Review

The landscape of targeted therapies for cholangiocarcinoma: current status and emerging targets

Dawn Q. Chong[1,2] **and Andrew X. Zhu**[1]

[1] Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA

[2] Division of Medical Oncology, National Cancer Centre Singapore, Singapore

Correspondence to: *Andrew X. Zhu,* ***email***: *azhu@partners.org*

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ABSTRACT

Cholangiocarcinoma (CCA) is a relatively rare malignancy that arises from the epithelial cells of the intrahepatic, perihilar and distal biliary tree. Intrahepatic CCA (ICC) represents the second most common primary liver cancer, after hepatocellular cancer. Two-thirds of the patients with ICC present with locally advanced or metastatic disease. Despite standard treatment with gemcitabine and cisplatin, prognosis remains dismal with a median survival of less than one year. Several biological plausibilities can account for its poor clinical outcomes. First, despite the advent of next generation and whole exome sequencing, no oncogenic addiction loops have been validated as clinically actionable targets. Second, the anatomical, pathological and molecular heterogeneity, and rarity of CCA confer an ongoing challenge of instituting adequately powered clinical trials. Last, most of the studies were not biomarker-driven, which may undermine the potential benefit of targeted therapy in distinct subpopulations carrying the unique molecular signature. Recent whole genome sequencing efforts have identified known mutations in genes such as epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), v-raf murine sarcoma viral oncogene homolog (*BRAF*) and tumor protein p53 (*TP53*), novel mutations in isocitrate dehydrogenase (*IDH*), BRCA1-Associated Protein 1 (*BAP1*) and AT-rich interactive domain-containing protein 1A (*ARID1A*), and novel fusions such as fibroblast growth factor receptor 2 (*FGFR2*) and ROS proto-oncogene 1 (*ROS1*). In this review, we will discuss the evolving genetic landscape of CCA, with an in depth focus on novel fusions (e.g. *FGFR2* and *ROS1*) and somatic mutations (e.g. *IDH1/2*), which are promising actionable molecular targets.

INTRODUCTION

Cholangiocarcinoma (CCA) comprises of malignancy arising from the intrahepatic, perihilar and distal biliary tree. Intrahepatic CCA (ICC) is the second most common primary hepatic malignancy, after hepatocellular carcinoma, and accounts for 10-20% of primary liver cancers [1, 2]. The incidence and mortality rates of ICC have been rising worldwide in the past decade, whereas those of extrahepatic CCA (ECC) are either stable or decreasing [2]. In the Western countries, the annual incidence of ICC is 2.1 per 100,000 person years [3]. Chronic inflammation from liver fluke infestation, hepatitis B and C infections, primary sclerosing cholangitis and inflammatory bowel disease are the main risk factors of CCA [4]. Other less common etiologic factors include hepatolithiasis, cirrhosis, alcohol, smoking, fatty liver disease and cholelithiasis [1].

Only 10-15% of the patients with CCA are amenable to potentially curative surgery, as majority present at an advanced stage due to lack of effective screening strategies [5]. Despite resection, high recurrence rates of 50-60% persist, conferring a five-year overall survival (OS) of only 30% [5, 6]. The high rate of relapse prompted a strong rationale for adjuvant therapies to improve survival. However, the available evidence remains conflicting as randomized adjuvant trials are still ongoing. A meta-analysis of 6,712 biliary tract cancer

(BTC) patients who received varying forms of adjuvant therapy (chemotherapy, radiotherapy, chemoradiotherapy) demonstrated no clear survival benefit with adjuvant treatment (Odds ratio (OR) 0.74, 95% Confidence interval (CI) 0.55-1.01; $P = 0.06$) [7]. Liver transplantation, though not considered as standard therapy for CCA, has also been explored in selected patients with early stage perihilar CCA, where complete resection is impossible due to vascular or biliary invasion. A meta-analysis of 605 CCA patients who underwent liver transplantation demonstrated a 5-year OS of 39%, with superior outcomes in those who underwent perioperative chemoradiotherapy (5-year OS 57%) [8].

Majority of the patients present at an advanced stage, with limited treatment options which include locoregional or systemic therapy. There has been a growing interest in various locoregional therapy modalities including transarterial chemoembolization, selective internal radiotherapy, external beam radiation or ablation in patients who present with liver-limited disease [9]. However, these therapies were evaluated in small retrospective series or single arm phase II trials, and thus limit generalizability. The current standard of care for first line treatment of unresectable CCA is the combination of gemcitabine and cisplatin, albeit with modest benefit [10]. The prognosis of patients with unresectable or metastatic CCA is universally poor, with a median OS of less than one year. The treatment complexity is further confounded by the presence of recurrent cholangitis or cholestasis, necessitating interventions for restoration of biliary drainage and long term antibiotics use, thus leading to delays in systemic treatment.

Notably, the conduct of phase III randomized controlled trials (RCTs) have been exceptionally challenging due to the rarity of CCA and its inherent anatomical, pathological and molecular heterogeneity. With the advent of whole genome sequencing, mutations in epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), v-raf murine sarcoma viral oncogene homolog (*BRAF*) and tumor protein p53 *(TP53*) were unraveled. More recently, novel mutations in isocitrate dehydrogenase (*IDH*), BRCA1-Associated Protein 1 (*BAP1*) and AT-rich interactive domain-containing protein 1A (*ARID1A*), and novel fusions such as fibroblast growth factor receptor (*FGFR2*) and ROS proto-oncogene 1 (*ROS1*) were revealed. In this review, we will discuss the evolving genetic landscape and summarize the targeted therapies in CCA.

SYSTEMIC CHEMOTHERAPY

The standard of care for first line chemotherapy for advanced CCA is the combination of gemcitabine and cisplatin. The pivotal United Kingdom National Cancer Research Institute Advanced Biliary Cancer (ABC)-02 study reported superior survival with gemcitabine and cisplatin (GC), with a median OS of 11.7 months *versus* 8.1 months, and median progression free survival (PFS) of 8.0 months *versus* 5.0 months, when compared to gemcitabine alone [10]. Despite intensified evaluation of other chemotherapy combinations with fluorouracil, oxaliplatin or irinotecan, the improvement in survival has been marginal [11]. Currently, there is no standard second-line chemotherapy. In a systemic review of 761 patients, treatment with second-line chemotherapy attained a mean OS of 7.2 months (95% CI 6.2-8.2), PFS of 3.2 months (95% CI 2.7-3.7), response rate (RR) of 7.7% (95% CI 6.5-8.9) and disease control rate (DCR) of 49.5% (95% CI 41.4-57.7) [12]. However, these results need to be interpreted with caution. First, patients who receive second-line chemotherapy have better performance status, which may be associated with improved prognosis [13]. Second, only 15-25% of patients will be fit enough to receive second-line treatment [14]. Third, no RCTs have been included in this systemic review. Given the marginal advances with chemotherapy, emphasis has been shifted to molecularly targeted therapies, either as a single agent or in combination with chemotherapy.

CURRENT GENETIC LANDSCAPE

CCA represents a molecularly diverse subgroup of BTCs. Genomic profiling with whole-exome and next-generation sequencing has identified multiple molecular aberrations that contribute to its multistep carcinogenesis [15-17]. Well established genomic alterations include overexpression of *EGFR* (5%-27%), vascular endothelial growth factor (*VEGF*) and its receptor (*VEGFR*) (55%-60%), human epidermal growth factor receptor 2 (*HER2*)/ erb-b2 receptor tyrosine kinase 2 (*ERBB2*) (0%-20%) [15-19], and MET proto-oncogene (*MET*) (7%-21%) [15, 17, 19, 20], mutations in *BRAF* (5%) and loss of function mutation in *TP53* (3%-45%) [15-17, 21]. Dysregulation of a plethora of key signaling pathways such as *RAS*/*RAF*/ mitogen-activated extracellular signal regulated kinase (*MEK*)/ extracellular signal-regulated kinases (*ERK*) and phosphatidylinositol 3-kinase (*PI3K*)/phosphatase and tensin (*PTEN*)/protein kinase B (*AKT*)/mechanistic target of rapamycin (*MTOR*) further contribute to its malignant transformation [15-17, 21]. The first whole exome sequencing study of 8 liver-fluke related CCA identified 206 somatic mutations in 187 genes, including novel genes (e.g. *SMAD4* (16.7%), roundabout guidance receptor 2 (*ROBO2*) (9.3%), *GNAS* (9.3%), *MLL3* (14.8%), Cyclin-dependent kinase inhibitor 2A (*CDKN2A)* (5.6%), paternally expressed 3 (*PEG3*) (5.6%), ring fingers proteins (*RNF*) (9.3%) [22]. Another study with genomic profiling on 209 CCA revealed that *SMAD4* and *TP53* were more frequent in Opisthorchis viverrini related CCA, and *IDH1/2* mutations were more frequent in non-Opisthorchis viverrini related CCA [23]. Furthermore, chromatin remodeling genes such as *BAP1*, *ARID1A*,

Table 1: Molecular aberrations in cholangiocarcinoma

	Intrahepatic cholangiocarcinoma	Extrahepatic cholangiocarcinoma	Reference
EGFR overexpression	11%-27%	5%-19%	[15-19]
KRAS mutation	9%-24%	40%	[15-17]
HER2 overexpression	0%-2%	5%-20%	[15-19]
VEGF overexpression	54%	59%	[15-19]
PIK3CA mutation or deletion	4%	NR	[15-19]
BRAF mutation	5%	NR	[21]
MET overexpression	7%-21%	0%	[15, 17, 19]
IDH1/IDH2 mutation	16%-36%	0%	[15-17, 21, 24-27, 54]
FGFR translocations	6%-50%	0-5%	[15-17, 28-30, 60]
TP53 mutation	3%-36%	45%	[15-17]
ARID1A mutation	19%-36%	5%	[16, 17, 24]
MCL1 amplification	16%-21%	NR	[16, 17]
PTEN mutation	1%-11%	NR	[15, 17, 21]
PBRM1 mutation	11%-17%	5%	[16, 24]
BAP1 mutation	9%-25%	10%	[16, 24]
SMAD4 mutation	4%	25%	[16]
FBXW7 mutation	6%	15%	[16]
CDKN2A mutation	7%	15%	[17]
CDK6 mutation	7%	NR	[17]
BRCA mutation	4%	NR	[17]
NF1 mutation	4%	NR	[17]
TSC1 deletion	4%	NR	[17]
ROS1 fusion	8.7% (all CCA)	NR	[15, 32]

Abbreviations: NR, not reported

Protein polybromo-1 (*PBRM1*), and *MLL3* were found to be highly mutated in CCA [24]. Other novel genetic signatures include *IDH* mutations (16%-36%) [15-17, 21, 24-27], *FGFR* (5%-50%) [15-17, 28-31] and *ROS1* fusions (9%) [15, 32]. The prevalence of these genetic aberrations vary widely across studies, anatomical sites and geographically, primarily attributed to the heterogeneity of BTCs, limited sample size, retrospective nature of majority of the studies, and different techniques used to identify the genomic mutations.

Next generation sequencing (NGS) of 46 cancer-related genes in 75 CC patients has highlighted anatomical variability in frequency of mutations [16]. Notably, it may be technically challenging to distinguish ICC and ECC based on pathology, and hence there may be inherent biases in these studies. The common genetic alterations in ICC include *TP53* (30%), *KRAS* (24%), *ARID1A* (20%), *IDH1* (18%) and *MCL1* (16%), whereas for extrahepatic CCA, common aberrations include *TP53* (45%), *KRAS* (40%), *ERBB2* (20%), *SMAD4* (25%), F-box/WD repeat-containing protein 7 (*FBXW7*) (15%) and *CDKN2A* (15%). Furthermore, there were significant differences with regards to the prognostic significance of the above molecular markers, with *TP53*, *KRAS* and *MTOR* alterations predicting a worse prognosis in ICC, and *BAP1*, *PBRM1* and chromatin modulating genes linked to a worse survival in ECC. A subsequent meta-analysis of 4,458 patients with the study of 102 individual markers revealed that genetic alterations of *HER2* and *TP53* were more common in ECC, and *BCL-2*, *EGFR*, *SMAD4*, *p16* and *VEGF-A* were more frequent in ICC [33]. Table 1 summarizes the molecular aberrations in CCA. In the following section, we will highlight the known molecular aberrations in conjunction with their targeted therapies. Figure 1 depicts the key signaling pathways in the pathogenesis of CCA, and novel targeted therapies in development in CCA.

Table 2: Clinical trials of targeted therapies in biliary tract cancers (including cholangiocarcinoma)

Drug	Study	Phase	Line of Rx	No. of pts	RR (%)	Median PFS (mths)	Median OS (mths)
Phase III study							
GEMOX + Erlotinib (A) vs. GEMOX (B)	Lee et al. [35]	III	1st	268	A: 30 B: 16	A: 5.8 B: 4.2	A: 9.5 B: 9.5
Phase I/II studies							
EGFR							
Erlotinib	Phillips et al. [97]	II	1st/ 2nd	42	8	2.6	7.5
Sorafenib + Erlotinib	El-Khoueiry et al. [42]	II	1st	30	7	2	6
GEMOX + Cetuximab	Gruenberger et al. [98]	II	1st	30	63	8.8	15.2
GEMOX + Cetuximab	Paule et al. [99]	II	2nd	9	33	Low EGFR: 4 High EGFR: 7	Low EGFR: 7 High EGFR: 9
GEMOX + Cetuximab (A) vs. GEMOX (B)	Malka et al. [36]	II	1st	150	A: 23 B: 29	A: 6 B: 5.3	A: 11 B: 12.4
GEMOX + Cetuximab (A) vs. GEMOX (B)	Chen et al. [37]	II	1st	122	A: 27 B: 15	A: 6.7 B: 4.1	A: 10.6 B: 9.8
Gemcitabine/ Capecitabine/ Cetuximab	Rubovszky et al. [100]	II	Any	34	17.6	8.6	15.7
Gemcitabine/ Cetuximab	Borbath et al. [101]	II	1st	44	20.4	6 month PFS: 47%	13.5
GEMOX/ Capecitabine/ Panitumumab	Jensen et al. [102]	II	Any	46	33	8.3	10
GEMOX + Panitumumab (KRAS WT)	Hezel et al. [103]	II	1st	31	45	10.6	20.3
Gemcitabine/ irinotecan/ Panitumumab	Sohal et al. [104]	II	1st	21	43	NR	12.7
HER-2							
Lapatinib	Ramanathan et al. [105]	II	1st/ 2nd	17	0	1.8	5.2
VEGF/ VEGFR							
GEMOX + Bevacizumab	Zhu et al. [39]	II	1st/ 2nd	35	40	7	12.7
Bevacizumab + Erlotinib	Lubner et al. [40]	II	1st	49	12	4.4	9.9
Gemcitabine + Capecitabine + Bevacizumab	Iyer et al. [41]	II	1st	50	72	8.1	11.3
Sorafenib	El-Khoueiry et al. [43]	II	1st	31	0	3	9
Sorafenib	Bengala [45]	II	Any	46	2	2.3	4.4

Gemcitabine + Sorafenib (A) vs. Gemcitabine (B)	Moehler et al. [44]	II	1st	102	A: 8 B: 6	A: 3 B: 4.9	A: 8.4 B: 11.2
Gemcitabine/ Cisplatin + Sorafenib	Lee et al. [46]	II	1st	39	NR	6.5	14.4
Sunitinib	Yi et al. [47]	II	2nd	56	9	1.7	4.8
Gemcitabine/ cisplatin + Cediranib (A) vs. Gemcitabine/ cisplatin (B)	Valle et al. [49]	II	1st	124	A: 44 B: 19	A: 8 B: 7.4	A: 14.1 B: 11.9
Vandetanib	Santoro et al. [48]	II	1st	173	4	105 days	228 days
C-MET							
Tivantinib + Gemcitabine	Pant et al. [52]	I	Any	20	20	NR	NR
Cabozanitib	Goyal et al. [53]	II	2nd & beyond	19	0	1.8	5.2
Others							
Selumetinib	Bekaii-Saab et al. [80]	II	1st/ 2nd	28	12	3.7	9.8
Selumetinib + Gemcitabine/ cisplatin	Bridgewater et al.[81]	I	1st	12	37.5% (8 evaluable pts)	6.4	NR
Bortezomib	Denlinger et al. [85]	II	2nd/ 3rd	20	5	1.6	9.5

Abbreviations: PFS, Progression free survival; OS, Overall survival; Rx, Treatment ; NR, Not reported; Pts, Patients

ESTABLISHED MOLECULAR ABERRATIONS AND TARGETED THERAPY

EGFR/HER2

The *EGFR* family comprise of *ERBB*1-4, with *ERBB1* (*EGFR*) and *ERBB2* (*HER2*) being frequently implicated in the multi-step carcinogenesis of CCA [15]. Binding of *EGF*-ligands to the receptors induce homodimerization or heterodimerization, which in turn activates downstream signaling pathways (*MAPK, PI3K/ AKT/MTOR* and *STAT*) that regulates cell differentiation, migration, angiogenesis and survival. *EGFR* overexpression occurs in 11-27% of ICC and 5-19% of ECC, and has been associated with tumor recurrence and worsened survival [15, 17, 18]. Majority (77-79%) of *EGFR* overexpression in BTCs exhibit copy number gain, with activating mutations in *EGFR* being extremely rare [15]. Although no mutations have been reported in *HER2*, *HER2* overexpression has been noted in 0-2% of ICC and 5-20% of ECC [18]. Preclinical studies have demonstrated that overexpression of *HER2* in transgenic mouse models and orthotopic transplantation BDEneu models enhance the development of CCA, and provided consistent evidence of the oncogenic potential of *EGFR* [34].

Despite the strong rationale of targeting *EGFR* in BTCs and early interesting results with single arm phase II trials suggesting the benefits of *EGFR* inhibitors either as single agents or in combination with chemotherapy (Table 2), four completed randomized studies have failed to confirm the benefits of targeting *EGFR* in advanced BTCs. The only phase III trial of 133 patients with BTCs demonstrated that the addition of erlotinib to gemcitabine-oxaliplatin (GEMOX) significantly improved RR, but did not demonstrate any benefit in survival, with a median OS of 9.5 months in both arms [35]. However, subgroup analyses showed that for patients with CCA, the addition of erlotinib to chemotherapy significantly prolonged median PFS by 2.9 months [5.9 months *vs*. 3.0 months (HR 0.73, 95% CI 0.53-1.00; $P = 0.049$)]. In a phase II study, the addition of cetuximab, a chimeric anti-*EGFR* monoclonal antibody to GEMOX did not confer a survival benefit in patients with advanced BTCs [36]. The median PFS was 6.1 months for the GEMOX and cetuximab

Table 3: *FGFR2* translocations in ICC

Study	No. of patients (n)	No. of patients with FGFR2 translocation (n, %)	Type of FGFR2 Translocations	Method
Wu et al. [59]	2	2 (100%)	*FGFR2-BICC1*	RNA, exome sequencing
Borad et al. [30]	6	3 (50%)	*FGFR2-TACC3* *FGFR2-BICC1* *FGFR2-MGEA5*	Genome-wide and whole transcriptome sequencing
Graham et al. [29]	96	12 (13%)	NR	Fluorescence in situ hybridization
Arai et al. [28]	66	9 (13.6%)	*FGFR2-AHCYL1* *FGFR2-BICC1*	Whole transcriptome sequencing
Ross et al.[17]	28	3 (10.7%)	*FGFR2-KIAA1598* *FGFR2-BICC1* *FGFR2-TACC3*	Next generation sequencing
Sia et al. [31]	107	48 (45%)	*FGFR2-PPHLN1* *FGFR2-BICC1*	RNA, exome sequencing
Nakamura et al. [60]	109	6 (5.5%)	*FGFR2-KCTD1* *FGFR2-TXLNA* *FGFR2-BICC1(Type 2)*	Exome sequencing

Abbreviations: NR, Not reported

arm, compared to 5.5 months in the GEMOX alone arm, and the median OS was 11.0 months and 12.4 months, respectively. In another study, patients who were stratified by *KRAS* status, received GEMOX with or without cetuximab [37]. The addition of Cetuximab to GEMOX was associated with a trend in improvement in PFS (6.7 months *vs*. 4.1 months; $P = 0.05$), but not OS (10.6 months *vs*. 9.8 months; $P = 0.91$). In addition, *KRAS* mutation did not predict for benefit in survival. The addition of another *EGFR* antibody, panitumumab to gemcitabine/cisplatin based chemotherapy did not improve survival in patients with advanced BTCs [38]. Additional biomarker-driven trials will provide further insight as most of the studies were conducted in patients who were unselected for *KRAS* mutation status or other signatures implicated in predicting response to *EGFR* therapy.

VEGF

The most potent angiogenic factor in perpetuating tumor growth and metastasis is the vascular endothelial growth factor. *VEGF* overexpression was observed in 54% of ICC and 59% of ECC, and has been shown to promote metastasis, tumor recurrence and confer a worse prognosis [15, 18].

The efficacy of *VEGF* inhibitors has been investigated in several trials (Table 2). Bevacizumab has been combined with GEMOX, erlotinib or gemcitabine and capecitabine, yielding a PFS of 4-8 months and OS of 10-13 months [39-41]. Five trials have investigated sorafenib, a multikinase inhibitor against *VEGFR-2*, *VEGFR-3*, *RAF*, platelet derived growth factor receptor (*PDGFR*) and stem cell factor (*KIT*), and did not report any significant benefit in survival [42-46]. Other *VEGF* inhibitors such as sunitinib [47] and vandetanib (ZD6474) [48] yielded disappointing results. Recently, Valle and colleagues reported the results of ABC-03 trial in which the addition of cediranib, a potent oral *VEGFR* 1-3 inhibitor, was evaluated in combination of gemcitabine/cisplatin in advanced BTCs in a randomized phase II trial [49]. Of the 124 patients enrolled (62 in each arm), the addition of cediranib improved the response rate (44% in the cediranib arm and 19% in the placebo arm, $P = 0.004$) but did not improve the median PFS (8.0 months in cediranib arm and 7.4 months in placebo arm, HR 0.93, $P = 0.72$) or OS (14.1 months in cediranib arm and 11.9 months in placebo arm, HR 0.86, $P = 0.44$). Whether other antiangiogenic agents have any benefits in BTCs and whether any biomarkers have any predictive values in BTCs remain to be investigated.

MET

Binding of hepatocyte growth factor (*HGF*) to *HGF* receptor (*c-MET*) activates multiple key downstream signaling pathways such as the *RAS/MAPK, PI3K/AKT* and *JAK/STAT*, which play critical roles in tumor proliferation and survival [50]. Activation of *MET* can arise *via* mutations or copy number amplification. Through gene expression profiling, increased *c-MET* expression was observed in 20-60% of ICC and 0-70% of ECC [20, 50]. Accumulating evidence has established that *MET* overexpression is associated with a poor prognosis. There is emerging evidence that suggest *MET* aberration to be one of the mechanisms responsible for *EGFR* resistance [51]. This led to the evolution of *MET* inhibitors for CCA,

Table 4: Targeted therapies in development

Drug	Target	Phase	Line of therapy	NCT number
AG-120	*IDH1*	I	2nd & beyond	NCT02073994
IDH305	*IDH1*	I	2nd & beyond	NCT02381886
AG-221	*IDH2*	I/II	2nd & beyond	NCT02273739
Dasatinib	*IDH1/2*	II	2nd & beyond	NCT02428855
BAY1187982	*FGFR2*	I	2nd & beyond	NCT02368951
ARQ087	*FGFR2*	I/II	2nd & beyond	NCT01752920
BAY1179470	*FGFR2*	I	Any	NCT01881217
AZD4547	*FGFR2*	I	Any	NCT00979134
BGJ398	*FGFR2*	II	2nd & beyond	NCT02150967
Ponatinib Hydrochloride	*FGFR2*	II	Any	NCT02265341
BLU-554	*FGFR4*	I	Any	NCT02508467
Erlotinib + Cetuximab	*EGFR*	I	Any	NCT00397384
GEMOX ± Cetuximab	*EGFR*	II	1st	NCT01267344
GEMOX ± Panitumumab	*EGFR*	II	1st	NCT01389414
GEMOX/Capecitabine ± Panitumumab	*EGFR*	II	Any	NCT00779454
GEMOX ± Panitumumab	*EGFR*	II	1st	NCT01389414
Gemcitabine/cisplatin + BIBW 2992	*EGFR/HER2*	I	1st	NCT01679405
Afatinib + Capecitabine	*EGFR/HER2*	I	2nd & beyond	NCT02451553
ASLAN001	*EGFR, HER2, HER4*	II	2nd & beyond	NCT02609958
Cediranib + mFOLFOX6	*VEGF*	II	1st	NCT01229111
Gemcitabine + Oxaliplatin + Capecitabine + Panitumumab/ Bevacizumab	*EGFR, VEGF*	II	1st	NCT01206049
Ramucirumab	*VEGFR*	II	2nd & beyond	NCT02520141
Lenvatinib	*VEGFR*	II	2nd & beyond	NCT02579616
LY2801653	*c-MET*	I	2nd & beyond	NCT01285037
Everolimus	*MTOR*	I	2nd & beyond	NCT00949949
Trametinib	*MEK*	II	2nd & beyond	NCT02042443
MK2206	*AKT*	II	2nd	NCT01425879
LDK378	*ROS1*	II	1st or 2nd	NCT02374489
Ceritinib	*ALK*	II	2nd & beyond	NCT02638909
Sorafenib + GEMOX	*VEGFR, PDGFR, RAF, KIT*	I/II	Phase 1: Any Phase II: 1st	NCT00955721
Regorafenib	*EGFR, Ras, Raf, VEGFR, PDGFR*	II	2nd	NCT02053376
Regorafenib	*EGFR, Ras, Raf, VEGFR, PDGFR*	II	2nd & beyond	NCT02115542
Pazopanib + GSK1120212	*VEGFR/ PDGFR /Raf /MEK*	I	Any	NCT01438554
Gemcitabine + Pazopanib	*c-KIT, FGFR, PDGFR and VEGFR*	II	1st	NCT01855724
Pembrolizumab	*PD-1*	II	2nd & beyond	NCT02628067
Pembrolizumab + mFOLFOX	*PD-1*	I/II	Any	NCT02268825
MEDI4736	*PD-L1*	I	2nd & beyond	NCT01938612

Gemcitabine/Cisplatin ± CX-4945	*CK2*	I/II	1st	NCT02128282
BBI503	Cancer stemness kinase	II	2nd & beyond	NCT02232633
DKN-01 and Gemcitabine/ Cisplatin	Dkk-1	I	1st	NCT02375880
ADH-1	*ICAM-1*	I	1st	NCT01825603

Abbreviations: *CK2*, Caesin kinase 2; *ICAM-1*, Intercellular adhesion molecule-1, *Dkk-1*, dickkopf *Wnt* signaling pathway inhibitor 1; mFOLFOX; Modified fluorouracil, folinic acid and oxaliplatin.

either alone or in combination with cytotoxic agents.

The combination of Tivantinib (ARQ 197) with gemcitabine was examined in 74 patients with solid tumors, with 20% (1 CCA patient) achieving partial response [52]. In another study, 19 CCA patients who were unselected for *MET* amplification or overexpression were treated with cabozantinib and exhibited no objective responses [53]. PFS and OS were 1.77 (95% CI 1.63-5.37) and 5.2 (95% CI 2.70-8.17) months, respectively.

NOVEL ONCOGENIC DRIVERS

The advent of next generation sequencing techniques has further shaped the genomic landscape of CCA and enhanced our understanding of its pathogenesis. Recent discoveries include *IDH1/2* mutations, *FGFR2* and *ROS1* fusions, and mutations in chromatin remodeling genes for example *ARID1A* and *BAP1*. We will further elaborate on these promising molecular targets.

IDH mutations

IDH1 and *2* alterations exist in several tumors including gliomas and more recently identified in BTCs through high throughput molecular profiling [15-17, 21, 25-27, 54]. *IDH1* and *IDH2* are metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate [55]. *IDH* mutations enhance the conversion of alpha-ketoglutarate to 2-hydroxyglutarate (2-HG), an oncometabolite that inhibits α-ketoglutarate-dependent enzymes responsible for DNA methylation, epigenetic regulation and call signaling. The accumulation of 2-HG in tumor tissue in turn promotes cell proliferation and survival.

The frequency of *IDH* mutations ranges from 16-36%, and is ubiquitously higher in ICC than ECC [15-17, 25-27, 54, 55]. *IDH* mutations were observed in 22-36% of ICC and only 0-7% of ECC, and may be associated with clear cell or poorly differentiated histology [26, 55]. The prognostic significance of *IDH* mutations remains conflicting. In a cohort of 326 patients with resected ICC, *IDH* mutation was associated with longer time to recurrence and OS [27]. In addition, the authors observed enhanced *p53* and DNA hypermethylation among patients with *IDH* mutations. In contrast, Jiao et al. demonstrated in a study of 32 patients with ICC that *IDH* mutations confer a worse prognosis when compared to those with *IDH* wild-type (3-year OS 33% *vs*. 81%; $P = 0.003$) [24]. However, this adverse finding may be due to the presence of a larger proportion of stage IV disease amongst the *IDH* mutants compared to *IDH* wild-type (50% *vs*. 15%). Two recent studies revealed no correlation between *IDH* mutation status and survival among 200 patients with resected ICC [21] and 104 patients with advanced ICC [54].

Two proof of concept studies illustrated the tumor suppressive effects of *IDH* inhibitors. Rohle et al. found that a selective R132H-IDH1 inhibitor (AGI-5198) impeded the growth of *IDH*-mutant glioma cells [56]. Similarly, Wang et al. showed that AGI-6780 selectively inhibits the leukemic cells harboring mutant *IDH2*/R140Q [57]. Current *IDH*-inhibitor studies are in early clinical development (NCT02073994, NCT02381886 and NCT02273739). The preliminary results of a phase 1 trial of AG120 (*IDH1* inhibitor) in 62 patients with *IDH1* mutation positive solid tumors who had progressed on standard treatment was reported at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics 2015. There were no dose limiting toxicities, with anemia being the most frequent Grade 3 AE (5%). 1/20 (5%) CC patients attained PR and 11/20 (55%) attained SD. Reduction in circulating 2-HG level was observed ranging from 73% to 99%, and reduction in Ki67 staining was seen from 22% - 96%. The expansion phase with 500 mg QD is underway (NCT02073994).

Fibroblast growth factor receptor (FGFR) 2 fusions

FGFR2, a member of the fibroblast growth factor family of receptors (*FGFR 1-4*), is located at chromosome 10q26 and mitigates cell differentiation, proliferation and apoptosis [58]. The oncogenic property of *FGFR2* has been linked to loss of the carboxy terminus and ligand independent dimerization, leading to *FGFR* protein overexpression.

Whole exome sequencing and fluorescence *in situ* hybridization (FISH) have identified *FGFR2* alterations primarily in 6%-50% of ICC and 0-5% of ECC [28-31, 59, 60]. Churi et al. analyzed 75 CCA patients with next generation sequencing, and found that genetic alterations

in the *FGFR* pathway occurred in 13% of intrahepatic CCA and 5% of extrahepatic CCA , and that these alterations were associated with improved survival [16].

More recently, *FGFR2* fusions have been detected in several studies (Table 3). These fusions are a product of the *FGFR* receptor (exons 1-19) and various partners (e.g. *AHCYL1*, *BICC1, KCTD1* and *TXLNA)*. The fusion protein is activated by the enforced dimerization of the respective partners with resultant intracellular domain tyrosine residue phosphorylation, and activation of downstream signaling pathways including *MAPK*, *PIK3/AKT/MTOR* and *JAK/STAT* pathways [59]. There are marked variability in the frequency of *FGFR2* fusions, ranging from 6-50% in ICC, and rarely in ECC. In a series of 102 patients with BTCs, Arai et al. observed *FGFR2* fusions (*FGFR2-AHCYL1* or *FGFR2-BICC1*) in 13.6% of ICC (9/66 ICC), and that inhibition of *FGFR2* impeded activation of *MAPK* pathway, which is responsible for uncontrolled tumor growth [28]. Another study evaluated 152 CCA and 4 intraductal papillary biliary neoplasm of the bile duct with FISH, and reported *FGFR2* translocation in 12/96 (13%) of ICC, with a female predominance [29]. Those who harbored *FGFR2* translocations had improved cancer-specific survival (123 *vs*. 37 months) and superior DFS (125 months *vs*. 26 months). Furthermore, cholangiocarcinoma harboring *FGFR2* translocation and concomitant *KRAS* mutation are only rarely reported [31]. Therefore, this association remains to be explored in larger cohorts to further assess if *FGFR2* translocation work in synergy with *KRAS* mutation in promoting carcinogenesis in CCA. In a study comprising of 109 ICC, 40 ECC and 11 gallbladder cases, novel *FGFR2* gene fusions (*FGFR2-KCTD1* and *FGFR2-TXLNA)* and a new variation of *FGFR2-BICC* (Type 2) were reported [60]. Using NIH3T3 clones that express either wild-type or kinase-inactive mutant forms of *FGFR2-KCTD1* or *FGFR2-TXLNA*, the Nakamura et al. showed that wild-type *FGFR* fusions, and not the mutant forms induce tumor growth *in vivo via* ligand-independent autophosphorylation and activation of the *MAPK* signaling pathway. In addition, there was marked inhibition of *FGFR* autophosphorylation and cell proliferation by the *FGFR* inhibitors (BGJ398 and

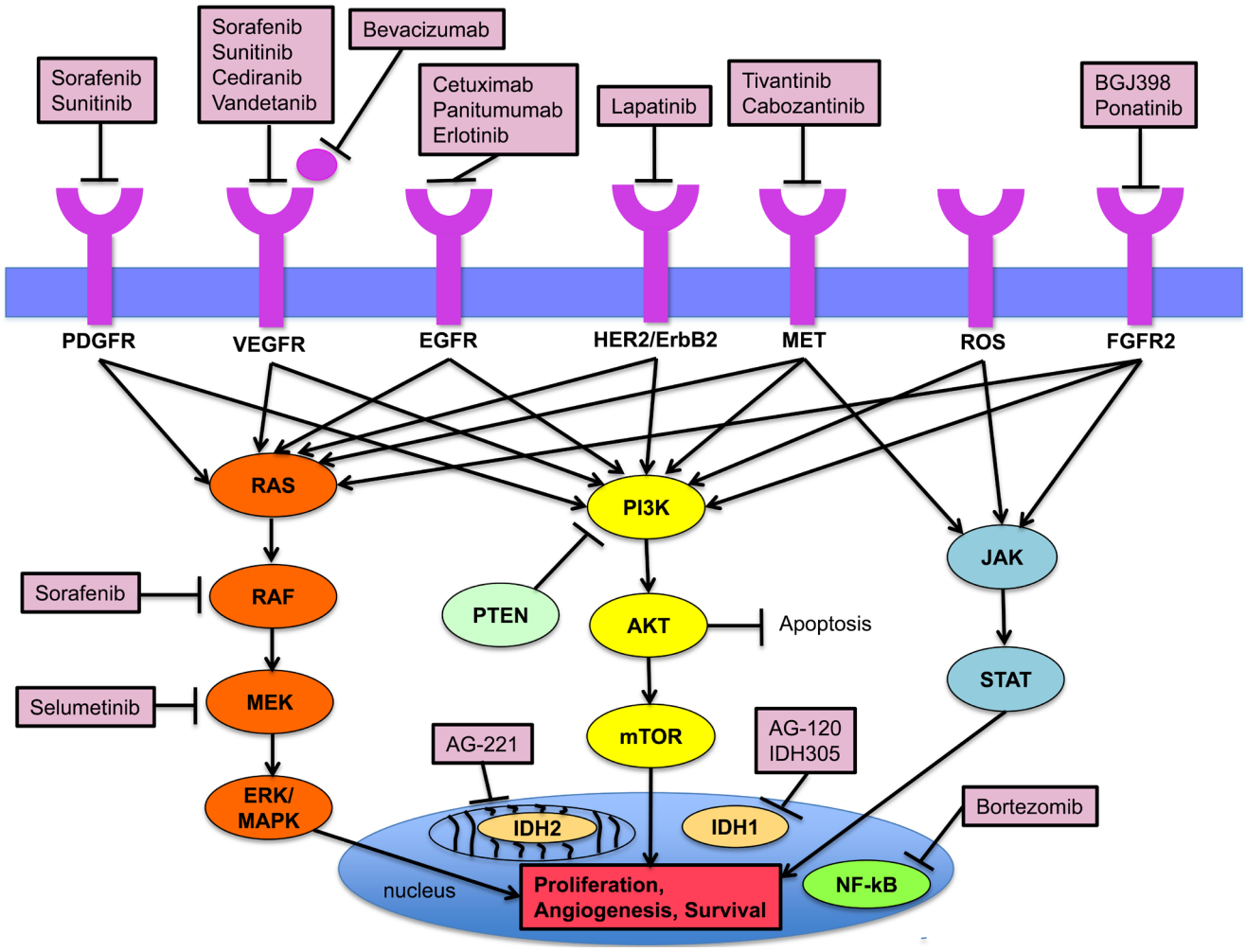

Figure 1: Key signaling pathways in the pathogenesis of cholangiocarcinoma and established targeted agents.

PD173474).

In a genome-wide and whole transcriptome sequencing on 6 ICC samples with *FGFR2* translocations in 3/6 (50%) patients, two out of three patients responded to *FGFR2* inhibitors [30]. One patient with *FGFR2-MGEA5* fusion was treated with ponatinib (a pan-*FGFR* inhibitor) and had a biochemical CA 19-9 response with shrinkage of tumor. Another patient with *FGFR2-TACC3* fusion who previously achieved a partial response with pazopanib, and subsequently received ponatinib attained stable disease. These encouraging results suggest that *FGFR2* has the potential to be an actionable molecular target, and that patients who harbor these alterations may benefit from tyrosine-kinase directed therapies. An ongoing phase 2 study of BGJ398 (a selective pan-*FGFR* inhibitor) in patients with advanced or metastatic CCA with *FGFR* genetic alterations reported promising efficacy (Javle MM et al, 2016 Gastrointestinal Cancer Symposium, J Clin Oncol 34, 2016 (suppl 4S; abstr 335)) The overall RR was 22% (8/36 evaluable patients) and DCR was 75% (27/26 patients). BGJ398 was generally well tolerated. The Grade 3/4 AEs include hyperphosphatemia (19%), hypophosphatemia (9%), hyponatremia (6%), and asymptomatic increased lipase (6%). This is a promising drug that warrants further investigation.

IMMUNE CHECKPOINT INHIBITORS

Immune checkpoints including cytotoxic T-lymphocyte-associated antigen (CTLA)-4, programmed cell death (*PD*)-1 receptor and its ligands (*PD-L1*, *PD-L2*) promotes T-cell anergy [61]. Increased levels of tumor-infiltrating CD8+ cytotoxic T cells and/or CD4+ T cells have been shown to be associated with improved prognosis in BTCs [62]. Given the success of ipilimumab (CTLA-4 monoclonal antibody), pembrolizumab and nivolumab (anti-*PD-1* antibodies) in the treatment of metastatic melanoma [63, 64], there has been growing interest of the benefit of immunomodulation in BTCs. In a preclinical study of intrahepatic CCA, Koido et al. showed that both gemcitabine and interferon -γ led to an upregulation of *PD-L1*, which suggest that treatment with *PD-L1* blockade may be beneficial [65]. Studies have suggested that mismatch repair (MMR) deficient tumors are more responsive to PD-1 blockade than are MMR proficient tumors [66]. A phase II study demonstrated that pembrolizumab led to high RR in colorectal cancer patients with genetic defects in mismatch repair (MMR) [66]. The phase II study with pembrolizumab in MMR deficient non-colorectal gastrointestinal cancers (ampullary (n = 4), pancreas (n = 4), biliary (n = 3), small bowel (n = 3), and gastric (n = 3) cancers) is ongoing. An interim analysis reported an ORR of 50% and DCR of 70% in 10 evaluable patients. The OS was 21 months and PFS was not reached (Le DT et al, 2016 Gastrointestinal Symposium, J Clin Oncol 34, 2016 (suppl 4S; abstr 195)). There are currently no studies evaluating the efficacy of *PD-1* inhibitors in CCA patients with microsatellite instability (MSI)-high *versus* MSI-stable tumors. The interim results of another phase 1b study of pembrolizumab (MK-3495) in patients with advanced BTC was presented at the European Cancer Congress 2015 (NCT02054806). Pembrolizumab was well tolerated with an ORR of 17.4% (95% CI, 5.0-38.8) in the 23 evaluable patients. 4/24 (16.7%) of the patients experienced a treatment-related grade 3 AE (anemia, autoimmune hemolytic anemia, colitis, and dermatitis). Currently, pembrolizumab is evaluated in combination with mFOLFOX6 in a phase 1/2 study at the University of Utah (NCT02268825).

LESS-ESTABLISHED MOLECULAR ABERRATIONS

There has been limited studies regarding the following molecular aberrations and additional studies are required to provide further insight.

ROS1

Elevated *ROS* expression has been observed in non-small cell lung cancer, glioblastoma and breast cancer [32]. *ROS* kinase fusions [between kinase domain of *ROS* and Fused in Glioblastoma (*FIG*) gene] has been described in 8.7% of patients with CCA [32]. These fusions further activate downstream effectors such as *STAT3* and *AKT*. The *FIG-ROS* fusion driver gene has been shown to accelerate tumor growth in an orthotopic allograft mouse model, and that inactivation of the gene portends an antitumor effect [67].

Notably, TAE684 (an *ALK* inhibitor) has been shown to inhibit *ROS* kinase activity, with consequent cell inhibition and cell death in BaF3 cells expressing this fusion protein [32]. Given the success of crizotinib in attaining an impressive response rate of 48% in *ROS1*-rearranged non-small cell lung cancer [68], similar studies in CCA are warranted to evaluate the potential benefit of targeted therapy in patients with *ROS* fusions. A phase II trial of crizotinib in patients with *ALK*, *MET* or *ROS1* alterations is underway (NCT02034981).

PI3K/AKT/MTOR

Constitutive activation of the *EGFR*, *HER2*, *MET* and Insulin growth factor (*IGF*) receptor or disruption of the *PTEN* and *SMAD4* triggers the downstream activation of *PI3K/PTEN/AKT/mTOR* signaling pathway [69, 70]. Dysregulation of this pathway subsequently stimulates cell proliferation, angiogenesis and survival. Activation of this pathway in patients harboring *EGFR*, *HER2* and *MET* overexpression has been reported in as high as 65%

of tumors. The incidence of *PIK3CA* (a subunit of PI3K) hotspot mutations in CCA ranges from 5% to 34% [71]. Furthermore, increased expression of phosphor-*AKT1* and phosphor-*MTOR* in intrahepatic CCA is positively correlated with prognosis and that this association was not modified by *PTEN* expression [72].

Dual inhibition of *AKT* and *MTOR* with MK-2206 and everolimus (RAD001) has been shown to enhance anti-proliferative effects in CCA [73]. More recently, increased efficacy was attained in-vitro by dual inhibition of the *PI3K/AKT/MTOR* and *RAF/MEK/ERK* pathway, which overcame resistance pathways [74]. A phase I trial of mFOLFOX6 and the oral *PI3K* inhibitor BKM120 in patients with advanced solid tumors (4/17 CCA) reported high toxicity rates, with 76 % of the patients experiencing a grade 3/4 AE [75]. The most common AEs were neutropenia, fatigue, leukopenia, hyperglycemia and thrombocytopenia. 1/4 of the CCA patients achieved SD. The combination of everolimus with gemcitabine and cisplatin was evaluated in 10 CCA and gallbladder cancers, of which 60% had SD [76]. Currently, MK2206 (*AKT* inhibitor) is being investigated in advanced refractory BTC (NCT01425879).

RAS/RAF/MEK/ERK

The *RAS/RAF/MEK/ERK* signal transduction pathway is frequently dysregulated in BTCs [77]. Activation of this pathway requires the binding of *EGF*, *PDGF* and cytokines to its receptors, with subsequent transactivation of downstream signaling cascade, leading to the end-phosphorylation of *MEK1* and *2* and *ERK-1* and *ERK-2*. *MEK* is an attractive target as *ERK -1* and *ERK-2* are the only known *MEK* substrates [70]. Gain of function mutations in *KRAS* constitutes one of the most frequent mutations in CCA, with the most frequent alteration in codon 12 [15]. The frequency of activating *KRAS* mutations ranges from 9%-40% [15-17]. *KRAS* has been associated with perineural invasion and poor prognosis [78]. In addition, there is marked anatomical variability in *KRAS* mutation, with *KRAS* mutations observed in 53.3% of perihilar-type, but only 16.7% of intrahepatic CCA. Notably, the incidence of *KRAS* mutations increases with disease stage.[79] Despite the recognized frequency of *KRAS* mutations, targeting this pathway remains challenging. Early evidence of efficacy of *MEK* inhibitor was reported in a single arm study of selumetinib in advanced BTCs [80]. Of the 28 patients enrolled, 3 patients had confirmed partial responses. In this study, no *BRAF* V600E mutations were found. Recently, the ABC-04 study of selumetinib in combination with gemcitabine and cisplatin in advanced or metastatic BTC (9/13 CCA) demonstrated a RR of 37.5%, a median PFS of 6.4 months and manageable toxicities [81].

BRAF

B-Raf is a proto-oncogene and is a key component of the *RAS/RAF/MEK/ERK* proliferation signaling pathway. The most common *BRAF* gene mutation found in human cancers is V600E, and exists in up to 22% of CCA in one report [82]. More importantly, *BRAF* and *KRAS* mutations are mutually exclusive. In a recent phase II "basket" study of vemurafenib in *BRAF* V600 mutated non-melanoma cancers, one patient with CCA achieved a durable PR of more than one year [83].

NFk-B

Several studies have suggested the *NF-kB*, a transcriptional nuclear factor, plays a critical role in tumor migration and treatment resistance in several tumors, although the evidence is not conclusive [84]. This stems from the observation that tumor proliferation can be kept in check *via* proteasome inhibition, which halts the clearance of pro-apoptotic factors. To date, the only proteasome inhibitor investigated was bortezomib and results were disappointing, with no objective response, median time to progression was 5.8 months and median OS was 9 months [85].

JAK/STAT cytokine pathway

Binding of pro-inflammatory cytokine, interleukin-6 (IL-6) to gp130 triggers the downstream activation of the *JAK/STAT* pathway, leading to the silencing of its inhibitor, suppressor of cytokine signaling-3 (*SOCS3*) [86]. This in turn accelerates inflammation, cell growth and tumor formation. This pathway has been noted in 70% of the inflammation subclass in ICC, characterized by activation of the *STAT3* and cytokine pathways and improved prognosis.[87] Furthermore, the *JAK2* inhibitor AZD1480 has been demonstrated to inhibit Stat3 signaling and exhibit anti-tumor efficacy in solid tumor cell lines [88].

Notch signaling pathways

The *Notch* signaling cascade is a highly conserved pathway, responsible for cell differentiation, apoptosis and cell survival. To date, there are four known *Notch* receptors and five ligands. Aberrant *Notch* signaling was first described in acute T-cell lymphoblastic leukemia, and subsequently in CCA [89, 90]. *Notch*-mediated conversion of hepatocytes into biliary lineage has been shown to promote intrahepatic CCA formation and progression in a mouse model of ICC [91]. *Notch* 1 and 4 were noted to be more frequently expressed in tumor cells compared to normal tissue. The frequency of *Notch* expression in

ICC for *Notch* 1, *Notch* 2, *Notch* 3 and *Notch* 4 were 82.9%, 56.1%, 39.0% and 34.1% respectively [92]. In addition, *Notch* 4 was found to be prognostic and *Notch* 1 overexpressed in large tumors. Furthermore, *Notch* overexpression has been demonstrated to predict sensitivity to 5-fluorouracil *in vivo*. The complex *Notch* signaling pathway warrants further understanding before the advent of novel *Notch* targeting agents.

Protein kinase A regulatory subunit 1 alpha (PRKAR1A) pathway

Protein kinase A is a cyclic AMP (*cAMP)*-dependent protein kinase and is part of the serine-threnonine protein kinase family. Activation of the *PRKAR1A/PKAI* pathway is found in various tumors, including CCA [93]. More recently, fusion genes comprising of *cAMP*-dependent protein kinase (*PKA*) and mitochondrial ATP synthase (*ATP1B-PRKACA* and *ATP1B-PRKACB*) were detected with resultant increased expression of *PRKACA* and *PRKACB* and activation of *MAPK* signaling [60]. The abrogation of *PRKAR1A* gene expression has been linked to significant cell inhibition and apoptosis of CC cells *via* suppression of the *JAK/STAT*, *MAPK*, *PI3K/AKT* and *WNT/β-catenin* pathway signaling. Drug evaluation with *PKA* inhibitor (isoquinoline H89) as well as site-specific *cAMP* analogs (8-Cl *cAMP* and 8-Br *cAMP*) showed promising anti-proliferative effect in CCA cells, supporting the notion that *PKA* can potentially contribute as a drug target in CCA.

Wnt/β-catenin pathway

Aberrant genetic alterations of the Wingless-type MMTV integration site family (*Wnt*)/*β-catenin* signaling cascade has been implicated in tumorigenesis in several studies [94]. The *Wnt* signaling pathway is highly activated in CCA, and an inflammatory milieu comprising of inflammatory macrophages is required for its sustainability [95]. Furthermore, tumor regression in mouse and rat models were prompted with the introduction of *Wnt* inhibitors. The *Wnt* signaling pathway has also been postulated as one of the mechanisms responsible for chemoresistance in CCA [94]. GSK3β, a "destruction complex" phosphorylates and degrade β-catenin, leading to downregulation of the *Wnt* survival pathway. Recently, Huang et al. showed that β-escin, an active compound in horse chestnut (*Aesculus hippocastanum*) seed, could inhibit the GSK3β/β-catenin pathway and thus terminate cell growth [96]. Hence, the *Wnt* signaling pathway may represent another alternative target for ICC treatment.

Clinical studies with novel agents in early development are summarized in Table 4.

CONCLUSIONS

Advanced CCA portends a dismal prognosis despite standard treatment with gemcitabine and cisplatin. Given the modest benefits with chemotherapy alone and the anatomical, pathological and molecular heterogeneity, there is an unmet and imperative need for comprehensive genomic profiling to improve the understanding of the pathogenesis of CCA, with the aim of personalized treatment. To achieve this aim, we must overcome the mounting challenges, which include a lack of RCTs due to the rarity of CCA, and the inherent complexity due to interactions of the signaling pathways. Extensive collaborative efforts will be required to formulate adequately powered biomarker-driven trials to improve clinical outcomes. Results of the *EGFR* inhibitors have been disappointing. As the majority of the trials are performed in unselected population, it will be informative to conduct trials in patients enriched for the presence of molecular signatures implicated in predicting *EGFR* sensitivity to determine its efficacy. Given the promising early evidence of efficacy signal with *IDH* and *FGFR2* inhibitors in early phase trials, additional studies should focus on novel strategies targeting *IDH* mutations and *FGFR2* fusions. Furthermore, the identification of oncogenic addiction loops, or novel combination strategy that targets critical molecular pathways simultaneously will be paramount to improve the clinical outcome in CCA.

Abbreviations

CCA, Cholangiocarcinoma; EGFR, Epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, V-raf murine sarcoma viral oncogene homolog; IDH, Isocitrate dehydrogenase, BAP1, BRCA1-Associated Protein 1; ARID1A, AT-rich interactive domain-containing protein 1A; FGFR, Fibroblast growth factor receptor; ROS1, ROS proto-oncogene 1; OS, Overall survival; BTC, Biliary tract cancer; OR, Odds ratio; CI, Confidence interval; RCT, Randomized controlled trials; GC, Gemcitabine and cisplatin; PFS, Progression free survival; HR, Hazard ratio; DCR, Disease control rate; VEGF, Vascular endothelial growth factor; VEGFR, Vascular endothelial growth factor receptor; HER2, Human epidermal growth factor receptor 2; ERBB2, erb-b2 receptor tyrosine kinase 2; MET, MET proto-oncogene; TP 53, Tumor protein p53; MEK, Mitogen-activated extracellular signal regulated kinase; ERK, Extracellular signal-regulated kinases; PI3K, Phosphatidylinositol 3-kinase; PTEN, Phosphatase and tensin; AKT, Protein kinase B; MTOR, Mechanistic target of rapamycin; NGS, Next generation sequencing; PBRM1, Protein polybromo-1; GEMOX, Gemcitabine-oxaliplatin; PDGFR , Platelet derived growth factor receptor; KIT,

Stem cell factor, HGF, Hepatocyte growth factor; IGF, Insulin growth factor; SMAD4, Mothers against Decapentaplegic Homolog ; IL-6, Interleukin-6; SOCS3, Suppressor of cytokine signaling-3; ROBO2, Roundabout guidance receptor 2; CDKN2A, Cyclin-dependent kinase inhibitor 2A; PEG3, Paternally expressed 3; RNF, Ring fingers proteins; 2-HG, 2-hydroxyglutarate; FISH, Fluorescence *in situ* hybridization; PRKAR1A, Protein kinase A regulatory subunit 1 alpha; Wnt, Wingless-type MMTV integration site family; CTLA, Cytotoxic T-lymphocyte-associated antigen, PD, Programmed cell death; PD-L, Programmed cell death ligand; MCL1, Induced myeloid leukemia cell differentiation protein Mcl-1; FBXW7, F-box/WD repeat-containing protein 7; CDK6, Cell division protein kinase 6; NF1, Neurofibromin 1; TSC1, Tuberous sclerosis 1; BRCA, Breast Cancer susceptibility gene; RR, Response rate; CK2, Caesin kinase 2; ICAM-1, Intercellular adhesion molecule-1.

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Both the authors contributed equally to this manuscript.

CONFLICTS OF INTEREST

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