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Novel investigational therapies for treating biliary tract carcinoma

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Abstract

Introduction: Cholangiocarcinoma (CCA) is an epithelial cell malignancy arising from bile ducts and/or peribiliary glands. Even though it is considered as a rare neoplasm, its incidence is raising, particularly in developed countries. Prognosis is generally poor with few patients who present the inclusion criteria for surgery (the mainstay treatment for this tumour). Several genetic alterations potentially driving tumour progression have been described, representing a possible target for new compounds.

Areas covered: A clinical trial search in Clinicaltrials.gov encompassing a literature search in PubMed and ASCO/ESMO Websites was undertaken in March 2016.

Expert opinion: Notwithstanding a large number of drug tested, results are still disappointing. The main reasons could be the low number of patients enrolled in trials, and the lack of a patient selection based on the biological profile of the tumours. Potential active drugs could have been discharged simply because beneficial in a particular subgroup of patients and not in un unselected population. The future direction of the research should consider biomarker evaluation in order to describe the genetic alteration/s that drive tumour progression and aggressiveness and the mechanisms of drug resistance. Finally, it will be of great interest to consider the results of immunotherapy whenever available.

Article highlights

- Cholangiocarcinoma is a rare cancer and its incidence is raising particularly in developed countries. Many genetic alterations have been described, giving rationale for the development of new treatment strategies
- Anti-EGFR therapies have been extensively studied in cholangiocarcinoma patients. Notwithstanding encouraging preliminary results, comparison trials did not demonstrate superiority of cetuximab or panitumumab plus chemotherapy versus chemotherapy alone. A new pan-HER oral inhibitor is being tested
- Several specific inhibitors directed to mTor, ABL, PI3K/AKT, Farnesyltransferase, proteasome, and VEGF failed to demonstrate significant clinical activity in unspecific patient population.
- Results from trial selecting patients with specific genetic alterations (FGFGR, IDH, ALK, ROS1, NTRK) or from trial that explore the role of drugs with new mechanisms of action (oxygen modulators, pan-IDH1 mutant inhibitor, PARP 1/2 inhibitor) will help researchers to shed light on the mechanisms underlying tumour progression
- The characterization of the genetic profile of each single tumour will pave the way for personalized and hopefully efficacious therapies.

1.1. Introduction

Cholangiocarcinoma (CCA) is an epithelial cell malignancy arising from intrahepatic (IH) and extrahepatic (EH) bile ducts and/or the peribiliary glands [1]. EH disease represents about 40%, perihilar disease 50% and IH disease less than 10% of cholangiocarcinoma cases. The incidence is globally raising, particularly in developed countries, accounting for up to 2 new cases every 100.000 inhabitants per year [1,2]. The mainstay treatment for this type of neoplasia is surgery. However, only approximately one half of the patients is suitable for resection and surgical techniques are rather complex, often necessitating lobar hepatic and bile duct resection, regional lymphadenopathy, and Roux-en-Y hepaticojejunostomy. Liver transplantation with neoadjuvant chemoradiation is a valid therapeutic alternative. However, only few patients present the strict inclusion criteria for transplantation. As a whole, prognosis of CCA is generally bad, with mortality rate of 1.4 per 100.000 inhabitants per year, an average.

After the publication of the ABC-02 study results [3] chemotherapy with Cisplatin and gemcitabine is considered the standard of care in locally advanced or metastatic CCA, with some alternatives equally considered as standard of care such as gemcitabine/oxaliplatin, or Gemcitabine/Capecitabine. Notwithstanding positive results with chemotherapy, median survival is poor with a life expectancy of no more than 12 months. Contemporary research techniques allowed the identification of several genetic changes contributing to the selective growth advantage of CCA cancer cells[1]. The most studied signalling networks in CCA biology is the RAS-MAPK pathway, followed by VEGF, PI3K/mTOR, HER2/neu and MET pathways. Thus, the number of clinical trials with targeted therapy alone or in combination with traditional chemotherapy is expanding, and new drugs are in early stages of investigation.

In this review we focus the attention on new drugs and new strategies currently under investigation in the treatment of biliary tract carcinomas. Where not otherwise specified, reported studies included patients with either IH, and EH, and peribiliary glands.

2. Anti HER family

2.1. Agents at later stage of development

2.1.1. Anti-EGFR monoclonal antibodies: cetuximab and panitumumab

The EGFR pathway has been identified as a promising molecular target in CCA. In fact, overexpression of EGFR was found in 38%–100% of the tumour samples, almost all in the gene sequence coding for the tyrosine kinase domain found in exon 21 [4].

Cetuximab (Erbitux™, Merck) is a recombinant human-mouse chimeric IgG1 monoclonal antibody binding EGFR with high affinity approved for the treatment of patients with metastatic colorectal cancer (CCR) and head and neck cancer [5-7].

The efficacy of cetuximab in CCA was firstly reported in a retrospective analysis evaluating the outcomes of five patients with stage IV or not completely resected tumours receiving cetuximab-containing therapy. After treatment, four patients experienced a clinical response (one complete) and one disease stabilization. [8]. Notwithstanding these encouraging results, subsequent and larger clinical trials reported contrasting results. In a multi-centre phase II trial 44 patients with CCA received cetuximab and gemcitabine. The study met its primary endpoint as six months progression free survival (PFS) was 47%. Median overall survival (OS) was 13.5 months and nine patients (20.4%) had partial response (PR) with an overall disease-control rate (DCR) of 79.5% [9]. In a non-comparative, open-label, randomised phase 2 trial, recruiting patients with non-resectable or metastatic cholangiocarcinoma, gallbladder carcinoma, or ampullary carcinoma, 150 patients were randomly assigned to receive gemcitabine and oxaliplatin (GEMOX) with or without cetuximab. Clinical outcomes resulted to be comparable in the two arms as median PFS was 6·1 months in the chemotherapy plus cetuximab group and 5.5 months in the chemotherapy alone group, whereas median overall survivals were 11 vs 12.4 months, respectively [10].

Cetuximab treatment seems to be beneficial particularly in patients with intrahepatic CCA. In fact in an open-label pilot study of nine patients progressing after GEMOX the addiction of cetuximab permitted to obtain a tumour shrinkage in two (22%) of them [11]. Gruenberger et al published a single-arm phase II study of GEMOX and cetuximab demonstrating an overall response rate of 63%, including 3 patients with a complete response. The majority of the patients in this trial had intrahepatic cholangiocarcinoma (60%). The study did not limit recruitment to patients with KRAS wild-type tumours, and interestingly, partial responses were observed in two of the three patients with KRAS mutant tumours. The interim analysis of the first 36 patients revealed an improvement in PFS rate at four months of the experimental arm vs placebo (61 vs. 44%) [12].

Recently, the results of a randomised, phase II study of GEMOX with or without cetuximab conducted in Asian countries confirmed that the combination was not statistically superior to chemotherapy alone. Response rate, primary endpoint of the study, did not differ between the two arms (27% vs 15% with vs without cetuximab, respectively, p=0.12). Median PFS were 6.7 vs 4.1 (p=0.05) and median OS 10.6 vs 9.8 months (p=0.91) [13]. Interestingly, KRAS status seemed not to influence clinical outcomes. Key results of the above described trials are summarized in Table 1.

Panitumumab (Vectibix™, Amgen) is a fully humanized IgG2 monoclonal antibody. It was generated in transgenic strains of mouse and modified to express human immunoglobulin genes (XenoMouse). Panitumumab is approved only for the treatment of metastatic colorectal cancer patients [14].

In CCA, encouraging results were reported in 42 patients with KRAS wild-type tumours enrolled in a phase II trial exploring the activity of the combination of GEMOX with capecitabine and panitumumab. Overall response rate (ORR) was 33% with a DCR of 86%, median PFS of 8.3 months,6-month PFS rate of 71%, and a median OS of 9.8 months [15]. These results were furtherly confirmed in a phase II study enrolling 31 patients with locally advanced or metastatic

KRAS wild-type CCA treated with GEMOX + panitumumab: ORR was 45%, median PFS 10.6 months and median OS 20.3 months [16].

A phase II study enrolling 35 patients with advanced CCA and treated with gemcitabine, irinotecan and panitumumab reported a ORR of 31%, a median PFS of 9.7 months with a 5-month PFS rate of 69% (primary aim of the study) and a median OS of 12.9 months. Interestingly, patients were recruited regardless the KRAS status of their tumours, obtaining clinical outcomes similar to those reported from patients with KRAS wild-type tumours [17].

These encouraging results were not confirmed in an Italian open-label phase II trial randomising patients with advanced KRAS wild-type CCA to receive GEMOX with (arm A) or without (arm B) panitumumab. Primary endpoint of the study was PFS. Eighty-nine patients (45 in arm A and 44 in arm B) were enrolled between June 2010 and September 2013. After a median follow-up of 10.1 months, the median PFS was 5.3 months in arm A and 4.4 months in arm B ($p = 0.27$). No survival difference was observed: median OSs were 9.9 vs 10.2 months, respectively ($p = 0.42$). In a pre-planned subgroup analysis, no difference in PFS was demonstrated according to the site of the primary tumour [18].

An ongoing trial conducted in Denmark aims to compare PFS of patients with advanced CCA randomly allocated to chemotherapy group (GEMOX + capecitabine) or to chemotherapy plus panitumumab. Patients are enrolled regardless the KRAS status of their tumours. Estimated enrolment is 70 patients, but the study is not yet recruiting (ClinicalTrials.gov Identifier: NCT00779454). Key results of the above described trials are summarized in Table 2.

2.1.2. Anti-EGFR TKIs: erlotinib

Erlotinib (Tarceva™) reversibly binds the intracellular kinase domain of EGFR inhibiting the ATP-dependent activation of downstream pathway enzymes. It is currently approved for the treatment of

EGFR mutant non-small cell lung cancer (NSCLC) as single agent and of pancreatic cancers in combination with gemcitabine [19].

A potential efficacy of erlotinib in CCA was firstly reported as single agent in a phase II study enrolling 43 patients at any line of therapy. Three partial responses were observed, for an overall response rate of 8% [20]. Data from a randomized, phase III, placebo controlled trial recruited a total of 285 patients who received GEMOX with erlotinib or placebo. Median PFS of the experimental arm was 5.8 months vs 4.2 months of the placebo group (p=0.08). Median OSs were 9.5 months and were similar in both groups [21]. The combination of erlotinib and bevacizumab was tested in a phase II, multicentre study enrolling 49 patients with advanced CCA. Six patients obtained confirmed PR, for an ORR of 12%, whereas median OS was 9.9 months, and median TTP was 4.4 months [22]. Finally, a phase II trial (NCT01093222) evaluated the combination of sorafenib with erlotinib in patients with locally advanced, unresectable or metastatic CCA. Treatment was administered to 34 patients resulting in a median PFS of 2 months and in a median OS of 6 months. Treatment was toxic, with an incidence of SAE in about 50% of the patients, including 17% of toxic deaths, 8 % of which for gastrointestinal toxicity or sepsis.

2.1.3. Anti HER2 monoclonal antibodies: trastuzumab

Trastuzumab is a humanized monoclonal antibody that selectively binds HER2 receptor. It is currently approved in the treatment of breast and gastric cancer patients [23,24].HER-2 overexpression has been found in about 5% of CCA, justifying the interest of the clinicians towards HER2 inhibitors in this setting [25,26].

Some sporadic experiences of trastuzumab treatment in patients with CCA have been reported in literature as single agent or in combination with paclitaxel [27,28]. After having screened 53 patients with locally advanced or metastatic gallbladder cancer or CCA, a phase II study submitted a total of four patients to trastuzumab monotherapy. Two out of the three evaluable patients

responded, with an ORR of 66.6%. No information about the histology of the recruited patients was available (NCT00478140). These results were not confirmed in a retrospective analysis of five patients with advanced gallbladder or CCA harbouring HER2 mutations or overexpression and treated with anti HER2therapy: while patients with gallbladder tumours beneficiated from therapy, no response among patients with CCA was observed and all the patients progressed while on treatment with trastuzumab [29].

2.1.4. Anti-HER2 TKIs: lapatinib

Lapatinib is an orally active, dual inhibitor of HER2 and EGFR approved in breast cancer patients. In *in vitro* studies lapatinib demonstrated superior inhibiting activity than trastuzumab in CCA cell lines [26].

Despite preclinical evidences, lapatinib did not show any activity in CCA. A phase II study with lapatinib was stopped early as no clinical response has been observed in the first nine enrolled patients [30], whereas in another study the response rate of 17 patients with CCA was 0%, with a median PFS of 1.8 months and a median OS of 5.2 months [31]. It has to be underlined, however, that in both studies no biomolecular selection has been made before patients enrolment.

2.2. Novel anti-HER agents

2.2.1. ASLAN001

ASLAN001 (Aslan Pharmaceuticals, Array-Biopharma), a novel oral pan-HER inhibitor, has shown clinical activity in both HER2-positive and EGFR-positive tumours. ASLAN001 is currently under development in HER2 expressing-cancers such as breast and gastric cancers.

A phase II multicentre study is recruiting patients with advanced or metastatic CCA (excluding peribiliary glands) who progressed after at least 1 line of systemic therapy to receive ASLAN001. Primary endpoint of the study is ORR, and secondary endpoints are the description of the safety

profile of the drug, duration of response, PFS, OS, and DCR according to EGFR/HER2 status of the tumours (NCT02609958). The study will enrol a total of 25 patients in Asian countries, and it will be stopped if any response is observed in the first 10 patients. Recruitment has started in October 2015 and it is estimated to end in June 2017.

No information on ASLAN001 safety profile is yet available.

3. mTOR inhibitors

3.1. Everolimus

The mTOR pathway is known to be up-regulated in many cancer types, and preclinical evidences indicate that its inhibition may be effective in the treatment of CCA [32,33]. To date, everolimus is authorized for the treatment of patients with advanced breast cancer, neuroendocrine tumour and renal cell carcinoma. [34-37].

Few experiences of anti mTOR therapy in patients with CCA have been reported. A phase II Italian study (EUDRACT 2008-007152-94) explored the role of everolimus 10mg daily in locally advanced or metastatic CCA patients refractory to standard chemotherapy. Primary end points of the study were DCR and ORR that resulted to be 44.7% and 5.1%, respectively. One patient had a partial response and one had a complete response lasting more than 8 months. Median PFS was 3.2 months, median OS was 7.7 months, and median TTP was 2.0 months [38]. A phase I study evaluated the combination of everolimus with gemcitabine (Cohort I) and with gemcitabine and cisplatin (Cohort II) in patients with advanced tumours (including CCA) refractory to standard chemotherapy. As an expansion cohort, 10 patients with advanced gallbladder cancers were treated with the maximally tolerated dose of cohort II: everolimus 5 mg on Monday/Wednesday/Friday, gemcitabine 600 mg/sqm, and cisplatin 12.5 mg/sqm. In this latter subgroup, six patients experienced a disease stabilization and four progressed [39]. A phase I trial conducted at Mayo

Clinic in Rochester (USA) is exploring the safety profiles of sirolimus with gemcitabine and cisplatin in patients at high risk for cholangiocarcinoma recurrence after liver transplant or surgery (NCT01888302). Trial is ongoing and estimated primary completion date is September 2016.Two phase II studies evaluating the efficacy of everolimus as monotherapy in CCA patients initiated in 2009 (NCT00973713) and in 2012 (NCT01525719). No information on the results of these two trials is yet available.

Everolimus is generally well tolerated, with most common adverse events being stomatitis, rash, fatigue, diarrhoea, nausea, and decreased appetite. A less common, life-threatening adverse event is non-infectious pneumonitis (presenting as an acute deterioration in respiratory function with ground glass–appearing or patchy opacities on computed tomography scans).

4. Anti neoangiogenic therapies

4.1. Monoclonal antibodies: bevacizumab and ramucirumab

Bevacizumab (Avastin™, Roche) is a recombinant, fully humanized iGg1 monoclonal antibody against VEGF A isoform approved for the treatment of patients with metastatic colorectal cancer, renal cell carcinoma, glioblastoma and ovarian cancer [40-43].

As far as CCA is concerned, the role of bevacizumab in combination with gemcitabine and oxaliplatin was explored in a phase II study. Study endpoint was not reached as 6-month PFS rate was 63% (target: 70%), whereas median PFS was 7 months, ORR 40%, and DCR 69% [44]. The combination of FOLFIRI (fluorouracil, folinic acid, and irinotecan) with bevacizumab as second-line treatment in patients with metastatic intrahepatic cholangiocarcinoma resulted in one complete response, four partial response, and six stable disease for an ORR of 38.4% and a DCR of 84.5%.The treatment was well tolerated [45].

The combination of bevacizumab and erlotinib was explored as first-line treatment in 53 patients with metastatic or unresectable biliary tumors. Among the 49 evaluable patients, six (12%) had a confirmed partial response, 25 a disease stabilization (51%), and 18 progressed. Median OS and TTP were 9.9 and 4.4 months, respectively [46]. The same combination was furtherly tested in 102 CCA patients with refractory tumours. ORR was 6%, median PFS 2.2 months and OS 4.3 months [47].

Concerning experimental studies, a multicentre phase II study of gemcitabine, capecitabine and bevacizumab as first-line treatment for locally advanced or metastatic adenocarcinoma of the gall bladder or biliary ducts recruited 50 patients in USA (NCT01007552). Study was terminated on May 2015 and results are not yet available. A phase I study aimed to evaluate safety profile of proton therapy and concurrent bevacizumab biotherapy (NCT00426829). This trial was terminated early due to low accrual and no safety data are available.

Ramucirumab (Cyramza™, Eli Lilly) is a fully human IgG1 monoclonal antibody targeting the extracellular domain of VEGFR-2. Preclinical models showed that ramucirumab might selectively bind to and inhibit the human VEGFR-2 with a much greater affinity than its natural ligand, resulting in significant antitumor activity in a wide range of malignancies [48]. Ramucirumab is approved for the treatment of NSCLC, gastric and colorectal cancer patients. A phase II trial is studying ramucirumab for advanced pre-treated biliary cancers. Recruitment of 50 patients started on December 2015 and is expected to end on December 2019 (NCT02520141).

4.2. Multi-TKI: cediranib, regorafenib, sorafenib, sunitinib, pazopanib

Cediranib (AstraZeneca) is a potent TKI inhibiting all three VEGF receptors (VEGFR-1, -2, -3), c-kit, and platelet derived growth factor receptor alpha and beta (PDGFR-α, PDGFR-β). It inhibits VEGF-induced angiogenesis showing anti-neoplastic effects in a range of tumour xenograft mouse models [49]. Cediranib has shown promising results in several phase-I clinical trials in patients with

various solid tumours [50]. A multicentre, placebo-controlled, randomised phase II study assessed the effect on PFS of the combination of cisplatin and gemcitabine with oral cediranib 20 mg once daily or placebo in 124 chemo-naïve patients with locally advanced or metastatic CCA. After a median follow-up of 12.2 months, median PFS was 8·0 months in the cediranib group and 7·4 months in the placebo group (HR 0·93, 95% CI 0·65-1·35; p=0·72). Patients who received cediranib had more grade 3-4 toxic effects: hypertension (37% vs21%; p=0·05), diarrhoea (13% vs 3%; p=0·05); and fatigue (24% vs 11%; p=0·04) [51].

Regorafenib (STIVARGA™, Bayer) is an oral multikinase inhibitors with activity against selected tyrosine kinases (VEGFR2-3, TIE-2, PDGFR, FGFR, RET and c-Kit) as well as a signal transduction inhibitor of the RAF/MEK/ERK pathway. It demonstrated preclinical and clinical activity in several tumours, including colorectal cancer for which treatment of refractory patients has been authorized [52,53]. Three phase II trials are now exploring the role of regorafenib in patients with metastatic or unresectable CCA as single agent in refractory tumours (NCT02115542), as second-line treatment (NCT02053376) or in combination with chemotherapy (GEMOX) in first-line setting (NCT02386397). Results are waited for the end of 2018. Toxicity profile of regorafenib is similar to that of other multi TKI targeting mainly VEGFR, and in particular fatigue, hypertension, and hand-foot skin reactions.

Sorafenib (Nexavar™, Bayer) is approved for the treatment of patients with hepatocarcinoma and metastatic renal cell cancer[54,55]. The activity of sorafenib as first-line treatment in patients with gallbladder carcinomas was evaluated in a phase II trial terminated after the first stage of accrual of 25 patients as no response was recorded. Median PFS was 3 months and median OS was 9 months [56]. A Phase I/II study explored the combination of GEMOX with sorafenib in CCA patients. Due to treatment toxicity, study was terminated after completion of the phase I (9 patients enrolled) and thus no clinical information is available. Toxicities were predominantly gastrointestinal (vomiting, diarrhoea, dehydration), fever, peritoneal infection and dyspnoea. (NCT00955721). The

concomitant administration of sorafenib and erlotinib in patients with advanced gallbladder carcinoma or CCA resulted toxic (SAE were reported in 50% of the patients including a toxic death) with poor anti-tumour activity as median PFS was 2 months and median OS 6 months (NCT 01093222).

Sunitinib (Sutent™, Pfizer) is a multi TKI mainly inhibiting VEGFR family [57] approved for the treatment of patients with GIST, metastatic renal cell carcinoma, and pancreatic neuroendocrine tumours [58-60]. A single phase II study of sunitinib as second-line treatment in patients with IH carcinomas has enrolled 51 patients in French institutions. Primary endpoint is OS and study completion date is estimated to be September 2016 (NCT01718327).

Pazopanib (Votrient™, Novartis) is approved for the treatment of patients with metastatic renal cell carcinoma and soft tissue sarcoma [61,62]. A phase II, single-arm study is assessing the impact of pazopanib and gemcitabine on the overall response rate of 46 patients with unresectable or metastatic CCA. This study is conducted in Greece, started on June 2009 as is expected to end in November 2016 (NCT01855724). A phase I study aims to describe the safety profile of pazopanib and trametinib, a MEK inhibitor in patients with advanced solid tumours, including CCA. The study is expected to enrol a total of 111 patients. Results are awaited as study was supposed to end on April 2016 (NCT01438554). The most common toxicities of pazopanib are similar to those reported for sorafenib and sunitinib, with gastrointestinal effects (nausea, vomiting, diarrhoea) and fatigue as the most frequent side effects

5. Anti FGFR therapy

5.1. BGJ398 (infigratinib)

Preclinical studies have demonstrated that aberration in fibroblast growth factor receptor (FGFR) activity is implicated in the development and progression of CCA and other malignancies. Thus, its

inhibition might have an important therapeutic potential as demonstrated by the pan-FGFR inhibitor BGJ398 (Infigratinib, Novartis Pharmaceuticals) either in CCA cell lines and in murine models [63]. Moreover, FGFR2 fusion events are present in up to 17% of intrahepatic CCAs and appear to predict sensitivity to FGFR inhibitors even after progression on chemotherapy [64]. On this basis, a phase II multicentre, single arm study of oral BGJ398 in patients with advanced or metastatic CCA with FGFR2 gene fusions or other FGFR genetic alterations who failed or are intolerant to platinum-based chemotherapy is ongoing. Primary endpoint is ORR and accrual of 55 patients is expected to end on July 2018 (NCT02150967).

5.2. ARQ087

ARQ087 (ArQule) is an orally bioavailable compound and a dual kinase inhibitor that binds to inactive form of FGFR1 and FGFR2 and potently inhibits the active form of FGFR1 and FGFR2. A phase 1/2 study evaluating the potential role of ARQ087 in patients with advanced solid tumours (including CCA) with FGFR genetic alterations is ongoing. The study is designed to explore the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary efficacy of ARQ087 as single agent in 120 patients refractory to standard therapies and is expect to end on June 2017 (NCT01752920).

6. Other oral inhibitors

6.1. ALK inhibitors

Ceritinib (Zykadia™, Novartis) is an oral, small-molecule, ATP-competitive, tyrosine kinase inhibitor of anaplastic lymphoma kinase (ALK) approved for the treatment of ALK-positive non-small cell lung cancer (NSCLC) patients failing crizotinib. The potential role of ceritinib in ALK-activated gastrointestinal tumours is being explored in two phase II studies, the first including patients with ALK-activated gastrointestinal malignancies refractory to standard chemotherapies,

the second including ROS1 and /or ALK over-expressed advanced intrahepatic or hilar CCA. (NCT02638909 and NCT02374489). Both studies started on 2015 and preliminary results are waited for the end of 2017-beginnig 2018. Ceritinib toxic effects included diarrhoea, vomiting, dehydration, elevated aminotransferase levels, and hypophosphatemia.

Another selective, ATP-competitive ALK TKI is Entrectinib (Ignyta). This small molecule is an orally bioavailable, pan-Receptor tyrosine kinases and ROS1 inhibitor. In pre-clinical models, entrectinib has shown antitumor efficacy in ALK- and ROS1-driven tumours [65]. In humans, a Phase I dose-escalation study (ALKA-372-001) in subjects with previously treated, advanced solid tumours harbouring neurotrophic receptor tyrosine kinase (NTRK)1, ROS1 or ALK alterations resulted in 22% of ORR [66]. A Phase 1/2 study is assessing the role of entrectinib in subjects with previously treated, locally advanced or metastatic solid tumours including CCA with NTRK1 -2 -3, ROS1 or ALK molecular alterations (NCT02568267). This study will enrol 300 patients and is expected to end in 2018.Entrectinibis generally well tolerated, with side effects comprising asthenia, paraesthesia, nausea, myalgia, dysgeusia, vomiting, arthralgia, diarrhoea and attention disturbance.

6.2. Anti ABL

Bosutinib (Bosulif™, Pfizer), a potent ATP-competitive dual Src/AblTKI, is approved for the treatment of Philadelphia chromosome-positive chronic myeloid leukaemia patients and demonstrated anti-tumour activity against several tumour xenograft [67-69]. In clinical trials, while bosutinib showed promising efficacy prolonging TTP in pre-treated patients with locally advanced or metastatic breast cancer [70], its unfavourable risk-benefit ratio and limited activity in other malignancies (including CCA) did not warrant further investigation [71,72].

Imatinib (Glivec™, Novartis) is the first TKI authorized for the treatment of patients with CML and GIST. Its impressive activity in those two malignancies led to explore its role in other tumours. A

phase II study is testing the association of imatinib with 5-FU/leucovorin in patients with advanced carcinoma of the gallbladder and bile duct. The study has been completed in 2010 but no results have been published, yet (NCT01153750). Finally, a bicentric phase II trial of imatinib as second-line treatment in CCA patients was terminated prematurely due to poor recruitment rate and very limited efficacy [73].

6.3. MEK inhibitors

Trametinib (Mekinist™, GlaxoSmithKline) is a mitogen-activated, extracellular signal-regulated kinase (MEK) inhibitor 1 and 2 approved for the treatment of patients with tumours harbouring BRAF V600E or V600K gene mutations. Focusing on patients with CCA, as already mentioned, a phase I study is exploring the association of trametinib with pazopanib (see pazopanib section; NCT01438554), whereas a randomized phase II trial is aiming to assess the role of trametinib alone or in combination with chemotherapy in patients with refractory CCA (NCT02042443). The study recruited 89 participants and results are awaited for the end of 2016. The most common adverse events associated with trametinib are rash, diarrhoea, peripheral oedema, fatigue, and dermatitis.

Another oral, selective inhibitor of the MEK1/MEK2 kinases is selumetinib (Astra Zeneca) in development particularly for NSLCL tumours [74,75]. Very recently, the results of a phase I study of selumetinib with cisplatin and gemcitabine in CCA patients have been published: dose limiting toxicity (DLT) was 75 mg twice daily, among eight evaluable patients three had a partial response and five stable disease, with a median PFS of 6.4 months [76]. Selumetinib is not currently under development in CCA patients. The most common toxicities observed were rash, mild to moderate diarrhoea, fatigue and oedema.

6.4. PI3K inhibitors/AKT inhibitors

Buparlisib (Novartis) is a pan-PI3K inhibitor preventing the PI3K–AKT–mTOR pathway activation [77] that demonstrated preliminary activity in preclinical models of solid tumours [78]. In clinical

studies, some results have been reported in lymphoma and in breast cancer patients. A phase II trial in patients with various tumours (including CCA) harbouring PI3K activating mutations has been proposed in 2011, but it was closed prematurely due to lack of accrual (NCT01501604). The most common treatment-related adverse events reported were rash, increased transaminase levels, increased blood insulin levels, increased eosinophil, and hyperglycaemia.

MK2206 (Merck) is an oral pan-AKT inhibitor showing some activity in biliary cancer tumour models [79,80]. Promising results have been reported in clinical studies enrolling patients with breast cancer and multiple myeloma. A phase II study in refractory CCA patients treated with MK2206 as monotherapy reported two disease stabilization out of eight treated patients. Authors concluded that even though therapy was well tolerated, MK2206 alone has limited activity in this patient subset and must be eventually be tested in combination with other target agents or standard chemotherapy [81].

6.5. Farnesyltransferase inhibitor

Lonafarnib (Merck) is an oral selective farnesyltransferase inhibitor (FTI) [82]. In preclinical studies, lonafarnib has shown to be active against a broad spectrum of tumour cell lines and tumour xenografts in nude mice [83]. Contrasting results have been reported in patients with breast cancer, glioblastoma, head and neck cancer, and ovarian cancer. A randomised phase II trial aiming to compare lonafarnib with surgery vs surgery alone in patients with primary liver cancers including CCA was proposed in 2011. The trial has been withdrawn prior to enrolment (NCT00020774)

Another orally bioavailable non-peptidomimetic FTI is Tipifarnib (Zarnestra™, Johnson & Johnson) which demonstrated preclinical and clinical activity against acute myeloid leukemia (AML) and breast cancer [84]. A phase I study of tipifarnib and herceptin in patients with advanced cancer including CCA enrolled 24 patients (NCT00005842). Preliminary safety results were

presented at the Annual meeting of the American Society of Clinical Oncology (ASCO) in 2001 [85]. The most common grade 3-4 toxicities included neutropenia, nausea, and vomiting.

6.6. Proteasome inhibitor

The proteasome inhibitor bortezomib (Velcade™, Janssen-Cilag), demonstrated tumour cell growth suppression and apoptosis in several tumour cell lines [86]. While it has been approved for the treatment of multiple myeloma (MM) and mantle cell lymphoma (MCL) patients, bortezomib treatment in solid tumours have generally produced less promising results as it failed to demonstrate clinical activity in NSCLC, head and neck cancer, ovarian cancer, hepatocellular cancer, gastric cancer, colorectal cancer, and prostate cancer patients. A phase II trial treated patients with unresectable or metastatic gallbladder or CCA with bortezomib as single agent. A Simon two-stage design was used. The trial was discontinued early because after the first 20 patients only one unconfirmed partial response was recorded. Median TTP was 5.8 months and median OS was 9 months with 6-month and 1-year survival rates of 70% and 38%, respectively. Author concluded that despite the treatment did not result in objective response, the rate of stable disease and time to progression benchmark is encouraging, so that further development of bortezomib in combination with other therapies should be considered in this setting [87]. The most frequently reported AEs were thrombocytopenia and peripheral neuropathy.

7. Multitarget inhibitors

Vandetanib (CAPRELSA™, AstraZeneca) is an orally available receptor TKI that inhibits EGFR, VEGFR-2, RET, BRK, Tie2, members of the EPH receptor and Src kinase families in tumour cells and endothelial cells and that demonstrated some anti-tumour activity in preclinical studies [88].

Vandetanib showed encouraging clinical activity in various tumours and it is approved for the treatment of patients with medullary thyroid cancer. Its role in CCA patients has been evaluated in

a phase II study randomizing advanced patients to receive vandetanib 300 mg monotherapy versus vandetanib 100 mg plus gemcitabine versus gemcitabine plus placebo. The primary end point was PFS; secondary end points were ORR, DCR, OS and safety outcomes. A total of 173 patients were recruited at 19 centres across Italy. Median PFS were 105 days, 114 days and 148 days, respectively ($p = 0.18$). No statistical difference between treatments was observed for secondary end points. Finally, the proportion of patients reporting adverse events was similar for the three groups (89.3%-96.6%) [89]. The most common adverse events include diarrhoea, rash, nausea, hypertension, fatigue, abdominal pain, hypocalcaemia, hypoglycaemia, and QT prolongation. More rarely, vandetanib administration could be linked to the onset of interstitial lung disease, ischemic cerebrovascular events, serious haemorrhagic events, heart failure, hypothyroidism, hypertension, and reversible posterior leukoencephalopathy syndrome.

Merestinib (LY2801653, Lilly) inhibits MST1R, FLT3, AXL, MERTK, TEK, ROS1, DDR1/2, MKNK1/2, MET, and HGF [90,91]. A phase II study is randomizing patients with advanced CCA to receive cisplatin, gemcitabine and either ramucirumab or merestinib as first-line treatment. The primary endpoint of the trial is PFS, the main secondary endpoints OS and ORR. Enrolment of 300 patients started on May 2016 and is expected to end on April 2018 (NCT02711553). Finally, a phase Ia/Ib study of ramucirumab in Combination With merestinib or abemaciclib in advanced cancers has been opened in April 2016 and results are waited for August 2019 (NCT02745769)

Cabozantinib (Cometriq™, Exelixis) is a TKI mainly inhibiting MET with activity against VEGFR2, RET, AXL, KIT, FLT-3 and TIE-2 kinases. Preclinical efficacy appears to be associated with its inhibitory effects against both MET and VEGFR [92]. Cabozantinib has demonstrated to be active in patients with HCC, NSCLC, metastatic castrate-resistant prostate cancer (CRPC) and metastatic medullary thyroid cancer for which this compound has been approved by regulatory agencies [93]. A phase II study administered cabozantinib as single agent in 44 pre-treated patients with locally advanced or metastatic IH or EH carcinomas. Results are expected on July 2016

(NCT01954745). Reported toxicities of cabozantinib included fatigue, mucositis, diarrhoea, anorexia, nausea/vomiting, dysphonia, hypertension, hand-foot syndrome and increased risk of haemorrhage.

Dasatinib (Sprycel™, Bristol-Myers Squibb) is a TKI inhibiting SRC/ABL, c-KIT, and PDGFR α and β [94]. In preclinical studies, it has been shown to be active against triple-negative breast cancer, gastric, pancreatic, head and neck, and lung cell lines [95,96]. Clinical studies have evaluated dasatinib activity in breast, prostate, melanoma, head and neck, and colorectal cancer patients. As far as CCA is concerned, a phase II trial of dasatinib in patients with isocitrate dehydrogenase (IDH)-mutant tumours is ongoing, primary endpoint being ORR, and main secondary endpoints being PFS and OS(NCT02428855). The study started on April 2015 and is expected to end in 2022, after having recruited 19 patients. Dasatinib was generally well-tolerated with side effects occurring in a small group of patients. Toxicities included abdominal pain, diarrhoea, nausea, vomiting, gastrointestinal bleeding, dyspepsia, pleural effusion, dyspnoea, cough, headache, peripheral neuropathy, left ventricular dysfunction, cardiac failure, cardiomyopathy, diastolic dysfunction, and QT-interval prolongation.

8. Novel agents

DKN-01 (Leap Therapeutics) is a humanized monoclonal antibody neutralizing Dickkopf-related protein 1 (Dkk-1) and thus inhibiting the Wnt/β-catenin pathway [97]. This drug has shown promising activity in preclinical studies in breast, melanoma and myeloid leukemia cell lines [98-100]. DKN-01 is under investigation in different clinical trials, including a phase I study designed to evaluate safety, tolerability, pharmacokinetics, and anti-tumour activity of DKN-01 in combination with gemcitabine and cisplatin in patients with CCA (NCT02375880). Preliminary safety results of the study (dose finding – Part A) have been presented at ASCO 2016 and indicated

300 mg as the MTD of DKN-01, with neutropenia as the main side effect. Among the three patients receiving the MTD of DKN-01, one obtained a clinical response and two a disease stabilization [101]. Study part B (expansion cohort) is ongoing and is expected to end in September 2017, after having enrolled 32 patients.

Saracatinib (AZD0530, Astra Zeneca) is a Src-family (including Fyn, Lyn, and Src) kinases inhibitor with *in vitro* activity against breast, gastric, ovarian, and sarcoma cell lines as well as in mouse models of prostate cancer [102-104]. Saracatinib on CCA cell lines and in mouse models demonstrated to counteract the activation of Src and of its downstream effectors, increasing the fraction of cells in G(0)-G(1) phase, and inhibiting cell migration, whereas at higher concentrations it inhibits CCA cell proliferation. In clinical setting, sarcatinib as single agent has been tested in patients with breast, NSCLC, colorectal, renal, gastric, pancreatic, melanoma, and head and neck cancers [105-107]. The results obtained in these studies, however, were not encouraging. Thus, sarcatinib is currently being evaluated in combination with other target agents or conventional chemotherapy. The combination of sarcatinib and cediranib has been tested in a phase I study enrolling patients with advanced, pre-treated solid tumours including CCA. All cediranib doses were tolerated; however with saracatinib 175 mg/day, cediranib 20 or 30 mg/day was more sustainable than 45 mg/day. The most common adverse events were hypertension (67%), diarrhoea (62%), dysphonia (46%) and fatigue (39%). Twenty-two out of 35 evaluable patients had stable disease as the best clinical response [108].

Exerin (ADH-1, Adherex Technologies) is an N-cadherin inhibitor that demonstrated some preclinical activity against breast, melanoma, and prostate cancer cells [109-111].In the clinical setting, a phase I study is evaluating the toxicity profile and the MTD of ADH-1 in combination with gemcitabine and cisplatin in patients with unresectable or metastatic pancreatic and CCA cancers (NCT01825603). The study will enrol 24 patients and it is estimated to end in 2018.

Silmitasertib (CX-4945, Senhwa Biosciences) is an orally available highly selective inhibitor of CK2, a constitutively active, ubiquitous serine/threonine kinase [112]. In *in vitro* studies it has been demonstrated to be active against breast cancer and NSCLC [113]. Silmitasertib can be orally administered safely with some reported encouraging clinical activity as a single agent with 15% of the patients with disease stabilization lasting more than 6 months [114]. A phase I/II study is evaluating the association of silmitasertib with gemcitabine and cisplatin in the frontline treatment of patients with advanced CCA (NCT02128282). Results are waited for the first part of 2017.

Mutations in mitochondrial isocitrate dehydrogenase (IDH) were shown in preclinical studies representing a potential target for anti-tumour agents. AG 881 (Agios and Cellgene), an orally available pan-IDH mutant inhibitor has shown to fully penetrate the blood-brain barrier and to inhibit isocitrate dehydrogenase-1 (IDH1) and IDH2 mutations in cancer models. A phase I, multicentre, open-label study, aimed to assess safety, pharmacokinetic, pharmacodynamics, and clinical activity of AG-881 in patients with advanced solid tumours, including CCA, with IDH1 and/or IDH2 mutation is recruiting patients (NCT02481154). The study started on May 2015 and is expected to end in October 2018, after having enrolled 150 patients. Similarly a selective IDH1 inhibitor (AG 120) is being evaluated in patients with advanced solid tumours (including CCA) harbouring IDH1 and/or IDH2 mutation (NCT02073994). Results of this study are expected for the end of 2016.

OXY111A (NormOxyx) is a novel allosteric modulator of affinity of oxygen to haemoglobin, enhancing oxygen delivery to hypoxic tissues. OXY111A demonstrated promising results in preclinical studies of colorectal and pancreatic cancer [115,116]. The safety profile and the activity of OXY111Ais currently investigated in a phase IB/IIA study enrolling patients with hepato-pancreato-biliary neoplasia. The trial is still recruiting patients and is expected to end on December 2016. (NCT02528526)

PLX 8394 is an orally available, potent and selective inhibitor of BRAF V600E exhibiting promising results in melanoma resistant cells [117]. Two phase I/II studies are ongoing to evaluated the safety profile and clinical activity of the drug in refractory, advanced solid tumours, including CCA. Results a are waited for 2017 (NCT02012231 - NCT02428712).

Veliparib (ABT-888, AbbVie) is an orally bioavailable PARP-1/2 inhibitor that significantly potentiated the anti-neoplastic effect of several cytotoxic agents including temozolomide, platinum, and irinotecan in preclinical models [118,119]. In clinical studies it showed a favourable safety profile with encouraging clinical outcomes in colorectal, breast, prostate, melanoma, NSCLC, and ovarian cancer patients [120-122]. A phase I study evaluating the efficacy and safety of veliparib, cisplatin and gemcitabine combination therapy in patients with advanced CCA, pancreatic, urothelial, or NSCLC has been terminated on February 2013, but results have never been published yet (NCT01282333). The most frequent adverse events are thrombocytopenia, anaemia, neutropenia, fatigue, nausea and vomiting.

RRx-001 (EpicentRx) is a structurally unique pharmacophore that inhibits multiple epi-enzymes and independently affects the apoptosis pathway and reactive oxygen and nitrogen species (RONS) production. RRx-001 is not cross-resistant with approved therapies and selectively targets and re-sensitizes hypoxic tumour cells to immunotherapy, chemotherapy and radiotherapy. Moreover, RRx-001 modulates tumour blood flow, hypoxia and vascular function triggering apoptosis in cancer cells [123]. In a phase I study, RRx-001 demonstrated encouraging antitumor activity including re-sensitization to formerly effective chemotherapy while exhibiting a benign safety profile in heavily pre-treated patients with relapsed/ refractory solid tumours [124]. CCA tumours initially responding to cisplatin and gemcitabine and then become resistant are the target population of a phase II study exploring whether therapy with RRx-001 may re-sensitize tumours to the same cisplatin and gemcitabine regimen. RRx-001 is administered intravenously weekly, for six weeks. At the end of this period, chemotherapy is introduced and in case of response, is continued as long

as tumour respond. The primary objective of this clinical trial is to evaluate PFS at 9 weeks after the reintroduction of gemcitabine and cisplatin. A total of 30 patients will be enrolled and the study is expected to end on May 2018 (NCT02452970).

Becatecarin (Helsinn Healthcare) is a synthetic diethylaminoethyl analogue of the indolocarbazole glycoside antineoplastic antibiotic rebeccamycin. Becatecarin intercalates into DNA and stabilizes the DNA-topoisomerase I complex, thereby interfering with the topoisomerase I-catalysed DNA breakage-reunion reaction and initiating DNA cleavage and apoptosis [125]. In clinical setting, becatecarin has shown promising results in colorectal, renal, and lung cancer patients as well as in CCA patients [126]. In a phase II study enrolling patients with advanced CCA it obtained an ORR of 5% and a DCR of 40% [127]. Following these encouraging results, a randomized, phase III multicentre, open label study of becatecarin versus 5-FU plus leucovorin in 248 patients with advanced CAA was terminated on November 2006. (NCT00090025). Results have never been reported. Becatecarin was well tolerable and hematologic toxicity was the most common side effects. Non-hematologic toxicities were moderate and included weakness/fatigue, nausea/vomiting, diarrhoea and anorexia.

9. Conclusion

A large number of target agents have been explored in patients with locally advanced or metastatic biliary cancers (Table 3). Notwithstanding the strong biomolecular rationale and preclinical results, none of them have demonstrated a sufficient clinical activity to be authorized in the clinical routine. Several reasons may account for these disappointing results. First of all, CCA is a rare neoplasms and thus enrolment of a large number of patients is arduous. As a consequence, it is easier to demonstrate a clinical activity of an antineoplastic compounds with a less specific mechanism of action rather than a target therapy that may be beneficial only in a selected subpopulation of

patients. In this sense, in an unselected population multitarget TKI should be more beneficial than specific inhibitors. However, preliminary results of vandetanib did not demonstrate superiority of the drug when compared to chemotherapy. Moreover, the low number of patients prompted researchers to enrol in their studies all CCA patients, regardless of the site of the tumour. This may jeopardize results as the mutational status of intrahepatic, extrahepatic and gallbladder cancers are not similar. In a recent work presented at ASCO 2016, in fact, it has been shown that MET overexpression was detected only in intrahepatic CCA, whereas extrahepatic CCA were more frequently RAS mutated and gall bladder carcinomas were more frequently HER2 amplified [128]. Thus, any target therapy administered without a patient selection according to the biological tumour profile may results in different outcomes simply on the basis of unbalances between these three groups of tumours.

In conclusion, while waiting for novel compounds with interesting mechanisms of action, no new agent improved clinical outcome of locally advanced or metastatic CCA patients either as single agent or combined to chemotherapy. A better biological characterization of these tumours is fundamental to guide new clinical trial.

10. Expert opinion

Cholangiocarcinoma is a rare neoplasm harbouring complex and multiple gene mutations and/or expressions. Thus, beside the well-established role of chemotherapy several specific inhibitors have been tested. The most studied compounds are cetuximab and panitumumab. Unfortunately, these anti-EGFR monoclonal antibodies did not confirm exciting preclinical findings. One possible explanation for these disappointing results may relay on the lack of a patient selection. Making a parallelism with the scientific history of anti-EGFR antibodies in the treatment of patients with advanced colorectal cancers, first results in an unselected population were unsatisfactory. However,

drug efficacy was dramatically improved after selection according to KRAS and then to NRAS status. In CCA patients, cetuximab was tested in patients regardless their RAS status. Some Authors tried to make a retrospective subgroup analysis, however comparison were made with very limited number of patients and thus results has to be considered as inconclusive. Panitumumab was tested in patients with KRAS wild type tumours. Notwithstanding this patient selection, results were negative. It is a good matter of debate wondering whether these disappointing findings would have been comparable in a RAS wild type population. Another possible explanation could be the choice of the biomolecular marker. It has been recently published a comprehensive review in which EGFR expression has been identified as a prognostic parameter [129]. This finding is very interesting as it gives rise to an intriguing question: should we continue to stratified CCA patients according to the RAS status or should we group patients according to EGFR expression? And in this latter case, which could be the positivity threshold? Future researches should be directed in defining predictive indicators for anti-EGFR therapies.

Beside some positive findings obtained with anti-EGFR therapy, no striking results are waited for the novel therapies currently tested and summarized in Table 3. No clinically relevant outcomes have been described for anti-HER2, anti-VEGF, anti-ABL agents and mTor inhibitors. Continuing the parallelism with colorectal cancer, some promising preliminary activity could be waited for the multitarget TKIs such as regorafenib, cabozantinib, merestinib, and dasatinib. In fact, while specific inhibitors (for instance anti-EGFR and anti-VEGF) had to be combined with chemotherapy, these multitarget compounds demonstrated to be active when administered as single agents. Finally, despite interesting mechanisms of action, no outstanding results are waited for the novel compounds listed at the end of Table 3.

In this rare neoplasm the future challenge is the discovery of biomolecular alterations which drive tumour progression and aggressiveness in order to focus on selected patients avoiding resource consuming and wasting. In this sense, next-generation sequencing (NGS) survey of biliary tract

cancers may help in determining which is the leading mutation of each single tumour, permitting physician to personalize therapy in the very next future. Some correlations between NGS and clinical outcome have already been reported [130]. The limitation of this techniques is mainly the cost. However, the standardization of a panel of genetic alteration automatically detected may reduce costs permitting to a larger spectrum of tumours to be analysed [131]. Some exciting data are already available for NSCLC patients [132]. This technique will also facilitate the description of possible multiple mutations driving tumour progression requiring various specific inhibitions and, maybe, the mechanisms of acquired drug resistance.

The main limitation of patient selection according to relative rare gene mutations is that accrual of a particular population of CCA patients is somehow impossible without the involvement of a large number of institutions. As a consequence, when designing a new trial researchers must choose whether collaborate with a large number of institutions (thing often difficult and expensive in practice) or enrol unselected patients (easier in practice). Testing a new compound in an unselected population, however, could result in negative results even if a particular agent is very active in a particular subgroup of patients. Thus, it could be hypothesized that some novel therapies already explored in unselected patients and considered as ineffective could be very active in selected ones. This was the case, for instance, for gefitinib in EGFR mutated adenocarcinoma of the lung. Thus, even though international collaborations are difficult, this will be the best way to explore particular gene inhibition. Some trials have been designed in this sense, aiming to explore the clinical usefulness of specific inhibitors in selected patients (such as those with tumours harbouring ALK, ROS1, FGFR, NTRK, and IDH genetic alterations). Although many of these studies will screen a large population to enrol a relative small number of patients, their results will be very interesting and will pave the way for new stimulating clinical trials.

Finally, immunotherapy has gain extreme importance in the last few years, especially in patients bearing metastatic melanoma, renal cell carcinoma and other several solid tumours. It will be of great interest to explore whether this class of agents may be beneficial also in patients with CCA.

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Table 1. Key results of trials exploring cetuximab in locally advanced or metastatic CCA patients

Author, year [ref]	Schedule	Phase	# patients	Results	Primary aim Met?
Chang, 2010 [8]	Cet-containing chemo	Retrospective	5	1 CR + 3 PR + 1 SD PFS 4-16 months	NA
Borbath, 2013 [9]	Cet + Gem	II	44	6 months PFS = 47% OS = 13.5 months ORR = 20.4% DCR = 79.5%	6 months PFS Met
Malka, 2014 [10]	GEMOX ± Cet	II Randomised	150	GEMOX vs GEMOX + Cet PFS = 5.5 vs 6.1 months OS = 12.4 vs 11.0 months	NA
Paule, 2007 [11]	GEMOX + Cet	Retrospective	9	In IH CCA only GEMOX pretreated 2 PR (ORR = 22%)	NA
Gruenberger, 2010 [12]	GEMOX + Cet	II	30	60% of the patients had IH CCA ORR = 63%	ORR Met
Chen, 2015 [13]	GEMOX ± Cet	II Randomised	122	GEMOX + Cet vs GEMOX ORR = 27% vs 15% (p=0.12) PFS = 6.7 vs 4.1 months (p=0.05) OS = 10.6 vs 9.8 months (p=0.91)	ORR Not Met

Cet = Cetuximab; Gem = Gemcitabine; GEMOX = Gemcitabine and oxaliplatin; ORR = Overall Response Rate; DCR = Disease Control Rate; CR = Complete Response; PR = Partial Response; SD = Stable Disease; OS = Overall Survival; PFS = Progression Free Survival; NA = Not Assessable

CCA = Cholangiocarcinoma; IH = Intrahepatic

Table 2. Key results of trials exploring panitumumab in locally advanced or metastatic CCA patients

Author, year [ref]	Schedule	Phase	# patients	Results	Primary aim Met?
Jensen, 2012 [15]	GEMOX + Pan + Capecitabine	II	46 KRAS wt	6-month PFS = 71% PFS = 8.3 months ORR = 33% DCR = 86% OS = 9.8 months	6-month PFS Met
Hezel, 2014 [16]	GEMOX + Pan	II	31 KRAS wt	ORR = 45% PFS = 10.6 months OS = 20.3 months	ORR Not Met
Sohal, 2013 [17]	Gem + Pan + irinotecan	II	35	5-month PFS = 69% ORR = 31% PFS = 9.7 months OS = 12.9 months	5-month PFS Met
Leone, 2016 [18]	GEMOX ± Pan	II Randomised	89 KRAS wt	GEMOX + Pan vs GEMOX PFS = 5.3 vs 4.4 months (p= 0.27) OS = 9.9 vs 10.2 months (p=0.42) ORR = 26.7% vs 18.2% (p=0.99)	PFS Not Met

Pan = Panitumumab; Gem = Gemcitabine; GEMOX = Gemcitabine and oxaliplatin; wt = wild type; ORR = Overall Response Rate; DCR = Disease Control Rate; CR = Complete Response; PR = Partial Response; SD = Stable Disease; OS = Overall Survival; PFS = Progression Free Survival; NA = Not Assessable

CCA = Cholangiocarcinoma; IH = Intrahepatic

Table 3. New agents under evaluation in CCA patients grouped according to inhibition target or novelty of action

Drug	Class Target	Stage of development in CCA	Note
HER Family			
Cetuximab	Ab	Random Phase II	Not superior to CT alone
Panitumumab	Ab	Random Phase II	Not superior to CT alone
Erlotinib	TKI	Phase III	Not superior to CT alone
Trastuzumab	Ab	Phase II	Ongoing. Slow accrual
Lapatinib	TKI	Phase II	Abandoned
ASLAN001	TKI	Phase II	Ongoing
mTOR			
Everolimus	Small mol	Phase II	Results not clinically relevant
VEGF			
Bevacizumab	Ab	Phase II	Not approved
Ramucirumab	Ab	Phase II	Ongoing. No result posted
Cediranib	TKI	Random Phase II	Not superior to CT alone
Regorafenib	TKI	Phase II	Ongoing
Sorafenib	TKI	Two-step Phase II	Terminated after first step. No clinical activity
Sunitinib	TKI	Phase II	Ongoing
Pazopanib	TKI	Phase II	Ongoing
FGFR			
Infigratinib	TKI	Phase II	Ongoing
ARQ087	TKI	Phase II	Ongoing in solid tumours including CCA
ALK			
Ceritinib	TKI	Phase II	Ongoing in ROS1/ALK over-expressed CCA
Entrectinib	TKI	Phase I/II	Ongoing in solid tumours with NTRK1-3, ROS or ALK molecular alterations
ABL			
Bosutinib	TKI	Phase II	Abandoned
Imatinib	TKI	Phase II	Low clinical activity. Abandoned
MEK			
Trametinib	TKI	Phase II	Waiting for results
Selumetinib	TKI	Phase I	Phase II not yet proposed
PI3K AKT			
Buparlisib	Small mol	Phase II	Lack of accrual in solid tumours. Not in development
MK2206	Small mol	Phase II	Limited clinical activity
FARNESYLTRANSFERASE			
Lonafarnib	Small mol	Phase II	Protocol withdrawn before enrolment
Tipifarnib	Small mol	Phase I	Not more in development
PROTEASOME			

Bortezomib	Small mol	Two-step Phase II	Terminated after first step. Limited clinical activity
MULTITARGET			
Vandetanib	TKI	Random Phase II	Not superior to CT alone
Merestinib	TKI	Phase II	Ongoing
Cabozantinib	TKI	Phase II	Ongoing
Dasatinib	TKI	Phase II	Ongoing in IDH-mutant CCA
NOVEL AGENTS			
DKN-01	Ab antiDkk-1	Phase I/II	Ongoing with cisplatin + gem
Saracatinib	TKI binding to Src Family	Phase I	MTD determined in solid tumours, including CCA
Exerin	N-cadherin inhibitor	Phase I	Ongoing with cisplatin + gem
Silmitasertib	CK2 (serine/threonine kinase) inhibitor	Phase I/II	Ongoing with cisplatin + gem
AG881	Pan-IDH mutant inhibitor	Phase I	Ongoing in solid tumours, including CCA
OXY111A	Oxygen modulator	Phase I/II	Ongoing
PLX 8394	BRAF inhibitor	Phase I/II	Ongoing
Veliparib	PARP 1/2 inhibitor	Phase I	With cisplatin + gem. No results have been published
RRx-001	Multiple epi-enzyme inhibitor	Phase II	Ongoing with cisplatin + gem
Becatecarin	Topoisomerase I inhibitor	Phase III	Trial terminated in 2006. No results available