

der treatment. We started a clinical trial with the aim to characterize, in each patient, an individual chemosensitivity profile, based on the expression of a panel of markers that are involved in the resistance to standard chemotherapy drugs.

#### **Conclusions**

Our results are encouraging in the view of an individualised therapeutic approach, to provide a higher treatment success rate while sparing patients unnecessary toxicity from drugs that are not suited for their tumors.

**P48**

#### **THE INTERACTION OF CELECOXIB WITH MDR TRANSPORTERS ENHANCES THE ACTIVITY OF MITOMYCIN C IN A BLADDER CANCER CELL LINE**

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#### **Aim of the study**

An *in vitro* model was developed to understand if Celecoxib may synergize with Mitomycin C (MMC), commonly used for the prevention of bladder cancer recurrence, and eventually elucidate if the mechanism of interaction involves multi drug resistance (MDR) transporters.

#### **Materials and methods**

UMUC-3, a non COX-2 expressing bladder cancer cell line, and UMUC-3-COX-2, a COX-2 overexpressing transfectant obtained through the stable introduction of a plasmid pSG5-COX2 DNA, were used in the present study. The expression of COX-2 and MDR pumps (P-gp, MDR-1 and BCRP) was explored through western blot and flow cytometry. The antiproliferative effect of Celecoxib and MMC was studied with MTT test. Three biological assays (Drug Transport Experiment, Substrate Transporter Inhibition, and ATP cell depletion) were combined to establish if there was an interaction between P-gp and Celecoxib. Finally, the ability of Celecoxib to restore MMC cell accumulation was investigated.

#### **Results**

Among MDR pumps, only BCRP levels were slightly increased in UMUC-3-COX-2 as compared to UMUC-3. The antiproliferative effects of Celecoxib and MMC were investigated alone and in co-

administration at 48 h, both in UMUC-3 and UMUC-3-COX-2 cells. When administered alone, Celecoxib displayed a comparable antiproliferative effect in both cell lines. However, in the same experimental conditions, the effect of MMC was eight fold greater in UMUC-3 as compared to UMUC-3-COX-2. The co-administration of 1 mM, 5 mM, and 10 mM Celecoxib to MMC did not cause a significant improvement to the antiproliferative effect of MMC alone in UMUC-3 cell line. By contrast, in UMUC-3-COX-2 cells, the antiproliferative activity of MMC was 2-3 fold improved by the co-administration of Celecoxib compared to MMC alone. As a result of all findings from the permeability experiments performed, Celecoxib was classified as P-gp unambiguous substrate: this result indicates that Celecoxib is transported by MDR pumps and interferes with the efflux of MMC.

#### **Discussion**

UMUC3COX-2 cells resulted more resistant to MMC killing. However, the administration of Celecoxib in combination to MMC caused a significant and dose dependent gain of the anti-proliferative effect. This result was paralleled by an increase in the intracellular concentration of MMC in UMUC3COX-2, as seen by flow cytometry. Although COX-2 enzyme inhibition cannot be excluded, we sought to investigate if the effect seen in UMUC3COX-2 cells after Celecoxib administration could be the result of a direct interaction between Celecoxib and any of the three transporters involved in MDR. Interestingly we found that Celecoxib is a substrate for MDR transporters and causes a time- and dose-dependent ATP cell depletion in our cells.

#### **Conclusions**

Based on our findings, Celecoxib is a substrate for MDR transporters and may cause a direct inhibition of BCRP. The combination of Celecoxib with MMC should be the focus of future *in vitro* and *in vivo* research.

**P49**

#### **TETRA BRANCHED NEUROTENSIN RESULTS AN EFFECTIVE TUMOR-TARGETING AGENT FOR SUPERFICIAL AND INFILTRATING BLADDER CANCER IN AN "IN VITRO" AND "EX VIVO" STUDY**

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**Aim of the study**

The aim of this study is to confirm the possibility to use the Neutrotensin-tetra-branched form (NT-4) as a targeting agent for tumor cells "in vitro" (Human bladder carcinoma HT1376 cell line) and "ex vivo" in 15 consecutive case-control bladder cancer patients.

**Materials and methods**

The sequence of NT (NT 1-13) was synthesized through a solid phase chemistry in a tetra-branched form (NT-4). Peptides synthesized in such an oligo-branched form offer the advantage of a multivalent binding and become extremely stable to proteolytic degradation. The "in vitro" phase consisted in the evaluation of binding and internalization of NT-4 TMR (Tetra metal rodamin) in human bladder carcinoma HT1376 cell lines. The "ex vivo" phase consisted in evaluating the fluorescence of fluorescein isothiocyanate (FITC) conjugated NT-4 molecules by confocal microscopy in transitional cell bladder cancer from 15 patients with either superficial or infiltrating bladder cancer (10 TURBTs plus healthy tissue biopsies and 5 radical cystectomies). Presence of tumor and healthy tissue was checked by standard staining procedure.

**Results**

Binding and internalization of fluorophore-conjugated branched NT were showed "in vitro". At time 0 the NT-4 is present at the level of the cell membrane and at 1 hour is internalized in the cytoplasm. In the "ex vivo" phase, a significant difference between tumor and healthy tissue samples was observed in all the 15 specimens treated with NT-4. Both tumor cells from superficial transitional cell carcinoma and from infiltrating transitional cell carcinoma had a significantly higher staining compared to healthy transitional cells of the bladder. Indeed, the median of each sample in the green scale of the RGB system results significantly higher in the cancer group.

**Discussion**

Receptors for different endogenous regulatory peptides like NT are over-expressed on plasma membrane of human cancer cells and might be targeted as selective tumor markers. Peptides have a very short half-life but their synthesis in branched form could represent a general feasible method to improve peptide stability. NT-4 branched peptides seem to be promising theranostic molecules in bladder cancer, thanks to the endocavitary utilization, to its stability and capacity to bind receptors over-expressed in bladder cancer cell lines,

which might allow a very precise cancer detection and selective therapy, by a simple switch of functional units.

**Conclusions**

NT-4, a protease-resistant tetra-branched form of NT, results an effective tumor-targeting agent. When NT-4 is conjugated to a fluorophore it is bound and internalized into the tumor cell cytoplasm "in vitro" in HT1376 cell lines and it can efficiently discriminate "ex vivo" between tumor (either superficial or infiltrating transitional cell bladder cancer) and healthy tissue.

**P50****GENOMIC CHARACTERIZATION OF CANCER STEM-LIKE CELLS FROM HUMAN BLADDER TRANSITIONAL CELL CARCINOMA SAMPLES**

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**Aim of the study**

Transitional cell carcinoma (TCC) comprises the majority of bladder cancers (more than 90%). TCC is grouped in high- or low-grade (HG or LG), noninvasive (NI, pT1) or invasive (IN, pTA) lesions, based on its histological appearance and clinical behavior. Emerging evidence has suggested that the capability of a tumor to grow, propagate and resist the conventional cancer therapies is dependent on a small sub-population of cells, called cancer stem cells (CSCs), therefore responsible for metastases formation and recurrence. TCC has a significant risk of recurrence (75% after 5 years) and a 10-30% risk of progression to HGIN lesions. The aim of this work was to draw a cytogenetic-genomic profile of TCC samples, comparing array-CGH data obtained from whole biopsies of TCCs, in order to identify recurrent copy number alterations (CNAs); in addition, we compared these genomic profiles with those resulting from DNA extracted from the corresponding isolated CSCs.

**Materials and methods**

A total of 48 TCC samples were collected from 2007 to 2011. We applied our published protocol for CSC isolation from Bladder Cancer on 33 biopsies; conventional cytogenetic analysis on fresh chromosome spreads immediately after the isolation was performed on