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Review

Unresectable biliary tract cancer: Current and future systemic therapy

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ABSTRACT

For decades, treatment of advanced biliary tract cancer (BTC) was confined to the use of chemotherapy. In recent years however, the number of therapeutic options available for patients with unresectable BTC have drastically increased, with immunotherapy and targeted treatment gradually joining the ranks of guideline-recommended treatment regimens. The aim of the present review is to summarise the current knowledge on unresectable BTC focusing on epidemiology, anatomical distribution and current strategies for systemic treatment. We further outline ongoing clinical trials and provide an outlook on future therapeutic interventions. In the realm of gastrointestinal malignancies, the increasing number of systemic treatment options for BTC is finally delivering on the longstanding commitment to personalised oncology. This emphasises the need for considering a comprehensive genomic-based pathology assessment right from the initial diagnosis to fully leverage the expanding array of therapeutic options that have recently become accessible.

1. Introduction

Biliary tract cancer (BTC) comprises a heterogeneous group of rare malignancies arising from epithelial cells of bile ducts or the gall bladder (GB). At diagnosis, the majority of patients are typically in an advanced disease stage, which significantly limits the feasibility of surgical and locoregional treatment approaches. The dismal prognosis of this disease in advanced stage points to a highly unmet medical need.

In the past, the therapeutic approach to systemic treatment of BTC was restricted to the use of chemotherapy. However, the recent development of immunotherapy and of novel agents for targeted treatment has rapidly changed the landscape of BTC treatment and revolutionised not only the therapeutic but also the diagnostic approach to this aggressive malignancy in late stage.

The present review focusses on the epidemiology, anatomical distribution and current treatment strategies of unresectable biliary tract cancer. We further outline ongoing clinical trials and provide an outlook on future therapeutic interventions.

2. Epidemiology

Incidence rates of BTC range from around 2/100,000 in Western countries to 80/100,000 in the North East of Thailand [1]. In Southeast Asia, high incidence rates are associated with trematode infections resulting from a diet rich in raw fish.

There has been a rise of intrahepatic cholangiocarcinoma (iCCA) observed in Western countries. This might be partly due to a more precise diagnosis of cancers of unknown primary (CUP) with hepatic involvement [2]. In most cases however, no clear cause can be identified, but common risk factors are chronic liver conditions, such as chronic cholangitis or cholestasis, chronic hepatitis B or C, alcohol induced cirrhosis or non-alcoholic fatty liver disease. Further, lifestyle

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and metabolic-related factors like consumption of alcohol, smoking, diabetes and obesity can increase the risk of developing BTC [3–5]. It appears some CUPs are in fact iCCA, that had initially been classified as the former [2,6].

3. Anatomy

CCAs are classified based according to their anatomical origin within the biliary tree [7] (Fig. 1).

iCCA originates from biliary ducts within the hepatic parenchyma, anatomically above the 2nd order bile ducts whereas extrahepatic CCA (eCCA) arises distal to the origin of the 2nd order biliary ducts. Extrahepatic CCA can be sub-divided into perihilar CCA (pCCA, historically also known as "Klatskin tumours") and distal CCA (dCCA). The latter originates between the cystic duct and the ampulla of Vater. Therefore, the 2nd order branches of the bile ducts are the anatomical point of distinction between iCCA and pCCA while the cystic duct defines the anatomical distinction between pCCA and dCCA. Morphologically, iCCA can be further distinguished according to its growth pattern and specifically, according to whether the tumour exhibits, as in the majority of cases, a mass-forming growth pattern, a periductal-infiltrating or (rarely) an intraductal-growing pattern (Fig. 2).

The latest International Classification of Diseases (ICD-11) provides specific codes for "malignant neoplasm of intrahepatic bile ducts" (2C12.1), "hilar CCA" (2C18.0), "adenocarcinoma of biliary tract, distal bile duct" (2C15.0) and "adenocarcinoma of the gallbladder" (2C13.0) [8].

In clinical practice, however, the established classification of BTC into the categories of GB cancer, iCCA and eCCA is regarded as a practical and established method for therapeutic stratification with regard to the presently available treatment options.

4. Symptoms & diagnosis

Age of incidence peaks at around 70 years and males are slightly more often affected than women. Usually asymptomatic at early stage, CCA is most often diagnosed at an unresectable stage [9]. Jaundice is the most frequent presentation symptom for eCCA owing to the predominantly intraductal growth pattern in extrahepatic tumours, but is rarely present at diagnosis in iCCA patients, where less specific symptoms, including abdominal pain, fever and weight loss are more frequent [10]. In many cases, CCAs are diagnosed in consequence of elevated liver enzymes or a liver mass detected on routine investigation leading to a diagnostic workup including a liver biopsy to eventually confirm the diagnosis.

Initial staging can be performed by CT-scan, MRI of the liver or PET-CT-Scans. Depending on tumour location and the need for intervention, there are different approaches to perform tumour biopsies: Tumour biopsies can be obtained through various methods, depending on the setting and the anatomical localisation of the tumour at diagnosis. If endoscopic drainage of the biliary tract is required, biopsies may be taken in the context of endoscopic retrograde cholangiopancreatography (ERCP) or cholangioscopy by forceps biopsy. For distal tumours endosonography (EUS), which is performed in the context of a gastroduodenoscopy, is often a valuable option which allows a better local staging and performing EUS-guided needle-biopsies.

In addition to the standard histological evaluation, next generation sequencing should be performed if possible. Detection of actionable alterations, i.e. FGFR2-fusion or IDH1-mutation, provide alternative treatment options in further lines and also offer enrolment into clinical trials. A limiting factor in this regard is the lack of sufficient tumour tissue to perform further analysis [11]. After obtaining all information, case presentation to the (molecular) tumour board is obligatory. A treatment recommendation is then given out by an interdisciplinary expert panel. Figure 3 outlines a standard patient journey from first discovery of BTC, over diagnostic approach to treatment.

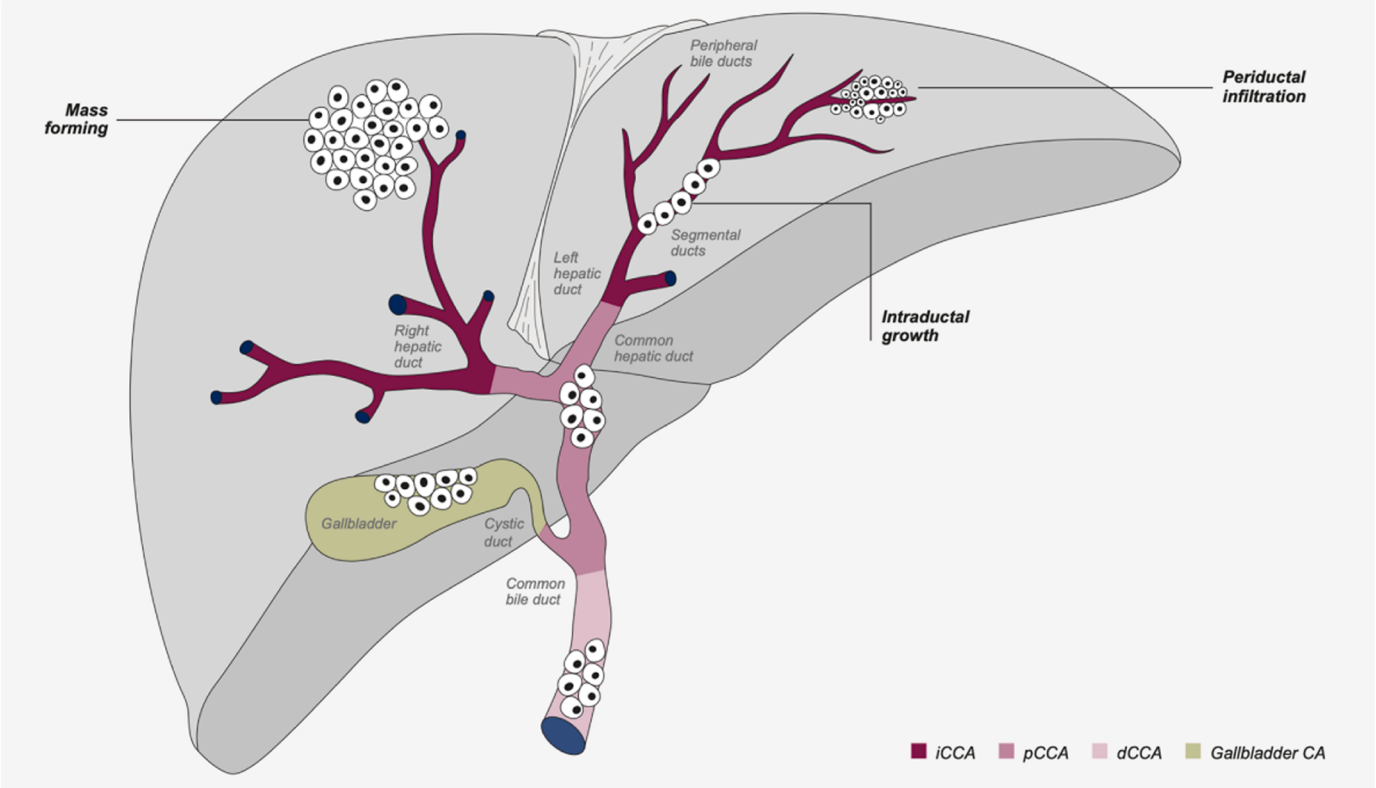

Fig. 1. Anatomy of BTCs.

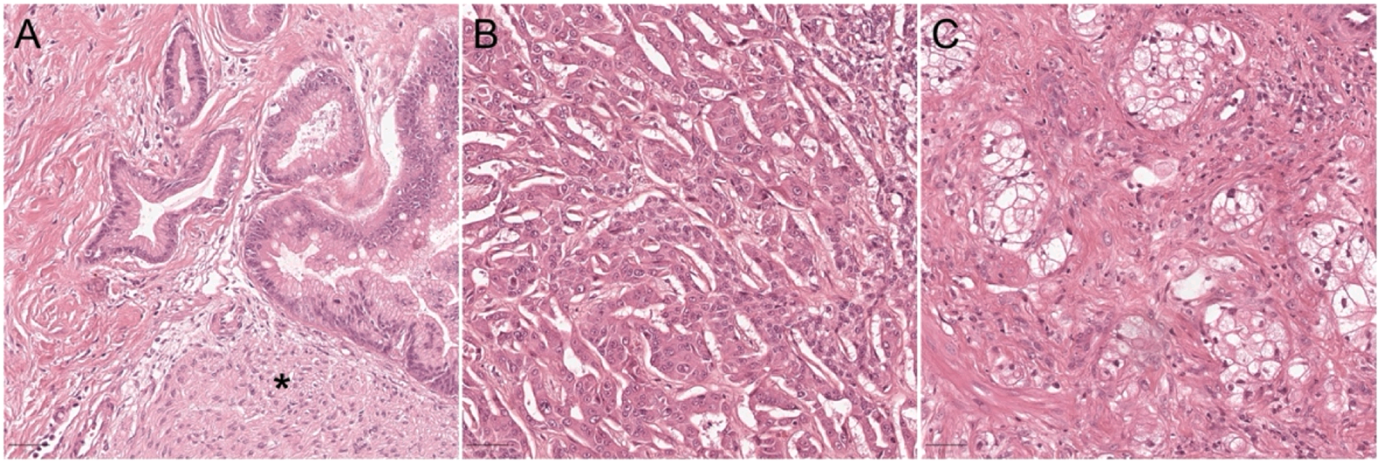

Fig. 2. Exemplary cases of a moderately differentiated perihilar cholangiocellular carcinoma (A), a poorly differentiated intrahepatic cholangiocellular carcinoma (B) and a poorly differentiated gall bladder carcinoma (C). Note the extensive perineural invasion (asterisk). Scale bars indicate 50 micrometers.

4.1. Molecular biology of BTC

With the rise of precision medicine, multiple actionable alterations have emerged in the last decade. The anatomical subtypes of BTC, iCCA, eCCA and GB cancer, harbour different genomic alterations [12–15]. The most frequent actionable alterations in iCCA are IDH1 and 2 mutations (10 – 20%), FGFR alterations (7 – 16%), followed by HER2-alterations (8%), PIK3CA (7%), BRAF mutations (5%) and *BRCA*-alterations (3 – 4%). In eCCA, alterations in HER2 (5 – 9%), PIK3CA (5%), IDH1/2 (2 – 3%) and BRCA (2 – 4%) are often detected. In GB cancer, the most commonly detected alterations are HER2/3 (20%), PIK3CA (10%), NTRK and BRAF (4% each), FGFR (3%) and IDH1/2 (2%).

4.2. Molecular diagnosis of actionable alterations

Targeted therapy requires prior molecular diagnosis and identification of actionable alterations. To this end, molecular pathologists may apply a variety of diagnostic assays. Here, we outline the different diagnostic approaches available.

4.3. Immunohistochemistry (IHC)

IHC is commonly used to detect overexpression of a specific target protein. In clinical routine, IHC testing of aberrant expression of *HER2 (ERBB2)* is reliable and well established. Further, overexpression of fusion proteins as a result of rearrangements could be detected by IHC, e.g. screening for NTRK gene fusions in selected solid tumours [16,17]. To date, no consistent IHC method for *FGFR2*- overexpression has been established [14,18]. IHC may also be used to detect the loss of expression of DNA mismatch repair enzymes such as MLH1, MSH2, MSH6 or PMS2, a commonly accepted surrogate marker for high grade microsatellite instability (MSI-H), which can alternatively be tested directly using PCR-based approaches.

4.4. Fluorescence in situ hybridisation (FISH)

In order to detect gene fusions, break-apart FISH is generally used on tumour tissue. In case of FGFR2-rearrangments however, about 50% are intrachromosomal rearrangements and will not be caught by FISH analysis [14,18]. Hence, PCR-based assays are used more broadly for the detection of alterations found in BTC.

4.5. PCR-based assays

PCR-based assays are efficient in detection of single mutations. DNA-based quantitative PCR assays allow screening for specific mutations and SNVs in exon sequences. In cases where both fusion partners and the location of the breaking point are known, RNA-based real-time-PCR can detect those gene fusions. However, if the fusion partner is unknown, as it is often the case in *FGFR2*-fusion-rearrangements, this method cannot be applied. Further, multiplex PCR enables simultaneous detection of pre-specified mutations [14,18]. For a point mutation in codon 132 of *IDH1* for example, a simple PCR might very well do the job. However, this would inform the pathologist and treating oncologist solely of the *IDH1* status of the tumour which occurs in only 14.3% of iCCAs [15].

4.6. Next-generation-sequencing (NGS)

Alternatively, genomic profiling with NGS can efficiently detect different alterations in multiple genes in a single analysis at the same time. Nowadays, NGS is widely applied in BTC as it allows a comprehensive genomic profiling (CGP) of selected and tumour-specific target alterations [11,18–20].

4.6.1. DNA-based NGS

DNA-based NGS panels can cover any genomic alteration: SNVs, indels, rearrangement, amplifications, TMB (tumour mutational burden) and can even detect MSI-H through specific algorithms. One limitation of a DNA-based NGS panel is the need for prediction of gene expression from the detected genetic alterations [21].

4.6.2. RNA-based NGS

To address the above-mentioned limitation of a DNA-based NGS approach, RNA-based NGS can analyse the transcriptomic changes resulting from genomic alterations, including the identification of splicing variants and complex gene fusions. Of major importance is the quality of the tissue biopsy as RNA is less stable than DNA [22,23].

Consequently, exploiting both DNA and RNA sequencing can provide the patient with the highest chance of identifying an actionable alteration. Thus, a combined DNA- and RNA-based sequencing approach using panels covering the range of currently actionable genetic and transcriptomic alterations should become standard of care, which is also reflected in the latest ESMO (European Society for Medical Oncology) guidelines [11,18–20].

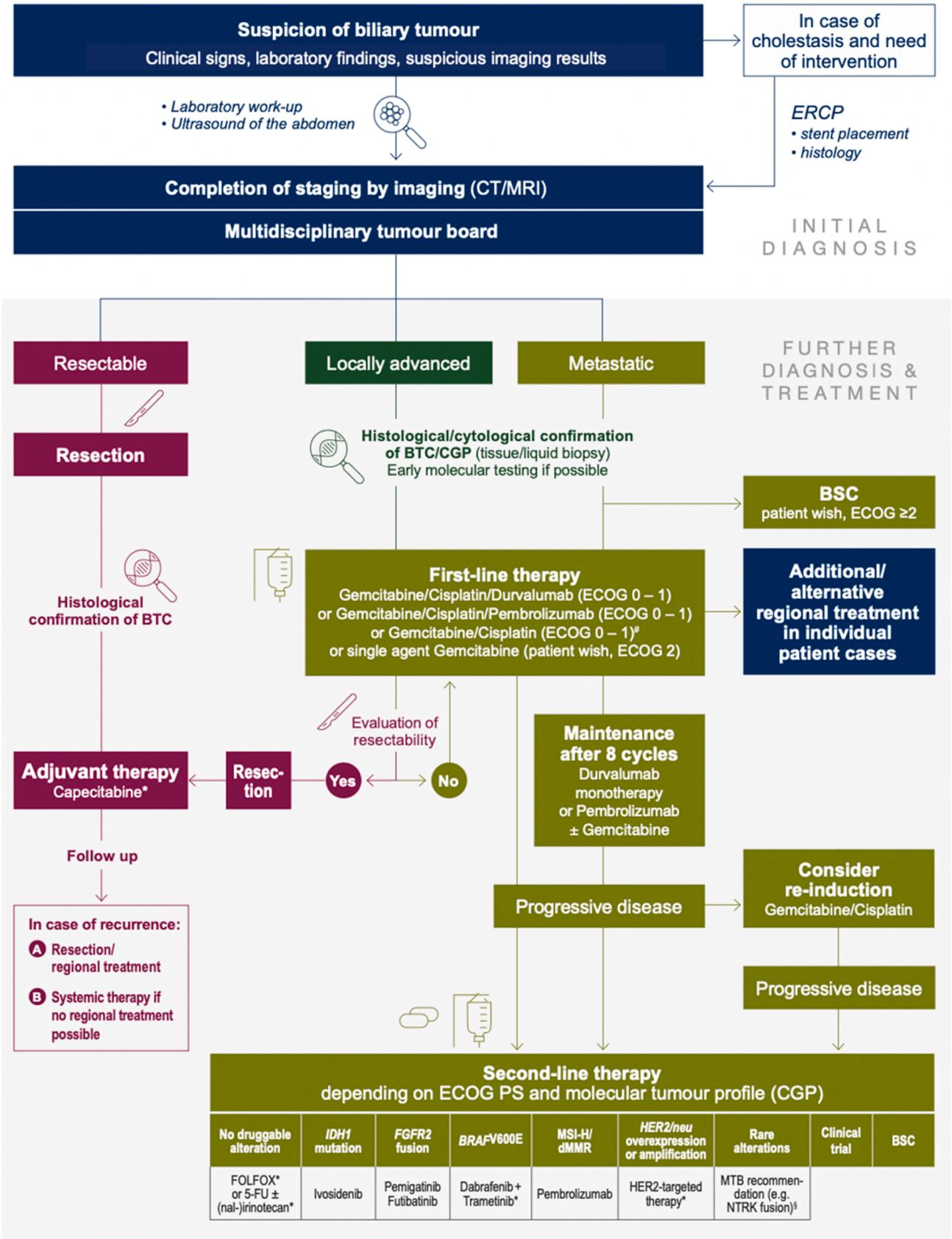

Fig. 3. Blueprint of a patient journey from diagnosis to treatment in accordance with the ESMO guidelines. * not FDA or EMA approved; potential HER2-targeted therapies: trastuzumab-deruxtecan, trastuzumab+pertuzumab, trastuzumab+tucatinib, zanidatamab. # in cases of contraindication to immunotherapy. § evaluation of personalised treatment according to MTB (molecular tumour board) recommendation. CGP: comprehensive genomic profiling.

4.7. Whole exome Sequencing (WES) / Whole transcriptome sequencing (WTS)

Theoretically, WES and WTS could be used for detection of alterations. But a long turn-around time, the need for more material, complex bioinformatic analysis and the detection of many alterations of unknown significance has shifted the focus to a more targeted approach with focused NGS-panels on clinically relevant molecular alterations.

4.8. Liquid biopsy

A prerequisite for all techniques mentioned above is to obtain a qualitatively sufficient tumour sample during biopsy. Unfortunately, acquisition of enough tumour tissue is often challenging. Here, liquid biopsy (including circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), exosomes, etc) might be a feasible alternative to obtain information on the molecular alterations of a disease. Although not recommended for daily clinical practice as of today, especially ctDNA might become a viable option in the future to detect actionable molecular alterations [24,25]. Additionally, ctDNA analyses of serially collected blood samples during therapeutic intervention may correspond with clinical course and could be exploited to tailor treatment regimens. This was shown for monitoring dynamic changes in IDH1- and IDH2-mutated ctDNA when treating with IDH inhibitors [26].

4.9. Locoregional therapies

Given the tumour anatomy, patients can present with localised complications, i.e. cholestasis and subsequent cholangitis or abscesses or pain due to local tumour growth. BTC also often presents with liver-limited, but unresectable disease. In all described cases, application of locoregional treatment can be considered to either prevent infection or control local disease in palliative intent but can also be used to achieve tumour shrinkage in neoadjuvant intent. There are different established loco-reginal treatments that can be used: ablation, radioembolisation (SIRT), transarterial chemoembolisation (TACE), stereotactic body radiation (SBRT), brachytherapy, hepatic arterial infusion (HAI) and photodynamic therapy (PDT) [27–30]. The application depends on the expertise of the hospital, as well as the treatment-related benefits and risks. A summary of techniques, indication and the risks associated with each of the locoregional therapies is outlined in Table 1. Given the delicate location of tumour site local treatment approaches might lead to severe toxicities. Accordingly, complications post intervention such as radiation-induced liver toxicity, necrosis, haemorrhages, liver abscesses or biliary strictures and cholangitis must be carefully monitored for [27,28].

5. Current systemic therapy of advanced disease

5.1. First line therapy

For more than a decade, the combination of gemcitabine and cisplatin represented the standard of care for first-line systemic therapy of BTC, which was established by the British phase III ABC-02 study [31] and the Japanese phase II BT22 study [32]. Further trials conducted in the following decade to assess different first-line treatment options in BTC failed to show significant additional clinical benefit [33].

Recently, the TOPAZ-1 trial demonstrated that the addition of the PD-L1 inhibitor durvalumab to this standard regimen led to a significant and clinically relevant improvement of overall survival (OS, primary endpoint: HR 0.80, $p = 0.021$), progression-free survival (PFS, HR 0.75, $p = 0.001$) and objective response rate (OR 1.60, $p = 0.011$) [34]. This combination of durvalumab and gemcitabine/cisplatin has thus been approved by the FDA and the EMA and is regarded as the new current standard of 1st-line treatment (Fig. 3).

The addition of immunotherapy to gemcitabine/cisplatin was also investigated in the KEYNOTE-966 study, which compared the combination of pembrolizumab plus gemcitabine/cisplatin to standard chemotherapy alone [35]. In contrast to the TOPAZ-1 protocol, according to which patients were transitioned to maintenance treatment with durvalumab monotherapy after 6 months, participants in the KEYNOTE-966 study continued to be treated with combined gemcitabine and pembrolizumab until clinical progression. This study showed that addition of pembrolizumab to chemotherapy induces a significant improvement of OS (HR 0.83, $p = 0.0034$), but had no statistically significant effect on PFS (HR 0.86, $p = 0.0225$) and overall response rate (ORR). It was subsequently approved by FDA and EMA (Fig. 3).

Both, TOPAZ-1 and KEYNOTE-966 included a large fraction of Asian patients (54.6% and 45%, respectively). While a subgroup analysis of the TOPAZ-1 study suggested that the addition of immunotherapy was more beneficial in Asian patients, this conclusion was not supported by the KEYNOTE-966 study.

In both trials, a subgroup analysis according to the tumour localisation showed that addition of immunotherapy did not improve clinical benefit for GB cancer patients (HR were 0.94 and 0.96 for TOPAZ-1 and KEYNOTE-966, respectively), suggesting a different underlying

Table 1
locoregional treatment options in BTC.

	Ablation,e.g. radio-frequency ablation (RFA) or microwave ablation (MWA)	Radioembolisation / Selective internal radiation therapy (SIRT)	Transarterial Chemoembolization (TACE)	Radiation: Stereotactic body radiation (SBRT); Brachytherapy	Hepatic arterial infusion (HAI)	Photodynamic therapy (PDT)
Technique	Application of thermal energy to tumour tissue	Catheter-guided application of Yttrium-90 microspheres to tumour tissue	Catheter-guided tumour embolisation by intraarterial application of lipiodol plus chemotherapy or by microembolic drug-eluted beats	Precise radiation to tumour tissue from external or catheter-guided	Hepatic arterial infusion of chemotherapy	i.v. infusion of photosensitising agent and subsequent endoscopic application of light

Indication	• Irresectable / locally advanced iCCC: disease control
	• eCCC: prevention of biliary stent occlusion
	• Palliation (in chemorefractory patients)
		• Irresectable / locally advanced iCCC:
		  • Downstaging
		  • Disease control
		• Palliation (in chemorefractory patients)
			• Irresectable / locally advanced iCCC:
			  • Downstaging
			  • Disease control
			• Palliation (in chemorefractory patients)
				• Irresectable / locally advanced iCCC: Disease control
				• Palliation (in chemorefractory patients)
					• Palliative therapy in selected patients	• eCCC: Prevention of stent occlusion

Risk/ Toxicity	Hemorrhage, infection, thermal injury, tumour seeding	Fatigue, abdominal pain, nausea, diarrhea, ascites, hepatic toxicity	Post-Embolisation Syndrome (PES): nausea, vomiting, pain, fever; infection, acute hepatic toxicity	Infection, hepatic toxicity, cholestasis	Infection, hepatic toxicity	Infection, cholangitis, liver abscess

microenvironment or molecular profile interfering with response to immunotherapy.

The randomised IMMUCHEC phase-II trial investigated the effect of double checkpoint inhibition with tremelimumab plus durvalumab in addition to standard chemotherapy of gemcitabine/cisplatin [36]. While imbalanced composition of subgroups limit comparability between treatment arms, this trial did not show an additional benefit of double-checkpoint inhibition with regard to ORR (primary endpoint). However, a trend for longer median OS favoured the cohort treated with gemcitabine/cisplatin plus durvalumab and a single dose of tremelimumab. Also, combination treatment with checkpoint inhibition and gemcitabine alone (without cisplatin) led to less favourable outcome in this trial, emphasising the importance of a platin derivative in the first-line therapy.

For patients not eligible for cisplatin treatment, an alternative option in first-line therapy might be represented by the combination of gemcitabine and oxaliplatin [37,38]. Notably, the triple combination FOLFIRINOX was not more effective than the doublet gemcitabine plus cisplatin in a randomised phase III trial [39].

Tyrosine kinase inhibitors (TKI) have also been tested as single agents or in combination with systemic therapy in the first line setting of advanced or metastasised BTC. However, neither a more specific anti-angiogenic TKI such as cediranib [40] nor a more promiscuous multi-kinase inhibitor such as sorafenib [41] outperformed traditional chemotherapy.

Although the preceding phase II trial reported promising results [42], adding nab-paclitaxel to standard chemotherapy with gemcitabine/cisplatin, did not provide any benefit in the unstratified population according to the phase III SWOG-1815 trial [43]. An exploratory subgroup analysis suggested that GB cancer patients might benefit from the addition of nab-paclitaxel to standard gemcitabine/cisplatin.

Although PD-L1 expression is predictive of response to immunotherapy in many solid tumours, it is not a reliable biomarker in BTC [44,45]. In fact, a subgroup analysis of the TOPAZ-1 study indicated that treatment response to immunotherapy occurred regardless of PD-L1 expression [34]. Thus, a predictive biomarker has yet to be identified in this regard.

5.2. Second line therapy

When laying out a strategy on sequential therapy, data from studies on patients with relapsed BTC must often be interpreted with caution. Many studies permitted recruitment of patients with more than 1 line of prior therapy making it difficult to decide on an optimal 2nd, 3rd and later line therapy based on their results. In general, there is no standard therapy beyond 2nd line. Table 2 gives an overview about selected clinical trials with respect to second line therapies in BTC.

Second-line therapy is initiated based on the mutational profile of the tumour (see section on targeted therapy). In patients without actionable alteration or contraindication to targeted treatment, chemotherapy remains the treatment of choice.

Although not formally approved, FOLFOX is considered a standard second-line therapy based on results of the British phase III ABC-06 study [46]. It remains debatable, whether an oxaliplatin-based therapy straight upon failure of cisplatin is most effective from a mechanism of action and from a toxicity point of view.

According to data from a randomised phase II trial, there was no difference in OS between FOLFOX and FOLFIRI [47]. However, toxicity profiles of both regimens were very different. More patients in the FOLFOX arm suffered from neuropathy and thrombocytopenia whereas more patients in the FOLFIRI arm suffered from vomiting and cholangitis.

Alternatively, the combination of liposomal irinotecan and 5-FU was shown to be more effective compared to 5-FU alone in the South Korean NIFTY study [48,49]. However, the recent German study NALIRICC failed to confirm this in a Western population based on a first read-out [50].

Although regorafenib, a multi-kinase inhibitor, did not prolong OS compared to best supportive care, it prolonged PFS [56].

Regarding immunotherapy, nivolumab monotherapy showed only modest efficacy in pre-treated patients [57]. However, data from a small phase II trial deemed the triple combination of pembrolizumab, capecitabine and oxaliplatin safe and effective [58]. On the other hand, combination of durvalumab and tremelimumab with paclitaxel led to an increase in anaphylactic adverse events during the safety lead-in phase which is why paclitaxel was dropped for the phase II part [59]. As shown in Phase I and II trials, pembrolizumab might lead to a better tumour control in PD-L1-positive tumours [60].

5.3. Targeted therapy

As described above, the anatomical subgroups of BTC are driven by

Table 2
Selected clinical trials for second-line therapy in biliary tract cancer.

Trial	Phase	Target	Treatment regimen	Study population	mPFS	mOS
ABC-06 (NCT01926236) [46]	III	all-comers	FOLFOX vs. BSC	UK population, progression after Gem/Cis	FOLFOX group: 4 months BSC group: not indicated	6.2 vs. 5.3 months (HR 0,69; p = 0·031)
NCT03464968[47]	II	all-comers	FOLFOX vs. FOLFIRI	South Korean population, progression after Gem/Cis	2.8 vs. 2.1 months (HR 1,0; p = 0.974)	6.3 vs 5.7 months (HR 1,1; p = 0.677)
NIFTY (NCT03524508) [48,49]	IIb	all-comers	Nal-Iri + FU/LV vs. FU/LV alone	South Korean population, progression after Gem/Cis	4.2 vs. 1.7 months (HR 0.61; p = 0.004)	8.6 vs. 5.3 months (HR 0.68; p = 0.02)
NALIRICC (NCT03043547) [50]	II	all-comers	Nal-Iri + FU/LV vs. FU/LV alone	German population, progression after Gem/Cis	2.6 vs. 2.3 months (HR 0.87)	6.9 vs. 8.21 months(HR 1.08)
FIGHT 202 NCT02924376) [51]	II	FGFR2 fusion or rearrange-ment	Pemigatinib single arm	North American and Western European population, progression following ≥ 1 previous systemic therapy	6.9 months (95% CI, 6.2–9.6)	17.5 months (95% CI, 14.4-22.9)
FOENIX-CCA2 (NCT02052778) [52]	II	FGFR2 fusion or rearrange-ment	Futibatinib single arm	North American, European, Asian population, progression following ≥ 1 previous systemic therapy	9.0 months (95% CI, 6.9 to 13.1)	21.7 months (95% CI, 14.5 to not reached)
ClarIDHy (NCT02989857) [53,54]	III	IDH1 mutation	Ivosidenib vs. Placebo (crossover allowed)	North American, European population, progression after at least 1 but no more than 2 prior treatment regimens	2.7 vs. 1.4 months (HR 0.37; p < 0.0001)	10.3 vs. 5.1 months (HR 0.49; p < 0.001), adjusted for crossover
KEYNOTE 158 (NCT02628067) [55]	II	MSI-H/dMMR	Pembrolizumab Single arm	solid tumors, progression on previous systemic treatment	4.2 (95% CI 2.1-24.9)	19.4 months (95% CI, 6.5-NR)

different genomic profiles and with molecular pathology it is possible to detect those actionable genomic alterations [61,62].

Table 3 outlines the efficacy of *HER2-, FGFR2-, IDH1-*, and *BRAF V600E*-targeted treatment in selected trials of advanced/metastatic BTC.

The first FDA and later EMA approved inhibitor for FGFR2 fusion/rearrangement, pemigatinib, propelled genomic testing of BTCs in daily clinical routine. Pemigatinib is approved for 2nd line therapy of CCA with FGFR2-rearrangment based on results of the single-arm phase II FIGHT-202 trial (Fig. 3, Table 3) [51]. Infigratinib and futibatinib followed promptly with their FDA approvals for the same indication also based on single-arm phase II trials [52,63]. For infigratinib, application for EMA approval has been withdrawn, though (ema.europa.eu/en/-medicines/human/withdrawn-applications/febseltiq). On the other hand, EMA recently approved futibatinib after standard of care first line treatment (Figure 3). Preliminary data on derazantinib was promising [64], but further data is awaited (NCT03230318). Further, RLY-4008, a highly selective and irreversible inhibitor targeting FGFR2-alterations (including those with common resistance mutations against 1st generation FGFR2 inhibitors) showed a high and durable response with an ORR of 88% in the Phase 1/2 ReFocus trial [65]. The trial is currently ongoing and its clinical endpoints will be of great interest.

Furthermore, ivosidenib received FDA and EMA approval for 2nd line therapy of *IDH1*-mutated CCA based on results of the double-blinded, randomised phase III ClarIDHy trial [53,54]. Clinically relevant IDH1 mutations occur in codon 132 at different rates (R132C 68.9%, R132L 15.6%, R132G 9.7%, R132S 3.5%, R132H 1.82%) [15]. Ivosidenib was shown to be effective against all of them (Fig. 3) [66].

As for other indications, tumours carrying an *NTRK*-fusion are sensitive to entrectinib and larotrectinib [67,68]. Although rare, this provides an additional treatment option approved by EMA and FDA.

Pembrolizumab received tissue-agnostic FDA approval for all MSI-H tumours and EMA approval for MSI-H BTCs and other cancers (Fig. 3) [55].

Clinical studies (Table 3) indicated efficacy of targeted therapy against BTCs with *HER2* amplification / overexpression [69–73]. Recently, zanidatamab, a bispecific antibody targeting two distinct HER2 domains, led to a clinical benefit after progression on gemcitabine-based first line treatment [72]. The SGNTUC-19 trial lately showed that the combination of two HER2 inhibitors, tucatinib and trastuzumab, led to a clear clinical benefit in pre-treated HER2 positive patients [73]. Also, in patients with $BRAF^{V600E}$ mutation [74] (Table 3), with *RET* fusions [75,76] or with $KRAS^{G12C}$ mutation [77] targeted therapy showed promising effects. *L-type amino acid transporter 1* (LAT1), a transporter for neutral amino acid for cancer cell energy, has emerged as a new potential target in BTC. In a randomised, placebo-controlled phase II trial LAT1 inhibitor nanvuranlat was associated with an improved clinical outcome, especially in patients with high LAT1-expression and in dCCA and GB [78]. The MEK inhibitor cobimetinib in combination with atezolizumab in previously treated BTC was well tolerated and effective in some patients [79]. Comparable results were observed with the MEK inhibitor binimetinib in a phase I/II study [80]. However, none of these inhibitors have been approved for BTC, yet.

Table 3
Efficacy of *HER2-*, *FGFR2-*, *BRAF V600E*-targeted treatment in advanced/metastatic BTC.

	N	Treatment regimen	ORR (%)	PFS (mo)	OS (mo)
Target: *HER2*					
HERIZON-BTC01	80	Zanidatamab	**41.3**	**5.5**	**na**
SGN-TUC-019	30	Tucatinib + Trastuzumab	**46.7**	**5.5**	**15.5**
HERB	22	TDX-d	**36.4**	**4.4**	**7.1**
DESTINY PANTUMOR02	41	TDX-d	**26.8**	**7.4**	**na**
Target: *FGFR2*					
INCB054828 – FIGHT 202 (Cohort A: *FGFR2* fusion/ rearrangement)	108	Pemigatinib	**37**	**7.0**	**17.5**
FOENIX-CCA2 (*FGFR2* rearrangement)	103	Futibatinib (TAS-120)	**42**	**9.0**	**21.7**
BGJ398 (Cohort 1: *FGFR2* fusion/ rearrangement)	108	Infigratinib	**23.1**	**7.3**	**12.2**
FIDES-01 (*FGFR2* fusion cohort)	103	Derazantinib	**21.4**	**7.8**	**15.5**
RAGNAR (*FGFR1-4* mut/fus)	35	Erdafitinib	**60**	**8.4**	**18.7**
Target: BRAF *V600E*					
ROAR	43	Dabrafenib + Trametinib	**47**	**9**	**13.5**

6. Ongoing trials

There is a great number of ongoing clinical trials that investigate various approaches to treatment of BTC. These range from novel combinations and individualised treatment to exploration of innovative targets. Here we summarise the most promising and innovative strategies (Table 4).

The combination of anti-VEGF and anti-PD1 therapy is being tested as a first line therapy by the phase II IMbrave151 study [81,82]. However, the addition of bevacizumab to atezolizumab failed to increase efficacy and the study did not reach its primary endpoint evaluating PFS. Further combination of immunotherapies and novel agents are currently being investigated (NCT03257761, NCT04941287, NCT04666688, NCT04068194, NCT04301778).

Combination of durvalumab and tremelimumab with ablative therapies (NCT02821754), with SIRT (NCT04238637) or with radiation (NCT03482102, NCT02866383) may also be an interesting strategy.

Approval of FGFR-targeted therapy in previously treated BTC patients spurred initiation of trials investigating this strategy in first line setting (NCT03656536, NCT04093362) and with next-generation FGFR inhibitors in early clinical development (NCT04526106, NCT03230318, NCT04083976).

Promising results of ivosidenib with immunotherapy in preclinical models [83] are currently being challenged in clinical trials (NCT04056910, NCT05921760).

In addition to the studies on liposomal irinotecan and 5-FU mentioned above, this combination is under investigation for untreated (NCT03044587) and previously treated (NCT04005339) BTC patients in two phase II studies.

KRAS has emerged as a potential target in many solid cancers. Several phase I and II studies include BTCs in their investigation to target KRAS-mutated solid cancers (NCT04965818, NCT04566133, NCT05002270).

Opaganib is a small molecule targeting SK2 involved in sphingolipid metabolism. For BTC, it is being tested in a phase II study (NCT03377179).

CPI-613 inhibiting pyruvate dehydrogenase and α-ketoglutarate dehydrogenase of the Krebs cycle within the mitochondria is currently under investigation (NCT04203160).

Regarding HER2 targeting, the phase II basket trial SUMMIT investigates the irreversible pan-HER tyrosine kinase inhibitor neratinib [84]. Trastuzumab deruxtecan is under investigation in the phase II HERB trial and has shown promising outcomes in HER2-expressing BTC [71,85].

The SAFIR ABC-10 umbrella study follows a more innovative study design (NCT05615818). Here, patients with BTC receive standard of care frontline therapy. In the meantime, tumours will be molecularly tested. After four cycles, patients will be randomised to receive a

Table 4
Selection of ongoing or active clinical trials or completed trials that await full publication for advanced/metastatic BTC.

Target / Strategy	Treatment Regimen	Patient population	Phase	Trial Number
Novel chemotherapy combination	Gemcitabine hydrochloride + Cisplatin alone or with nab-Paclitaxel	First-line treatment in advanced/metastatic BTC	III	NCT03768414 (SWOG 1815)
Novel chemotherapy combination	Nanoliposomal Irinotecan with 5-fluorouracil (5-FU) + Leucovorin or Gemcitabine / Cisplatin	First-line treatment in advanced/metastatic BTC	II	NCT03044587 (NIFE)
Novel chemotherapy combination	Fluorouracil + Leucovorin + Nanoliposomal Irinotecan	Advanced/metastatic BTC patients after prior standard first-line treatment	II	NCT04005339 (NAPOLI-2)
Molecular targeted maintenance therapy	Targeted maintenance therapy vs. standard first-line therapy	BTC with actionable alterations; start of targeted maintenance after 4 cycles of standard treatment	III	NCT05615818 (SAFIR-ABC10)
***FGFR*-alteration**				
FGFR2-rearrangement	Pemigatinib vs. Gemcitabine / Cisplatin	First-line treatment in patients with *FGFR2*-rearrangement	III	NCT03656536 (FIGHT 302)
FGFR2-rearrangement	Futibatinib vs. Gemcitabin / Cisplatin	First-line treatment in iCCC patients with *FGFR2*-rearrangement	III	NCT04093362 (FOENIX-CCA3)
FGFR2-fusion/ rearrangement	Atezolizumab + Derazantinib	iCCA with *FGFR2* fusion/rearrangement after at max. one previous treatment line (excluding *FGFR2* targeted therapy)	II	NCT05174650 (ADVANCE)
FGFR2-fusion	E7090	Patients with *FGFR2*-fusion with at least one prior gemcitabine-based chemotherapy	II	NCT04238715
FGF2-fusion/mutation/ amplification	Derazantinib	Patients with *FGFR2*-mutation or alteration with at least one prior therapy	II	NCT03230318 (FIDES-01)
FGFR2-alteration	Pemigatinib + Gemcitabine / Cisplatin	First-line treatment in patients with *FGFR2*-alteration	I	NCT04088188
FGFR2-alteration	RLY-4008	BTC patients after standard therapy, independent of previous *FGFR* inhibition	I/II	NCT04526106 (REFOCUS)
FGFR2-alteration	TT00420 (Tinengotinib)	3 cohorts: Patient with *FGFR2*-fusions and previous *FGFR*-targeted treatment; other *FGFR2*-alterations; *FGFR2* wildtype	II	NCT04919642 (TT420C1206)
FGFR2 and/or *FGFR3* alterations	KIN-3248	Advanced solid tumours, including iCCC	I/Ib	NCT05242822
FGFR-fusion/mutation	Erdafitinib	Patients with *FGFR* mutations/fusions after first-line therapy	II	NCT04083976 (RAGNAR)
***IDH*-mutation**				
IDH1-mutation	Ivosidenib + Gemcitabine / Cisplatin	First-line treatment in patients with *IDH1*-alteration	I	NCT04088188
IDH1-mutation	Ivosidenib + Nivolumab	Advanced solid tumours with *IDH1* alteration after standard therapy	II	NCT04056910
IDH1-mutation	Ivosidenib + Nivolumab + Ipilimumab	Nonresectable or metastatic cholangiocarcinoma with an *IDH1* mutation	I/II	NCT05921760
IDH1	• Olutasidenib + Nivolumab (hepatobiliary tumours) • Olutasidenib + Gemcitabine / Cisplatin (iCCA)	Advanced solid tumours with *IDH1* alteration after standard therapy	I/II	NCT03684811
IDH1 or *IDH2*-mutation	Olaparib	Advanced/metastatic solid tumor with *IDH1* / *IDH2*-mutation after standard therapy	II	NCT03212274
IDH1 or *IDH2*-mutation	LY3410738	Advanced/metastatic solid tumor with *IDH1* / *IDH2*-mutation after standard therapy	I	NCT04521686 (LOXO-IDH-20002)
IDH2	Enasidenib	Advanced/metastatic solid tumor with *IDH2*-mutation after standard therapy	I/II	NCT02273739
IDH-mutation	HMPL-306	Advanced/metastatic solid tumor with *IDH*-mutation after standard therapy	I	NCT04762602
***HER2*-alteration**				
HER2 expression	Trastuzumab-deruxtecan (T-Dx)	Advanced solid tumours progressed on prior treatment, prior *HER2* targeted treatment allowed	II	NCT04482309 (DESTINY-PanTumor02)
HER2 expression	Zanidatamab (ZW25) + standard first-line chemotherapy	*HER2*-expressing GI-tumours	II	NCT03929666
HER2 expression	ZW49	Advanced/metastastic solid tumours after failure of standard therapy *HER2* expression	I	NCT03821233
HER2 amplification	Zanidatamab (ZW25)	Advanced/ metastatic BTC progressed on at least 1 gemcitabine base therapy	II	NCT04466891 (HERIZON-BTC-01)
HER2-alteration	Tucatinib + Trastuzumab	Previously treated solid tumours with *HER2* alterations	II	NCT04579380
***KRAS* mutation**				
*KRAS*mut	Futibatinib + Binimetinib	Advanced/metastatic *KRAS* mutated solid tumours	Ib/II	NCT04965818
KRAS G12C	GDC-6036 alone or in combination with other anti-cancer treatments	Advanced/metastatic *KRAS G12C* solid tumours	Ia/Ib	NCT04449874
KRAS G12C	JAB-21822 alone or in combination with Cetuximab	Advanced/metastatic *KRAS G12C* solid tumours after at least 1 prior treatment	I/II	NCT05002270
DNA damage repair				
DNA damage repair	Ceralasertib (AZD6738) + Durvalumab orCeralasertib (AZD6738) + Olaparib	Advanced BTC with DNA damage repair after first-line treatment	II	NCT04298021
Aberrant DNA repair gene mutation	Olaparib	BTC with aberrant DNA repair gene mutation	II	NCT04042831
***MDMD2* amplification**				
MDM2 amplification	Brigimadlin	Advanced/metastatic solid tumours with *MDM2* amplification	Ia/Ib	NCT03449381
Immunotherapy + other				

(*continued on next page*)

Table 4 (*continued*)

Target / Strategy	Treatment Regimen	Patient population	Phase	Trial Number
Immunotherapy + chemotherapy + VGEF-inhibition	Atezo + Bev+CisGem, followed by Atezo + Bev vs. Atezo + Placebo + CisGem, followed by Atezo + Placebo	First-line treatment in advanced/metastatic BTC	II	NCT04677504 (IMbrave-151)
Immunotherapy + local regional treatment	Durvalumab + Tremelimumab + TACE/RFA/ Cryoablation	Inoperable, BTC must be technically amenable to local treatment	II	NCT02821754
Immunotherapy + local regional treatment	Durvalumab + Tremelimumab + SIRT	Inoperable, BTC must be technically amenable to local treatment	II	NCT04238637 (IMMUWHY)
Immunotherapy + local regional treatment	Durvalumab + Tremelimumab + Radiation	Inoperable, HCC/BTC must be technically amenable to local treatment, for BTC: progression after at least one prior therapy	II	NCT03482102
Immunotherapy + local regional treatment	Bintrafusp Alfa + Hypofractionated radiation therapy	Advanced/metastatic iCCC after at least one prior therapy	I	NCT04708067
Immunotherapy + PARP-inhibition	Rucaparib + Nivolumab	Advanced/metastatic BTC following platinum-based first-line therapy	II	NCT03639935
Immunotherapy + TKI	Pembrolizumab + Lenvatinib	Advanced/metastatic solid tumours after at least one prior therapy	II	NCT03797326 (LEAP-005)
Immunotherapy + hypomethylating agent	Durvalumab + guadecitabine	BTC; second-line therapy	Ib	NCT03257761
Immunotherapy + CD27 agonist + MEK-inhibition	Atezolizumab and CDX-1127 (Varlilumab) with or without addition of Cobimetinib	Advanced/metastatic BTC after least 1 prior line of systemic therapy, and rno more than 2 prior lines of therapy	II	NCT04941287 (ETCTN 10476)
Immunotherapy + DKK1-inhibition	DKN-01 + Nivolumab	Advanced/metastatic BTC after at least one prior therapy	II	NCT04057365
Immunotherapy and galectin-9 protein inhibition	LYT-200 alone or in combination with chemotherapy or Tislelizumab	Advanced/metastatic solid tumours	I/II	NCT04666688
Immunotherapy + DNA-dependent protein kinase inhibition	Peposertib (M3814) and Avelumab in combination with hypofractionated radiation	Advanced/metastatic solid tumours after standard therapy	I/II	NCT04068194
Novel agents				
B7-H4 expression	AZD8205	Advanced/metastatic solid tumours with B7-H4 expression after standard therapy	I/IIa	NCT05123482
B7-H4 expression	SGN-B7H4V	Advanced/metastatic solid tumours with B7-H4 expression after standard therapy	I	NCT05194072
Mitochondrial metabolism inhibition	Gemcitabine / Cisplatin alone or with Demivistat (CPI-613)	First-line therapy for advanced/metastatic BTC	Ib/II	NCT04203160 (BilT-04)
Enzyme sphingosine kinase-2 (SK2) inhibition	Opaganib alone and in combination with Hydroxychloroquine Sulfate (HCQ)	Advanced/metastatic BTC with no more than 2 prior treatments	II	NCT03377179 (ABC-108)
Therapeutic autologous dendritic cells	Pneumococcal 13-valent Conjugate Vaccine	Unresectable iCCC treated with high-dose conformal external beam radiotherapy (EBRT)	I/II	NCT03942328
Lymphnode targeting vaccine	ELI-002 (amphiphile (Amph)-modified G12D and G12R mKRAS long peptides and Amph-modified Toll-like receptor 9 (TLR9) agonistic CpG-7909 DNA)	Positivity for minimal residual mKRAS disease (ctDNA and/or serum tumor antigen) after locoregional treatment in PDAC and other solid tumours	I	NCT04853017 (AMPLIFY-201)

targeted therapy depending on the genomic alteration found or to continue with standard of care.

7. Conclusion

After a decade without clinically meaningful innovations, the therapeutic armoury for the treatment of BTC patients has been filled rapidly within the last few years. Chemotherapy still represents the backbone of systemic treatment of BTC. Its combination with immunotherapy represents the new standard of care for front-line treatment, though. Furthermore, the identification of actionable alterations has paved the way to a new era of personalised therapy aiming to extend the benefit of systemic treatment to and for an increasingly larger segment of patients. Ongoing clinical trials are also opportunities for patients to receive targeted treatment. As soon as possible after diagnosis of an advanced staged BTC, a comprehensive genomic panel of the tumour should be obtained to guarantee appropriate subsequent targeted treatment. If feasible, a comprehensive DNA- and RNA-based NGS panel is ideally applied to identify potential alterations. Alternatively, a combination of IHC, FISH, and/or PCR-based assays with the focus of actionable alterations can be performed.

Inferring therapeutic consequences from genomic data however is not always straight forward and interdisciplinary discussion in the context of a molecular tumour board is therefore recommended. This further highlights the need for crosstalk between office-based oncologists, hospitals and specialised centres.

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