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Article

The impact of molecular alterations in patients with advanced biliary tract cancer receiving cisplatin, gemcitabine, and durvalumab: a large, real-life, worldwide population

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Abstract

Background: Cisplatin, gemcitabine, and durvalumab combination is a standard first-line treatment for advanced biliary tract cancer. This study aimed to assess the impact of genetic alterations on outcomes in patients with advanced biliary tract cancer treated with cisplatin, gemcitabine, and durvalumab in real-world clinical practice.

Methods: Patients with unresectable, locally advanced, or metastatic biliary tract cancer treated with cisplatin and gemcitabine plus durvalumab across 39 centers in 11 countries in Europe, the United States, and Asia were included in this analysis.

Results: The cohort included 513 patients with advanced biliary tract cancer. The 5 most frequently altered genes were *TP53* (22.1%), *KRAS* (13.7%), *CDKN2A/B* (13.6%), *ARID1A* (12.2%), and *IDH1* (9.2%). In multivariate analysis, *SMAD4* mutations were associated with improved progression-free survival (PFS) (hazard ratio [HR] = 0.49, $P = .018$) and overall survival (HR = 0.11, $P = .023$), while *TP53* mutations were linked to worse PFS (HR = 1.62, $P = .0047$) and *TERT* mutations to worse overall survival (HR = 8.92, $P = .0012$). No other genomic alterations were statistically associated with outcomes. Subgroup analysis showed that *TP53* mutations negatively affected PFS and overall survival in intrahepatic cholangiocarcinoma, while *KRAS* mutations were associated with poorer PFS in extrahepatic cholangiocarcinoma. No gene alterations were linked to outcomes in gallbladder cancer.

Conclusions: This large-scale analysis, with comprehensive molecular profiling, supports the positive prognostic impact of *SMAD4* mutations for PFS and overall survival and highlights the negative prognostic roles of *TP53* (PFS) and *TERT* (overall survival) mutations, providing valuable insights for personalized treatment strategies in biliary tract cancer.

Introduction

Advanced biliary tract cancer remains a major clinical challenge due to its aggressive behavior, poor prognosis, and limited response to systemic therapies.[1-4] Recently, a better understanding of the molecular biology of biliary tract cancer has led to the identification of targetable genomic alterations, such as *IDH1* mutations and *FGFR2* fusions, which have been successfully addressed by biomarker-driven treatments in select patients.[5-12] More recently, immunotherapy has been introduced into the first-line setting. The phase 3 Durvalumab or Placebo in Combination With Gemcitabine/Cisplatin in Patients With 1st Line Advanced Biliary Tract Cancer (TOPAZ-1; ClinicalTrials.gov ID NCT03875235) and Pembrolizumab (MK-3475) Plus Gemcitabine/Cisplatin Versus Placebo Plus Gemcitabine/Cisplatin for First-Line Advanced and/or Unresectable Biliary Tract Carcinoma (BTC) (KEYNOTE-966; ClinicalTrials.gov ID NCT04003636) trials demonstrated a survival benefit by adding immune checkpoint inhibitors (durvalumab and pembrolizumab, respectively) to the standard cisplatin-gemcitabine chemotherapy backbone.[13,14] In parallel, real-world studies have confirmed the feasibility, safety, and potential clinical benefit of these chemoimmunotherapy combinations in broader patient populations.[15,16] Despite these advancements, biomarkers capable of predicting which patients would benefit most from immunotherapy remain unidentified, and data on the clinical impact of genomic features in this context are limited. Notably, the TOPAZ-1 trial found no significant interaction between molecular alterations and treatment efficacy. In this exploratory analysis using the FoundationOne panel (Foundation Medicine, Inc), 441 patients were tested. A trend in favor of cisplatin plus gemcitabine plus durvalumab was found in all clinically actionable mutant subtypes except *ERBB2* alterations.[17] A recent real-world analysis identified 3 molecular clusters with different impacts on overall response rate: cluster 2, with multiple pathway alterations, showed the highest responses; cluster 3, involving RTK/RAS and cell-cycle pathways, had none; and cluster 1, marked by chromatin modifications, showed intermediate activity.[18] Nevertheless, no conclusive data are available, and no molecular criteria are currently used in clinical practice to select patient candidates for first-line systemic treatment.

A deeper understanding of the genomic landscape and its clinical impact in this setting could help identify molecular signatures associated with treatment response or resistance, thereby guiding therapeutic decisions. Based on these premises, the present study aimed to assess the prognostic and predictive value of recurrent somatic alterations in a large, multinational, real-world cohort of patients with advanced biliary tract cancer treated with cisplatin, gemcitabine, and durvalumab as first-line therapy. In particular, the study was designed to explore potential differences in the distribution and impact of genetic alterations across biliary tract cancer anatomical subtypes and to evaluate whether specific alteration could predict differential benefit from chemoimmunotherapy, thereby informing more precise therapeutic strategies. This is one of the first and largest real-life analyses to integrate molecular profiling with clinical outcomes in this population.

Methods

Study population

The study population consisted of patients with unresectable, locally advanced, or metastatic biliary tract cancer who received treatment at any of 39 clinical institutions across 11 countries (Italy, Germany, Austria, Spain, Portugal, the United Kingdom, the United States, the Republic of Korea, China, the Hong Kong Special Administrative Region of China, and Japan) from July 2021 to December 2023.

All patients treated with first-line cisplatin, gemcitabine, and durvalumab at participating centers during the study period were retrospectively screened for inclusion. Molecular testing was performed according to local practice on available tumor tissue samples. Of the 666 patients included in the clinical analysis, 513 (77.0%) underwent molecular profiling and were evaluable for genomic data analyses. Molecular profiling of primary tumors was locally performed using either comprehensive multigene next-generation sequencing panels or targeted assays focusing on select actionable genes in accordance with local diagnostic routine practice. Commercial assays included FoundationOne CDx Clinical Trial Assay, Myriadpod NGS Cancer panel RNA kit (Diatech Pharmacogenetics), Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific), AmoyDx HANDLE Classic NGS Panel, MI Tumor Seek Hybrid (Caris Life Sciences), Tempus xT Solid Tumor, and TruSight Oncology 500 (Illumina). We excluded variants of uncertain significance by including only those classified as pathogenic or likely pathogenic. In the case of commercial tests this classification was already provided in the results report. Conversely, gene alterations detected by in-house panels were classified as pathogenic or likely pathogenic based on OncoKB (Memorial Sloan Kettering Cancer Center; https://www.oncokb.org). The reference range of each next-generation sequencing panel was revised to check whether biliary tract cancer–related genes were covered.

Patients included in the analysis were treated with first-line cisplatin (25 mg/m^2, days 1 and 8), gemcitabine (1000 mg/m^2, days 1 and 8), and durvalumab (1500 mg, day 1), administered intravenously on a 21-day cycle for up to 8 chemotherapy cycles, followed by durvalumab monotherapy every 4 weeks until disease progression or unacceptable toxicity.

To find potential molecular markers predictive of response, the cohort of patients treated with chemotherapy plus durvalumab was tested vs a historical cohort of patients receiving only chemotherapy. For this cohort, data were prospectively collected from 17 centers in Italy from March 2006 to December 2022. Patients who received treatment before the publication of the TOPAZ-1 results received the previous standard combination of cisplatin 25 mg/m^2 plus gemcitabine 1000 mg/m^2 on days 1 and 8 of each 21-day cycle for up to 8 cycles, according to the Gemcitabine With or Without Cisplatin in Treating Patients With Unresectable Locally Advanced or Metastatic Cholangiocarcinoma or Other Biliary Tract Tumors (ABC-02) trial (ClinicalTrials.gov ID NCT00262769). To identify patients with actionable alterations, European Society for Molecular Oncology Scale for Clinical Actionability of molecular Targets criteria were used, as recommended by European Society for Molecular Oncology guidelines,[19] and level I and II alterations according to European Society for Molecular Oncology Scale for Clinical Actionability of molecular Targets were defined as targetable.

The study received approval from the San Raffaele Ethics Committee (No. 113/INT/2021) and the local ethics committees at each participating center and adhered to Good Clinical Practice guidelines, the Declaration of Helsinki, local laws, and the European Union General Data Protection Regulation 2016/679.

Statistical analysis

The primary endpoint of the study was the evaluation of the prognostic significance of the most commonly mutated genes (>3% of patients) in terms of overall survival and progression-free survival (PFS) in a cohort of patients who received cisplatin, gemcitabine, and durvalumab as first-line therapy. First, the whole patient cohort was evaluated, independent of primary tumor location. Subsequently, subgroup analyses were performed according to the primary tumor location (intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer). This stratification was justified by the well-recognized biological, molecular, and clinical differences across biliary tract cancer subtypes. The distinct genomic landscapes and disease behaviors of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer could differentially influence the prognostic significance of genomic alterations and thus warranted separate analyses.

The secondary endpoints included the impact in terms of overall response rate and disease control rate of the most commonly mutated genes (>3% of patients) in the whole cohort of patients.

For the selected genes, we used the false discovery rate approach to mitigate the risk of false positives associated with multiple testing. Initially, univariate analyses were conducted. To account for multiple testing in this context, we applied false discovery rate correction to the results of the univariate analysis, ensuring a more robust interpretation of our findings. Following this, a multivariate analysis was performed on the significant variables identified in the univariate analysis for all characteristics with a P value threshold below .2.

To find potential molecular markers predictive of response, the cohort of patients treated with chemotherapy plus durvalumab was tested vs a historical cohort of patients receiving only chemotherapy as first-line treatment, and an interaction test was performed. To reduce the risk of bias in this analysis, a propensity score was calculated representing the likelihood of being assigned to the historical cohort conditional on covariates. All clinical and tumor variables available at treatment initiation were used for propensity score calculation to avoid imbalances related to other parameters not associated with the probability of

receiving the historical cohort treatment but with an unknown effect on the outcome. The obtained propensity score was then used to generate stabilized inverse probability of treatment weights through appropriate mathematical methods that were applied to weight each clinical feature as well as measured outcomes for each patient in both groups. After weighting baseline characteristics, d values were recalculated, and adequate balance was declared if all variables showed $d < 0.1$. Finally, a prognostic index was built that integrated prognostic clinical and molecular markers.

Overall survival was defined as the duration from treatment initiation to death, while PFS was the duration from treatment initiation to disease progression, death, or last follow-up, whichever occurred first. Overall response rate was assessed by the investigator as the proportion of patients achieving complete response or partial response. The disease control rate was the proportion of patients achieving an overall response rate or stable disease. Treatment responses were evaluated using computed tomography scans and categorized as complete response, partial response, stable disease, or progressive disease according to Response Evaluation Criteria in Solid Tumors 1.1.

Statistical analysis was performed using MedCalc, version 20.2, statistical software (MedCalc Software, Ltd).

Results

Overall results

Overall, the patient cohort comprised 666 individuals with advanced biliary tract cancer treated with cisplatin and gemcitabine plus durvalumab; of these, 513 (77.0%) underwent gene alteration analysis on tumor tissue samples. Patients' characteristics are presented in Table 1. Table S1 illustrates all the genes analyzed, along with the number of cases tested and their respective percentages of alterations.

Testing assays employed for tumor individual samples were FoundationOne CDx (193/513 patients [37.6%]), a mixture of commercial or in-house in-depth next-generation sequencing methods (156/513 patients [30.4%]), Myriapod NGS Cancer Panel (63/513 patients [12.3%]), and Oncomine (49/513 patients [9.6%]). A single-gene alterations assay (*FGFR2, IDH1, MSI, BRAF, NTRK, RET*, and *HER2*) was used in 52 patients (10.1%). Table S2 reports the genes analyzed with a mixture of commercial and in-house, in-depth next-generation sequencing methods.

Gene alterations and clinical outcome in the overall population

Outcomes for the 513 patients who underwent genomic testing were as follows: median PFS was 8.4 months (95% CI = 7.6 to 9.0), and median overall survival was 15.9 months (95% CI = 13.9 to 29.1).

We conducted a descriptive molecular analysis and identified genomic alterations present in at least 3% of patients. These alterations involved 22 genes, and their respective percentages are illustrated in Figure 1. Among the 513 patients who underwent molecular profiling, the most frequently altered genes were *TP53* (22.1%), *KRAS* (13.7%), *CDKN2A/B* (13.6%), *ARID1A* (12.2%), and *IDH1* (9.2%). Additional recurrent alterations included *HER2* amplifications (7.2%), *BRCA2* (6.8%), *PBRM1* (6.6%), *MTAP* (5.7%), *BAP1* (5.1%), *MDM2* (5.1%), *SMAD4* (4.8%), *ATM* (4.5%), *NF1* (4.4%), and *PIK3CA* (4.3%). Less frequent but notable alterations were *FGFR2* fusions (4.3%), *KMT2D/MLL2* (3.8%), *TERT* (3.8%), *MYC* (3.5%), *CCNE1* (3.1%), *BRAF* V600E (2.4%), and non-V600E *BRAF* mutations (1.5%).

We subsequently analyzed outcomes of patients with any alteration in the 22-gene panel selected. On univariate analysis for PFS, *SMAD4* mutation (all specified *SMAD4* alterations were loss of function) was associated with longer median PFS (not reached vs 8.7 months: hazard ratio [HR] = 0.49, 95% CI = 0.27 to 0.88, $P = .018$) (Figure 2, A). Conversely, *TP53* mutation (all reported *TP53* alterations were known to be oncogenic, but the majority were loss of function [69.4%] mutations and the minority gain of function [30.6%]) was associated with shorter median PFS (6.6 vs 9.4 months: HR = 1.62, 95% CI = 1.16 to 2.26, $P = .0047$) (Figure 2, B). Figure S1A presents the forest plot illustrating outcomes for all evaluated genes.

On univariate analysis for overall survival, *SMAD4* mutation was associated with longer median overall survival (not reached vs 15.1 months: HR = 0.11, 95% CI = 0.02 to 0.20, $P = .023$) (Figure 2, C), while *TERT* (all *TERT* mutations were gain of function on the promoter region [124C>T, 112C>T, 146C>T]) and *CDKN2A* alterations (all alterations are loss of function; promoter hypermethylation was observed in 54% of patients for p16INK4a and 46% of patients for p14ARF) were associated with shorter median overall survival (5.3 vs 15.9 months: HR = 8.92, 95% CI = 2.36 to 33.70, $P = .0012$; 9.9 vs 16.1 months: HR = 2.21, 95% CI = 1.20 to 4.05, $P = .0105$, respectively) (Figure 2, D and E, respectively). Figure S1B presents the forest plot illustrating outcomes for all evaluated genes.

To minimize the risk of obtaining false-positive results, the false discovery rate approach was applied for both PFS and overall survival outcomes in the univariate analysis. After applying the false discovery rate correction with $\alpha = .05$, all the P values remained statistically significant. After adjustment for variables with a prognostic impact in the univariate analysis (including factors with $P < .2$), the multivariate analysis (234 patients were included) for PFS confirmed the positive prognostic role of *SMAD4* mutation and the negative prognostic role of *TP53* mutation (Table 2). After adjustment for variables with a prognostic impact in the univariate analysis (including factors with $P < .2$), the multivariate analysis (which included 203 patients) for overall survival confirmed the positive prognostic role of *SMAD4* mutation and the negative prognostic role of *TERT* mutation (Table 3). No other genomic alterations among the cases analyzed showed statistically significant associations with PFS or overall survival in the multivariate analysis.

Regarding the disease control rate, *TERT* and *TP53* mutations were associated with lower rates than their wild-type counterparts (53.8% vs 80.1% and 71.0% vs 81.8%, respectively); *BRAF* mutations were associated with lower overall response rates than were wild-type tumors (16.7% vs 37.5%) (Table S3).

Finally, we identified 42 patients with actionable alterations. Among these patients, 24 (57.1%) received a matched targeted therapy in the second line according to their molecular profile (ivosidenib, anti-ERBB2 [formerly HER2] agents, pemigatinib, or dabrafenib plus trametinib), while 18 patients (42.9%) received chemotherapy alone. These 18 patients did not receive matched targeted therapy despite harboring actionable alterations, primarily due to lack of access. Only patients who received second-line treatment after progression on cisplatin and gemcitabine plus durvalumab were included in this comparison. Matched targeted therapy was associated with an improvement in overall survival compared with chemotherapy alone in patients with actionable alterations (not reached vs 4.2 months: HR = 0.12, 95% CI = 0.04 to 0.37, $P = .003$).

To test the predictive or prognostic impact of genes that were positive in the multivariate analysis, we compared this cohort

Table 1. Baseline characteristics of the study population, stratified by availability of molecular data and tumor subtype (intrahepatic and extrahepatic cholangiocarcinoma, gallbladder).[a]

Parameter	Population with genetic analysis, No. (%)	Population without genetic analysis, No. (%)	P	Intrahepatic tumor (population with genetic analysis), No. (%)	Intrahepatic tumor (population without genetic analysis), No. (%)	P	Extrahepatic tumor (population with genetic analysis), No. (%)	Extrahepatic tumor (population without genetic analysis), No. (%)	P	Gallbladder tumor (population with genetic analysis), No. (%)	Gallbladder tumor (pPopulation without genetic analysis), No. (%)	P
	n = 513 (77.0%)	*n* = 153 (23.0%)		*n* = 286 (42.9%)	*n* = 77 (11.5%)		*n* = 120 (18.0%)	*n* = 48 (7.2%)		*n* = 107 (16.0%)	*n* = 28 (4.2%)	
Age, y												
>70	206 (40.1)	60 (39.2)	.92	110 (38.4)	26 (33.7)	.89	37 (30.8)	19 (39.5)	.004	59 (55.1)	15 (53.5)	>0.99
≤70	292 (56.9)	83 (54.2)		172 (60.1)	44 (57.1)		76 (63.3)	27 (56.2)		44 (41.1)	12 (42.8)	
Unknown	15 (2.9)	10 (6.5)		4 (1.39)	7 (9.0)		7 (5.8)	2 (4.1)		4 (3.7)	1 (3.5)	
Sex												
Male	277 (53.9)	78 (50.9)	.51	152 (53.1)	39 (50.6)	.70	76 (63.3)	30 (62.5)	>0.99	49 (45.7)	9 (32.1)	.20
Female	236 (46.0)	75 (49.0)		134 (46.8)	38 (49.3)		44 (36.6)	18 (37.5)		58 (54.2)	19 (67.8)	
Primary tumor site												
Intrahepatic cholangiocarcinoma	286 (55.7)	77 (50.3)	.13	—	—		—	—		—	—	
Extrahepatic cholangiocarcinoma	120 (23.3)	48 (31.3)										
Gallbladder	107 (20.8)	28 (18.3)										
Disease stage												
Locally advanced	116 (22.6)	41 (26.7)	.28	59 (20.6)	19 (24.6)	.43	39 (32.5)	15 (31.2)	>0.99	18 (16.8)	7 (25.0)	.41
Metastatic	397 (77.3)	112 (73.2)		227 (79.3)	58 (75.3)		81 (67.5)	33 (68.7)		89 (83.1)	21 (75.0)	
Carcinoembryonic antigen												
Normal value	260 (50.6)	73 (47.7)	.76	155 (54.1)	35 (45.4)	.33	62 (51.6)	27 (56.2)	.45	43 (40.1)	11 (39.2)	.82
Above normal value	202 (39.3)	61 (39.8)		108 (37.7)	32 (41.5)		45 (37.5)	14 (29.1)		49 (45.7)	15 (53.5)	
Unknown	51(9.9)	19(12.4)		23(8.0)	10(12.9)		13(10.8)	7(14.5)		15(14.0)	2(7.1)	
Carbohydrate antigen 19-9												
Normal value	158 (30.7)	45 (29.4)	.76	92 (32.1)	23 (29.8)	.67	29 (24.1)	11 (22.9)	.84	37 (34.5)	11 (39.2)	.65
Above normal value	326 (63.5)	101 (66.0)		178 (62.2)	51 (66.2)		82 (68.3)	35 (72.9)		66 (61.6)	15 (53.5)	
Unknown	29 (5.6)	7 (4.5)		16 (5.5)	3 (3.8)		9 (7.5)	2 (4.1)		4 (3.7)	2 (7.1)	
Neutrophil to lymphocyte ratio												
>3	256 (49.9)	75 (49.0)	.76	172 (60.1)	45 (58.4)	.88	42 (35.0)	18 (37.5)	.43	42 (39.2)	12 (42.8)	.81
≤3	198 (38.5)	54 (35.2)		91 (31.8)	25 (32.4)		63 (52.5)	19 (39.5)		44 (41.1)	10 (35.7)	
Unknown	59 (11.5)	24 (15.6)		23 (8.0)	7 (9.0)		15 (12.5)	2 (4.1)		21 (19.6)	6 (21.4)	
ECOG-ACRIN performance state												
0	272 (53.0)	56 (36.6)	.0004	149 (52.0)	32 (41.5)	.12	66 (55.0)	19 (39.5)	.08	57 (53.2)	5 (17.8)	.001
>0	241 (46.9)	97 (63.3)		137 (47.9)	45 (58.4)		54 (45.0)	29 (60.4)		50 (46.7)	23 (82.1)	

[a] Categorical variables compared using the Fisher exact test.

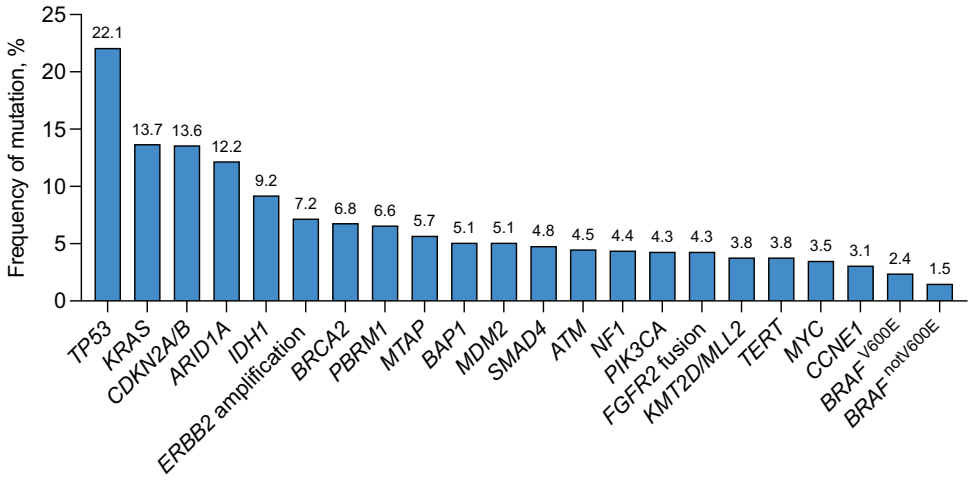

Figure 1. Gene alterations in the overall population.

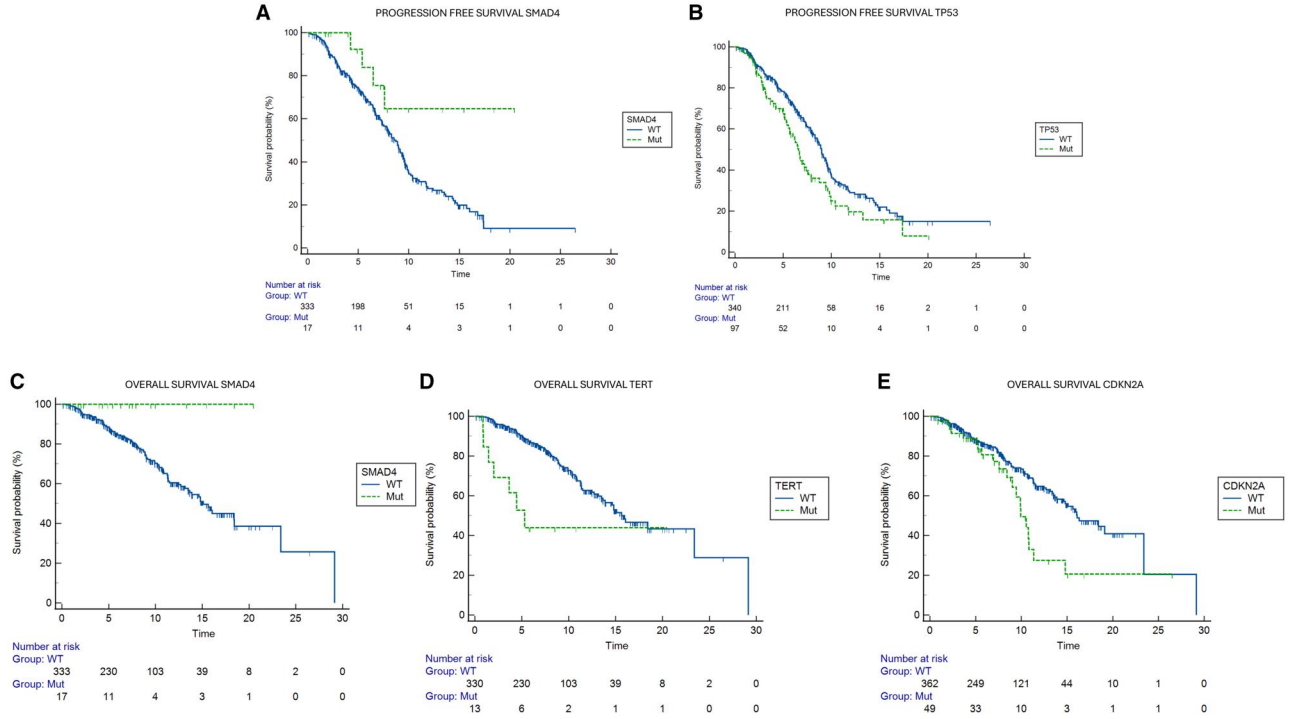

Figure 2. Kaplan Meier curves of progression-free survival (PFS) according to *SMAD4* (**A**) and *TP53* (**B**) in the overall population. Kaplan-Meier curves of overall survival according to *SMAD4* (**C**), *TERT* (**D**), and *CDKN2A* (**E**) in the overall population. $P < .05$ was considered statistically significant (Kaplan-Meier estimates and log-rank test).

with a historical cohort of 207 patients treated with cisplatin plus gemcitabine. No baseline differences in somatic mutations and patient characteristics were found in the 2 cohorts (Table S4). Figure 3, A and B present forest plots illustrating the different outcomes between the 2 groups in terms of PFS and overall survival.

The interaction test highlighted *SMAD4* mutation as a positive predictive factor for overall survival ($P = .03$) and PFS ($P = .04$) on cisplatin and gemcitabine plus durvalumab. After inverse probability of treatment weights adjustment, baseline clinical and tumor characteristics were similar between the 2 groups (Table S5), as indicated by $d < 0.10$ in all cases. Figure 3, C presents forest plots illustrating the different outcomes between the 2 groups in terms of overall survival, confirming the previous data before inverse probability of treatment weights adjustment. The interaction test highlighted *SMAD4* mutation as a positive predictive factor for overall survival ($P = .023$) and PFS ($P = .046$) on cisplatin and gemcitabine plus durvalumab.

Subgroup analysis in each tumor type

Figure S2 provides the complete heatmap, including all analyzed genes, stratified by tumor location. When comparing intrahepatic cholangiocarcinoma to extrahepatic cholangiocarcinoma, alterations in *KRAS*, *SMAD4*, and *TP53* were more prevalent in extrahepatic cholangiocarcinoma. Conversely, *BAP1*, *IDH1*, and *IDH2* were more frequently altered in intrahepatic cholangiocarcinoma. When comparing intrahepatic cholangiocarcinoma with gallbladder cancer, alterations in *ELF3*, *ERBB2*, *MAP2K4*, *SMAD4*, and *TP53* were more prevalent in gallbladder cancer. Conversely,

Table 2. Univariate and multivariate analysis of progression-free survival according to baseline characteristics and gene alterations.

	Univariate			Multivariate		
Parameter	**Hazard ratio**	**95% CI**	**P[a]**	**Hazard ratio**	**95% CI**	**P[a]**
SMAD4						
Wild type	**1**			**1**		
Mutated	**0.49**	**0.27 to 0.88**	**.018**	**0.19**	**0.05 to 0.82**	**.0255**
TP53						
Wild type	**1**			**1**		
Mutated	**1.62**	**1.16 to 2.26**	**.0047**	**1.73**	**1.12 to 2.67**	**.0139**
CDKN2B						
Wild type	**1**			**1**		
Mutated	**1.65**	**0.90 to 3.00**	**.10**	**1.34**	**0.74 to 2.40**	**.33**
BRCAness						
Wild type	**1**			**1**		
Mutated	**0.76**	**0.50 to 1.15**	**.19**	**0.60**	**0.42 to 1.23**	**.25**
ERBB2						
Not amplified	**1**			**1**		
Amplified	**1.44**	**0.86 to 2.40**	**.16**	**2.47**	**0.73 to 8.31**	**.14**
Age, y						
>70	1					
≤70	0.86	0.67 to 1.10	.25			
Sex						
Male	1					
Female	1.04	0.82 to 1.33	.71			
Primary tumor site						
Intrahepatic cholangiocarcinoma	**1**			1		
Extrahepatic cholangiocarcinoma	**0.65**	**0.49 to 0.85**		0.72	0.48 to 1.09	
Gallbladder	**1.17**	**0.84 to 1.63**	**.0036**	1.21	0.78 to 1.93	.64
Disease stage						
Locally advanced	**1**			**1**		
Metastatic	**1.82**	**1.39 to 2.38**	**<.0001**	**1.78**	**1.10 to 2.13**	**.024**
Carcinoembryonic antigen						
Normal value	**1**			**1**		
Above normal value	**1.55**	**1.20 to 2.01**	**.0009**	**1.39**	**1.03 to 2.01**	**.043**
Carbohydrate antigen 19-9						
Normal value	**1**			1		
Above normal value	**1.34**	**1.03 to 1.73**	**.02**	1.65	1.02 to 1.97	.062
Neutrophil to lymphocyte ratio						
>3	**1**					
≤3	**0.61**	**0.47 to 0.79**	**.0002**	0.82	0.59 to 1.27	.53
ECOG-ACRIN performance status						
0	1			**1**		
>0	1.82	1.42 to 2.34	**<.0001**	**1.75**	**1.16 to 2.64**	**.0079**

[a] $P < .05$ was considered statistically significant (Kaplan-Meier estimates and log-rank test).
Bold represent statistically significant results.

BAP1 and *IDH1* were more frequently altered in intrahepatic cholangiocarcinoma. Finally, when comparing extrahepatic cholangiocarcinoma with gallbladder cancer, alterations in *KRAS* were more prevalent in extrahepatic cholangiocarcinoma.

Intrahepatic cholangiocarcinoma

The patient cohort comprised 364 individuals with advanced intrahepatic cholangiocarcinoma treated with cisplatin and gemcitabine plus durvalumab. Of these patients, 287 (78.8%) underwent gene alteration analysis on tumor tissue samples (Table S1).

The testing methods used for individual tumor samples included FoundationOne CDx for 117 patients (40.8%); a combination of commercial and in-house, in-depth next-generation sequencing methods for 92 (30.4%) patients; and the Myriapod NGS Cancer Panel for 31 (10.8%) patients. In addition, a single-gene alteration assay (for *FGFR2*, *IDH1*, *MSI*, *BRAF*, *NTRK*, *RET*, and *ERBB2*) was performed on 27 patients (9.4%).

Outcomes for the 364 patients who underwent gene alteration analysis were as follows: median PFS was 8.0 months (95% CI = 7.1 to 8.9), and median overall survival was 14.8 months (95% CI = 11.3 to 29.1). A descriptive molecular analysis was conducted, identifying genomic alterations in at least 3% of patients. A total of 28 genes were affected, and their respective percentages are depicted in Figure S3A. Next, outcomes for patients with any alteration in the 28-gene panel were evaluated. On univariate analysis for PFS, *TP53* and *CDKN2A* alterations were associated with shorter median PFS (5.7 vs 8.9 months: HR = 2.01, 95% CI = 1.19 to 3.40, $P = .0089$; 5.5 vs 8.9 months: HR = 2.33, 95% CI = 1.20 to 4.51, $P = .0125$, respectively) (Figure 4, A and B). Figure S1C presents the forest plot illustrating outcomes for all evaluated genes.

On univariate analysis for overall survival, *TP53* and *CDKN2A* alterations were associated with shorter median overall survival (9.9 vs 14.8 months: HR = 2.21, 95% CI = 1.17 to 4.15, $P = .0142$; 9.0 vs 16.0 months: HR = 3.42, 95% CI = 1.53 to 7.63, $P = .0026$, respectively) (Figure 4, C and D). Figure S1D presents the forest plot illustrating outcomes for all evaluated genes. Following adjustment for variables with a prognostic impact at univariate analysis, multivariate analysis for PFS and overall survival confirmed the negative prognostic role of *TP53* mutation (Table S6 and Table S7).

Table 3. Univariate and multivariate analysis of overall survival according to baseline characteristics and gene alterations.[a]

	Univariate			Multivariate		
Parameter	**Hazard ratio**	**95% CI**	**P**	**Hazard ratio**	**95% CI**	**P**
SMAD4						
Wild type	**1**			**1**		
Mutated	**0.11**	**0.02 to 0.20**	**.023**	**0.17**	**0.03 to 0.45**	**.035**
TERT						
Wild type	**1**					
Mutated	**8.92**	**2.36 to 33.70**	**.0012**	**3.79**	**1.03 to 13.92**	**.045**
CDKN2A						
Wild type	**1**			1		
Mutated	**2.21**	**1.20 to 4.05**	**.0105**	1.60	0.83 to 3.05	.16
TP53						
Wild type	**1**			1		
Mutated	**1.40**	**0.89 to 2.19**	**.14**	1.30	0.69 to 2.44	.41
ATM						
Wild type	**1**			1		
Mutated	**0.50**	**0.22 to 1.14**	**.098**	0.96	0.69 to 2.56	.96
Age, y						
>70	1					
≤70	0.90	0.64 to 1.25	.52			
Sex						
Male	1					
Female	1.07	0.77 to 1.48	.70			
Primary tumor site						
Intrahepatic cholangiocarcinoma	**1**			1		
Extrahepatic cholangiocarcinoma	**0.47**	**0.32 to 0.69**		0.56	0.37 to 1.18	.66
Gallbladder	**0.87**	**0.58 to 1.36**	**.0062**	0.91	0.43 to 1.72	.43
Disease stage						
Locally advanced	**1**			**1**		
Metastatic	**2.52**	**1.74 to 3.64**	**<.0001**	**1.84**	**1.35 to 2.25**	**.01**
Carcinoembryonic antigen						
Normal value	**1**			**1**		
Above normal value	**2.14**	**1.50 to 3.04**	**<.0001**	**1.45**	**0.89 to 1.93**	**.22**
Carbohydrate antigen 19-9						
Normal value	**1**			1		
Above normal value	**1.32**	**0.92 to 1.87**	**.12**	1.54	0.54 to 2.01	.32
Neutrophil to lymphocyte ratio						
>3	**1**			1		
≤3	**0.43**	**0.31 to 0.60**	**<.0001**	0.69	0.45 to 1.04	.09
ECOG-ACRIN performance status						
0	**1**			**1**		
>0	**2.23**	**1.60 to 3.12**	**<.0001**	**2.24**	**1.38 to 3.42**	**.001**

[a] $P < .05$ was considered statistically significant (Kaplan-Meier estimates and log-rank test).
Bold represent statistically significant results.

In conclusion, we identified 30 patients with actionable alterations: 17 patients (56.7%) who received second-line targeted therapy according to their molecular alteration (8 patients on ivosidenib, 6 patients on pemigatinib, and 3 patients on anti-ERBB2 drugs) vs 13 patients (43.3%) who did not receive targeted therapy according to their molecular alteration but received only chemotherapy. Patients treated with targeted therapy showed better outcomes in terms of overall survival compared with patients treated without targeted therapy (not reached vs 4.2 months: HR = 0.18, 95% CI = 0.076 to 0.63, $P = .0065$).

Extrahepatic cholangiocarcinoma

The patient cohort comprised 168 individuals with advanced extrahepatic cholangiocarcinoma treated with cisplatin and gemcitabine plus durvalumab. Of these patients, 120 (71.4%) underwent gene alteration analysis on tumor tissue samples.

Assays used for genetic testing in individual samples included FoundationOne CDx (47 patients [39.2%]); a mixture of commercial and in-house, in-depth next-generation sequencing methods (37 patients [30.8%]), Myriapod NGS Cancer Panel (18 patients [15.0%]), and Oncomine (7 patients [5.8%]). A single-gene alterations assay (*FGFR2*, *IDH1*, *MSI*, *BRAF*, *NTRK*, *RET*, and *ERBB2*) was used in 11 patients (9.2%). We performed a descriptive molecular analysis, as well, identifying genomic alterations occurring in at least 3% of the patients. These alterations affected 26 genes, with the corresponding percentages shown in Figure S3B. Following this analysis, we examined the outcomes of patients who had any alterations within the selected 26-gene panel.

On univariate analysis for PFS, *KRAS* alterations were associated with shorter median PFS (8.7 vs 10.0 months: HR = 2.86, 95% CI = 1.13 to 7.23, $P = .0265$) (Figure 4, E). Figure S1E presents the forest plot illustrating outcomes for all evaluated genes. On univariate analysis for overall survival, no genomic alterations were found to be linked to patient outcomes. Figure S1F displays the forest plot summarizing the outcomes for all the genes evaluated. After adjusting for prognostic variables identified on univariate analysis, multivariate analysis for PFS confirmed that *KRAS* mutations had a negative prognostic impact (Table S8).

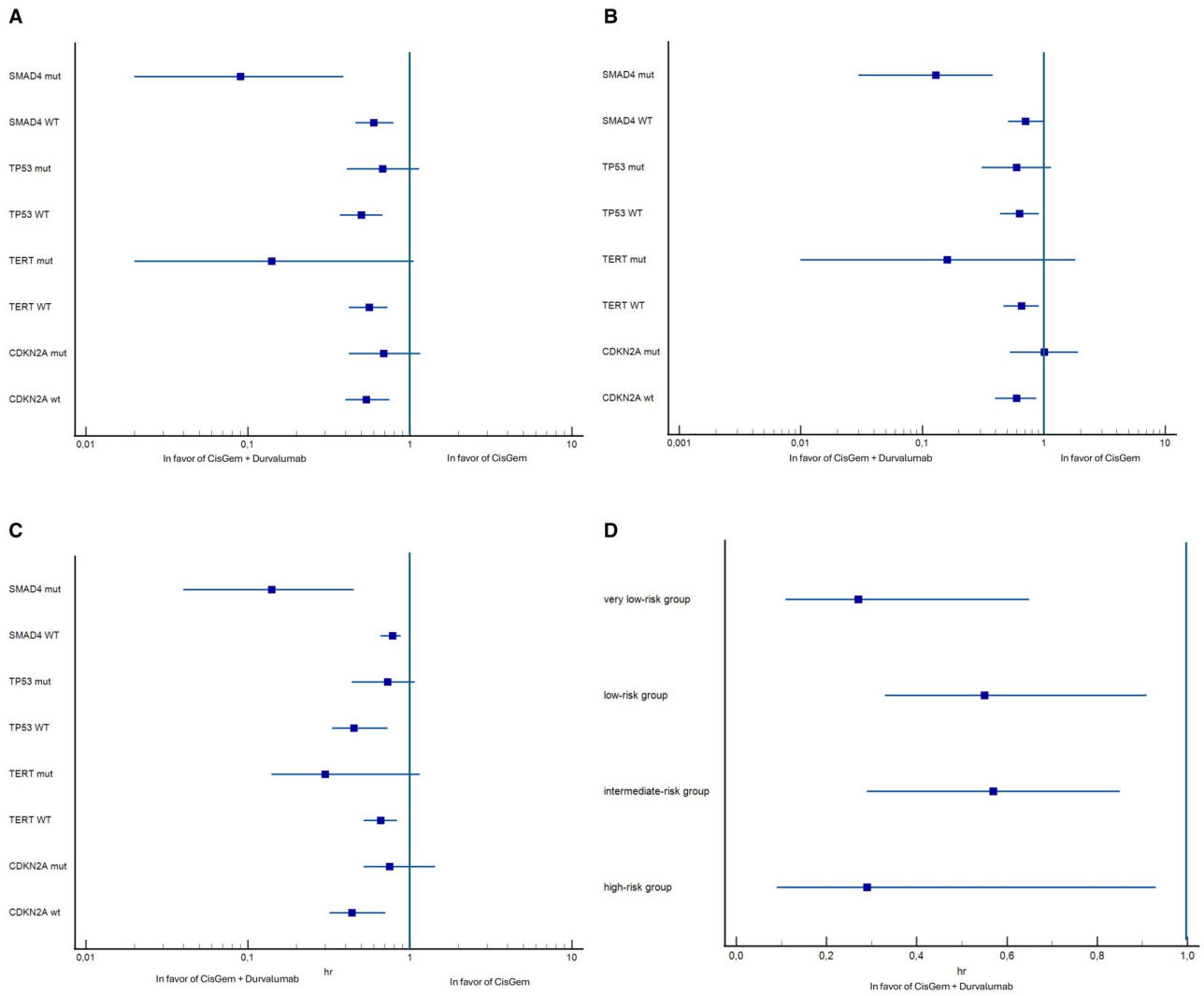

Figure 3. Forest plot of PFS (**A**) and OS (**B**) according to all evaluated genes in the overall population. Forest plot of OS according to all evaluated genes in the overall population after IPTW-adjustment. Forest plot of OS (**C**) according to subgroup of index. Forest plot illustrating the different outcomes across the four groups in terms of OS (**D**). On the x-axis, the HR of each analyzed gene.

Gallbladder cancer

The patient cohort comprised 135 individuals with advanced gallbladder cancer treated with cisplatin and gemcitabine plus durvalumab; of these patients, 107 (79.2%) underwent gene alteration analysis on tumor tissue samples. Testing assays employed for tumor individual samples were FoundationOne CDx (30 patients [28.0%]); a mixture of commercial and in-house, in-depth next-generation sequencing methods (45 patients [42.0%]), Myriapod NGS Cancer Panel (14 patients [13.1%]), and Oncomine (12 patients [11.2%]). A single-gene alterations assay (*FGFR2*, *IDH1*, *MSI*, *BRAF*, *NTRK*, *RET*, and *ERBB2*) was used in 12 patients (11.2%).

Outcomes for the 107 patients who underwent gene alteration analysis were as follows: median PFS was 7.3 months (95% CI = 6.0 to 8.5), and median overall survival was 15.1 months (95% CI = 11.5 to 27.7). First, we conducted a descriptive molecular analysis and identified genomic alterations present in at least 3% of patients. These alterations involved 32 genes, and their respective percentages are illustrated in Figure S3C. We subsequently analyzed outcomes of patients with any alteration in the 32 gene-panel selected. On univariate analysis for PFS and overall survival, no gene alteration was found to be associated with outcome. Figures S1G and S1H present the forest plot illustrating outcomes for all evaluated genes.

Mixed prognostic index based on molecular and clinical characteristics

With the aim to build a mixed prognostic index based on molecular and clinical characteristics, we used the model of multivariate testing in the overall population. We developed the prognostic model by combining the 4 identified prognostic variables on multivariate analysis in Table 3 (*SMAD4* status, *TERT* status, disease stage, and ECOG-ACRIN performance status) and assigning a weight of 1 to each of the following: *SMAD4* wild type, *TERT* mutant, metastatic stage (with extrahepatic spread, including nonlocoregional lymph nodes or with liver lesions in addition to the primary lesion), and ECOG-ACRIN performance status above 0. Accordingly, patients were stratified into 4 risk groups: very low risk (1 negative prognostic factor), low risk (2 negative prognostic factors), intermediate risk (3 negative prognostic factors), and high risk (4 negative prognostic factors). Overall, 342

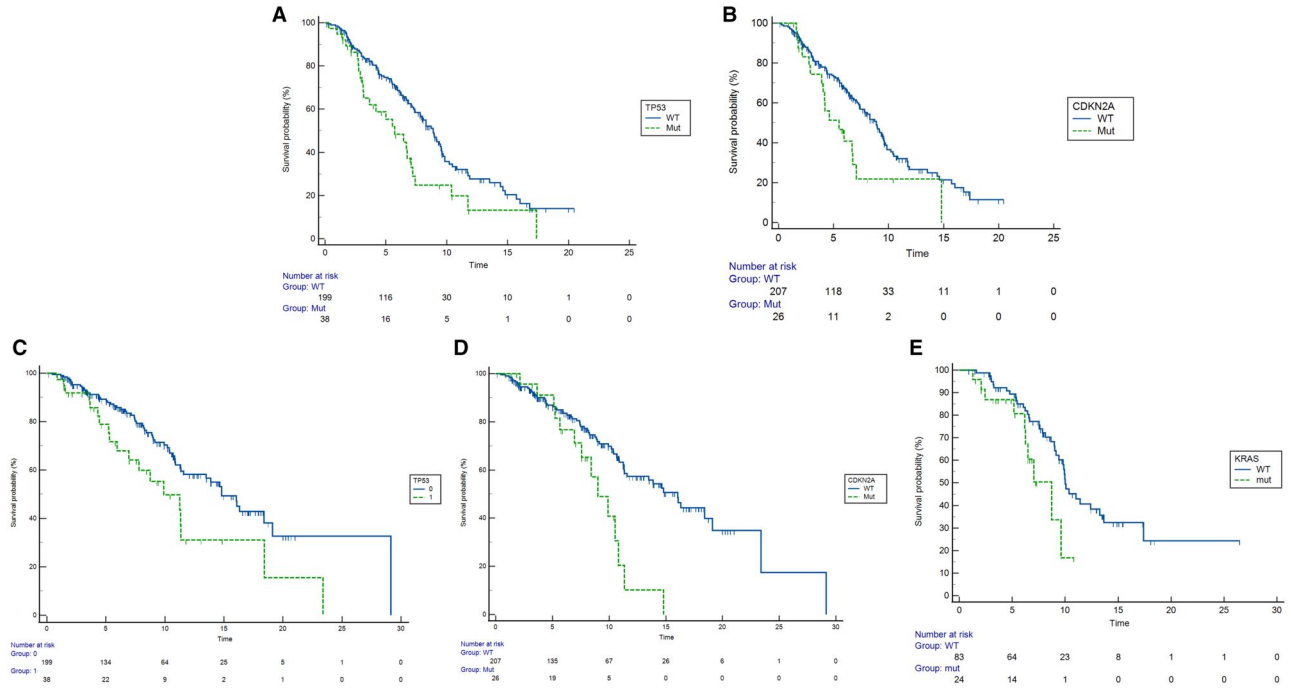

Figure 4. Kaplan Meier curves of progression-free survival according to *TP53* (**A**) and *CDKN2A* (**B**) in patients with intrahepatic cholangiocarcinoma. Kaplan-Meier curves of overall survival according to *TP53* (**C**) and *CDKN2A* (**D**) in patients with intrahepatic cholangiocarcinoma. (**E**) Kaplan-Meier curves of progression-free survival according to *KRAS* in patients with extrahepatic cholangiocarcinoma. Level of significance $P < .05$ was considered statistically significant (Kaplan-Meier estimates and log-rank test).

patients were analyzed, with 59 patients categorized as very low risk, 154 as low risk, 121 as intermediate risk, and 8 as high risk.

Survival curves according to the prognostic model are shown in Figure S4. The median PFS was 13.3 months in the very low-risk group, 9.2 months in the low-risk group, 7.1 months in the intermediate-risk group, and 2.7 months in the high-risk group. Median overall survival was not reached in the very low-risk group, 20.5 months in the low-risk group, 11.2 months in the intermediate-risk group, and 3.6 months in the high-risk group (Figures S4A and S4B). Differences in PFS and overall survival were statistically significant among all 4 groups ($P < .001$). Differences in overall response rate were statistically significant among the very low-risk group (42.9%), low-risk group (38.5%), intermediate-risk group (30.2%), and high-risk group (0%) ($P < .0001$). To test the predictive impact of the index, we compared this cohort with a historical cohort of 114 patients treated with cisplatin and gemcitabine.

Figure 3, D presents the forest plot illustrating the different outcomes across the 4 groups in terms of overall survival. The results highlighted an advantage of cisplatin and gemcitabine plus durvalumab across all 4 subclasses of our index.

Discussion

To the best of our knowledge, the present analysis constitutes one of the first real-world analyses focused on the prognostic impact of somatic genomic alterations in a cohort of patients who received cisplatin, gemcitabine, and durvalumab for advanced biliary tract cancer. One of the first considerations is the proportion of patients with advanced biliary tract cancer who underwent genomic and molecular testing as well as the heterogeneity of tests used. Despite international guidelines recommending comprehensive molecular testing for all patients with advanced biliary tract cancer, only 77% of our cohort received the test. This discrepancy may result from limited test reimbursement and a lack of awareness about its importance, highlighting the need for public health policies to ensure the test for each patient.

This aspect could have influenced the proportion of patients found to have a targetable genomic alteration, which is known to be significantly lower than previous international evidence.[20,21] The next-generation sequencing technique could have a role, too, because different techniques are characterized by different sensitivity and specificity. Another remarkable issue is the access to targeted therapies. To date, considerable heterogeneity exists in terms of access to targeted therapies worldwide based on differences in reimbursement policies. Thus, even for patients suitable for a targeted therapy, the possibility of receiving such treatment is not guaranteed and could be influenced by the reimbursement policies of different countries.

Analyzing baseline characteristics, the only difference between tested and untested patients was in ECOG-ACRIN performance status. Patients with a performance status above 0 were tested less often than patients with a performance status of 0. This finding may imply that patients with poorer ECOG-ACRIN performance status have less access to further therapies or that, upon disease progression on first-line treatment, they may not have adequate conditions for additional treatments, leading to less frequent testing.

In terms of molecular profiling, the present work confirmed previous evidence from smaller cohorts of patients with biliary tract cancer. Several differences in terms of molecular landscape based on primary tumor site have been highlighted: a higher incidence of *KRAS*, *SMAD4*, and *TP53* mutations has been reported in extrahepatic cholangiocarcinoma than in intrahepatic cholangiocarcinoma; a higher incidence of *BAP1* and *IDH1/2* mutations has been shown in intrahepatic cholangiocarcinoma than in extrahepatic cholangiocarcinoma and gallbladder cancer; a higher

incidence of *ELF3*, *ERBB2*, *MAP2K4*, *SMAD4*, and *TP53* alterations has been observed in gallbladder cancer than in intrahepatic cholangiocarcinoma; and, a higher incidence of *KRAS* mutations has been detected in extrahepatic cholangiocarcinoma than in gallbladder cancer. Previous evidence reported similar results,[22-24] reinforcing the value of the present data. Recently, Kendre and collaborators[25] published a large retrospective analysis conducted on 6130 patients with intrahepatic cholangiocarcinoma from the FoundationCORE database, with a special focus on the co-mutational spectra. They highlighted a negative selection of the *RTK/RAS/ERK* pathway co-alterations and an enrichment in epigenetic modifiers, including *BAP1* and *ARID1A*, in patients carrying *IDH1/2* mutations and *FGFR2* alterations. Unlike the present study, the above-mentioned work was focused on intrahepatic cholangiocarcinoma and was aimed at providing a highly representative cartography of the genomic landscape of intrahepatic cholangiocarcinoma without a focus on the prognostic and predictive role of genomic alterations. Moreover, patients were homogeneously tested using the same next-generation sequencing technique, unlike the present investigation, which studied real-world patients who received different types of next-generation sequencing tests.

The results from the TOPAZ-1 and KEYNOTE-966 trials led to the introduction of immunotherapy to the therapeutic armamentarium for patients with advanced biliary tract cancer. Not all patients respond to immunotherapy, however, and biomarkers that can identify patients who could benefit better represent an unmet need in this setting. The current analysis revealed that *SMAD4* alteration is a prognostic and predictive factor for the efficacy of durvalumab. Although patients with and without *SMAD4* alteration benefited from the addition of durvalumab, this result suggests that *SMAD4* mutation may only help identify individuals who could achieve greater benefit from immunotherapy. *TP53* mutation is a negative prognostic factor for PFS, whereas *TERT* mutation is a negative prognostic factor for overall survival, regardless of primary tumor site. Data about the prognostic role of *SMAD4* genomic alterations in biliary tract cancer are scarce and inconclusive. *SMAD4* is regarded as a tumor suppressor gene that codes for a protein known to be a crucial mediator of transforming growth factor β signaling and regulates several vital processes, including fibrosis coordination, tumor development, immune function, and wound healing.[26-28] Loss of SMAD4 expression has been associated with chemoresistance and worse prognosis in several oncology settings, including colorectal cancer, non–small cell lung cancer, and pancreatic adenocarcinoma.[29-31] In the biliary tract cancer setting, only scarce evidence reported poor prognosis and reduced response to chemoradiation therapy in patients with *SMAD4*-mutated cancer.[32,33] Regarding its role in the tumor microenvironment and immune response, *SMAD4* has been found to have a multifaceted role, with data varying across different oncologic settings.[34] In particular, the transforming growth factor β/SMAD signaling pathway has been noted to interfere with the activity of cytotoxic T cells and natural killer cells by promoting the recruitment of FOXP3-positive regulatory T cells and by altering the function of antigen-presenting cells from immune activation to tolerance,[35,36] as confirmed in in vivo studies.[37] A crucial role of *SMAD4* in suppressing immunogenicity in pancreatic cancer was uncovered.[38] *SMAD4* deficiency was shown to promote spontaneous DNA damage, which in turn stimulates type I interferon signaling, leading to CD8-positive T-cell responses against cancer cells.[38]

In contrast, in other oncologic settings, the loss of *SMAD4* has been noted to drive immune evasion, leading to scarce response to immunotherapy.[39,40] Preclinical evidence has demonstrated that the loss of *SMAD4* in gastric cancer cells induced immune evasion and conferred resistance to immune checkpoint inhibitor monotherapy, suggesting the potential role of combined immunotherapy treatments in this setting.[40] Other previous evidence has supported the role of *SMAD4* in mediating T-cell activities and tumor infiltration, which are known to be related to longer survival and better outcomes with immunotherapy.[41] These data contrast with the results of the present analysis. The observed discrepancies could be explained by the complex and multifaceted function of *SMAD4* in immune cells, which might be influenced by the oncologic setting and different treatments. Interestingly, *SMAD4* loss has been associated in preclinical models with increased genomic instability and activation of innate immune pathways, particularly type I interferon signaling, ultimately leading to enhanced CD8-positive T-cell infiltration and cytotoxicity.[38] This phenomenon, described particularly in pancreatic cancer models, suggests that *SMAD4* deficiency could paradoxically increase tumor immunogenicity under certain conditions. In the context of biliary tract cancer, it is plausible that *SMAD4* mutation results in a more immunogenic tumor microenvironment, improving immune-mediated tumor recognition and potentiating the efficacy of checkpoint inhibitors such as durvalumab. Although the exact mechanisms have not yet been fully elucidated, these observations suggest that *SMAD4* mutations may define a molecular subset of biliary tract cancer with increased susceptibility to immunotherapy, meriting further prospective validation.

As mentioned, *TP53* mutations have been shown to have a negative prognostic role for overall survival, whereas *TERT* mutations have been shown to have a negative prognostic role for PFS. In addition, both gene mutations have been shown to correlate with a lower disease control rate than their wild-type counterparts. The negative prognostic role of *TP53* mutations is not surprising,[42] and their correlation with more aggressive biological behavior[43] has been well established. As highlighted in the subgroup analysis of the TOPAZ-1 trial, however, our data confirm that adding durvalumab to the treatment regimen for patients with both mutant and wild-type cancers results in improved outcomes compared with patients treated with chemotherapy alone.

Conversely, the negative prognostic role of *TERT* mutations could be considered a new finding and deserves consideration. *TERT* encodes the catalytic subunit of telomerase, maintaining genomic integrity. TERT expression is usually repressed in somatic cells, but overexpression through gene rearrangements, amplification, or promoter mutation is common in cancer.[44] *TERT* promoter mutations have been suggested to negatively affect prognosis in several cancers, although data are inconclusive. TERT also affects the immune system as a self-antigen in many tumors.[45-48] Recently, Li and colleagues[49] found that *TERT* mutations may predict immunotherapy response, reporting higher tumor mutation burden and proinflammatory immune activation in *TERT*-mutated tumors. Moreover, a better prognosis was observed in the subgroup of patients who received anti-CTLA4 treatment, but no clear data are available for patients who received anti–programmed cell death 1 ligand 1 treatment. The underlying pathways that could justify the negative prognostic role shown by the present analysis should be investigated in further studies. Notably, no genomic alterations with a prognostic significance were detected in gallbladder cancer in the present

study. The molecular heterogeneity of this subgroup along with the small sample size have likely made the identification of molecular aberrations with prognostic significance more difficult. Finally, patients with *BRAF*-V600 mutated tumors had a significantly lower overall response rate than their wild-type counterparts (16.7% vs 37.5%).

Concerning the impact of molecular profile on the clinical outcomes of patients with advanced biliary tract cancer who received durvalumab plus cisplatin and gemcitabine, only a few previous exploratory analyses have been reported. Valle and colleagues[49] reported the first exploratory analysis on the impact of mutational status in patients who received durvalumab plus cisplatin and gemcitabine in the TOPAZ-1 trial. They highlighted that patients with alterations in *KRAS, TP53*, and *CDKN2A/2B/MTAP* loss had a reduced risk of cancer growth, spread, or worsening after treatment with durvalumab plus chemotherapy vs placebo plus chemotherapy. More recently, Bouattour and colleagues[50] presented their analysis, aimed at assessing clinical characteristics, outcomes, and genomic profiles of long-term survivors treated with durvalumab plus cisplatin and gemcitabine. Interestingly, a higher proportion of patients with *BRCA1/2* mutated tumors was included in the long-term survival subgroup, suggesting that the prevalence of these mutations may be associated with long-term survival. The different results observed between the above-mentioned studies and those reported in the present analysis could be explained by the different study design and methodology. Moreover, the different next-generation sequencing techniques used in the studies could be at the root of discrepancies due to the different sensitivities and sensibilities. Our research group previously analyzed 51 patients with advanced biliary tract cancer treated with cisplatin and gemcitabine plus durvalumab, identifying 3 molecular clusters. Cluster 1 showed chromatin modification mutations, cluster 2 displayed alterations in multiple pathways (DNA damage repair, chromatin modification, *RTK/RAS*, cell-cycle apoptosis, *TP53, PI3K*), and cluster 3 mainly involved *RTK/RAS* and cell-cycle apoptosis mutations. Cluster 2 had the highest overall response rate, while cluster 3 showed none ($P = .0188$). Unlike the smaller, Italy-based cohort of the previous study focused on molecular clusters and clinical outcomes, this larger, multiethnic study investigated the impact of individual gene alterations without finding survival correlations.

As a final point, we built a combined score that included *SMAD4* mutations, *TERT* mutations, disease stage, and ECOG-ACRIN performance status. This combined score stratified patients with statistically significant differences in overall survival and PFS among 4 risk groups. Notably, patients included in the very low-risk and the low-risk groups showed remarkable median overall survival (>20 months), almost double the overall survival shown in the TOPAZ-1 trial. The identified index groups were compared with a historical cohort of patients treated with chemotherapy alone (cisplatin plus gemcitabine), and the results showed a benefit from adding durvalumab across all subcategories, demonstrating that all patients benefited. This finding suggests that the newly developed index may have a prognostic value and, if validated, could be used to better stratify patients in the future.

The present study has several limitations. First, the retrospective nature of the work could not exclude potential selection biases. Indeed, we performed multivariate adjustment, but that cannot replace level I evidence derived from prospective randomized clinical trials. Second, molecular analysis was carried out adopting several diagnostically available external and in-house next-generation sequencing assays affected by variations in terms of technical parameters, including reference range. We confirm that all technical solutions were aligned to cover all biliary tract cancer–related genes, harmonizing molecular records. In addition, we excluded variants of uncertain significance by including only those classified as pathogenic or likely pathogenic. The classification was provided directly by the tests used, however, and it is possible that the variants of uncertain significance classification differed slightly across tests. Moreover, our database did not collect time frame information regarding the molecular evaluation in relation to the start of treatment with cisplatin and gemcitabine plus durvalumab. Nevertheless, the main goal of the present study was to re-collect and analyze molecular data derived from the real-world context to draw conclusions that could be transferred to clinical practice. In addition, no universally accepted consensus exists about which next-generation sequencing tests should be used for patients with advanced biliary tract cancer. Third, limitations derived from the multinational and multi-institutional nature of the work must be considered. For instance, the PFS analysis has to account for differences in terms of tumor assessment modalities and time points, according to institutions' protocols. Another important limit is represented by the lack of information about the variant allele frequency, which could constitute a substantial bias for the descriptive molecular analysis and the survival analysis. Finally, in the descriptive molecular analysis, no direct comparison was made between perihilar and distal cholangiocarcinoma. Despite these important limitations, the present work reported 1 of the first molecular analyses focused on a large cohort of patients with advanced biliary tract cancer treated with cisplatin and gemcitabine plus durvalumab and whose tumors have been tested in clinical practice with a next-generation sequencing test. The present study highlighted possible prognostic and predictive biomarkers based on the molecular profile of this complex and heterogeneous group of diseases. Moreover, a combined clinical and molecular score has been proposed to improve patient stratification and better select patients who are most likely to respond to the new standard of care. If validated in larger prospective cohorts, the results presented in this work could make an important contribution to understanding the prognostic implications of the molecular profile of patients with advanced biliary tract cancer who may be candidates for immunotherapy and, consequently, to improve the stratification of our patients.

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Conflicts of interest

None declared.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of each institution involved in the project. Under the condition of retrospective archival tissue collection and patients' data anonymization, our study was exempted from the acquisition of informed consent from patients by the institutional review board. Written informed consent for treatment was, however, obtained for all patients.

Data availability

The data underlying this article are not publicly available due to privacy and ethical restrictions. Deidentified data can be requested from the corresponding author, however. Requests will be formally evaluated and approved by the Ethics Committee of San Raffaele Scientific Institute in accordance with institutional and General Data Protection Regulation regulations. Requestors will be asked to provide contact details and a description of the intended use for the purpose of logging and data use tracking. Data access will not be subject to discretionary approval by the study investigators.

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