New molecular and immunotherapeutic approaches in biliary cancer

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ABSTRACT

Biliary tract carcinoma is a collective term for a group of rare gastrointestinal cancers. This overview outlines the key pathways and specialised therapeutics in biliary cancer and the emerging role of immunotherapy by highlighting the rationale and selected examples of studies in each area.

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Biliary tract carcinoma (BTC) is a collective term for a group of rare gastrointestinal cancers. The intrahepatic cholangiocarcinomas (IHCC) arise from the small ducts within the liver. The more common extrahepatic cholangiocarcinomas (EHCC) include hilar and perihilar carcinomas, more distal tract tumours, and gall bladder carcinomas (GBCs). The incidence of cholangiocarcinomas is rising in the Western world, with reports of up to 2/100 000. By contrast, in Asian countries the incidence is much higher and reflects the endemic liver fluke infection as a key risk factor, as opposed to chronic inflammation from hepatitis C and primary sclerosing cholangitis in the West. GBC also has an incidence of 2/100 000, but its etiology is primarily related to cholecystitis and cholelithiasis, as well as some chronic infections, and is much more prevalent in parts of South America. Collectively these cancers present late in the majority of patients. Long-term outcomes for resectable patients are poor (about 30% 5-year survival) and survival in the advanced setting is short with a median survival of less than 1 year.1–4

Historically for unresectable disease, radiation and systemic chemotherapy have been the mainstay of treatment. Drug regimens with activity include gemcitabine and oxaliplatin, gemcitabine and cisplatin, 5-fluorouracil and oxaliplatin, and single agent options including gemcitabine and capecitabine.5 Although the outlook has been dismal for these diseases, the molecular genomics revolution, which has changed the paradigm of treatment in many cancers, has also led to novel approaches in biliary cancer Table 1. A number of clinical trials with targeted therapies have been completed in recent years (table 1). A key problem that has emerged, however, is the breadth of driver mutations with small patient subsets for each target and key differences across IHCC, EHCC and GBC. This combined with the rarity of the disease creates challenges with testing novel therapies.6 The development of international networks for rare cancers such as the International Rare Cancers Initiative, a consortium involving the USA, Canada, Europe and Australasia,6 is key to translating the identification of targets into trials to test and validate efficacy. Additionally the concept of basket trials accepting multiple anatomical sites with shared genetic changes has the potential to accelerate identification of active targeted agents.7

Whole genomic tumour profiling studies have identified a wide range of mutations, amplifications and deletions, many of which have targetable options.2 Important driver mutations reported in other tumours have also been documented, including the epidermal growth factor (EGF) pathway with EGF receptor (EGFR), kras and braf mutations or overexpression, as well as alteration in the mitogen-activated protein kinase and PI3K/mammalian target of rapamycin pathways, as well as TP53. Mutations in chromatin-remodelling genes BAP1 (encoding a nuclear deubiquitinase), ARID1A (encoding a subunit of the SWI/SNF chromatin-remodelling complexes) and PBRM1 (encoding a subunit of the ATP-dependent SWI/SNF chromatin-remodelling complexes) have been reported in frequencies of 10%–25%. Mutations in the metabolic pathway involving isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) are also seen. Amplifications in c-MET, FGF 19, cyclin-dependent kinase 6 and cyclin d1, as well as deletions in cyclin-dependent kinase inhibitors 2A and 2B, are all documented.3,8
Overall Nakamura et al identified five molecular modules, with alterations varying according to anatomical location (figure 1). They uncovered potentially targetable genetic alterations in 38.9% of BTC cases (93/239). These potential targets included kinases (FGFR1, FGFR2, FGFR3, PIK3CA, ALK, EGFR, ERBB2, BRAF and AKT3), other oncogenes (IDH1, IDH2, CCND1, CCND3 and MDM2) and tumour suppressor genes (BRCA1 and BRCA2). They also identified four molecular subgroups of gene expression which clustered with clinical prognosis. Of particular interest, in one group they identified positive enrichment for genes involved in the immune system, in cytokine activity and in antiapoptotic genes. In addition there were cases where a high mutation load created abundant tumour-specific neoantigens, which were also significantly enriched in this group.

In another analysis restricted to IHCC, profiling identified two different molecular-defined subclasses with distinctive clinical behaviour. An ‘inflammation’ class (38% of patients) characterised by activation of inflammatory signalling pathways, overexpression of cytokines and STAT3 activation, and a ‘proliferation’ class (62% of patients) were characterised by activation of oncogenic signalling pathways (eg, (RAS-Kirsten rat sarcoma viral oncogene homolog and MET-mesenchymal-epithelial transition factor (MET) receptor tyrosine kinase gene)), DNA amplifications at 11q13.2, deletions at 14q22.1, and mutations in KRAS and BRAF.

A significant feature of these genetic changes is the variation in targets by anatomic site. Bridgewater et al have summarised these in table 2, showing that apart from CDK 2A/B deletions and ARID1A, there is quite a distinct variation between IHCC, EHCC and GBC. To add to this complexity, significant differences in frequency of mutations are reported in liver fluke-related cholangiocarcinomas compared with non-liver fluke in Asians and compared with Western studies. Some of these may be related to anatomic site variations as well as aetiology.

Overall the ability of genomic sequencing to appropriately segment patients into groups for which targeted treatments would be most likely to improve outcomes has been supported by the work of Javle et al. They showed a similar spread of genetic changes in a large cohort of patients, but in particular in a subgroup of 321 with clinical annotation. A multivariate analysis showed that TP53 and kras mutations were indicators of inferior survival and FGFR2 of improved survival. In an analysis restricted to intrahepatic cholangiocarcinoma (IHCC), they showed that patients receiving experimental targeted therapies had a numerically better outcome than those on standard therapy (241 vs 186 weeks, p=0.07). They also showed that targeting a specific genomic association can have a major impact. They identified a number of fibroblast growth factor receptor (FGFR) aberrant expression in 54 patients. Twenty of those patients received appropriate targeted therapy and the overall survival exceeded those receiving non–FGFR-targeted therapy (25 vs 80 months, p=0.006).

### Table 1 Incidence of molecular mutations in biliary tract cancer as determined by genomic sequencing

| Mutation                  | Intrahepatic cholangiocarcinoma (%) | Extrahepatic cholangiocarcinoma (%) | Gall bladder cancer (%) |
|---------------------------|-------------------------------------|-------------------------------------|------------------------|
| ERBB2 amplification       | 3                                   | 11                                  | 16                     |
| BRAF substitution          | 5                                   | 3                                   | 1                      |
| KRAS                      | 15–22                               | 42–47                               | 11–19.2                |
| PI3KCA substitution        | 5                                   | 7                                   | 14                     |
| FGFR1-3 fusion            | 11–12.5                             | 0                                   | 3                      |
| CDKN2A/B loss             | 18                                  | 17                                  | 19                     |
| IDH1/2 substitution        | 15–23                               | 3–4                                 | 0                      |
| ARID1A alteration         | 11–20                               | 12                                  | 11–13                  |
| MET                       | 4                                   | 0                                   | 0                      |
| BAP1                      | 9–25                                | 0                                   | 4–13                   |

**Figure 1** Molecular spectra of BTC. In addition to subtype-specific characteristics, alterations were identified that were common to ICCite targetable traehepatic cholangiocarcinoma and ECC or common to all three subtypes. Blue symbols indicate genes.
| Therapeutic regimen (target) | Authors          | Patients (n) | End points     |
|-------------------------------|------------------|--------------|----------------|
| EGFR                          |                 |              |                |
| Cetuximab                     | Borbath et al\[14\] | 44           | RR 20.4, 6mPFS 47 | 13.5 |
| GEM and cetuximab First-line ph2 |                 |              |                |
| GEMOX and cetuximab First-line, single-arm ph2 | Gruenberger et al\[15\] | 30          | 63% 8.8, OS 15.2 |
| First-line versus GEMOX ph2    | Malka et al\[16\] | 150         | 23 vs 29, 6 vs 5.3 | 11 vs 12.4 |
| First-line versus GEMOX ph2    | Chen et al\[17\]  | 122         | 27 vs 15, 6.7 vs 4.1 | 10.6 vs 9.8 |
| Second-line ph2               | Paule et al\[18\] | 9           | 33% EGFR low: 4 vs high: 7 | EGFR low: 7 vs high: 9 |
| GEMCAP versus cetuximab       | Rubovszky et al\[19\] | 34          | 17.6, EGFR low: 7 vs high: 9 | 15.7 |
| Panitumumab                   |                 |              |                |
| GEMOX and panitumumab First-line ph2 KRAS WT | Hezel et al\[20\] | 31         | 45%, 10.6, 20.3 |
| GEMOX-CAP and panitumumab     | Jensen et al\[21\] | 46          | 33, 8.3, 10 |
| Anyline ph2                   |                 |              |                |
| GEM-IRINO and panitumumab     | Sohal et al\[22\] | 35          | 39, 9.7, 12.9 |
| First-line ph2                |                 |              |                |
| GEMCIS and panitumumab        | Vogel et al\[23\] | 93          | 45 vs 39, pNS 6.7 vs 8.2, pNS 12.8 vs 21.4, pNS |
| versus GEMCIS in KRAS WT      |                 |              |                |
| First-line ph2                |                 |              |                |
| Erlotinib                     | Lee et al\[24\]  | 268         | 30 vs 16, 5.8 vs 4.2 | 9.5 vs 9.5 |
| GEMOX and erlotinib versus    |                 |              |                |
| GEMOX First-line ph3          | Chioorean et al\[25\] | 11         | 0, 5.7 |
| Docetaxel and erlotinib       | Philip et al\[26\] | 42          | 8, 2.6, 7.5 |
| Ph2                           |                 |              |                |
| Her2                          |                 |              |                |
| Lapatinib                     | Ramanathan et al\[27\] | 17         | 0, 1.8, 5.2 |
| First-/Second-line ph2        | Kaseb\[28\] | 4 | 50, NR |
| Trastuzumab                   |                 |              |                |
| Second-line ph2               |                 |              |                |
| GEMCIS and afatinib           | Moehler et al\[29\] | 9 | NR 158 days, 235 days |
| First-line ph1b               |                 |              |                |
| VEGF and multitarget          |                 |              |                |
| Bevacizumab                   | Zhu et al\[30\]  | 35          | 40, 7, 12.7 |
| GEMOX and bevacizumab         |                 |              |                |
| First/Second ph2              | Iyer et al\[31\] | 50          | 72, 8.1, 11.3 |
| GEMCAP and bevacizumab        |                 |              |                |
| First-line ph2                |                 |              |                |
| Sorafenib                     | El-Khoueiry et al\[32\] | 31 | 0, 3, 9 |
| First-line ph2                | Bengala et al\[33\] | 46 | 2, 2.3, 4.4 |
| Anyline ph2                   |                 |              |                |
| GEM-sorafenib versus GEM      | Moehler et al\[34\] | 102 | 8 vs 6, 3 vs 4.9, 8.4 vs 11.2 |
| First-line ph2                |                 |              |                |
| GEMCIS and sorafenib          | Lee et al\[35\]  | 39          | NR 6.5, 14.4 |
| First-line ph2                |                 |              |                |
| Sunitinib                     | Yi et al\[36\]   | 56          | 9, 1.7, 4.8 |
| Second-line ph1               | Neuzillet et al\[37\] | 53 | 15, DCR: 85 |
| Second-line ph2               |                 |              |                |

Continued
## Table 2  Continued

| Therapeutic regimen (target) | Authors | Patients (n) | End points |
|-----------------------------|---------|--------------|------------|
| Cediranib                    | Valle et al<sup>6</sup> | 124 | 44 vs 19 | 8 vs 7.4 | 14.1 vs 11.9 |
| GEMCIS and cediranib versus GEMCIS | | | | | |
| First-line ph2/3            | | | | | |
| Vandetanib                   | Santoro et al<sup>6</sup> | 173 | 4 | 105 days | 228 days |
| FGFR                         | | | | | |
| BGJ398 in CC with FGFR2      | Javie et al<sup>60</sup> | 26 | 14 DCR: 82 | NR | NR 50% pts on study for >120 days |
| >Second-line phase 2         | | | | | |
| ODM-203                      | Ahnert et al<sup>61</sup> | 24 (1 CC with FGFR fusion) | 8 | NR | NR (>40 weeks for CC pt) |
| MAPK pathway                 | Bekali-Saab et al<sup>62</sup> | 28 | 12 | 3.7 | 9.8 |
| Selumetinib                  | Bridgewater et al<sup>63</sup> | 12 | 37.5 | 6.4 | NR |
| First-/Second-line ph2       | | | | | |
| GEMCIS and selumetinib       | | | | | |
| First-line ph1               | Ioka et al<sup>64</sup> | 20 | 5 | 10.6 weeks | NR 1 pt>9m |
| Trametinib                   | Lowery et al<sup>65</sup> | 12 | 50 | 6.4 | 9.1 |
| Binimetinib and GEMCIS       | Ahn et al<sup>66</sup> | 8 | NR | 1.7 | 3.5 |
| First-line ph1               | | | | | |
| MK-2206                      | Pant et al<sup>67</sup> | 20 | 20 | NR | NR |
| >Second-line, ph2            | | | | | |
| c-MET                        | Goyal et al<sup>68</sup> | 19 | 0 | 1.8 | 5.2 |
| Tivantinib + gemcitabine     | | | | | |
| Anyline ph1                  | | | | | |
| Cabozanitib                  | | | | | |
| >Second-line ph2             | | | | | |
| Multiagent/Other             | | | | | |
| Bevacizumab and erlotinib    | Lubner et al<sup>69</sup> | 49 | 12 | 4.4 | 9.9 |
| First-line ph2               | | | | | |
| Sorafenib and erlotinib      | El-Khoueiry et al<sup>70</sup> | 34 | 7 | 2 | 6 |
| First-line ph2               | | | | | |
| Bortezomib                   | Denlinger et al<sup>71</sup> | 20 | 5 | 1.6 | 9.5 |
| Second-/Third-line ph2       | | | | | |
| Pazopanib and trametinib     | Shroff et al<sup>72</sup> | 25 | 5 DCR: 75 | 4.3 | 6.7 |
| >Second-line ph1b            | | | | | |
| GEMOX-CAP and panitumumab    | Jensen et al<sup>73</sup> | 88 | 46 vs 18 | 6.1 vs 8.2 | 9.5 vs 12.3 |
| versus bev                   | | | | | |
| First-line with crossover at PD | | | | | |
| IDH1                         | Burris et al<sup>74</sup> | 20 | 5 DCR: 60 | NR | 5 SD>6 months |
| WNT pathway                  | | | | | |
| DKN-01                       | Eads et al<sup>75</sup> | 22 ongoing abstract | 33% (3 from 9 evaluable) | NR | NR |
| First-line ph1               | | | | | |
| Solid tumour studies         | Nayak et al<sup>76</sup> | 19 (1 CC) | NR | NR | 1 CC SD>225 days |

CAP, capcitabine; CIS, cisplatin; DCR, disease control rate; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GEM, gemcitabine; HER2, human epidermal growth factor receptor 2; IDH, isocitrate dehydrogenase; IRINO, irinotecan; KRAS, Kirsten rat sarcoma viral oncogene homologue; MAPK, mitogen-activated protein kinase; OS, overall survival; OX, oxaliplatin; PD, progressive disease; PFS, progression-free survival; ph, phase; RR, response rate; VEGF, vascular endothelial growth factor; WT, wild-type.
KEY PATHWAYS AND SELECTED STUDIES

Epidermal growth factor receptor
A number of key targets have been identified, including EGFR mutation and amplification, bRAF mutation, and HER2/neu amplification. EGFR blockade has been less successful in four randomised trials, with erlotinib, cetuximab and panitumumab not showing a survival gain (table 1) despite promising progression-free survival. However, a study in a more molecularly defined all RAS wild-type population may still be indicated. Identification of HER2/neu in 13% of Gb and 8% of EHCC and some retrospective data provide an impetus for more defined study. BRAF mutations occur in a small number of BTC cases [7], and given the significant survival advantage that bRAF inhibition gives in the melanoma setting, dual blockade with bRAF and MEK inhibitors merits evaluation in this subset. Further trials with such targets are outlined in table 3.

Fibroblast growth factor receptor
FGF mutations and fusions predominate in IHCCs in about 16% of cases. There are now FGF-targeted therapies undergoing clinical evaluation. These include multilargeted tyrosine kinase inhibitor (TKIs) that also inhibit FGF (such as ponatinib, nintedanib, dovitinib and brivanib), as well as specific FGF-directed small molecule TKI (eg, BGJ398), FGF antibodies and FGF trap molecules. A recent phase II interim report was presented of BGJ398. Fifty patients with BTC having FGF genetic alterations were enrolled, the majority being intrahepatic cholangiocarcinoma (IHCC). The overall response rate was 15% and the disease control rate was 95% with progression-free survival of 6 months, supporting further development of FGF-directed therapy for FGF mutated cholangiocarcinoma.

IDH1 and IDH2 mutations in BTC
Mutations in IDH1 and IDH2 have been identified in cholangiocarcinoma. In IHCC, an estimated 20% have IDH1, whereas 5% have IDH2 mutations. These mutations are not seen in EHCC or GBC. The mutated IDH1 and IDH2 proteins have a gain-of-function activity, catalysing the reduction of α-ketoglutarate to 2-hydroxyglutarate (2HG) by NADPH. Cancer-associated IDH mutations block normal cellular differentiation and promote tumorigenesis via the abnormal production of the oncometabolite 2HG, although their prognostic significance is controversial. Preliminary results from phase I clinical trials with IDH inhibitors in patients with advanced haematological malignancies have demonstrated an objective response rate ranging from 31% to 40% with durable responses (>1 year) observed. Furthermore, the IDH inhibitors have demonstrated early signals of activity in solid tumours with IDH mutations, including cholangiocarcinomas and low-grade gliomas. Recently, Burris et al reported the findings of a dose escalation study of AG-120 in various cancer types having these mutations. Of the 20 cholangiocarcinoma patients enrolled, response or stability was noted in 12 patients, with disease stability seen beyond 6 months.

Mutations in chromatin remodelling genes
Chromatin remodelling allows genomic DNA to access regulatory transcriptional proteins and thereby controls gene expression. Inactivating genetic alterations in ARID, BAP1, PBRM and MLH that are responsible for chromatin remodelling have been implicated in the development of BTC. Jiao et al observed that mutations in at least one of these genes occurred in almost half of the BTCs sequenced in their study. The prognostic role of mutations in chromatin remodelling genes is currently unknown, although BAP1 mutations were associated with aggressive disease resulting in bony metastases. It is hypothesised that histone deacetylase inhibitors such as vorinostat and panobinostat may offer therapeutic value in this setting.

Vascular endothelial growth factor (VEGF)
VEGF expression is increased in many biliary tract cancers, and its expression is associated with metastasis and poor survival. In one retrospective study of 239 cholangiocarcinomas, VEGF was overexpressed in 53.8% and 59.2% of intrahepatic and EHCC, respectively. On this basis, the multitargeted kinase inhibitors of VEGF receptors, such as sorafenib and sunitinib, have been studied without encouraging results. Similarly, a recent randomised study with cediranib and a study with the multitargeted kinase vandetanib have also failed to demonstrate a survival advantage, also outlined in table 1. The major stumbling block remains the absence of a reliable biomarker of efficacy for VEGF inhibitors.

DNA repair mutations in BTC
DNA repair mechanisms are essential for maintaining genomic stability and defects in these occur in BTC. Gene mutations leading to defective DNA mismatch repair (MMR) are commonly seen in several solid tumours like colorectal cancer, endometrial and gastric cancer. Report on 321 BTCs who underwent mutational profiling, and DNA repair mutations (MSH6, BRCA1, BRCA2, ATM, MLH1 or MSH2 genes) occurred in 13% IHCCA, 26% in EHCCA and 6% of GBC cases. The subset of cancers with MMR system defects is very sensitive to programmed cell death protein 1 (PD-1) blockade using checkpoint inhibitor agents like pembrolizumab. BTC patients with mutations in the DNA repair pathways can represent a subset where specific DNA repair inhibitors in addition to immunotherapy may be effective.

Immunotherapy
Biliary tract cancers represent a potentially attractive target for immune-based therapies given the background association with chronic inflammation and conditions such as cholecystitis, sclerosing cholangitis and primary biliary cirrhosis.
## Table 3  New therapies under evaluation in biliary tract cancers

| Therapeutic regimen                                                                 | Target                                                                 | Phase | ClinicalTrials.gov identifier | Status                      |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------|-------|-------------------------------|-----------------------------|
| **VEGF and multitarget**                                                            |                                                                       |       |                               |                             |
| Ramucirumab                                                                         | VEGFR2                                                                | 2     | NCT02520141                   | Recruiting                  |
| GEMCIS and ramucirumib or merestinib                                                | VEGFR2, c-MET                                                         | 2     | NCT02711553                   | Recruiting                  |
| GEMOX and sorafenib                                                                  | VEGFR-2/3, PDGFR-β, B-Raf, C-Raf                                     | 1/2   | NCT00955721                   | Completed (efficacy not reported) |
| GEM and sorafenib                                                                    | VEGFR-2/3, PDGFR-β, B-Raf, C-Raf                                     | 1     | NCT01229111                   | Recruiting                  |
| FOLFOX and cediranib                                                                | VEGFR                                                                 | 2     | NCT01229111                   | Not recruiting (no results) |
| **MAPK**                                                                            |                                                                       |       |                               |                             |
| Trametinib versus CAP/5 FU                                                           | MEK 1/2                                                               | 2     | NCT02042443                   | Ongoing (not recruiting)    |
| Binimetinib (MEK162) and GEMCIS                                                     | MEK 1/2                                                               | 2     | NCT01828034                   | Active (not recruiting)     |
| Binimetinib and GEMOX                                                                | MEK 1/2                                                               | 1     | NCT02105350                   | Suspended (no results)      |
| Binimetinib (MEK162) and capecitabine                                               | MEK                                                                   | 1     | NCT02773459                   | Recruiting                  |
| Selumetinib (at different doses) and GEMCIS                                         | MEK 1/2                                                               | 2     | NCT02151084                   | Recruiting                  |
| MEK162 and capecitabine                                                             | MEK                                                                   | 1     | NCT02773459                   | Recruiting                  |
| **mTOR**                                                                            |                                                                       |       |                               |                             |
| Everolimus                                                                          | mTOR                                                                  | 2     | NCT00973713                   | Unknown (not verified on ClinicalTrials.gov) |
| GEMCIS and everolimus                                                                | mTOR                                                                  | 1     | NCT00949949                   | Not recruiting (no results) |
| **Other**                                                                           |                                                                       |       |                               |                             |
| ARG 087                                                                             | FGFR                                                                  | 1/2   | NCT01752920                   | Recruiting                  |
| Cabozantinib                                                                        | c-MET, VEGFR2                                                         | 2     | NCT01954745                   | Active, not recruiting      |
| AG-221                                                                              | IDH2 mutation                                                         | 1/2   | NCT02273739                   | Completed (not reported)    |
| GEMCIS and ADH1 (Exherin)                                                           | N-cadherin                                                           | 1     | NCT01825603                   | Recruiting                  |
| **Immunotherapy**                                                                   |                                                                       |       |                               |                             |
| Nivolumab                                                                           | PD1                                                                   | 2     | NCT02829918                   | Recruiting                  |
| Pembrolizumab and GM-CSF                                                             | PD1                                                                   | 2     | NCT02703714                   | Recruiting                  |
| Ipilimumab and nivolumab                                                            | CTLA4 and PD1                                                         | 2     | NCT02923934                   | Not yet recruiting          |
| Dendritic cell-precision T cells for neoantigen (DC-PNAT) and gemcitabine           | Personalised neoantigens                                              | 2     | NCT02632019                   |                             |
| **Multiagent**                                                                       |                                                                       |       |                               |                             |
| Multiple arms based on molecular profiling (cetuximab, trastuzumab, gefitinib,      | Multiple (EGFR, HER2, mTOR, VEGF/EGFR/PDGFR, ALK/ROS1)               | 2     | NCT02836847                   | Recruiting                  |
| lapatinib, everolimus, sorafenin, crizotinib)                                       |                                                                       |       |                               |                             |
| Ramucirumab and pembrolizumab                                                       |                                                                       | 1     | NCT02443324                   | Recruiting                  |

All cited from ClinicalTrials.gov on 21 November 2016.  
5FU, 5-fluorouracil; ALK, anaplastic lymphoma kinase; BRAF, v-Raf murine sarcoma viral oncogene homologue B; CAP, capecitabine; CIS, cisplatin; C-Raf, RAF proto-oncogene serine/threonine protein kinase; CTLA4, cytotoxic T-lymphocyte associated protein 4; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FOLFOX, 5-fluorouracil and oxaliplatin; GEM, gemcitabine; GM-CSF, granulocyte macrophage colony stimulating factor; HER2, human epidermal growth factor receptor 2; IDH, isocitrate dehydrogenase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; OX, oxaliplatin; PDGFR, platelet-derived growth factor receptor; PD1, programmed cell death protein 1; ROS1, c-ros oncogene 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
Recognition that an activated tumour microenvironment exists in biliary cancers encourages a focus on adoptive therapy. There are identified tumour antigens, and the presence of both CD4+, CD8+ and Fox3+ T lymphocytes and macrophages suggests both that response to antigen may occur in selected patients and that relevant cells can be isolated and stimulated ex vivo. The correlation of activated immune cell infiltration and better outcomes supports a focus on this area.\(^1\)

Approaches to modulating the immune system include:

- vaccination with putative tumour antigens either as peptides or loaded within dendritic cells to enhance recognition;
- adoptive immunotherapy where patients’ own T cells are expanded ex vivo and reinfused;
- reversing tumour cell-induced immune suppression.

Vaccination against tumour-associated antigens is attractive as a number of proteins that are overexpressed have been identified.\(^2\) At least two tumour-related antigens have been identified with moderate to high expression in biliary cancers – Wilms tumour 1 (WT1) and mucin-1 (MUC-1).\(^3\) Trials of both a dendritic-based cell vaccine against both antigens\(^4\) as well as a randomised trial of chemotherapy and a WT1 vaccine in patients with advanced biliary cancer have been described,\(^5\) as has a trial of combining a dendritic cell pulsed vaccine plus ex vivo activated T cells in the postoperative setting.\(^6\) The future of antigen-based therapy may require more refinement as both the distribution of the antigens varies, as does the degree of immune response to them. An approach that identifies those most likely to be responded to in each patient is one such method.\(^7\)

Recently the unpacking of the mechanisms behind tumour-induced immunosuppression has created optimism throughout the cancer community. Data on melanoma, non-small cell lung cancer and renal cancer have all sparked the search for identification of suitable patients for PD-1, PD-L1 and CTLA4 therapies, which can reverse immune suppression. The study by Nakamura et al\(^8\) found that the worst prognosis for BTC patients was in those with relatively hypermutated tumours and elevated expression of checkpoint molecules such as CTLA-4 and PD-L1. In total, 45.2% of cases showed an increase in the expression of immune checkpoint molecules. In Keynote-026, a trial of pembrolizumab in advanced biliary tract patients, Bang et al\(^9\) reported interim results, that of 89 screened patients 37 (42%) had PD-L1-positive tumours, of those 24 were studied. Eight patients (34%) had response or stable disease lasting 40+ weeks. The variation in immune predictors by anatomic site suggests a need for appropriate selection to trials.\(^10\) In addition there is potential for augmenting tumour immunity with both chemotherapy and radiation. A number of immunotherapy studies are currently recruiting, some of which are outlined in table 3. Ultimately combination studies that use all three approaches to immunotherapy in the context of standard therapy are the most likely to provide sustained benefit.

**Conclusions**

Biliary tract cancers represent a key model of a rare cancer with complex genetic associations. Increasingly it is clear that this anatomic site is a collection of quite disparate genomically distinct neoplasms. Although easily viewed as an intractable problem with a multiplicity of small subgroups, a new approach agnostic to tissue of origin may represent a significant way forward. Instead of dedicated studies in each of intrahepatic, extrahepatic and gall bladder cancer, it may be possible to focus on the new basket trial designs, where a study of a particular targeted therapy directed at a specific mutation is identified and different histological subtypes with the right target are enrolled. If activity is shown, then an expansion study in that indication would follow. When patients are treated in a defined/personalised approach with the right target chosen, informed by their genomic landscape, we can expect to finally make some movement in dealing with this difficult group of diseases.

**Competing interests**

None declared.

**Provenance and peer review**

Commissioned; externally peer reviewed.

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