

Targeted Therapies in Advanced BTCs

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Biliary tract cancers (BTCs) are a heterogeneous group of adenocarcinomas characterized by presentation with advanced disease and a poor prognosis. Chemotherapy is the mainstay of the current treatment that provides limited survival benefit, underscoring the need for novel therapeutic agents and strategies. Next-generation sequencing-based molecular profiling has shed light on the underpinnings of the complex pathophysiology of BTC and has uncovered numerous actionable targets, leading to the discovery of new therapies tailored to the molecular targets. Therapies targeting fibroblast growth factor receptor (FGFR) fusion, isocitrate dehydrogenase (IDH) mutations, the human epidermal growth factor receptor (HER) family, DNA damage repair (DDR) pathways, and *BRAF* mutations have produced early encouraging results in selected patients. Current clinical trials evaluating targeted therapies, as monotherapies and in combination with other agents, are paving the way for novel treatment options. Genomic profiling of cell-free circulating tumor DNA that can assist in the identification of an actionable target is another exciting area of development. The present article provides an overview of a precision medicine centered evolving paradigm of BTC treatment.

Keywords: Biliary tract cancer, Cholangiocarcinoma, ctDNA,

Biliary tract cancers (BTCs) are a heterogeneous group of adenocarcinomas that originate from the epithelial lining of the biliary tree. BTCs are characterized by presentation with advanced disease precluding curative surgery, rising global incidence, and a poor prognosis. Chemotherapy is the mainstay of the current treatment, which results in a median overall survival of less than one year, underscoring the need for novel therapeutic agents and strategies. Next-generation sequencing-based molecular profiling has shed light on the underpinnings of the complex pathophysiology of BTC and has uncovered numerous actionable targets, leading to the discovery of new therapies tailored to the molecular targets. Therapies targeting fibroblast growth factor receptor (FGFR) fusion, isocitrate dehydrogenase (IDH) mutations, the human epidermal growth factor receptor (HER) family, DNA damage repair (DDR) pathways, and *BRAF* mutations have produced early encouraging results in selected patients. Current clinical trials evaluating targeted therapies, as monotherapies and in combination with other agents, are paving the way for novel treatment options. Genomic profiling of cell-free circulating tumor DNA that can assist in the identification of an actionable target is another exciting area of development.

1. Introduction

Biliary tract cancers (BTCs) are a heterogeneous group of adenocarcinomas that originate in the epithelial lining of the bile ducts and gallbladder. Based on the anatomical location, BTCs are classified into two major categories—cholangiocarcinoma (CCA), which arises from the bile ducts, and gall bladder carcinoma (GBC). CCAs are subdivided into intrahepatic CCA (iCCA) and extrahepatic CCA (eCCA), with the eCCA further split into perihilar (Klatskin's tumor) and distal CCA ^[1]. Each of these anatomic subgroups have their unique natural history and treatment nuances ^[1].

BTCs, although relatively uncommon in the United States, with 23,000 new cases diagnosed annually ^[2], constitute approximately 3% of all gastrointestinal malignancies ^[3]. Advanced BTC has a dismal prognosis, with a five-year survival rate of less than 10% ^[4]. The majority of patients with BTC (>65%) present with unresectable disease, and patients who undergo potentially curative surgery experience a high rate of relapse ^{[5][6]}, underscoring the importance of systemic therapy. The current standard therapy of advanced BTC primarily consists of systemic chemotherapy, which results in a median overall survival (OS) of approximately 12 months ^[7]. However, targeted therapies are emerging as a promising treatment option, encouraged by the recent Food and Drug Administration (FDA) approval of pemigatinib for treatment of adults with chemotherapy-refractory CCA, i.e., advanced CCA harboring a fibroblast growth factor receptor (*FGFR*) 2 fusion.

As the understanding of the genomic landscape of BTC is growing, therapies targeting actionable alterations are being evaluated in numerous clinical trials, often combined with chemotherapy, both in frontline and chemotherapy-refractory settings [8]. Herein, we review the recent advancements and the emerging novel targeted therapies for BTC that will likely improve patient outcomes in the near future.

2. Targeted Therapies

Studies utilizing NGS to elucidate the molecular profile of BTC have highlighted that BTCs are target-rich malignancies with therapeutically relevant genetic alterations identified in approximately half of the patients [8][9], which has led to a plethora of clinical trials with targeted therapies. Although no randomized study reported to date has confirmed the superiority of the targeted approach in advanced BTC, the MOSCATO-01 trial provides early supportive evidence [10]. In this trial, patients who received targeted therapy (18 out of 43 patients) had an improved median OS compared to those treated with unselected therapies (median OS 17 vs. 5 months; $p = 0.008$).

Currently, the most promising targets in advanced BTCs consist of *IDH* mutations, *FGFR* aberrations, *BRAF* mutations, the DNA damage repair (DDR) pathway, and the *HER2* pathway. Table 1 summarizes the published study results of the targeted therapies in advanced BTC.

Table 1. Published study results of targeted therapies in patients with advanced biliary tract carcinoma (BTC).

Study Drug	<i>n</i>	Study Phase	Study Population	Pathway Targeted	ORR (%)	Median PFS (Months)	Median OS (Months)	Comments
Multiple targeted agents (MOSCATO-01) [10]	43	Prospective, molecular triage trial	Refractory BTC	-	33	5.2	17	18 of 43 patients received targeted agents resulting in improved median OS of 17 months vs. 5 months in patients who did not have an actionable target. Targetable alterations present: <i>IDH1/2</i> (18%), <i>FGFR1/2</i> (16%), <i>EGFR</i> , <i>ERBB2</i> or <i>ERBB3</i> (16%), <i>PTEN</i> (14%), <i>MDM2</i> (10%) and <i>PIK3CA</i> (10%).
Pemigatinib [11]	107	II (FIGHT-202)	Refractory iCCA	<i>FGFR</i>	35.5	6.9	21.1	Recently approved by FDA in the second-line setting
Infigratinib [12] (BGJ398)	61	II	Refractory iCCA	<i>FGFR</i>	14.8	5.8	-	Disease control rate was 75.4%
Futibatinib [13] (TAS-120)	45	I	Refractory iCCA including patients who failed other <i>FGFR</i> inhibitor	<i>FGFR</i>	25	-	-	Median duration of treatment 7.4 months

Futibatinib [14]	67	II (FOENIX-CCA2)	iCCA	FGFR	34.3	-	-	Median duration of response was 6.2 months (range, 2.1–14.2).
Derazantinib (ARQ 087) [15]	29	I/II	Refractory iCCA	FGFR	20.7	5.7	-	The disease control rate was 82.8%. OS not reached after a median follow up of 20 months
Erdafitinib [16]	17	II	Refractory iCCA	FGFR	47	5.6	-	In patients with <i>FRFR2/3</i> fusion: ORR 67%, median PFS 12.6 months
Debio 1347 [17]	9	I	Refractory solid tumors	FGFR	22	-	-	The median time on treatment was 24 weeks (range, 4–57 weeks)
Ivosidenib [18]	73	I	Refractory iCCA	IDH1	5	3.8	13.8	MTD was not reached; 500 mg daily was selected for expansion.
Ivosidenib (AG-120) [19] vs. placebo	185	III (ClarIDHy)	Refractory iCCA	IDH1	2.4	2.7 vs. 1.4, $p < 0.001$	10.8 vs. 9.7, $p = 0.06$	PFS rates at 6 and 12 months were 32.0% and 21.9% in ivosidenib arm. Grade 3 adverse events: 46% in ivosidenib arm vs. 36% with placebo.
Dabrafenib + Trametinib [20]	33	II (ROAR)	Refractory solid tumors including CCA	Combined <i>BRAF</i> + <i>MEK</i> inhibition	41	7.2	11.3	7 of 13 responders (54%) had a duration of response ≥ 6 months.
Neratinib [21]	20	II (SUMMIT)	Refractory BTC	<i>EGFR</i> , <i>HER2</i> , and <i>HER4</i>	10	1.8	-	Most common grade 3 or 4 adverse event was diarrhea (20%)
Regorafenib [22]	68	Randomized phase II (REACHIN)	Refractory BTC	VEGF	0	3 vs. 1.5, $p = 0.004$	5.3 vs. 5.1, $p = 0.21$	Median treatment duration is 10.9 weeks for regorafenib vs. 6.3 weeks for placebo ($p = 0.004$).

Varlitinib plus capecitabine vs. placebo + capecitabine [23]	127	Randomized phase II (TreeTopp)	Refractory BTC	EGFR, HER2, and HER4	9.4 vs. 4.8 ($p =$ 0.42)	2.8 vs. 2.8	7.8 vs. 7.5	Exploratory analyses suggested that female patients and patients with gall bladder cancer achieved comparatively higher median PFS with the study agent
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Abbreviations: ORR, overall response rate; PFS, progression-free survival; OS, overall survival; CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; BTC, biliary tract cancer.

2.1. FGFR Pathway

Targeting FGFR fusions, almost exclusively seen in iCCA, has shown clinically meaningful benefits in recent clinical trials. FGFR gene fusions are present in approximately 20% of patients with iCCAs. FGFRs consist of four transmembrane receptors (FGFR 1–4) with intracellular tyrosine kinase domains that regulate cell proliferation, migration, differentiation, and angiogenesis via binding to the ligands (fibroblast growth factors, FGFs) [24]. FGFR2 fusion is the most common FGFR aberration, which activates multiple oncogenic canonical signaling events downstream of FGFR [25]. FGFR2 fusions are therapeutically more relevant than FGFR mutations.

Among the *FGFR* tyrosine kinase inhibitors, Pemigatinib (INCB054828), Infigratinib (BGJ398), and Futibatinib (TAS-120) have shown encouraging results in phase I and II studies with manageable toxicity profiles (Table 1). These selective *FGFR* inhibitors are associated with an ORR of 15–35%, and a median PFS of around six months in previously treated patients with *FGFR2* fusions. Pemigatinib, a selective oral inhibitor of *FGFR1-3*, has been evaluated in a phase II study (FIGHT-202) that enrolled chemotherapy-refractory patients with a variety of *FGFR* alterations. This study reported an ORR of 35.5%, a median PFS of 6.9 months, and a preliminary OS of 21.1 months in patients with *FGFR2* fusions. This study result led to the FDA approval of pemigatinib for the treatment of chemotherapy-refractory advanced iCCA, the first approval of a targeted agent for advanced BTC. Of note, pemigatinib had no significant activity in patients with *FGFR* amplification or mutations (ORR 0% and a median PFS of 2.1 months). Adverse events were manageable, with hyperphosphatemia being the most common any-grade adverse event (60%). Grade ≥ 3 adverse events were reported in 64% of patients, which included hypophosphatemia (12%), arthralgia (6%), stomatitis (5%), hyponatremia (5%), abdominal pain (5%), and fatigue (5%). Serious adverse events occurred in 45% of patients, but no treatment-related deaths were reported. Hyperphosphatemia appears to be related to the alteration of vitamin D and phosphorus metabolism mediated via fibroblast growth factor 23 (FGF23) [26]. Currently, Pemigatinib, infigratinib, and futibatinib are being investigated in treatment-naïve patient cohorts in phase III trials against the gemcitabine and cisplatin combination (Table 2). ARQ087 (derazantinib), a nonselective multikinase inhibitor with activity on *FGFR*, has also recently entered phase III trial in pretreated patients after the publication of encouraging phase II data. The study results of other *FGFR* inhibitors, including Debio 1347 and Erdafitinib, are summarized in Table 1.

Table 2. Selected ongoing and upcoming clinical trials evaluating targeted therapies in patients with advanced biliary tract cancer *.

Clinical Trials	Targeted Agent	Study Phase	Signaling Pathway Targeted	Primary Endpoint	Study Identifier (ClinicalTrials.gov Identifier)
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First Line	Pemigatinib vs. gemcitabine+cisplatin	III	<i>FGFR</i>	PFS	NCT03656536 (FIGHT-302)
	Infigratinib vs. gemcitabine+cisplatin	III	<i>FGFR</i>	PFS	NCT03773302 (PROOF)
	Futibatinib vs. gemcitabine+cisplatin	III	<i>FGFR</i>	PFS	NCT04093362 (FOENIX)
	Varlitinib + gemcitabine + cisplatin	IB/II	EGFR, HER2, HER4	MTD, PFS	NCT02992340
	Copanlisib + gemcitabine + cisplatin	II	mTOR	PFS	NCT02631590
	Olaparib	II	DNA damage repair	ORR	NCT04042831

Second or Later Line	Derazantinib	II	<i>FGFR</i>	ORR, and PFS at 3 months	NCT03230318 (FIDES-01)
	BGJ398	II	<i>FGFR</i>	ORR	NCT02150967
	Futibatinib	II	<i>FGFR</i>	ORR	NCT02052778
	JNJ-42756493 (Erdafitinib)	II	<i>FGFR</i>	ORR	NCT02699606
	Ivosidenib or pemigatinib in combination with Gemcitabine and cisplatin	I	<i>FGFR</i> and <i>IDH1</i>	Tolerability	NCT04088188
	Rucaparib + nivolumab	II	<i>PARP</i> + PD-1	PFS rate at 4 months	NCT03639935
	Olaparib	II	<i>IDH</i> mutation associated 'BRCAness'	ORR	NCT03212274
	Niraparib	II	<i>BAP1</i> and DDR pathway	ORR	NCT03207347
	Entrectinib	II	<i>NTRK</i> , <i>ROS1</i> , or <i>ALK</i>	ORR	NCT02568267
	PLX8394	I/II	<i>BRAF</i>	Pharmacokinetics, ORR	NCT02428712
	Gemcitabine + selumetinib vs. gemcitabine	II	<i>MEK</i>	ORR	NCT02151084
	AG-881	I	<i>IDH</i>	Safety, MTD, RP2D	NCT02481154
	FT 2102	Ib/II	<i>IDH1</i>	MTD, RP2D, ORR	NCT03684811
	BAY1436032	I	<i>IDH1</i>	Safety, MTD, RP2D	NCT02746081
	Afatinib + capecitabine	I	<i>EGFR</i>	Safety, MTD, RP2D	NCT02451553
	Trastuzumab deruxtecan (DS-8201)	II	<i>HER2</i>	ORR	JMA-IIA00423 (HERB)
	KA2507	Ib/II	HDAC	MTD, PFS rate at 4 months	NCT04186156
	Fruquintinib	II	VEGFR	PFS	NCT04156958

Abbreviations: PFS, progression-free survival; MTD, maximal tolerated dose; ORR, overall response rate; RP2D, recommended phase II dose. * Information obtained from ClinicalTrials.gov (<https://clinicaltrials.gov/>), accessed between 17 May 2020, and 21 June 2020.

FGFR inhibitors are subject to a variety of resistance mechanisms, including polyclonal point mutation in the *FGFR2* kinase domain and the development of novel *FGFR2* fusions [27]. Futibatinib (TAS-120), a highly selective pan-*FGFR* inhibitor, has activity against *FGFR2* resistance mutations and has shown promising clinical activity in *FGFR*-aberrant iCCA patients in an early-phase study, including in patients progressing on prior *FGFR* inhibitors. The phase II FOENIX-101 study with futibatinib is currently recruiting patients with iCCA-harboring *FGFR2* gene rearrangements after progression on first-line treatment (Table 2).

2.2. Isocitrate Dehydrogenase (IDH) Mutations

Approximately 20% of patients with iCCA harbor *IDH1* or *IDH2* mutations (Table 1). *IDH1* mutations are more frequent than *IDH2* mutations and are generally found in iCCAs not related to hepatitis and fluke infection [28]. These somatic mutations cause an increase in *IDH1/2* activity resulting in modulation of cell metabolism and accumulation of 2-hydroxyglutarate (2-HG), an oncometabolite that interferes with normal cell differentiation and promotes tumorigenesis [29]. *IDH1* and *IDH2* mutations do not have any prognostic implications, unlike *FGFR* alterations [30].

Ivosidenib (AG-120), an oral *IDH1* inhibitor, has shown encouraging activity in patients with advanced *IDH1*-mutant, chemotherapy-refractory CCA (Table 1

). The phase I study with ivosidenib in refractory patients reported an ORR of 5%, a median PFS of 3.8 months, and a median OS of 13.8 months. Although the response rate was low, a significant proportion of patients were progression-free at 12 months (21.8%), and the tolerability of ivosidenib was remarkable—only 5% of patients experienced grade 3 or higher toxicities, and there were no dose-limiting toxicities. The most frequent side effects, including fatigue and nausea, were manageable. Subsequently, a phase III, placebo-controlled trial (ClarIDHy) was conducted in patients with *IDH1*-mutant advanced iCCA who progressed on at least one line of chemotherapy. The primary endpoint was PFS, and crossover from placebo to ivosidenib was permitted after progression. This study demonstrated a small median PFS improvement with ivosidenib over placebo, 2.7 months vs. 1.4 months (HR 0.37; 95% CI, 0.25–0.54; $p < 0.0001$). Although the short PFS improvement is a cause for concern, 32% of patients on the ivosidenib treatment arm had not progressed at six months, and 22% had not progressed at one year. Conversely, no patient on the placebo arm was progression-free at six months. The median OS in the ivosidenib arm was 10.8 months vs. 9.7 months in the placebo arm, which was not statistically significant. However, when adjusted for the crossover, the median OS in the placebo arm dropped to six months, and the OS difference was significant (HR 0.46; $p = 0.0008$). The toxicity profile was consistent with the previous report.

Given the modest activity of *IDH* inhibitors, novel strategies targeting *IDH* mutations are being explored in clinical trials. iCCA cell lines showed remarkable sensitivity to dasatinib, a multikinase inhibitor, in a preclinical study utilizing a high-throughput drug screening method, which was confirmed in a xenograft model [31]. Currently, dasatinib is being investigated in a phase II trial in patients of advanced iCCA harboring *IDH*-mutations (ClinicalTrials.gov Identifier: NCT02428855). Another preclinical study demonstrated that 2-HG produced as a result of *IDH* mutations suppresses homologous recombination and induces PARP (poly ADP ribose polymerase) inhibitor sensitivity ('BRCAness') [32], which led to a phase II trial with Olaparib in solid tumors, including CCA (ClinicalTrials.gov Identifier: NCT03212274). Furthermore, a phase I study is planned with a combination of ivosidenib and cisplatin/gemcitabine in patients with advanced CCA (ClinicalTrials.gov Identifier: NCT04088188).

2.3. Human Epidermal Growth Factor Receptor (HER) Pathway

The HER family receptors consist of four distinct receptors: epidermal growth factor receptor (EGFR) or HER1, HER-2, HER-3, and HER-4. In normal cells, binding of the ligands to the extracellular domain of these receptors leads to the dimerization of the receptors with eventual phosphorylation of the intracellular tyrosine kinase domain and activation of the downstream pathways, which include MAPK, PI3K/AKT/mTOR, and STAT pathways [33].

Therapies targeting HER2 have demonstrated modest activity thus far. Among the 5–15% of BTCs expressing HER2, most are gallbladder cancers or eCCA [34]. The combination of HER2 directed monoclonal antibodies pertuzumab and trastuzumab was investigated as a part of the MyPathway multi-basket study in 11 patients with refractory BTC [35]. At a median follow-up of 4.2 months, four patients had partial responses (PR), and three had stable disease (SD) for about four months. Neratinib, an oral tyrosine kinase inhibitor of *EGFR*, *HER2*, and *HER4*, was studied in *HER2*-mutant

advanced solid tumors in the phase II SUMMIT basket trial . An ORR of 10% was reported among the 20 patients with HER2-mutant BTC. Trastuzumab deruxtecan (DS-8201), an antibody-drug conjugate targeting HER2, is under evaluation in HER2-positive patients with advanced BTC in a clinical trial (Table 2).

EGFR (HER1) overexpression is common among among patients with CCA and is associated with poor prognosis, particularly in iCCA [36]. A phase III study investigated the activity of an EGFR inhibitor, erlotinib, plus chemotherapy (gemcitabine and oxaliplatin) versus chemotherapy alone in therapy-naïve patients with advanced BTC [37]. The combination of erlotinib and chemotherapy was associated with a significantly increased ORR (30% vs. 16%; $p = 0.005$), but an improvement in PFS or OS was not demonstrated. A post-hoc analysis of this study showed that the subgroup of patients with CCA had an improved median PFS with the combination therapy (5.9 months vs. 3.0 months; $p = 0.049$). Studies with a combination of chemotherapy and anti-EGFR antibodies cetuximab [38] and panitumumab [39] failed to show an improvement in ORR, PFS, or OS. Studies with other HER pathway inhibitors, including afatinib [40] and Lapatinib [41], also showed disappointing results. Conversely, varlitinib, a pan-HER inhibitor, demonstrated a somewhat encouraging result, with a partial response rate of 27%, stable disease of 43%, and a disease control rate of 70% in 37 patients with refractory BTC in a pooled analysis of three phase I studies [42]. Currently, varlitinib, in combination with gemcitabine and cisplatin, is being evaluated in patients with treatment-naïve advanced BTC (ClinicalTrials.gov Identifier: NCT02992340) (Table 4).

2.4. DNA Damage Repair (DDR) Mechanisms and BAP1 Mutations

Somatic or germline mutations in *BRCA* are detected in about 3.5% of patients with BTC and result in an immunogenic tumor profile characterized by a higher TMB and a higher rate of microsatellite instability (MSI)-high tumors [43]. Furthermore, *IDH* mutations confer sensitivity to PARP inhibitors, as discussed above. DDR pathway mutations or *IDH1* mutations were reported to be present in about half of patients with BTC [44]. Based on this rationale, a phase II study is underway to test the combination of rucaparib (a PARP inhibitor) and nivolumab (a PD-1 blocker) in a cohort of patients who progressed on chemotherapy (Table 2). Patients of CCA (mostly iCCA) with mutations in *BAP1*, a tumor suppressor gene involved in DNA double-strand break repair, are reported to have an aggressive disease and poor response to standard therapies [45]. A phase II basket trial is currently investigating the clinical benefit of the PARP inhibitor niraparib in patients with *BAP1* mutations and other DDR-deficient solid tumors, including CCA (Table 2).

2.5. RAS/RAF/MEK/ERK Pathway

Although *RAS* mutations serve as oncogenic drivers in many different types of malignancies, targeting *RAS* has mostly been unsuccessful because of its intricate interactions with other signaling pathways. Consequently, inhibiting targets downstream of *RAS*, such as *BRAF* and *MEK*, has been attempted, but with limited clinical success thus far. Selumetinib, a *MEK* inhibitor, in combination with cisplatin/gemcitabine has been studied in a phase Ib study (ABC-04) in therapy-naïve patients with advanced BTC [46]. Among eight evaluable patients, three had a partial response, and five had stable disease with a median PFS of 6.4 months. A phase II study with selumetinib, which included 39% of chemotherapy-refractory patients, showed an ORR of 12%, a median PFS of 3.7 months, and a median OS of 9.8 months with an acceptable safety profile [47].

BRAF mutations have been described in less than 5% of patients with BTC, primarily in the cohort with intrahepatic disease . Dual targeting with dabrafenib (*BRAF* inhibitor) and trametinib (*MEK* inhibitor) within the ROAR trial, a phase II basket trial of 178 patients harboring the *BRAF*^{V600E} mutations, which included 33 patients with advanced refractory CCA, showed encouraging efficacy with an ORR of 41%, a median PFS of 7.2 months, and a median OS of 11.3 months . PLX8394, a *BRAF* inhibitor, is currently being evaluated in patients with refractory solid tumors, including BTC (ClinicalTrials.gov Identifier: NCT02428712).

2.6. PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway regulates cellular proliferation and angiogenesis through its interactions with the RAS/RAF/MEK pathway and the mTOR signaling pathway [48] (Figure 1). Mutations in *PIK3CA*, resulting in upregulation of the PI3K/AKT/mTOR pathway, have been identified in many cancer types, including in BTC [49]. Everolimus, an mTOR inhibitor, has shown modest activity in patients with previously treated BTC in a phase II trial, which reported a median PFS of 3.2 months and a median OS of 7.7 months [50]. A phase II study with everolimus in the first-line setting demonstrated a PFS of 5.5 months and OS of 9.5 months [51]. A combination of cisplatin/gemcitabine and copanlisib, a pan-PI3K inhibitor, has shown modest activity in a phase I study with an ORR of 17.4% [52]. A phase II study with this combination in the first-line setting is planned (Table 2).

2.7. Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) overexpression in BTC is a poor prognostic factor, particularly in iCCA [66]. However, targeting VEGF has not been a successful strategy so far. A meta-analysis of seven randomized trials involving 964 patients reported a lack of PFS or OS benefit with anti-VEGF therapy in combination with chemotherapy compared to chemotherapy alone [53]. Cediranib, a potent VEGF receptor tyrosine kinase inhibitor, did not show a survival benefit in therapy-naïve patients when combined with gemcitabine/cisplatin in comparison to gemcitabine/cisplatin plus placebo in the randomized phase II ABC-03 trial [54]. Studies with other multikinase VEGF receptor inhibitors, including sorafenib [55], lenvatinib [56], vandetinib [57], and regorafenib [58], have also been disappointing. Alternative strategies, for example, a combination of VEGF inhibition (lenvatinib) and immune checkpoint inhibition (pembrolizumab), have reported encouraging results (detailed in Section 5.9).

2.8. Miscellaneous Pathways

NTRK (neurotropic tyrosine kinase receptor) fusions, identified in 3.5% of patients with iCCA [59], are targetable with currently approved first-generation TRK (tropomyosin receptor kinase) inhibitors larotrectinib and entrectinib, which have produced an impressive ORR of 57% to 75% in advanced solid tumors harboring *NTRK* fusions [60][61]. Of note, TRK inhibitors have activity against *ROS1* and *ALK* fusions, which are reported to be present in 0–8.7% and 2.7% of patients with CCA, respectively [62].

c-MET regulates cell proliferation, migration, and invasion [63]. A phase II study with cabozantinib, an oral *MET* inhibitor, reported a median PFS of 1.8 months and a median OS of 5.2 months in patients with previously-treated advanced CCA [64]. A phase I study of an oral *c-MET* inhibitor, tivantinib, in combination with gemcitabine, demonstrated a modest activity with an ORR of 19% [65]. Merestinib, a small molecule inhibitor of *MET*, did not improve ORR, PFS, or OS when added to gemcitabine and cisplatin in a recently reported randomized phase II trial [66].

2.9. Targeted Therapy and Immunotherapy Combinations

Although a detailed account of immunotherapy in BTC is beyond the scope of this article, a discussion of novel immunotherapy/targeted therapy combinations is relevant. The modest activity of single-agent immunotherapeutic agents in advanced BTC, reporting an ORR of 5%–20% [67], led to the exploration of a variety of combination strategies, including immunotherapy/targeted therapy combinations. The combination of pembrolizumab (a PD-1 inhibitor) and ramucirumab (a VEGF receptor 2 inhibitor) investigated in a phase I trial in patients with previously treated advanced BTC reported a modest median PFS and median OS of 1.6 and 6.4 months, respectively [68]. A promising result has been reported with the combination of a multikinase inhibitor, lenvatinib, and pembrolizumab in 32 patients who had received at least two prior anticancer therapies [69]. This study reported an ORR of 25%, a median PFS of 4.9 months, and a median OS of 11 months. A multicenter randomized phase II trial ($n = 86$) of atezolizumab (a PD-L1 blocker) as monotherapy or in combination with cobimetinib (a MEK inhibitor) in refractory advanced BTC has recently reported superior median PFS with the atezolizumab/cobimetinib combination—3.65 months vs. 1.87 months ($p = 0.027$); OS data are not mature at this time [70]. A combination of PD-1 inhibitor and DNA repair modulators, as described in section 5.4, is another area of exploration. These early results suggest that the immunotherapy/targeted therapy combinations will likely be a new frontier for further exploration.

References

1. Razumilava, N.; Gores, G.J. Classification, diagnosis, and management of cholangiocarcinoma. *Clin. Gastroenterol. Hepatol.* 2013, 11, e13–e14.
2. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2020. *CA A Cancer J. Clin.* 2020, 70, 7–30.
3. Charbel, H.; Al-Kawas, F.H. Cholangiocarcinoma: Epidemiology, risk factors, pathogenesis, and diagnosis. *Curr. Gastroenterol. Rep.* 2011, 13, 182–187.
4. Banales, J.M.; Cardinale, V.; Carpino, G.; Marzioni, M.; Andersen, J.B.; Invernizzi, P.; Lind, G.E.; Folseraas, T.; Forbes, S.J.; Fouassier, L.; et al. Cholangiocarcinoma: Current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat. Rev. Gastroenterol. Hepatol.* 2016, 13, 261–280.
5. Valle, J.W. Advances in the treatment of metastatic or unresectable biliary tract cancer. *Ann. Oncol.* 2010, 21 (Suppl. 7), vii345–vii348.

6. Wang, Y.; Li, J.; Xia, Y.; Gong, R.; Wang, K.; Yan, Z.; Wan, X.; Liu, G.; Wu, D.; Shi, L.; et al. Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy. *J. Clin. Oncol.* 2013, 31, 1188–1195.
7. Valle, J.; Wasan, H.; Palmer, D.H.; Cunningham, D.; Anthoney, A.; Maraveyas, A.; Madhusudan, S.; Iveson, T.; Hughes, S.; Pereira, S.P.; et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N. Engl. J. Med.* 2010, 362, 1273–1281.
8. Lowery, M.A.; Ptashkin, R.N.; Jordan, E.J.; Berger, M.F.; Zehir, A.; Capanu, M.; Kemeny, N.E.; O'Reilly, E.M.; El Dika, I.; Jarnagin, W.R.; et al. Comprehensive molecular profiling of intra- and extrahepatic cholangiocarcinomas: Potential targets for intervention. *Clin. Cancer Res.* 2018, 24, 4154–4161.
9. Nakamura, H.; Arai, Y.; Totoki, Y.; Shirota, T.; Elzawahry, A.; Kato, M.; Hama, N.; Hosoda, F.; Urushidate, T.; Ohashi, S.; et al. Genomic spectra of biliary tract cancer. *Nat. Genet.* 2015, 47, 1003–1010.
10. Verlingue, L.; Malka, D.; Allorant, A.; Massard, C.; Ferte, C.; Lacroix, L.; Rouleau, E.; Auger, N.; Ngo, M.; Nicotra, C.; et al. Precision medicine for patients with advanced biliary tract cancers: An effective strategy within the prospective MOSCATO-01 trial. *Eur. J. Cancer* 2017, 87, 122–130.
11. Abou-Alfa, G.K.; Sahai, V.; Hollebecque, A.; Vaccaro, G.; Melisi, D.; Al-Rajabi, R.; Paulson, A.S.; Borad, M.J.; Gallinson, D.; Murphy, A.G.; et al. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: A multicentre, open-label, phase 2 study. *Lancet. Oncol.* 2020, 21, 671–684.
12. Javle, M.; Lowery, M.; Shroff, R.T.; Weiss, K.H.; Springfield, C.; Borad, M.J.; Ramanathan, R.K.; Goyal, L.; Sadeghi, S.; Macarulla, T.; et al. Phase II Study of BGJ398 in Patients With FGFR-Altered Advanced Cholangiocarcinoma. *J. Clin. Oncol.* 2018, 36, 276–282.
13. Meric-Bernstam, F.; Arkenau, H.; Tran, B.; Bahleda, R.; Kelley, R.K.; Hierro, C.; Ahn, D.; Zhu, A.; Javle, M.; Winkler, R.; et al. Efficacy of TAS-120, an irreversible fibroblast growth factor receptor (FGFR) inhibitor, in cholangiocarcinoma patients with FGFR pathway alterations who were previously treated with chemotherapy and other FGFR inhibitors. *Ann. Oncol.* 2018, 29 (Suppl. 9), ix46–ix66.
14. Goyal, L.; Meric-Bernstam, F.; Hollebecque, A.; Valle, J.; Morizane, C.; Karasic, T. FOENIX-CCA2: A phase II, open-label, multicenter study of futibatinib in patients (pts) with intrahepatic cholangiocarcinoma (iCCA) harboring FGFR2 gene fusions or other rearrangements. *J. Clin. Oncol.* 2020, 38 (Suppl. 15), 108.
15. Mazzaferro, V.; El-Rayes, B.F.; Droz dit Busset, M.; Cotsoglou, C.; Harris, W.P.; Damjanov, N.; Masi, G.; Rimassa, L.; Personeni, N.; Braitheh, F.; et al. Derazantinib (ARQ 087) in advanced or inoperable FGFR2 gene fusion-positive intrahepatic cholangiocarcinoma. *Br. J. Cancer* 2019, 120, 165–171.
16. Park, J.O.; Feng, Y.-H.; Chen, Y.-Y.; Su, W.-C.; Oh, D.-Y.; Shen, L.; Kim, K.-P.; Liu, X.; Bai, Y.; Liao, H.; et al. Updated results of a phase IIa study to evaluate the clinical efficacy and safety of erdafitinib in Asian advanced cholangiocarcinoma (CCA) patients with FGFR alterations. *J. Clin. Oncol.* 2019, 37, 4117.
17. Ng, M.C.H.; Goyal, L.; Bang, Y.-J.; Oh, D.-Y.; Chao, T.-Y.; Cleary, J.M.; Voss, M.H.; Meric-Bernstam, F.; Iyer, G.; Heist, R.S.; et al. AB065. P-36. Debio 1347 in patients with cholangiocarcinoma harboring an FGFR gene alteration: Preliminary results. *Hepatobiliary Surg. Nutr. (3rd Asia-Pacific Cholangiocarcinoma Conference)* 2019, 8 (Suppl. 1), AB065.
18. Lowery, M.A.; Burris, H.A., 3rd; Janku, F.; Shroff, R.T.; Cleary, J.M.; Azad, N.S.; Goyal, L.; Maher, E.A.; Gore, L.; Hollebecque, A.; et al. Safety and activity of ivosidenib in patients with IDH1-mutant advanced cholangiocarcinoma: A phase 1 study. *Lancet Gastroenterol. Hepatol.* 2019, 4, 711–720.
19. Abou-Alfa, G.K.; Macarulla, T.; Javle, M.M.; Kelley, R.K.; Lubner, S.J.; Adeva, J.; Cleary, J.M.; Catenacci, D.V.; Borad, M.J.; Bridgewater, J.; et al. Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): A multicentre, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet. Oncol.* 2020, 21, 796–807.
20. Wainberg, Z.A.; Lassen, U.N.; Elez, E.; Italiano, A.; Curigliano, G.; De Braud, F.G.; Prager, G.; Greil, R.; Stein, A.; Fasola, A.; et al. Efficacy and safety of dabrafenib (D) and trametinib (T) in patients (pts) with BRAF V600E-mutated biliary tract cancer (BTC): A cohort of the ROAR basket trial. *J. Clin. Oncol.* 2019, 37, 187.
21. Harding, J.J.; Cleary, J.; Shapiro, G.; Braña, I.; Moreno, V.; Quinn, D.; Borad, M.; Loi, S.; Spanggaard, I.; Stemmer, S.M.; et al. Treating HER2-mutant advanced biliary tract cancer with neratinib: Benefits of HER2-directed targeted therapy in the phase 2 SUMMIT “basket” trial. In *Proceedings of the ESMO World Congress on Gastrointestinal Cancer, Barcelona, Spain, 3–6 July 2019*. Abstract Number O-005.
22. Demols, A.; Borbath, I.; Eynde, M.V.D.; Houbiers, G.; Peeters, M.; Maréchal, R.; Delaunoit, T.; Goeminne, J.C.; Laurent, S.; Holbrechts, S.; et al. Regorafenib after failure of gemcitabine and platinum-based chemotherapy for locally advanced (nonresectable) and metastatic biliary tumors: A randomized double-blinded placebo-controlled phase II trial. *J. Clin. Oncol.* 2019, 37, 345.
23. Javle, M.; Oh, D.-Y.; Ikeda, M.; Yong, W.; McIntyre, N.; Lindmark, B.; McHale, M. Results from TreeTopp: A randomized phase II study of the efficacy and safety of varlitinib plus capecitabine versus placebo in second-line (2L) advanced or m

etastatic biliary tract cancer (BTC). *J. Clin. Oncol.* 2020, 38, 4597.

24. Touat, M.; Ileana, E.; Postel-Vinay, S.; André, F.; Soria, J.C. Targeting FGFR Signaling in Cancer. *Clin. Cancer Res.* 2015, 21, 2684–2694.
25. Helsten, T.; Elkin, S.; Arthur, E.; Tomson, B.N.; Carter, J.; Kurzrock, R. The FGFR Landscape in Cancer: Analysis of 4,853 Tumors by Next-Generation Sequencing. *Clin. Cancer Res.* 2016, 22, 259–267.
26. Perwad, F.; Zhang, M.Y.; Tenenhouse, H.S.; Portale, A.A. Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxyvitamin D-1 α -hydroxylase expression in vitro. *Am. J. Physiol. Ren. Physiol.* 2007, 293, F1577–F1583.
27. Young Kim, S.; Ahn, T.; Bang, H.; Soo Ham, J.; Kim, J.; Tae Kim, S.; Jang, J.; Shim, M.; Young Kang, S.; Hoon Park, S.; et al. Acquired resistance to LY2874455 in FGFR2 -amplified gastric cancer through an emergence of novel FGFR2-ACSL5 fusion. *Oncotarget* 2017, 8, 15014.
28. Chan-on, W.; Nairismägi, M.-L.; Ong, C.K.; Lim, W.K.; Dima, S.; Pairojkul, C.; Lim, K.H.; McPherson, J.R.; Cutcutache, I.; Heng, H.L.; et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. *Nat. Genet.* 2013, 45, 1474–1478.
29. Dang, L.; Yen, K.; Attar, E.C. IDH mutations in cancer and progress toward development of targeted therapeutics. *Ann. Oncol.* 2016, 27, 599–608.
30. Boscoe, A.N.; Rolland, C.; Kelley, R.K. Frequency and prognostic significance of isocitrate dehydrogenase 1 mutations in cholangiocarcinoma: A systematic literature review. *J. Gastrointest. Oncol.* 2019, 10, 751–765.
31. Saha, S.K.; Gordan, J.D.; Kleinstiver, B.P.; Vu, P.; Najem, M.S.; Yeo, J.-C.; Shi, L.; Kato, Y.; Levin, R.S.; Webber, J.T.; et al. Isocitrate Dehydrogenase Mutations Confer Dasatinib Hypersensitivity and SRC Dependence in Intrahepatic Cholangiocarcinoma. *Cancer Discov.* 2016, 6, 727–739.
32. Sulkowski, P.L.; Corso, C.D.; Robinson, N.D.; Scanlon, S.E.; Purshouse, K.R.; Bai, H.; Liu, Y.; Sundaram, R.K.; Hegan, D.C.; Fons, N.R.; et al. 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. *Sci. Transl. Med.* 2017, 9, eaal2463.
33. Yarden, Y. The EGFR family and its ligands in human cancer: Signalling mechanisms and therapeutic opportunities. *Eur. J. Cancer* 2001, 37 (Suppl. 4), 3–8.
34. Yang, X.; Wang, W.; Wang, C.; Wang, L.; Yang, M.; Qi, M.; Su, H.; Sun, X.; Liu, Z.; Zhang, J.; et al. Characterization of EGFR family gene aberrations in cholangiocarcinoma. *Oncol. Rep.* 2014, 32, 700–708.
35. Javle, M.M.; Hainsworth, J.D.; Swanton, C.; Burris, H.A.; Kurzrock, R.; Sweeney, C.; Meric-Bernstam, F.; Spigel, D.R.; Bose, R.; Guo, S.; et al. Pertuzumab + trastuzumab for HER2-positive metastatic biliary cancer: Preliminary data from MyPathway. *J. Clin. Oncol.* 2017, 35, 402.
36. Yoshikawa, D.; Ojima, H.; Iwasaki, M.; Hiraoka, N.; Kosuge, T.; Kasai, S.; Hirohashi, S.; Shibata, T. Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. *Br. J. Cancer* 2008, 98, 418–425.
37. Lee, J.; Park, S.H.; Chang, H.-M.; Kim, J.S.; Choi, H.J.; Lee, M.A.; Chang, J.S.; Jeung, H.C.; Kang, J.H.; Lee, H.W.; et al. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: A multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* 2012, 13, 181–188.
38. Malka, D.; Cervera, P.; Foulon, S.; Trarbach, T.; De la Fouchardière, C.; Boucher, E.; Fartoux, L.; Faivre, S.; Blanc, J.F.; Viret, F.; et al. Gemcitabine and oxaliplatin with or without cetuximab in advanced biliary-tract cancer (BINGO): A randomised, open-label, non-comparative phase 2 trial. *Lancet. Oncol.* 2014, 15, 819–828.
39. Leone, F.; Marino, D.; Cereda, S.; Filippi, R.; Belli, C.; Spadi, R.; Nasti, G.; Montano, M.; Amatu, A.; Aprile, G.; et al. Panitumumab in combination with gemcitabine and oxaliplatin does not prolong survival in wild-type KRAS advanced biliary tract cancer: A randomized phase 2 trial (Vecti-BIL study). *Cancer* 2016, 122, 574–581.
40. Moehler, M.; Maderer, A.; Ehrlich, A.; Foerster, F.; Schad, A.; Nickolay, T.; Ruckes, C.; Weinmann, A.; Sivanathan, V.; Marquardt, J.U.; et al. Safety and efficacy of afatinib as add-on to standard therapy of gemcitabine/cisplatin in chemotherapy-naïve patients with advanced biliary tract cancer: An open-label, phase I trial with an extensive biomarker program. *BMC Cancer* 2019, 19, 55.
41. Peck, J.; Wei, L.; Zalupski, M.; O’Neil, B.; Villalona Calero, M.; Bekaii-Saab, T. HER2/neu may not be an interesting target in biliary cancers: Results of an early phase II study with lapatinib. *Oncology* 2012, 82, 175–179.
42. Tan, A.C.; Oh, D.-Y.; Chao, Y.; Hsieh, C.-Y.; Chang, W.-L.; Isanto, F.; Chen, Y.-C.; McHale, M.; Lindmark, B.; Ng, M.C. Efficacy and safety of varlitinib, a reversible pan-HER tyrosine kinase inhibitor, in combination with platinum-based regimens in biliary tract cancers: A pooled analysis from three phase I studies. *J. Clin. Oncol.* 2019, 37, 331.

43. Spizzo, G.; Puccini, A.; Xiu, J.; Goldberg, R.M.; Grothey, A.; Shields, A.F.; Arora, S.P.; Khushman, M.d.M.; Salem, M.E.; Battaglin, F.; et al. Frequency of BRCA mutation in biliary tract cancer and its correlation with tumor mutational burden (TMB) and microsatellite instability (MSI). *J. Clin. Oncol.* 2019, 37, 4085.
44. Sahai, V.; Tran, N.H.; Griffith, K.A.; Zalupski, M. A multicenter phase II trial of rucaparib in combination with nivolumab as maintenance therapy for patients with advanced biliary tract cancer. *J. Clin. Oncol.* 2019, 37, TPS4153.
45. Al-Shamsi, H.O.; Anand, D.; Shroff, R.T.; Jain, A.; Zuo, M.; Conrad, C.; Vauthey, J.-N.; Javle, M.M. BRCA-associated protein 1 mutant cholangiocarcinoma: An aggressive disease subtype. *J. Gastrointest. Oncol.* 2016, 7, 556–561.
46. Bridgewater, J.; Lopes, A.; Beare, S.; Duggan, M.; Lee, D.; Ricamara, M.; McEntee, D.; Sukumaran, A.; Wasan, H.; Valle, J.W. A phase 1b study of Selumetinib in combination with Cisplatin and Gemcitabine in advanced or metastatic biliary tract cancer: The ABC-04 study. *BMC Cancer* 2016, 16, 153.
47. Bekaii-Saab, T.; Phelps, M.A.; Li, X.; Saji, M.; Goff, L.; Kauh, J.S.; O'Neil, B.H.; Balsom, S.; Balint, C.; Lierseemann, R.; et al. Multi-institutional phase II study of selumetinib in patients with metastatic biliary cancers. *J. Clin. Oncol.* 2011, 29, 2357–2363.
48. Wu, C.E.; Chen, M.H.; Yeh, C.N. mTOR Inhibitors in Advanced Biliary Tract Cancers. *Int. J. Mol. Sci.* 2019, 20, 500.
49. Deshpande, V.; Nduaguba, A.; Zimmerman, S.M.; Kehoe, S.M.; MacConaill, L.E.; Lauwers, G.Y.; Ferrone, C.; Bardeesy, N.; Zhu, A.X.; Hezel, A.F. Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. *BMC Cancer* 2011, 11, 60.
50. Buzzoni, R.; Pusceddu, S.; Platania, M.; Barone, G.; Valente, M.; Guardo, L.D.; Bajetta, E. Efficacy and safety of RAD001 in advanced biliary tract cancer (BTC) patients (pts) progressing after first-line chemotherapy: A phase II study. *J. Clin. Oncol.* 2010, 28, e14500.
51. Lau, D.K.; Tay, R.Y.; Yeung, Y.H.; Chionh, F.; Mooi, J.; Murone, C.; Skrinos, E.; Price, T.J.; Mariadason, J.M.; Tebbutt, N.C. Phase II study of everolimus (RAD001) monotherapy as first-line treatment in advanced biliary tract cancer with biomarker exploration: The RADiChol Study. *Br. J. Cancer* 2018, 118, 966–971.
52. Kim, R.D.; Alberts, S.R.; Pena, C.; Genvresse, I.; Ajavon-Hartmann, A.; Xia, C.; Kelly, A.; Grilley-Olson, J.E. Phase I dose-escalation study of copanlisib in combination with gemcitabine or cisplatin plus gemcitabine in patients with advanced cancer. *Br. J. Cancer* 2018, 118, 462–470.
53. Zhuang, X.; Xiao, Y.P.; Tan, L.H.; Wang, L.T.; Cao, Q.; Qu, G.F.; Xiao, S.; Duan, H.X. Efficacy and safety of chemotherapy with or without targeted therapy in biliary tract cancer: A meta-analysis of 7 randomized controlled trials. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 2017, 37, 172–178.
54. Valle, J.W.; Wasan, H.; Lopes, A.; Backen, A.C.; Palmer, D.H.; Morris, K.; Duggan, M.; Cunningham, D.; Anthoney, D. A.; Corrie, P.; et al. Cediranib or placebo in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer (ABC-03): A randomised phase 2 trial. *Lancet Oncol.* 2015, 16, 967–978.
55. Bengala, C.; Bertolini, F.; Malavasi, N.; Boni, C.; Aitini, E.; Dealis, C.; Zironi, S.; Depenni, R.; Fontana, A.; Del Giovane, C.; et al. Sorafenib in patients with advanced biliary tract carcinoma: A phase II trial. *Brit. J. Cancer* 2010, 102, 68–72.
56. Ikeda, M.; Sasaki, T.; Morizane, C.; Mizuno, N.; Nagashima, F.; Shimizu, S.; Hayata, N.; Ikezawa, H.; Suzuki, T.; Nakajima, R.; et al. 722P—A phase 2 study of lenvatinib monotherapy as second-line treatment in unresectable biliary tract cancer: Primary analysis results. *Ann. Oncol.* 2017, 28, v246.
57. Santoro, A.; Gebbia, V.; Pressiani, T.; Testa, A.; Personeni, N.; Arrivas Bajardi, E.; Foa, P.; Buonadonna, A.; Bencardino, K.; Barone, C.; et al. A randomized, multicenter, phase II study of vandetanib monotherapy versus vandetanib in combination with gemcitabine versus gemcitabine plus placebo in subjects with advanced biliary tract cancer: The VanGogh study. *Ann. Oncol.* 2015, 26, 542–547.
58. Sun, W.; Patel, A.; Normolle, D.; Patel, K.; Ohr, J.; Lee, J.J.; Bahary, N.; Chu, E.; Streeter, N.; Drummond, S. A phase 2 trial of regorafenib as a single agent in patients with chemotherapy-refractory, advanced, and metastatic biliary tract adenocarcinoma. *Cancer* 2019, 125, 902–909.
59. Ross, J.S.; Wang, K.; Gay, L.; Al-Rohil, R.; Rand, J.V.; Jones, D.M.; Lee, H.J.; Sheehan, C.E.; Otto, G.A.; Palmer, G.; et al. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. *Oncologist* 2014, 19, 235–242.
60. Doebele, R.C.; Drilon, A.; Paz-Ares, L.; Siena, S.; Shaw, A.T.; Farago, A.F.; Blakely, C.M.; Seto, T.; Cho, B.C.; Tosi, D.; et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: Integrated analysis of three phase 1-2 trials. *Lancet Oncol.* 2020, 21, 271–282.
61. Drilon, A.; Laetsch, T.W.; Kummar, S.; DuBois, S.G.; Lassen, U.N.; Demetri, G.D.; Nathanson, M.; Doebele, R.C.; Farago, A.F.; Pappo, A.S.; et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N. Engl. J. Med.* 2018, 378, 731–739.

62. Gu, T.L.; Deng, X.; Huang, F.; Tucker, M.; Crosby, K.; Rimkunas, V.; Wang, Y.; Deng, G.; Zhu, L.; Tan, Z.; et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS ONE* 2011, 6, e15640.
63. Peruzzi, B.; Bottaro, D.P. Targeting the c-Met signaling pathway in cancer. *Clin. Cancer Res.* 2006, 12, 3657–3660.
64. Goyal, L.; Zheng, H.; Yurgelun, M.B.; Abrams, T.A.; Allen, J.N.; Cleary, J.M.; Knowles, M.; Regan, E.; Reardon, A.; Khatryan, A.; et al. A phase 2 and biomarker study of cabozantinib in patients with advanced cholangiocarcinoma. *Cancer* 2017, 123, 1979–1988.
65. Pant, S.; Saleh, M.; Bendell, J.; Infante, J.R.; Jones, S.; Kurkjian, C.D.; Moore, K.M.; Kazakin, J.; Abbadessa, G.; Wang, Y.; et al. A phase I dose escalation study of oral c-MET inhibitor tivantinib (ARQ 197) in combination with gemcitabine in patients with solid tumors. *Ann. Oncol.* 2014, 25, 1416–1421.
66. Valle, J.W.; Bai, L.-Y.; Orlova, R.; Cutsem, E.V.; Alfonso, J.A.; Chen, L.-T.; Obermannova, R.; Ettrich, T.J.; Chen, J.-S.; Wasan, H.S.; et al. Ramucirumab (RAM) or merestinib (MER) or placebo (PL) plus gemcitabine (GEM) and cisplatin (CIS) as first-line treatment for advanced or metastatic biliary tract cancer (BTC): A randomized, double-blind, phase II study. *J. Clin. Oncol.* 2020, 38, 477.
67. Jakubowski, C.D.; Azad, N.S. Immune checkpoint inhibitor therapy in biliary tract cancer (cholangiocarcinoma). *Chin. Clin. Oncol.* 2020, 9, 2.
68. Arkenau, H.T.; Martin-Liberal, J.; Calvo, E.; Penel, N.; Krebs, M.G.; Herbst, R.S.; Walgren, R.A.; Widau, R.C.; Mi, G.; Jin, J.; et al. Ramucirumab Plus Pembrolizumab in Patients with Previously Treated Advanced or Metastatic Biliary Tract Cancer: Nonrandomized, Open-Label, Phase I Trial (JVDF). *Oncologist* 2018, 23, 1407–e136.
69. Lin, J.; Yang, X.; Long, J.; Zhao, S.; Mao, J.; Wang, D. Pembrolizumab combined with lenvatinib as non-first-line therapy in patients with refractory biliary tract carcinoma. *Hepatobiliary Surg. Nutr.* 2020.
70. Yarchoan, M.; Cope, L.; Anders, R.A.; Noonan, A.; Goff, L.W.; Goyal, L. CT043—A multicenter randomized phase 2 trial of atezolizumab as monotherapy or in combination with cobimetinib in biliary tract cancers (BTCs): A NCI Experimental Therapeutics Clinical Trials Network (ETCTN) study. In Proceedings of the AACR Virtual Annual Meeting, Los Angeles, CA, USA, 28 April 2020