involved in the reward circuit.

METHODS: Adult male rats lacking serotonin transporter (SERT-/-) and wild-type animals (SERT+/+) underwent 19 days of amphetamine self-administration. Animals were exposed daily to a Short Access (ShA, 1 h/day), or a Long Access (LgA, 6 h/day) protocol. Then, 24 hours after last amphetamine-self administration, rats were sacrificed, and brains were collected and frozen. Core and shell subregions of nucleus accumbens were collected through punches. Protein expression of glutamate determinants was measured via western blot in the whole homogenate.

RESULTS: From a behavioural point of view, we found that SERT-/- rats showed a significant increase in amphetamine infusions compared to the SERT+/+ animals in the LgA, but not in ShA, condition. In the nucleus accumbens, we found an altered homeostatic dysregulation of glutamatergic neurotransmission in rats that underwent LgA AMPH self-administration, depending on the NAc subregion analysed (cNAc

vs sNAc). In particular, in the cNAc of SERT-/-- LgA animals we found an overexpression of the main subunits of NMDA and AMPA receptors that was not accompanied by an enhancement of the respective scaffolding proteins, presumably leading to unstable receptors. In the sNAc of SERT-/--LgA animals the increase in GluN1 expression was not accompanied by an increase in the accessory subunits GluN2A and GluN2B and of its anchoring protein SAP102. Moreover, both GluA1 and SAP97 expression was significantly increased following LgA drug exposure, suggesting the formation of GluA2-lacking AMPARs, a mechanism reported to drive addiction.

CONCLUSIONS: In conclusion, our findings show that the deletion of SERT alters amphetamine intake motivation that could be mediated by changes in the homeostasis of glutamatergic synapse in the cNAc. The perturbation of serotonin activity may, via dysregulation of glutamate homeostasis in these NAc regions, contribute to drive the transition from goal- directed drug intake toward addictive states.

ACKNOWLEDGEMENTS: Sponsored by Dipartimento delle Politiche Antidroga, Presidenza del Consiglio dei Ministri, Progetto STAND-UP (ERANID).

COMPARISON OF HALLUCINOGENS DOB, 2C-B AND 25B-NBOME ON SENSORIMOTOR AND PPI RESPONSES IN MICE

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BACKGROUND: In the illegal European market of New Psychoactive Substances, hallucinogens are always present despite their use prevalence levels remain low. The hallucinogenic phenethylamines DOB, 2C-B and 25B-NBOMe (derivatives of mescaline) are widely popular among recreational drug users, especially when they attend "rave" and psychonaut experiences. DOB was the first synthetized and largely utilized by consumers. Subsequently, to circumvent the laws and put more potent hallucinogens in the market, the 2C-X and their more powerful derivatives NBOMes were also synthesized. These compounds derive from the predecessor class 2C-X, in which a N-methoxybenzyl substitution has been performed, which has increased their power by about 100 times. Psychonaut users reported that DOB, 2C-B and 25B-NBOMe induce euphoria, well-being, change of perception and light stimulating properties and hallucinations. All these compounds act mainly as agonists at 5HT2A receptors. The purpose of this study was to compare in vivo effects induced by administration of DOB, 2C-B and 25B-NBOMe in mice by assessing sensorimotor and sensory gating alterations.

METHODS: Adult male ICR mice were used for this study. DOB, 2C-B and 25B-NBOMe (0.01-30 mg/ kg) were administered by intraperito-

neal injection at a volume of 4 µl/g. The sensorimotor (visual, acoustic and tactile) responses were video recorded and analyzed off-line by different trained operator. All experiments were performed between 8.30 AM and 2.00 PM. Visual responses were assessed by two different tests investigating whether the mouse was able to capture visual information either when was stationary (visual object response) or when moving (visual placing response). Tactile responses were verified by touching whiskers, cornea and ear pinna of the mouse (right and left) with a thin hypodermic needle. Acoustic response measured the reflex of the mouse in response to four acoustic stimuli of different intensity and frequency: a snap of the fingers (four snaps repeated in 1.5 sec), a sharp click produced by a metal instrument (four clicks repeated in 1.5 sec), an acute sound produced by an audiometer (frequency: 5.0-5.1 kHz) and a severe sound produced by an audiometer (frequency: 125-150 Hz). Lastly, mice were tested on pre-pulse inhibition test (PPI) in startle chambers consisting of a sound-attenuated, lighted and ventilated enclosure holding a transparent non-restrictive cage (90 x 45 x 50 mm). Acoustic startle test sessions consisted of startle trials (pulse-alone) and pre-pulse trials (pre-pulse + pulse). Prepulse input consisted of three different signal of intensity (68, 75 and 85 dB). The amount of PPI was expressed as the percentage decrease in the amplitude of the startle reactivity caused by the presentation of the pre-pulse (% PPI).

RESULTS: Systemic administration of DOB, 25B-NBOMe and 2C-B induced a dose-dependent decrease of all sensory responses (visual, tactile and acoustic) in mice. Comparison of the maximal effect of the substances revealed that the effect of 25B-NBOMe was stronger than those induced by DOB and 2C-B at both visual placing and object tests. Instead, at tactile and acoustic tests, comparison of the maximal effect of DOB, 25B-NBOMe and 2C-B was minimal. Lastly, administration of DOB, and 25B-NBOMe at 10 mg/kg reduced startle amplitude in mice both at 15 (25B-NBOMe) and 120 min (DOB and 25B-NBOMe) respectively, while 2C-B did not reduce startle amplitude. All compounds inhibited the PPI in mice. DOB (at 68, 75 and 85 dB) and 2C-B (at 75 dB) at 10 mg/kg inhibited the PPI after 15 min from injection. DOB also impaired PPI after 120 min (at 75 and 85 dB) while 2-CB was ineffective. 25B-NBOMe was the most powerful compound and inhibited PPI in mice at lower dose (0.1 mg/kg) both at 15 min (only at 85 dB) and at 120 min (at 68 and 75 dB).

CONCLUSIONS: The results obtained in this study demonstrate that all three phenethylamine tested (DOB, 2C-B and 25B-NBOMe) caused significant impairment of sensorimotor responses (visual and acoustic, slightly on tactile responses), and sensory gating alterations. In particular, the 25B-NBOMe was the most powerful compound in impairing visual sensorimotor responses and acoustic sensory elaboration (startle/PPI impairment). These data highlight the dangerousness of these phenethylamines, in particular their impact on driving skills (driving under the influence of drugs) and/or workers who are involved in jobs with risky tasks.

PAEDIATRIC DRUG REPURPOSING IN EUROPE AFTER THE PAEDIATRIC REGULATION

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BACKGROUND: Since many years drug repurposing, the process of marketing previously approved drugs for new indications, is of growing interest for European academia and industry because it reduces time and costs of R&D. As far as paediatrics is concerned, this process takes hold in a scenario affected by the lack of medicines specifically designed for children. To this aim not only the European Paediatric Regulation (EC) N° 1901/2006 (Paediatric Regulation) has introduced a Paediatric Use Marketing Authorisation (PUMA) for off-patent products but also Directive 2001/83/EC is applicable in the paediatric framework. In this paper we analysed both the drug repurposing procedures, by considering the number and the main features of off-patent paediatric medicinal products (p-MPs) marketed for new indications (or with new dosage or formulation) since 2007.
METHODS: The search for EMA off-patent p-MPs receiving a marketing authorisation (MA) from January 2007 to December 2019 was performed on EPMD the European Paediatric Medicines Database managed by TEDDY - European Network of Excellence for Paediatric Research. The following data were collected: year of approval, drug type (i.e. chemical or biological), paediatric indication, age for which the drug is intended, Anatomical Therapeutic Chemical (ATC) code (first-level), orphan drug status, number and type of paediatric studies supporting the MA.
RESULTS: In the period 2007-2019, 34% of EMA medicines were approved for children. Out of 296 MPs, a new paediatric indication (or dosage or formulation) was granted for 53 off- patent MPs (18%): 6 under the Paediatric Regulation and 47 under the Directive 2001/83/EC. 70% of these drugs is chemicals, while 30% is biologicals and all p-MPs belong to 8 ATC first-level categories. Besides, A-ATC and V-ATC (alimentary and various, respectively) represent the groups with the highest ratio. 51% was authorised for adolescents versus 6% for neonates. The orphan p-MPs are the 30%. A total of 76 studies were found in the first MA dossiers, with a trials/medicines' ratio equal to 1.6. Almost one-third of the p-MPs were approved based on a satisfactory drug developmental plan including PK/PD/Efficacy/Safety paediatric studies.
CONCLUSIONS: Based on these results, the paediatric drug repurposing in Europe seems to be particularly implemented and most repurposed p-MPs are approved according with the provisions of Directive 2001/83/EC while PUMA applications are residual. However, repurposed p-MPs, for which a Paediatric Investigational Plan is not mandatory, are less studied in comparison with p-MPs approved under art. 7 and 8 of the Paediatric
REGULATION: It is questionable if a special repurposing procedure involving the EMA-Paediatric Committee's opinion but assuring limited regulatory burdens would foster the development of more and high- quality p-MPs.

MGLUR5 AS A TARGET TO MODULATE THE REACTIVE PHENOTYPE OF ASTROCYTES IN THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder due to motor neurons (MNs) and glial cells degeneration in the spinal cord, brainstem, and motor cortex. One major cause for MNs degeneration in ALS is represented by glutamate (Glu)-mediated excitotoxicity. Group I metabotropic glutamate receptors (mGluR1, mGluR5) play a role in ALS, since they are largely over-expressed during disease progression and are involved in the altered neuronal and glial cellular processes. We here investigated the consequences of reducing mGluR5 expression in SOD1G93A mice (SOD1G93AGrm5-/+) on the reactive phenotype of spinal cord astrocyte cell cultures from late symptomatic SOD-1G93A, age matched SOD1G93AGrm5-/+ and WT mice.

METHODS: Primary adult astrocytes were isolated from spinal cord of WT, SOD1G93A, Grm5-/+ and SOD1G93AGrm5+/- mice at 120 days of life. For immunofluorescence (IF) analyses, astrocytes were fixed, permeabilized and incubated with appropriate antibodies. For western blot analyses, astrocytes were detached, lysed, and labeled for the target proteins. To evaluate IL-6, IL-1β and TNF-α secretion, astrocytes supernatant was analyzed by commercial ELISA kits. To measure the [Ca2+]c, cells were incubated with the fluorescent dye FURA-2AM (10μM, 45 min, at 37°C), in the presence of absence of 3,5-DHPG (30μM). Astrocytes were also treated in-vitro with the mGluR5 negative allosteric modulator CTEP (100nM) for six days and detached at day 8 for IF analyses.
Astrocytes supernatant was analyzed for the glycolytic pathway, while the mitochondria function was evaluated studying O2 consumption and the ATP synthesis. Spinal motor neurons (MNs) form SO1G93A embryos (E13,5) were co-cultured with adult astrocytes from SO1G93A or SOD1G93AGrm5-/+ mice and counted for cell survival.

RESULTS: [Ca2+]C was increased in SOD-1G93A astrocytes in absence or presence of 3,5-DHPG, to activate mGluRs. The mGluR5 down-regulation reduced the excessive [Ca2+]C. GFAP, Vimentin and S100β, three astrogliosis markers, were increased in SOD1G93A astrocytes and this over-expression was reduced in SOD1G93AGrm5-/+ astrocytes. mGluR5 down-regulation resulted in a lower presence of misfolded SOD1. The overexpression and excessive secretion of IL-6, IL-1β and TNF-α in SOD1G93A astrocytes were strongly reduced in SOD1G93AGrm5-/+ astrocytes.
Mitochondria function was impaired in SOD-1G93A astrocytes and the impairment was recovered in SOD1G93AGrm5-/+ astrocytes. Notably, the viability of spinal MNs was increased when co- cultured with SOD1G93AGrm5-/+ astrocytes, instead of SOD1G93A astrocytes. Treatment of SOD1G93A astrocytes with CTEP reduced the expression of S100β and GFAP.

CONCLUSIONS: These results show that mGluR5 activity reduction in SOD1G93A mice has a positive impact on astrocyte functions. This supports the idea that mGluR5 may be a potential therapeutic target aimed at preserving MNs death, possibly by modulating the reactive astroglia phenotype in ALS.

THE ROLE OF PHARMACOGENETICS IN THE CHEMOTHERAPY OF CO-ADMINISTERED DRUGS WITH FLUOROPYRIMIDINES

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BACKGROUND: The limiting phase of fluoropyrimidines (FP) catabolism is catalysed by dihydropyrimidine dehydrogenase (DPD), encoded by DPYD gene. The FP-pharmacogenetics, based on DPYD polymorphisms identification, is recommended to personalize the therapy.
Also, polymorphisms in genes codifying enzymes for metabolism of drugs commonly co-administered with FP as irinotecan (i.e. UGT1A1*28) are predictive factors of toxicity. Other variants, including CYP3A4*1B (-392 A>G) and CYP3A5*3 (6986 A>G), are involved in the variability of irinotecan metabolism. Even in ABCB1, a gene encoding P-glycoprotein, there are polymorphisms (e.g. ABCB1 C3435T) that may change its activity, influencing the excretion.
Oxaliplatin is detoxified by glutathione S-transferase P (encoded by GSTP1 gene). Enzymes involved in DNA damage repair (XRCC1 and ERCC1) affect its cytotoxic activity. GSTP1 313GG genotype increases the risk of neurotoxicity. XRCC1 28152AA may increase damage and failure to repair DNA. ERCC1 T19007C polymorphism leads to a reduction in gene expression while ERCC1 8092CC is associated to increased patients' survival. To estimate the contribution of aforementioned polymorphisms to variability response to FP-based chemotherapy regimen is very important.
METHODS: Patients (pts) admitted to the Oncology Unit of the University Hospital of Salerno, eligible for FP-based therapy, were enrolled and blood samples were collected from all pts. A case report form (CRF) was implemented to record clinical, anamnestic and demographic data, including administered chemotherapeutic agents. Also ADRs were recorded on the CRF. Pharmacogenetic analysis was performed by pyrosequencing at the Clinical Pharmacology Unit.
RESULTS: More than half of the genotyped pts treated with irinotecan were carriers of UGT1A1*28 variant (50% heterozygous and 8% homozygous). All the pts were wt for CYP3A4 polymorphism. 75% of pts were CYP3A5*3/*3 carriers, while 25% were CYP3A5*1/*3. 21% of pts were wt for ABCB1 C3435T, 37% were heterozygous (3435CT) and 42% had 3435TT genotype.

In addition, 42% of pts treated with oxaliplatin were wt, 51% heterozygous and 7% homozygous GG for GSTP1 A313G. 54% of pts were wt, 37% heterozygous and 9% were homozygous AA for XRCC1 G28152A. 66% of pts were wt, 32% heterozygous and 2% homozygous AA for ERCAA1 C8092A. Finally, 47% of pts were wt, 48% heterozygous and 5% homozygous CC for ERCC1 T19007C. 71% of pts treated with irinotecan reported haematotoxicity and 83% gastrointestinal toxicities. 74% of pts treated with platinum derivatives showed neurotoxicity and 76% reported haematological toxicity.

CONCLUSIONS: The application of pharmacogenetics consists in the possibility of predicting the response to a specific therapy on the basis of specific genetic tests, contributing to the so-called "personalized medicine". The use of pharmacogenetics could ward off the occurrence of several chemotherapy-related ADRs thereby ameliorating patient care.

EFFECT OF PALMITOYLETHANOLAMIDE IN THE CROSSTALK BETWEEN MAST CELLS AND ASTROCYTES AGAINST MORPHINE-INDUCED ALTERATIONS

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BACKGROUND: The clinical use of opioids is limited by the onset of tolerance in consequence to repeated treatments. Multiple mechanisms underlie this side effect involving tissue, receptor plasticity, intracellular signals of both nervous and immune cells. Results obtained *in vivo* by our laboratory showed that the use of palmitoylethanolamide (PEA), in combination with morphine, is able to delay the onset of tolerance and increases the effectiveness of the opioid allowing the use of lower doses to achieve a good analgesic effect. The mechanism by which PEA influences the morphine signal involves the regulation of glial cells, but given the homeostatic role of PEA, interaction with different cell types must not be excluded. In particular, a large literature describes a powerful regulatory action of PEA on mast cells.
The purpose of the study will be to evaluate the effect of morphine with or without PEA in the complex signaling exchange between mast cells and astrocytes.

METHODS: Primary astrocytes were isolated from cortex of new-born rats (day 1-3) and RBL-2H3 cell line was used as a model of rat mast cells. The RBL-2H3 degranulation was evaluated by β-hexosaminidase assay and dosage of histamine by HPLC. The activation state of astrocytes was evaluated analysing by RT-PCR the expression of markers of pain mediation and inflammation such as ccl2, IL-1α, IL-1β, Il-6, EAAT2, Serpina and PTX3. Co-culture of astrocytes and mast cells was performed using transwell system with micropore of 0.4 μm.

RESULTS: RBL-2H3 cells need to be pre-treated for 24h with IgE (0,5 μg/mL) and after incubation with the drug for 30 minutes, they have to be stimulated with DNP-BSA (625 ng/mL) to perform β-hexosaminidase assay at 5,10,15 and 30 minutes of DNP-BSA stimulus. 30 min-

utes of morphine 30 μM treatment leads to an increase of 10-15% of degranulation after 5 and 10 minutes of DNP-BSA stimulus. PEA, tested at two doses (10 and 100 μM) for 24h (in pre-treatment with IgE) revealed a reduction up to 80% of degranulation especially at 100 μM. The same strong degranulation-preventive effect was demonstrated by PEA in the presence of morphine. Moreover, PEA 100 μM strongly decreased the histamine release. The effect of morphine on astrocytes was studied by RT-PCR: 30 μM morphine significantly increased the expression of genes involved in inflammation and pain mediation such as ccl2, IL-1α, IL-1β, IL-6 and PTX3.
In astrocytes co-cultured with RBL-2H3 in transwell, the expression level increase of ccl2, IL-1α, IL-1β, IL-6 and EAAT2 induced by morphine was controlled by PEA.
CONCLUSIONS: PEA reduced the degranulation of mast cells, completely reverting the effect of morphine. PEA exerted a regulatory effect on astrocyte gene expression profile of inflammation- and pain-related signals. In astrocyte/mast cell co-culture, this PEA effect was able to counteract morphine-dependent alterations.

SERPINB3: A NOVEL TARGET FOR TREATING HEPATIC ISCHEMIA-REPERFUSION INJURY

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BACKGROUND: Liver ischemia/reperfusion (I/R) injury occurs in liver transplantation, complex liver resection or hemorrhagic shock. The pathophysiological process of liver I/R injury is accompanied by the overproduction of free radicals and an increase in cytokine production. The modulation of specific survival pathways by appropriate drugs may be useful in reducing liver I/R injury. Serine protease inhibitor B3 (SerpinB3) is undetectable in the normal liver; recently, using an in vitro model, it has been found to be induced by oxidative stress in hypoxic conditions (Cannito S. et al, Oncotarget, 2015). Aim of the present study was to investigate whether the molecular expression of SerpinB3 was affected by oxygen deprivation also in in vivo models of hepatic ischemia and I/R injury.
METHODS: Male Wistar rats (n=15) were subjected to partial hepatic ischemia (60, 120, 180 min) by clamping of the hepatic artery and portal vein; sham operated rats (n=12) were used as control group. A restricted group of animals (n=5) were also subjected to 60-min ischemia followed by 60- min reperfusion; sham operated rats (n=5) were used as control group. At the end of the procedure, liver and blood samples were collected. Hepatic enzymes were analyzed in serum, while gene expression of SerpinB3 and inflammatory cytokines as well as ATP levels and thiobarbituric acid-reactive substance (TBARS) formation were assessed in the liver.
RESULTS: Liver ischemic injury was confirmed by increased hepatic enzymes (ALP, ALT, gGT) in the group submitted to ischemia when compared with the sham group. A time-dependent decrease in tissue ATP and a significant increase in TBARS occurred at 180-min ischemia. A significant time-dependent increase in liver SerpinB3 expression was found, reaching an 18-fold increase at 180 min. The peak expression of IL-1β was observed at 60 min, while

IL-6 and TNF-α peaked at 120 min. No significant modifications were observed in the liver of sham-operated rats. In animals subjected to 60-min ischemia and also 60-min reperfusion, SerpinB3 mRNA was undetectable after a 60-min reperfusion interval.
CONCLUSIONS: The present data show that a time-dependent increase in SerpinB3 occurs during hepatic ischemia. This change is oxygen-dependent, since, after reoxygenation, SerpinB3 values are back to undetectable levels. Although other studies are requested, the ischemia-induced increase in SerpinB3, in association with the peaks of inflammatory cytokines, may reflect an unknown pathological process occurring during ischemia. Early administration of SerpinB3 analogues may be considered as a new strategy against hepatic I/R injury.

CHARACTERIZATION OF THE GPR17 RECEPTOR EXPRESSION IN HUMAN LESIONS OF MULTIPLE SCLEROSIS PATIENTS AND POSSIBLE CLINICAL IMPLICATIONS

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BACKGROUND: Alzheimer's disease (AD) is a chronic disorder characterized by progressive neurodegeneration due to abnormal protein aggregation, deposition of extracellular β-amyloid proteins (Aβ), beside an increase of oxidative stress. Amniotic fluid stem cells (AFSC) should have a therapeutic potential for neurodegenerative disorders, mediated by exosomes (exo). Aim of the study has been to examine the effect of exo derived from human AFSC (AFSC-exo) in preventing the disease phenotypes in an AD model in vitro, a neuron primary culture.
METHODS: The AFSC were obtained from 4 amniotic fluids collected from healthy pregnant women at the 16th week of gestation who underwent amniocentesis. Cortical and hippocampal neurons from neonatal wild type (WT) and 5xFAD (FAD) transgenic mice for AD were collected within 24 h from birth and treated with AFSC-exo up to 14 days. In this study we evaluated cellular morphology, intracellular ROS levels and secreted Aβ42 from primary cultured neurons. Finally, to evaluate the effect of AFSC-exo on apoptotic and autophagic pathways and the reduction of AD markers in the presence of AFSC-exo we performed western blot analysis, immunofluorescence and confocal microscopy.
RESULTS: FAD neurons appeared unhealthy, compared to WT neurons, since neurodegeneration in FAD neurons was prominent. FAD neurons displayed a higher ROS level compared to WT cells: the expression of proteins related to ROS modulation demonstrated that antioxidant enzymes (SOD1, TrxR, TrxR2 and Gpx1) were all more present in FAD + exo neurons than in FAD cells. FAD neurons displayed a higher level of Nox4, a ROS producing enzyme constitutively active. The analysis of apoptosis by annexin V and propidium iodided (PI) demonstrated that the presence of exo significantly restored cell viability and reduced PI staining. Immunoblotting

data demonstrated increased activation of Akt, signaling molecule for neuronal survival, and an activation of Bcl-2, an anti-apoptotic marker, in FAD + exo neurons. The cleavage of PARP, significantly higher in FAD neurons, compared to WT, was reduced by the exo-treatment. Autophagic pathways were also modulated through AFSC-exo content as demonstrated by the increased level of SIRT1, a NAD- dependent class III histone and non-histone protein deacetylase, and LC3β protein. WB analysis and immunofluorescence images showed that levels of APP, Aβ oligo and p-Tauser422 were reduced in exo-treated AD neurons. The quantitative detection of secreted Aβ42 demonstrated the positive effect of AFSC-exo on FAD neurons, since the production of Aβ in AD neurons was decreased after treatment.

CONCLUSIONS: Here we demonstrated that the administration of MSC exosomes ameliorated the progression of Aβ-induced neuronal death and AD, supporting the idea that exosomes could exert disease-modulating effects. Furthermore, we showed the ROS regulation involvement in the exosome therapeutic role in neurodegenerative diseases, such as AD.

VERY ELDERLY PATIENTS IN NSCL TREATED WITH IMMUNOTHERAPY: REAL WORLD EFFICACY/SAFETY AND BIOLOGIC DATA OF PREDICTIVE OUTCOMES

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BACKGROUND: Detrimental changes to immune function with age are predominantly illustrated by the increased susceptibility of older adults to infectious disease, and the increased severity thereof. The reasons for this are not exclusively immunological, but it is commonly perceived that waning immunity is primarily responsible. Many studies over the years have sought to compare immune parameters in people of different ages, usually perforce by means of cross-sectional studies and accessing only peripheral blood. On the basis of these assumptions, we evaluated the activity and safety data in the cohort of patients treated at our institution. In the last 5 years the natural histoy of NSCL has been deeply changed and started to news therapeuthics frontiers for the introduction of immuno check point inhibitors targeting (ICI) PD1/ PDL1 and CTLA4. However, it is unclear whether the efficacy and safety of ICI is similar in elderly (>75 age) to that in non ederly patients (<75 age). In fact in most of the clinical trials randomized with ICI for NSCLC, patients > 75 years of age were included only in small subgroups, therefore there is an absolute need for data regarding the efficacy and safety of ICI in very elderly.

METHODS: Between December 2015 and April 2020, 164 patients with NSCL receveid second line with antiPD1/PDL1 (Nivolumab / Atezolizumab). Treatment efficacy and safety, and treatment related or sierologic autoimmunity was evalueted. Baseline characteristics, efficacy, safety and predictive outcomes were recorded. We have correlated autoimmune adverse events with efficacy in terms of OS and PFS. Overall survival both from diagnosis and from the second line were recorded.

RESULTS: Of the 164 patients, 64 (32.2%) were older than 75 years (very elderly patients) . The median follow-up time was 27 months. The OS in the two groups was 11.4 and 8.5 months

respectively (p = 0.88), while the PFS was 5.1 and 6.2 months (p = 0.96). The assessment of overall survival according to both age and autoimmunity showed the following data: very elderly patients with or without autoimmunity the overall survival was 7.2 months and 30.8 months respectively, while in patients less than 75 years autoimmunity correlated OS in the opposite way, that is 14.3 vs 11.4 months.

CONCLUSIONS: This pooled analysis of elderly patients >75 years with NSCLC had efficacy and safety profile in second line confirm the effectiveness of ICI of other institutions. While, with the limitations of the retrospective evaluation and as opposed to the data that are increasingly consolidated in the literature, in real elderly patients autoimmunity would inversely correlate the response and survival of ICI.

ADHERENCE TO TREATMENT IN DIABETES: WHAT'S THE MATTER?

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BACKGROUND: Type 2 Diabetes Mellitus (DM2) is a common disease related with frequent morbidity and mortality1. Although effective oral hypoglycaemic agents and insulin have been developed, nonadherence to diabetes medication is still a major issue in the management of patients with DM2. Nonadherence to prescribed medications is associated with a poor glycaemic control, leading to microvascular and macrovascular complications with consequent decrease in quality of life and an increased healthcare cost2. Inadequate adherence could be due to several reasons including complex polytherapy, dosing frequency and treatment emergent adverse events (TEAEs). Aim of this study was to evaluate adherence to diabetes treatment and identify factors that predate poor adherence among patients with DM2.

METHODS: A cross-sectional observational pharmacovigilance study was conducted from September 2018 to December 2019. Consecutive outpatients with DM2, age> 18 years and ongoing pharmacological treatment from "Mater Domini" University Hospital have been enrolled. Drug adherence was assessed by the Italian version of Morisky's Eight-Item (MMAS-8), a self-reported measure of adherence structured in 8 questions.

RESULTS: Overall 670 patients have been enrolled, 352 (52.5%) males and 318 (47.5%) females. Mean age was 56.6 (range: 18-99) years and mean duration of diabetes 12.8±9.4 years. Most patients were treated with metformin 62.7% (420), insulin 39.4% (264) and sulfonylurea 12.2% (82). 42.1% of patients were in monotherapy (282) and 57.9% were in polytherapy (388).

By the analysis of MMAS-8 questionnaires, we observed ~60% (405) of patients adherent to treatment, 25% (168) showed medium adherence and 15% (97) were non-adherent to therapy. Patients with low/high adherences significantly differ by the age of DM2 onset (p=0.03) and the number of diabetes medications prescribed (median 2; p=0.15). Non-adherent patients presented 41.2% (40) of TEAEs and 6.2% (6) of severe TEAEs, whereas adherent patients 20% (84) of TEAEs and 2.2% (9) of STEAEs. No statistical factors associated with low adherence were found in the multivariate logistic regression model.

CONCLUSIONS: This real-life cohort of diabetic patients found an overall high prevalence of adherence. Patient compliance to drug therapy is one of the most relevant issues in clinical practice as the success of a therapeutic intervention depends on the actual patient adherence to therapy. Therapeutic adherence should therefore always be investigated, in our context the use of the Morisky questionnaire represented a rapid and useful screening tool that allowed to identify the patients most in need of greater clinical monitoring.

GILZ CONTROLS HEMATOPOIETIC STEM CELL AND PROGENITOR PROLIFERATION IN EXPERIMENTAL MODEL OF SEPSIS

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INTRODUCTION: The maintenance of hematopoietic stem cells (HSC) is linked to their quiescent state, while HSC proliferation is associated with differentiation and a loss of long-term stem cell potential. The balance between HSC quiescence and proliferation is tightly regulated by intrinsic and extrinsic cues in the bone marrow. Pro-inflammatory stimuli like infection trigger the HSC proliferation to ensure adequate production of leukocytes, however with negative impact on HSC maintenance. In fact, stimulation of mice with lipopolysaccharide (LPS) that mimics bacterial infection in sepsis is associated with enhanced HSC cycling and an expansion of phenotypic HSC associated with a functional decrease in the long term. Endogenous glucocorticoid hormones (GC) regulate HSC homing via control of CXCR4 expression. Glucocorticoid-Induced Leucine Zipper (GILZ) is a gene rapidly induced by GC. It mediates many of GC' anti-proliferative and anti-inflammatory effects in several cell types. GILZ was found to limit LPS-triggered lethality in the mouse model of sepsis. The role of GC and GILZ in the control of HSC proliferation and function at steady state and upon LPS challenge is not yet defined.
METHODS: We have addressed the role of GILZ in HSC homeostasis using age-matched WT, GILZ knock-out (KO) and transgenic mice overexpressing GILZ (GILZ-TG) mice. Sepsis was achieved by LPS challenge through intraperitoneal injection of 35 ug to 50 ug of LPS, corresponding to 35 to 50 CFU (Escherichia coli 011:B4), 48 hours before collection of bone marrow samples. HSC frequency and number was evaluated using flow cytometry; differential gene expression was analyzed by RNASeq and qPCR methods; statistical analysis were performed using GraphPad Prism.
RESULTS: We found that GILZ mRNA is expressed at higher levels in HSC compared to myeloid progenitors. At steady state, young GILZ-KO mice did not show alteration in HSC number and lineage commitment compared to WT mice. However, competitive transplantation studies revealed a significant decrease in the frequency and number of GILZ-KO compared to WT HSC, suggesting that GILZ-deficient HSC have competitive disadvantage compared to WT cells. RNAseq analysis of gene expression revealed that several cellular pathways implicated in HSC function were deregulated upon GILZ deletion. Importantly, Gene Set Enrichment Analysis showed a significant depletion of the HSC signature HSC

and an enrichment of the mTOR signalling signature in GILZ deficient compared to WT HSC. Consistently, GILZ deficient HSC showed enhanced proliferation as revealed by flow cytometry analysis of Ki67 expression and DNA content. As previously reported, LPS stimulation enhanced HSC proliferation in WT mice. Interestingly, GILZ over expression prevented the LPS-induced HSC expansion in GILZ-TG mice.

CONCLUSIONS: Overall, these data identify GILZ is a novel regulator of HSCs function.

REMOTE POSTCONDITIONING AMELIORATES STROKE DAMAGE BY PREVENTING NEURONAL LET7A AND ASTROCYTIC MIR-143 UP-REGULATION

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BACKGROUND: Remote limb ischemic postconditioning (RLIP) is a well-established neuroprotective strategy able to protect the brain from a previous harmful ischemic insult through a sub-lethal occlusion of the femoral artery. Neural and humoral mechanisms have been proposed as mediators required to transmit the peripheral signal from limb to brain. Moreover, different studies suggest that protection observed at brain level is associated to a general genetic reprogramming involving also microRNAs (miRNAs) intervention.

METHODS: Brain ischemia was induced in male rats by transient occlusion of the middle cerebral artery (tMCAO), whereas RLIP was achieved by one cycle of temporary occlusion of the ipsilateral femoral artery after tMCAO. The expression profile of 810 miRNAs was evaluated in ischemic brain samples from rats subjected either to tMCAO or to RLIP. Those brain ischemic miRNAs that were up-regulated after stroke were exogenously intracerebroventricularly perfused by osmotic pumps in rats subjected to remote ischemic postconditioning. Brain damage was evaluated by measuring infarct volume and neurological deficit scores at definite time intervals after stroke induction.

RESULTS: Twenty-one miRNAs, whose expression was significantly affected by tMCAO and by tMCAO plus RLIP, were selected based on microarray microfluidic profiling. Our data showed that: (1) stroke induced an up-regulation of neuronal let-7a and of astrocytic miR-143 and (2) these two miRNAs were involved in the protective effects induced by RLIP. Indeed, their expression was reduced after RLIP and the exogenous intracerebroventricularly infusion of let-7a and miR-143 mimics prevented neuroprotection conferred by RLIP. Blocking let-7a activity but not miR-143 in stroke induced neuroprotection.

CONCLUSIONS: Preventing neuronal let-7a and astrocytic miR-143 up-regulation emerges as new potential strategies in stroke intervention, althoug

MANAGEMENT OF TOCILIZUMAB FOR THE TREATMENT OF PATIENTS WITH COVID19 DURING THE EMERGENCY: THE EXPERIENCE OF THE REFERENCE CENTRE FOR EASTERN SICILY

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BACKGROUND: The monoclonal antibody tocilizumab emerged as potential off-label treatment for severe COVID19. During the emergency phase, the drug was provided free of charge by the manufacturer. On March 13 the University Hospital of Catania was identified as the reference Centre (according to a Hub-and-Spoke model) for the management of tocilizumab in order to centralize the procedures for the requests, collection and distribution of the drug to the COVID19 Hospitals of the Eastern Sicily, avoiding potential waste of drug and ensuring an equal and rapid access to all patients. On March 18, the clinical trial TOCIVID19 was approved by AIFA with the aim to produce clinical evidence and to control the systematic use of tocilizumab in this setting. The University Hospital of Catania maintained its role of managing the drug access for the clinical centers of the Eastern Sicily involved in the study.

METHODS: We analyzed the off-label prescriptions of tocilizumab made by 13 centers before and after the start of the TOCIVID19 study. A specific procedure was defined by the reference Centre including the following steps: 1. a nominal request for single patient according to the off-label regulation; 2. a request to the manufacturer with delivery of the drug to the Hub; 3. distribution to the spokes.

RESULTS: From March 14 to May 5, 124 patients with COVID19 were treated with tocilizumab, equal to approximately 60% of the requests received (n=211). For 87 patients, the explanation of non administration was clinical improvement (n=26, 29.9%), death (n=5, 5.7%), refusal of treatment (n=2, 2.3%), unknown (n=54, 62.1%). The average time between request and delivery to the Spoke was approximately 2 days (range 0–8 days) between March 14 and March 24 (date of the first delivery of tocilizumab as part of the TOCIVID19 study). Subsequently, each center received the drug within 24 hours from the request. All treated patients received 8 mg/kg iv at T0 up to a maximum of 800 mg and after 12 hours at the same dose in the absence of improvement (average dosage used 1,252 mg). Specifically, 15.3% of patients received the maximum dose, 48.4% an intermediate dose (between 600 and 800 mg) and 36.3% a dose <600 mg. The manufacturer delivered to the Hub the maximum dose of the drug. Therefore, almost 43,200 mg (21.8%) of tocilizumab were not administered and could be used for the treatment of further 35 patients at the average dose.

CONCLUSIONS: The centralization of the management of tocilizumab ensured the appropriateness of off-label drug prescriptions and the optimization of the available drug in the emergency. The high number of non-administration was probably related to the waiting time in the early phase, after consuming the in-stock tocilizumab intended for the treatment of rheumatic patients. The TOCIVID19 trial certainly reduced the waiting time for treatment. Nevertheless, even in this phase the role of the Hub was critical to manage drug access in the eastern Sicily.

DATA INTEROPERABILITY AND FAIRIFICATION: A KEY TECHNOLOGY IN THE CONTEXT OF THE EUROPEAN PAEDIATRIC TRANSLATIONAL RESEARCH INFRASTRUCTURE-EPTRI

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BACKGROUND: The ID-EPTRI project was funded by the European Commission to create a framework for a new Research Infrastructure aimed at enhancing technology-driven paediatric research to facilitate the translation of research findings for the evaluation, approval and therapeutic use of paediatric medicines. EPTRI will also support the paediatric research community by providing services, competence, and access to key technologies (biological advancements and data science) specifically implemented or adapted to the paediatric setting. The operational capability, scientific excellence, and sustainability of EPTRI have been already tested in the feasibility phase of the project. Here, we report results of EPTRI capacity building in the sector of data interoperability, representing one of the most relevant innovative technology for drug development.
METHODS: Paediatric research groups were invited via an open call to submit their requests for support on a topic within the scope of EPTRI*. After assessment of the feasibility of each proposal by the Advisory Board, a dialogue was initiated with each group regarding the specific requirements and technical details of their research resulted in the definition of quality, quantity, timing and costs of services, and identification of service providers (Research Units dislocated in Europe and/or Centralised Services).
RESULTS: 4/10 proposals were focused on data interoperability:
- Omics data integration to understand how the differentially expressed proteins, metabolites and RNAs interact (Paediatric beta thalassaemia patients responding to drug Hydroxyurea).
- Secondary use of therapeutic drug monitoring data in children with suspected sepsis treated with antibiotics (MoSe2).
- Bioinformatics analyses and omics data integration (Mitochondrial regulation of functional pathways).
- Data sharing and integration (Precision Personalized Paediatric neuroPsychiatry).

For each proposal, a preliminary study phase was concluded with the identification of available clinical/preclinical setting including data to be shared and interoperability model.
CONCLUSIONS: Data interoperability is fundamental to support sharing and re-use of data for

research purposes, and the repeated requests for data re-use demonstrate the need of a paediatric data interoperability service focused on discovery, access, integration and analyses of biological data. This requirement is included in the proposed collaboration between EPTRI and ELIXIR, the distributed infrastructure for life-science information. To support interoperability, different issues need to be addressed, as the development of new standards, formats and ontologies for heterogeneous data integration and querying. The setup of specific methods providing Uniform Resource Identifiers would contribute to identify data for the paediatric scientific community.

*Paediatric Medicines Discovery, Paediatric Biomarkers and Biosamples, Developmental Pharmacology, Paediatric Medicines Formulations

A LC-MS/MS QUANTIFICATION METHOD FOR SORAFENIB, REGORAFENIB AND THEIR ACTIVE METABOLITES IN HUMAN PLASMA FROM CANCER PATIENTS

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BACKGROUND: Sorafenib (SORA) and regorafenib (REGO) belong to the multikinase inhibitors family. SORA is approved for the treatment of advanced hepatocellular carcinoma (HCC), for advanced or metastatic renal cell cancer and for locally advanced or metastatic differentiated thyroid carcinoma refractory to radioactive iodine. Instead, REGO is used for the treatment of metastatic colorectal cancer, III line treatment of unresectable or metastatic gastrointestinal stromal tumors, II-line treatment in advanced HCC and relapsed glioblastoma. SORA and REGO are usually administered at fixed dose according to the tumor type, but a wide inter-patients variability is reported. The Therapeutic Drug Monitoring (TDM) should be recommended to personalize the drug dosage in patients.
The aim of this work was to develop and validate a new LC-MS/MS method for the simultaneous quantification of SORA, REGO and their active metabolites (SORA-N-oxide, REGO-N-oxide and N-desmethyl-REGO-N-oxide) in patients' plasma.
METHODS: The quantification was performed using liquid chromatography coupled to a triple quadrupole with a negative ESI source. The analytes separation was obtained with C 18 Synergi Fusion RP (4 µm, 50x2.0 mm) by gradient elution of 10 mM AmAc plus 0.1% HCOOH and MeOH:iPrOH (9:1, v/v) with 0.1% HCOOH. The method could be easily used for TDM due to the short runtime (7 min), very small plasma volume required by the analysis (5 µL) and the fast sample preparation based on protein precipitation. This LC-MS/MS method was applied to quantify the C_{min} of SORA, REGO and their metabolites in 66 plasma samples collected from patients enrolled in a clinical study (CRO-

2018-83) ongoing at the National Cancer Institute of Aviano.
RESULTS: The method was fully validated according to FDA and EMA guidelines. This method covered adequately the therapeutic range with calibration curves that resulted linear ($R^2 \geq 0.998$, for all the analytes) between 50-8000 ng/mL for SORA and REGO and 30-4000 ng/mL for all metabolites. Moreover, the method demonstrated a good intra- and inter-day precision and accuracy (CV%≤8.7% and accuracy from 89.4 to 108.8%), selectivity, sensitivity, reproducibility, analytes stability under various conditions and the absence of matrix effect. The application of this LC-MS/MS method to quantify 66 plasma samples from patients treated with SORA or REGO demonstrated a wide variability in C_{min} values among patients treated with the same drug dosage: a mean SORA C_{min} of 3139 ng/mL (range: 1016-6669 ng/mL at the dose of 200 mg/die) and a mean REGO C_{min} of 1440 ng/mL (range: 813-2552 ng/mL at the dose of 80 mg/die).
CONCLUSIONS: A new LC-MS/MS quantification method for SORA, REGO and their active metabolites was developed and validated. The application of the method to a pool of cancer patients demonstrated its clinical utility to evidence a wide inter-patients variability of plasma concentrations suggesting the clinical benefit of the use of TDM in clinical practice.

EFFECTS OF EXOSOMES DERIVED FROM IFNΓ-PRIMED MESENCHYMAL STEM CELLS ON THE PHENOTYPE OF ASTROCYTES CULTURED FROM LATE SYMPTOMATIC SOD1G93A MICE

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects upper and lower motor neurons (MNs) leading to muscle atrophy and paralysis. Invariably, it results in patient death, generally within 3 to 5 years from onset, due to respiratory failure. ALS is a non-cell autonomous disease, in which astrocytes play a central role in clinical progression. We previously demonstrated that the intravenous administration of mesenchymal stem cells (MSCs) in the SOD1G93A mouse model of ALS prolonged survival, improved motor skills and reduced reactive gliosis. These beneficial effects were not associated with MSC differentiation, being possibly mediated through paracrine mechanisms. We postulated that MSC-derived exosomes can be a mode to sustain the paracrine effects of MSCs. We studied here the effects of MSC-derived exosomes on the phenotype of astrocytes from SOD1G93A mice.
METHODS: Primary astrocyte cell cultures were prepared from 120-day-old spinal cord WT and SOD1G93A mice. Spinal cord tissue was me-

chanically dissociated in DMEM high Glucose medium and astrocytes were cultured at 37°C and 5% CO2 for 20 DIV before experiments. Exosomes were isolated by Total Exosome Isolation Kit (Invitrogen) from the supernatant of MSCs previously stimulated with IFN-γ for 24h. Astrocytes were exposed to exosomes for 24h. Exosomes from 6x105 MSC / 1x105 astrocytes were used. Motor neurons (MNs) were prepared from the spinal cord of E13,5 SOD1G93A embryos and isolated by Optiprep gradient. MNs were counted for viability starting at day 4 after seeding until day 14. Cytokines concentration was measured in astrocyte- conditioned medium at 24 h and 48h after exosomes treatment by commercial ELISA kits.

RESULTS: GFAP, S100β and vimentin expression was increased in 120 days-old SOD1G93A astrocytes compared to age-matched WT astrocytes. In vitro exposure to MSC-derived exosomes significantly reduced the overexpression of the three astrocyte activation markers. The expression of the inflammation complex NLRP3 was increased in SOD1G93A astrocytes and the increase was reversed after exposure to exosomes. Accordingly, the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 were significantly more expressed and more efficiently released in SOD1G93A astrocytes and exosomes significantly decreased their overexpression and release. Conversely, the expression of the anti-inflammatory cytokine IL-10 was decreased in SOD1G93A astrocytes and normalized after exposure to exosomes. The viability of embryonic SOD1G93A mouse-derived MNs was significantly increased when seeded on exosome-treated adult SOD1G93A astrocytes, compared to non-treated astrocytes.

CONCLUSIONS: Our results suggest that the reactive phenotype and the inflammation state of SOD1G93A mouse astrocytes are ameliorated by exosomes derived from IFNγ-primed-MSCs. Spinal MNs viability was also improved. These results open to the possibility to in vivo preclinical trials with exosomes in SOD1G93A mice.

PACSIN2 AS A MODULATOR OF AUTOPHAGY AND THIOPURINE SENSITIVITY: IN VITRO EVALUATIONS IN LYMPHOID AND INTESTINAL CELLS

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BACKGROUND: Thiopurines, such as mercaptopurine (MP), are immunosuppressants used to treat acute lymphoblastic leukemia (ALL) and inflammatory bowel disease (IBD). Thiopurinemethyltransferase (TPMT) is an enzyme involved in thiopurines inactivation; its function is affected mainly by known TPMT polymorphisms and by Protein Kinase C And Casein Kinase Substrate In Neurons 2 (PACSIN2) through a less characterized molecular mechanism. The aim of the present study is to clarify the role of PACSIN2 in autophagy and on thiopurine cytotoxicity in leukemic and intestinal models.

METHODS: Human B-ALL NALM6 were stably transfected with a plasmid for TPMT*1 overexpression (*1); NALM6, NALM6*1 and colorectal adenocarcinoma LS180 cell lines were engineered to obtain stable PACSIN2 KD. Protein levels of TPMT, PACSIN2 and the autophagic

marker LC3-II were quantified by immunoblotting in presence or absence of 30 μM of the autophagic inhibitor chloroquine (CL). PACSIN2 and LC3-II levels were evaluated also in paired inflamed and non- inflamed colon biopsies of 5 IBD pediatric patients; PACSIN2 expression was quantified by TaqMan assay in 15 paired colon samples. Cell sensitivity to MP and tunicamycin, an endoplasmic reticulum stress and autophagy inducer was evaluated by MTT assay.

RESULTS: Compared to control cells (MOCK transfected with empty vector), KD showed significant lower PACSIN2 levels (NALM6: fold change 0.11 ± 0.11, P=0.013; LS180: 0.15 ±0.03, P=0.0006) and NALM6 *1 presented higher TPMT levels (2.6 ± 0.49, P=0.0311); TPMT levels decreased in NALM6 KD*1 (0.56 ± 0.17, P=0.021). When autophagy was investigated, KD cells showed increased LC3-II levels compared to MOCK (NALM6: fold change 2.14 ± 0.18, P=0.0031; LS180: 1.19 ± 0.04, P=0.0032); CL increased LC3-II both in NALM6 MOCK and KD, but further in KD cells (1.48 ± 0.13, P=0.02), suggesting that PACSIN2 could inhibit autophagy acting at the beginning of the autophagic flux. In colon samples, higher amount of LC3-II (1.85 ± 0.05, P=0.0034) and lower PACSIN2 mRNA (P=0.0084) and protein (0.65 ± 0.09, P=0.02) were observed in inflamed compared to non inflamed colon biopsies, suggesting an inverse correlation between PACSIN2 and autophagy levels, as observed in cell lines. PACSIN2 KD increased MP sensitivity in LS180 (KD vs MOCK (-Log IC50): 6.64 ± 1.77 vs 5.71 ± 0.74; 2-way ANOVA, P<0.0001) but not in NALM6. In NALM6 *1 MP sensitivity was higher than in MOCK (*1 vs MOCK: 6.41 ± 0.08 vs 5.92 ± 0.04; 2-way ANOVA, P<0.0001) but was decreased by PACSIN2 KD to control level, suggesting a possible cell-dependent role of PACSIN2 on thiopurine exposure related also to TPMT levels. NALM6 KD cells were more sensitive to tunicamycin than MOCK (KD vs MOCK: 7.06 ± 0.06 vs 6.80 ± 0.05; 2-way ANOVA, P<0.0001); tunicamycin MTT on LS180 are ongoing.

CONCLUSIONS: Taken together our findings suggest that PACSIN2 is a negative regulator of autophagy. However, further investigations are needed to confirm that autophagy is the molecular mechanism affecting TPMT activity.

PRENATAL EXPOSURE TO HEAVY METALS AND NEURODEVELOPMENTAL PATHOLOGIES CHELATING THERAPY?

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BACKGROUND: High levels of xenobiotics have been associated with several pathological conditions. The hypothesis of their etiopathogenetic role in the large family of neurodevelopmental diseases is increasingly frequent. For this study, it was essential to have, first of all, suitable matrices, accurate measurement systems and a significant and representative statistical population. The study looked at a large panel of heavy metals, reporting accurate analytical data obtained in mass spectrometry, using the most suitable biological matrices for the purpose. The study aimed at verifying any correlations between metallic pollution of the mother and the new-

born, providing further information on the clinical outcome for chelating therapy.
METHODS: A prospective study was conducted in 217 mother-infant couples, selected in Sicily. For each mother-infant couple, metal levels were measured in the mother's hair, just before delivery, and in the umbilical cord blood at the birth of the baby. Blood was obtained by using a syringe without needle and collected on metal-free tubes after discarding the first 3 ml, to limit any possible contamination. The measurement of the metals was performed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). All the laboratory materials used were made by polyethylene and/or Teflon. Each sample was subjected to a preliminary mineralization process. An instrumental Performance Report Test was obtained in order to verify the operative conditions both in standard and KED modes by using a TUNE A solution at 10 µg/L (Li, Be, Bi, Ce, Co, In) in HNO3 (1%) for covering the entire range of masses to be analyzed. Data analysis was performed by Regional Quality Laboratory Center, of the Sicilian Government Health Department, using geometric mean and categorical variables.
RESULTS: Levels of metals in umbilical cord blood samples were not closely related to mother levels. The distribution data of the metals always showed a positive correlation for higher concentration in the hair, except for the Hg.
CONCLUSIONS: The comparison of the data identified the couples most at risk, in order to study the feasibility of a biological chelation therapy, suggesting that each placenta has a peculiar permeability to the passage of metals.

NOVEL THERAPEUTIC OPTIONS FOR MYOTONIA: FROM PHARMACOGENETICS TO DRUG REPURPOSING

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Non-dystrophic myotonias (NDM) are genetic disorders characterized by sarcolemma over-excitability inducing muscle stiffness, which may greatly affect patients' quality of life.
The NDM encompass several disorders with distinct features but clinical overlap, which are myotonia congenita (MC), paramyotonia congenita (PMC), and sodium-channel myotonia (SCM).
PMC and SCM are caused by gain-of-function mutations of the Nav1.4 sodium channel, whereas MC is due to loss-of-function mutations of the ClC-1 chloride channels.
These disorders have been empirically treated with sodium channel blockers because these drugs reduce abnormal action potential firing. The antiarrhythmic mexiletine represents the first line therapy in NDM, used in both chloride and sodium channel myotonia. Unfortunately, about 30% of patients are intolerant or obtain unsatisfactory response to mexiletine.
Thus, alternative drugs are required to address the unmet needs of myotonic patients. The challenge in NDM is to develop a personalized mechanism-based therapy, looking for drugs able to correct selectively the molecular defect of channel mutants.
Regarding sodium channel myotonia, myotonic Nav1.4 mutations can modify channel sensitivity to mexiletine, due to alteration of binding site or channel gating. For instance, patch-clamp experiments showed that the Nav1.4 mutants with a rightward shift of fast inactivation voltage dependence are generally less sensitive to mexiletine, whereas sensitivity to the antiarrhythmic flecainide is not impaired. Patients carrying such mutations and refractory to mexiletine were successfully treated with flecainide, which demonstrates the translatability of in vitro data to patient side.
In chloride channel myotonia, loss-of-function mutations cause a reduction of chloride currents by altering the gating of ClC-1 channel (gating defect) or by decreasing its cell surface expression (trafficking defect). No direct ClC-1 channel activator is currently available. In order to identify drugs specifically targeting ClC-1 channels, we used well-known potent and reversible ClC-1 inhibitors to define binding sites and better understand effects on channel gating. In parallel, we performed proof-of-concept studies to verify the ability of pharmacological chaperones to restore sarcolemma expression of trafficking-deficient ClC-1 mutants. These results will pave the way for the rational design and screening of novel ClC1-specific compounds useful for the treatment of MC. Altogether, these studies improve our understanding of the molecular mechanisms underlying NDM and define a pharmacogenetics strategy to address precision medicine in myotonic individuals.
Supported by French AFM-Telethon and Italian-Telethon.

NEUROINFLAMMATION AND GUT MICROBIOTA: ALTERNATIVE THERAPEUTIC TARGETS FOR ALZHEIMER'S DISEASE?

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Alzheimer disease (AD) is the most common form of dementia affecting almost 50 million people worldwide urgently calling for a cure capable of arresting its progression. Despite neurotoxic aggregates of the β-amyloid (Aβ) peptide remain the primary culprits of AD, neuroinflammation has recently emerged as an intimate associated factor contributing to the initiation of the neuropathological process. In AD neuroinflammation is chronic and induces a perpetuating loop of neurodegenerative events. Its modulation is, unfortunately, extremely difficult to achieve, since immune cells modify their state of activation in an uncontrollable manner, under the influence of specific pathological events, occurring within specific time frames. Notably, a series of compelling "omics" studies recently indicated that microglia develop a specific disease-related signatures in AD brains of mice and humans. Through a sophisticated single-nucleus transcriptomic analysis, the development of different microglial cell subpopulations were described, highlighting a novel microglia type associated with AD. Immune cells are also significantly implicated in mediating detrimental actions originating from the gut microbiota (GM). The GM started to receive particular attention in the last years as a target for AD prevention, since increasing evidence indicates that alterations in the GM favor AD development. Bidirectional communication between the central and the enteric nervous system, called gut-brain axes, is largely influenced by GM, and the immune system is a potential key mediator of this interaction. Growing evidence is pointing to the influence of GM in the maturation and activation of host microglia and peripheral immune cells, brain development and cognitive functions. Several recent studies have found abnormalities in GM, namely dysbiosis, in AD patients and mice. Apparently, these alterations, would associate with raptures of the intestinal mucosa and blood brain barrier, with the consequent efflux of bacteria in the circulation and the brain leading to an increase of brain amyloids and the initiation of adverse neuroinflammatory responses. Of note, germ-free, healthy mice implanted with GM derived from AD mice develop memory impairment, brain amyloidosis and neuroinflammation. In contrast treatment of AD mice with wide spectrum antibiotics reduced amyloidosis and gliosis. All this evidence suggests that beside Aβ, new therapeutic targets at both central and peripheral level must be considered. The identification of disease-associated glial signatures and a more careful patient selection would likely increase treatment efficacy. In addition, well established strategies preserving a healthy GM would represent a new and easily accessible road for AD prevention, allowing to overcome the enormous difficulty of recognizing AD at initial stages and to cure it when it is too late.

REDOX DYSREGULATION AND DRUG-INDUCED PSYCHOSIS: UNRAVELLING NEW PATHWAYS AND PHARMACOLOGICAL TARGETS

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BACKGROUND: Recent studies reported the involvement of redox dysregulation, defined as an imbalance between Reactive Oxygen Species (ROS) and their degradation enzymes, in the pathogenesis of drug addiction and its related comorbidities. In particular, a key role has been attributed to the Nicotinamide Adenosine Dinucleotide Phosphate (NADPH) oxidase NOX enzymes that, together with mitochondria, are one of the major ROS producers. Moreover, several evidence reported a strong correlation between drug addiction and the development of psychiatric disorders.
METHODS: In this study, we investigated the role of redox dysregulation in the pathogenesis of drug-induced psychiatric disorders, by firstly using an animal model of drug-induced psychosis and then analyzing human blood samples from drug-addicted subjects with psychiatric comorbidities.
RESULTS: Our results showed a decrease in NOX1 cortical levels, as well as an increase in cortical NOX2 contents and lipid peroxidation, accompanied by an enhancement of indirect oxidative-stress markers levels (8-hydroxy-2-deoxyguanosine- 8OHdG, 4-Hydroxynonenal- 4HNE), in an animal model of early ketamine-induced psychosis. In this regard, the antioxidant treatment with celastrol, a natural compound derived from the root of Tripterygium wilfordii, was able to prevent ketamine-induced redox imbalances, such as the increase in ROS production, lipid peroxidation, and neuroinflammation markers expression, via the enhancement of the antioxidant defenses.
From a translational point of view, we evaluated the expression of ROS producing and degrading enzymes, together with the expression of indirect markers of oxidative stress, in peripheral blood samples of drug-addicted subjects in dual diagnosis with psychiatric disturbances and related non drug-addicted healthy controls. Our results showed an increase of NOX2 and 8OHdG levels, together with lipid peroxidation, in drug-addicted psychotic patients compared to controls.
CONCLUSIONS: Taken together, our data suggest that the modulation of redox system, through the enhancement of the antioxidant defenses, may represent a new pharmacological target to treat drug-induced psychiatric comorbidities.

CHRONIC STRESS EXPOSURE AFFECTS COGNITIVE PERFORMANCE: SEARCHING FOR THE UNDERLYING MOLECULAR MECHANISMS

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INTRODUCTION: Exposure to chronic stress is one of the main environmental factors responsible for the development of major depressive disorders (MDD). Cognitive dysfunctions are an intrinsic characteristic of stress-related pathologies and contribute to the impairment in multiple psychological domains and disruption in instrumental activities of daily living. Moreover, they may also play a critical role in increasing individuals' vulnerability for the first onset and recurrence of depression.

In this context, it is important to understand the molecular mechanisms underlying the relation between impairment in cognition in the different stages of the pathologies and deregulation of emotions that are features of depressive disorders.

The employment of animal models useful for the study of MDD based on the exposure to chronic stress paradigms at adulthood is a valuable approach to characterize the possible molecular pathways altered by prolonged stress exposure in animals showing cognitive deficits, in specific brain regions connected with learning and memory mechanisms, such as the prefrontal cortex and the dorsal counterpart of the hippocampus.

RESULTS: By exposing adult male rats to the chronic mild stress (CMS) paradigm we demonstrated that independently from the development of the anhedonic like behavior, both vulnerable and resilient rats had cognitive deficits in the novel object recognition test, memory decline associated with the "de-novo" protein synthesis at synaptic level in the rat dorsal hippocampus, brain region strictly connected with learning and memory functions.

Furthermore, known that the function of the hypothalamic pituitary adrenal (HPA) axis is impaired in psychiatric disorders and that the glucocorticoid receptors (GR) in the brain are essential for the regulation of memory-related mechanisms, we investigated the changes occurring in the genomic and non-genomic pathways of the glucocorticoid receptor (GR) and their potential involvement in the cognitive impairment caused by the exposure to the CMS. Moreover, we found that chronic stress induced persistent epigenetic modification of genes related to the glucocorticoids signaling in the prefrontal cortex that may be connected with the behavioral deficits we observed in chronically stressed animals.

CONCLUSIONS: These findings suggest that several molecular mechanisms, in specific brain regions, may be involved in the detrimental effect of chronic stress exposure on memory performance, suggesting that pharmacological intervention able to normalize such alterations may be helpful in the improvement of functions that are affected in patients with stress-related disorders.

DRUGS OF ABUSE-INDUCED NEGATIVE EMOTIONAL STATE: INTERACTION BETWEEN SEROTONIN AND GLUTAMATE IN THE TRANSITION TOWARD ADDICTION

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BACKGROUND: Rats lacking the serotonin transporter (SERT$^{-/-}$) show increased sensitivity to environmental stimuli, in line with the personality trait sensory processing sensitivity. This trait, observed in humans and animals extremely vulnerable to both positive and negative stimulations, may lead to increased anxiety and depression-like behavior in response to stressful stimuli as well as increased cocaine intake.
Given the pivotal role of glutamate in environmental sensitivity and in cocaine seeking behaviour, we investigate the expression of proteins implicated in glutamate neurotransmission in reward-related brain areas of naïve and SERT-/- exposed to repeated short-access (ShA, 1h/day) or long-access (LgA, 6h/day) cocaine (COC) or amphetamine (AMPH) self-administration (SA).
METHODS: Male SERT-/- rats, generated by induced mutagenesis, were subjected to COC or AMPH SA through jugular vein catheters. Seven days after surgery, rats were allowed to self-administer COC (0.5 mg/kg/infusion) or AMPH (0.03 mg/kg/infusion) during daily ShA or LgA session, for a total of 17 days. 24 hours following the last SA session, we analysed the expression of glutamate system components in the nucleus accumbens shell (sNAc) and core (cNAc).
RESULTS: SERT-/- rats self-administer more cocaine under both ShA and LgA conditions, while they display increased AMPH intake under LgA, but not ShA, conditions. SERT gene deletion increases the motivational and psychomotor effects of COC and AMPH, respectively.
Deletion of SERT determined an overall reduction of NMDA and AMPA receptor subunits and their scaffolding proteins in the cNAc, mimicking the COC and AMPH-induced changes in wild-type animals. LgA, but not ShA, in SERT-/- rats increased glutamatergic signaling in cNAc, but not in sNAc, suggesting that SERT removal reorganizes the glutamate synapse, contributing to the escalation of cocaine seeking.
CONCLUSIONS: These results suggest that the liability of SERT-/- rats to compulsive COC and AMPH intake may, at least in part, depend upon lack of SERT. Hypersensitivity of the glutamatergic synapse in the cNAc, a subregion involved in the incubation of drug seeking, may contribute to the increased vulnerability to addiction observed in SERT-/- rats. Since enhanced glutamatergic neurotransmission in the NAc has been implicated in the pathophysiology of depressive-like behaviors, our data suggest that serotonin-glutamate interactions may contribute to the negative emotional state observed in drug users after drug discontinuation.
Sponsored by ERANID Grant 'STANDUP' project.

VULNERABILITY AND RESILIENCE TO CHRONIC STRESS ALTER THE RESPONSE TO AN ACUTE NOVEL STRESSOR: INVOLVEMENT OF THE HYPOTHALAMIC-PITUITARY ADRENAL AXIS

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BACKGROUND: Stress is one of the main precipitating factors for psychiatric disorders. However, there are differences in the individual susceptibility to stress, with some individuals displaying vulnerability following stress exposure and others showing resistance to its maladaptive effect. Moreover, the effect of stress may depend on its duration and intensity and exposure to stressful events may alter the response to a subsequent stressor. In this study, we investigated the effect of the exposure to an acute restraint stress (ARS) immediately after 2 weeks of chronic mild stress (CMS) in both vulnerable (anhedonic) and resilient (non-anhedonic) rats, to evaluate the responsiveness of the two subpopulations of rats to the ARS. Moreover, we aimed to establish how long the anhedonic phenotype induced by CMS takes to normalize and we studied the response to these rats to the ARS once recovered. At molecular level, we focused on the contribution of the hypothalamic-pituitary-adrenal (HPA) axis functionality, the primary system mediating the stress response, by evaluating the corticosterone levels and the expression of genes responsive to the glucocorticoid receptor in dorsal (dHip) and ventral (vHip) hippocampus, brain regions strictly implicated in mood disorders and related to the emotional behavior.

METHODS: Adult male Wistar rats were exposed for 14 days to CMS and subjected to ARS (1h) after the last episode of CMS, whereas a subgroup of vulnerable animals was left undisturbed until the recovery of the behavioral phenotype and than acutely stressed with one hour of ARS. Sucrose consumption test was performed at weekly intervals to assess the development of anhedonia. dHip and vHip were dissected, frozen on dry ice and stored at -80°. Total RNA was isolated and the analyses of mRNA were carried out by real time-PCR, whereas corticosterone (CORT) plasma levels were measured with a commercial ELISA kit.

RESULTS: CMS induced anhedonia in a subpopulation of stressed rats (vulnerable), while the remaining were resilient since they consumed the same amount of sucrose as controls. The anhedonic phenotype in vulnerable rats normalized after 3 weeks of rest from stress procedure. We found that CORT levels were increased in vulnerable but not in resilient rats and that the ARS induced an upregulation of its plasma levels in controls as well as in resilient animals. In vHip the acute challenge upregulated Gadd45β, Sgk1 and Dusp mRNA levels in control, resilient and vulnerable+washout rats, effects completely blunted in vulnerable animals. By contrast in dHip all the genes were positively modulated by the restraint independently from CMS pre-exposure.

CONCLUSIONS: These data suggest that CMS altered the ability of the HPA axis to deal with a challenging condition and indicate a different implication of the two hippocampal sub-regions in modulating the effect of a novel acute challenge in vulnerable and resilient animals.

PHARMACOLOGICAL TARGETING OF KEY PROXIMAL DRIVERS OF METAFLAMMATION IN DIET-INDUCED METABOLIC DISEASES

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Low-grade, chronic inflammatory response, known as "metaflammation", exerts a key role in promoting diet-related metabolic disorders. Causative relationships between exposure to hypercaloric diets and quantitative and qualitative changes in intestinal commensal bacteria communities, which contribute to the over-production of inflammatory mediators, driving the development of dysglycemic and dyslipidemic states, have been recently documented. We contributed to demonstrate the peculiar impact of the heterogeneous class of diet-derived advanced glycation end products (AGEs) on "metaflammation". Here we will discuss our findings on the molecular pathways activated by dietary AGEs chronic exposure at organ and tissue levels, and the potential role of salivary AGEs as early and easily accessible biomarkers of risk factors for diet-related diseases. Treatments that halt or induce regression of metaflammation have potential to provide an immense clinical, social and economic benefit. However, only limited experience is available regarding the identification of inflammatory pathways activated by the metabolic, biochemical and hemodynamic derangements known to exist in metabolic diseases. Using in vivo models of diet-induced metabolic diseases, we recently demonstrate the key pathogenetic role of the pro-inflammatory NLRP3 inflammasome pathway as well as the contribution of Annexin A1, an endogenously produced anti-inflammatory protein, in dampening the development of both the diabetic phenotype and the associated hepatic steatosis and nephropathy. We also extended our investigation to the potential repurposing of Jak inhibitors and BTK inhibitors, already approved for rheumatoid arthritis and chronic lymphatic leukaemia, respectively, within the therapeutic context of diet-related metabolic diseases. Here our findings will be presented at the light of most recent literature evidence to critically discuss the cross-talk mechanisms linking pharmacological modulation of metaflammation-related signalling pathways to the improvement in metabolic cascades as well as the attractive perspective to identify approved medications potentially suitable for drug repurposing into the treatment of metabolic inflammation, in diseases such as type-2 diabetes.

SOCE CHANNELOPATHIES: A FOCUS ON TAM

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Store-Operated Ca2+ Entry (SOCE) is a skeletal muscle Ca2+-entry process activated by the depletion of intracellular stores. It is a pivotal mechanism in cellular calcium homeostasis (Cho et al., 2017) and it is coordinated by the stromal interaction molecule 1 (STIM1) and ORA1 (Baba et al., 2006) proteins. STIM1 is the main Ca2+ sensor in the endoplasmic reticulum and acts as a key factor in SOCE mechanism. Upon Ca2+ store depletion of the sarcoplasmic reticulum, STIM1 is activated, then it aggregates and interacts with the tetrameric Ca2+ -permeable channel of the surface membrane, ORAI1, forming an active and selective channel for Ca2+ entry from the extracellular environment (Cho et al., 2017). SOCE terminates following the reuptake of Ca2+ by sarco/endoplasmic reticulum Ca2+-ATPases (SERCA protein) and, upon store refilling, STIM1 and ORAI1 revert to their diffuse distributions. Physiologically, SOCE in skeletal muscle has the crucial role to regulate long-term skeletal muscle functions such as muscle development, growth and cellular remodelling, via the activation of various Ca2+-dependent pathways and via changes of intracellular Ca2+ levels (Skiber et al., 2011, Kiviluoto et al., 2011). SOCE gained increasing attention when the lack of, or mutation in, STIM1 and/or Orai1, and the consequential altered calcium influx, was suggested to have severe consequences for muscle function. Gain-of-function (GoF) or Loss-of-function (LoF) mutations in STIM1 and ORAI1 have been linked to multiple human diseases including various forms of immunodeficiency and myopathy. Particularly, GoF mutations in both STIM1 and ORAI1 genes, which result in constitutively activated SOCE, were found to be linked to three overlapping diseases: Tubular Aggregate Myopathy (TAM)(Bohm et al., 2013; Endo et al., 2015), Stormorken Syndrome (Misceo et al., 2014) and York Platelet Syndrome (Markello et al., 2015).

TAM is a hereditary ultra-rare progressive muscle disorder, clinically heterogeneous and involving either proximal muscle weakness, muscle cramps or myasthenic features. Despite the clinical heterogeneity of this disorder among TAM patients, a consistent histopathological feature is represented by the presence of tubular aggregates (TAs), which are inclusions within muscle fibers originated from sarcoplasmic reticulum likely due to altered Ca2+ homeostasis (MH et al.,1998, Bohm et al., 2018). TAM is caused by GoF mutations in STIM1 (Bohm 2013) or ORAI1 (Nesin 2014). So far there is no cure for TAM and there is little information in literature regarding a therapy of this disorder. Analyzing myoblasts and myotubes deriving from TAM patients' biopsy carrying Leu96Val STIM1 mutation, we demonstrated that STIM mutation lead to an increased resting Ca2+ concentration and an augmented SOCE activation. Importantly, considering that post-natal myogenesis relies on Ca2+influx through SOCE process, we demonstrated that differentiating Leu96Val STIM1 myoblasts persisted in a mononuclear state and resulted in a reduced number of multinucleated myotubes with distinct morphology and different geometry of mitochondrial network. Our study provides novel evidences about the correlation between SOCE activation, mitochondrial sufferance and defecting myogenesis in TAM suggesting that STIM1 and/or ORAI1 can be considered promising target in drug discovery due to their key role in calcium homeostasis.

GENDER ANALYSIS ON COVID-19 DATA IN PIEMONTE: THE VIRUS PREFERS MEN

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BACKGROUND: Several important gender differences in clinical manifestation and response to treatments for many diseases are known since a long time, although they continue to be underestimated and not really considered in clinical practice. The recent Covid-19 pandemic has provided a further evidence of the importance of gender-based approach. Many fields of study, such as medicine, law, psychology, sociology, as well as sciences applied to data analysis, highlight the importance of a gender perspective in studying the effects of the Covid-19 pandemic, and the actions to contain it. The gender factor is present also in this health crisis: Covid-19 mainly affects men, with a worse symptomatology and a general exacerbation of the disease. Mechanisms underlying these gender differences are varied (including socio-behavioral, immune and viral factors) and not yet fully clarified. Aim of the work was to analyze data on Covid-19 testing in Piedmont region, northwest of Italy, from people admitted to Amedeo di Savoia hospital, regional referral center for infectious diseases.

METHODS: Data are referred to a period of two months (March-April 2020), i.e. the beginning of pandemic. We performed analysis on 38018 testing records: 77.99% was suitable (N = 29653) and 22.01% (N = 8365) was not evaluable (empty, not received or still awaiting results).

RESULTS: Among suitable sample, N = 21466 was negative for Covid-19 testing (about 72.3%) and N = 8187 was positive for Covid-19 testing (about 27.7%). Of suitable sample, N = 26361 was attributable to unique subjects; on this number we performed disaggregation by sex: 10208 were males (about 38.7%) and 16153 females (about 61.3%). Median age for males was 57 (range 0-101); median age for females was 54 (range 0-111). At the symposium we will show analyses performed on the suitable sample concerning local distribution of data, recurrence of Covid-19 testing in the same person and correlation of testing with motivation for hospital admission (i.e. first symptoms, health surveillance, disease monitoring), always in a gender perspective.

CONCLUSIONS: Time is ripe: gender approach should be recognized as pivotal part of the medical knowledge. Medicine without any categorization by sex and gender is methodologically incorrect. National and international health policies are still likely to be flagged as partial and discriminatory if they do not take account of the cultural and scientific implication of gender-specific medicine. A gender-based approach to clinical practice also in the context of this pandemic seems to be mandatory, as it could significantly contribute to health promotion by improving the effectiveness of diagnostic and/or therapeutic approaches and, therefore, leading to important benefits primarily for the patients but also for the sustainability of the National Health System.

ADVERSE REACTIONS TO HERBAL EXTRACTS USED FOR OVERWEIGHT AND OBESITY

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BACKGROUND: The prevalence of overweight and obesity is increasing worldwide. Lifestyle change is a critical aspect in addressing the problem; nevertheless, sometimes a pharmacotherapeutic approach is also needed. In Italy, orlistat, liraglutide, bupropion/naltrexone are approved for the treatment of obesity and overweight, but the adverse effects limit their use. An alternative strategy is represented by the use of dietary supplements and galenic preparations based on herbal extracts. These products have no approved therapeutic effects, but they are perceived as safe due to their natural origin. With the increase of natural products popularity, also reports of adverse events associated with them became more frequent. Therefore, monitoring their safety is an urgent need.

METHODS: A comprehensive search was made using PubMed and SCOPUS electronic databases and selecting English as preferred language, although neither language limitations nor filters were applied. For more specific requirements, Google Scholar was considered too. The following searching keywords and their combinations through the Boolean logical operators were used: "overweight", "obesity", "phytochemicals", "nutraceuticals", "medicinal plants", "herbal extracts", "botanicals", "weight loss", "weight control", "adverse effects", "side effects", "adverse reaction", and "safety".

RESULTS: Several herbal preparations used for weight control have been associated with adverse reactions (ARs). Particularly, cardiovascular ARs have been reported for *Citrus aurantium*, owing to its active substance synephrine, although concomitant use of other compounds (e.g., caffeine) could contribute. Based on this evidence, the Ministry of Health has established that synephrine is not allowed in food supplements and in magistral galenic preparations. Conversely, *C. aurantium* extract is allowed in both of them, but in food supplements the daily intake of synephrine cannot be higher than 30 mg. Green tea (*Camellia sinensis*) has been associated with hepatic ARs. Most reports of hepatotoxicity were related to products containing extracts enriched in catechins. These compounds are believed the culprit for the liver injury, but recently association with HLA-B*35:01 polymorphism has been showed. Liver damage, sometimes requiring transplantation, has been also reported for *Garcinia cambogia*. Although some green tea-based products have been recalled, *G. cambogia* has never been banned; indeed, often products involved in the ARs contained other components, so making difficult to establish a causal relationship. Recently, turmeric (*Curcuma longa*) supplements used for slimming purposes have been involved in liver injury. In many products, turmeric was present in particular formulations (inclusion in liposomes and/or nanoparticles, combination with piperine) able to increase the curcumin bioavailability. Furthermore, in some cases, products contained enriched extracts (*C. longa* dry extract with 95%). Such preparations resemble a pure substance rather than a traditional preparation, so their safety is completely unknown.

CONCLUSIONS: Serious ARs have been reported from the use of herbal weight loss preparations, although to establish a causal relationship is often difficult. Considering the poor clinical evidence supporting their use, the safety concerns become more relevant. In this context, since 2017, as established in a Decree issued by the Ministry of Health (DM 31/03/2017), the Italian National Institute of Health has the task to monitor the use and safety of magistral prescriptions for weight control (often containing herbals) in order to point out potential risks associated with their consumption.

ADENOSINE ORCHESTRATES OLIGODENDROGLIOGENESIS AND MYELINATION IN VITRO: FOCUS ON A2A AND A2B RECEPTORS

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BACKGROUND: Oligodendrocyte-formed myelin sheaths allow fast synaptic transmission in the brain and their degeneration leads to demyelinating diseases such as multiple sclerosis (MS). Remyelination requires the differentiation of oligodendrocyte progenitor cells (OPCs) into mature oligodendrocytes (OLs). Adenosine and its receptors (A1, A2A, A2B and A3 receptors: A1R, A2AR, A2BR and A3R) are crucial mediators in remyelination processes. Remarkably, A1Rs facilitate OPC maturation (Stevens et al., Neuron 2002 Dec 5;36(5):855-68) and migration (Othman et al., Glia 2003 Nov;44(2):166-72) whereas the A3Rs initiates apoptosis in OPCs (Gonzalez-Fernandez et al., Glia 2014 Feb;62(2):199-216). Sphingosine kinase/sphingosine1-phosphate signaling axis (SphK/S1P) are also important players in remyelination and the S1P receptor modulator fingolimod (FTY720), the first oral agent for MS treatment, facilitates OPC differentiation (Miron et al., Ann Neurol 2008 Jan;63(1):61-71).
METHODS: By using patch-clamp recordings coupled to quantitative Real Time PCR (qRT-PCR), Western Blot (WB) and small-interference RNA (siRNA) techniques in primary rat OPC cultures, we investigated the role of A2ARs and A2BRs in modulating voltage-dependent K+ currents necessary to cell differentiation and the expression of myelin markers in OLs like myelin associated glycoprotein (MAG) and myelin basic protein (MBP).
RESULTS: We demonstrated that adenosine the A2AR-selective agonist CGS21680 (100 nM) inhibits in vitro OPC maturation by decreasing tetraethylammonium (TEA)-sensitive K+ currents (IK) and the effect is prevented by the A2AR antagonist SCH58261 (100 nM). Similarly, the prototypical A2BR agonist BAY60-6583 reduces IK and also IA (4-aminopyridine-sensitive, transient K+) conductances. The effects of BAY60-6583 are mimicked by a new, not commercially available, A2BR agonist P453 and are prevented by the adenylate cyclase activator forskolin (20 μM) and by the A2BR antagonist MRS1706 (10 μM). Of note, WB analysis showed that SphK1 phosphorylation in OPC cultures is enhanced by BAY60-6583, whereas qRT-PCR demonstrated that MAG and MBP expression after 7 days of in vitro differentiation to OLs are significantly inhibited by A2BR agonists. Furthermore, A2BR upregulation during oligodendrogliogenesis is completely prevented by the SphK1/2 inhibitor VPC96047 (0.5 μM). Finally, OPCs transfected with the A2BR-selective siRNA showed increased cell maturation, decreased SphK1 expression and enhanced S1P lyase levels.
CONCLUSIONS: Our data show that A2AR and A2BR activation inhibit TEA-sensitive K+ currents in cultured OPC and prevents their differentiation into mature OLs and may represent a valuable target in demyelinating pathologies such as MS. Concerning the A2BR subtype, an interaction with the SphK/S1P pathway is involved in these effects.

TARGETING ADENOSINE TONE IN NIEMANN PICK TYPE C DISEASE

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BACKGROUND: Niemann Pick type C (NPC) disease is a rare, genetic and fatal disorder caused by the accumulation of unesterified cholesterol, sphingolipids and other lipids in cells.In the Central Nervous System (CNS) the disease is characterized by a massive loss of Purkinje neurons and by neuroinflammation, dysmyelination and Alzheimer disease-like lesions, which cause serious neurological symptoms such as ataxia, tremor, epilepsy and depression.So far, only one drug is approved in EU (miglustat), thus the discovery of new therapeutic targets is urgent.We focused our attention on adenosine, a homeostatic modulator of neuronal and non-neuronal cell functions, which levels are finely tuned by the orchestrated action of enzymes and transmembrane transporters (ENTs) that ensure the extracellular level necessary to exert its proper signaling.Interestingly, an imbalance of the adenosine signaling has been reported to occur in NPC (namely, reduced levels of adenosine have been found in the brain of NPC mice), and our working hypothesis is that targeting such an imbalance could represent a promising therapeutic approach.In line with this hypothesis, we found that the treatment of NPC1-/- mice with the compound T1-11, able to weakly stimulate the adenosine A2A receptor and to increase the level of adenosine in the brain by inhibiting its transporter ENT1, significantly ameliorated their cognitive deficits, Purkinje neuron loss, and extended their survival. Considering that T1-11 is not an approved drug and that clinically used ENT1 inhibitors exist, we wanted to confirm our data by testing one of them; we focused on dipyridamole (DIP), which is used in humans to reduce platelet aggregation and was already tested in some pathologies of the CNS such as schizophrenia and restless leg syndrome.

METHODS: We treated NPC1 fibroblasts from three different patients (GM17911, GM17926, GM18415) with DIP at the concentration of 20μM for 48h and evaluated its effect on cholesterol accumulation by Filipin III fluorescence (which represent the main criterion to identify new potential drugs for NPC).

RESULTS: Our results clearly demonstrated a significant reduction ($p<0.05$) in mean fluorescence intensity (MFI) indicative of a reduced cholesterol load in all the three samples examined: MFI: 90.50±15.64 in Veh- vs. 56.80±8.86 DIP-treated GM17911 cells, (n=6 independent experiments); MFI: 98.37±8.33 in Veh- vs. 65.10±10.66 DIP-treated GM17926 cells (n=3 independent experiments); MFI: 123.1±16.07 in Veh- vs. 73.88±11.35 DIP-treated GM18415 cells (n=3 independent experiments).

CONCLUSIONS: These data converge to strongly support that an impairment in adenosine signaling occurs in NPC and that increasing its levels by the inhibition of ENT1 transporter may represent a new approach to treat the disease.The next step will be to evaluate efficacy of DIP in in vivo models of NPC.

IMMUNOPHARMACOLOGICAL MODULATION IN ALLERGIC DISEASES: CAN WE BE HAPPIER THAN WE WERE?

Francesca Levi Schaffer

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Allergic inflammation (AI) is a reaction occurring in asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, etc. AI is triggered by the cross-linking of IgE and FcεRI on the surface of mast cells, the main effector cells of allergy, which orchestrate the early phase of AI. Subsequently, the late phase takes place in which other cells, notably the eosinophils, are recruited. The late phase is usually followed by resolution, but most of the time allergic diseases undergo chronicization, which is still an unmet clinical need. Over the years, several approaches have been developed to treat allergic diseases but mostly their symptoms. More recently, the use of monoclonal antibodies such as anti-IgE, anti-IL-5, anti-IL-5R, and anti-IL-4/IL-13Rα has brought to the concept of personalized therapy also of allergic diseases and especially of asthma and atopic dermatitis. Together with that our understanding of the pathologic mechanisms of asthma and atopic dermatitis, both characterized by allergic inflammation and labelled by WHO unmet clinical needs, has greatly improved. However, it is evident that both diseases present with high heterogeneity, which complicates the diagnosis and the therapeutic approach of the patients. Therefore, there is a need for better therapeutic targets, and we propose inhibitory receptors, such as CD300a and Siglec-7, and specialized pro-resolving lipid mediators, such as LXA4-B4 and RvD1, as novel approaches to possibly treat chronic allergic inflammation. In my lecture I shall present some in vitro and pre-clinical data on the potential of activating monoclonal antibodies for inhibitory receptors and of pro-resolving lipid mediators in prevention and treatment of allergy.

LONG-TERM EXPOSURE TO COCAINE DURING ADOLESCENCE MODULATES THE REWARDING THRESHOLD FOR COCAINE IN ADULTHOOD

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INTRODUCTION: Adolescence is indeed a period of life characterized by unique sensitivity to drug abuse and, therefore, it represents a risk factor for drug addiction. It has been demonstrated that adolescent exposure to cocaine leads to increased drug use in adulthood, although evidence exists showing a reduction of incubation of cocaine seeking in rats that self-administered cocaine during adolescence. Accordingly, we decided to investigate 1) whether exposure to cocaine during adolescence may modulate its rewarding properties during adulthood; 2) whether the modality of drug exposure might influence such effect and 3) whether behavioral changes in adulthood could be reflected and/or be sustained by changes in neuroplasticity.

MATERIALS AND METHODS: A yoked-control operant paradigm was used in which one rat controls cocaine delivery (SA) and the other two passively received cocaine (YC) or saline (YS). Rats were exposed to cocaine (1.0 mg/kg/infusion) self-administration on a FR1TO20s or vehicle from PND37-PND50. Following 50 drug-free days, animals underwent a conditioned place preference (CPP) protocol in an unbiased apparatus (PND105-PND114). Rats were then sacrificed 96 hours after the evaluation of CPP and the nucleus accumbens (NAc, including both shell and core subregions) dissected. GluA2-lacking Ca2+-permeable AMPA (CP-AMPA) receptors formation, a signature of drug-associated behaviors, were investigated by means of western blot.

RESULTS: During adolescence cocaine produced robust reinforcing effect as determined by self-administration experiments. Cocaine self-administration during adolescence increases the rewarding threshold in adulthood, CPP for cocaine was observed at the higher (20 mg/kg), but not at the lower (10 mg/kg), dose employed. an effect that does not depend upon the modality of drug exposure (SA vs YC). Of note, this appears to be due, at least in part, upon the formation of CP-AMPA receptors and the consequent increase of αCaMKII activity in the NAc.

CONCLUSIONS: To sum up, our results indicate that exposure to cocaine during adolescence might cause tolerance to a rewarding dose of cocaine injected in adulthood, leading to an increase in the rewarding threshold necessary to drive CPP. We hypothesize that the formation of GluA2-lacking Ca2+-permeable AMPA receptors and increased activity of αCaMKII may represent critical mechanisms to drive CPP after exposure to cocaine self-administration during adolescence.

BIOMARKERS AND PRECISION THERAPY FOR PRIMARY IMMUNODEFICIENCIES: AN IN VITRO STUDY BASED ON INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS

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INTRODUCTION: Ataxia telangiectasia (AT) and Aicardi–Goutières syndrome (AGS) are primary immunodeficiencies with common neurological symptoms caused by an inappropriate immune activation. Both syndromes are associated with defects in DNA repair mechanisms, cell cycle progression and regulation. Accessible cures are mainly supportive and the prognosis is very poor. Induced pluripotent stem cells (iPSCs) are obtained by reprogramming patient somatic cells, preserving the donor individual genetic heritage and creating patient-specific disease models, useful to understand the underlying pathophysiologic mechanisms of diseases, pharmacological effects of drugs potentially effective on them and to personalize the therapy of the patient.

RESULTS: The purpose of the work was to analyse the cytotoxic effects of a group of immunomodulatory drugs already clinically used or potentially effective in AT or AGS employing iPSCs of patients affected by these diseases. iPSCs were obtained by reprogramming AT and AGS patients' cells and, as a control, the BJ normal human fibroblast line, using Sendai virus. Cytotoxicity of dexamethasone and mepacrine, drugs suggested to treat respectively AT and AGS was examined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after 72 hours of iPSCs exposure. Data were acquired also for other immunomodulators (thioguanine, mercaptopurine, thalidomide, and lenalidomide) potentially effective in these disorders. Relative expression of genes implied in the investigated drug pathways were evaluated. AGS7-derived iPSCs exhibited higher viability with respect to the BJ-iPSCs control when treated with low doses of mepacrine. For this line also higher expression of cyclic guanosine monophosphate–adenosine monophosphate synthase, which is the main target for mepacrine action, with respect to the BJ control was identified. AGS7-derived iPSCs were also more sensitive to thioguanine, while AGS2 and AT iPSCs were less sensitive to this treatment than the BJ-iPSC. All iPSCs were equally sensitive to mercaptopurine and resistant to dexamethasone, thalidomide, and lenalidomide.

CONCLUSIONS: This study sets an innovative patient-specific in vitro model that is functional to evaluate the mechanisms of drugs used or potentially effective in AT and AGS treatment.

DOES MANIPULATING THE GUT MICROBIOTA HOLD THE KEY TO VISCERAL PAIN RELIEF?

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BACKGROUND: Chronic abdominal pain affects a large percentage of patients who have suffered an intestinal injury and represents a clinical burden due to the lack of effective and safe treatments. Recent findings highlight dysbiosis of gut microbiota as a common denominator in gastrointestinal disorders characterized by persistent pain. Our research aimed to evaluate the power of gut microbiota as a visceral sensitivity modulator and, consequently, the relevance of its manipulation as a therapy for post-inflammatory abdominal pain.
METHODS: Colitis was induced in rats by the intra-rectal injection of 2,4-dinitrobenzene sulfonic acid (DNBS). The effect of faecal microbiota transfer (FMT) from viscerally hypersensitive DNBS and naïve donors was evaluated in control rats after an antibiotic-mediated microbiota depletion. The effects of FMT on visceral sensitivity to colorectal distension, gut histology, permeability and immune response were studied. Short-chain fatty acids and monoamines within the colon, as well as plasma tryptophan metabolites, were analysed by chromatography. The reverse protocol was used to evaluate the therapeutic effect of FMT from healthy rats to DNBS treated animals.
RESULTS: FMT from DNBS donors induced a long-lasting visceral hypersensitivity in control rats. Pain threshold trend correlated with major modifications in the composition and structure of the gut microbiota at phylum and family levels, evaluated by 16S rRNA sequencing. Acetic acid was significantly increased in the recipients FMT from DNBS donors. Gut cytokine profile, as well as tryptophan metabolism were similarly altered after FMT from both DNBS and naïve donors. By contrast, no significant alterations of colon histology, permeability and monoamines levels were detected. Finally, a counteraction of persistent visceral pain was achieved after 4 cycles of FMT from healthy donors to DNBS-treated animals.
CONCLUSIONS: These results provided novel insights about the relationship between intestinal microbiota and visceral hypersensitivity, highlighting the therapeutic potential of microbiota manipulation to treat post-inflammatory abdominal pain.

IMMUNE DYSREGULATION IN CARDIOVASCULAR DISEASE - FROM NOVEL MECHANISMS TO THERAPEUTIC TARGETING

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Data from basic science experiments and observational studies in humans are overwhelmingly supportive of the causal role of immune-inflammatory response(s) at the core of cardiovascular diseases. The CANTOS (Canakinumab Anti-inflammatory Thrombosis and Outcomes Study) and the COLCOT (Colchicine Cardiovascular Outcomes Trial) studies have recently demonstrated the potential of targeting inflammation for reducing secondary cardiovascular events. However, still, the adoption of anti-inflammatory therapies in patients with residual inflammatory risk is limited by the risk of impaired host defense.
At the same time, technological advances in particular in characterizing molecular heterogeneity at the single-cell level have enabled a deeper understanding of the biological diversity of immune cells present in the vascular tissues.
In this talk, I will discuss key therapeutic targets under investigation in my lab for the treatment of vascular inflammation, placing basic research in a wider clinical perspective, as well as identifying outstanding questions.

THE ROLE OF THE ENDOCANNABINOID SYSTEM IN SUBSTANCE USE DISORDERS

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BACKGROUND: The brain's endocannabinoid system (ECS) consists of endogenous lipid-based retrograde neurotransmitters that modulate neural activity by binding to cannabinoid receptors. Preclinical studies have found that the ECS plays a critical role in normal brain development and is implicated in reward regulation and addiction-like behaviors. Moreover, epidemiological studies have found a link between early cannabis use and increased risk for psychopathology, including substance use disorders (SUDs). However, to date, no genome-wide association studies (GWAS) have uncovered a significant link between ECS-related genes and substance abuse in humans. This points to a complex system that warrants additional investigation in order to understand how the ECS modulates the risk for SUDs and be able to turn this knowledge into novel pharmacological interventions or preventive measures.
METHODS: To this end, we posed two questions: (1) Since epidemiological data suggest a critical role for the developmental period during which the brain is exposed to cannabis, can we gain novel molecular insights into the modulatory role of the ECS by comparing adolescent versus adult preclinical models of addiction? – and (2) Since GWAS of substance use have failed to uncover significant loci in ECS-related genes, can this be attributed to "missing heritability" due to understudied gene-by-environment interactions? To provide answers to these

questions, we utilize a translational approach to examine (1) whether cannabinoid exposure in rat adolescence (versus adulthood) alters the molecular, epigenetic and behavioral response to cocaine, and (2) whether functional single nucleotide polymorphisms (SNPs) in the human cannabinoid receptor 1 gene (CNR1/CB1R) interact with stressful life events to predict alcohol abuse.
RESULTS: Rodent behavioral data confirm an association between adolescent (but not adult) cannabinoid exposure and increased sensitivity to cocaine's stimulatory effects, which associates with distinct molecular and epigenetic changes in key areas of the mesocorticolimbic system. Moreover, human data provide evidence for a gene-by-environment interaction between CNR1 and stressful life events, which predicts an increased risk for problematic alcohol use.
CONCLUSIONS: Our results support an involvement of the endocannabinoid system in substance use disorders. Guided by these findings, we also present a newly designed project that employs a translational GWAS approach, including genome-environment-wide interaction studies (GEWIS), in order to study the underlying genetic architecture of substance use.

EARLY MICROGLIAL RESPONSE TO Aβ AS A TARGET FOR NEUROPROTECTION

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INTRODUCTION: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that develops slowly over the years, remaining asymptomatic for up to two decades before diagnosis. By this time, neurodegeneration is advanced and chances for treatment reduced. Abnormal accumulation of oligomeric beta-amyloid peptide (Aβ), in the years that precede symptomatic appearance, has been proposed to trigger neurotoxic and inflammatory events. Interestingly, glial cells initially respond to abnormal Aβ levels with the activation of pathways aimed at opposing both its buildup and neurotoxicity. Microglia in particular, are the main effectors of Aβ clearance and can release neuroprotective cues through phenotypic polarization towards a "M2-like" phenotype. It is then predictable that microglia represent an important early target for strategies to enhance/prolong its beneficial functions and halt/delay pro-inflammatory polarization. We studied the role of microglia in different models mimicking the early phases of Aβ build-up, to identify mediators of the anti-inflammatory phenotype acquisition as well as pharmacological interventions affecting microglial states of activation.
RESULTS: In organotypic hippocampal cultures (OHC) exposed to low concentrations of Aβ oligomers BDNF expression was significantly increased and microglial cells were relocated to the sites of Aβ deposition at early time points. Neuronal cells concurrently underwent a compensatory increase of pre-synaptic vesicle component synaptophysin (SYP), which appeared significantly reduced at later time points, coincident with increasing neuronal death. In cell cultures, only microglia responded to Aβ with increased BDNF expression. Addition of Aβ to neuronal cells in conditioned medium from Aβ-activated microglia was sufficient to induce the compensatory increase of SYP and delayed neuronal death. Given its established protective role in neurodegenerative conditions, including AD, the deacetylase SIRT1 was examined as a candidate mediator of microg-

lial protective polarization in human HMC3 microglia. Low concentrations of Aβ rapidly but transiently increased expression, nuclear localization and activity of SIRT1. The enzyme directly mediated the increase of BDNF expression, with involvement of the AMP-activated protein kinase pathway. At later experimental time points of Aβ treatment, when SIRT1 levels dropped, microglial expression of the neuroinflammatory marker NF-kB appeared increased. Finally, melatonin could beneficially affect microglia supporting its M2-like state for a prolonged time.

CONCLUSIONS: Our studies provide evidence for an early beneficial role played by microglia in AD and point to the involvement of the pAMPK-SIRT1-BDNF axis. This may represent a valuable therapeutic target for early pharmacological intervention aimed at sustaining protective microglial activation.

ANTI-VEGFR-1 IMMUNOBLOCKING RELIEVES NEUROPATHIC PAIN MULTIPLE SCLEROSIS PATIENTS AND POSSIBLE CLINICAL IMPLICATIONS

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BACKGROUND: The vascular endothelial growth factor A (VEGF-A) has emerged as a biomarker of neuropathy but the VEGF-A-dependent pain signaling needs to be investigated for individuating new therapeutic approaches.

METHODS: CD-1 male mice were intrathecally injected with 3, 10 and 30 ng/5 µl of recombinant ligands VEGF165b, PlGF2 and VEGF-E and with the specific monoclonal antibody anti-VEGF-Rs (D16F7 anti-R1, 5 µg; DC101 anti-R2, 100 pg – 6 ng). The mice pain threshold was measured by the Cold Plate test up to 6h after treatment. The same ligands were administered in mice after the silencing of VEGF-R1 and VEGF-R2 by specific siRNAs. Therefore, a knock down of astrocytic VEGF-A by a spinal viral injection of AAV-GFAP-shRNA was performed in oxaliplatin-treated mice. Moreover, immunohistochemical analysis for VEGF-R1 and VEGF-A localization in the CNS were performed.

RESULTS: In mice the intrathecal infusion of VEGF-A (VEGF165b isoform) induced a dose-dependent noxious hypersensitivity. This effect was mediated by the VEGFR-1 as demonstrated by behavioral analysis with selective agonists, receptor blocker monoclonal antibodies and receptors' silencing by siRNA against VEGFR-1 or VEGFR-2. In electrophysiological studies, VEGF-A strongly stimulated the spinal nociceptive neurons activity through VEGFR-1. In the dorsal horn of the spinal cord,

immunofluorescence staining and confocal microscopy analysis indicated that VEGF-A was found to increase in astrocytes of animals affected by oxaliplatin-induced neuropathy and that VEGFR-1 was mainly detected in neurons, suggesting a VEGF-A-mediated astrocyte-neuron cross-talk through VEGFR-1 in neuropathic pain pathophysiology. Accordingly, the selective knockdown of astrocytic VEGF-A by intraspinal injection of shRNAmir blocked the development of oxaliplatin-induced neuropathic hyperalgesia and allodynia. Interestingly, the novel anti-VEGFR-1 monoclonal antibody D16F7 reverted oxaliplatin-induced neuropathic pain by both intrathecal and systemic administrations. Besides, D16F7 effectively relieved hypersensitivity induced by other chemotherapeutic agents, such as paclitaxel and vincristine. Therefore, based on these finding and on its previously described anti-tumour efficacy, D16F7 might represent a suitable candidate for the relief of neuropathic pain in cancer patients receiving neurotoxic chemotherapeutic agents.

CONCLUSIONS: astrocyte-released VEGF-A is a new player in the complex neuron-glia network that oversees physiological and pathological pain and VEGFR-1 blockade by D16F7 is an innovative strategy to counteract chemotherapy-induced neuropathic pain.

IN-VITRO AND IN-VIVO STUDIES DEPICT METABOTROPIC GLUTAMATE RECEPTOR 5 AS A POTENTIAL PHARMACOLOGICAL TARGET TO MODULATE DISEASE PROGRESSION IN ALS

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is fatal neurodegenerative disease due to a progressive degeneration of motor neurons (MNs). The aetiology is still largely obscure, and several mechanisms have been proposed, including glutamate (Glu)-mediated excitotoxicity. In this context, group I metabotropic Glu receptors (mGluR1/5) play an active role, since their expression and functions are altered, especially in glial cells. Moreover, we previously showed that group I mGluRs sustains the excessive Glu release in the spinal cord of the SOD-1G93A mouse model of ALS, and this alteration is already present at the onset of the pathology.

METHODS: We first investigated in-vivo the effect of mGlu5 receptor genetic ablation in SOD1G93A mice (SOD1G93A-mGluR5-/-). We then tested in-vivo the pharmacological blockade of mGluR5 by oral administration of the mGluR5 negative allosteric modulator CTEP (4 mg/kg every 24h, from 90 days of life). Finally, we studied in-vitro the effects of the mGluR5 genetic downregulation, on the reactive phenotype of spinal cord astrocytes cultured from symptomatic SOD1G93A mice. Histological,

functional and biochemical experiments have been performed to characterise astrocytes and their cytotoxicity towards MNs.
RESULTS: The in-vivo genetic ablation of mGluR5 demonstrates that SOD1G93A-mGluR5-/- mice showed a delayed disease onset and a significant prolonged survival probability. These effects were paralleled by a significant MNs preservation, a decreased astrocyte and microglia activation and by a normalization of the excessive Glu release, compared to age matched SOD1G93A mice.
Subsequently, we tested the pharmacological modulation of mGluR5 by CTEP. Behavioural studies showed that, as for the genetic ablation, also CTEP in-vivo treatment significantly slowdown the clinical progression of the pathology and increased the survival probability in SOD1G93A mice. Moreover, we also observed a reduced glial activation and a significant MNs preservation.
In-vitro experiments with primary spinal cord astrocytes showed that the elevated cytosolic calcium concentration as well as the over expression of astrogliosis markers (GFAP, S100, Vimentin, NLRP3) were significantly reduced in cells genetically lacking the mGluR5 (SOD-1G93A_mGluR5+/-) compared to SOD1G93A astrocytes. Most importantly, the modulation of the reactive phenotype in SOD1G93A_mGluR5+/-astrocytes translates into a reduced release of neuroinflammatory cytokines (IL1b, IL-6, TNFa), improved bioenergetics and a less toxic effect toward co-cultured MNs.
CONCLUSIONS: Our results demonstrate that a lower constitutive level of mGluR5, or the pharmacological blockade of the receptor by CTEP, has a positive clinical outcome in SOD-1G93A ALS mice. The in-vivo effects can be mainly ascribed to a reduced reactive astrogliosis, supporting the role of mGluR5 as a therapeutic target to obtain a shift from a pathological toward a less noxious phenotype of astrocytes in SOD1G93A mice.

ACTIVITY-BASED ANOREXIA ALTERS THE STRESS RESPONSE IN ADOLESCENT FEMALE RATS

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INTRODUCTION: Anorexia Nervosa (AN) is a psychiatric disorder with major incidence in pubescent females. It begins with a restrictive diet to lose weight that, in combination with intense physical exercise, progresses towards an *out-of-control spiral*. Although the aetiology of AN is still poorly understood, anorexic patients have shown a dysregulation of the hypothalamic-pituitary-adrenal axis (HPA), the system responsible for the stress response. Our major aim was to investigate the molecular mechanisms underlying stress response in AN as the critical drive of weight loss seeking in the activity-based anorexia (ABA) rat model, a well-established animal model of AN.
MATERIALS AND METHODS: In order to pursue this aim, adolescent female rats were individually housed starting from postnatal day (P) 35 and divided in four groups: controls (CTRL, food ad libitum–sedentary), FR (food restricted–sedentary), EXE (food ad libitum-exercise) and ABA (food restricted-exercise). On P38, the ABA group was food-restricted (2h/d) till P42, when ABA rats reached the anorexic phenotype. Animals were tested with the Spatial Order Recognition Test (SORT) to evaluate spatial memory capacity, and then sacrificed at different time-points: during the acute phase of the phenotype (P42) and after 7-days of body weight recovery (P49); trunk blood and hippocampus (Hip) were then collected. Hip mRNA and protein expres-

sion levels were analyzed by means of Real-time PCR and Western blot, respectively
RESULTS: After three days of AN induction, body weight was reduced in ABA rats significantly more than in FR rats. Unlike EXE animals that maintained a stable performance, wheel activity of ABA rats constantly increased over days. Corticosterone levels were enhanced in the plasma of ABA rats at P42 and reduced at P49, an effect paralleled by an altered trafficking toward the nucleus of the glucocorticoid receptor in the hippocampus of these rats. While recovery restored body weight of ABA rats, hormonal levels and hippocampal molecular changes were still present. In line with that, ABA rodents also exhibit spatial memory deficits in the acute phase of the pathology, highlighting their inability in the discrimination of displaced objects, an effect which persists till P49.
CONCLUSIONS: Notably, peripheral and central molecular alterations induced by the anorexic phenotype are paralleled by enduring spatial memory cognitive deficits. Based on our results, we speculate that AN condition dysregulates the HPA axis leading to long-lasting changes in stress-related mechanisms.
Supported by Cariplo Foundation, Italy

FROM DYING BACK TO SAVING BACK: HOW TO RESTORE THE MOLECULAR INTERPLAY BETWEEN MUSCLE AND NERVE IN THE AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Despite several pathological mechanisms have been elucidated, ALS remains an invariably fatal disease, for which no effective therapy is known. There is now growing consensus in the field that motor neurons are not the only primary target of the disease, and increasing evidence suggests that the earliest presymptomatic functional and pathological changes are occurring distally at the neuromuscular junctions (NMJ). Moreover, ALS affects whole body physiology and induces severe metabolic changes in several tissues, including skeletal muscle. The result is a loss of neuronal homeostasis and progressive die-back of motor axons culminating in death of the afflicted motor neurons. Nevertheless, whether alterations in the plasticity, heterogeneity, and metabolism of muscle fibers are the result of motor neuron degeneration or alternatively occur independently of it remain to be elucidated. Similarly, controversy exists over whether NMJ dismantlement is a pathogenic event directly associated with the primary defects occurring in motor neurons or whether it occurs independently from motor neuron degeneration.
METHODS: To address these issues, we made use of a mouse model (MLC/SOD1G93A) that overexpresses the SOD1 mutant gene selectively in skeletal muscle.
RESULTS: We found an alteration in the metabolic properties of skeletal muscle characterized by alteration in fiber type composition and metabolism. Moreover, we disclosed the molecular mechanism that triggers NMJ dismantlement and functional denervation associated with the toxic activity of SOD1G93A expression.
CONCLUSIONS: Our studies provides new insights into the mechanisms that trigger functional denervation associated withth neuromuscular diseases, and suggests pharmacological intervention to attenuate muscle dysfunction, NMJ loss, and eventually disease progression.

CANCER TRANSCRIPTOMIC PROFILES AS BIOMARKERS OF RESISTANCE TO DRUG TREATMENT: FROM BENCH TO BEDSIDE

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Tumor drug resistance is the main determinant of pharmacological treatment failure in cancer. Cancer cells acquire mutations in the drug target or other molecular or cellular differences that render treatment ineffective or redundancies among signaling networks fueling cancer progression. Differences in tumor microenvironment components may also affect drug treatment. These alterations can be investigated by pathological, biochemical, molecular and 'omics' methodologies (e.g. genomics, epigenomics, transcriptomics, phosphoproteomics). Expression level analysis of single, multiple or whole genome transcripts has been successfully applied to the identification of drug resistance mechanisms in *in vitro/in vivo* tumor models and in human tumor explants. Gene expression profiling has also become a clinically applied prognostic classifier for various tumors (e.g. high-grade lymphomas, breast and colorectal cancer). However, its application as a tool for discovering predictive signatures of drug resistance is still scanty.

Colorectal cancer (CRC), one of the most frequent and lethal cancers, represents a relevant example of an integrated research approach for predictive biomarker (BM) discovery. Combinations of cytotoxic agents and biological targeted drugs have significantly improved overall survival of CRC patients. However, clinical outcome is highly variable among patients receiving the same drug treatment at the same disease stage.

Genomic mutation analysis has provided relevant contributions to the clinical diagnosis of resistance for anti-EGFR MoAbs and immune checkpoint inhibitors. Also, NGS analysis has the promise to provide further knowledge on resistant mechanisms to these and other targeted drugs. Instead, there is still a high level of unmet need for the discovery of epigenetic and transcriptomic BMs of resistance to these drugs and to cytotoxic agents. Gene expression analysis has been widely used to detect drug resistance mechanisms in the context of pyrimidine metabolism for fluoropyrimidines (FPs), of DNA repair pathways for oxaliplatin and of DNA replication enzymes for irinotecan. These studies led to the identification of various determinants of resistance in experimental tumor models (e.g. *TYMS, TP, DPYD*). Translational studies in which the stratification of drug treatment was based on the application of these BMs obtained only partial results, not adequate to validate them from the clinical point of view.

RNA seq technology has allowed rapid and accurate analysis of whole genome expression profiles leading to the identification of gene expression signatures with prognostic value (e.g. CMS, CRIS). Preliminary studies have been performed to verify if these classifiers may also predict drug response. Our group has recently identified and preliminarily validated BMs of FP resistance in the adjuvant treatment of CRC (e.g. *PNN, KCNQ1OT1*). Examples of gene expression analysis and transcriptomic research in this field will be presented.

TEACHING OLD DRUGS NEW TRICKS: EFFECTS OF CARBONIC ANHYDRASES MODULATORS ON FEAR MEMORY EXTINCTION

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BACKGROUND: Fear extinction is defined as the learned inhibition of retrieval of a previously acquired aversive response. It has been as the cognitive therapy for the treatment of several disorders such as anxiety, phobias and post-traumatic stress disorder. Despite being highly effective, exposure therapy has also some limitations such as the long time required, high costs, and frequently observed relapse episodes. In this context, pharmacotherapy can be used as adjunctive therapy to overcome these fragilities by bolstering the formation and persistence of extinction memories. Therefore, a key goal is to identify new targets to improve the extinction learning process. Recent evidence indicates a role for brain carbonic anhydrases (CAs) in fear memory acquisition and consolidation, but nothing is know about its role on extinction.
METHODS: To fill this gap in this gap we evaluated the effects of CAs inhibitors acetazolamide (ACZ) and compound 18 (C18) or the CA activator D-phenylalanine (D-PHE) in the extinction of contextual fear conditioning paradigm in rats. In brief, the animals were placed in the conditioning chamber and 3 footshocks (0.5 mA, 2s 30s interval) were delivered. Twenty-four hours later they were placed in the same apparatus for 15 or 30 min depending on the experimental setting, in the absence of punishments. Drugs were given immediately after this session. In the following day, a 3 min retention session was performed.
RESULTS: A dose-dependent effect was observed after systemic administration of acetazolamide: no differences in the time the rats spent freezing were registered between animals receiving vehicle or ACZ at a low dose (10 mg/Kg, i.p.); on the contrary rats treated with the higher dose (30 mg/kg, i.p.) spent more time freezing than controls during the test, indicating a fear extinction impairment. No behavioural alterations were observed after systemic treatment with C18 (30 mg/kg, i.p.), a CA inhibitor that does not cross the blood brain barrier, therefore excluding the participation of peripheral CAs in ACZ-induced impairment. Systemic injection of the CA activator D-PHE (300 mg/kg, i.p.) facilitated the learning of fear extinction memory using an experimental design that did not allow the formation of extinction memory per se. Co-treatment with ACZ prevented D-PHE-induced effect. Local infusion with ACZ (10 nmol/side) or D-PHE (50 nmol/side) into the hippocampus, the basolateral amygdala or the ventromedial prefrontal cortex recapitulated the effects observed when the drugs were injected systemically. On the contrary, both drugs were ineffective when infused into the substantia nigra.
CONCLUSIONS: These are the first report of the involvement of central CAs in specific brain areas on fear extinction learning. Therefore, CAs can be considered an innovative target for the development of new compounds for the treatment of disorders characterized by maladaptive fear responses.

BEYOND HYPHOPHAGIC ACTION: A PAS DE DEUX BETWEEN OLEOYLETHANOLAMIDE AND HISTAMINE

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INTRODUCTION: Many gut- and adipose tissue-derived peptides and neurotransmitters are recruited to orchestrate feeding behavior, including the lipid-derived satiety factor oleoylethanolamide (OEA) and histamine (HA). Exogenous administration of OEA suppresses food intake by activating PPAR-α and engaging oxytocin neurotransmission through a mechanism mediated by the vagus nerve. Extensive evidence inticate that high neuronal histaminergic activity induces hypofagia while low activity stimulate food consumption. In the last then years, our research group in Florence have been investigating the interaction between these two factors. Regarding food consumption, we found that OEA-induced hypophagic effect was significantly decreased in mice deficient in the histamine-synthesizing enzyme histidine decarboxylase (HDC-KO) or acutely depleted of histamine via interocerebroventricular infusion of the HDC blocker α-fluoromethylhistidine (α-FMH). On the contratry, increasing histamine release in the CNS with ABT239 (an H3R antagonist) potentiated OEA-induced effects. Consistently, OEA augmented histamine release of fasted mice within a time window compatible to its anorexic effects. Moreover, OEA increased c-Fos expression in the oxytocin neurons of the paraventricular and sopraoptic nuclei and oxytocin secretion in the neurohypophysis of WT but not HDC-KO mice. Recently we have been expanded the evidence of this interaction to several functions beyond feeding behaviour. For instance, we demonstrated that the depletion of releasable histamine in the brain by α-FMH infusion into the lateral ventricles or infusion directly into the basolateral amygdala (BLA) of pyrilamine or zolantidine, an H1R and an H2R antagonist, respectively, prevented the memory-enhancing effects of OEA measured in the contextual fear conditioniong in rats. Consistently, we found that OEA increased histamine release from the BLA when systemically administered at the same dose that improved animals' memory. Using the tail suspension test we observed an antidepressant-like effect in animals receiving OEA sub-chornic treament. However, such effect was not observed following either acute (α-FMH-treated mice) or chronic (HDC-KO) histamine deprivation. Accordingly, OEA-induced increase in hippocampal and cortical CREB phosphorylation was not observed in histamine-deprived mice. Given the important role of the transcription factor CREB in signaling pathways relevant for pathogenesis and therapy of depression we believe that disruption of CREB activation may be responsible, at least in part, for the inefficacy of OEA in histamine-deprived mice. In conclusion, our results strongly support the hypothesis that these two ancient systems converge and interact at several levels. However, it is clear that several pieces are missing to solve the puzzle and further work is required to clarify all the mediators and to completely describe the neuronal circuitry involved in this beautifull pas de deux between OEA and neuronal HA.being highly effective, exposure therapy has also some limitations such as the long time required, high costs, and frequently observed relapse episodes. In this context, pharmacotherapy can be used as adjunctive therapy to overcome these fragilities by bolstering the formation and persistence of extinction memories. Therefore, a key goal is to identify new targets to improve the extinction learning process. Recent evidence indicates a role for brain carbonic anhydrases (CAs) in fear memory acquisition and consolidation, but nothing is know about its role on extinction.

METHODS: To fill this gap in this gap we evaluated the effects of CAs inhibitors acetazolamide (ACZ) and compound 18 (C18) or the CA activator D-phenylalanine (D-PHE) in the extinction of contextual fear conditioning paradigm in rats. In brief, the animals were placed in the conditioning chamber and 3 footshocks (0.5 mA, 2s 30s interval) were delivered. Twenty-four hours later they were placed in the same apparatus for 15 or 30 min depending on the experimental setting, in the absence of punishments. Drugs were given immediately after this session. In the following day, a 3 min retention session was performed.
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CONCLUSIONS: These are the first report of the involvement of central CAs in specific brain areas on fear extinction learning. Therefore, CAs can be considered an innovative target for the development of new compounds for the treatment of disorders characterized by maladaptive fear responses.

INTESTINAL INFLAMMATION, SEIZURES AND ANTIEPILEPTIC DRUG EFFICACY

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Neuroinflammation is clearly linked to seizure and epilepsy pathophysiology with a bidirectional interaction, since increased brain inflammation has been associated with the occurrence of seizures and epileptogenesis and, on the other hand, seizures themselves can induce inflammatory responses in the brain. Many studies have indicated that inflammatory mediators and pathways can be considered a suitable target for the development of novel drugs effective in the treatment of epilepsy; furthermore, mounting evidence suggests that these mediators can also be considered reliable biomarkers. Less is known about the role of peripheral inflammation and whether it has a role in the pathophysiology of epilepsy. It is known that administration of the pro-inflammatory bacterial endotoxin lipopolysaccharide can lower seizure threshold or increase seizures in some animal epilepsy models. However, it is known that seizures are highly associated with some systemic diseases characterized by peripheral inflammation such as systemic lupus erythematosus, Behcet's disease and Chron's disease. This latter, along with other inflammatory bowel diseases, have been suggested to be an increased risk factor

for neurological complications including seizures and epilepsy. Accordingly, it was previously reported that intestinal inflammation in animal epilepsy models lowers seizure threshold, most likely through increased circulating levels of some cytokines or other inflammatory mediators. Furthermore, intestinal inflammation is able to reduce drug efficacy. The gut microbiota, is a complex intestinal microbial ecosystem essential to health, made of bacteria, archaea and eukarya that colonizes the gastrointestinal tract. Many studies demonstrate a microbiota-gut-brain bidirectional connection via neural, endocrine, metabolic and immune pathways. Recent studies examined the effects of a ketogenic diet in two mouse models of refractory epilepsy; gut microbiota was found to be necessary for the diet to effectively reduce seizures. In other studies, changes in the gut microbiota of children with drug-resistant epilepsy were found in comparison to healthy children. Accordingly, it was recently demonstrated that dysbiosis associated with chronic stress enhances kindling epileptogenesis, and FMT from sham-stressed animals transplanted to chronically stressed rats counteract proepileptic effects of restraint stress. Moreover, probiotic supplementation reduced seizure severity in the pentylenetetrazole-kindling model; this latter result is in agreement with a pilot study with probiotic supplementation in epileptic patients. Gut microbiota alteration may increase inflammatory cytokines and bacterial metabolites may alter the gut- and blood-brain barriers permeability causing neuroinflammation; however, several other mechanisms may be involved in this not yet completely explored link.

UNRAVELING THE COMPLEX GLIAL RESPONSE IN AGING AND ALZHEIMER'S DISEASE

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BACKGROUND: Old age is the main risk factor for sporadic late-onset Alzheimer's disease (AD). Besides deposits of beta amyloid peptides in the extracellular space and neurofibrillary tangles inside neurons, alterations of both glia morphology and activities have been observed. Among the diverse glial cells, astrocytes emerge as central elements in AD etiology and/or progression, mainly because of their ability to maintain central nervous system homeostasis at molecular, cellular, organ, and system levels of organization. Multiple and disparate changes in astrocytes have been found in AD. Are these alterations due to AD or are they a consequence of aging?
METHODS: We studied the expression of specific astrocytic and microglial structural and functional proteins in different preclinical models of Alzheimer's disease. Moreover, we performed MRI/MRS experiments to evaluate brain metabolism in young and adult 3xTg-AD mice.
RESULTS: Our data suggest that aging, rather than AD progression, importantly affects the morphology and functions of rat hippocampal glial cells as well as cerebral metabolism. In our experimental conditions, astrocytes appeared the most vulnerable cells, whose structure and functions were profoundly modified.
CONCLUSIONS: These data open novel perspectives in the field of astrocyte functions in health and disease, suggesting their potential as pharmacological targets.

ROLE OF ENTERIC GLIA AS BRIDGING ELEMENT BETWEEN ACUTE GUT INFLAMMATION AND CHRONIC VISCERAL PAIN

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BACKGROUND: Intestinal inflammation and abdominal pain are gastrointestinal issues commonly associated with both functional bowel disorders and inflammatory bowel disease. Neuronal plasticity and broad alterations of enteric neuronal circuits involved in relaying pain signals contribute to the development of visceral hyper-sensibility, but the specific underlying mechanisms remain completely unknown. Increasing evidence suggests that astrocytes and microglia in the central nervous system and satellite glia in dorsal root ganglia (DRG) contribute to develop inflammatory-related pain states through a persistent reactive gliosis and neuromodulators release. This modifies neuronal circuits involved in conveying pain signals across the nervous system and pain perception. Within the enteric nervous system (ENS), enteric glia are a unique population of peripheral neuroglia involved in maintain the intestinal homeostasis through interactions with neurons and immune cells. However, enteric glia exhibit a reactive phenotype during intestinal inflammation that actively contributes to amplify the inflammatory response. Similarly to central and peripheral neuroglia, whether enteric glia also participate to alter visceral perception in acute intestinal inflammation by affecting neurons activity and neurotransmission is currently unknown. Here, we showed that 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis drives a glial-dependent inflammatory response in the colon characterized by persistent post-inflammatory abdominal pain state.
METHODS: Sprague Dawley male rats were treated by intrarectal injection of DNBS (30 mg of DNBS was dissolved in 0.25 ml of 50% ethanol) or ethanol alone for controls. Enteric glial function perturbation was induced in both DNBS and control mice by pre-treatment with a single injection (IP) of fluorocitrate (FC, 10 μmol/Kg) before colitis induction. Visceral sensitivity was assessed by recording abdominal withdrawal reflex (AWR) to colorectal distensions (CRDs) during the resolution phase of colitis at day 7. Then, animals were sacrificed and tissues collected to assess the overall severity of acute colitis and phenotypical changes along the gut-brain axis.
RESULTS: Acute inflammation caused hyperalgesia and increased the magnitude of AWRs to all noxious pressures in DNBS animals ($p<0.01$ for DNBS vs Veh; 1-way ANOVA with Bonferroni post-test). Visceral pain associated with acute DNBS-induced inflammation required glial component since heightened AWRs were significantly reduced in FC-treated DNBS rats ($p<0.05$ and $p<0.01$ for DNBS+FC vs DNBS; 1-way ANOVA with Bonferroni post-test). DNBS induced macroscopic and histological damages to the mucosa and submucosal layers with a marked infiltration of inflammatory

cells ($p< 0.01$ for DNBS vs Veh in either mascoscopic and histological damage scores; 1-way ANOVA with Bonferroni post-test). Enteric glia appear to drive inflammatory response in the context of DBNS colitis, as deleting glial component by FC improved the overall severity of colitis at day 7, as reflected by improvement in macroscopic and histological damage scores ($p<0.05$ and $p<0.01$ for DNBS+FC vs DNBS in macroscopic and histological damage scores, respectively; 1-way ANOVA with Bonferroni post-test). Further, DNBS rats displayed a parallel increase S100B expression in colonic myenteric ganglia (+72.7% for DNBS vs Veh), DRG (+85.7% for DNBS vs Veh), and periaqueductal grey area (+105.9% for DNBS vs Veh) at day 7 after colitis induction, but not DNBS FC-treated rats (-36.8% for DNBS+FC vs DNBS in the colonic myenteric ganglia, -39.7% for DNBS+FC vs DNBS in the DRG, -32.3% for DNBS+FC vs DNBS in the periaqueductal grey area; 1-way ANOVA with Bonferroni post-test). Interestingly, a significant increase in the co-expression of S100B and TRPV1 in PLP1-expressing cells was observed along the gut-brain axis in DNBS animals (+200% for DNBS vs Veh in the colonic myenteric ganglia, +185.7% for DNBS vs Veh in the DRG, and +182.3% for DNBS vs Veh in the periaqueductal grey area; 1-way ANOVA with Bonferroni post-test), which was countered by FC treatment (-46.7% for DNBS+FC vs DNBS in the colonic myenteric ganglia, -52.2% for DNBS+FC vs DNBS in the DRG, and -35% for DNBS+FC vs DNBS in the periaqueductal grey area; 1-way ANOVA with Bonferroni post-test).

CONCLUSIONS: Our results show that enteric glial activation is associated with an increased visceral hypersensitivity and intestinal damage in the context of acute intestinal inflammation. An increase in the expression of reactive gliosis markers correlates with the increased expression of TRPV1 along the gut-brain axis, suggesting that the inflammatory driving force for neuroplastic changes in visceral sensory neurons involves the glial component.

DRUG REPURPOSING IN CANCER RESEARCH

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BACKGROUND: In recent years, oncology research gave the bases for the marketing of new drugs, developed using costly and time-consuming technologies. Besides innovative drugs, a great opportunity to explore is the repurposing of 'old' drugs to treat diseases other than those for which they are authorized. This is increasingly becoming an advantage, as it involves the use of de-risked compounds, re-entering the development process from a phase-II clinical trial, substantially lowering development costs and shortening timelines. The drug repurposing is of particular interest for rare cancers, where the search for new drugs is often neglected. A paradigm is represented by the Adrenocortical carcinoma (ACC), a very rare aggressive cancer with overall 5-year survival rate less than 15% in patients with metastatic disease. Mitotane represents the only approved drug since many decades, although its mechanism is still not fully understood. The standard chemotherapy regimen to be added is the combination of etoposide, doxorubicin, and cisplatin (EDP), that however, has a limited efficacy and burdened by significant toxicity.

RESULTS: Approximately 50% of ACC are hormone-secreting tumors that produce cortisol or multiple steroids. Cortisol excess negatively influences the outcome of ACC patients, in particular for the hypercortisolism-secondary

immune defects. Abiraterone acetate (AA) is an inhibitor of CYP17A1, a key enzyme for steroid hormone synthesis, currently used in metastatic castration-resistant prostate cancer. Besides reducing androgen levels, we demonstrated that the drug rapidly impairs cortisol synthesis both in in vitro and in vivo ACC experimental models and the clinical study ABACUS is currently recruiting cortisol-secreting ACC patients, in order to confirm this hypothesis in humans as well. Interestingly, we observe an AA cytotoxic effect in our in vitro and in vivo experimental models, and this effect is mainly mediated by the increase of progesterone (Pg) levels. Pg, interacting with its receptors, through both genomic and non-genomic effects, induces a direct increase of cytotoxicity. The combination of Pg/mitotane on ACC cells exerts a synergic cytotoxic activity and an additive/synergic activity is as well demonstrated when Pg was added to each EDP drug. Thus, based on these results, we would like to repurpose Pg as drug endowed with direct anticancer activity in ACC. **CONCLUSIONS:** These results allowed us to design a phase-II randomized clinical trial (PESETA) having as primary objective the activity of the combination regimen EDP-M plus Pg (EDP-MP) vs EDP-M plus placebo in advanced/metastatic ACC patients. The safety profile of Pg and its analogue megestrol acetate is already known, as they are commonly used in cancer patients as supportive therapy, so their administration in association with EDP-M could be further beneficial, counteracting the treatment-induced asthenia and improving the tolerability.

THE ROLE OF REAL-WORLD EVIDENCE IN HEALTHCARE EMERGENCY FOR INFORMED DRUG REGULATORY DECISION MAKING

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The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or COVID-19) highlights the need to rapidly generate evidence that can rapidly support the "Informed decision making" process of the Drug Regulatory Agencies. In such a situation, it is more important than ever to study drug exposure, chronicity and multimorbidity to define and estimate the correlation with the potential risk of infection, and to make appropriate decisions based on sound scientific evidence. This also applies to healthcare settings that are not necessarily related to viral pandemics but require a timely response in any case. In addi-

tion to the need to rapidly conduct controlled and randomized experimental clinical trials, it is clear that the role of real world evidence (RWE) is of strategic importance in the management of health emergencies such as COVID-19. Indeed, RWE studies can be carried out with lower costs and especially in a very short time span. With regard to COVID-19, in addition to the existing databases, data sources based on COVID-19 regional patient registers can be used, with the coordination of the Istituto Superiore di Sanità. These registers can be linked to claims databases, thus creating a linkage database of great value that can provide timply evidence on the above exposure to pharmacological treatments, chronicity and multimorbidity and correlation with the risk of infection. The role of the pharmacologist includes prioritizing research needs, designing the required studies and interpreting results, but is expected to evolve to meet the needs of the healthcare emergency.

HERBAL PREPARATIONS FOR WEIGHT LOSS AND WEIGHT OF CLINICAL EVIDENCE

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INTRODUCTION: Overweight, obesity and the resulting metabolic syndrome are continuously increasing all over the world. The desire to lose weight without too much effort is widespread, but the few drugs available on the market have numerous side effects. For this reason, people recourse to herbal medicine, ignoring the real effectiveness and underestimating the safety profile of use of the available products.

MAIN TEXT: The medicinal plants used for weight loss are included in the following categories: 1. plants with thermogenic effects (Citrus aurantium var. amara L., Fucus vesiculosus L., Camellia sinensis L., etc.); 2. plants acting on appetite, fullness and/or satiety (Garcinia cambogia Desr., Amorphophallus konjac K. Koch, Phaseulus vulgaris L., etc.); 3. plants reducing waist and hip circumferences (Caralluma fimbriata Wall., Cinnamomum verum J Presl, etc.); 4. adjuvant plants in (metabolic, nervous, vascular) problems related to overweight (Euterpe oleracea Mart., Valeriana officinalis L., Ginkgo biloba L.). Moreover, there are also few pure compounds, utilized as new "nutraceuticals", isolated from various medicinal plants, including: 4-Hydroxyisoleucine, isolated from Trigonella foenum-graecum seeds; chlorogenic acid, one of the major compounds isolated in the green coffee [Kamburov et al., Gastroenterology Hepatology Open Access, 2018]; 6-gingerol and 6-shogaol obtained from Zyngiber officinalis; capsaicin from Capsicum annuum [Behl & Nijhawan, Obesity Medicine, 2020].

In clinical practice, many of the properties attributed to the plants/compounds listed above are not always confirmed. The problems in evaluating the efficacy (and safety) of a medicinal plant are manifold due to their variability. The factors that play a role in this context can be summarized as follows: 1) Herbal extracts are often not standardized in their phyto-constituents, making their dosage and the relative pharmaco-toxicological effect unforeseeable. 2) The type of herbal preparation used (extract, tincture) is often poorly described. 3) The property of purified active compounds could be different from the phytocomplex and from the activities of different plants eventually associated between them in the same weight loss product. 4) The mechanisms of action of herbal products are generally not known and their potential of interaction with other herbal preparations or pharmaceutical drugs is often

unclear. 5) Only some herbal extracts/supplements used for weight loss have been tested for their effectiveness in clinical trials, so that the extrapolation of preclinical evidence to clinical practice is not immediate [Farrington et al., Journal of Integrative Medicine, 2019].
For some medicinal plants, there is clinical evidence (sometimes contradictory or not univocal) of efficacy. For example, there is evidence that G. cambogia may act as a weight loss agent, but severe adverse events (hepatotoxicity, psychosis, etc.) have also been reported. On the other hand, various clinical studies have failed to demonstrate an effect on weight control using Camellia sinensis, Hoodia gordonii. etc. There is minimal objective evidence that consumption of Citrus aurantium causes weight loss in humans. At the same time, there is an indication of cardiovascular effects (hypertension, migraines, etc.) [Farrington et al., Journal of Integrative Medicine, 2019]. There is also an emerging body of clinical evidence that water extract of Phaseolus vulgaris reduces post-prandial glucose and promotes weight loss. In this case, side effects are not serious and they are mainly gastrointestinal [Barrett & Udani, Nutr Journal, 2011].
CONCLUSIONS: The weight of the scientific evidence of efficacy of herbal preparations is often compromised by the complexity of the preparations (which are frequently not standardized). Finally, in terms of safety, medicinal plants are not free from undesirable effects and drug interactions. Being herbal products used as self-medication, it is important to monitor their risk/benefits profile.

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