Adjuvant therapy of biliary tract cancers

**Joanna Kefas, John Bridgewater, Arndt Vogel, Alexander Stein
and John Primrose**

Abstract: Biliary tract cancers (BTCs) are rare and heterogeneous malignant tumours including cholangiocarcinoma and gallbladder cancer. They are very aggressive, often refractory to chemotherapy and associated with an overall poor prognosis. Surgical resection remains the only potentially curative treatment option but less than 35% present with resectable disease. Adjuvant treatments have been widely used but until recently, supportive data were limited to non-randomised, non-controlled retrospective studies. Recent evidence from the BILCAP trial has established adjuvant capecitabine as the standard of care. But there are still unanswered questions as to the role of adjuvant therapy. Further prospective data and translational research with reproducible evidence of clinical benefit are needed. In this review of adjuvant therapy in resectable BTCs, we will summarise the latest evidence setting current treatment standards and highlight future prospects.

Background and current practice

Biliary tract cancers (BTCs) are rare and whilst being the second most common primary hepatic malignancy, they account for less than 1% of all cancers, 3% of all gastrointestinal cancers, and typically present in the seventh decade with a slight male predominance.[1] They represent a distinct and heterogeneous group of cancers arising from the bile duct epithelium and are subdivided into gallbladder cancer (GBC) and cholangiocarcinoma (CCA). CCAs are classified by anatomical origin as intrahepatic CCA (iCCA) and extrahepatic CCA (eCCA) – the latter further subdivided into perihilar CCA (pCCA) and distal CCA (dCCA) (Figure 1).[2]

These subgroups demonstrate distinct epidemiological variations with a low incidence in Western countries (0.3–6 cases per 100,000 per year) but higher in specific regions of Southeast Asia (7.1–14.5 per 100,000 per year).[3] Overall incidence is rising specifically for iCCA, whereas rates are stable or falling for eCCA. Worldwide mortality rates have increased over the last 20 years with mortality higher in men and the Asian population.[4] These shifts are probably explained by differences in risk factor exposure and genetic predispositions. Identified risk factors differ relative to anatomical origin but are largely based on the principle of chronic inflammation and bile stasis such as chronic parasitic infection (in regions of high endemicity), primary sclerosing cholangitis and hepatolithiasis. Other potential risk associations include hepatitis and smoking with iCCA[5]; and obesity, inflammatory bowel disease and diabetes with GBC.[6] But most patients do not have an identifiable risk factor nor do these factors offer a clear etiologic explanation for global trends and so largely, the underlying pathogenesis remains unknown.[7] At a molecular level, through comprehensive whole-exome and transcriptome sequencing, heterogeneity is again observed in the different molecular profiles of iCCA, eCCA and GBC. Genetic alterations in potential therapeutic targets occur in nearly 40%, particularly in iCCAs and most commonly as fibroblast growth factor receptor (FGFR) fusions and isocitrate dehydrogenase 1 mutations.[8] Correlation of molecular profiling with prognosis and/or potential benefits from adjuvant treatment are currently lacking, but translational analysis of the large randomised adjuvant trials is on the way.

Correspondence to:
Joanna Kefas
University College London Hospital NHS trust, 250 Euston Road, London NW1 2PG, UK.
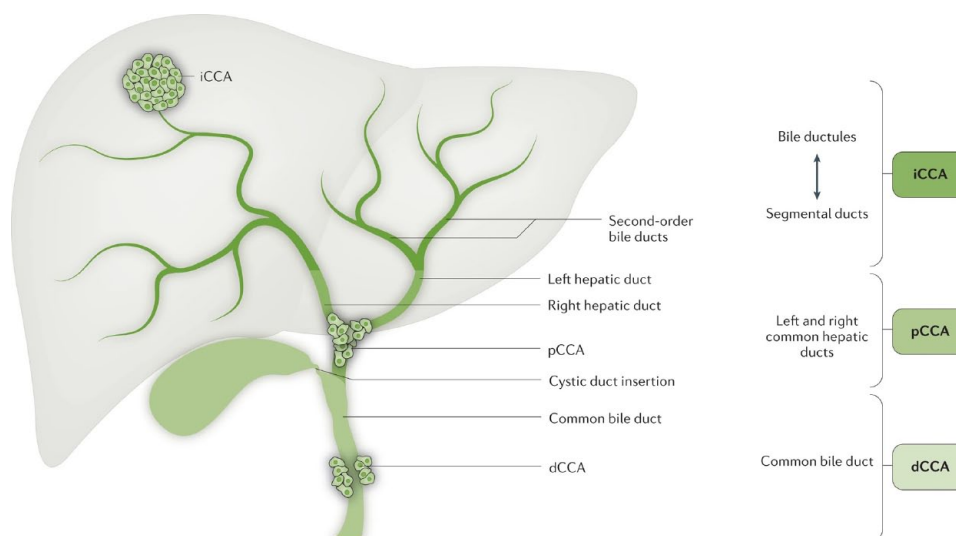**Joannakefas@nhs.net**

John Bridgewater
UCL Cancer Institute, London, UK

Arndt Vogel
Hannover Medical School, Hanover, Germany

Alexander Stein
Hematology-Oncology Practice Eppendorf, University Cancer Center Hamburg, Hamburg, Germany

John Primrose
University of Southampton, Southampton, UK

Figure 1. Anatomical classification of cholangiocarcinoma.
Reprinted with permission from 2. Copyright © 2021, Springer Nature Limited.
CCA is best classified according to the primary, anatomic subtype as iCCA, pCCA and dCCA. iCCA is located proximally to the second-order bile ducts within the liver parenchyma. pCCA is localised between the second-order bile ducts and the insertion of the cystic duct into the common bile duct. dCCA is confined to the common bile duct below the cystic duct insertion.
CCA, cholangiocarcinoma; dCCA, distal CCA; iCCA, intrahepatic CCA; pCCA, perihilar CCA.

The only curative management and the best chance for prolonged survival is radical surgical resection. Unfortunately, less than 35% of patients present with resectable disease at diagnosis, and postoperative recurrence and relapse rates are around 50–70%.[9–11] Despite advances in diagnostics and therapeutic interventions, prognosis remains poor with an overall 5-year survival of less than 20%.[12] As most patients present with advanced or metastatic disease, treatment strategies currently used include systemic chemotherapy, targeted therapy or locoregional management.[1] First-line chemotherapy with gemcitabine and cisplatin (GemCis) was standardised based on the ABC-02 phase III clinical trial, with a median overall survival (OS) of 11.7 months and an objective response rate (ORR) of 26.1%.[13] More recently, the TOPAZ-1 trial has reported an improvement of all efficacy endpoints with the addition of durvalumab to GemCis and the immuno-oncology-based combination will most likely be the primary choice in the first-line setting once approved by European Medicines Agency (EMA; already approved by Food and Drug Administration).[14] Pemigatinib, the FGFR2 inhibitor, is the first approved molecularly targeted therapy and is recommended for patients harbouring FGFR2 fusions or rearrangements who have progressed after one or more lines of therapy.[15]

For the favourable patients presenting with resectable disease, support and recommendations for adjuvant therapy followed the 2012 Horgan *et al.*[16] meta-analysis which included 20 trials and 6712 patients with resected BTC who had adjuvant therapy ($n = 1797$) or surgery alone ($n = 4915$). Overall, there was a non-significant comparative improvement in survival with any adjuvant therapy (OR: 0.74, 95% CI: 0.55–1.01, $p = 0.06$). In terms of treatment modality, adjuvant chemotherapy alone (OR: 0.39, 95% CI: 0.23–0.66, $p < 0.001$) or chemoradiotherapy (OR: 0.61, 95% CI: 0.38–0.99, $p = 0.049$) was of greater benefit than radiotherapy alone (OR: 0.98, 95% CI: 0.67–1.43, $p = 0.90$). Prespecified subgroup analyses found the greatest benefit was in those with node positive (OR: 0.49, 95% CI: 0.30–0.80, $p = 0.004$) and R1 disease (OR: 0.36, 95% CI: 0.19–0.68, $p = 0.002$). For radiotherapy only, there was a reported significant benefit in R1 patients (OR: 0.33, 95% CI: 0.14–0.81, $p = 0.01$). Besides this, available review data could not distinguish or compare the diverse types of chemotherapy and chemoradiotherapy regimens and schedules. The included studies were retrospective and therefore likely to represent an inherent selection bias towards high-risk patients. Additionally, being limited to non-randomised and non-controlled studies makes the conclusive evidence of a tangible benefit from adjuvant

therapy less reliable. Over the last 5 years and notably since the BILCAP trial,[17] chemotherapy-based adjuvant strategies are offered to patients with macroscopically complete resected BTCs. In this review, we will summarise available evidence for adjuvant therapy from clinical trials, as well as the role and rationale of other potential therapeutic options.

Randomised trials since 2017

A literature search was performed using PUBMED, American Society of Clinical Oncology (ASCO) Gastrointestinal, European Society of Medical Oncology (ESMO) and ClinicalTrials.gov. The following search terms were used in combination: 'biliary tract cancer', 'cholangiocarcinoma', 'gallbladder cancer' AND 'adjuvant' from inception to the last search on 26th July 2022. Figure 2 reports the flow chart of trials included in this review. The previously mentioned meta-analysis included only one randomised phase III trial,[18] and so this search identified four completed phase III randomised trials for discussion since this time point.

The BCAT open-label multicentre phase III trial was the first published trial to explore adjuvant chemotherapy in BTC with long-term follow-up over 5 years.[19] Set up in Japan, patients with resected eCCA were randomised to gemcitabine *versus* observation alone. Based on known favourable effects of gemcitabine in the metastatic setting, a total of 225 patients were randomised 1:1 to receive six cycles of adjuvant gemcitabine ($n = 117$) *versus* observation ($n = 108$). Patients were stratified by known prognostic factors of resection margin status, lymph node involvement and primary tumour site. The OS did not differ significantly at 62.3 months *versus* 63.8 months (HR: 0.93, 95% CI: 0.66–1.32, $p = 0.693$; Figure 3(a)). Relapse-free survival (RFS) also did not differ significantly at 36.0 months *versus* 39.9 months (HR: 1.01, 95% CI: 0.70–1.45, $p = 0.964$; Figure 3(b)). There was no significant subgroup postoperative survival difference in resection margin involvement or node-positive status. The 50% 5-year survival in both gemcitabine and observation cohorts observed was better than the anticipated rate of 30%, likely due to advancing surgical techniques and the trial's selection criteria.[20] Although gemcitabine is widely used in metastatic disease, the BCAT trial found no benefit in the adjuvant setting and so

the standard postoperative care approach at this point remained observation.

Similar negative findings were reported in the PRODIGE 12-ACCORD 18 (UNICANCER GI) phase III trial comparing adjuvant chemotherapy to observation.[21] A total of 196 patients diagnosed with iCCA/eCCA/GBC were enrolled following R0 or R1 surgery and randomised 1:1 to 12 cycles of gemcitabine oxaliplatin (GEMOX) ($n = 195$) or observation ($n = 99$). Co-primary endpoints were RFS and quality of life (QoL). Median RFS was 30.4 months (95% CI: 15.4–43.0) in the GEMOX arm and 18.5 months (95% CI: 12.6–38.2) in the observation arm (HR: 0.88, 95% CI: 0.62–1.25, $p = 0.47$; Figure 4(a)). Planned subgroup analyses similarly found no subgroup benefit and a similar proportion of the observation and GEMOX arm developed metastatic recurrence. With no significant difference found in RFS (despite a difference in medians), OS or time to deterioration of QoL, there was no evidence of superiority of GEMOX compared to surveillance in resected BTC – although the study was significantly underpowered.

Both the BCAT and PRODIGE trial findings contrast with the BILCAP trial published in 2019 which is so far the largest prospective phase III randomised adjuvant BTC study.[17] The BILCAP trial ran over 8 years and recruited from 44 centres. A total of 447 patients were randomised to eight cycles of adjuvant oral capecitabine *versus* observation after surgical resection. Patients with CCA and GBC were included. The median OS in the intention-to-treat (ITT) population was 51.1 months (95% CI: 34.6–59.1) in the capecitabine arm and 36.4 months (95% CI: 29.7–44.5) in the observation arm (HR: 0.81, 95% CI: 0.63–1.04; $p = 0.097$). In the per-protocol (PP) specified analysis, there was a clear significant difference in mean OS of 53 months (95% CI: 40–NR) in the capecitabine arm compared to 36 months (95% CI: 29.6–44.2) (HR: 0.75, 95% CI: 0.58–0.97, $p = 0.028$). RFS in the ITT population was 24.4 months (95% CI: 18.9–35.9) in the capecitabine arm and 17.5 months (95% CI: 12.0–23.8) in the observation arm (HR: 0.76, 95% CI: 0.58–0.99, $p = 0.039$). The ITT OS was not statistically significant but the PP OS and RFS demonstrated statistically and clinically meaningful benefits. The results of the long-term 5-year survival analysis published this year are illustrated in Figure 5.[9] In this analysis, the median OS in the
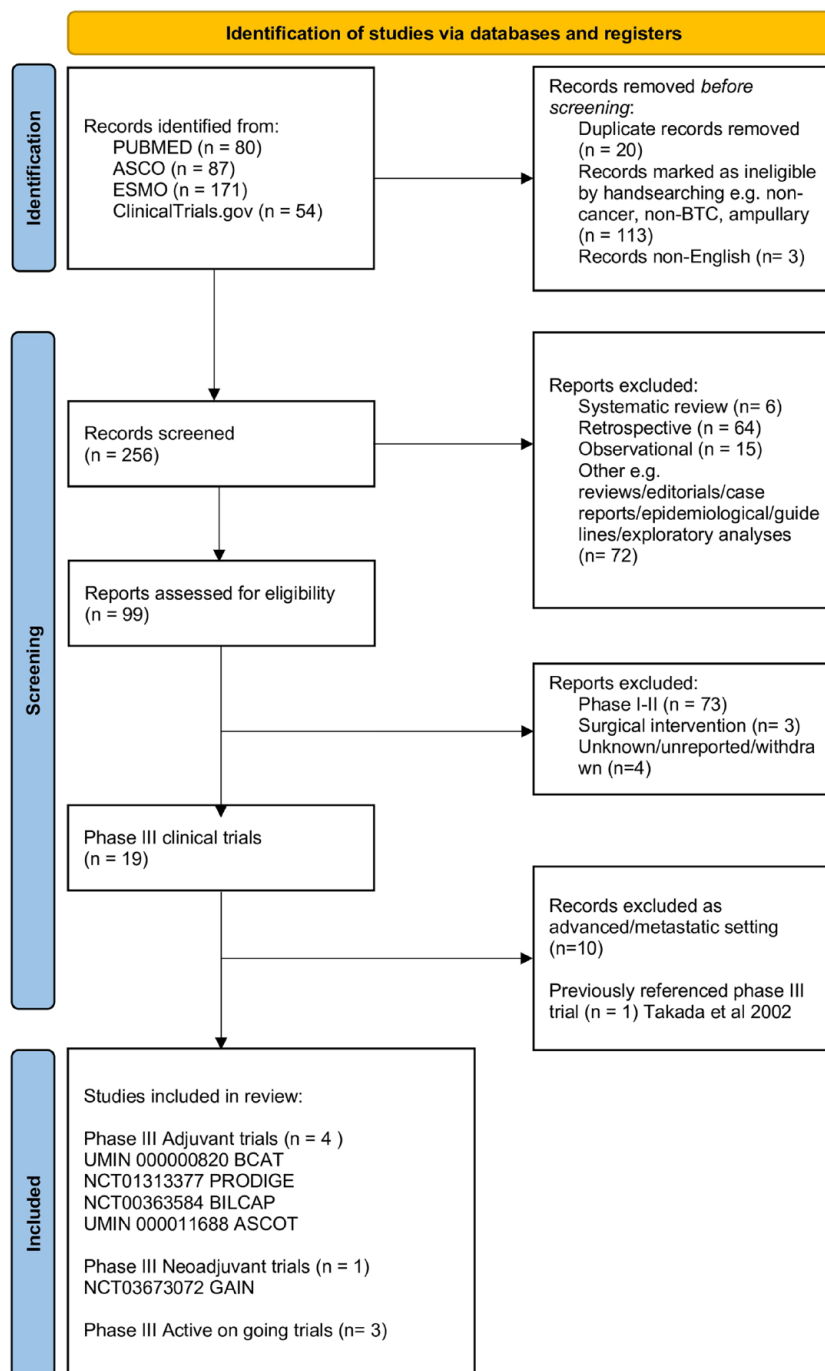
Figure 2. Flow chart of literature search.
ASCO, American Society of Clinical Oncology; BTC, biliary tract cancer; ESMO, European Society of Medical Oncology.

ITT capecitabine arm was 49.6 months (95% CI: 35.1–59.1) compared to 36.1 months (95% CI: 29.7–44.2) in the observation arm (HR: 0.84, 95% CI: 0.67–1.06). In the long-term PP sensitivity analyses (adjusted for nodal status, grade of disease and gender), median OS was 52.3 months in the capecitabine arm *versus* 36.1 months in the observation arm (HR: 0.79, 95% CI: 0.63–1.00). In the ITT analyses, the median RFS was 24.3 months *versus* 17.4 months (HR: 0.81, 95% CI: 0.65–1.01). The PP analyses median RFS was 25.3 months *versus* 16.8 months (HR: 0.77, 95%
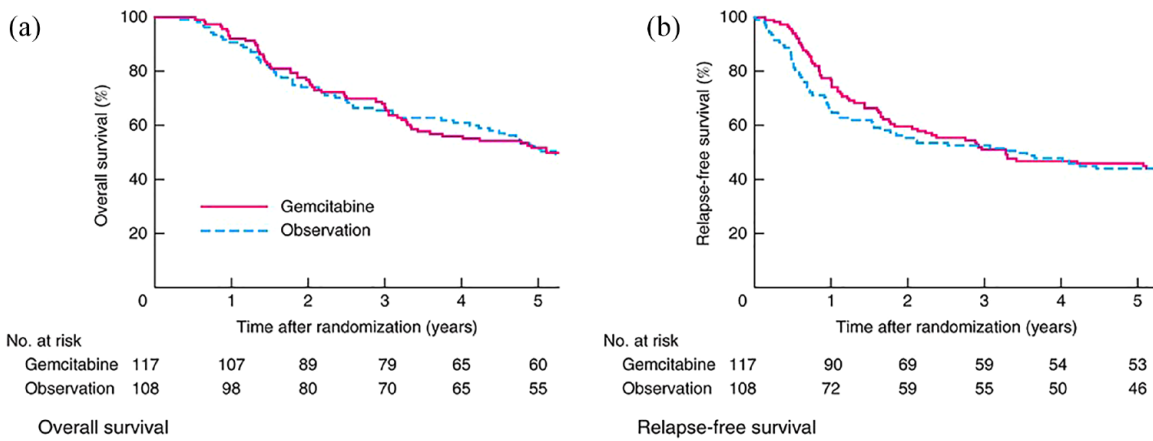
Figure 3. Kaplan–Meier estimates of (a) OS and (b) RFS in gemcitabine and observation groups. (a) *p* = 0.997 and (b) *p* = 0.717 (log rank test).
Reprinted with permission from 19. Copyright 2018 BJS Society Ltd Published by John Wiley & Sons Ltd.
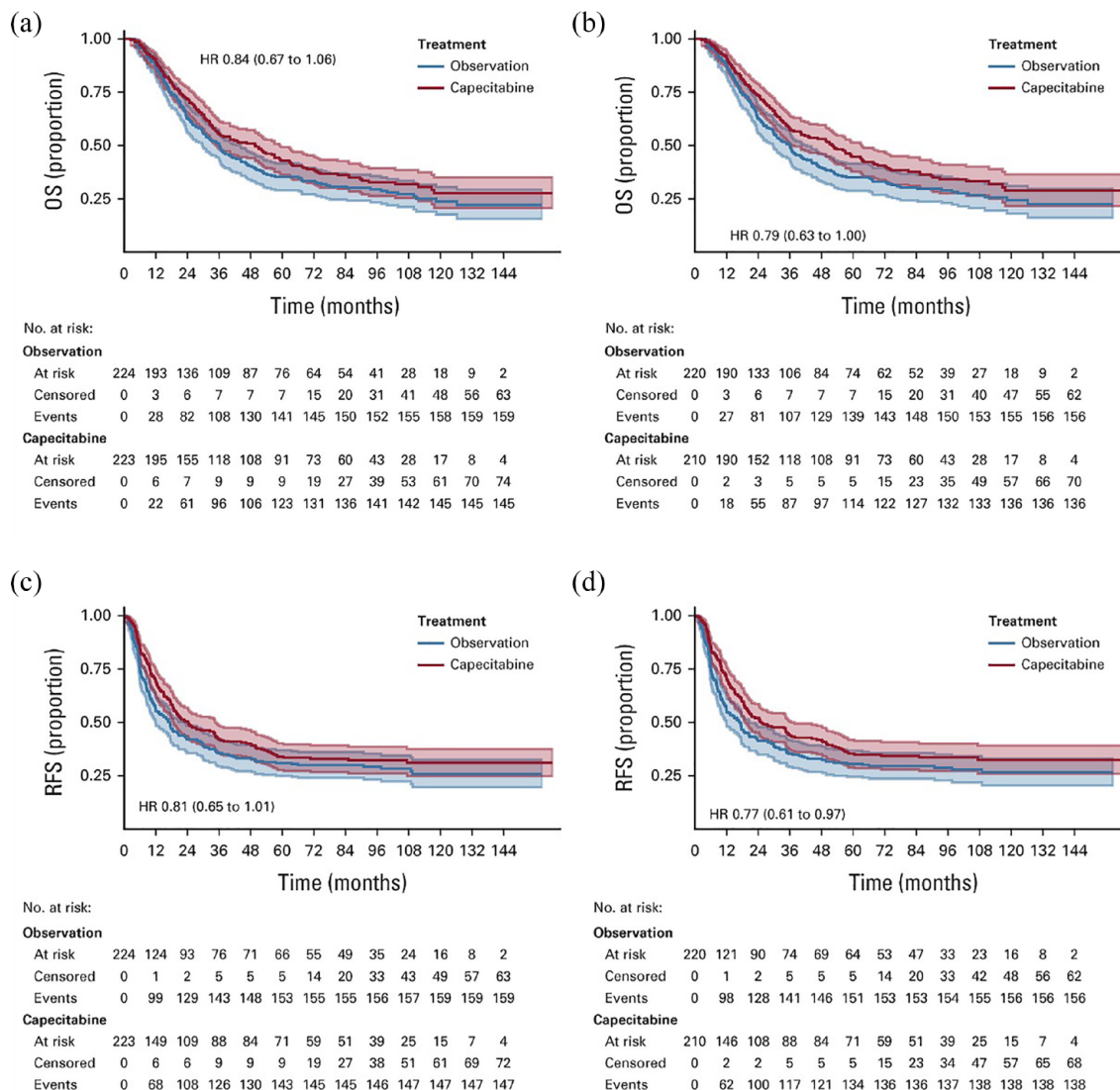OS, overall survival; RFS, relapse-free survival.
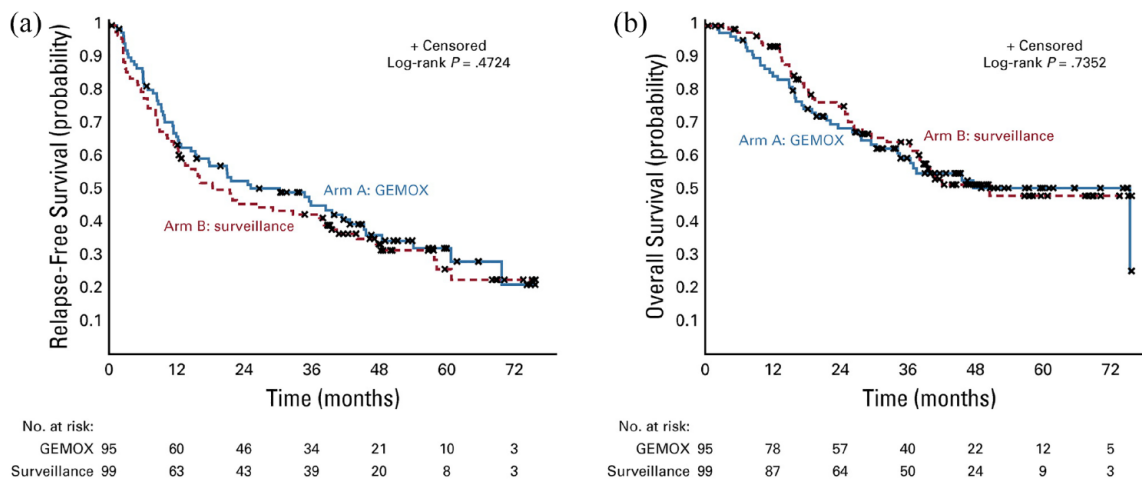
Figure 4. (a) RFS and (b) OS Kaplan–Meier curves.
Reprinted with permission from 21. Copyright © 2019 by the American Society of Clinical Oncology.
GEMOX, gemcitabine and oxaliplatin; OS, overall survival; RFS, relapse-free survival.

CI: 0.61–0.97). Exploratory analysis of prognostic factors confirmed significantly poorer survival in R1, nodal positive and poorly differentiated cohorts. But no significant capecitabine benefit was found in any of these subgroups, and the trial was not powered to do so. There was no observed difference in local recurrence rates besides a delay with capecitabine and so recurrence remains a barrier to improving clinical outcomes. The molecular profiling of the study participants is in progress.

These three randomised controlled trials differ in sample size calculation, statistical power of study design, maturity of data and follow-up. Whilst attempting comparison is complex, these differences, as outlined in Table 1, may explain the conflicting positive results of the capecitabine-based BILCAP trial and the negative results of gemcitabine-based BCAT and PRODIGE trials. First comparing study design, all three were similarly randomised open-label phase III trials. The PRODIGE-12 trial was ambitiously designed to detect an improvement in RFS with GEMOX at an HR of 0.60 and therefore a 40% reduction in risk. No statistical difference was found; however, as significantly underpowered, a role for GEMOX

Figure 5. Long-term outcomes and exploratory analyses of the randomised phase III BILCAP Study. (a) Kaplan–Meier OS curves for ITT population, (b) OS for PP population, (c) RFS for ITT, and (d) RFS for PP. Reprinted with permission from 9. Copyright © 2022, by the American Society of Clinical Oncology.
HR, hazard ratio; ITT, intention-to treat; OS, overall survival; PP, per-protocol; RFS, relapse-free survival.

in this setting cannot be excluded. When comparing time to treatment, it is noted that although not consistently reported, for pre-2017 studies the time from surgery to adjuvant therapy was within 12 weeks, with one trial giving adjuvant chemotherapy at the time of resection.[18] The 'modern' trials started adjuvant therapy up to 12 weeks post-surgery and continued for up to 6 months. The BCAT trial enrolled patients within 10 weeks of surgery and the median interval between surgery and randomisation was 50 days. Gemcitabine was discontinued in 53 patients (45.3%) for reasons such as intolerance, patient refusal, disease relapse, physician decision

and dosing delays. So, 61 patients (52.1%) completed protocol therapy. The PRODIGE trial similarly enrolled patients who had curative resections less than 3 months before randomisation and started adjuvant treatment within 7 days of random assignment. The included patients received a median of 12 cycles of gemcitabine and 10 cycles of oxaliplatin. In the BILCAP trial, the original start to chemotherapy was 8 weeks but this extended to 16 weeks following multiple protocol amendments, and 55% of included patients completed the protocolled eight cycles of capecitabine. Next comparing study patient selection, the BCAT trial patients have exclusively eCCA

Table 1. Adjuvant therapy randomised trials in resectable BTC: published trials since 2017.

Study	BCAT	PRODIGE-12	BILCAP	ASCOT	STAMP
Phase	Randomised phase III open-label	Randomised phase III open-label	Randomised phase III open-label	Randomised phase III open-label	Randomised phase II open-label
Location	Japan	France	UK	Japan	Korea
Number of sites	48	33	44	38	3
Recruitment period	1 September 2007 and 31 January 2011	July 2009 and February 2014	15 March 2016 and 4 December 2014	September 2013 to June 2018	July 2017 and November 2020
Experimental arm	Gemcitabine 1000 mg/m^2 iv days 1, 8 and 15 every 4 weeks for six cycles	GEMOX (gemcitabine 1000 mg/m^2 iv on day 1 and oxaliplatin 85 mg/m^2 iv on day 2 of a 2-week cycle) for 12 cycles	Capecitabine oral 1250 mg/m^2 twice daily on days 1–14 of a 21-day cycle, for eight cycles	S-1 oral 40 mg/m^2 twice daily for 4 weeks, followed by 2 weeks rest, for 4 cycles	GemCis (gemcitabine 1000 mg/m^2 iv and cisplatin 25 mg/m^2 iv on days 1 and 8, every 3 weeks for eight cycles
Control arm	Observation	Observation	Observation	Observation	Capecitabine oral 1250 mg/m^2 twice daily on days 1–14 every 3 weeks for eight cycles
Sample size	225 patients (gemcitabine: $n=117$, observation: $n=108$)	196 patients (GEMOX: $n=95$, observation: $n=99$)	447 patients (capecitabine $n=223$, observation $n=224$)	440 patients (S-1 $n=218$ and observation $n=222$)	101 patients (GemCis $n=50$, capecitabine $n=51$)
Primary endpoint	OS	RFS	OS	OS	RFS
Secondary endpoints	RFS, subgroup analysis and toxicity	OS, toxicity and exploratory translational endpoints	PP analysis of outcomes, RFS, toxicity, health economics and QoL	RFS, incidence of adverse events and proportion of treatment completion	OS, safety and health-related QoL
Enrolment timeline	Enrolled within 10 weeks after surgery and median interval between surgery and randomisation was 50 days	Resection less than 3 months before randomisation	Treatment within 16 weeks of surgery	Treatment within 2 and 10 weeks after resection	Resection less than 12 weeks before randomisation
		Treatment arm within 7 days of randomisation			
Randomisation method	1:1 modified minimisation method	1:1 with minimisation method	1:1 randomisation with minimisation method	1:1 randomisation with minimisation method	1:1 randomisation
Stratification groups	Resection status, nodal status, tumour location and enrolment centre	Primary site, resection status, nodal status and enrolment centre	Primary site, resection status, nodal status, ECOG PS	Primary site, nodal status	Primary site and resection status
Exploratory subgroup analyses	Planned according to stratification parameters	Planned according to stratification parameters	Planned according to stratification parameters	Planned according to stratification parameters	Planned according to stratification parameters
Patient characteristics	ECOG PS 0-1	ECOG PS 0-2	ECOG PS 0-2	ECOG PS 0-1	ECOG PS 0-1

(Continued)

Table 1. (Continued)

Study	BCAT	PRODIGE-12	BILCAP	ASCOT	STAMP
Tumour subtype	eCCA only	iCCA 44%	iCCA 19%	iCCA 13%	pCCA 44.6%
	pCCA 45%	pCCA8%	pCCA 29%	eCCA 56%	dCCA 55.4%
	dCCA 55%	dCCA 28%	dCCA 35%	GBC 14%	
		GBC 20%	GBC 18%	Ampullary 17%	
Nodal status	N0 65%	N0 50%	N0 53%	N0 60%	N1 78%
	N1 35%	N1 36%	N1 47%	N1 40%	N2 22%
Resection margins	R0 89%	R0 87%	R0 62%	R0 86%	R0 68%
	R1 11%	R1 13%	R1 38%	R1 14%	R1 32%
OS (months) and HR (95% CI)	62.3 *versus* 63.8 (1.01, 0.70–1.45, $p = 0.964$)	75.8 *versus* 50.8 (1.08, 0.70–1.66, $p = 0.74$)	49.6 *versus* 36.1 (0.84, 0.67–1.06)	77.1% *versus* 67.6% 3 year OS (0.694, 0.514–0.935, $p = 0.008$)	35.7 *versus* 35.7 (1.08, 0.72–1.64, $p = 0.81$)
RFS (months) and HR (95% CI)	36.0 *versus* 39.9 (0.93, 0.66–1.32, $p = 0.693$)	30.4 *versus* 18.5 (0.88, 0.62–1.25, $p = 0.48$)	24.3 *versus* 17.4 (0.81, 0.65–1.01)	62.4% *versus* 50.9% 3-year RFS (0.797, 0.613–1.035)	14.3 *versus* 11.1 (0.96, 0.71–1.30, $p = 0.86$)
Results in planned subgroup analyses	No significant difference	No significant difference	Poorly differentiated and male sex associated with greater benefit	Favourable OS and RFS in N1 subgroup for adjuvant S-1 arm	Favourable DFS in R1 subgroup with control capecitabine arm ($p = 0.03$)
Median follow-up period (months)	79.4	46.5	106	45.4	28.7
Scientific impact/ trial outcome	Negative	Negative	Positive	Positive	Negative

BTC, biliary tract cancer; CCA, cholangiocarcinoma; dCCA, distal CCA; DFS, disease-free survival; GEMOX, gemcitabine oxaliplatin; ECOG PS, Eastern Cooperative Oncology Group performance status; iCCA, intrahepatic CCA; pCCA, perihilar CCA; OS, overall survival; QoL, quality of life; RFS, relapse-free survival.

tumours, whereas the PRODIGE trial was predominantly iCCA (44%). As well as differences in the BTC subtypes, there was heterogeneity in the populations enrolled in these three adjuvant trials based on nodal involvement and resection margins. Both the BCAT and PRODIGE trials had similar-sized node-positive populations of 35% and 36%, respectively, but this was higher at 47% in the BILCAP study. The genomic profile varies based on tumour location and so therefore could explain differences in chemotherapy response rates and prognosis. Also, R0 clear resection margin was again similar in BCAT and PRODIGE at 89% and 86%, respectively, but lower in the BILCAP trial at 62%. Notably, the BILCAP trial included the largest number of patients, and so statistical power, as well as a high proportion with poor prognostic factors which

partly explains why it was the only positive study. Primary endpoints differed between the trials, with PRODIGE-12 powered for RFS. Although a treatment effect on RFS would be expected to show an effect on OS, it is not an optimal surrogate and ideally, studies should be adequately powered to detect significance in both to appropriately determine clinically meaningful benefits.

The OS in the control arms was clearly different between the three studies with BCAT at 63.8 months, PRODIGE 50.8 months and BILCAP 36.0 months. Although there were also differences in the control arms' survival rates, it is not clear whether these differences affected trial outcomes and statistical power. Of the modern randomised phase III trials, only the BILCAP trial indicated a survival benefit. It did not meet

its primary endpoint and disease recurrence was high at 65% in the observation cohort and 60% in the treatment cohort. Despite this, it changed clinical practice and current ASCO and recently updated ESMO guidelines recommend adjuvant capecitabine as the standard of care for resectable BTC[22,23].

Included in Table 1 for comparison are the more recently completed phase III ASCOT and phase II STAMP trials. A favourable outcome with adjuvant therapy has been described in the unpublished Japanese JCOG1202 (ASCOT) [UMIN00001166888] open-label multicentre randomised phase III trial comparing adjuvant S-1 (oral fluoropyrimidine derivative only used in Japan) with observation alone in resected CCA, GBC and ampullary cancer.[24] Presented at ASCO Gastrointestinal Symposium 2022, 440 patients were enrolled and randomised to observation ($n = 222$) or four cycles of adjuvant S-1 ($n = 218$) with a median follow-up of 45.4 months. Similar baseline characteristics were observed in the two arms and the majority of participants had eCCA, stage II disease, R0 resection and N0 status. The primary endpoint was OS and was significantly improved with adjuvant S-1 therapy compared to surgery alone (HR: 0.694, 95% CI: 0.514–0.935, $p = 0.008$). 3-year OS was 77.1% in the adjuvant arm (95% CI: 70.9–82.1%) and 67.6% for surgery alone (95% CI: 61.0–73.3%). The 3-year RFS was 62.4% for the adjuvant arm (95% CI: 55.6–68.4%) and 50.9% for surgery alone (95% CI: 44.1–57.2%). Pre-planned subgroup analyses all showed a favourable outcome and so probable that adjuvant S-1 will become standard of care in Japan.

In contrast, the relatively small STAMP multi-centred randomised phase II trial [NCT03079427] comparing adjuvant GemCis with capecitabine for resected lymph node-positive pCCA and dCCA was statistically negative.[25] A total of 101 patients were included and randomised within 12 weeks of resection. The 2-year disease-free survival was 25.1% in the capecitabine cohort (17.4–33.5) compared to 38.5% in the GemCis cohort (29.5–47.4), HR: 0.96 (90% CI: 0.71–1.30) after a median follow-up of 33 months. There was no improvement in secondary end-points including OS. The clinical implications of this small trial in a high-risk eCCA cohort are not yet clear, but it is the first randomised trial to include GemCis.

In the ongoing work to determine the value of adjuvant therapy, further insights are also awaited from the largest adjuvant phase III trial ACTICCA-1 [NCT02170090] which is currently enrolling patients with CCA and GBC in seven countries (Australia, Austria, Denmark, Germany, Italy, the Netherlands and the United Kingdom).[26] The trial initially randomised biliary tract patients to observation with or without GemCis and was paused after the initial presentation of the BILCAP results with 187 patients randomised. After protocol adjustment, the trial was continued and randomised a further 594 patients to GemCis *versus* capecitabine. The trial has completed recruitment and will reach the primary endpoint of disease-free survival beginning 2025. Blinded interim analysis of the first cohort ($n = 187$) was conducted in March 2020 by the independent data management committee, which recommended continuation of the trial.

Role of neoadjuvant treatment

Although not a standard approach in resectable BTCs, early systemic therapy as neoadjuvant treatment is already standard of care in other cancers such as oesophageal, gastric and rectal. The rationale is to control systemic spread as early as possible, optimise surgery by downstaging the tumour and increasing the likelihood of resectable disease, reducing recurrence rates, and thus improving OS.[27] Evidence so far supporting neoadjuvant treatment for BTCs comes from retrospective analyses indicating a higher likelihood of attaining an R0 resection in those who receive neoadjuvant chemotherapy, but the evidence is conflicting and insufficient.[28] There are no completed phase III randomised controlled trials assessing the survival benefit of neoadjuvant treatment in BTC. Several single-arm or randomised trials are currently recruiting with neoadjuvant chemo- and /or immunotherapy or transarterial approaches in iCCA (Table 2). The phase II single-arm NEO-GAP trial administered preoperative combination gemcitabine, cisplatin and nab-paclitaxel in 30 patients with resectable high-risk iCCA.[29] The primary outcome of feasibility was met with 77% of patients completing all preoperative chemotherapy and surgical resection. The partial response rate was 23%, the disease control rate was 90% and there is ongoing follow-up for RFS and OS. Other trials underway include the randomised phase II/III trial ECOG-ARCIN (EA2197) evaluating the role of neoadjuvant

GemCis before re-resection of incidental GBC compared to adjuvant only GemCis after re-resection [NCT04559139]; the phase II DEBATE trial of preoperative GemCis with or without durvalumab [NCT04308174]; phase III GAIN trial on perioperative GemCis *versus* standard of care [NCT03673072]; and the next-generation sequencing (NGS)-incorporated OPTIC phase II trial of preoperative nab-paclitaxel, cisplatin and gemcitabine with or without infigratinib.

Role of immunotherapy

Immunogenic features of BTC include programmed cell death ligand 1 (PD-L1) upregulation, mismatch repair deficiency (dMMR/MSI-H) and HLA class I antigen downregulation.[30] dMMR is associated with sensitivity to immune checkpoint inhibition due to the accumulation of somatic mutations.[31] In BTC, dMMR is reported in 5–10% of tumours, being more common in iCCA than eCCA or GBC. This implies a potential role for checkpoint inhibition, but data are limited, and efficacy has only really so far been assessed in advanced/metastatic settings. Recently, the EMA approved the programmed cell death protein 1 (PD-1) inhibitor pembrolizumab in pre-treated dMMR/MSI-H BTC based on the results of the phase II KeyNote 158 basket trial.[32] This is one of the largest published immunotherapy trials assessing the efficacy of pembrolizumab in solid tumours. Subgroup analysis of BTC MSI-H subgroup found an ORR of 40.9%, median PFS 4.2 months and mOS 24.3 months. In considering the role of immunotherapy in systemic therapy, the TOPAZ-1 phase III trial was presented at the ASCO Gastrointestinal symposium 2022, showing a significant albeit modest improvement in survival with anti-PD-1 durvalumab as first-line treatment in combination with GemCis.[33] Now published, 685 patients were randomised 1:1 double-blind to GemCis plus durvalumab or placebo.[14] Durvalumab treatment was well tolerated, and median OS was 12.8 months compared to 11.6 months with GemCis alone (HR: 0.80, 95% CI: 0.66–0.97, $p=0.021$). Median PFS was significantly improved with durvalumab 7.2 months *versus* placebo 5.7 months (HR: 0.75, 95% CI: 0.64–0.89, $p=0.001$). ORR was 26.7% with durvalumab and 18.7% with placebo (OR: 1.60, 95% CI: 1.11–2.31). Differences in continued response have also been noted at over 9 months (32.6% in durvalumab arm *versus* 25.3% in placebo arm) and at over 12 months (26.1% *versus* 15.0%). The trial is ongoing and so will evaluation of the

differences in duration of response and OS. But the benefits seen so far go beyond microsatellite instability as only 1.5% of those with MSI evaluable disease had MSI-H tumours. The similarly designed KeyNote 966 trial [NCT04003636] with GemCis plus pembrolizumab or placebo has completed enrolment and data are awaited. The data in conjunction with further follow-up analysis of TOPAZ-1 may give further insights into the role of PD-1/L1 inhibitors in BTC and further potentially responsive subgroups (as dMMR).

Role of radiotherapy

No prospective randomised trials have compared adjuvant sequential radiotherapy and chemotherapy to chemotherapy alone. The feasibility of such a regimen was established in a single-arm trial; however, due to the lack of comparative data, this has not become a standard of care.

SWOG S0809, a single-arm phase II multi-centre study, recruited 79 patients with eCCA and GBC post-resection who were deemed high risk due to advanced T stage, nodal status and/or involved resection margins.[34] Patients received four cycles of gemcitabine–capecitabine chemotherapy followed by chemoradiotherapy 45 Gy/25 fractions to regional lymph node bed and 54–59 Gy to the preoperative tumour bed. Three-dimensional planning and intensity-modulated radiotherapy was included and found to be effective, tolerable and safe. The predetermined threshold of activity was exceeded by the 65% 2-year OS (67% for R0 and 60% for R1) and median OS of 35 months. This trial established a protocol of sequential chemotherapy and chemoradiation and showed good survival rates, particularly in R1 resected patients. However, without a control group, it is of limited application as the efficacy of radiotherapy remains unclear. Its relevance in the current and evolving perioperative treatment landscape remains open. Future randomised prospective studies are needed to evaluate the role of radiotherapy in the perioperative setting.

Role of locoregional treatment

Locoregional treatment strategies for selected patients with locally advanced or liver-predominant disease are being increasingly explored including transarterial embolisation, hepatic artery infusion, transarterial chemoembolisation and radiofrequency ablation (RFA). Edeline *et al*.'s[35] systematic review and pooled analysis of

Table 2. Ongoing randomised phase II/III clinical trials including adjuvant and neoadjuvant therapy for resectable BTC.

Trial name	Country	Phase	Estimated enrolment	Target tumour subtypes	Arms and interventions	Primary endpoint	Secondary endpoints	Estimated completion date
Adjuvant setting								
ACTICCA-1 (NCT02170090)	Germany (multi-centred with 66 study locations)	Phase III randomised open-label	781	CCA, GBC	Experimental arm: GemCis	DFS	RFS, OS, QoL, safety and tolerability	April 24
					Comparison arm: capecitabine			
NCT04401709	Korea	Phase III randomised open-label	490	BTC	Experimental arm: gemcitabine plus capecitabine	DFS	OS	March 25
					Comparison arm: capecitabine			
AdBTC-1 (NCT03779035)	China	Phase III randomised open-label	460	CCA and GBC	Experimental arm: gemcitabine plus capecitabine	DFS	OS	December 23
					Comparison arm: capecitabine			
Neoadjuvant setting								
NEO-GAP (NCT03579771)	United States	Phase II single arm open-label	31	CCA	Experimental arm: neoadjuvant GemCis plus Nab-paclitaxel	Feasibility	RECIST response, R0 resection rate, RFS, OS	September 23
ECOG-ARCIN (NCT04559139)	United States	Phase II/III randomised open-label	186	GBC	Experimental arm: neoadjuvant and adjuvant GemCis	OS	Residual disease incidence, Resectability rate, PFS	September 23
					Comparison arm: adjuvant GemCis			
DEBATE (NCT04308174)	Korea	Phase II randomised open-label	45	BTC and GBC	Experimental Arm: neoadjuvant and adjuvant durvalumab plus GemCis	R0 resection rate	OS	December 23
					Comparison arm: neoadjuvant GemCis and adjuvant durvalumab			
GAIN (NCT03673072)	Germany	Phase III randomised open-label	300	BTC and GBC	Experimental arm: neoadjuvant and adjuvant GemCis	OS	QOL, PFS, R0 resection rates	November 24

(Continued)

Table 2. (Continued)

Trial name	Country	Phase	Estimated enrolment	Target tumour subtypes	Arms and interventions	Primary endpoint	Secondary endpoints	Estimated completion date
OPTIC (NCT05514912)	United States	Phase II non-randomised	40	Intrahepatic CCA	Comparison arm: standard of care, no preoperative chemotherapy / Experimental arm A: Nab-paclitaxel, GemCis and infigratinib if FGFR2 fusion/translocation positive / Active comparator arm B: Nab-paclitaxel plus GemCis in FGFR2 fusion/translocation negative	Feasibility, safety and tolerability	RECIST, ctDNA, RFS and OS	June 27
POLCAGB (NCT02867865)	India	Phase II/III randomised open-label	314	GBC	Experimental arm: neoadjuvant chemoradiation (GemCis and 45–55 Gy/25 fractions) / Comparison arm: adjuvant GemCis	OS	Resection rates, PFS, QoL	September 27

BTC, biliary tract cancer; CCA, cholangiocarcinoma; ctDNA, circulating tumour DNA; GBC, gallbladder cancer; DFS, disease-free survival.

mostly single-centre retrospective studies found insufficient evidence to support the recommendation of these locoregional treatments. Evidence for RFA was more consistent as a minimally invasive option for unresectable hepatic lesions in setting of localised recurrence with response rates and OS comparable to surgery albeit in a different population subset.[35,36]

Patient stratification and biomarkers for adjuvant chemotherapy

The currently accepted classification of BTC is anatomical but does not offer predictions of the natural history of the disease or its underlying pathogenesis, therefore nor does it offer insight into treatment response. Treatment decisions so far are based on clinical and pathological features such as tumour location and subtype, staging and the patient's performance status and comorbidities. There is a well-established emphasis on pathology in predicting likely outcomes by considering the recognised negative prognostic indicators of tumour size, positive resection margins and nodal involvement.[37] Lymph node involvement is independently associated with a high risk of early relapse and adequate lymphadenectomy should be standard in patients undergoing liver resection due to the survival benefit, even in clinically node negative iCCA.[38] Recently, Lamarca *et al.*[39] proposed a modification to staging classifications to account for the significantly worse prognosis observed in iCCA if liver metastases present, irrespective of lymph node status. But these staging factors cannot reliably predict outcomes and therapeutic response. Emerging molecular distinctions through integrative genomic, epigenomic and transcriptomic analyses are identifying cluster subtypes with distinct molecular and clinicopathological features independent of anatomical location.[40] A current and future challenge is determining which patients are more likely to benefit from adjuvant systemic treatments beyond existing clinicopathological features. Considering the disease heterogeneity and limited benefit of systemic chemotherapy, sensitive and specific predictive and prognostic biomarkers are necessary and will drive a more personalised approach.

Serum tumour markers such as carcinoembryonic antigen and carbohydrate antigen (CA) 19-9 have been used for diagnosis, prognostic value and disease monitoring.[41] Although a non-specific diagnostic marker, CA 19-9 is the most studied and widely used in predicting resectability.[42]

CA 19-9 levels are indicative of unresectable disease with a sensitivity of 76.3% and a specificity of 70.8%.[43] It can also act as a surrogate marker for treatment efficacy as persistently elevated CA 19-9 levels post-curative resection are a predictor of worse survival.[44] During gemcitabine chemotherapy, a reduction in CA19-9 equal to or more than 50% correlated with improved therapy response and increased survival but its sensitivity and specificity is 72% and 84%, respectively.[45] Changes in serum CA 19-9 could therefore be used to identify high-risk patients and therefore those who are more likely to benefit from adjuvant therapy.[44] Rather than absolute levels, an observed change may be more applicable but approximately 10% of the general population are Lewis blood group antigen negative and unable to produce CA 19-9.[46] Another potentially useful serum tumour marker, cytokeratin-19 fragment (CYFRA 21-1), is significantly upregulated in BTC and is negatively correlated with postoperative outcomes in iCCA and GBC.[47] Concentrations of CYFRA 21-1 vary according to tumour size and vascular invasion; and as an independent predictor of RFS and OS, it is therefore a potentially suitable biomarker. The prognostic value of other serum biomarkers for example, preoperative inflammatory indices such as cytokines, circulating nucleic acids including microRNA and other molecular factors are being investigated but so far in small subsets and as of yet, none with clinical applicability.[48]

In view of the standardisation of adjuvant capecitabine, few available publications so far have explored potential predictors of capecitabine response. Thymidine phosphorylase (TP) is the rate-limiting enzyme in the activation of 5′-deoxy-5-fluorouridine and capecitabine. Retrospective studies have analysed its potential as a predictor of response. Higher TP expression, although associated with a worse prognosis, is seen predominantly in GBC and may be linked to their higher response rates seen in clinical trials.[49]

Tumour tissue biomarkers in resectable disease can have both prognostic and predictive implications.[45] Pre-clinical and early-phase research have identified molecular alterations with potential prognostic implications and therapeutic options including B-raf proto-oncogene serine/threonine kinase (BRAF) V600E, neurotrophic tyrosine receptor kinase fusions, dMMR, tumour mutational burden (TMB), human epidermal growth factor-2 (HER2/ERBB2) amplifications and mutations, BRCA1/2 and DNA damage repair mutations, PIK3C, Akt, RSPO fusions, RNF43 mutations and RET fusions.[50] Overexpression of p53, KRAS mutations, amplifications of HER2, and overexpression of EGFR are associated with recurrence and poor survival.[48] But none can predict response to chemotherapy.

Predictors of response to immunotherapy in specific tumour types includes PD-L1 status, TMB and MSI. Although not yet used in the adjuvant setting, for BTCs the only current relevant marker is dMMR/MSI-H. But the role of immunotherapy is yet to be defined and available data on biomarker predictors are conflicting and limited.[30]

Overall, no predictive or prognostic biomarkers are currently validated for use in clinical practice with exploration so far limited to a few studies in small cohorts with inconclusive results. CA19-9 remains the most used serum biomarker. Large cohort prospective biomarker-embedded studies in early-stage disease are needed to establish clinically applicable predictors of treatment response, gain understanding of primary and acquired resistance and identify suitable future lines of therapy including immunotherapy and targeted. As an adjunct to clinical trials, pre-clinical models could also further clarify the underlying molecular pathogenesis, discovery of biomarkers as well as testing therapeutic efficacy.[51] In clinical practice, this is all crucial for stratifying decision-making regarding likelihood of surgical response, timing and duration of adjuvant treatment, follow-up schedules and identifying likely poor responders who may benefit from neoadjuvant therapy. Evidence-supported patient selection is important in improving survival and directing treatment strategies to those most likely to benefit. Results of translational studies from clinical trials, including the use of circulating tumour DNA (ctDNA) sequencing for relapse mechanisms are awaited, but an affordable, sensitive and specific biomarker for BTC is yet to be found.

Efforts to improve patient selection face the well-known constraint of a lack of sufficient tissue. Emerging use of liquid biopsies overcomes this challenge and offers the potential to guide treatment selection for example in detecting post-resection residual disease or early relapse; tracking therapeutic response and emerging acquired resistance mechanisms.[52] Liquid biopsies are a method of detecting and analysing tumour-derived material such as circulating tumour cells

(CTCs), ctDNA, circulating exosomes and micro-RNAs from biofluids. The tumour genome is isolated from accessible sources of serum, urine, bile or saliva, and then sequencing can be done from a range of whole-genome sequencing to targeted or mutation-specific analyses. The ABC-03 trial was the first to demonstrate CTCs and to correlate this with a poor prognosis in patients with BTC.[53] Subsequent genotyping studies have also found ctDNA shares over 70% concordance with tumour tissue and is an independent predictor of survival.[54,55] The clinical validation and relevance of liquid biopsies for more accessible profiling are still being defined but promising evidence so far advocates already for integration alongside standard tumour biopsies.

Summary of recommendations and conclusion

The scope of treatment for BTCs has made slow progress over the last 10 years. Surgical resection is only a beneficial option for the small proportion of those with resectable BTC and the approved standard of care remains 6 months of adjuvant capecitabine after the positive RFS and OS results of the BILCAP trial, despite its limitations. Adjuvant therapy offers the best opportunity to impact patient outcomes with systemic therapy; however, several issues and inconsistent trial outcomes remain, most likely due to a lack of consideration for the heterogeneity of BTC seen in its epidemiology, aetiology, molecular characteristics, surgical intervention and prognosis. This complexity calls for personalised surveillance, treatment and de-escalation strategies matched to tumour subtype, molecular alterations and high-risk cohorts. To get there, better patient selection and further studies of the role of combination therapy, immunotherapy, targeted treatment and radiotherapy in neoadjuvant, adjuvant and oligo-progression settings are necessary. Neoadjuvant strategies could address the issue of early recurrence. The application of immunotherapy from basket cohort trials has the potential to bring durable clinical responses into the adjuvant setting. Although consideration of chemoradiotherapy is reasonable when addressing those at high risk of recurrence, such as R1 disease, there is so far low-level evidence supporting this. Optimal large prospective randomised trials need to be rationally designed for biologically selected patients and adequately powered with standardised risk factors, relevant pre-specified analyses and sufficient follow-up to provide evidence of clinical efficacy. With patients stratified, it is possible to have a 'low-risk' observation arm and randomised 'high-risk' comparative active treatment arms. They also need to be open to potentially significant amendments as new data become available.

Molecular profiling, understanding its relevance and identifying suitable robust biomarkers are integral to precision oncology and should be offered to all patients. And whilst being validated in an advanced setting, the incorporation of molecular profiling will be informative to subgroup analyses, predictive biomarkers, prognostic variability and drive a move into targeted therapeutic options. For this, accessible liquid biopsies and fast and affordable sequencing technologies should be adopted. BTC is a rare and challenging cancer, so encouraging trial participation, multi-centre cross-study collaboration and dedicated expert centres are essential for the evaluation and development of meaningful evidence-based recommendations that go beyond OS. Internationally collaborative databases of the unprecedented wealth of information from sequencing such as NGS are needed to determine clinical significance and actionability. A dynamic synchronicity is needed between broadening our understanding of the natural history and genomic spectra of BTC alongside translational research and new drug development.

Declarations

Author contribution(s)
Joanna Kefas: Conceptualisation; Investigation; Writing – original draft; Writing – review & editing.

John Bridgewater: Conceptualisation; Resources; Supervision; Writing – review & editing.

Arndt Vogel: Conceptualisation; Resources; Writing – review & editing.

Alexander Stein: Conceptualisation; Resources; Writing – review & editing.

John Primrose: Conceptualisation; Resources; Writing – review & editing.

References

1. Valle JW, Borbath I, Khan SA, *et al.* Biliary cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; 27(suppl 5): v28–v37.

2. Brindley PJ, Bachini M, Ilyas SI, *et al.* Cholangiocarcinoma. *Nat Rev Dis Primers* 2021; 7(1): 65.

3. Izquierdo-Sanchez L, Lamarca A, La Casta A, *et al.* Cholangiocarcinoma landscape in Europe: diagnostic, prognostic and therapeutic insights from the ENSCCA Registry. *J Hepatol* 2022; 76(5): 1109–1121.

4. Patel T. Worldwide trends in mortality from biliary tract malignancies. *BMC Cancer* 2002; 2: 10.

5. Tyson GL and El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology* 2011; 54(1): 173–184.

6. Sharma A, Sharma KL, Gupta A, *et al.* Gallbladder cancer epidemiology, pathogenesis and molecular genetics: recent update. *World J Gastroenterol* 2017; 23(22): 3978–3998.

7. Blechacz BR and Gores GJ. Cholangiocarcinoma. *Clin Liver Dis* 2008; 12(1): 131–150.

8. Nakamura H, Arai Y, Totoki Y, *et al.* Genomic spectra of biliary tract cancer. *Nat Genet* 2015; 47(9): 1003–1010.

9. Bridgewater J, Fletcher P, Palmer DH, *et al.* Long-term outcomes and exploratory analyses of the randomized phase III BILCAP study. *J Clin Oncol* 2022; 40(18): 2048–2057.

10. Komaya K, Ebata T, Shirai K, *et al.* Recurrence after resection with curative intent for distal cholangiocarcinoma. *Br J Surg* 2017; 104(4):426–433.

11. Lamarca A, Edeline J, McNamara MG, *et al.* Current standards and future perspectives in adjuvant treatment for biliary tract cancers. *Cancer Treat Rev* 2020; 84: 101936.

12. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov). SEER 17 Research Data (2012-2018), Age-standardised incidence rates, one- and five-year survival, all patients diagnosed with upper gastrointestinal cancers. National Cancer Institute 2015. Accessed 16 June 2022.

13. Valle J, Wasan H, Palmer DH, *et al.* Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; 62(14): 1273–1281.

14. Oh Do-Youn, Aiwu RH, Shukui Q, *et al.* Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer. *NEJM Evidence* 1(8), EVIDoa2200015.

15. Rizzo A, Ricci AD and Brandi G. Pemigatinib: hot topics behind the first approval of a targeted therapy in cholangiocarcinoma. *Cancer Treat Res Commun* 2021; 24: 100337.

16. Horgan AM, Amir E, Walter T, *et al.* Adjuvant therapy in the treatment of biliary tract cancer: a systematic review and meta-analysis. *J Clin Oncol* 2012; 30(16): 1934–1940.

17. Primrose JN, Fox RP, Palmer DH, *et al.* Capecitabine compared with observation in resected biliary tract cancer (BILCAP): a randomised, controlled, multicentre, phase 3 study. *Lancet Oncol* 2019; 20(5): 663–673.

18. Takada T, Amano H, Yasuda H, *et al.* Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A phase III multicenter prospective randomized controlled trial in patients with resected pancreaticobiliary carcinoma. *Cancer* 2002; 95(8): 1685-1695.

19. Ebata T, Hirano S, Konishi M, *et al*. Randomized clinical trial of adjuvant gemcitabine chemotherapy versus observation in resected bile duct cancer. *Br J Surg* 2018; 105(3): 192–202.

20. Nagino M, Ebata T, Yokoyama Y, *et al*. Evolution of surgical treatment for perihilar cholangiocarcinoma: a single-center 34-year review of 574 consecutive resections. *Ann Surg* 2013; 258(1): 129–140.

21. Edeline J, Benabdelghani M, Bertaut A, *et al*. Gemcitabine and oxaliplatin chemotherapy or surveillance in resected biliary tract cancer (PRODIGE 12-ACCORD 18-UNICANCER GI): a randomized phase III study. *J Clin Oncol* 2019; 37(8): 658–667.

22. Shroff RT, Kennedy EB, Bachini M, *et al*. Adjuvant therapy for resected biliary tract cancer: ASCO clinical practice guideline. *J Clin Oncol* 2019; 37(12): 1015–1027.

23. Vogel A, Bridgewater J, Edeline J, *et al*. Biliary tract cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2022; 34(2): 127–140.

24. Ikeda M, Nakachi K, Konishi M, *et al*. Adjuvant S-1 versus observation in curatively resected biliary tract cancer: a phase III trial (JCOG1202: ASCOT). *J Clin Oncol* 2022; 40(4_suppl): 382.

25. Yoo C, Jeong H, Kim K, *et al*. Adjuvant gemcitabine plus cisplatin (GemCis) versus capecitabine (CAP) in patients (pts) with resected lymph node (LN)-positive extrahepatic cholangiocarcinoma (CCA): a multicenter, open-label, randomized, phase 2 study (STAMP). *J Clin Oncol* 2022; 40(16_suppl): 4019.

26. Stein A, Arnold D, Bridgewater J, *et al*. Adjuvant chemotherapy with gemcitabine and cisplatin compared to observation after curative intent resection of cholangiocarcinoma and muscle invasive gallbladder carcinoma (ACTICCA-1 trial) – a randomized, multidisciplinary, multinational phase III trial. *BMC Cancer* 2015; 15: 564.

27. Versteijne E, Suker M, Groothuis K, *et al*. Preoperative chemoradiotherapy versus immediate surgery for resectable and borderline resectable pancreatic cancer: results of the Dutch randomized phase III PREOPANC trial. *J Clin Oncol* 2020; 38(16): 1763–1773.

28. Hakeem AR, Papoulas M and Menon KV. The role of neoadjuvant chemotherapy or chemoradiotherapy for advanced gallbladder cancer – a systematic review. *Eur J Surg Oncol* 2019; 45(2): 83–91.

29. Maithel SK, Javle MM, Mahipal A, *et al*. NEO-GAP: a phase II single-arm prospective feasibility study of neoadjuvant gemcitabine/cisplatin/nab-paclitaxel for resectable high-risk intrahepatic cholangiocarcinoma. *J Clin Oncol* 2022; 40(16_suppl): 4097.

30. Rizzo A, Ricci AD and Brandi G. PD-L1, TMB, MSI, and other predictors of response to immune checkpoint inhibitors in biliary tract cancer. *Cancers (Basel)* 2021; 13(3): 558.

31. Silva VW, Askan G, Daniel TD, *et al*. Biliary carcinomas: pathology and the role of DNA mismatch repair deficiency. *Chin Clin Oncol* 2016; 5(5): 62.

32. Marabelle A, Le DT, Ascierto PA, *et al*. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol* 2020; 38(1): 1–10.

33. Oh D, He AR, Qin S, *et al*. A phase 3 randomized, double-blind, placebo-controlled study of durvalumab in combination with gemcitabine plus cisplatin (GemCis) in patients (pts) with advanced biliary tract cancer (BTC): TOPAZ-1. *J Clin Oncol* 2022; 40(4_suppl): 378.

34. Ben-Josef E, Guthrie KA, El-Khoueiry AB, *et al*. SWOG S0809: a phase II intergroup trial of adjuvant capecitabine and gemcitabine followed by radiotherapy and concurrent capecitabine in extrahepatic cholangiocarcinoma and gallbladder carcinoma. *J Clin Oncol* 2015; 33(24): 2617–2622.

35. Edeline J, Lamarca A, McNamara MG, *et al*. Locoregional therapies in patients with intrahepatic cholangiocarcinoma: a systematic review and pooled analysis. *Cancer Treat Rev* 2021; 99: 102258.

36. Brandi G, Rizzo A, Dall'Olio FG, *et al*. Percutaneous radiofrequency ablation in intrahepatic cholangiocarcinoma: a retrospective single-center experience. *Int J Hyperthermia* 2020; 37(1): 479–485.

37. Nooijen LE, Banales JM, de Boer MT, *et al*. Impact of positive lymph nodes and resection margin status on the overall survival of patients with resected perihilar cholangiocarcinoma: the ENSCCA registry. *Cancers (Basel)* 2022; 14(10): 2389.

38. Sposito C, Ratti F, Cucchetti A, *et al*. Survival benefit of adequate lymphadenectomy in patients undergoing liver resection for clinically node negative intrahepatic cholangiocarcinoma. *J Hepatol* 2022; 78(2): 356–363.

39. Lamarca A, Santos-Laso A, Utpatel K, *et al.* Liver metastases of intrahepatic cholangiocarcinoma: implications for an updated staging system. *Hepatology* 2021; 73(6): 2311–2325.

40. Jusakul A, Cutcutache I, Yong CH, *et al.* Whole-genome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. *Cancer Discov* 2017; 7(10):1116–1135.

41. Macias RIR, Banales JM, Sangro B, *et al.* The search for novel diagnostic and prognostic biomarkers in cholangiocarcinoma. *Biochim Biophys Acta Mol Basis Dis* 2018; 1864(4 Pt B): 1468–1477.

42. Grunnet M and Mau-Sørensen M. Serum tumor markers in bile duct cancer–a review. *Biomarkers* 2014; 19(6): 437–443.

43. Liu F, Wang J, Ma W, *et al.* Clinical value of preoperative CA19-9 levels in evaluating resectability of gallbladder carcinoma. *ANZ J Surg* 2019; 89(3): E76–E80.

44. Yamashita S, Passot G, Aloia TA, *et al.* Prognostic value of carbohydrate antigen 19-9 in patients undergoing resection of biliary tract cancer. *Br J Surg* 2017; 104(3): 267–277.

45. Pavicevic S, Reichelt S, Uluk D, *et al.* Prognostic and predictive molecular markers in cholangiocarcinoma. *Cancers (Basel)* 2022; 14(4): 1026.

46. Gundín-Menéndez S, Santos VM, Parra-Robert M, *et al.* Serum CA 19.9 levels in patients with benign and malignant disease: correlation with the serum protein electrophoretic pattern. *Anticancer Res* 2019; 39(2): 1079–1083.

47. Huang L, Chen W, Liang P, *et al.* Serum CYFRA 21-1 in biliary tract cancers: a reliable biomarker for gallbladder carcinoma and intrahepatic cholangiocarcinoma. *Dig Dis Sci* 2015; 60(5): 1273–1283.

48. Montalvo-Jave EE, Rahnemai-Azar AA, Papaconstantinou D, *et al.* Molecular pathways and potential biomarkers in gallbladder cancer: a comprehensive review. *Surg Oncol* 2019; 31: 83–89.

49. García P, Lamarca A, Díaz J, *et al.* Current and new biomarkers for early detection, prognostic stratification, and management of gallbladder cancer patients. *Cancers (Basel)* 2020; 12(12):3670.

50. Valle JW, Lamarca A, Goyal L, *et al.* New horizons for precision medicine in biliary tract cancers. *Cancer Discov* 2017; 7(9): 943–962.

51. Massa A, Varamo C, Vita F, *et al.* Evolution of the experimental models of cholangiocarcinoma. *Cancers (Basel)* 2020; 12(8): 2308.

52. Corcoran RB and Chabner BA. Application of cell-free DNA analysis to cancer treatment. *N Engl J Med* 2018; 379(18): 1754–1765.

53. Valle JW, Wasan H, Lopes A, *et al.* Cediranib or placebo in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer (ABC-03): a randomised phase 2 trial. *Lancet Oncol* 2015; 16(8): 967–978.

54. Yang JD, Campion MB, Liu MC, *et al.* Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. *Hepatology* 2016; 63(1): 148–158.

55. Ettrich TJ, Schwerdel D, Dolnik A, *et al.* Genotyping of circulating tumor DNA in cholangiocarcinoma reveals diagnostic and prognostic information. *Sci Rep* 2019; 9(1): 13261.

Visit SAGE journals online
journals.sagepub.com/home/tam

§SAGE journals