Genomic Profiling of Biliary Tract Cancers and Implications for Clinical Practice

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Opinion statement

Biliary tract cancers are relatively uncommon, have an aggressive disease course and a dismal clinical outcome. Until recently, there have been very few clinical advances in the management of these patients and gemcitabine-based chemotherapy has been the only widely accepted systemic therapy. The advent of next generation sequencing technologies can potentially change the treatment paradigm of this disease. Targeted therapy directed against actionable mutations and identification of molecular subsets with distinct prognostic significance is now feasible in clinical practice. Mutation profiling has highlighted the genomic differences between the intra, extrahepatic cholangiocarcinoma, and gallbladder cancer. The mutational spectrum of intrahepatic cholangiocarcinoma differs according to geographic location and ethnicity. There is a higher incidence of chromatin modulating gene mutations in Western patients as compared with Asian patients with liver fluke-associated cholangiocarcinoma. *KRAS* and *p53* mutations are associated with an aggressive disease prognosis while *FGFR* mutations may signify a relatively indolent disease course of intrahepatic cholangiocarcinoma. *FGFR* and *IDH* mutations have promising agents in clinical trials at this time. An estimated 15 % of gallbladder cancers have *Her2/neu* amplification and can be targeted by trastuzumab. On the other hand, an estimated 10–15 % of cholangiocarcinomas have DNA repair mutations and may be candidates for immune therapies with checkpoint inhibitors. The promise of targeted therapies for biliary tract cancers can be fulfilled with well-designed, prospective, and multi-center clinical trials.

58 Page 2 of 13

Curr. Treat. Options in Oncol. (2016) 17: 58

Introduction

A genomic era for cancer therapy is rapidly emerging, largely incentivized by next-generation sequencing (NGS) technologies that detect actionable mutations with high sensitivity, deep coverage, and at a fraction of the cost required for traditional sequencing methods just two decades ago. Targeted sequencing panels that investigate high frequency, actionable mutations in cancers are now available at the major cancer centers in the USA. This technology has greatly impacted the management of hematologic malignancies for the past decade and more recently has revolutionized therapy for some solid tumors such as non-small cell lung cancer and melanoma [1]. Biliary tract cancers (BTC), in particular cholangiocarcinoma have a relatively large number of actionable mutations as compared with other gastrointestinal cancers. Recent mutational profiling studies in BTC have indicated that genomic profiling is of profound value for prognostic stratification and for targeted therapy options [2, 3]. In this review, we will discuss mutational spectrum of BTC, clinical correlative findings, and preliminary results from trials of targeted agents.

Epidemiology of biliary tract cancers

As per the American Cancer Society, 39,230 cases of primary liver cancer [which includes intrahepatic cholangiocarcinoma (IHCCA) and hepatocellular cancer] and 11,420 cases of gallbladder cancer (GBC) and extrahepatic cholangiocarcinoma (EHCAA) were diagnosed in the year 2015 [4]. The clinical and molecular epidemiology of these three types of BTC varies markedly.

Higher incident rates of cholangiocarcinoma (CCA) occur in Southeast Asia as compared with the Western population (>80/100,000 vs. 0.3/100,000 in Northeast Thailand and Canada, respectively) [5]. This geographic difference can at least partly be attributed to etiological factors. In western countries, primary sclerosing cholangitis (PSC) is a well-recognized risk factor that is associated with CCA. In Southeast Asia, CCA is more commonly associated with parasitic infection by the liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis,* hepatitis B infection and hepatolithiasis [6, 7]. GBC on the other hand occurs more commonly in South Asia (Northern India and Pakistan), Latin America (including Chile that has the highest incidence rates in the world) and in the Native American, Alaskan Indian, and the Hispanic population in the USA [8, 9]. Risk factors associated with GBC include cholelithiasis, obesity, and diabetes [9].

In most of the western world, GBC is decreasing in incidence in the Caucasian population largely due to increased usage of laparoscopic cholecystectomy [10]. Within the USA, however there is a slight increase in GBC incidence in the younger population [8]. This increase has been attributed to migratory trends. However, IHCCA was previously reported to be increasing in incidence. [11–13]. This increased incidence is being disputed as it is at least partly attributable to a change in the International Classification of Disease classification system wherein cancers of unknown primary in the liver are now being re-classified as IHCCA. Furthermore, Klatskin tumors were previously incorrectly characterized as IHCCA and correction of this anomaly suggests that the incidence of IHCCA is relatively stable from 2000 to 2007 while EHCCA has increased over the same period [14].

Not only are there major epidemiological differences between the three types of BTC, but there are also significant molecular differences as illustrated below.

Molecular heterogeneity of BTC

Significant differences exist in mutational spectrum of GBC vs. CCA. For instance, Isocitrate Dehydrogenase 1 or 2 (*IDH1/2*), *BAP1* mutations and *FGFR* fusions are more likely to occur in IHCCA while *KRAS*, *p53*, and *SMAD4* mutations are more common in EHCCA. GBC on the other hand has the high frequency of *ERBB2* mutations [2, 15]. Furthermore, mutational aberrations vary with etiology of CCA. In patients with non-liver fluke-related CCAs, *BAP1* and *IDH1*, *IDH2* are more commonly seen whereas in liver fluke–related CCA, *p53 and SMAD4* mutations are observed [16]. *IDH1/2* mutation frequency varies depending upon ethnicity—lower in Asians (7.5 %) than in Caucasians (25 %) [17]. A difference in the mutational landscape is also observed when IHCCA is associated with chronic liver disease as compared with IHCCA that occurs in normal liver. In chronic liver disease associated IHCCA, higher frequency of *EGFR* and lower *KRAS*, *MLH1*, and *GNAS* mutations are observed as compared with normal liver associated IHCCA [18].

Genomic profiling in clinical practice

Since 2012, a rapidly increasing pace of genetic sequencing studies have begun to illuminate the landscape of deregulated genes and pathways in cholangiocarcinoma. As a result of technical advances in molecular pathology, high-throughput sequencing, also referred to as next- generation sequencing (NGS) has now become feasible in standard clinical practice. Standard formalin-fixed paraffin-embedded specimens can now be used for NGS. Several academic centers and commercial vendors have developed analytic protocols for the interpretation of NGS data. Simultaneous developments of novel therapeutics that target the somatic mutations (drivers) have together made significant advances towards precision oncology. A significant caveat in this field is the need for core needle biopsies for high-throughput NGS studies. Biopsies in BTC are often limited to fine needle aspirates and cytology and therefore NGS is feasible in less than 50 % of the new BTC cases in our clinical experience.

Before the advent of NGS, tumor genotyping was performed for few select oncogenes (hotspots). The present assay options include cancer 'gene panels', whole exome genome, or transcriptome sequencing. There are several potential uses for NGS besides targeted therapeutics: these include diagnostics, identification of resistance mechanisms, and prognostic stratification. The most recurrent and functionally validated mutations discovered in IHCCA occur in five broad categories are as follows: (1) fusions affecting the receptor tyrosine kinase *FGFR2*; (2) MAPK pathway mutations, particularly *KRAS*, *NRAS*, *BRAF*, and *ARAF*; (3) *IDH1* and *IDH2* mutations; (4) chromatin-modifying genes, particularly *ARID1A*, *ARID1B*, *PBRM1*, *BAP1*, and *MLL3*; and (5) cell cycle-related genes including *TP53*, mutation or deletion of *CDKN2A*, and amplification of *CCND1*. Tables 1 and 2 illustrate the most common mutations found in BTCs.

Table 1. Most frequently seen mutations in IHCCA

Reference	ICGC 2015 [51]	Zou 2014 [52]	Churi 2014 [2]	Jiao 2013 [3]	Simbolo 2014 [53]	TCGA [54]	IHCCA Total
Country	Japan	China	USA	USA	Italy	USA/Canada	
N	135	102	55	64	70	32	458
TP53	22 %	38 %	29 %	6 %	9 %	6 %	21 %
KRAS/NRAS	27 %	18 %	22 %	11 %	23 %	3 %	20 %
IDH1/2	10 %	5 %	24 %	20 %	20 %	19 %	14 %
ARID1A	17 %	7 %	20 %	14 %	11 %	16 %	14 %
BAP1	10 %	1 %	5 %	20 %	14 %	38 %	12 %
PBRM1	9 %	1 %	7 %	13 %	14 %	25 %	9 %
FGFR2 fusion	4 %	ND	5 %	ND	ND	16 %	6 %
PIK3CA	9 %	3 %	5 %	3 %	6 %	6 %	6 %
PTEN	0 %	7 %	5 %	3 %	1 %	3 %	3 %
BRAF hotspot	0 %	1 %	5 %	0 %	4 %	3 %	2 %
ARAF hotspot	0 %	1 %	ND	3 %	ND	6 %	1 %
RB1	1 %	5 %	0 %	0 %	0 %	0 %	1 %

Mutational spectrum and prognosis

Correlation between mutational burden and prognosis has the potential of guiding clinical interventions. Our group recently profiled 321 cases with BTC and correlated the mutational spectrum with clinical outcome [19]. In this study, 224 were IHCCA, 42 were EHCCA, and 55 had GBCA. Treatments administered included surgery ($n = 130$), radiation ($n = 83$), while the rest received systemic therapy. In IHCCA, p53 and KRAS mutations were associated with poor overall survival (OS), whereas FGFR mutations were associated with a significantly improved OS. On the other hand, IDH1 or IDH2 mutations were not prognostic in this study confirming a similar prior report from Goyal et al. [20]. CDKN2A mutations were significantly associated with poor progression-free survival (PFS) in both IHCCA and EHCCA. On multivariate analysis, only p53 and FGFR mutations were significantly associated with OS, with a HR of 1.64 ($p = 0.015$) and 0.478 ($p = 0.03$), respectively. The FGFR mutant subgroup in particular appears to be a unique and relatively indolent subtype of CCA, occurring more frequently in women, some <40 years of age at diagnosis. BAP1 mutant CCA appears to be an aggressive subtype, with a predilection for bony metastases although the number of cases were limited in our series [2].

Table 2. Most frequently seen mutations in EHCCA and GBC

	EHCCA				
Reference	Lee 2015 [55]	Churi 2014 [2]	Simbolo 2014 [53]	Chan-on 2013 [16]	Total EHCCA
Country	USA	USA	Italy	Singapore	
N	99	20	57	14	190
TP53	45 %	45 %	18 %	14 %	35 %
KRAS	43 %	40 %	47 %	21 %	43 %
ARID1A	13 %	5 %	12 %	7 %	12 %
PBRM1	9 %	5 %	4 %	NA	6 %
PIK3CA	7 %	10 %	9 %	NA	7 %
CDKN2A	28 %	15 %	0 %	NA	16 %
ERBB2	9 %	25 %	0 %	NA	7 %
SMAD4	15 %	25 %	11 %	21 %	15 %
PTEN	7 %	5 %	4 %	NA	5 %
	GBC				
Reference	Li 2014 [56]	Jiao 2013 [3]	Simbolo 2014 [53]	Stephens 2014 [57]	Total GBC
Country	China	USA	Italy	USA	
N	57	8	26	83	174
TP53	47 %	63 %	46 %	63 %	55 %
KRAS	8 %	0 %	19 %	NA	6 %
ARID1A	0 %	0 %	12 %	18 %	10 %
PBRM1	0 %	25 %	8 %	NA	2 %
PIK3CA	6 %	13 %	8 %	12 %	9 %
CDKN2A	6 %	0 %	4 %	49 %	26 %
ERBB2	10 %	0 %	4 %	17 %	12 %
BAP1	0 %	0 %	4 %	NA	1 %
SMAD4	4 %	13 %	8 %	11 %	8 %

NA Not Applicable

Actionable mutations and targeted therapy

BTC may have more actionable mutations than any other gastrointestinal malignancy. In IHCCA in particular, over 30 % of cases have *FGFR*, *IDH* or *BRAF* mutations, all of which have small molecule inhibitors in clinical trials [2, 21, 22]. In GBC, 15 % of cases have *HER2/Neu* amplification that can be

targeted by trastuzumab and newer inhibitors of this pathway [23]. These mutations and early clinical trial results are discussed below.

FGFR signaling pathway

The FGFR pathway is a complex signaling pathway consisting of four trans-membrane tyrosine kinase receptors FGFR (1 to 4) along with 18 FGF ligands. These ligands are polypeptide growth factors that bind to receptors expressed on the surface of target cells. Upon binding, the receptor-ligand combination is responsible for the regulation of several key cell processes including cell proliferation, survival, migration, and angiogenesis [24, 25]. For instance, FGF mediated activation of MAPK signaling pathway is responsible for cellular proliferation and activation of the PI3K/AKT pathway which prevents apoptosis [26]. Aberrant FGFR pathway activity caused by genetic alterations such as activating mutations, amplifications, or chromosomal translocation can initiate malignant transformation.

FGFR1 amplifications have been implicated in several human solid tumors like squamous and adenocarcinoma non-small cell lung cancer (NSCLC), small cell carcinoma lung, squamous carcinoma head and neck, breast, ovarian, and esophageal cancer. Additionally, hepatocellular carcinoma is associated with *FGFR4* overexpression [25]. In IHCCA, *FGFR* fusions have been observed more commonly [21]. About 13–14 % of IHCCAs have *FGFR2* fusions/ translocations and these may be mutually exclusive with *KRAS* mutations [2, 3, 21, 27]. *FGFR2* fusions have been observed more commonly in women, with an improved disease-free and overall survival and relatively indolent disease course [2, 27]. Common *FGFR2* fusions observed are *FGFR2-AHCYL1*, *FGFR2-BICC1*, *FGFR2-PARK2*, *FGFR2-MGEA5*, *FGFR2-TACC3*, and *FGFR2-KIAA1598* [2, 21, 28–30] Cancers with *FGFR2* gene fusions have demonstrated an enhanced susceptibility to FGFR inhibitors as compared to cancers having *FGFR* point mutations [21, 30]. On the other hand, *FGFR4* overexpression is associated with proliferation, invasion and epithelial-mesenchymal transition leading to a more aggressive disease type with poorer survival in IHCCA [24]. Anti-FGFR agents include small molecule tyrosine kinase inhibitors (TKIs) that act at the receptor level inhibiting downstream signaling and the FGFR antibody drug conjugate LY3076226. Selective FGFR-targeted TKIs include BGJ398 and JNJ42756493. BGJ398 is a selective pan-FGFR inhibitor with potent activity against *FGFR*1-3 and has been the furthest along in clinical development in CCA. A recent phase II study of BGJ398 in CCA was reported at the Gastrointestinal Cancers Symposium. This study showed that BGJ398 was well tolerated other than for hyperphosphatemia and resulted in a response rate of 14 % with the disease control rate of 82 % [31]. Non-selective FGFR inhibitors include brivanib, nintedanib, pazopanib, regorafenib, dovitinib, and ponatinib. Phase II trial of ponatinib for advanced IHCAA is ongoing [24].

IDH1/2 mutations in CCA

IDH1 and IDH2 are the cytosolic and mitochondrial variants of metabolic enzymes, respectively and both have very similar functions. These enzymes

normally lead to the conversion of a-ketoglutarate (aKG) into isocitrate but the mutant enzymes convert aKG to D-2-hydroxyglutarate (2-HG), which is oncogenic [22]. Wang et al., established that *IDH1* mutations in cholangiocarcinomas impair the activity of TET family of DNA dioxygenases which are aKG-dependent dioxygenases, resulting in a decrease of cytosine hydroxymethylation with a concurrent increase of DNA methylation [17]. The epigenetic alterations caused by *IDH1/2* mutations lead to a blockade of cellular differentiation, causing an increase in the progenitor cells, which eventually results in tumorigenesis, most commonly in gliomas and acute myeloid leukemia. A similar mechanism may be responsible in IHCCA [17]. *IDH* mutations occur primarily in IHCCA (ranging from 10 to 43 % cases) and rarely in EHCCA and GBC [32]. Several studies have demonstrated the presence of *IDH1/2* mutations exclusively in IHCCA. A meta-analysis demonstrated that *IDH1/2* mutations are mutually exclusive with *KRAS*/*NRAS* mutations in CCA ($p = 0.055$) but may co-exist frequently with *BAP1* mutations ($p = 0.002$) [33]. *IDH* and *FGFR* mutations also almost never co-exist and may be mutually exclusive. The most common *IDH* mutations in IHCCA are *IDH1-R132C* and *IDH1-R132G* (44 and 14 %, respectively) [33]. Circulating 2-HG level has been reported as a surrogate biomarker for IDH-mutant CCA. IDH inhibitors AG-120, AG-881, and IDH-305 are in clinical trials at the current time for the treatment of IDH-mutant CCA, acute myeloid leukemia, and glioma [34]. Burris et al., recently presented the clinical efficacy data of the IDH1 inhibitor AG-120, in patients with *IDH1* mutant cancers, including IHCCA [35]. In their study, one out of 20 patients with IDH1-mutant IHCCA had a partial response while 11 patients had sustained stable disease. While the preliminary signal from IDH1-directed therapies is encouraging, further studies are required to clarify their role in the management of BTC.

DNA repair mutations in CCA

DNA repair mechanisms play a critical role in the maintenance of genomic stability. Germline mutations are the cause of cancer, for instance in hereditary breast and ovarian cancer, Fanconi anemia, and xeroderma pigmentosum syndromes. Somatic mutations in DNA repair genes are present quite commonly in tumors and may represent targets for therapeutic intervention with specific inhibitors or DNA damaging chemotherapeutic agents. The presence of incorrect DNA repair in tumor cells predisposes them to accumulate even more genetic alterations. For example, colorectal and endometrial cancers with defective DNA mismatch repair (MMR) due to mutations in the *MLH1* and *MSH2* genes have very high single nucleotide changes and small insertions/deletions. This high mutational burden makes Microsatellite Instability-high (MSI-H) tumors susceptible to immune blockade using checkpoint inhibitors [36]. In our series of 321 BTC with mutational profiling discussed earlier, DNA repair mutations (in *MSH6*, *BRCA1*, *BRCA2*, *ATM*, *MLH1*, *or MSH2* genes) occurred in 12 % IHCCA, 26 % in EHCCA, and 5 % of GBC cases [19]. Data in regards to MMR in BTC is very limited. Goyal et al., reported a 9 % rate of MMR protein loss in CCA patients on immunohistochemistry [37]. The incidence is even lower in Asia, particularly in liver fluke-associated IHCCA in Thailand [38]. These results suggest that DNA repair mutations may be an important area of

investigation for targeted therapeutics with specific DNA repair inhibitors or immunotherapy in a subset of IHCCA patients.

HER2/Neu in BTC

HER2/neu gene is a key driver of oncogenesis and its overexpression as a result of gene amplification is a critical target for therapy in breast cancer and gastric cancer. *HER 2/neu* overexpression has also been reported in BTC. Our group recently studied *HER2/neu* expression in 187 cases of gallbladder cancer; this is the largest reported series to date using the commonly accepted American Society of Clinical Oncology criteria [15]. We noted that 13 % of patients have *HER2/neu* overexpression (3+ by immunohistochemistry) and radiological partial responses were noted with HER2/neu directed therapies [23]. *HER2/neu* amplification is uncommon in CCA, although mutations in the kinase domain (V777 L) or in the extracellular domain (S310F) were noted in EHCCA [39]. *HER2/neu* amplifications can be successfully targeted by trastuzumab reversible small molecule tyrosine kinase inhibitors (TKIs), including afatinib, neratinib, and dacomatinib. Fig. 1 illustrates the benefits of *HER 2/neu* targeted therapy, noted in a case of GBC that was invading the liver arising in a 61-year-old female. After 3 months of treatment with trastuzumab and FOLFOX, follow-up scans demonstrated resolution of the gallbladder mass and decrease in the size of the liver mass. Other EGFR pathway-directed agents include erlotinib, cetuximab, and panitumumab. EGFR overexpression is common in BTC and these agents have been investigated in prospective clinical trials. Philip et al., demonstrated the benefits of erlotinib as a single agent in 42 patients with advanced BTC. A 10 % response rate was noted [40]. Lee at al, studied the effect of gemcitabine, oxaliplatin + erlotinib in a phase III randomized trial conducted in 268 South Korean patients with BTC. They noted a significantly higher response rate with the addition of erlotinib. However, this did not translate to a survival improvement [41]. In the gemcitabine, oxaliplatin, with or without
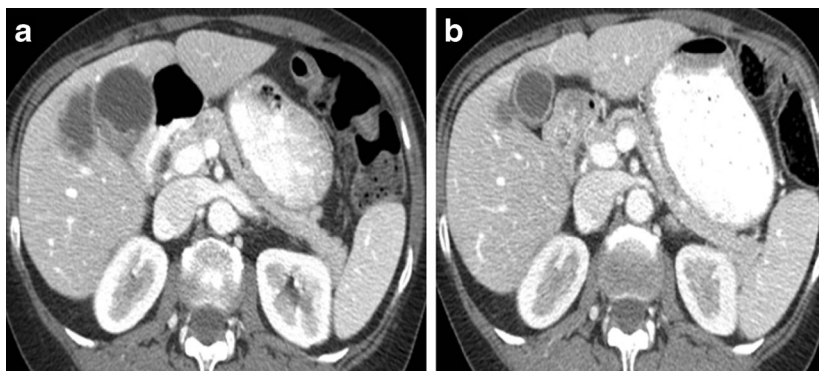
Fig. 1. Her2/neu directed therapy for advanced gallbladder carcinoma. Axial contrast-enhanced CT images demonstrate **a.** a mass in the gallbladder neck causing gallbladder obstruction. There is contiguous extension from the mass to the liver **b** after 3 months of Her2/neu directed therapy with trastuzumab, the polypoid gallbladder mass is no longer visualized on the scans, and the liver mass is decreased. The patient was then treated with en bloc cholecystectomy and extended right hepatectomy followed by adjuvant chemotherapy

cetuximab, in advanced biliary-tract cancer (BINGO) phase II randomized trial, the effect cetuximab addition to standard first-line chemotherapy was explored. Unfortunately, Malka et al. noted that cetuximab did not improve the patient outcome as measured by 4-month progression survival [42]. Other EGFR-directed therapies are discussed in detail by Merla et al. [34].

Mutations in chromatin remodeling genes

Chromatin remodeling allows the genomic DNA to access regulatory transcriptional proteins and thereby controlling gene expression. Genes involved in chromatin remodeling including *ARID*, *BAP1*, *PBRM*, *and MLL* are mutated in several cancer types and the resultant alterations induce carcinogenesis. *BAP1* encodes for a nuclear deubiquitinase involved in chromatin remodeling and mutations in this gene are associated with uveal melanoma, mesothelioma, and renal cancers, while PBRM1 and ARID1A both encode a subunit of the ATP dependent SWI/SNF chromatin remodeling complexes [43, 44]. Jiao et al., performed exome sequencing in 32 IHCCA cases and reported frequent mutations in *BAP1*, *ARID1A*, *and PBRM1* [3]. In fact, mutation in at least one of these genes occurred in almost half of the carcinomas sequenced. Interestingly, exome sequencing studies by Ong et al., in liver fluke-associated CCA did not report a high incidence of these mutations, thereby further highlighting the differences between these two types of CCA (liver fluke-associated CCA in Asia and sporadic CCA in western patients) [45••]. Furthermore, a Chinese study of IHCCA detected very low *BAP1* and *PBRM1* mutations (1 % each) compared to other studies, suggesting possible geographic/demographic influences as seen in Table 1. In our series, we found no correlation between mutations in chromatin remodeling genes and prognosis. However, *BAP1* mutation was associated with an aggressive outcome in some cases of IHCCA and a predilection for bone metastases [2]. Germline *BAP1* mutations confer increased susceptibility for the above cancers as well as epithelioid atypical Spitz tumors and cutaneous melanomas [46]. At the current time, there are no proven therapies for cancers having these mutations. However, histone deacetylase (HDAC) inhibitors may offer therapeutic value and need further investigation.

Linking genomics with immunotherapy in BTC

There is a well-recognized link between tumor genomics and immune response. Hypermutated tumors, such as those with microsatellite instability result in a heavy burden of neoantigens that contribute significantly to the success of clinical immunotherapies in this setting [47]. DNA repair mutations in CCA including those resulting in MSI were discussed earlier. Investigators at National Institute of Health used a unique approach to immunotherapy for CCA. They used a whole-exomic-sequencing-based approach to demonstrate that tumor-infiltrating lymphocytes (TIL) from a patient with metastatic cholangio-carcinoma contained CD4+ T helper 1 (TH1) cells recognizing a

58 Page 10 of 13

Curr. Treat. Options in Oncol. (2016) 17: 58

mutation in erbb2 interacting protein (ERBB2IP). After adoptive transfer of TIL containing mutation-specific polyfunctional TH1 cells, the patient achieved prolonged partial response. Upon disease progression, the patient was retreated with mutation-reactive TH1 cells and again experienced tumor regression [48]. These results provide evidence that a CD4+ T-cell response against a mutated antigen can lead to tumor regression. Identification of immunogenic epitopes in the mutations seen in CCA will be key to success with checkpoint inhibitors. From an immunological perspective, IDH1 (R132H) represents a potential target for immunotherapy as it is a tumor-specific potential neoantigen expressed in tumor cells. Schumacher et al., demonstrated that IDH1 (R132H) contains an immunogenic epitope suitable for mutation-specific vaccination. Peptides from the mutated region induce CD4+ immune response that can potentially be exploited by mutation-specific anti-IDH1 (R132H) vaccines [49]. Further immunologic profiling of CCA and correlation with mutational spectrum of this cancer is required. Recent results with pembrolizumab in pre-treated BTC having Programmed death-ligand 1 (PD-L1) expression indicated that 17 % of patients have a meaningful response to checkpoint blockade [50]. These results have reinforced the potential role of immune therapy in this disease.

Conclusions

BTC are enriched with actionable mutations and with the advent of modern NGS technologies, precision medicine may soon become a reality and alter the treatment landscape of these cancers. The use of NGS is no longer limited to research settings and this technology can now be incorporated into standard clinical practice for the management of BTC patients. Given the genomic heterogeneity between the different BTC tumors, NGS argues for a need for molecular classification of BTC as against the traditional classification based on the site of the primary. The current American Joint Committee on Cancer (AJCC) classification is based on tumor location and extent and is an effective tool for estimating prognosis and treatment selection. In the case of BTC, we hypothesize that molecular information could add to the clinical utility of the AJCC classification. For instance, KRAS mutations and FGFR fusions may represent polar ends of the clinical prognostic spectrum at a given stage of intrahepatic CCA. An integrated clinical and molecular classification system consisting of AJCC staging in addition to validated molecular biomarkers could alter the treatment paradigm for BTC, and an international collaborative effort is required to advance this field. Our charge now is to make NGS feasible using limited biopsy samples and accessible to a larger population of BTC patients at a lower cost.

Compliance with ethical standards

Conflict of interest
The authors declare that they have no conflict of interest.

Human and animal rights and informed consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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