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Young-onset biliary tract cancers: Characteristics, treatment patterns, and patient outcomes

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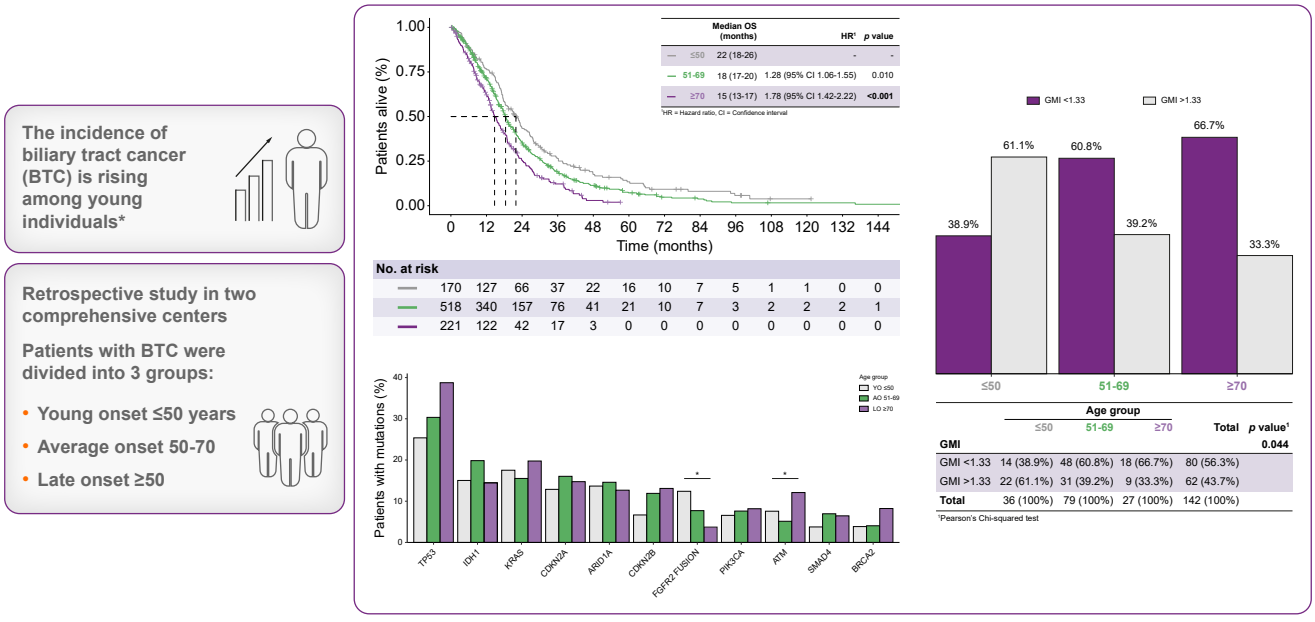
Graphical abstract

Patients with young onset metastatic biliary tract cancer have improved outcomes, more *FGFR2* fusions and seems to response better to molecularly-matched treatments



Highlights:

- Patients with young-onset BTC have improved outcomes and more treatable disease in the metastatic setting.
- *FGFR2* fusions are more frequent in patients with young-onset BTC, paving the way for precision oncology-based approaches.
- Patients with young-onset BTC could benefit from targeted treatment of ESCAT I-IIIA alterations.

Impact and implications:

The study underscores the scientific justification for investigating age-related differences in biliary tract cancers, revealing that patients with young-onset biliary tract cancer have improved survival outcomes and a higher prevalence of actionable molecular alterations, particularly *FGFR2* fusions. Physicians can apply these results by incorporating molecular profiling and targeted therapies earlier in the treatment plan for younger patients, potentially improving their prognosis and quality of life. However, it is crucial to consider the study's limitations, such as the retrospective design and potential selection bias, to avoid overgeneralization and ensure appropriate application of the findings in clinical practice and future research.

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Research article

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Young-onset biliary tract cancers: Characteristics, treatment patterns, and patient outcomes

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Background & Aims: The incidence of biliary tract cancers (BTC) among young individuals (≤50 years) is currently rising. We aimed to investigate the clinical, therapeutic and molecular characteristics and outcomes of young-onset BTC (YO-BTC).

Methods: Patients with histologically confirmed BTC treated at Gustave Roussy (France) and Vall d'Hebron Institute of Oncology (Spain) were categorized as YO-BTC (≤50 years old), average-onset (AO-BTC; 51-69 years old), and late-onset (LO-BTC; ≥70 years old). The primary endpoint was overall survival (OS). The secondary endpoint was the growth modulation index (GMI), *e.g.*, the ratio of progression-free survival (PFS) with the targeted therapy line to the PFS of the n-1 line.

Results: Among 1,023 patients with BTC, 184 (18%) had YO-BTC, 561 (54.8%) had AO-BTC, and 278 (27.2%) had LO-BTC. Median OS in metastatic patients was longer in the YO group (22 months; 95% CI 18–26) than in the AO group (18 months; 95% CI 17–20; $p = 0.010$) or LO group (15 months; 95% CI 13–17; $p < 0.001$), despite a higher tumor burden in YO-BTC. *FGFR2* fusions were more frequent in YO-BTC (12% *vs*. 5.7% AO and 4.3% LO; $p = 0.038$). Patients with YO-BTC received more targeted therapies as second or later lines (48%, 37%, and 29% for YO, AO, and LO; $p = 0.020$). Among patients receiving molecular-matched treatments, GMI >1.33 was more frequent in YO-BTC (61.1%, 39.2%, and 33.3% for YO, AO, and LO; $p = 0.044$), although no differences in PFS or OS were observed.

Conclusion: Patients with YO-BTC have improved outcomes in the metastatic setting. The YO-BTC group is enriched for *FGFR2* fusions, highlighting opportunities for precision oncology-based approaches.

Introduction

Over the past decade, the incidence of gastrointestinal cancers has risen alarmingly among individuals aged 50 years or younger, referred to as young-onset (YO) patients..[1] Biliary tract cancers (BTCs) are malignant tumors arising from epithelial cells of the bile duct (cholangiocarcinomas [CCA]) or the gallbladder,[4,5] and they are characterized by their aggressive nature and poor prognosis.[6] Historically considered to be a disease affecting older individuals, there is a growing body of evidence suggesting an increasing incidence of YO-BTCs.[2,3]

This shift in age distribution has raised concerns among clinicians and researchers, as the clinical presentation, the molecular characteristics, and the underlying risk factors for YO-BTCs may differ significantly from those observed in older patients.[3,7] Intrahepatic CCA mortality in younger adults has shown an increasing trend since 1980.[8] The etiology of these tumors in younger adults remains elusive. Recent changes in the exposome, such as diet, environmental pollution, and obesity, along with their interaction with genetic susceptibility, are suspected factors.[9]

Understanding the molecular pattern of the tumor and finding targetable alterations are two main challenges in cancer treatment.[10] In advanced BTCs, targeted therapies, including FGFR2 inhibitors, IDH1 inhibitors, anti-HER2 agents, or anti-BRAF/anti-MET agents, are currently recommended in the second-line setting in international guidelines.[11,12]

This study aimed to investigate the clinical and molecular characteristics of YO-BTCs, focusing on genomic and transcriptomic characteristics compared to adults with older-onset BTC.

Patients and methods

Patients

All consecutive patients with a histologically confirmed BTC who were followed in two European tertiary centers between 2001 and 2023 (for the Vall d'Hebron Institute of Oncology

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[VHIO], Barcelona, Spain) or between 2015 and 2022 (for Gustave Roussy [GR], Villejuif, France) were included. All eligible patients who underwent genomic analysis signed informed consent. Clinical characteristics, treatment, and outcomes were retrospectively collected from the hospital chart review. Study data were collected and managed using *REDCap* (electronic data capture tools) hosted at GR and VHIO. This retrospective study complies with the French MR004 methodology regarding general data protection regulation for non-interventional retrospective health research (N° 2018-155 3rd of May 2018) and was approved by the GR institutional review board in compliance with the Helsinki declaration. The study was also approved by the Ethics Committee of the VHIO, which waived the need for written informed consent (PR(AG)228/2023).

Molecular sequencing

Patients with BTC were offered molecular profiling by next-generation sequencing (NGS) as part of their clinical management in an attempt to identify actionable molecular alterations. For the GR cohort, either tumor tissue analysis (for 80% of the cases), liquid biopsies (for 7% of the cases), or both (for 13% of the cases) were performed. For the VHIO cohort, NGS from tumor samples was performed for all cases.

The different molecular panels used included (Table S1; Fig. S1): in-house GR panel (75 oncogenes and tumor suppressor genes);[13] in-house VHIO p300 panel (300 genes); FoundationOne®CDx panel, including 324 oncogenes, tumor suppressor genes, or gene rearrangements, as well as the microsatellite stability status and tumor mutational burden, used in both centers; at VHIO, the molecular panels Amplicon® (60 genes), OncoMine® (161 genes).

The molecular profiles of patients were reviewed, interpreted, and discussed by a molecular tumor board, which met on a weekly basis. Actionability of molecular alterations was categorized based on the European Society of Medical Oncology scale for Clinical Actionability of Molecular Target (ESCAT) classification.[14] Considerations for molecularly matched treatments (MMTs) were based on variant annotation databases, such as OncoKB, CIViC, My Cancer Genome, and the literature, as well as on approval of the European Medicines Agency, data from ClinicalTrials.gov, and clinical trials available at both institutions.

Study endpoints and evaluation

The primary endpoint was overall survival (OS), defined as the time between the diagnosis of advanced BTC and death or loss to follow-up. Secondary endpoints included: i) progression-free survival (PFS), defined as the time between treatment initiation and disease progression according to RECIST 1.1 criteria or death, or the date of last follow-up for patients alive without progression; and ii) the growth modulation index (GMI), defined as the PFS under the MMT (PFSn, *e.g.* oriented treatment line) to the PFS under treatment received prior to MMT (PFSn−1). A GMI ≥1.33 was considered to indicate a clinically meaningful benefit.[15]

Patients were only included in the outcome analysis for molecular alterations and targeted treatments if they had already undergone molecular profiling and initiated at least one line of therapy in the advanced setting.

RNA-seq analyses

For the GR cohort, frozen tumor samples from BTC tumors were selected for combined RNA extraction; the presence of neoplastic cells was confirmed by pathological analysis. RNA was extracted for all samples using the RNeasy® Micro Handbook kit (Qiagen®), and mRNA profiles were obtained using Illumina NovaSeq 6000 Sequencing System after polyA enrichment. Quantification of transcript abundance was performed using the Salmon method.

Differential expression analysis between cellular groups was performed using the DESeq2 package. Adjusted $p < 0.05$ indicates significant differential gene regulation between the *KRASG12* gene and other *KRAS* genes. Gene set-enrichment analysis (GSEA) between the YO and older-onset CCA groups was performed using the fgsea package. Stromal components were assessed using MCPCounter. Adjusted p <0.05, and normalized enrichment scores above or below –2 were used to indicate significant differential signaling pathways between the YO and older-onset CCA groups.

Statistics

Collected data were summarized using appropriate descriptive statistics, including i) the mean, standard deviation, median, interquartile range, minimum, and maximum values for continuous variables; and ii) numbers and percentages for categorical variables. Comparisons among groups were performed with non-parametric statistics: Kruskal-Wallis rank sum test for continuous variables and Pearson's $\chi 2$ or Fisher's exact tests for categorical data.

Survival analyses were performed using Kaplan–Meier analyses (for median estimates) and univariate Cox proportional hazards models (for hazard ratio [HR] estimates), seven of which were stratified by all age groups and three by ESCAT category (for a single age group). The three age groups were: YO-BTC (≤50 years old), average-onset (AO) BTC (51–69 years old), and late-onset (LO) BTCs (≥70 years old). Age-stratified analyses were: i) OS from diagnosis; ii) OS from metastasis; iii) PFS from 1st line; iv) PFS from MMT for any ESCAT; v) PFS from MMT for ESCAT IA-IIIA; vi) PFS from MMT for ESCAT IIIB-IV; and vii) OS from MMT for any ESCAT. Analyses stratified by ESCAT (IA-IIIA or IIIB-IV) were: viii) OS from metastasis for YO-BTC; ix) OS from metastasis for AO-BTC; and x) OS from metastasis for LO-BTC. A multivariate Cox model for OS since diagnosis was also performed. The initial proposed factors to include were: i) age group (YO-BTC, AO-BTC, LO-BTC); ii) sex (female, male); iii) stage (resectable, locally advanced, metastatic); iv) location (intrahepatic, extrahepatic, gallbladder); v) tobacco use (ex, yes, never); vi) diabetes (yes, no); vii) ECOG-performance status (0-1, 2-3); viii) grade (G1, G2, G3, undefined); ix) extrahepatic (no, yes); x) multiple liver metastases (no, yes); xi) mismatch repair status (microsatellite stable, microsatellite instability); xii) surgery (yes, no); and xiii) adjuvant treatment (yes, no). To avoid collinearity issues, all factors were explored pairwise, and their strength of association was determined with the Cramer's V value. After this exploration, the factor stage and adjuvant treatment were excluded, as there was a strong association between them and surgery.

The reported Wald test p values indicated that each level's HR (compared to the reference level) significantly differed from 1. The proportional hazards assumption was checked for all

models. The reported time unit is months, and all provided CIs are at the 95% level. Statistical analyses were performed with R v4.3.3.[16]

Results

Characteristics of evaluated patients

We identified 1,023 patients treated for BTC between 2001 and 2023 (Fig. S2). Of these, 184 (18%) were YO-BTCs, aged 50 years or younger; 561 (54.8%) were AO-BTCs, aged between 51 and 69 years; and 278 (27.2%) were LO-BTCs, aged 70 years or older (Fig. 1A).

BMI was lower in the YO-BTC group (median: 23.0, 25.5, and 24.9 for the YO, AO, and LO groups, respectively; p <0.001); diabetes mellitus was less frequent in the YO group (2.2%, 13% and 22% for the YO, AO, and LO groups, respectively; p <0.001). There were no differences in terms of tobacco use (Table 1).

The distribution of BTC subtypes differed significantly among the three groups (p = 0.021). Overall, intrahepatic CCA was the most common subtype (62.2%), followed by extrahepatic CCA (26.5%) and gallbladder carcinoma (10.3%). The proportion of intrahepatic CCA was higher in the YO group (69%) than in the LO group (55%). Conversely, extrahepatic CCA and gallbladder carcinoma were more frequent in the LO group compared to the YO group (33% vs. 22% and 12% vs. 8.9%, respectively).

At a localized stage, there were no differences in resection of the primary tumor between the YO, AO, and LO groups (p = 0.8). Likewise, there were no differences in the groups for patients who underwent resection in terms of adjuvant chemotherapy administration (p = 0.7).

At the metastatic stage, multifocal disease was more frequent in YO-BTC (72%) compared with AO-BTC (62%) and LO-BTC (53%) (p = 0.033) (Table 1).

Outcomes and clinical benefits of treatments

In the overall cohort, the median OS from initial diagnosis was 28 months (95% CI 23–32) in the YO-BTC group, 25 months (95% CI 23-27; p = 0.14) in the AO-BTC group, and 20 months (95% CI 17-23; p <0.001) in the LO-BTC group (Fig. 1B). In metastatic patients, the median OS from metastatic diagnosis

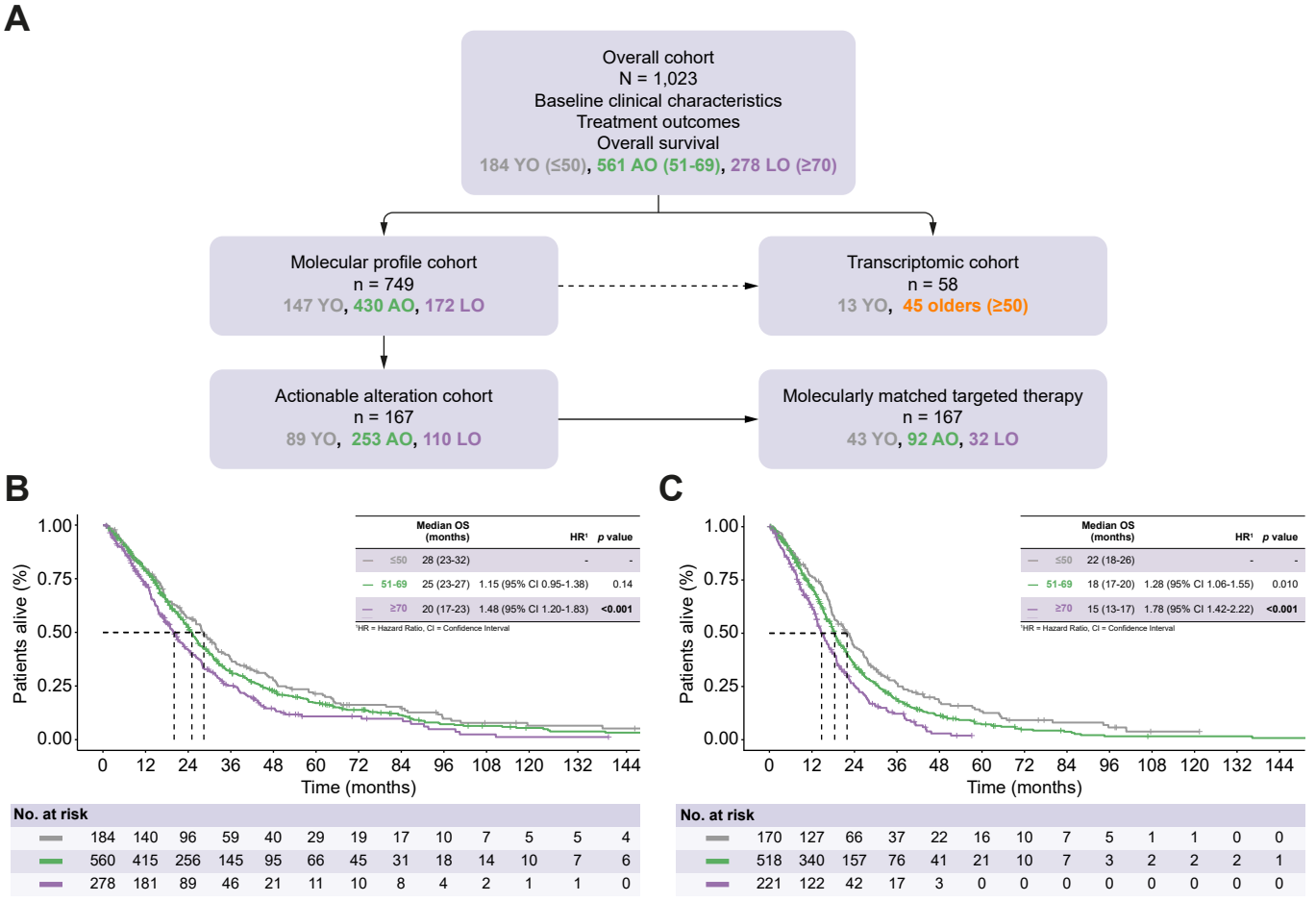


Fig. 1. Patient outcomes in the whole cohort. (A) Study flow chart. (B) Median OS from initial diagnosis in the whole cohort. Levels of significance: YO *vs.* AO, HR = 1.15, p = 0.14; YO *vs.* LO HR = 1.48, p < 0.001. (C) Median OS from the metastatic diagnosis in metastatic patients. Levels of significance: YO *vs.* AO, HR = 1.28, p = 0.010 (Cox model); YO *vs.* LO HR = 1.78, p <0.001 (Cox model). AO, average-onset; HR, hazard ratio; LO, late-onset; OS, overall survival; YO, young-onset.

Table 1. Clinical characteristics of evaluated patients.

Characteristics	≤50, n = 184[1]	51-69, n = 561[1]	≥70, n = 278[1]	*p* value[2]
Gender				**0.043**
Female	107 (58%)	270 (48%)	132 (47%)	
Male	77 (42%)	290 (52%)	146 (53%)	
Stage				**<0.001**
Resectable	69 (38%)	207 (37%)	101 (37%)	
Locally advanced	27 (15%)	76 (14%)	69 (25%)	
Metastatic	88 (48%)	275 (49%)	105 (38%)	
Location				**0.021**
Intrahepatic	125 (69%)	360 (65%)	151 (55%)	
Extrahepatic	39 (22%)	142 (25%)	90 (33%)	
Gallbladder	16 (8.9%)	55 (9.9%)	34 (12%)	
Cirrhosis				**0.018**
No	175 (98%)	508 (92%)	249 (92%)	
Yes	4 (2.2%)	46 (8.3%)	22 (8.1%)	
HBV				0.6
No	176 (97%)	546 (98%)	270 (99%)	
Yes	5 (2.8%)	11 (2.0%)	4 (1.5%)	
HCV				0.076
No	179 (99%)	535 (96%)	268 (98%)	
Yes	2 (1.1%)	23 (4.1%)	6 (2.2%)	
Tobacco				**<0.001**
Ex	14 (9.8%)	91 (22%)	38 (21%)	
Yes	24 (17%)	86 (21%)	18 (9.9%)	
No	105 (73%)	238 (57%)	125 (69%)	
Diabetes				**<0.001**
No	177 (98%)	482 (87%)	212 (78%)	
Yes	4 (2.2%)	75 (13%)	61 (22%)	
BMI				**<0.001**
Mean (SD)	23.8 (4.4)	25.8 (4.9)	25.1 (4.3)	
Median (IQR)	23.0 (20.5, 26.4)	25.5 (22.8, 28.4)	24.9 (22.1, 27.7)	
Range	16.2, 36.9	15.2, 46.2	16.4, 37.3	
Lynch syndrome				0.13
No	181 (100%)	552 (99%)	274 (100%)	
Yes	0 (0%)	5 (0.9%)	0 (0%)	
ECOG				**<0.001**
0	55 (34%)	150 (29%)	49 (19%)	
1	102 (63%)	333 (65%)	169 (67%)	
2	2 (1.2%)	23 (4.5%)	26 (10%)	
3	2 (1.2%)	3 (0.6%)	9 (3.6%)	
CA 19.9				0.10
Mean (SD)	31,488 (146, 102)	3,958 (25, 919)	3,448 (12, 242)	
Median (IQR)	201 (49, 2,113)	116 (15, 670)	85 (17, 867)	
Range	1, 999,999	0, 375,180	1, 81,411	
Grade				0.9
G1 well differentiated	21 (24%)	48 (21%)	35 (25%)	
G2 moderately differentiated	41 (47%)	113 (48%)	63 (45%)	
G3 poorly differentiated	25 (29%)	71 (30%)	41 (29%)	
Undifferentiated	0 (0%)	2 (0.9%)	2 (1.4%)	
MMR status				**0.043**
MSS	115 (100%)	313 (97%)	136 (94%)	
MSI	0 (0%)	11 (3.4%)	8 (5.6%)	
If metastatic (n = 468), extrahepatic metastases				0.5
No	18 (21%)	72 (27%)	26 (25%)	
Yes	69 (79%)	199 (73%)	78 (75%)	
If metastatic (n = 468), liver metastases/multifocal disease				**0.033**
No	23 (28%)	102 (38%)	47 (47%)	
Yes	60 (72%)	163 (62%)	54 (53%)	
Surgery				0.8
No	105 (57%)	337 (61%)	164 (59%)	
Yes	78 (43%)	220 (39%)	112 (41%)	
Adjuvant				0.7
No	83 (67%)	273 (71%)	127 (71%)	
Yes	41 (33%)	113 (29%)	53 (29%)	

Values in bold denote statistical significance.
MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable.
[1]n (%).
[2]Pearson's Chi-squared test; Kruskal-Wallis rank sum test, level of significance $p < 0.05$.

was 22 months (95% CI 18-26, reference) in the YO-BTC group, 18 months (95% CI 17-20; $p = 0.010$) in the AO-BTC group, and 15 months (95% CI 13-17; $p < 0.001$) in the LO-BTC group (Fig. 1C). In a multivariate analysis, the prognostic factors for OS were the presence of multiple liver metastases (related to a poor prognosis: HR 1.80; 95% CI 1.06-3.06; $p = 0.030$) and surgery of the primary tumor (HR 0.30; 95% CI 0.12-0.72; $p = 0.007$) (Table S2).

In the metastatic population, the proportion of patients who received first-line chemotherapy significantly differed among groups (YO, 96%; AO, 93%; LO, 79%; $p < 0.001$). A cisplatin-gemcitabine doublet was the most frequently administered chemotherapy regimen (54%, 57%, and 44% of patients in the YO, AO, and LO groups, respectively) (Fig. S3A).

The median PFS for the first-line of systemic chemotherapy was 5.8 months (95% CI 5.0-7.6, reference) for the YO-BTC group, 6.2 months (95% CI 5.8-7.0; $p = 0.4$) for the AO-BTC group, and 5.5 months (95% CI 4.6-7.0; $p = 0.9$) for the LO-BTC group (Fig. S3B).

Molecular data

The proportion of patients with molecular profiling was different among the groups (80% for YO, 77% for AO, and 62% for LO; $p < 0.001$) (Table S1). Notably, all the patients with microsatellite instability were in the YO-BTC group. With respect to the ESCAT I-IIIA targetable alterations, the proportions were similar between the groups (30%, 29%, and 22% for the YO, AO, and LO groups, respectively; $p = 0.089$), with a trend in favor of patients with YO-BTC (Table 2). *FGFR2* fusions were more frequent in the YO-BTC group (12%) than in the AO-BTC (5.7%) or LO-BTC (4.3%) groups ($p = 0.038$). *ATM* mutations were less frequent in the YO-BTC group than in the LO-BTC group (7.4% for YO and 5.1% for AO *vs.* 12% for LO, $p = 0.022$). The rates of the other main actionable molecular alterations (*e.g. IDH1/2, BRAF-V600E, FGFR2* mutations, *NTRK* fusion, *HER2*, and microsatellite instability) did not differ between the YO-BTC group and the older-onset (AO and LO) groups (Figs 2 and S4; Table S3).

Outcomes and clinical benefits of targeted therapies and molecularly matched treatments

Overall, 167 patients (16.3%) received an MMT. Most MMTs were administered in the second-line setting (62%) (Table S4). When molecular profiling was performed, the most frequent actionable molecular alteration was an *IDH1* pathogenic variant, occurring in 121 patients (16.2%) (Table S2). The most frequently prescribed MMT was ivosidenib for 24 patients (20%) with mutated *IDH1*. Other MMTs included anti-HER2 treatments for *HER2* amplifications, erdafitinib for *FGFR2* alterations, and dabrafenib and trametinib for the *BRAF* V600E mutation (Table S5).

Overall, 43 patients (48%) in the YO-BTC group had a targetable alteration, and the administration of MMTs in this group was more frequent than in the two other groups (23% for YO, 16% for AO, and 12% for LO; $p = 0.003$) (Table 2). Further, with respect to MMT administration, we observed significant differences across age groups for patients in the ESCAT IA-IIIA cohort (71% in YO, 50% in AO, and 44% in L; $p = 0.006$) (Fig. 3A; Table S6) but not for patients in the ESCAT IIIB-IV cohort (9.1% in YO, 13% in AO, and 5% in LO; $p = 0.8$) (Table 2; Table S6).

The PFS following an MMT, independently of the ESCAT alteration, was similar for all three groups (YO [reference], 5.7 months; 95% CI 3.9-9.3; *vs.* AO, 5.2 months; 95% CI 3.8-8; $p > 0.9$; and LO, 4.4 months; 95% CI 2.0-10; $p = 0.4$) (Figs. S5 and 6).

Patients in the YO-BTC group had a significantly higher rate of GMI >1.33 after receiving MMT compared to the older-onset patients (61% for YO, 39% for AO, and 33% for LO; $p = 0.044$) (Fig. 3B).

For patients who received an MMT, the OS was higher overall in the YO-BTC group, independently of the ESCAT alteration: YO (reference), 34 months; 95% CI 26-42; *vs.* AO, 27 months; 95% CI 23-36; $p = 0.4$; and LO, 23 months; 95% CI 19-33; $p = 0.038$ (Fig. 3C). Notably, the benefit was higher for each age category in the ESCAT IA-IIIA population (35, 27, and 21 months, for YO, AO, and LO, respectively) compared to the ESCAT IIIB-IV population (19, 25, and 28 months, for YO, AO, and LO, respectively) (Fig. 3D). Focusing on patients with YO-BTC, the OS also tended to be higher in the ESCAT I-IIIA subgroup than in the IIIB-IV subgroup (35 *vs.* 19 months; $p = 0.057$).

Transcriptomic analyses

To understand the differences between YO and the older-onset patients, we performed RNA-sequencing for a subset of 58 patients with BTC from the GR cohort, with a differential gene analysis between 13 patients with YO-BTC and 45 AO- or LO-BTC.

Among the top differentially expressed genes, several genes upregulated in YO-BTCs are involved in extracellular matrix remodeling (*CTSK, DPT,* and *MFAP4*), angiogenesis (*ANGPTL1*), Wnt signaling (*LGR6* and *FRZB*), or Th2 induction (*PTGER2* and *IL19*), and 17 of these genes are in regions of the immunoglobulin variable domain. On the other hand, genes upregulated in the older-onset patients (AO and LO) were primarily involved in TGFβ signaling (*VASN* and *KLF14*), malignant transformation (*CCN6, S100A2,* and *LIN28B*), and neuronal activity (*DENND1, LHFPL4, CSMD3,* and *KCNH3)* (Fig. S7A).

Using GSEA, we observed a significant upregulation in YO-BTC of immune pathways involved in B-cell receptor (BCR) signaling, Fc gamma receptor–dependent phagocytosis, Fc epsilon receptor (FCERI) signaling, complement cascade cytokine signaling, and PD-1, and TCR signaling, as well as in pathways involved in hemostasis and ECM remodeling. In contrast, patients in the AO and LO groups showed significant upregulation in pathways involved in the cell cycle and DNA repair (Fig. S7B).

We assessed whether BTC stromal components differed according to the onset age. Consistent with the GSEA results, cancer-associated fibroblasts, endothelial cells, and immune cells (including B cells, CD8+ T cells lymphocytes, and NK cells) were enriched in the YO-BTC group compared to the other two groups (Fig. S7C).

Discussion

Patients with YO-BTC have a rare but increasingly recognized condition.[17,18] They can benefit from precision-based medicine, as they have an enrichment for *FGFR2* fusions, especially

Table 2. Molecular alteration according to ESCAT.

Characteristic	≤50, n = 184[1]	51-69, n = 561[1]	≥70, n = 278[1]	*p* value[2]
Targetable alteration				0.6
No	63 (41%)	186 (42%)	68 (38%)	
Yes	89 (59%)	253 (58%)	110 (62%)	
ESCAT				**0.035**
I-A	21 (24%)	70 (28%)	22 (20%)	
I–B	24 (27%)	37 (15%)	10 (9.1%)	
I–C	3 (3.4%)	25 (10.0%)	15 (14%)	
II-B	2 (2.2%)	12 (4.8%)	6 (5.5%)	
III-A	6 (6.7%)	16 (6.4%)	9 (8.2%)	
III-B	19 (21%)	46 (18%)	21 (19%)	
IV	14 (16%)	45 (18%)	27 (25%)	
ESCAT I-IIIA3				0.089
No	128 (70%)	401 (71%)	216 (78%)	
Yes	56 (30%)	160 (29%)	62 (22%)	
Targeted therapy[3]				**0.003**
No	141 (77%)	469 (84%)	246 (88%)	
Yes	43 (23%)	92 (16%)	32 (12%)	

Values in bold denote statistical significance.
ESCAT, European Society of Medical Oncology Scale for Clinical Actionability of Molecular Targets.
[1]n (%).
[2]Pearson's Chi-squared test, level of significance p <0.05.
[3]*vs*. whole population.

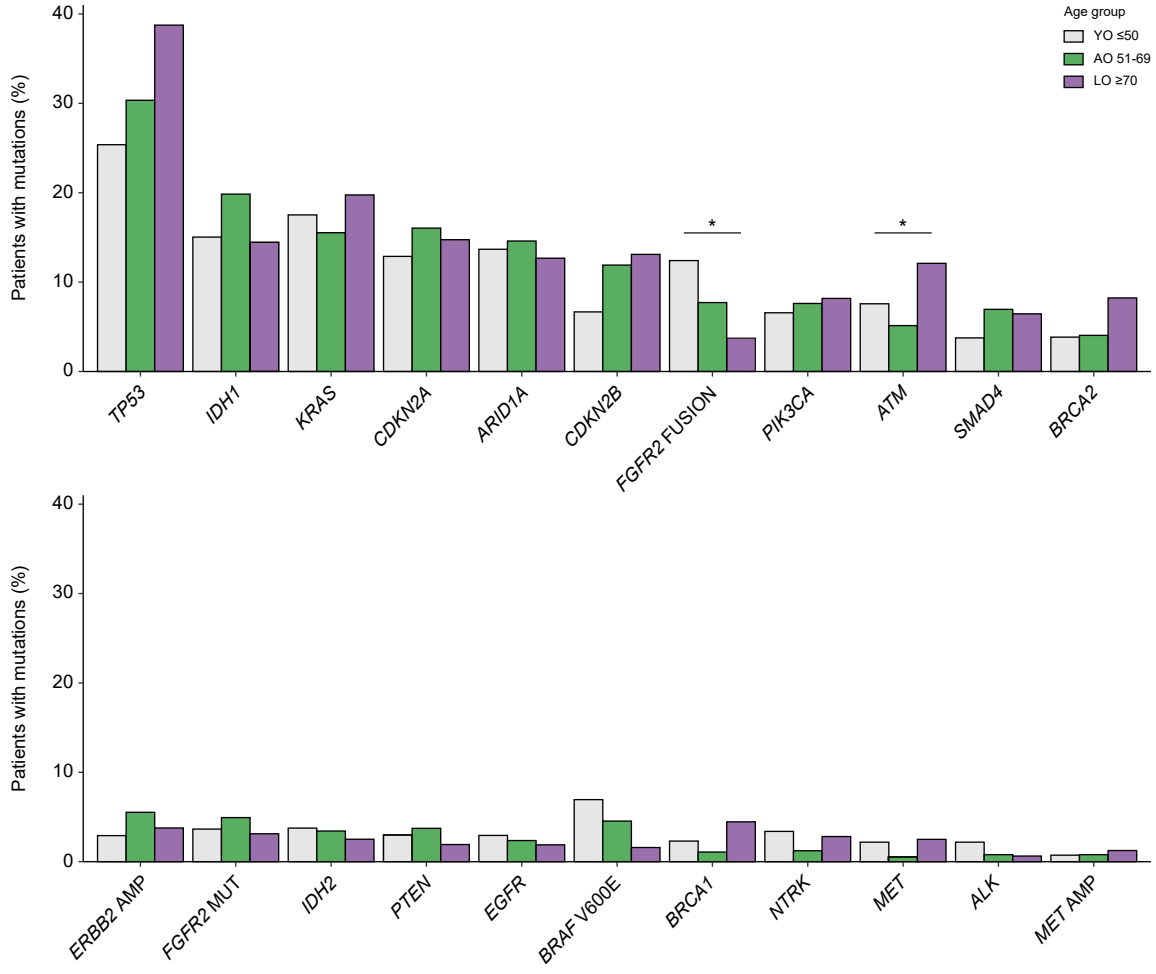
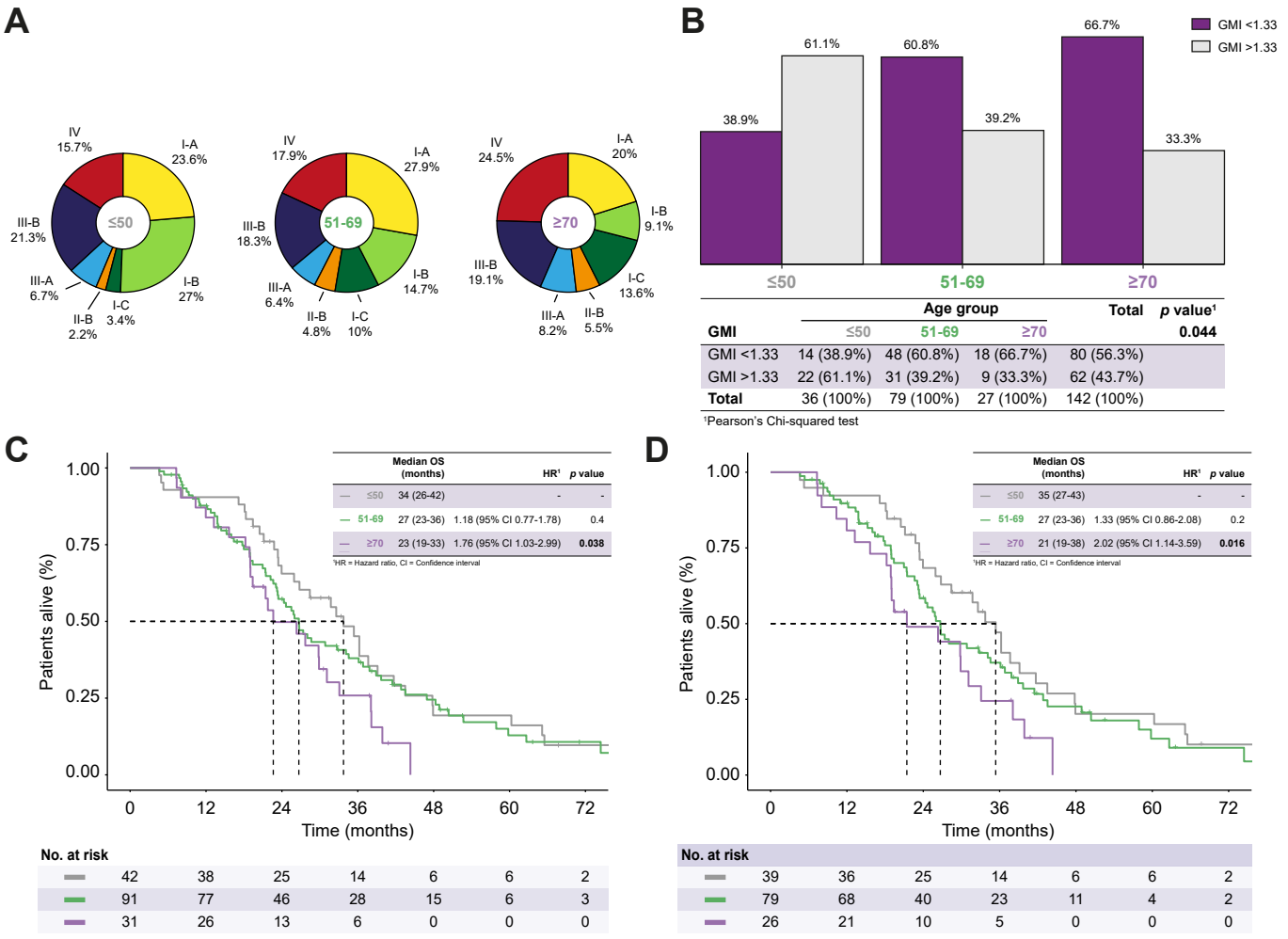


Fig. 2. Molecular alterations according to age group. Levels of significance: *FGFR2* fusion: p = 0.038 (Chi-square test); ATM: p = 0.022 (Chi-square test). GMI, growth modulation index; HR, hazard ratio; OS, overall survival.



GMI	Age group ≤50	51-69	≥70	Total	p value¹
					0.044
GMI <1.33	14 (38.9%)	48 (60.8%)	18 (66.7%)	80 (56.3%)	
GMI >1.33	22 (61.1%)	31 (39.2%)	9 (33.3%)	62 (43.7%)	
Total	36 (100%)	79 (100%)	27 (100%)	142 (100%)	

¹Pearson's Chi-squared test

Fig. 3. Patient outcomes following targeted therapies. (A) MMT according to ESCAT alterations in each age category. (B) GMI* of patients receiving MMT in each age category. Levels of significance: $p = 0.044$ (Chi-square test). (C) OS for patients receiving MMT. Levels of significance: YO *vs.* AO, HR = 1.18, $p = 0.4$ (Cox model); YO *vs.* LO HR = 1.76, $p = 0.038$ (Cox model). (D) OS for patients receiving MMT with respect to an ESCAT I-IIIA alteration. Levels of significance: YO *vs.* AO, HR = 1.33, $p = 0.2$ (Cox model); YO *vs.* LO HR = 2.02, $p < 0.016$ (Cox model). *Ratio of PFS with the targeted therapy line to the PFS of the n-1 line. AO, average-onset; ESCAT, European Society of Medical Oncology Scale for Clinical Actionability of Molecular Targets; GMI, growth modulation index; HR, hazard ratio; LO, late-onset; MMT, molecularly matched treatment; OS, overall survival; PFS, progression-free survival; YO, young-onset.

treatable with the recent development of FGFR inhibitors in clinical trials. Importantly, YO patients demonstrated improved responses to MMT, with a greater proportion achieving GMI >1.33 compared with patients over 50 years. Additionally, patients receiving an MMT for ESCAT IA-IIIA actionable alterations had prolonged survival.

In our cohort, patients with YO-BTC represented 18% of the overall population of BTC cases; this is comparable to observations from the CITY study (15%), which also assessed YO-CCAs.[19] However, contrary to the CITY study, we chose to divide the population into three categories. BTCs are highly heterogeneous, and it appears that older patients have different prognoses and tumor characteristics, as previously reported in the literature and supported by our clinical experience in both centers. Therefore, it seemed interesting to divide our cohort into three categories to avoid bias in the non-early onset group due to geriatric considerations. This methodology has previously been used to assess patients with YO-pancreatic cancer.[20] Of note, our population was selected from two tertiary centers, which might be biased toward younger patients.

Our findings suggest that, in terms of clinical features, patients with YO-BTCs had more advanced disease at diagnosis. In particular, they had more metastatic disease at onset with liver metastasis. Nevertheless, the OS from diagnosis was significantly longer in the YO-BTC group than in the LO-BTC group and was comparable to that of the AO-BTC groups (with an onset at 51-69 years); this has also been observed for other gastrointestinal tumors, such as pancreatic adenocarcinoma.[20] In our cohort, patients with YO-BTC received more first-line advanced-stage treatments, indicating a generally better overall condition of the younger patients. This is supported by their better ECOG-performance status, lower rate of diabetes, and lower BMI. YO-BTC patients may receive more intensive treatment due to their higher tumor burden and may also exhibit greater resistance to treatment-related toxicity. The higher tumor burden could also be linked to the more frequent distribution of iCCA in the YO group in our study.

Similar clinical and therapeutic results were found in the two other recently published cohorts, the American CITY cohort[19] and the French ACABI-PRONOBIL cohort.[21]

As in other studies, patients with YO-BTC were more likely to undergo molecular profiling than older patients, suggesting that clinicians caring for these patients are more inclined to propose a precision medicine approach to find potentially actionable alterations, especially in iCCA. The enrichment for *FGFR2* fusions in YO-BTC, especially in iCCA, also aligns with the molecular results from the American and French cohorts.[13,14] In our study, *BRAF* mutation rates were not different between the groups, but *ATM* mutations were less frequent in the YO group.

Patients with YO-BTC seemed to respond better to MMT, especially when they had an ESCAT I-IIIA actionable alteration. This underlines the urgent need for systematic molecular screening for patients with BTC, as treatment efficacy depends on the early metastatic stages[12] and a precision oncology-based therapeutic approach. Notably, this approach is currently being explored in the ongoing international SAFIR-ABC-10 trial (NCT05615818). This will pave the way for more refined treatment.

To elucidate the underlying biology, we performed a transcriptomic analysis based on a subset of patients who underwent gene expression analysis. GSEA revealed significant upregulation of immune, hemostasis, and ECM remodeling pathways in the YO-BTC group. In contrast, the AO- and LO-BTC had a significant upregulation in pathways involved in the cell cycle and DNA repair. This would suggest a potentially higher efficacy of immune checkpoint inhibitors in YO-BTC compared to older-onset patients. On the other hand, older-onset patients would benefit from platinum-based chemotherapy. No patient in our cohort received immune checkpoint inhibitors, as patients were included before the approval of durvalumab and pembrolizumab in the first-line metastatic setting; therefore, more data are needed to clearly answer this point. Subgroup analyses of the TOPAZ-1 and KEYNOTE-966 trials based on age would also be interesting.[22]

In a recent study, YO-CCA and AO-CCA patients underwent molecular analysis using a real-world multi-omics dataset and were compared using whole-exome and whole-transcriptome analyses.[23] This study, like ours, found differences between YO-CCA and AO-CCA groups, such as a higher prevalence of *FGFR2* fusions and notable distinctions in immunotherapy markers, angiogenesis enrichment, and inflammatory response. Surprisingly, patients with YO-CCA experienced better outcomes with immunotherapy even though immune-oncology-relevant markers favored patients with AO-CCA.[23]

A limit of our study comes from having a bicentric cohort from tertiary centers, where a high number of patients received first- and second-line chemotherapy. This probably suggests a selection bias compared to real-life populations.[24] Indeed, the median OS in our cohort was higher than those of other published cohorts for YO-BTCs.[13,14] However, analyzing this cohort allowed us to address the molecular profiles of YO-BTCs, which is not possible in centers that cannot afford NGS. Secondly, due to having very few cases, we used a long period of retrospective patient inclusion that started in 2001. Nonetheless, most patients were included and treated between 2015 and 2021, which limits the heterogeneity of treatment modalities for BTCs during this period.

Finally, for some patients in the GR cohort, the molecular testing was based on liquid biopsies, which may have a lower sensitivity for detection of some alterations, particularly for copy number alterations and fusions.[25] However, in recently published results, a good concordance has been observed between tumor and liquid molecular profiling in a large cohort of patients with BTCs concerning actionable alterations, such as *FGFR2* fusions and *IDH1* mutations.[26] Liquid biopsies can serve as a complementary or alternative strategy to tissue-based testing, particularly when tumor tissue is unavailable, as is frequently the case in extrahepatic CCA.[27] In our study, the majority of genetic tests performed in both cohorts were from tumor samples, and any discrepancies observed between liquid-based and tissue-based approaches for actionable variant detection may be limited.

Patients with YO-BTC are more often diagnosed at the metastatic stage, especially with liver metastases, and exhibit a higher tumor burden. These tumors are more likely to be enriched for *FGFR2* gene fusions, highlighting opportunities for precision oncology-based therapeutic approaches. In our study, this is more generally the case in the context of ESCAT IA-IIIA alterations, whereby YO metastatic patients had improved outcomes compared to older-onset patients. Additional prospective clinical and translational studies are required to more precisely characterize this patient subgroup and to elucidate the biological mechanisms underlying these rare tumors of increasing incidence.

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Abbreviations

AO-BTC, average-onset biliary tract cancer; BTC, biliary tract cancer; CCA, cholangiocarcinoma; ESCAT, European Society of Medical Oncology Scale for Clinical Actionability of Molecular Targets; GMI, growth modulation index; GR, Gustave Roussy; HR, hazard ratio; LO-BTC, late-onset biliary tract cancer; MMT, molecularly matched treatment; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; VHIO, Vall d'Hebron Institute of Oncology; YO-BTC, young-onset biliary tract cancer.

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Conflict of interest

TP has received travel and accommodation fees from Servier and Viatris outside the submitted work. AT has received personal fees from Servier, Viatris, Incyte Bioscience, BMS, and Merck, as well as grants and personal fees from AstraZeneca and MSD outside the submitted work. MD declares conflicts of interest with Merck Serono, MSD, AMGEN, Roche, Bayer, Ipsen, Pfizer, Servier, Pierre Fabre, HalioDx, Lilly, Sanofi, and BMS. TM reports advisory roles for Ability Pharmaceuticals SL, Arcus Bioscience Inc., AstraZeneca, Basilea Pharma, Baxter, BioLineRX Ltd, Celgene, Eisai, , Incyte, Ipsen Bioscience Inc; speaker fees from Janssen, Lilly, Esteve, Daïchi, Biontech, Novartis, Jazz Pharmaceuticals; research grants from MSD, Novocure, QED Therapeutics, Roche Farma, Sanofi-Aventis, Servier, Zymeworks; travel and accommodation fees from Servier, AstraZeneca, Sanofi, Incyte, Lilly, MSD and Roche. VB reports compensation from Amgen, Bayer Schering Pharma, Ipsen, Merck Serono, MSD Oncology, and Roche/Genentech; for consulting or an advisory role from Bayer Schering Pharma, Eisai, Ipsen, Merck Serono, and Roche/Genentech, outside the submitted work; research funding from Merck Serono (Inst); travel, accommodations, and expenses from Bayer Schering Pharma, Ipsen, Merck Serono, Roche/Genentech, and Sanofi/Aventis outside the submitted work. DM reports honoraria and non-financial support from Amgen, Bayer, Ipsen, Merck, Merck Serono, Roche, Sanofi, and Servier; honoraria from Shire, HalioDx, and Agios. CS reports personal fees from Roche and Servier, as well as funding from Merck outside the submitted work. MH reports personal fees from Pierre Fabre and Amgen outside the submitted work. FC reports speaker fees from AstraZeneca, Eisai, and Roche, as well as travel and accommodation fees from Roche and Servier. TVT reports research grants from AstraZeneca, Incyte, Alentis, Servier, LOXO oncology, and personal fees from AstraZeneca, Incyte. AB has received personal fees from Merck Serono and Servier and grants from Ipsen outside the submitted work. AH reports consulting fees from Amgen, Sanofi, BMS, Basilea, Incyte, Servier, Relay Therapeutics, Taiho, and MSD, and honoraria for lecture presentations from Servier, Incyte, Seagen outside of the submitted work. AL reports research grant from AstraZeneca, Bayer, BMS, Merck, MSD, Pharmamar. Other authors report no conflict of interest.

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Authors' contributions

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary data

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