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Clinical Trial

Regorafenib–avelumab combination in patients with biliary tract cancer (REGOMUNE): a single-arm, open-label, phase II trial

Sophie Cousin [a], Coralie Cantarel [b], Jean-Philippe Guegan [c], Thibault Mazard [d], Carlos Gomez-Roca [e], Jean-Philippe Metges [f], Carine Bellera [b,g], Antoine Adenis [d], Iphigenie Korakis [e], Pierre-Guillaume Poureau [f], Kevin Bourcier [a], Maud Toulmonde [a], Michèle Kind [h], Christophe Rey [c], Céline Auzanneau [i], Alban Bessede [c], Isabelle Soubeyran [i], Antoine Italiano [a,j,k,*]

[a] *Early Phase Trials Unit, Institut Bergonié, 229 Cours de L'Argonne, 33000, Bordeaux, France*
[b] *Clinical and Epidemiological Research Unit, INSERM CIC1401, Institut Bergonié, Comprehensive Cancer Center, F-33000, Bordeaux, France*
[c] *Explicyte, 229 Cours de L'Argonne, Bordeaux, France*
[d] *Department of Medical Oncology, Institut Regional Du Cancer de Montpellier, 208 Avenue des Apothicaires, 34298, Montpellier, France*
[e] *Department of Medical Oncology, IUCT, 1 Avenue Irène Joliot-Curie, 31100, Toulouse, France*
[f] *Department of Medical Oncology, CHRU de Brest - Hôpital Morvan, 2 Avenue Foch, 29609, Brest, France*
[g] *Bordeaux, Inserm, Bordeaux Population Health Research Center, Epicene Team, UMR 1219, F-33000, Bordeaux, France*
[h] *Department of Radiology, Institut Bergonié, 229 Cours de L'Argonne, 33000, Bordeaux, France*
[i] *Department of Biopathology, Institut Bergonié, 229 Cours de L'Argonne, 33000, Bordeaux, France*
[j] *University of Bordeaux, Bordeaux, France*
[k] *Gustave Roussy, Villejuif, France*

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Abstract ***Background:*** Regorafenib has shown substantial clinical activity in patients with advanced biliary tract cancers (BTCs). Preclinical data suggested that this drug modulates antitumour immunity and is synergistic with immune checkpoint inhibition.
Patients and methods: This is a single-arm, multicentric phase II trial. Regorafenib was given 3 weeks/4, 160 mg quaque die (once a day) (QD); avelumab 10 mg/kg IV was given every two weeks, beginning at C1D15 until progression or unacceptable toxicity. The primary end-point

* *Corresponding author*: Early phase trials unit, Institut Bergonié, 229 Cours de l'Argonne, 33000, Bordeaux, France. Fax: +33.(0)5.47.30.60.83.
E-mail address: a.italiano@bordeaux.unicancer.fr (A. Italiano).

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was the confirmed objective response rate under treatment, as per Response Evaluation Criteria in Solid Tumours 1.1. The secondary end-points included the following: 1-year non-progression rate; progression-free survival (PFS) and overall survival; safety and biomarkers studies performed on sequential tumour samples obtained at baseline and at cycle 2 day 1.

Results: Thirty-four patients were enrolled in four centres. Twenty-nine patients were assessable for efficacy after central radiological review. The best response was partial response for four patients (13.8%), stable disease for 11 patients (37.9%) and progressive disease for 14 patients (48.3%). The median PFS and overall survival were 2.5 months (95% confidence interval [CI] [1.9–5.5]) and 11.9 months (95%CI [6.2–NA]) respectively. The most common grade 3 or 4 clinical adverse events related to treatment were hypertension (17.6%), fatigue (14.7%) and maculopapular rash (11.8%). High baseline levels of programmed cell death ligand 1 and of indoleamine 2, 3-dioxygénase expression were associated with improved outcomes.

Conclusions: Regorafenib combined with avelumab has antitumour activity in a subset of heavily pretreated biliary tract cancer population. Further investigations are needed in patients selected based on tumour microenvironment features.

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1. Introduction

Biliary tract cancers (BTCs) represent a group of aggressive malignancies classified based on their site of origin across the biliary tree as intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA) and gallbladder cancer.

Patients diagnosed with BTC have a poor prognosis because most patients are not eligible for curative surgery [1]. In the advanced disease setting, systemic therapy combining cisplatin with gemcitabine represents the current standard of care [2].

After the failure of first-line therapy, therapeutic options are limited [2]. Given the importance of angiogenesis in BTC tumourigenesis, several antiangiogenic agents have been investigated in this setting [3]. Regorafenib, a small molecule that targets several protein kinases including platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor (VEGFR), fibroblast growth factor receptor (FGFR), FMS-like tyrosine kinase 3 (FLT-3), REarranged during Transfection (RET) and c-kit receptor tyrosine kinase (KIT), is the sole antiangiogenic agent that has shown significantly improved clinical benefit compared with placebo in the context of a randomised study [4]. Indeed, regorafenib significantly improved progression-free survival (PFS) and tumour control in comparison to place in a randomised phase II study including patients with previously treated metastatic/unresectable BTC in the second- or third-line setting [4].

Inflammation and immune modulation have also been shown to play a crucial role in BTCs [5,6]. Clinical trials investigating immune checkpoint inhibitors in patients with BTC have reported only modest results [7], underscoring the need for novel approaches including combination therapies and the identification of efficacy biomarkers.

There are several lines of evidence indicating that targeting VEGF and its receptor may be synergistic with immune checkpoint inhibition in human tumours [8,9]. Therefore, we hypothesised that combining an anti-angiogenic agent with anti-programmed cell death 1 (anti-PD-1)/anti-programmed cell death ligand 1 (anti-PD-L1) antibodies may be associated with significant clinical benefit in patients with advanced BTC who have not responded to previous standard chemotherapy regimens.

Here, we report the clinical and biomarker results of a phase II study investigating the combination of regorafenib plus the anti-PD-L1 antibody, avelumab, in patients with advanced BTC.

2. Methods

2.1. Study design and participants

REGOMUNE is a single-arm, multicentre phase II basket study for which patients were recruited from four French sites. In the BTC cohort, patients were eligible if they were at least 18 years old and had histologically proven advanced or metastatic BTC, an Eastern Cooperative Oncology Group performance status of 0–1, measurable disease as per Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 [10], at least one previous line of systemic treatment and adequate haematological, renal, metabolic and hepatic functions (see study protocol for a full list of eligibility criteria). Blood tests included the assessment of blood cell count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, bilirubin, lipase, creatinine phosphokinase,

coagulation test, creatinine and urea nitrogen. The main exclusion criteria included previous treatment with avelumab or regorafenib, previous treatment with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-cluster of differentiation 137 (CD137) or anticytotoxic T-lymphocyte-associated antigen-4 antibody, and are detailed in the protocol. As required by French regulations, the protocol was approved by a central institutional review board (Comité de Protection des Personnes Sud-Est II, Lyon, France), which reviewed the appropriateness of the clinical trial protocol and the risks and benefits to study participants. All patients provided written informed consent.

2.2. Procedures

After assessing eligibility, the patients received regorafenib, 160 mg per day on a 3-week on/1-week off schedule, in cycles of 28 days. Avelumab treatment began on cycle 1 day 15, by intravenous infusion once every two weeks at a dose of 10 mg/kg. Treatment was continued until disease progression, unacceptable toxicity, the investigator's decision to discontinue or withdrawal of patient consent. Participants were monitored for adverse events at every follow-up assessment. Adverse events were graded as per the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. Laboratory assessments were conducted at baseline and every two weeks thereafter until treatment discontinuation. Regorafenib dose modifications to manage adverse events were allowed (see study protocol). The regorafenib dose could be reduced to 120 mg and then to 80 mg. Dose interruptions were allowed based on the clinical situation. Patients requiring a delay of >4 weeks because the last dose of regorafenib had to permanently discontinue regorafenib but could continue avelumab if it was deemed appropriate. No dose reduction of avelumab was allowed. Dose interruptions were allowed based on the severity of immune-related adverse events. Patients requiring two or more consecutive cancellations of avelumab injection had to permanently discontinue avelumab but were allowed to continue regorafenib. Tumour lesions were assessed as per RECIST version 1.1 at baseline (within four weeks before cycle 1 day 1) and every eight weeks until disease progression or the start of another treatment. Tumour samples were collected at baseline and on cycle 2 day 1 for all consenting patients to assess the impact of treatment on the tumour microenvironment and to identify potential biomarkers associated with outcomes.

2.3. Outcomes

The primary end-point was the objective response rate defined as the proportion of patients with objective response (confirmed or unconfirmed) undertreatment based on adapted RECIST 1.1 after centralised radiological review.

Secondary objectives included best overall response, 1-year PFS, 1-year overall survival (OS) and safety. The best overall response was defined as the best response across all time points. Durable clinical benefit was defined as the proportion of patients with objective response and/or stable disease lasting more than six months. PFS was defined as the time from study treatment initiation to death from any cause or the first occurrence of disease progression based on RECIST 1.1 after centralised radiological review. OS was defined as the time from study treatment initiation to death from any cause. Safety was graded as per the common toxicity criteria stated in National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI CTC-AE v5.0).

2.4. Statistical analysis

This study was based on a Bayesian adaptive phase II design approach and followed an adaptive trial design. The primary end-point was the objective response rate under treatment. The probability of success was estimated from a beta-binomial model. The maximum response probability threshold and minimum response probability threshold were defined as 40% and 20%, respectively [11]. The maximum sample size was set at 50 patients. The analysis of the primary end-point was carried out sequentially, with interim analyses planned after a 16-week follow-up for the first 10 patients and then every five patients.

At each interim analysis, stopping rules recommended that the trial be stopped owing to inefficacy (if there was a high predictive probability [≥80%] that the objective response rate was less than or equal to 20%, the minimum response probability threshold) or efficacy (if there was a high predictive probability [≥80%] that the objective response rate was greater than or equal to 40%, the maximum response probability threshold).

The efficacy population included all participants who met the eligibility criteria and who received at least one complete or two incomplete treatment cycles. All enrolled patients who initiated the study treatment were included in the safety analysis.

The median follow-up was calculated using the reverse Kaplan–Meier method, and survival end-points were described using the Kaplan–Meier method. Data for patients who were alive and event-free were censored at the date of the last follow-up. Quantitative variables were described using the median and range. Qualitative variables were described using frequency, rates and the 95% CI (binomial law). Estimated parameters are reported with two-sided 95% CIs. P values less than 0.05 (typically ≤0.05) were considered to be statistically significant. The statistical analyses were performed using Statistical Analysis System (SAS) software (version 9.4).

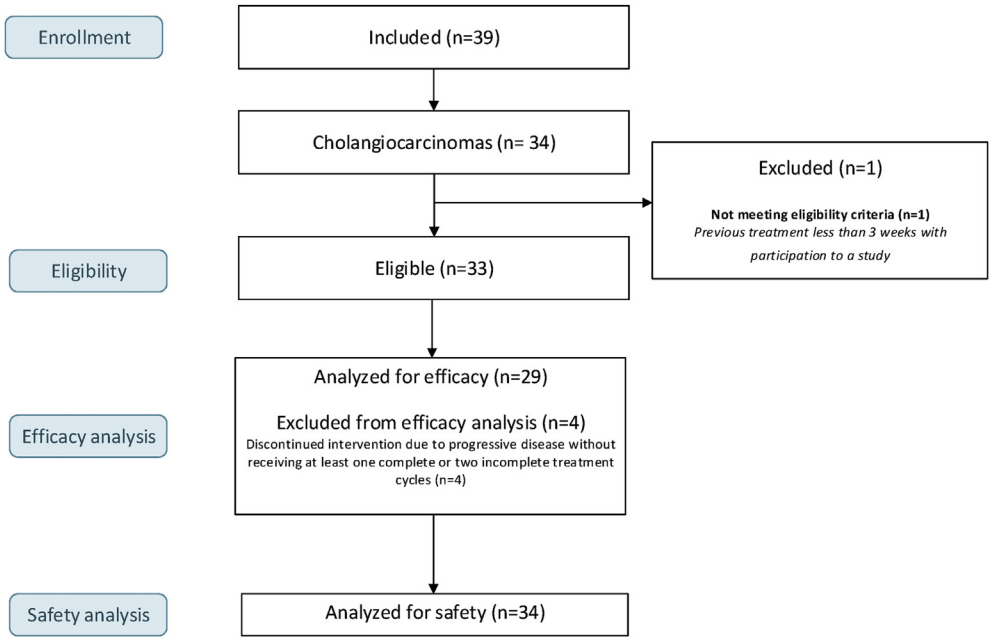

Fig. 1. Flow chart of patients included in the REGOMUNE STUDY.

This study is registered with ClinicalTrials.gov, number NCT03475953.

2.5. Tissue sample analysis

Tumour biopsies were collected at baseline and on cycle 2 day 1. These samples were analysed to characterise the impact of regorafenib combined with avelumab on the tumour microenvironment and to identify potential predictive biomarkers of clinical benefit. Immunohistofluorescence analysis was performed on the automated Ventana Discovery XT staining platform (Ventana Medical Systems). Slides of tumour tissue were deparaffinised by heating to 69 °C combined with the application of Discovery Wash solution for 8 min. This operation was repeated three times. The slides were incubated with the following primary antibodies: anti-CD8 (clone C8/144B, Dako); anti-CD163 (clone 10D6, Leica) anti-PD-L1 (clone QR1, Diagomics) and anti-cytokeratine 19 (clone A53-B/A2.26, Ventana). Bound primary antibodies were detected using either OmniMap anti-mouse (Ms) or rabbit-horseradish peroxidase (Rb-HRP) with the Opal detection kit (Akoya). The slides were counterstained with spectral DAPI (Akoya) and cover-slipped. The stained slides were imaged on a multispectral imaging system (Vectra Polans, Akoya) and analysed using Inform image analysis software (Akoya, version 2.4.1) to segment the tissue into tumour and stroma and to phenotype the cells.

Table 1
Patient characteristics.

	Eligible patients for safety N = 34
Median age (years, range)	63.1 (36–80)
Gender	
Female	16 (47.1%)
Male	18 (52.9%)
Performance status ECOG	
0	16 (47.1%)
1	17 (50.0%)
Unknown	1 (2.9%)
Tumour location	
Intrahepatic	26 (76.5%)
Extrahepatic	7 (20.6%)
Gallbladder	1 (2.9%)
Number of metastatic sites	
Single	6 (17.6%)
Multiple	28 (82.4%)
Metastatic sites	
Liver	27 (79.4%)
Lung	20 (58.8%)
Peritoneum	14 (41.2%)
Node	18 (52.9%)
Other	11 (32.4%)
Previous chemotherapy treatment	
Platinum-based	34 (100%)
Gemcitabine-based	34 (100%)
Topoisomerase I or II inhibitor	13 (38.2%)
Taxanes	3 (8.8%)
Previous lines of treatment for advanced disease	
1	14 (41.2%)
2	12 (35.3%)
>2	8 (23.5%)

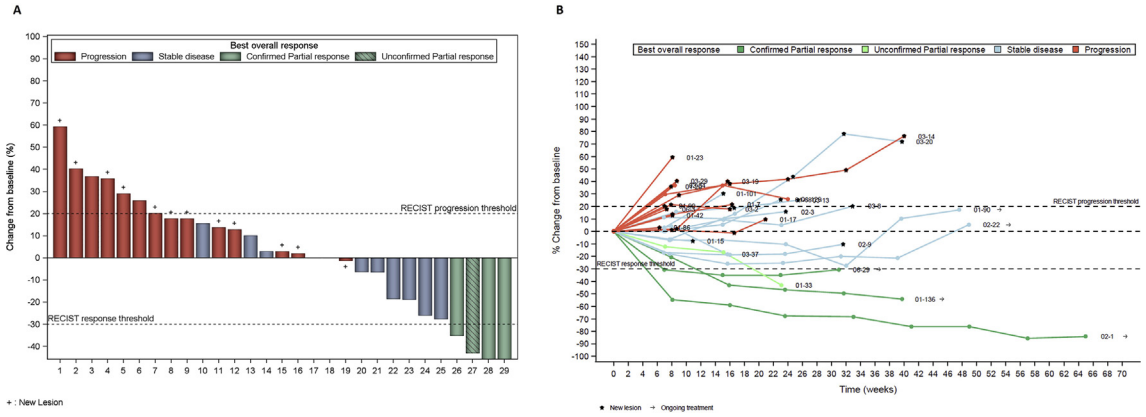

Fig. 2. Waterfall plot (**A**) and spider plot (**B**) of best overall response in patients with biliary tract cancer treated with regorafenib plus avelumab (n = 29, response based on central review assessment as per RECIST 1.1). RECIST, Response Evaluation Criteria in Solid Tumours.

2.6. Role of the funding source

The study was sponsored by the Institut Bergonié, Comprehensive Cancer Centre (Bordeaux, France). The data were collected with the sponsor data management system and were analysed and interpreted by representatives of the sponsor in collaboration with the investigators. SC, CC, CB and AI had access to the raw data. The funders of the study (Bayer and Merck) had no role in the study design, data collection, data interpretation or writing of the report. The corresponding author had full access to all of the data in the study and had the final responsibility for deciding to submit the article for publication.

3. Results

A total of 34 patients were recruited for the study between 21st November 2018 and 13th November 2019. As per the Bayesian adaptive design, inclusion was stopped prematurely, as there was a high predictive probability (98.6%) that the objective response rate was less than or equal to 20%. Twenty-nine patients were eligible and included in the efficacy end-point. Five patients were excluded from efficacy analysis for reasons prespecified in the protocol: one owing to protocol deviation regarding the inclusion criteria and four because of early discontinuation without any tumour assessment (Fig. 1). The patient characteristics are summarised in Table 1. The median age was 63 years (range 36–80), and 47.1% of the patients were women. All of the patients had already received systemic treatment for advanced disease, with a median of two (range 1–4) previous lines of treatment.

Of the 29 patients included in the efficacy assessment, four patients (13.8%) achieved a partial response; as such, the primary efficacy criterion was not reached. Regarding the best overall response as per RECIST, 11 (37.9%) patients demonstrated stable disease, including 10 patients (34.5%) with tumour shrinkage (range from

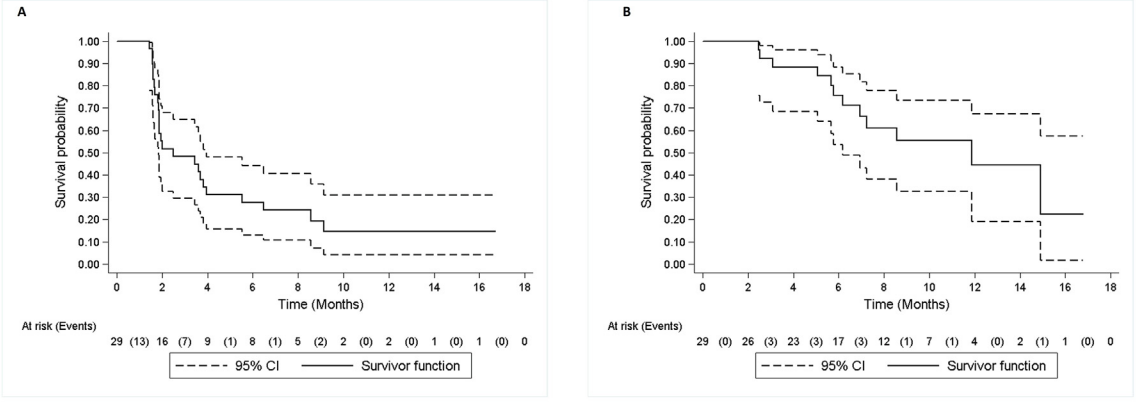

Fig. 3. **Kaplan–Meier curves** of progression-free survival after central review (**A**) and overall survival (**B**) in patients with biliary tract cancer treated with regorafenib and avelumab (29 patients eligible for efficacy analysis).

Table 2
Treatment-related adverse events during the treatment period in >5% of patients (N = 34).

Adverse event	Grade 1-2	Grade 3	Grade 4
Fatigue	22 (65%)	5 (15%)	
Infusion-related reaction	16 (47%)	1 (2.9%)	
Palmoplantar erythrodysesthesia	14 (41%)	3 (9%)	
Diarrhoea	13 (38%)	2 (6%)	
Dysphonia	12 (35%)		
Oral mucositis	11 (32%)		
Anorexia	10 (29%)	2 (6%)	
Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) increased	8 (24%)	4 (9%)	
Muscle cramp	8 (24%)		
Nausea	8 (24%)	1 (3%)	
Fever	8 (24%)		
Alkaline phosphatase and/or GGT increased	7 (21%)	3 (9%)	
Thrombopenia	7 (21%)	3 (9%)	
TSH increased	7 (21%)		
Vomiting	6 (18%)	1 (3%)	
Pruritus	6 (18%)		
Hypertension	5 (15%)	6 (18%)	
Maculopapular rash	5 (15%)	4 (12%)	
Dry skin	5 (15%)		
Alopecia	5 (15%)		
Hypophosphatemia	5 (15%)		
Anaemia	4 (12%)	1 (3%)	
Hypothyroidism	4 (12%)		
Abdominal pain	4 (12%)		
Headache	4 (12%)		
Dry mouth	4 (12%)		
Erythema multiforme	3 (9%)	2 (6%)	
Constipation	3 (9%)		
Lipase increased	3 (9%)		
Myalgia	3 (9%)		
Peripheral sensory neuropathy	3 (9%)		
Epistaxis	3 (9%)		
Dysphagia	3 (9%)		
Chills	3 (9%)		
Lymphocyte count decreased	2 (6%)	1 (3%)	
Blood bilirubin increased	2 (6%)	2 (6%)	
Gastrointestinal pain	2 (6%)		
Haemorrhoids	2 (6%)		
Periodontal disease	2 (6%)		
Blood lactate dehydrogenase increased	2 (6%)		
CPK increased	2 (6%)		
Arthralgia	2 (6%)		
Back pain	2 (6%)		
Proteinuria	2 (6%)		
Dyspnoea	2 (6%)		
Hoarseness	2 (6%)		
Hyperkeratosis	2 (6%)		
Rash acneiform	2 (6%)		
Hyponatremia	1 (6%)	2 (6%)	
Neutrophil count decreased		2 (6%)	
Left ventricular systolic dysfunction		1 (3%)	
Oesophageal ulcer		1 (3%)	
Biliary tract infection		1 (3%)	
Hyperglycemia		1 (3%)	

−1.2% to −85.6%) (Fig. 2). Fourteen patients (48.3%) had progressive disease. Among 15 patients with objective response or stable disease at six months, 14 patients had an iCCA. The median duration of response was 10.4 months.

The median follow-up was 9.8 months (95%CI 6.6−12.4). The median PFS was 2.5 months (95%CI 1.9−5.5). The 6- and 12-month PFS were 27.6% (95%CI 13.1−44.3) and 6.9% (95%CI 0.6−24.3), respectively.

At the time of analysis, 24 patients discontinued treatment. Reasons for treatment discontinuation were disease progression for 23 patients (95.8%) and alteration of general status for one patient (4.2%). Seventeen patients (58.6%) patients were still alive, with five patients (17.2%) still under treatment. The median OS was 11.9 months (95%CI 6.2−NA) (Fig. 3). The 6- and 12-month OS rates were 75.7% (95%CI 53.5−88.3) and 44.4% (95%CI 19.0−67.3), respectively.

Thirty-four patients received at least one dose of regorafenib and/or avelumab and were therefore evaluated for safety. The treatment was generally well-tolerated. Treatment-related adverse events and laboratory abnormalities that were reported in more than 5% of patients for grades 1−2 and any for grades 3 and 4 are shown in Table 2. The most common clinical treatment-related adverse events were fatigue, infusion-related reaction, palmoplantar erythrodysesthesia, diarrhoea, dysphonia and mucositis. The most common treatment-related laboratory abnormalities were transaminitis and thyroid-stimulating hormone increase. At least one serious adverse event was reported in 21 patients (62%). Twenty-six (76.5%) and 19 (55.9%) patients experienced treatment modifications with regorafenib (19 patients with temporary discontinuation, 12 patients with dose reduction and three patients with permanent discontinuation) and avelumab (12 patients with perfusion interruption, 10 patients with administration cancellation but not permanent discontinuation), respectively. No patient died from drug-related toxicity.

Analysis of the tumour biopsies revealed that high expression by tumour cells of PD-L1 and indoleamine 2,3-dioxygenase 1 (IDO1) was associated with a better durable clinical benefit rate and improved PFS (46.15% for high PD-L1 expression vs 7.14% for low PD-L1 expression, 85.7% for high IDO1 expression vs 7.14% for low IDO1 expression; 5.45 [1.68−not attained (NA)] months for high PD-L1 expression vs 2.28 [1.87−5.78] months for low PD-L1 expression, 5.78 [2.00−NA] months for high IDO1 expression vs 1.91 [1.84−5.45] months for low IDO1 expression) (Fig. 4). A comparison of the tumour microenvironment between iCCA and eCCA revealed a higher infiltration of eCCA by tumour-associated macrophages (TAMs) ($p = 0.052$) (Supplementary Fig. 1).

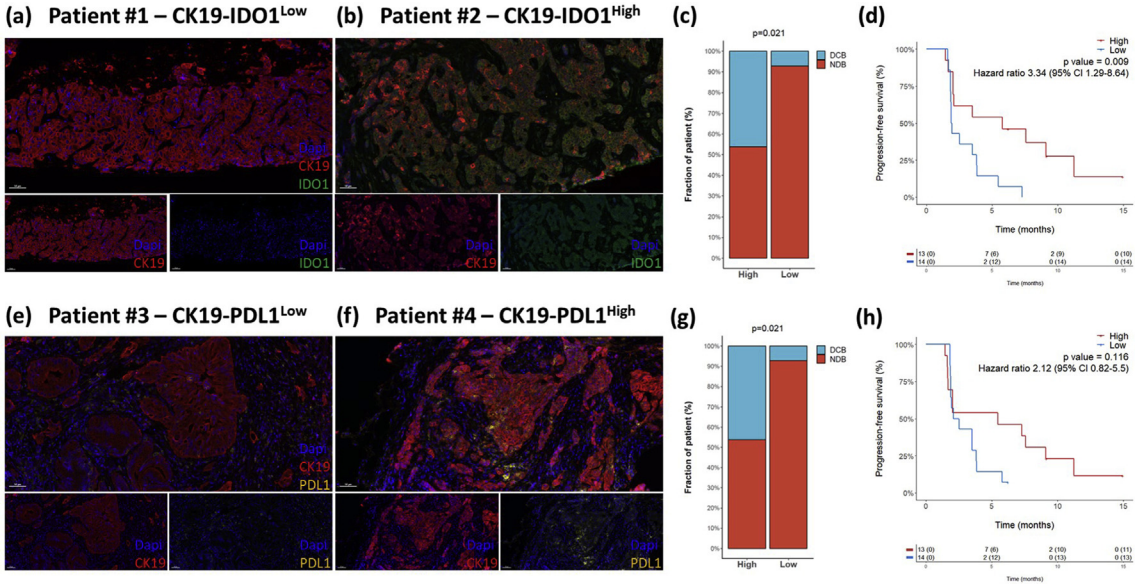

Fig. 4. **IDO1 and PD-L1 expressing tumours are associated with a favourable clinical outcome in patients with BTC treated with regorafenib combined with velumab.** Illustration of IDO1 and cytokeratin 19 (CK19) stainings in tumour samples – collected before treatment onset – from (**a**) patient #1 with negative/low IDO1 expression and a poor clinical outcome (PFS: 1,8 mo) and (**b**) patient#2 with high IDO1 expression and a favourable clinical outcome (PFS: 7,5 mo). (**c**) Rates of durable clinical benefit as per the expression level of IDO1 within CK19-positive tumour cells. (**d**) Kaplan–Meier curves of progression-free survival as per the expression level of IDO1 in CK19 positive cells; blue curve: low IDO1 expression and red curve: high IDO1 expression. Illustration of PD-L1 and cytokeratin 19 (CK19) stainings in tumour samples – collected before treatment onset – from (**e**) patient #3 with low tumour expression of PD-L1 and a poor clinical outcome (PFS: 3,8 months) and (**f**) patient#4 with high PD-L1 expression by tumour cells and a favourable clinical outcome (PFS: 14,9 months). (**g**) Rate of clinical benefit as per the expression level of PD-L1 within tumour cells. (**h**) Kaplan–Meier curves of progression-free survival as per the expression level of PD-L1 in tumour cells; blue curve: low PD-L1 expression and red curve: high PD-L1 expression. BTC, biliary tract cancer; IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival.

4. Discussion

BTCs are a heterogeneous group of adenocarcinomas for which next-generation sequencing has revealed several targetable alterations including FGFR fusion, isocitrate dehydrogenase (IDH) mutations, the human epidermal growth factor receptor family, DNA damage repair pathways and BRAF mutations with promising clinical results [12].

Although the first objective of our study was not reached, nearly 50% of patients experienced clinical benefit resulting in objective response (13.8%) or stable disease with tumour shrinkage (34.5%).

Several studies have shown that molecules such as VEGF and fibroblast growth factor that play a crucial role in tumour angiogenesis are upregulated in BTC [3,13]. Interestingly, preclinical experiments investigating antiangiogenic drugs such as bevacizumab [14], sorafenib [15], vandatenib [16] and axitinib [17] have shown that such agents can reduce both tumour growth and neoangiogenesis in xenograft models of BTC.

Regorafenib is the most-investigated antiangiogenic drug in patients with advanced BTC who have had progression despite standard therapy [4,18,19]. Demols et al. [4] reported the first double-blind, placebo-controlled clinical trial investigating regorafenib in this setting. A total of 66 patients with refractory BTC were randomised to receive regorafenib or a placebo. The study met its end-point by showing an improvement from 1.5 to 3 months (hazard ratio [HR], 0.49; 95%CI, 0.29–0.81; p = 0.005). Despite demonstrating some level of regorafenib activity in patients with refractory BTC, these studies showed that such patients have a very poor outcome, with a median PFS and OS ranging from 3 to 3.9 months and 5.3–7.9 months, respectively [4,18,19].

Monoclonal antibodies targeting PD-1 and PD-L1 represent one of the most important breakthroughs in cancer treatment for several tumour types. Studies that have evaluated these agents in patients with BTC report activity in a subset of patients, with response rates ranging from 3 to 11%, a median PFS of 1.4–3.7 months and a median OS of 5.2–14.2 months. Interestingly, when responses occurred, they were durable – all lasting more than six months [7,20].

Antiangiogenic agents can reduce immunosuppression. Conversely, immunotherapies can impact the vasculature and have antivascular effects; thus, immunotherapy and antiangiogenic drugs have the potential to create a virtuous cycle of immunostimulation and vascular remodelling within tumours [8,9]. This potential synergy has been shown in several preclinical models

and in the clinical setting, as illustrated by the recent approval of axitinib plus avelumab or pembrolizumab as a first-line treatment for advanced or metastatic renal cell carcinoma [21].

Little is known about the tumour microenvironment of BTC. Recent data have shown that the immunological profile of BTC is not dependent on the isocitrate déhydrogénase 1 (IDH1)/2 mutational status [22]. In iCCA, two subgroups have been defined based on transcriptomic analysis; one of which includes up to 40% of cases and is characterised by an immunological signature resulting from strong T lymphocyte infiltration and the activation of immune checkpoints [5]. Integrative molecular characterisation of eCCA has also revealed that only a minority of BTCs (11%) are characterised by an inflammation signature [6]. Interestingly, in our study, all but one patient with clinical benefit (objective response or stable disease) had an iCCA. Moreover, we found that eCCA had higher levels of TAM infiltration. Several lines of evidence suggest the important role of TAMs in cholangiocarcinoma progression, and their presence has been associated with poor outcomes [23].

In our cohort, patients with high PD-L1 expression were more likely to benefit from the treatment. These results agree with those of previous studies showing that high PD-L1 expression was associated with improved outcomes in patients with BTC treated with immune checkpoint inhibitors [24]. We also observed that high IDO1 expression was significantly associated with improved outcomes. IDO1 is a rate-limiting enzyme involved in the metabolism of the essential amino acid tryptophan and plays a crucial role in modulating the tumour microenvironment [25]. Our results are also in line with those of previous studies revealing that certain subsets of patients with solid tumours exhibiting IDO1 overexpression had favourable outcomes on anti-PD-1 therapy. A possible explanation is that IDO1 overexpression is, in this context, a surrogate of the presence of tumour-reactive T cells within the tumour microenvironment. For instance, Hamid, et al. [26] found that high IDO1 expression correlated with an improved efficacy of ipilimumab in patients with metastatic melanoma. Analyses of other patient cohorts, including patients with non-small cell lung cancer, have shown similar results [27].

Most of the patients required dose reductions of regorafenib owing to adverse events. A recent open-label trial that included 116 evaluable patients with metastatic colorectal cancer showed that a regorafenib dose-escalation strategy (starting dose of 80 mg/day with weekly escalation in 40 mg increments to 160 mg/day if no significant drug-related adverse events occurred) may represent an alternative approach for optimising regorafenib dosing with comparable activity and a lower incidence of adverse events compared with a standard dose strategy (160 mg/day for 21 days of a 28-day cycle) [28]. Such a strategy may be worth investigating when combining regorafenib with immune-oncology agents.

Although one limitation of our study is the limited sample size in relation to the heterogeneity of BTC, our results demonstrated clinical activity of the combined regorafenib and avelumab therapy in a subset of patients with advanced BTC. Further studies should investigate such a regimen in a population selected based on a favourable tumour microenvironment signature (high PD-L1 and/or high IDO1 expression).

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Author contribution

AI and CB contributed to the design of the study. SC, AI, CB, CC, SA, CA, AB, JPG and SMP contributed to the development of the methods. SC, TM, CGR, JPM, AA, IK, PGP, KB, MT, MK, CR and AI contributed to data collection. SC, CB, CC, AB, JPG and AI contributed to data analysis and interpretation. CC, CB and JPG contributed to statistical analysis. AI contributed to obtaining funding. All authors contributed to the writing of the article and approved the final version.

Conflict of interest statement

AB, CR and JPG: employees from Immusmol/Explicyte.

AI: research grants MSD, BMS, ROCHE and personal fees: Epizyme, Bayer, Lilly, Roche, Springworks; non-financial support: Merck.

All remaining authors have declared no conflicts of interest.

Appendix A. Supplementary data

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