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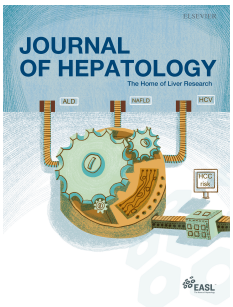

Molecular profiling and matched targeted treatment in cholangiocarcinoma: results from the Italian dataset (ANITA)

Virginia Genovesi, Francesca Salani, Margherita Rimini, Mario Domenico Rizzato, Tiziana Pressiani, Chiara Pircher, Lisa Salvatore, Lorenzo Antonuzzo, Lucrezia Silvestro, Bruno Daniele, Francesco Leone, Caterina Vivaldi, Silvia Camera, Rita Balsano, Francesca Bergamo, Monica Niger, Silvia Foti, Giulia Tesini, Giacomo Di Paolo, Federico Rossari, Sara Sperotto, Gianluca Masi, Sara Lonardi, Lorenza Rimassa, Andrea Casadei-Gardini, Lorenzo Fornaro

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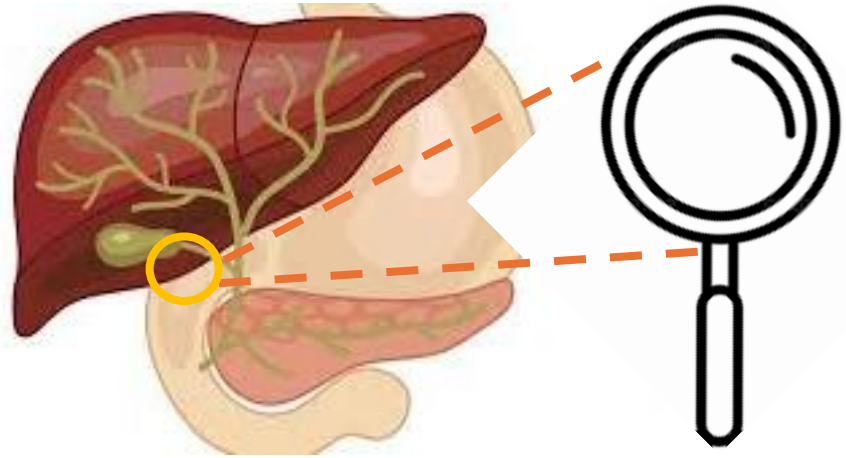
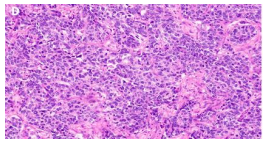
Molecular profiling and matched targeted treatment in cholangiocarcinoma: results from the Italian dataset (ANITA)

Overall population:
621patients

- n=368 iCCA
- n=105 pCCA
- n=148 dCCA





Extended molecular profiling

(local NGS, multigene platforms)

N=285

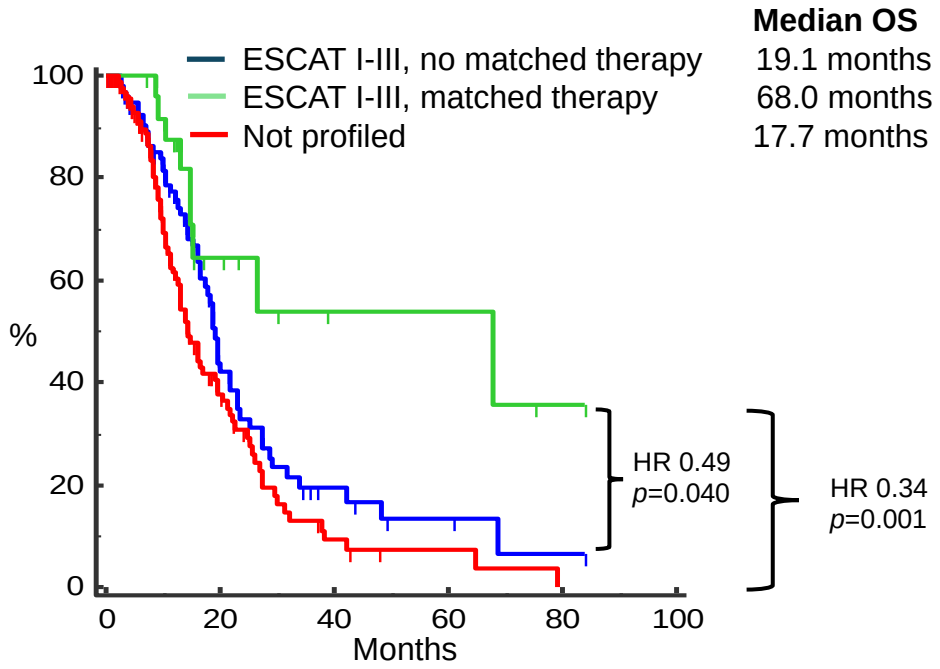
ESCAT I-III treated with targeted therapy (n=26)

ESCAT I-III not treated with targeted therapy (n=113)

Not profiled (n=201)

Therapeutic opportunities

- 16 patients with *FGFR2* fusion/rearrangement → pemigatinib, futibatinib or derazantinib
- 6 patients with *IDH1* mutation → ivosidenib
- 3 patients with dMMR disease → pembrolizumab
- 2 patients with *BRAF* V600E mutation → dabrafenib + trametinib



Molecular profiling and matched targeted treatment in cholangiocarcinoma: results from the Italian dataset (ANITA)

Short title: Molecular profiling in cholangiocarcinoma.

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Data availability statement: Data available on request from the authors.

Authors contributions: Conceptualisation: ACG, LF. Data curation: all authors. Formal analysis: VG, FS, LF. Funding acquisition: ACG, LF. Investigation: all authors. Methodology: VG, FS, MR, CV, GM, ACG, LF. Project administration: ACG, LF. Resources: ACG, LF. Software: ACG. Supervision: ACG, LF. Writing-original draft: VG, FS, LR, ACG, LF. Verified the data: VG, FS, ACG, LF. All authors had access to the data.

Abstract

Background and Aims: Extended molecular profiling (EMP) is recommended to tailor targeted treatment for advanced cholangiocarcinoma (CCA). However, neither the impact of EMP nor the availability of targeted therapies has been extensively described in a real-world setting.

Methods: ANITA is an observational (retrospective and prospective) study enrolling 621 patients with CCA from 10 tertiary Italian cancer centers between 2017 and 2023. Current analyses are focused on access to EMP, evolving rate of EMP over time, access to as well as outcomes with targeted treatment. Moreover, we explore the prognostic and predictive role of major driver molecular alterations.

Results: EMP was performed in 79.9% of the patients with advanced CCA. EMP rate significantly increased over the years (2017 to 2023: +25.8%): 139 patients with ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) I-III alterations were identified and 18.7% received matched tailored therapies. No differences were seen in overall survival (OS) and progression-free survival (PFS) according to EMP availability. Patients receiving targeted treatments reported the best OS, significantly longer than those untreated with targeted therapies (HR 0.49, 95% CI 0.28-0.86) or without EMP available (HR 0.34, 95% CI 0.21-0.54). Among ESCAT I-III positive cases, 42 had *FGFR2*-fused/rearranged and 59 had *IDH1*-mutated disease: after excluding patients treated with targeted treatment, only *FGFR2* fusions/rearrangements confirmed their prognostic role for OS.

Conclusions: Despite a broader availability of EMP in advanced CCA, patient access to targeted treatments remains suboptimal. Our results demonstrate that such treatments can dramatically impact on OS, Therefore, strategies aiming at increasing the access to (and accelerate the availability of) targeted treatments are warranted.

Impact and implications

- ANITA is the largest observational, retrospective and prospective, study exploring the impact of molecular profiling and matched targeted treatment in a large cohort of patients with advanced cholangiocarcinoma.
- While the availability of molecular profiling increased in the latest years, access to targeted therapy is still limited in routine practice.
- Administration of targeted treatment has a significant impact on patient overall survival, stressing the need of promoting timely and early access to new agents in this disease.

Introduction

Biliary tract cancers (BTCs) are aggressive malignancies, comprising cholangiocarcinoma (CCA), either intrahepatic (iCCA), perihilar CCA (pCCA) or distal CCA (dCCA), and gallbladder carcinoma (GBC). In most cases, BTCs are diagnosed at advanced stage or recur after an apparently radical surgery, and the aim of treatment is therefore palliative.[1] In recent years, a distinct molecular profile has been recognized among the specificities of different primary BTCs locations, thus paving the way toward the development of targeted treatments and making BTCs a paradigm of personalized cancer medicine.[2,3]

DNA and RNA analyses by next-generation sequencing (NGS) techniques and protein expression assessment by immunohistochemistry (IHC) are the recommended methods for the definition of BTC somatic profile, allowing for the identification of actionable targets.[3,4]. In particular, the 2022 European Society of Medical Oncology (ESMO) guideline strongly recommends [I,A] to carry out molecular analyses in patients with advanced BTCs deemed suitable for systemic treatment at the time of first-line treatment decision.[4] Due to the paucity of available tissue in many cases, parallel sequencing with NGS granting EMP is preferred over single gene testing.[5] EMP should at least include the identification of *fibroblast growth factor receptor 2* (*FGFR2*) and *neurotrophic tyrosine receptor kinase* (*NTRK*) rearrangements, *isocitrate dehydrogenase 1* (*IDH1)* R132x and *V-raf murine sarcoma viral oncogene homolog B1* (*BRAF*) V600E mutations, microsatellite instability/mismatch repair (MSI/MMR) status and *human epidermal growth factor receptor 2* (*HER2*) amplification: these alterations are classified as ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) I, *i.e.* driving a clinical benefit when targeted by tailored treatments within prospective clinical trials.[6]

On the one hand, national-wise retrospective cohorts of targeted-treated- patients confirm the activity and efficacy of the tailored systemic strategies.[7,8] On the other hand, several limitations to the wide application of EMP are identified in daily clinical practice throughout Europe: complex pre-analytical steps, high rate of test failure in low cancer-cells samples, prolonged turnaround time, increased costs and the necessity of a dedicated molecular

tumor board (MTB) for the correct interpretation of the biological data all contribute to make EMP and its clinical application a hard road to travel in BTCs.[5,9]

As of 2023, the above-mentioned limitations to EMP implementation in the Italian BTC scenario were confirmed by national scientific societies.[10] This is particularly relevant, since in Italy mortality from BTCs has increased by 5% in both sexes since 2008, mainly due to the increased incidence of iCCA.[1]

ANITA is a nationwide observational study, describing the clinical presentation and management of patients with BTCs at referral Institutions across the country. Here, we report the first results of the study, focusing on the clinical characteristics and access to tailored treatments among EMP-assessed patients. Specifically, we will present the EMP rate over time, its impact on access to targeted treatments, and how the latter influences patients' outcomes. Moreover, we will explore in our cohort the prognostic value of the most commonly found actionable alterations, to account for a potential confounding effect when assessing the survival impact of target therapies.

Material and methods

Study design and data collection

ANITA is an observational, multi-center, retrospective and prospective dataset aimed at describing the real-world characteristics and clinical outcomes of patients with CCA diagnosed from January 2017 to June 2023 in Italy. The retrospective cohort included patients diagnosed from January 2017 to March 2022, while the prospective cohort included patients diagnosed from April 2022 to June 2023. Patient data were collected from 10 Italian referral centers (*i.e.*, Azienda Ospedaliero-Universitaria Pisana in Pisa, IRCCS San Raffaele Hospital in Milan, IRCCS Humanitas Research Hospital in Rozzano, National Cancer Institute in Milan, Veneto Institute of Oncology in Padua, Azienda Ospedaliero-Universitaria Careggi in Florence, IRCCS Fondazione Policlinico Universitario Agostino Gemelli in Rome, IRCCS Fondazione G. Pascale in Naples, Ospedale del Mare in Naples and Nuovo Ospedale degli Infermi in Biella). Referral Institutions were identified by the presence of at least 10 out of 15 features provided in the ideal setup of the multidisciplinary team (MDT) for BTCs as developed by the

European Network for the Study of Cholangiocarcinoma (ENS-CCA),[11] with the mandatory presence of selected higher-weight items (*i.e.*, hepatobiliary surgeon, medical oncologist with expertise in BTCs, interventional radiologist and dedicated gastrointestinal pathologist with access to EMP locally).

Patients with cyto-histological diagnosis of CCA were eligible if: *i*) clinical and survival data about patient and disease characteristics were available; *ii*) results of the molecular analyses (if performed) were available; *iii*) they gave written informed consent.

Individual patient data were obtained from the medical records of each participating institution and, subsequently, were collected using the anonymized electronic format "CGP-Cohort Genomics Platform" hosted by IRCCS San Raffaele Hospital. In February 2024, data extraction was conducted and subjected to harmonization and completeness checks. See CTAT methods table in the Supplementary data for all methods undertaken in this study.

The study was sponsored by Fondazione GONO (Gruppo Oncologico Nord-Ovest) (https://gonogroup.org/). AstraZeneca provided financial support for study conduction but was not involved in drafting of the protocol, data collection, data analyses and interpretation of the results (protocol number: ESR-21-21387). The protocol was centrally approved by the Ethics Committee of Azienda Ospedaliero-Universitaria Pisana (approval number 21861) on 28th April 2022, as well as by local Ethics Committees from participating Institutions.

Sample collection and molecular profiling

Data regarding molecular profiling were collected independently of the type of sample analyzed (either surgical specimens or tumor core biopsies) and techniques used: indeed, the assessment was performed locally as *per* clinical practice or by a commercially available platform in central laboratories. All the analyses were performed on tissue samples, and no plasma samples were collected to perform molecular profiling.

For the single gene evaluation, IHC was used to assess HER2, NTRK and MMR protein expression, fluorescence *in situ* hybridization (FISH) to evaluate *FGFR2* rearrangements and *HER2* gene amplification (in case of intermediate expression, *i.e.* 2+ at IHC). In case of *NTRK* rearrangements identified with IHC, confirmation by polymerase chain reaction

(PCR) was carried out. EMP was carried out using local NGS (with individual specifications for each center: a list of genes covered by each local platform is provided in **Supplementary Table 1**) or FoundationOne® CDx platform (a 324-gene DNA-based assay for both mutations and rearrangements) if performed centrally. In some cases both EMP evaluation and single gene evaluation were carried out by IHC and/or FISH techniques. The threshold used in FoundationOne® CDx and in every local-panel for identifying a copy number amplification is 4 for *ERBB2* and 6 for all other genes.

Molecular alterations were classified, as *per* ESMO Clinical Practice Guidelines, according to tier I-III of the ESCAT scale.[6,12]

Data analyses and outcomes

Primary aim of the ANITA registry was to assess the management of patients with BTCs in Italy, in different disease settings.

Baseline patient characteristics were summarized using descriptive statistics. Continuous variables were reported as median (interquartile range, IQR), while categorical variables as proportions (number, %). Kruskal-Wallis test and Pearson Chi-squared test were used for continuous and categorical variables, respectively.

Survival endpoints were calculated using the Kaplan-Meier method and compared among different groups with a log-rank test. Multivariate Cox models were calculated to obtain hazard ratios (HRs) with 95% confidence intervals (CIs), after adjusting for potential confounders (*i.e.*, adding to the multivariate equation only significant variables at the univariate analysis for each endpoint of efficacy).

Overall survival (OS) for advanced disease was defined as the time from first-line chemotherapy start to the date of death or last follow-up. Progression-free survival (PFS) was calculated from the first cycle of first-line chemotherapy administration to the date of progression or death, which one occurred first. Relapse-free survival (RFS) for localized, resectable disease was defined as the time between radical-intent surgery and first evidence of disease recurrence. When the prognostic role of *FGFR2* fusions/rearrangements and *IDH1* mutations were explored among patients treated with first-line chemotherapy, patients who had received matched targeted treatments were excluded.

Statistical analyses were performed using MedCalc® Software Ltd (version 14.8.1).

Results

Patient characteristics and tumor molecular profile

A total of 621 patients were enrolled in the study. **Figure 1** shows the study flowchart. Baseline characteristics are reported in **Supplementary Table 2**. As shown, the majority were diagnosed with iCCA (368, 59.3%) and nearly half (302, 48.6%) underwent surgery as the first treatment for BTC.

Focusing on patients with advanced disease (486, 78.3%), 341 (70.2%) presented with synchronous metastases, while 129 (26.5%) recurred after surgery. **Table 1** shows the main characteristics of all patients with advanced BTCs included. Of note, most of them had visceral involvement (intended as liver or lung) (354, 72.8%), and liver (318, 65.4%) and lymph nodes (190, 39.1%) were the most frequently affected sites. Almost all patients received at least one line of systemic treatment (481, 99.0%): gemcitabine plus cisplatin was the most widely used combination in first line (237, 48.8%). About 80% (388, 79.9%) of the patients diagnosed with advanced disease underwent EMP, the vast majority through FoundationOne© CDx (343, 70.6%). One hundred and fifty-six (32.1%) patients underwent single gene testing. Notably, we reported an increase in EMP rate over the years (from 2017 to 2023: +25.8%, $p<0.001$): in particular, the rate of EMP in iCCA remained substantially stable over the last seven years, despite a progressive increase in EMP rate for pCCA and dCCA (**Supplementary Figures 1A-1B**). See **Supplementary Table 3** and **4** for a detailed description of patients without EMP.

Of the EMP-profiled tumors, 139 (28.6%) presented an ESCAT I-III alteration; this was significantly more frequent in iCCA (115, 36.9%) as compared to pCCA (13, 15.3%) and dCCA (11, 12.4%), $p<0.001$). Molecular profiling among the 312 enrolled iCCA revealed *IDH1* mutation as the most common druggable alteration, with a prevalence of 15.1% (47), followed by *FGFR2* fusions/rearrangements (33, 10.6%), *IDH2* mutation (10, 3.2%), MSI-high/deficient MMR status (6, 1.9%), and *BRAF V600E* mutations (4, 1.3%). MSI-high/deficient MMR (4, 4.7%) status and *IDH1* (3, 3.5%) or *BRCA1/2* mutations (2, 2.4%) were the most frequent alterations found in pCCA. On the contrary, dCCA had a

substantially different molecular profile, with *HER2* alterations (amplification: 2, 2.2%; mutation: 3, 3.4%) being the most frequent ones. The prevalence of specific alterations among the ESCAT I-III profiled BTC patients is portrayed in **Figure 2A** according to primary tumor sites**.**

Figure 2B summarizes the ESCAT alteration rate (classified as I vs. II-III), according to the metastatic sites, independently of the site of origin of BTC and the tissue on which EMP was performed. Of note, tier I alterations were mainly found in patients with liver (77, 42.5%) and nodal (31, 21.0%) metastases.

Access to targeted treatments

Of 139 advanced patients harboring a druggable alteration according to ESCAT I-III tiers, only 26 (18.7%) received one or more corresponding tailored treatments. Among the explored reason for restricted treatment access, the main cause resided in limitations in drug availability (**Supplementary Figure 1C**). The majority (16, 61.5%) received an FGFR2 inhibitor [*i.e.* pemigatinib (14), derazantinib (2), futibatinib (1)], followed by the IDH1 inhibitor ivosidenib (6, 23.1%), the anti-PD-1 pembrolizumab (3, 11.5%) and BRAF plus mitogen-activated protein kinase kinase (MEK) inhibitors combination (dabrafenib + trametinib: 2, 7.7%). **Supplementary Table 5** and **Figure 3** detail targeted treatments characteristics, timing of administration and mode of access.

Patients receiving a tailored treatment differed significantly from those treated with chemotherapy alone in terms of primary site location and number of systemic lines for advanced disease (**Table 2**). In fact, the former harbored more frequently iCCA (96.1% vs. 73.1%, p=0.011) and received a higher number (3 or more) of palliative therapies compared to the latter (53.8% vs. 33.6%, p=0.025). No differences were found between the two cohorts in terms of sites of metastatic lesions, timing of molecular profiling, choice of first-line chemotherapy and the other clinical and/or pathological analyzed factors (all p>0.05). Of note, rapid deterioration of patient PS after progression seems not to represent the main reason in our series.

Of note, 7 out of 26 (26.9%) patients treated with targeted therapies had a metachronous disease, 1 patient (3.8%) underwent curative liver transplantation at the time of best

response with tailored treatment and 3 patients (11.5%) received transarterial radio-embolization of liver lesions while on targeted agents.

Outcomes of advanced patients

After a median follow-up of 16.6 months (95% CI 13.8-19.4), among the 486 patients with advanced disease, survival data of 30 patients were not available due to limited follow up. Median OS of the included 456 patients with unresectable/metastatic disease was 16.6 months (95% CI 15.4-19.4) and median PFS on first-line was 6.6 months (95% CI 6.2-7.6) **(Supplementary Figures 2A-2B)**.

Determinants of OS and PFS in the overall advanced disease population are shown in **Table 3.** In particular, median OS was significantly longer in patients who underwent resection (20.5 months, 95% CI 16.6-26.9 vs. 16.0 months, 95% CI 14.4-18.5; *p*=0.004), in patients with metachronous disease, *i.e.*, relapsed at least 6 months after surgery (21.9 months, 95% CI 18.4-27.5 vs. 16.2 months; 95% CI 14.4-18.5; *p*=0.004), in patients without nodal metastases (19.4 months, 95% CI 16.6-22.3 vs. 14.6 months, 95% CI 11.9-16.4, *p*=0.002) and in those who had an ORR as best response to first-line treatment (22.3 months, 95% CI 18.7-27.2 vs. 15.4 months, 95% CI 13.1-17.4; *p*=0.001). Interestingly, after matching for potentially confounding variables (surgery, nodal metastases, number of lines received and *FGFR2* status) we observed that median OS did not differ according to the timing of molecular profiling: 16.2 months (95% CI 13.3-18.9) for patients profiled at diagnosis or before starting first-line systemic treatment vs. 21.6 months (95% CI 15.1-26.1) for those who underwent EMP after progression to first-line treatment (p=0.092). In order to investigate whether earlier EMP availability could facilitate access to targeted treatment, we also calculated the time from progression to first-line treatment to targeted treatment initiation among the 17 out of 26 included patients. Notably, we noticed that median time to targeted treatment initiation for patients with EMP performed at baseline or during first-line treatment was 0.9 months (95% CI 0.2-1.5), while it was 3.0 months (95% CI 0.6-9.7) for patients with EMP performed after progression to first-line: this difference, despite numerically relevant, did not achieve statistical significance (p=0.113).

Among 139 patients with ESCAT I-III alterations, survival data of 9 patients were not available; consequently, survival analyses were performed on a cohort of 130 patients. Median OS was significantly longer in patients with ESCAT I-III alterations (19.5 months, 95% CI 17.4-23.6) than in those who did not harbor any potential target (16.2 months, 95% CI 13.7-20.8) (HR 0.60, 95% CI 0.42-0.84; *p*=0.002) **(Figure 4A)**. Of all the individual gene alterations, only *FGFR2* fusions/rearrangements showed a significant association with longer OS (26.5 months, 95% CI 17.4-48.2 vs. 17.0 months, 95% CI 15.4-19.5; *p*=0.006) at univariate assessment. Interestingly, median OS of patients with ESCAT I-III alterations who did not receive a matched targeted treatment was quite alike that of non-profiled patients (19.1 months, 95% CI 16.6-23.0 vs. 17.7 months, 95% CI 13.0-19.4, respectively). Of note, both these patient subsets showed significantly shorter OS compared to patients treated with targeted therapies (68.0 months, 95% CI 15.4-NR) (HR 0.49, 95% CI 0.28-0.86, *p*=0.040 and HR 0.34, 95% CI 0.21-0.54, *p*=0.001, respectively) **(Figure 4B).** Nonetheless, multivariate analysis for OS failed to confirm the presence of ESCAT I-III alterations as independent determinants of better outcomes. Indeed, only nodal metastases (HR 1.85, 95% CI 1.29-2.64, *p*=0.001), ORR to first-line treatment (HR 0.40, 95% CI 0.26-0.61, *p*<0.001) and *FGFR2* fusion/rearrangement (HR 0.54, 95% CI 0.30-0.95, *p*=0.035) were proved as independent prognostic factors in our dataset.

In terms of first-line PFS, significantly better outcomes (*p*=0.024) were witnessed for patients with pCCA (8.8 months, 95% CI 6.5-11.1), compared to iCCA (6.5 months, 95% CI 5.8-7.2) and dCCA (6.1 months, 95% CI 6.2-7.6). In addition, visceral metastases led to a shorter PFS of 6.3 (95% CI 5.5-6.8) months vs. 8.0 (95% CI 6.7-11.0) months (*p*=0.044). Only ORR confirmed its positive prognostic role at multivariate analysis (HR 0.47, 95% CI 0.37-0.61, *p*<0.0001). No differences were seen among patients with ESCAT I-III alterations who did or did not receive a matched targeted treatment (5.8 months, 95% CI 2.9-8.7 vs. 6.6 months, 95% CI 5.9-8.3, respectively; *p*=0.809).

Of note, considering the 84 patients with available EMP showing ESCAT I-III alterations who received second-line treatment, median OS from second-line systemic therapy was significantly longer in those treated with (43.6 months, 95% CI 10.2-43.6) than in those who did not receive (10.8 months, 95% CI 7.8-14.4) a targeted agent (HR 0.48, 95% CI

0.3-0.9; *p*=0.040). The same benefit was seen in median PFS for second-line treatment: 5.4 months (95% CI 4.4-8.2) for patients who received a targeted treatment and 4.0 months (95% CI 2.8-4.6) for those who did not (HR 0.57, 95% CI 0.4-0.9, *p*=0.035) **(Figure 4C-4D)**.

Role of *FGFR2* and *IDH1* alterations in all-stage BTCs

The ANITA dataset comprised 42 patients with *FGFR2*-fused/rearranged disease and 59 patients with *IDH1*-mutated CCA, accounting for 11.4% and 15.2% of the profiled population, respectively. In both cases, iCCA was the most common site of origin (*FGFR2*: 95.2%; *IDH1*: 94.9%). In **Supplementary Table 6** and **Supplementary Table 7** we reported baseline characteristics of patients with CCA harboring *FGFR2* fusion/rearrangements and *IDH1* mutations.

The prognostic and predictive role of *FGFR2* fusions/rearrangements and *IDH1* R132x mutation among patients with advanced disease were tested in the population of patients not treated with targeted therapies, in order to account for the positive predictive value of these alterations for the matched treatments' outcome. A total of 34 patients with *FGFR2*-fused/rearranged and 46 patients with *IDH1*-mutated disease were then included in the analysis. Median OS from advanced disease diagnosis was 26.5 months (95% CI 17.4-48.2) and 17.7 months (95% CI 13.3-21.9), respectively, for patients with *FGFR2* fusion/rearrangements and *IDH1* R132X mutation positive disease (**Supplementary Figure 3A-3B**).

At univariate analysis, *FGFR2* alterations demonstrated a positive correlation with OS (HR 0.49, 95% CI 0.33-0.74, *p*=0.006), confirmed at multivariate analysis (HR 0.54, 95% CI 0.30-0.95, *p*=0.035) after adjusting for confounders, such as carbohydrate antigen 19-9 (CA19-9) levels (>500 UI/mL) before first-line chemotherapy, liver metastases, synchronous metastatic disease and multiple sites of metastases. No association with OS was reported for *IDH1* status (HR 0.93, 95% CI 0.61-1.41, *p*=0.738). Moreover, *FGFR2* fusions/rearrangements and *IDH1 R132x* mutation were tested as putative determinants of first-line platinum-based chemotherapy benefit. No role was found for

either alteration (*FGFR2*: HR 0.96, 95% CI 0.65-1.41, *p*=0.834; *IDH1:* HR 0.99, 95% CI 0.70-1.40, *p*=0.946).

Finally, the putative prognostic role of *FGFR2* and *IDH1* alterations was exploratory assessed in the cohort of radically resected patients. Seventeen patients with *FGFR2*-fused/rearranged and 28 patients with *IDH1*-mutated BTCs were included in the analysis for RFS. Median RFS was 10.3 months (95% CI 2.0-18.9) in the former and 11.4 months (95% CI 6.3-24.0) in the latter: no significant difference was observed compared to the wild-type counterpart [12.3 (95%CI 11.1-14.6) months and 12.5 (95% CI 11.0-15.0) months for *FGFR2* and *IDH1* wild type, respectively (both *p*>0.05)].

Discussion

BTCs represent a global health problem, due to their well-known aggressiveness, limited treatment options and rising incidence.[13,14] Among patients with advanced disease, systemic chemotherapy has long represented the standard of care, providing a significant despite limited OS benefit.[2] More recently, the introduction of immune checkpoint inhibitors in first-line has further improved patient outcomes, with modest advantage in unselected patient population.[15–19] On the other hand, we have witnessed a concomitant increasing understanding of the biological background of different BTC subtypes, which have been translated into the development of active and effective targeted treatments in molecularly selected subgroups.[20] This approach currently represents the most promising alternative to standard second-line cytotoxic chemotherapy and maximizes the benefit of tailored treatments. However, due to the incidence of the disease and the low prevalence of each molecular alteration in different BTC subtypes, formal demonstration of a definitive impact of targeted treatments on outcome may be challenging, as demonstrated by the early closure of randomized phase 3 clinical trials with FGFR inhibitors.[21–23] National and international guidelines therefore support molecular profiling as a recommended procedure for patients with advanced BTCs and candidates to systemic therapy,[12,24–26] but a clear proof of the impact of EMP and matched targeted treatments on outcome is lacking in the real-world setting. Moreover, how guideline recommendations have been implemented in Italian routine practice has not been

explored, yet. Therefore, we designed ANITA in order to answer these questions, ultimately providing stronger evidence in support of routine use of EMP and subsequent access to targeted treatments in advanced BTCs.

Our results show that a larger proportion of patients with BTCs have had access to EMP in recent years, with a clear increase of 25.8%, from 43.2% in 2017 to 69.0% in 2023. Notably, 70.6% of the patients had the disease profiled with a commercially available test within the context of a clinical trial, confirming that, at least at referral institutions, indications from guidelines have been rapidly implemented in practice. However, the lower percentage of BTC cases profiled by in-house NGS platforms suggests that criticisms might exist in developing a shared and validated profile for molecular characterization, outside the framework of company-driven study protocols.

In ANITA, only access to targeted treatment and not EMP *per se* provided an impact on patient survival. Notably, patients with BTCs with ESCAT I-III alterations but not receiving tailored agents had similar OS outcomes compared to patients who did not have their tumor profiled (median, 19.1 and 17.7 months, respectively, similar to what we can expect from recently selected series from tertiary centers with routinely available treatments).[28-30] On the contrary, patients whose tumor was profiled by EMP and received targeted treatments had a dramatically improved outcome, with a median OS of 68.0 months, which compares favorably with prior studies.[31]

In line with other literature reports,[32,33] our data support a positive prognostic role for *FGFR2* fusion/rearrangements, but not for *IDH1* mutations. However, not all published data confirm the same prognostic value of these alterations.[34] This discrepancy is possibly due to the heterogeneity of the considered populations across different publications and the outcome definition (in our study, OS was defined since first-line treatment initiation). While debate on this topic is still open, our findings also suggest a central prognostic role of multiple treatments on OS (presumably comprising locoregional therapies after response to targeted treatments: indeed, 26.9% of patients receiving targeted treatment patients had also consolidative/potentially curative treatments). Taken together, these data prompt us to speculate that the OS benefit of targeted treatments might be even greater than it could be anticipated from previous studies. Indeed, the higher number of lines of systemic therapies received by patients treated with targeted

treatments (in some patients, sequential tailored agents were also administered) might contribute to prolong disease control and ultimately OS among patients treated with targeted agents. In fact, more active treatments could help preserving patients' conditions as well as organ functions longer, thus allowing the administration of multiple lines and making the continuum of care a real possibility for selected patients with BTCs. In this regard, it is not surprising that 20 out of 26 (76.9%) patients receiving targeted treatments were treated in first or second line: in our opinion, data from ANITA (as well as literature results about the high attrition rate across subsequent treatment lines in BTCs)[35] suggest that patients with disease harboring ESCAT I-III alterations should receive targeted treatments in earlier lines, in order to avoid the risk of not receiving them at all.

Regarding EMP results, it is not surprising that *FGFR2* and *IDH1* were the most frequently altered targetable genes in ANITA, with a clear prevalence of iCCA as the most frequent disease site among patients receiving targeted treatments. However, as the number of newly recognized targets is increasing also for extrahepatic CCA (e.g., *HER2* alterations), we agree with international/ESMO guidelines and believe that available evidence supports the extension of EMP to all disease sites in advanced BTCs.

Among the key messages from ANITA, the low percentage of patients with ESCAT I-III alterations definitively treated with tailored agents (26/139, 18.7%) prompts the need for novel strategies to implement new treatment options in routine practice. This could be even more relevant, as our results were reported from referral institutions, with access to clinical trials and advanced expertise in BTCs. Actions should be therefore taken at any level to increase early accessibility to targeted treatments[36], particularly for a relatively rare and aggressive disease such as BTCs. Taking into account the difficulties in completing randomized trials in patient subpopulations selected by low-prevalence molecular alterations, real-world data such as those from ANITA might be helpful to provide support for accelerated targeted treatment approval and negotiations with regulatory agencies.[37–39]

Potential limitations should be considered when interpreting our results. As described, ANITA is a retrospective/prospective study: as a consequence, potential bias of retrospectively collected data should be acknowledged. However, restricting the analyses to patients treated since 2017 in referral cancer centers could have partly limited this

criticism. Secondly, only centers with adequately proven experience in BTC management were included. Therefore, we cannot reassure that a similar trend in EMP rate increase over time can be registered also at spoke centers. Even more importantly, the percentage of patients with access to targeted treatments could have been even lower with less stringent selection criteria.

As previously mentioned, the median OS reported among patients receiving targeted therapies (68.0 months) outperforms the one reported in other real-word data case series.[31-40] This result could be partially related to a selection bias deriving from the retrospective nature of the study, from the exclusion of gallbladder cancers from our dataset (conversely commonly included in randomized trials together with cholangiocarcinoma and characterized by a unique spectrum of molecular alterations), and from the unavailability of data of EMP tests failed due to technical issues. However, the importance of targeted therapies as a positive and independent prognostic factor in driving patient survival is confirmed by the above-mentioned case series. Moreover, when comparing data from observational studies from different countries with different healthcare models, the impact of other potential confounding factors should be considered (e.g., socioeconomic aspects, drug pricing and reimbursement policies, access to early supportive and palliative care). Keeping these limitations in mind, the rate of patients accessing targeted therapies on the basis of EMP we reported in ANITA is similar to that observed in other European countries.[40,41] Finally, only a minority of the patients included in ANITA received the current standard of care in first-line,[16-19] *i.e.* the combination of durvalumab or pembrolizumab and cisplatin plus gemcitabine because of the availability of durvalumab through an Expanded Access Program only after the publication of the TOPAZ-1 trial pembrolizumab since March 2025. However, there is currently no evidence that recognized molecular alterations could impact the efficacy of first-line immunotherapy in BTCs, while previous immune checkpoint inhibition seems not to influence the efficacy of subsequent targeted treatments.[42] Therefore, we can safely state that the value of EMP in all patients with BTCs should be considered retained, even after the introduction of immunotherapy in the treatment armamentarium.

Conclusions

To conclude, we report the first results of the ANITA study showing that, despite an increased availability of EMP in advanced CCA, ultimate access to targeted treatments remains challenging, even at referral institutions. When tailored treatment, matched to EMP results, is available, there is a clinically significant impact on outcome, with a median OS exceeding 5 years since the diagnosis of advanced disease. Strategies aiming at facilitating access to EMP and subsequent targeted treatments are therefore warranted.

Abbreviations:

BRAF: V-raf murine sarcoma viral oncogene homolog B1
BRCA: Breast cancer gene
BTCs: Biliary tract cancers
CA19-9: Carbohydrate antigen 19-9
CCA: Cholangiocarcinoma
CDx: Companion diagnostics
CEA: Carcinoembryonic antigen
CGP: Cohort genomics platform
CI: Confidence interval
dCCA: Distal cholangiocarcinoma
DNA: Deoxyribonucleic acid
ECOG-PS: Eastern cooperative oncology group performance status
EMP: Extended molecular profiling
ENS-CCA: European network for the study of cholangiocarcinoma
ESCAT: ESMO scale for clinical actionability of molecular targets
ESMO: European Society of Medical Oncology
FGFR2: Fibroblast growth factor receptor 2
FISH: Fluorescence *in situ* hybridization
GBC: Gallbladder carcinoma
GONO: Gruppo Oncologico Nord-Ovest
HBV: Hepatitis B virus

HCV: Hepatitis C virus
iCCA: Intrahepatic cholangiocarcinoma
IDH1: Isocitrate dehydrogenase 1
IDH2: Isocitrate dehydrogenase 2
IHC: Immunohistochemistry
IRCCS: Istituto di ricovero e cura a carattere scientifico
IQR: Interquartile range
HER2: Human epidermal growth factor receptor 2
HR: Hazard ratio
MDT: Multidisciplinary team
MEK: Mitogen-activated protein kinase kinase
MMR: Mismatch repair
MSI: Microsatellite instability
MTB: Molecular tumor board
NASH: Nonalcoholic steatohepatitis
NGS: Next-generation sequencing
NTRK: Neurotrophic tyrosine receptor kinase
ORR: Objective Response Rate
OS: Overall survival
PALB2: Partner and localizer of BRCA
pCCA: Perihilar cholangiocarcinoma
PD: Progressive disease
PD-1: Programmed cell death protein 1
PFS: Progression-free survival
PSC: Primary sclerosing cholangitis
RNA: Ribonucleic acid
RFS: Relapse-free survival
UI/mL: International unit/milliliter

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Tables

Table 1. Clinical, molecular and treatment characteristics of patients diagnosed with advanced disease.

Characteristics, n (%)	iCCA (n=312)	pCCA (n=85)	dCCA (n=89)	*p*	Overall (n=486)
Clinical presentation					
● Locally advanced	63 (20.2)	16 (18.9)	20 (22.5)	0.774	99 (20.4)
● Metastatic	246 (78.8)	66 (77.6)	67 (75.3)		379 (78.0)
● Missing data	3 (1.0)	3 (3.5)	2 (2.2)		8 (1.6)
Timing of metastases					
● Synchronous	242 (77.6)	47 (55.3)	52 (58.4)	**<0.001**	341 (70.2)
● Metachronous	59 (18.9)	35 (41.2)	35 (39.4)		129 (26.5)
● Missing data	11 (3.5)	3 (3.5)	2 (2.2)		16 (3.3)
Adjuvant chemotherapy*	62 (62.6)	30 (58.8)	34 (73.9)	**0.047**	126 (64.3)
Distant involvement					
● Visceral (liver, lung)	245 (78.5)	49 (57.6)	60 (67.4)	**0.001**	354 (72.8)
● Non-visceral	56 (17.9)	31 (36.5)	22 (24.7)		109 (22.4)
● Missing data	11 (3.6)	5 (5.9)	7 (7.9)		23 (4.8)
Site of metastatic lesions					
● Liver	225 (72.1)	42 (49.4)	51 (57.3)	**<0.001**	318 (65.4)
● Lung	64 (20.5)	11 (12.9)	12 (13.5)	0.121	87 (17.9)
● Nodes	140 (44.9)	31 (36.5)	19 (21.3)	**0.001**	190 (39.1)
● Bone	27 (8.7)	3 (3.5)	4 (4.5)	0.074	34 (7.0)
● Peritoneum	50 (16.0)	32 (37.6)	30 (33.7)	**<0.001**	112 (23.0)
● Other	4 (1.3)	1 (1.2)	4 (4.5)	0.113	9 (1.9)
Number of systemic lines					
● 1	308 (98.7)	84 (98.8)	89 (100)	**0.009**	481 (99.0)
● 2	167 (53.5)	39 (45.9)	31 (34.8)		237 (48.8)
● 3 or more	80 (25.6)	13 (15.3)	16 (18.0)		109 (22.4)
Type of first-line chemotherapy					
● Gem-or cape-monotherapy	28 (9.0)	8 (9.4)	17 (19.1)	**0.032**	53 (10.9)
● Cisplatin-gemcitabine	170 (55.2)	35 (41.2)	32 (35.9)		237 (48.8)
● Cisplatin-gemcitabine + ICI	78 (25.3)	28 (32.9)	32 (35.9)		138 (28.4)
● Other platinum-based combination	23 (7.5)	7 (8.2)	5 (5.6)		35 (7.2)
● Other treatment	9 (2.9)	3 (3.5)	3 (3.4)		15 (3.1)
● Missing data	0 (0.0)	3 (3.5)	0 (0)		3 (0.6)

Type of second-line chemotherapy					
● FOLFOX	64 (20.5)	15 (17.6)	15 (16.9)	0.252	94 (19.3)
● Other doublet	50 (16.0)	15 (17.6)	11 (12.4)		76 (15.6)
● Monochemotherapy	21 (6.7)	6 (7.1)	4 (4.5)		31 (6.4)
● Other	32 (10.3)	3 (3.5)	1 (1.1)		36 (7.4)
Type of extended molecular profiling	32 (10.3)	6 (7.1)	7 (7.9)	0.504	45 (9.3)
● DNA and RNA NGS	217 (69.6)	50 (58.8)	76 (85.4)		343 (70.6)
● FoundationOne® CDx					
ESCAT alteration					
● Yes	115 (36.9)	13(15.3)	11(12.4)	**<0.001**	139(28.6)
● IA	47(15.1)	3 (3.5)	1 (1.1)		51(10.5)
● IB	34(10.9)	2 (2.4)	1 (1.1)		37(7.6)
● IC	13 (4.2)	4 (4.7)	4 (4.5)		21 (4.3)
● IIB	6 (1.9)	0 (0.0)	3 (3.4)		9(1.9)
● IIIA	15 (4.8)	4 (4.7)	2 (2.2)		21 (4.3)
Type of ESCAT alteration					
❖ ESCAT I					
● *IDH1* mutation	47 (15.1)	3 (3.5)	0 (0.0)	**<0.001**	50 (10.3)
● *FGFR2* fusion/rearrangement	33 (10.6)	1 (1.2)	0 (0.0)	**<0.001**	34 (7.0)
	3 (1.0)	0 (0.0)	2 (2.2)	0.174	4(0.8)
● *HER2* amplification	4 (1.3)	0 (0.0)	1 (1.1)	0.223	6 (1.2)
● *BRAF* V600E mutation	2 (0.6)	1 (1.2)	1 (1.1)	0.779	4 (0.8)
● *KRAS* G12C	3 (1.0)	1 (1.2)	1 (1.1)	0.223	5 (1.0)
● *NTRK* fusion	6 (1.9)	4 (4.7)	2 (2.2)	0.368	12 (2.5)
● dMMR/MSI					
❖ ESCAT II					
● *FGFR2* mutation	6 (1.9)	0 (0.0)	1 (1.1)	0.131	7 (1.4)
● *HER2* mutation	0 (0.0)	0 (0.0)	3 (3.4)	0.097	3 (0.6)
❖ ESCAT III					
● *IDH2* mutation	10 (3.2)	0 (0.0)	0 (0.0)	**0.009**	10 (2.1)
● *BRCA1* mutation	2 (0.6)	0(0.0)	0 (0.0)	0.368	2(0.4)
● *BRCA*2 mutation	1 (0.3)	2 (2.4)	1 (1.1)	0.476	4 (0.8)
● *PALB2* mutation	1 (0.3)	2 (2.4)	0 (0.0)	0.779	3 (0.6)

Abbreviations: iCCA: intrahepatic cholangiocarcinoma, pCCA: perihilar cholangiocarcinoma, dCCA: distal cholangiocarcinoma, Gem: gemcitabine, Cape: capecitabine, NGS: next-generation sequencing, ESCAT: ESMO scale for clinical actionability of molecular targets.

* relative percentages of patients receiving adjuvant chemotherapy were calculated on the total amount of resected patients: 196 for the overall cohort divided into 99 iCCA, 51 pCCA and 46 dCCA.

Significant values are highlighted in bold.

Threshold for significance: $p \leq 0.05$ (Pearson Chi-squared test).

793 **Table 2.** Characteristics comparison of patients with disease harboring ESCAT alterations and
794 treated vs. non-treated with targeted therapies

Characteristics, n (%)	ESCAT I-III treated (n=26)	ESCAT I-III not treated (n=155)	*p*
Gender			
• Male	10 (38.5)	71 (45.8)	0.629
• Female	16 (61.5)	84 (54.2)	
ECOG-PS at diagnosis			
• 0	16 (61.5)	106 (68.4)	0.346
• 1	7 (26.9)	33 (21.3)	
• 2	3 (11.5)	16 (10.3)	
Age at diagnosis, years			
• <65	17 (65.4)	75 (48.4)	0.164
• ≥65	9 (34.6)	80 (51.6)	
CA19-9 at diagnosis			
• ≥ 500 UI/mL	1 (3.9)	9 (5.8)	0.701
• < 500 UI/mL	8 (30.8)	61 (39.4)	
• Missing data	17 (65.4)	85 (54.8)	
Liver disease			
• HBV infection	1(3.9)	7 (4.5)	0.754
• HCV infection	0 (0)	7(4.5)	
• Cirrhosis from other etiology	0 (0)	1 (0.6)	
• NASH	0 (0)	1 (0.6)	
• PSC	0 (0)	0 (0)	
• Missing data	25 (96.1)	139 (89.8)	
Site of primary tumor			
• iCCA	25 (96.1)	114 (73.5)	**0.011**
• pCCA	1 (3.9)	17 (11.0)	
• dCCA	0 (0)	24 (15.5)	
Surgery			
• Yes	14 (53.8)	88 (56.8)	0.857
• No	12 (46.2)	67 (43.2)	
Grading			
• G1-G2	6 (23.1)	24 (15.5)	0.751
• G3-G4	2 (7.7)	15 (9.7)	
• Missing data	18 (69.2)	116 (74.8)	
Adjuvant chemotherapy			
• Yes	9 (34.6)	51 (32.9)	0.271
• No	3 (11.5)	4 (2.6)	

• Missing data	2 (7.7)	33 (21.3)	
Clinical presentation			
• Locally advanced	5 (19.2)	27 (17.4)	0.819
• Metastatic	21 (80.8)	87 (56.1)	
• Missing data	0 (0)	1 (0.6)	
Timing of metastases			
• Synchronous	19 (73.1)	80 (51.6)	0.908
• Metachronous	7 (26.9)	35 (22.6)	
• Missing data	0 (0)	6 (3.9)	
Distant involvement			
• Visceral (liver, lung)	25 (96.1)	87 (56.1)	0.067
• Non-visceral	1 (3.9)	24 (15.5)	
• Missing data	0 (0)	4 (2.6)	
Site of metastatic lesions			
• Liver	22 (84.6)	78 (50.3)	0.435
• Lung	1 (3.8)	6 (3.9)	0.850
• Nodes	8 (30.8)	42 (27.1)	0.699
• Peritoneum	1 (3.8)	22 (14.2)	0.075
• Other	2 (7.7)	3 (1.9)	0.510
Number of systemic lines			
• 1	26 (100)	113 (72.9)	**0.025**
• 2	23 (88.5)	64 (41.3)	
• 3	14 (53.8)	38 (24.5)	
Type of first-line chemotherapy			
• Platinum-based	20 (76.9)	96 (61.9)	0.420
• Other monotherapies or doublet	6 (23.1)	14 (9.0)	
• Missing data	0 (0)	3 (1.9)	
ECOG PS after progression to 1st line treatment			
• 0	14 (53.8)	81 (52.3)	0.796
• 1	6 (23.1)	38 (24.5)	
• 2	2 (7.7)	10 (6.5)	
• Not available	4 (15.4)	26 (16.7)	
Timing of molecular profiling			
• During first-line chemotherapy	17 (65.4)	114 (73.6)	0.067
• After PD to first-line chemotherapy	9 (34.6)	23 (14.8)	
• Missing data	0 (0)	18 (11.6)	

Method of assessment			
Single gene evaluation	20 (76.9)	136 (87.7)	0.165
● IHC	15 (57.7)	125 (80.6)	
● FISH	7 (26.9)	39 (25.2)	
Extended molecular profiling	23 (88.5)	137 (88.4)	0.770
● FoundationOne® CDx	22 (84.6)	123 (79.4)	
● Local DNA and RNA NGS	1 (3.9)	14 (9.0)	
Type of ESCAT alteration			
❖ ESCAT I	24 (92.4)	117 (75.5)	0.099
● *IDH1* mutation	6 (25.0)	53 (45.3)	
● *FGFR2* fusion/rearrangement	16 (66.7)	26 (22.2)	
● *HER2* amplification	0 (0.0)	12 (10.3)	
● *BRAF* V600E mutation	1 (4.2)	5 (4.3)	
● *KRAS* G12C	0 (0.0)	4 (3.4)	
● *NTRK* fusion	0 (0.0)	6 (5.2)	
● dMMR/MSI	1 (4.2)	11 (9.3)	
❖ ESCAT II	1 (3.8)	15 (7.1)	0.871
● *FGFR2* mutation	1 (100.0)	6 (40.0)	
● *HER2* mutation	0 (0.0)	9 (60.0)	
❖ ESCAT III	1 (3.8)	23 (16.8)	0.164
● *IDH2* mutation	0 (0.0)	12 (52.2)	
● *BRCA1 mutation*	1 (100.0)	3 (13.0)	
● *BRCA2* mutation	0 (0.0)	6 (26.1)	
● *PALB2* mutation	0 (0.0)	2 (8.7)	

Abbreviations: ESCAT: ESMO scale for clinical actionability of molecular targets, BTC: biliary tract cancer, ECOG PS: Eastern Cooperative Oncology Group Performance Status, CA19-9: carbohydrate antigen 19-9, HBV: hepatitis-B virus, HCV: hepatitis-C virus, NASH: non-alcoholic steatohepatitis, PSC: primary sclerosing cholangitis, iCCA: intrahepatic cholangiocarcinoma, pCCA: perihilar cholangiocarcinoma, dCCA: distal cholangiocarcinoma, IHC: immunohistochemistry, FISH: fluorescence in situ hybridisation, NGS: next-generation sequencing.

Significant values are highlighted in bold.

Threshold for significance: $p \leq 0.05$ (Pearson Chi-squared test).

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812 **Table 3.** Prognostic factors for overall survival and progression-free survival among patients
813 with advanced disease

	Overall survival				Progression-free survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
ECOG PS ● 0 ● 1 ● 2	0.58 (0.28-1.17) 0.88 (0.66-1.17) 1	0.141	-	-	0.96 (0.59-1.54) 1.01 (0.62-1.65) 1	0.898	-	-
Age at diagnosis, years ● <65 ● ≥65	0.92 (0.70-1.95 1	0.617	-	-	0.94 (0.76-1.17) 1	0.579	-	-
Gender ● Female ● Male	0.94 (0.72-1.22) 1	0.621	-	-	0.95 (0.76-1.17) 1	0.604	-	-
Primary tumor site ● eCCA ● iCCA	0.84 (0.64-1.11) 1	0.234	-	-	0.74 (0.59-0.92) 1	**0.008**	0.80 (0.63 - 1.03) 1	0.080
CA19-9 at diagnosis ● <500 UI/mL ● ≥500 UI/mL	0.76 (0.50-1.16) 1	0.170	-	-	0.86 (0.60-1.23) 1	0.393	-	-
Surgery ● Yes ● No	0.61 (0.47-0.79) 1	**<0.001**	0.92(0.55-1.53) 1	0.744	0.81 (0.65-1.00) 1	0.056	-	-
Adjuvant chemotherapy ● Yes ● No	0.30 (0.10-0.96) 1	0.207	-	-	0.38 (0.19-0.74) 1	0.055	-	-
Clinical presentation ● Locally advanced ● Metastatic	0.92 (0.67-1.25) 1	0.574	-	-	0.88 (0.68-1.14) 1	0.349	-	-
Distant involvement ● Non-visceral		0.066	-	-		**0.044**		0.226

• Visceral (liver, lung) 0.72 (0.53-0.99) 1					0.75 (0.58-0.97) 1		1.19 (0.90-1.58) 1	
Timing of metastases • Metachronous • Synchronous 0.62 (0.47-0.83) 1	**0.004**	0.63 (0.35-1.14) 1	0.128		0.81 (0.64-1.02) 1	0.093	-	-
Type of first-line chemotherapy • Platinum combos • Monotherapy 0.96 (0.62-1.48) 1	0.847	-	-		1.15 (0.82-1.62) 1	0.434	-	-
Nodal metastases • Yes • No 1.51 (1.14-2.00) 1	**0.002**	1.85 (1.29-2.64) 1	**0.001**		1.11 (0.88-1.39) 1	0.364	-	-
ORR to 1st line • Yes • No 0.56 (0.42-0.73) 1	**0.0001**	0.40 (0.26-0.61) 1	**<0.0001**		0.49 (0.39-0.61)	**<0.0001**	0.47 (0.37-0.61) 1	**<0.001**
ESCAT I-III • Yes • No 0.60 (0.42-0.84) 1	**0.002**	0.69 (0.47-1.02) 1	0.062		1.08 (0.82-1.41) 1	0.588	-	-
ESCAT I-III • Treated with targeted therapy • Not treated with targeted therapy 0.49 (0.28-0.86) 1	**0.040**	(*)	-		0.95 (0.59.1.51) 1	0.809	-	-
FGFR2 status • Fusion/rearrangement • Wild type 0.49 (0.33-0.74) 1	**0.006**	0.54(0.30-0.95) 1	***0.035***		0.96 (0.65-1.41) 1	0.834	-	-
IDH1 status • Mutated • Wild type 0.93 (0.61-1.41) 1	0.738	-	-		0.99 (0.70-1.40) 1	0.946	-	-

(*) Not included in Cox regression number of events too low to compute COX regression

Note: analyses conducted in 265 patients

Abbreviations: HR: hazard ratio, CI: confidence interval, ECOG PS: Eastern Cooperative Oncology Group Performance Status, iCCA: intrahepatic cholangiocarcinoma, eCCA: extrahepatic cholangiocarcinoma, CA19-9: carbohydrate antigen 19-9, CT1: first-line chemotherapy, ESCAT: ESMO scale for clinical actionability of molecular targets.

Significant values are highlighted in bold.

Threshold for significance: p≤0.05 (log-rank test for univariate model, Cox proportional-hazard regression for multivariate model).

Figure legends

Figure 1. Study flow chart.

Figure 2. (**A**) Prevalence of specific alterations among the ESCAT I-III profiled BTCs according to primary tumor site. (**B**) Occurrence of ESCAT alterations (tier I vs. II-III) according to the different BTCs metastatic sites.

Figure 3. Flow diagram with detailed timing of administration of targeted treatments in patients with ESCAT alterations.

Figure 4. (**A**) Kaplan-Meier curves for OS according to the presence of ESCAT alterations. (**B)** Kaplan-Meier curves for OS according to molecular profiling and exposure to targeted treatments. (C) Kaplan-Meier curves for OS from 2nd line systemic therapy according to molecular profiling and exposure to targeted treatments. (D) Kaplan-Meier curves for PFS from 2nd line systemic therapy according to molecular profiling and exposure to targeted treatments. Threshold for significance: $p \leq 0.05$ (log-rank test for univariate model)

Supplementary materials

Supplementary Table 1. List of genes covered by every local NGS platform.

Supplementary Table 2. Baseline patients' characteristics according to different primary tumor sites. Threshold for significance: p ≤ 0.05 (Pearson Chi-squared test).

Supplementary Table 3. Characteristics of overall population according to access to EMP.

Supplementary Table 4. Characteristics of advanced population according to access to EMP.

Supplementary Table 5. Targeted treatments administered according to ESCAT alterations.

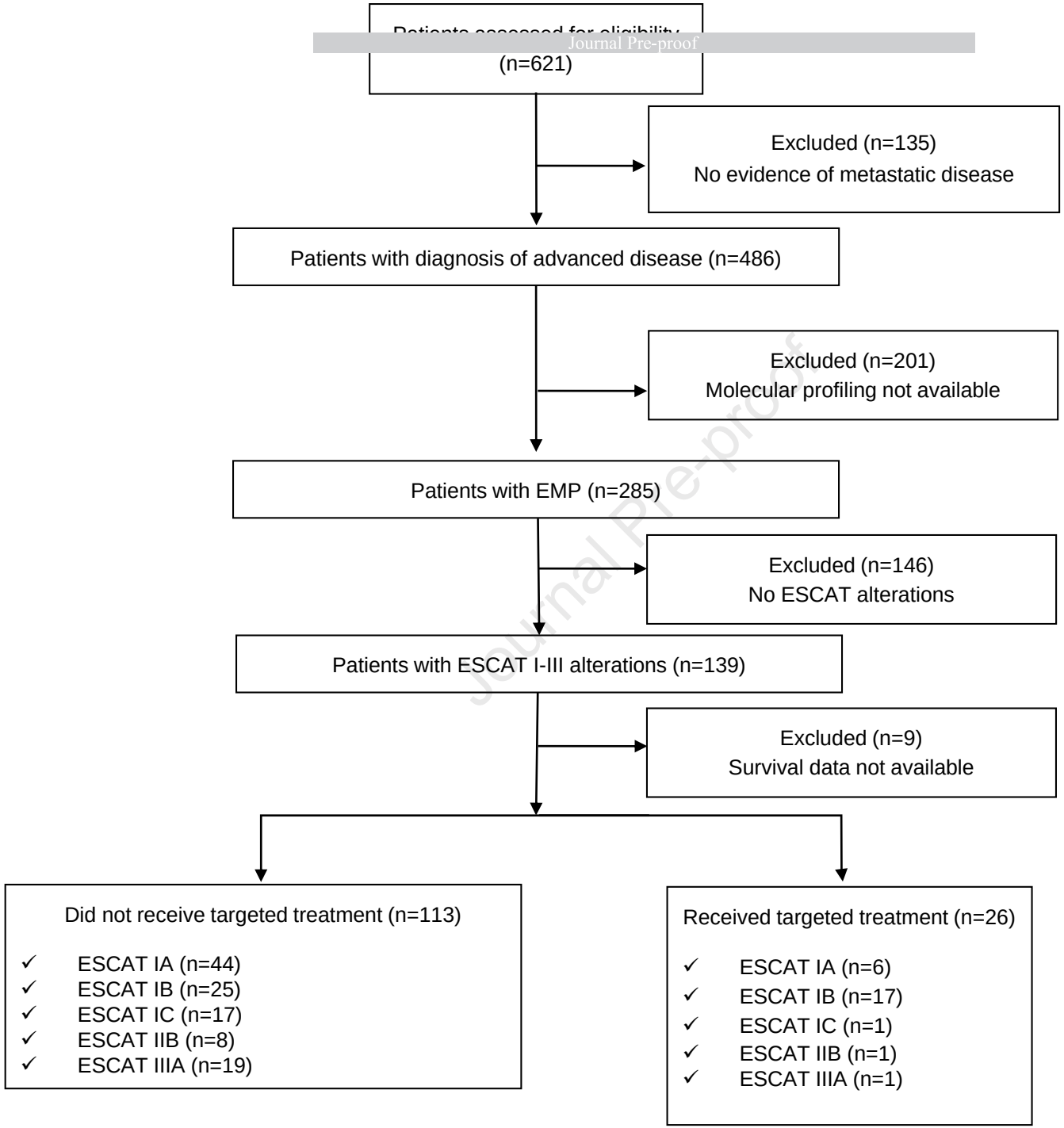
Supplementary Table 6. Baseline characteristics of patients with *FGFR2* fusion/rearrangement compared to *FGFR2* wild type. Threshold for significance: p ≤ 0.05 (Pearson Chi-squared test).

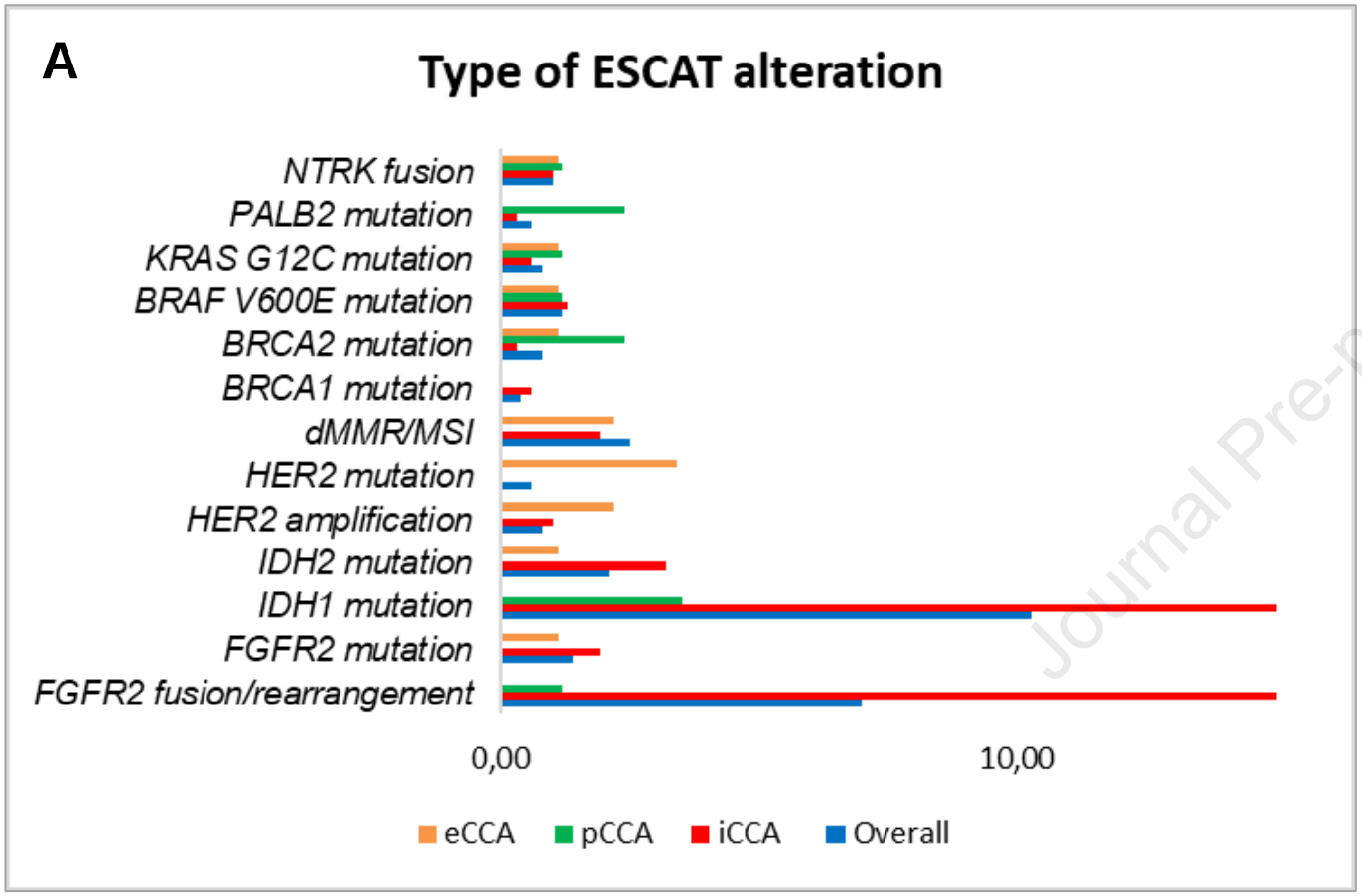
Supplementary Table 7. Baseline characteristics of patients with *IDH1* R132X mutations compared to *IDH1* wild type. Threshold for significance: p ≤ 0.05 (Pearson Chi-squared test).

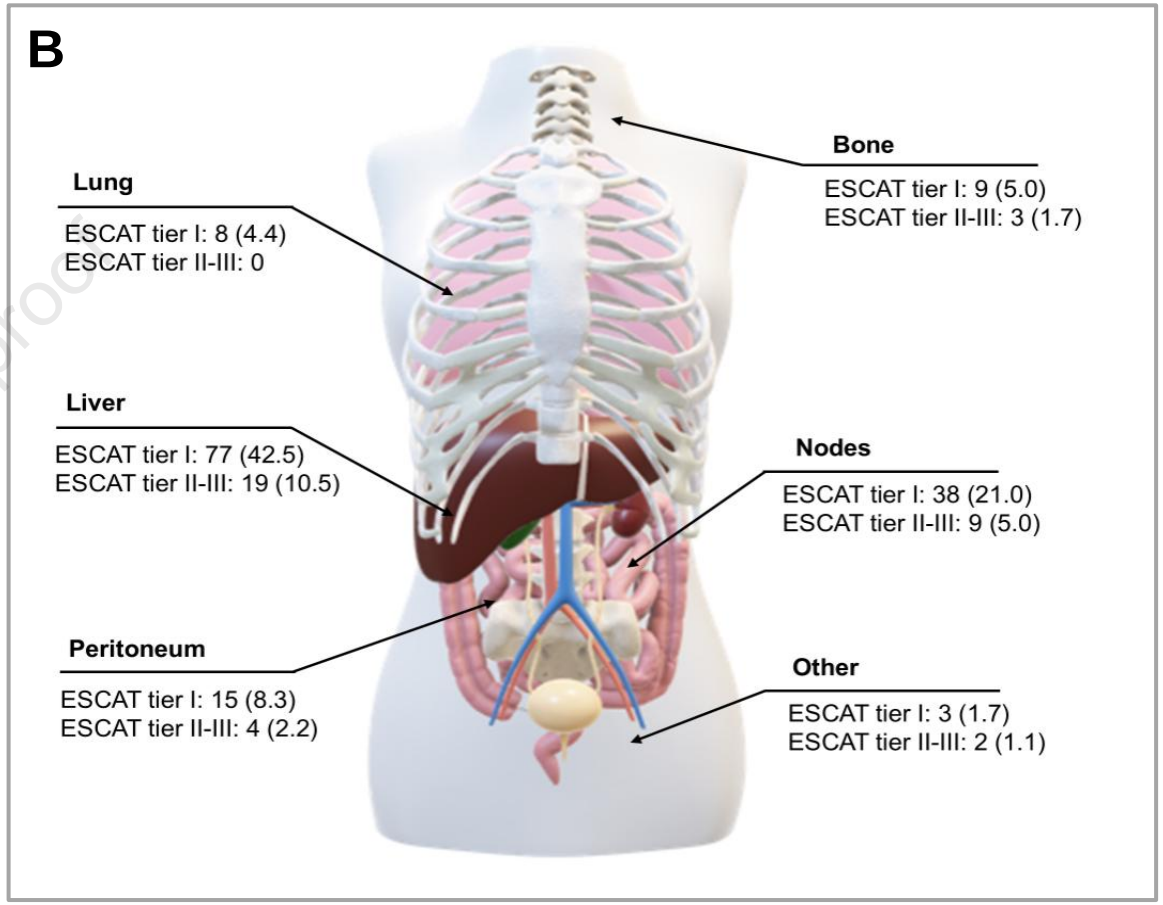
Supplementary Figure 1. (**A**) EMP rate over years in the overall population. (**B**) EMP rate over years according to primary tumor location. (C) Rate of advanced patients treated with targeted treatment among those with EMP.

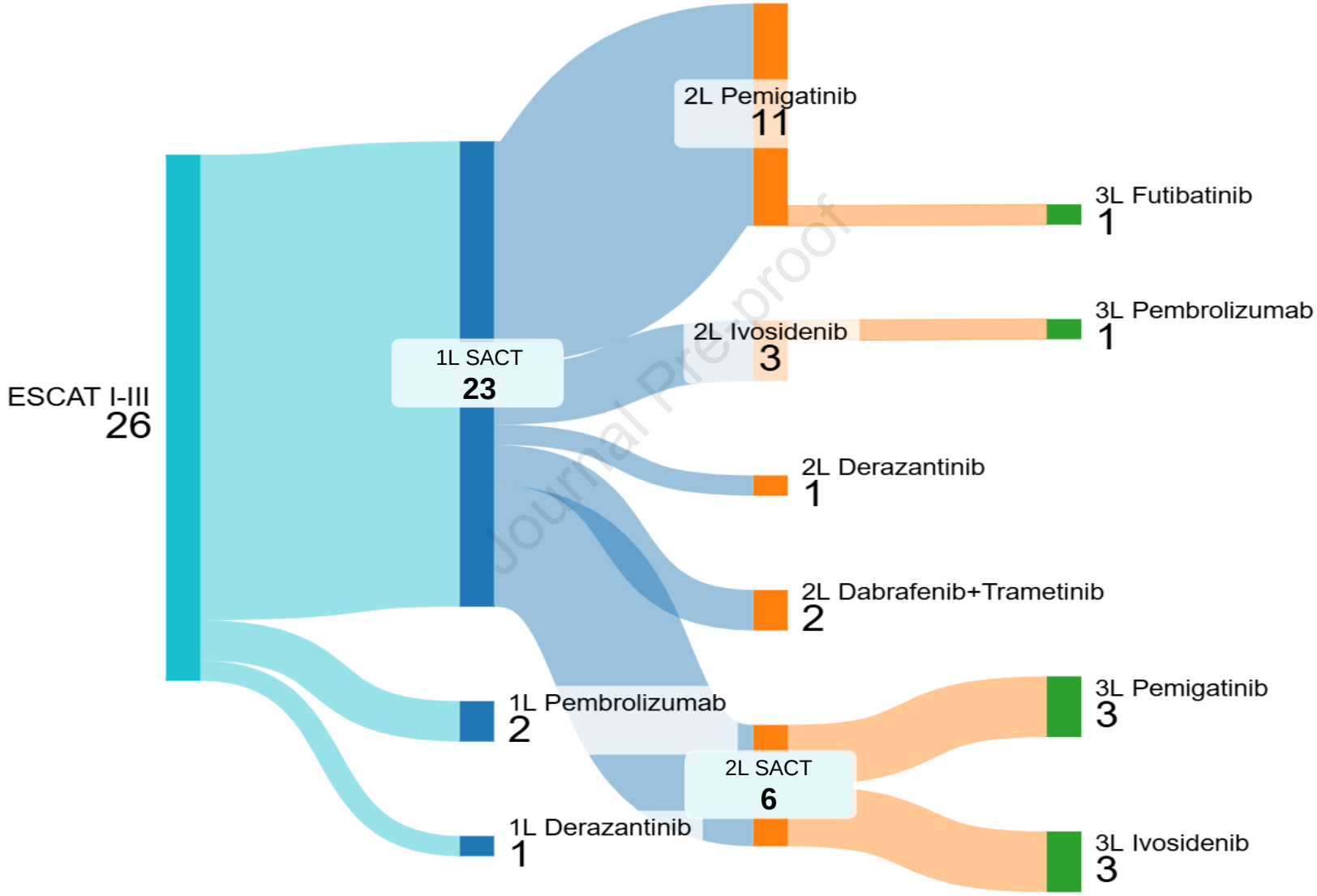
Supplementary Figure 2. (**A**) Median OS of patients with unresectable/metastatic disease. (**B**) Median PFS of patients with unresectable/metastatic disease who received first-line systemic therapy. Threshold for significance: p≤0.05 (log-rank test for univariate model)

Supplementary Figure 3: (A) Median OS of patients with unresectable/metastatic disease harbouring *FGFR2* fusions/rearrangements. (B) Median OS of patients with unresectable/metastatic disease harbouring *IDH1* R132X mutations. Threshold for significance: p≤0.05 (log-rank test for univariate model)









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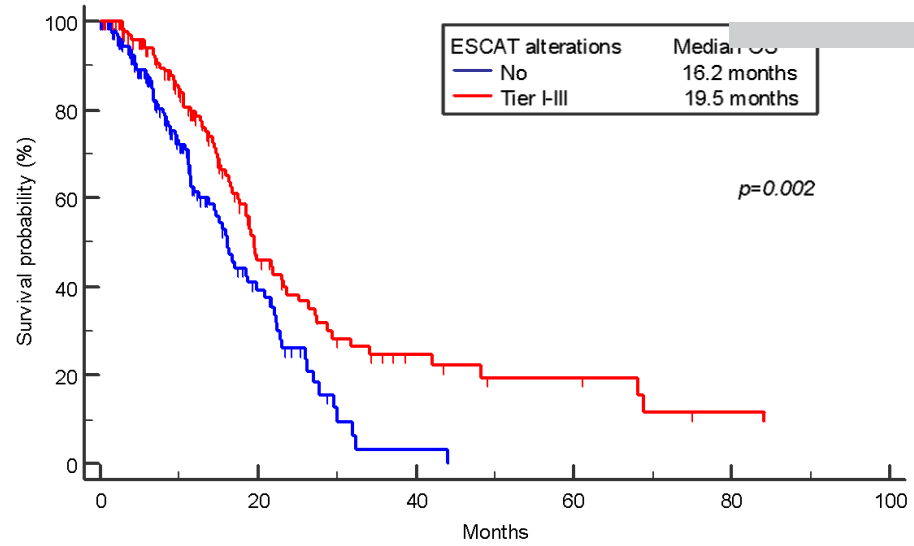
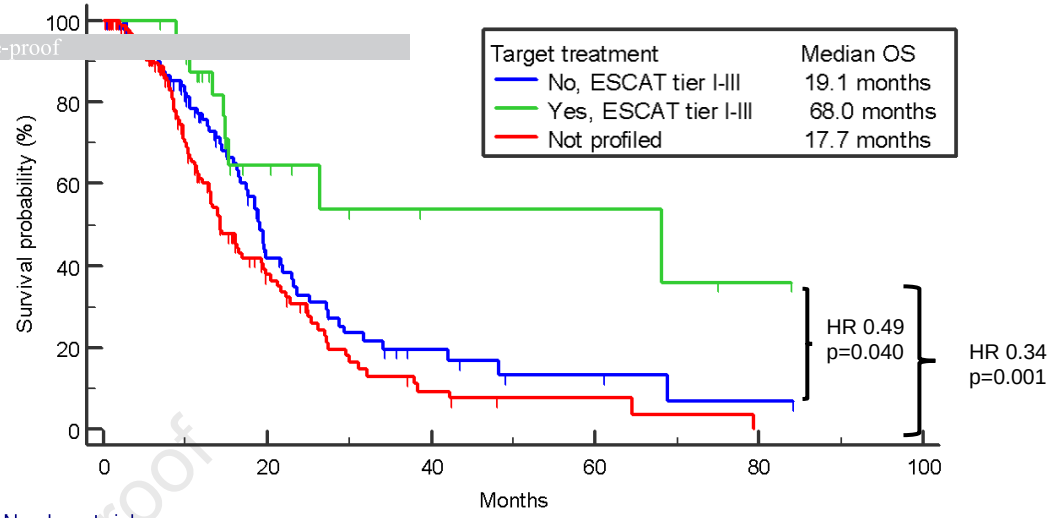


Number at risk

Group: No	16.2 months				
154	22	1	0	0	0
Group: Tier I-III	19.5 months				
130	32	10	6	2	0

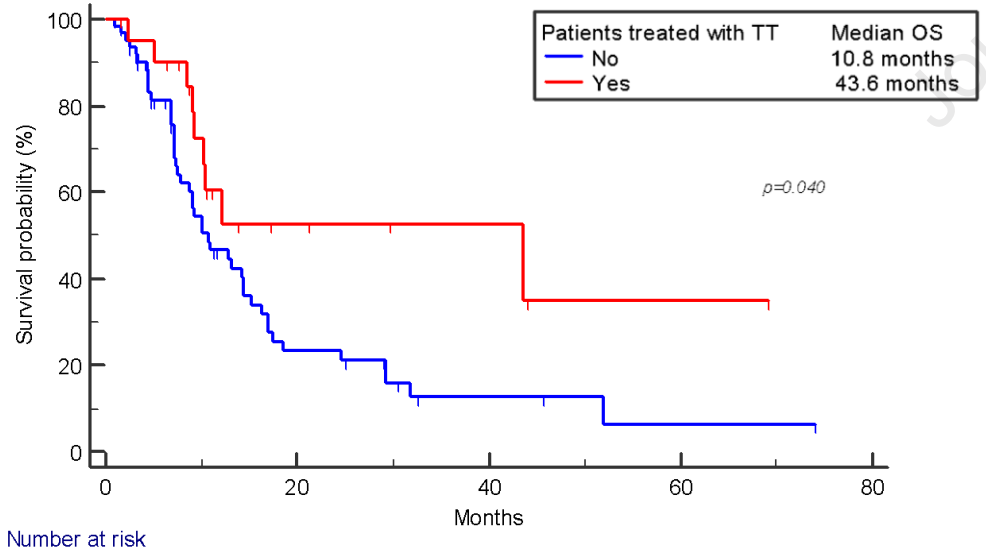
B



Number at risk

Group: No, ESCAT tier I-III	19.1 months				
104	24	7	3	1	0
Group: Yes, ESCAT tier I-III	68.0 months				
26	8	3	3	1	0
Group: Not profiled	17.7 months				
168	27	5	2	0	0

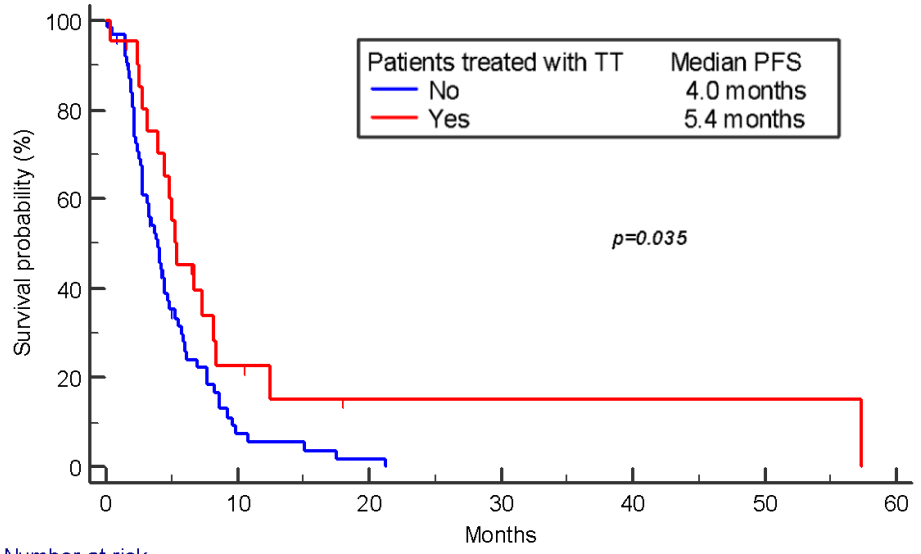
C



Number at risk

Group: No	10.8 months			
63	11	3	1	0
Group: Yes	43.6 months			
21	5	3	1	0

D



Number at risk

Group: No		4.0 months				
63	4	1	0	0	0	0
Group: Yes		5.4 months				
21	4	1	1	1	1	0

Highlights

- ANITA is the largest observational, retrospective and prospective, study exploring the impact of molecular profiling and matched targeted treatment in a large cohort of patients with advanced cholangiocarcinoma.
- Availability of molecular profiling increased in the latest years.
- Access to targeted treatment remains limited in routine practice for patients with advanced disease.
- Administration of targeted treatment has a significant impact on the overall survival of patients with advanced disease.
- Strategies aiming at promoting timely access to new agents in this disease are warranted.