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REVIEW

Advances in the systemic treatment of therapeutic approaches in biliary tract cancer

O. Mirallas, MD, MSc*, D. López-Valbuena, D. García-Illescas, C. Fabregat-Franco, H. Verdaguer, J. Tabernero & T. Macarulla

Medical Oncology Department, Vall d'Hebron Hospital Campus and Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Available online 10 June 2022

Introduction: Biliary tract cancers (BTCs) are a rare and heterogenous group with an increasing incidence and high mortality rate. The estimated new cases and deaths of BTC worldwide are increasing, but the incidence and mortality rates in South East Asia are the highest worldwide, representing a real public health problem in these regions. BTC has a poor prognosis with a median overall survival <12 months. Thus, an urgent unmet clinical need for BTC patients exists and must be addressed.

Results: The backbone treatment of these malignancies is chemotherapy in first- and second-line setting, but in the last decade a rich molecular landscape has been discovered, expanding conceivable treatment options. Some druggable molecular aberrations can be treated with new targeted therapies and have already demonstrated efficacy in patients with BTC, improving clinical outcomes, such as the *FGFR2* or *IDH1* inhibitors. Many other molecular alterations are being discovered and the treatment of BTC will change in the near future from our current clinical practice.

Conclusions: In this review we discuss the epidemiology, molecular characteristics, present treatment approaches, review the recent therapeutic advances, and explore future directions for patients with BTC. Due to the rich molecular landscape of BTC, molecular profiling should be carried out early. Ongoing research will bring new targeted treatments and immunotherapy in the near future.

Key words: biliary tract cancer, cholangiocarcinoma, targeted therapies, molecular testing, next-generation sequencing

INTRODUCTION

Biliary tract cancers (BTCs) are a rare and heterogenous group comprising various aggressive malignancies emerging in the biliary tree. BTC includes intrahepatic cholangiocarcinoma (iCCA), extrahepatic CCA (eCCA), comprised of perihilar CCA (pCCA) and distal CCA (dCCA), ampullary cancer (AC), and gallbladder cancer (GBC).[1] CCA represents ~15% of primary intrahepatic tumors, and after hepatocellular carcinoma, is the most frequent diagnosis of primary liver cancer.[2] CCAs are typically adenocarcinomas, more frequent in males, and multiple risk factors explain the variable incidence of CCAs, such as alcohol consumption, tobacco smoking, bile duct morphological anomalies, primary sclerosing cholangitis, Lynch syndrome, *Opisthorchis viverrini*, *Clonorchis sinensis*, obesity, or diabetes. Major liver diseases such as hepatitis B and hepatitis C virus infections, alcohol and non-alcoholic fatty liver have a stronger association with iCCA than eCCA.[2-4] GBC are more frequent in women and the more common risk factors are: gallstones, gallbladder polyps, chronic infections, drugs (methyldopa), obesity, and diabetes.[5]

The estimated new cases of and deaths from BTCs in the USA by the end of 2021 are 11 980 and 4310, respectively, and they are expected to increase in the next decades. The incidence and mortality rates are highest in South East Asia, representing a real public health problem in these regions (Figure 1).[6,7]

BTCs have a poor prognosis with a median overall survival (OS) <12 months. More specifically, the 5-year relative survival rate ranges from 9% to 25% for iCCA, 10% to 15% for eCCAs, and 15% to 35% for GBC, conditional to stage.[8-11] Cisplatin plus gemcitabine (CisGem) is the current approved first-line therapy for unresectable or advanced BTC, and second-line FOLFOX, but there is no strong evidence about what to do in following lines as chemotherapy regimens have poor survival outcomes.[12,13] There is an urgent unmet clinical need for BTC patients and

*Correspondence to: Oriol Mirallas, Medical Oncology Department, Vall d'Hebron Hospital Campus and Vall d'Hebron Institute of Oncology (VHIO), Passeig de la Vall d'Hebron, 119-129, Barcelona 08035, Spain. Tel: +34-628224189

E-mail: omirallas@vhebron.net (O. Mirallas).

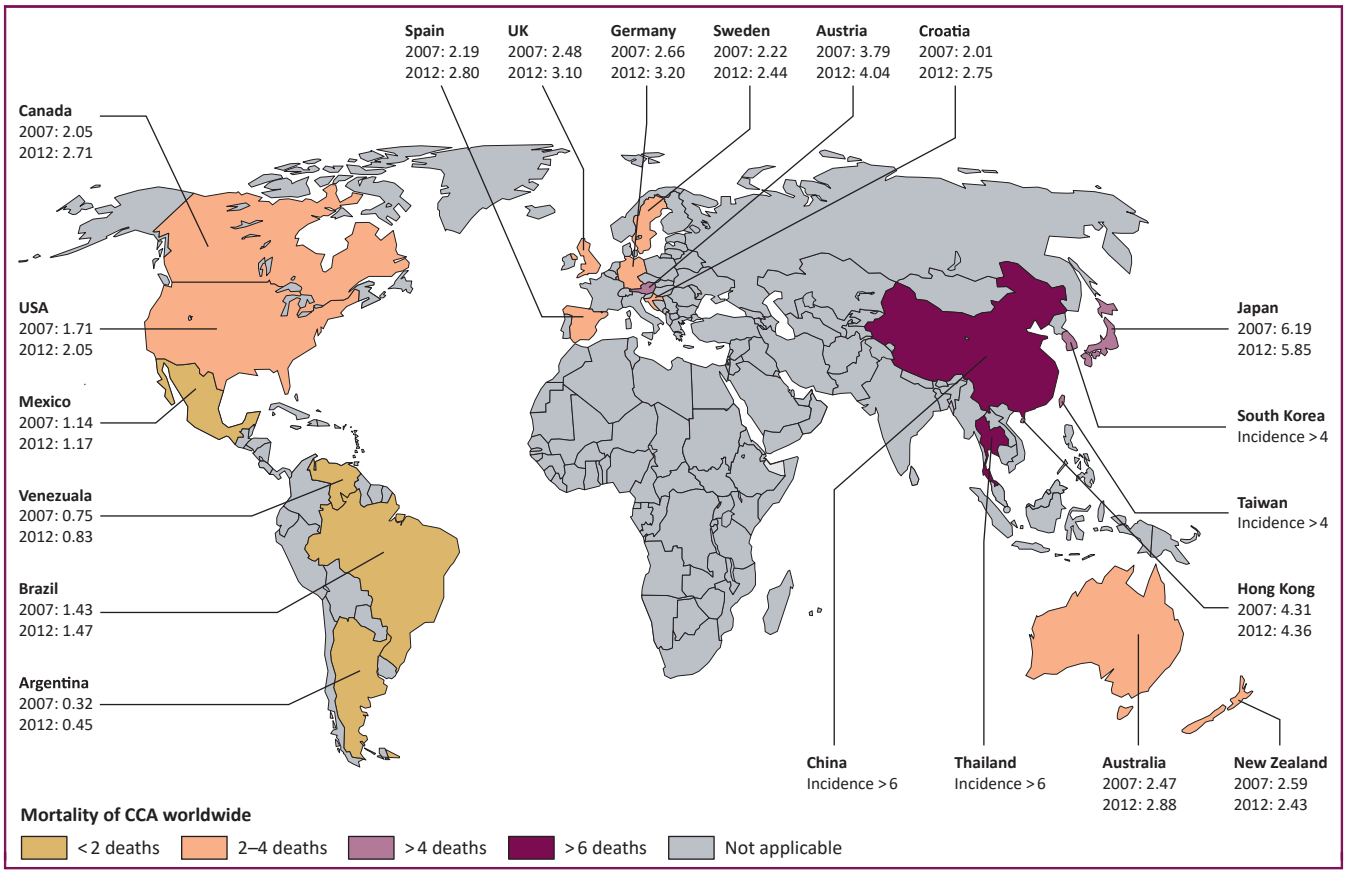

Figure 1. Mortality of cholangiocarcinoma worldwide. Age-standardized annual mortality rates for CCA in deaths per 100 000 person-year in the age group 45-64, according to country. Data from the time periods 2005-2009 (2007), 2010-2014 (2012). Dark red indicates countries with high mortality (>6 deaths per 1 000 000 people), red indicates high mortality (>4 deaths per 100 000 people), orange indicates countries with mortality between 2 and 4 deaths per 100 000 people, and yellow indicates countries with low mortality (<2 deaths per 100 000 people). Figure adapted from Bertuccio et al., 2019.[7] CCA incidence is shown for Asian countries, where mortality has not yet been reported.
CCA, cholangiocarcinoma.

here we review the recent therapeutic advance approaches.

ANATOMIC CLASSIFICATION

BTC can be classified based on the anatomical origin of the primary tumor. BTCs arising from the bile ductules to the second-order ducts are classified as iCCA, those arising between second-order ducts and the beginning of the cystic duct are pCCA, and those that originated after the insertion of the cystic duct are dCCA. The concept eCCA compiles both pCCA and dCCA. AC comprises those tumors that originated at the end of the bile duct, GBCs arise from the cystic duct or the gallbladder itself (Figure 2).[14,15] Three growth patterns have been described for iCCA: (i) mass-forming, the most common growth pattern, (ii) periductal-infiltrating, which infiltrates along the lumen wall, and (iii) intraductal growing, the least common subtype.[14,16]

MOLECULAR CHARACTERISTICS

CCAs are particularly molecularly rich, especially iCCA which has the highest genetic alterations per tumor frequency of all BTCs. A study published by Nakamura et al.,[17] which analyzed 260 BTCs by whole-exome sequencing, described a median number of non-silent somatic mutations in iCCA, eCCA, and GBC of 39, 35, and 64, respectively. Both iCCA and eCCA presented significant differences on the number of non-silent somatic mutations compared with GBC, but not between iCCA and eCCA. Overall, this analysis detected targetable genetic alterations in 39% of BTC cases, being the most frequent molecular alteration in the kinase-RAS domain (52%). Another study published by Javle et al.,[18] which analyzed 412 BTCs by hybrid capture-based comprehensive genomic profiling, detected non-significant genetic alterations differences between the three tumor subtypes. Detailed molecular alterations by BTC location can be found in Table 1.

TREATMENT

Surgery and adjuvant treatment

BTC management and prognosis depends on the resectability of the tumor. The expected outcomes for patients who undergo surgery and adjuvant chemotherapy for 6 months are: a median OS of 51.1 months, a median relapse-free survival (RFS) of 24.4 months, and a relapse rate of 60%.[24-27] The established adjuvant treatment after surgery for BTCs is fluoropyrimidine-based, being preferred after

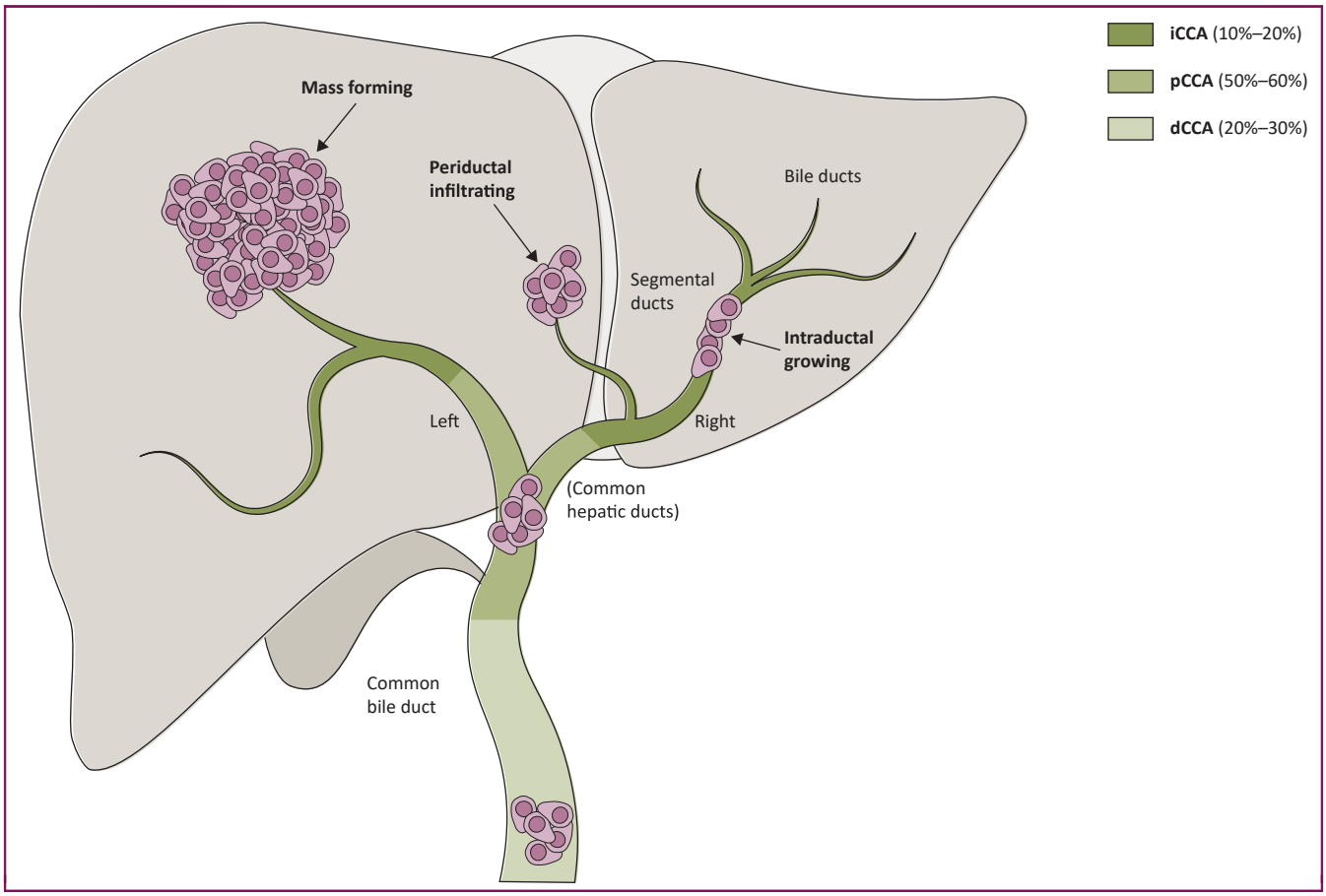

Figure 2. Anatomical classification of cholangiocarcinoma. Cholangiocarcinoma (CCA) is further subclassified into intrahepatic CCA (iCCA), extrahepatic CCA (eCCA), comprised of perihilar CCA (pCCA) and distal CCA (dCCA). Three growth patterns have been described for iCCA: mass-forming, periductal-infiltrating, and intraductal growing. Most common molecular alterations are detailed for iCCA and eCCA.

Table 1. Molecular alterations and frequencies by biliary tract cancer subtype (in bold the most frequent alteration)[17-23]

	iCCA (%)	eCCA (%)	GBC (%)
TP53 mutations	18-35	40-48	**50-59**
KRAS mutations	20-25	**12-42**	0-8
IDH1 mutations	**16-29**	0-7	0-2
CDKN2A/B mutations	**6-26**	6-17	4-19
ARID1A mutations	**15-21**	7-19	4-20
BAP1 mutations	**15**	0-6	2
FGFR2 fusions	**5-14**	3	1
FGFR1 mutations	**7**	0	0
FGFR2 mutations	**8**	0	0
FGFR3 mutations	**5**	3	**0**
SMAD4 mutations	10-12	**21-24**	**4**
PIK3CA mutations	8	0-4	**7-14**
MET amplification	**2-7**	0	1
BRAF mutations	4.4	**5.4**	4.9
IDH2 mutations	**4**	0	0-1
HER2 amplification	2.5-3	8-11	**7-16**
ARID1B mutations	2-3	**3-7**	4-6
MYC amplification	2.5	**5.4**	3.9
TET 1-3 mutations	1.9	**5.4**	1
NTRK 1-3 mutations	1.3	2.7	**5.5**
PTEN loss	1	1	**1-7**
RET mutations	0	**2.7**	0

the positive results in RFS and OS of the BILCAP trial. This was a randomized, controlled, multicenter, phase III trial across specialized centers in the UK, including patients with macroscopically resected CCA or muscle-invasive GBC and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1. Participants were randomized 1 : 1 to observation or capecitabine (1250 mg/m^2 twice a day on days 1-14 of a 21-day cycle for eight cycles) within 16 weeks of surgery. The primary outcome was OS. Notably, RFS was statistically significant in both the intention-to-treat analysis and in the per-protocol analysis [hazard ratio (HR) = 0.75, 95% confidence interval (CI) 0.58-0.98; $P = 0.033$, and HR = 0.70, 95% CI 0.54-0.92; $P = 0.009$, respectively]. Median OS difference, however, was statistically significant in the per-protocol analysis (HR = 0.75, $P = 0.028$), but not in the intent-to-treat analysis; the OS HR was 0.81 ($P = 0.010$).[27] Negative results have been reported in two randomized phase III trials. In the phase III PRODIGE 12-ACCORD, patients were randomized to gemcitabine and oxaliplatin (gemcitabine 1000 mg/m^2 on day 1 and oxaliplatin 85 mg/m^2 infused on day 2 of a 2-week cycle for 12 cycles) or observation alone, and no statistically significant differences

in OS or RFS were found between the study arms.[28] In the phase III BCAT trial from Japan evaluating the efficacy of gemcitabine in monotherapy (1000 mg/m^2, administered on days 1, 8, and 15 every 4 weeks for six cycles) compared with observation in 226 patients with eCCA, no statistically significant differences in RFS or OS were found either.[29] Despite the fact that gemcitabine-based chemotherapy can also be considered according to the clinical National Comprehensive Cancer Network (NCCN) guidelines,[30] it has not demonstrated efficacy in the adjuvant setting and it is not recommended according to the European Society for Medical Oncology (ESMO) guidelines.[31] More data will be gathered after the results of the ongoing phase III ACTICCA-1 trial evaluating the role of gemcitabine and cisplatin compared with standard of care (SOC) capecitabine.[32] S-1 is an oral fluoropyrimidine combination consisting of tegafur, gimeracil, and oteracil.[33,34] In the phase III ASCOT trial from Japan evaluating the efficacy of S-1 (four cycles of 40 mg/m^2 if BSA was $<$1.25 m^2, 50 mg if BSA was 1.25 to 1.5 m^2 and 60 mg if BSA was $>$1.50 m^2 twice daily for 4 weeks, followed by 2 weeks of rest) compared with observation in 440 patients with eCCA, iCCA, GBC, or AC, statistically significant differences in OS and RFS were reported. Adjuvant S-1 may be considered as a valid option in the Asian population.[35]

First-line setting

In patients with unresectable or metastatic disease, curative treatment is no longer an option and systemic treatment is recommended. ABC-02 phase III clinical trial randomly assigned 410 untreated patients with locally advanced or metastatic BTCs, including iCCA, eCCA, GBC and AC, to receive either cisplatin and gemcitabine (Cisplatin 25 mg/m^2 and gemcitabine 1000 mg/m^2 on days 1 and 8 every 21 days cycle for eight cycles) or gemcitabine alone (1000 mg/m^2 on days 1, 8, and 15, every 4 weeks for six cycles) as first-line treatment. The median OS was 11.7 months in the CisGem group and 8.1 months in the gemcitabine group (HR $=$ 0.64, $P < 0.001$). The median progression-free survival (PFS) was 8.0 in the experimental group and 5.0 months in the gemcitabine group (HR $=$ 0.63, $P < 0.001$)[12] (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100503). In Japan, another phase III trial named BT22 evaluated the same treatment as ABC-02, which gave the approval of this regime for Japanese patients after its positive results.[36] A difference between both trials was that CisGem was continued for up to 16 cycles and gemcitabine up to 12 cycles. Despite the fact that gemcitabine is maintained after eight cycles in routine clinical practice, a cross-trial comparison did not show a median PFS or OS improvement of prolonging the treatment beyond 6 months. Although the increased dosage of gemcitabine did not show a clear benefit, the KEYNOTE-966 study uses this approach as opposed to TOPAZ-1, which used a maximum of eight cycles.[37,38] Thus, the recommended treatment of unresectable BTCs is CisGem after these positive results and is the preferred regime in Europe and the United States for fit patients with PS $\leq$1. The mechanism of action by which this combination is active is the halt of DNA replication by gemcitabine and DNA breaks done by cisplatin bonds to DNA[39] (Figure 3). Gemcitabine monotherapy may be considered for PS 2 patients, since a meta-analysis of ABC-02 and BT22 showed no benefit of CisGem in this subgroup of patients.[40] Platinum-based chemotherapy strategies have shown efficacy in CCA. Recently, a French group compared FOLFIRINOX with the SOC. The PRODIGE38-AMEBICA phase III clinical trial randomly assigned 191 patients with locally advanced or metastatic BTCs to receive either oxaliplatin, irinotecan, and infusional 5-fluorouracil (5-FU) without bolus [modified FOLFIRINOX (mFOLFIRINOX)], or CisGem for a maximum of 6 months. The study did not meet the primary endpoint for OS, obtaining a median OS of 11.7 months for mFOFLIRINOX versus 13.8 months in the CisGem arm, remaining the SOC in the first-line setting.[41] In this line, the development of S-1 combined with platinum is active in BTC. S-1 has been tested in the FUGA-BT study, a phase III randomized trial in Japan including 354 chemotherapy-naive patients with recurrent or unresectable BTC with ECOG of 0 or 1. FUGA-BT is a non-inferiority study comparing gemcitabine plus S-1 (S-1 30 mg/m^2 orally twice a day on days 1-14 and gemcitabine 1000 mg/m^2 on days 1 and 8, every 3 weeks) versus CisGem (same schedule as ABC-02), with OS as the primary outcome. Gemcitabine combined with S-1 was non-inferior to CisGem-based chemotherapy in Japanese patients. The median OS of patients assigned to the CisGem group was 13.4 months, and 15.1 months in the S1-gemcitabine group. The combination of S-1 and gemcitabine was non-inferior to CisGem (HR 0.945; one-sided P for non-inferiority $=$ 0.046). The proportion of adverse effects did not differ significantly between treatment arms and were globally well tolerated[42] (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100503). The authors concluded that this new combination approach should be considered as a new option for SOC and has the advantage that it does not require hydration. While both regimes can be used in Asian patients, CisGem remains the SOC for the first-line treatment of good-PS patients with advanced BTC, regardless of ethnicity, as showed in a meta-analysis carried out by Valle et al in 2014.[40]

Second-line setting and beyond

In the second-line setting, combination regimens including fluoropyrimidines, platinum salts, and other chemotherapies have been tested. Results from a randomized phase III trial in second line have been recently reported. The ABC-06 clinical trial, an open-label, phase III trial included a UK population with locally advanced or metastatic BTC (including CCA, GBC, and AC) after progression to first-line CisGem chemotherapy, with an ECOG 0-1. A total of 162 participants were randomized 1 : 1 to receive active symptom control plus FOLFOX or active symptom control alone. FOLFOX chemotherapy consisted of oxaliplatin (85 mg/m^2) as a 2-h infusion on day 1 and a

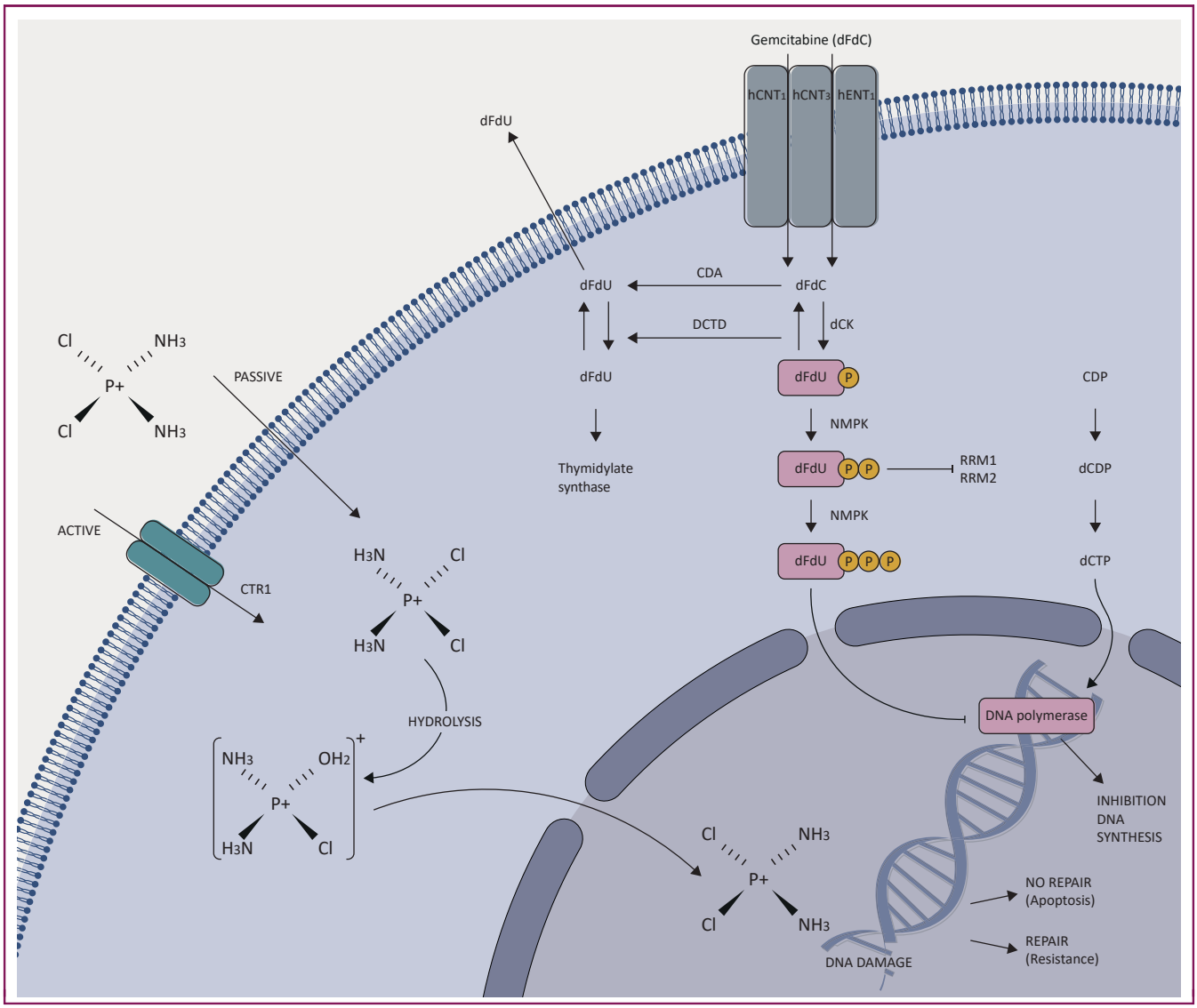

Figure 3. Gemcitabine and cisplatin transport, intracellular activation/deactivation and mechanism of action. Both gemcitabine diphosphate (dFdU) and gemcitabine triphosphate inhibit processes required for DNA synthesis, causing cell death.

CDA, cytidine deaminase; CDP, cytidine diphosphate; CTR1, copper transporter; dCDP, deoxycytidine diphosphate; dCTP, deoxycytidine triphosphate; dCK, deoxycytidine kinase; DCTD, deoxycytidylate deaminase; dFdC, 2′,2′-difluorodeoxycytidine; dFdU, 2′,2′-difluorodeoxyuridine; hCNTs, human concentrative nucleoside transporters; hENTs, human equilibrative nucleoside transporters; NDPK, nucleoside diphosphate kinase; NMPK, nucleoside monophosphate kinase; RR(M1/M2), ribonucleotide reductase; 5′-NT, 5′-nucleotidase.

2-h infusion of leucovorin (LV) (175 mg/m^2/day) followed by a 5-FU bolus [400 mg/m^2/day] and 46-h infusion of 5-FU (2400 mg/m^2) every 2 weeks. The primary outcome was OS in the intention-to-treat population. FOLFOX modestly improved median OS with 6.2 months versus 5.3 months in the control arm (HR = 0.69, P = 0.031). Grade 3/4 toxicities, such as fatigue and neutropenia, were reported in 59% of patients in the experimental arm versus 39% in the control arm. In the subgroup analysis, all subtypes of BTC tumors benefited similarly from FOLFOX.[13] Despite the modest absolute median OS difference between the two arms, there was a clinically meaningful increase in OS rates at 6 and 12 months in patients who received second-line treatment with FOLFOX and the trial has provided clinical evidence for the first time in this setting.

Other novel chemotherapy combinations are being tested in the second-line setting for advanced BTCs. A phase II trial randomized 120 patients to mFOLFOX (oxaliplatin 100 mg/m^2 over 2 h, LV 100 mg/m^2 over 2 h, 5-fluorouracil 2400 mg/m^2 over 46 h, every 2 weeks) or mFOLFIRI (irinotecan 150 mg/m^2 over 2 h, LV 100 mg/m^2 over 2 h, 5-fluorouracil 2400 mg/m^2 over 46 h). The mFOLFOX group reported a superior overall response rate (ORR) (5.9%), median PFS (2.8 months), and median OS (6.6 months).[43] FOLFOX should be offered as the new SOC for second-line therapy for BTC patients, especially those patients with no driver mutations.

Another regimen, platinum-free, liposomal irinotecan (nal-IRI) combined with 5-FU/LV has been tested in second-line BTC and the results were recently reported. In a randomized, open-label, phase IIb trial (NIFTY), 174 patients from Korea were randomized 1 : 1 to nal-IRI (70 mg/m^2 plus intravenous (i.v.) LV 400 mg/m^2 and i.v. fluorouracil at 2400 mg/m^2 for 46 h) or 5-FU/LV both every 2 weeks after progression to CisGem. The nal-IRI plus 5-FU/LV significantly improved PFS and OS compared with 5-FU/LV. The nal-IRI plus 5-FU/LV group reported a median PFS of 3.9 months and a median OS of 8.6 months versus a median PFS of 5.5 months versus 1.4 months in the 5-FU/LV group. Despite being a phase IIb clinical trial, the patient number does not differ from that in the only phase III trial available to date (ABC-06). Grade $\geq$3 adverse events, such as neutropenia and asthenia, were reported in 77% patients in the nal-IRI plus 5-FU/LV group versus 31% of patients in the 5-FU/LV group. These results are promising, but they will need to be tested in international phase III clinical trials.[44] Other irinotecan-based regimens have been studied after progression to SOC. A phase II trial carried out in China randomized 64 patients to either irinotecan 180 mg/m^2 on day 1 plus capecitabine 1000 mg/m^2 twice daily on days 1-10 versus irinotecan 180 mg/m^2 on day 1, with both treatments on a 14 days cycle. The combination resulted in superior median OS (10.1 months for the combination versus 7.3 months for the single agent) and disease control rate (DCR).[45]

Targeted therapies

In the metastatic and unresectable setting, additional molecular testing and inclusion into a clinical trial is highly encouraged. In the MOSCATO-01 trial, out of 1013 patients screened for molecular aberrations, 199 patients could receive a matched treatment (19%), but only 7% of those successfully screened benefited from the targeted therapy.[46] If the 43 BTC patients included in the trial are analyzed, however, 23 (50%) had druggable molecular aberrations and 18 patients (42%) could receive a matched therapy. Among these patients, the DCR was 88% and the median OS was 17 months compared with 5 months for those who did not receive a targeted therapy (HR = 0.29, P = 0.008).[47] Thus, molecular screening is highly recommended in patients affected by BTC, allowing personalized and effective targeted therapy to be offered.

Fibroblast growth factor receptors fusions. Genomic alterations, including fibroblast growth factor receptors (*FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, or *FGFR19*) activate the *FGFR* pathway in ~20% of iCCAs.[48] Chromosomal fusions of *FGFR2* exons 1 to 17 are the most common alterations, found in 10%-16% of iCCAs.[49,50] The resulting chimeric *FGFR2* proteins constitutively activate the pathway and promote proliferation (Figure 4). *FGFR2* can also present point mutations and amplification or overexpression.[57] First-generation *FGFR* inhibitors target multiple receptors, lacking a profound anti-*FGFR* inhibition and presenting multiple deleterious adverse events.[58] Thus, numerous new inhibitors of *FGFR* isoforms 1-3 have demonstrated benefit in advanced CCAs harboring *FGFR2* gene fusions, including various ATP-competitive, reversible inhibitors (erdafitinib, infigratinib, pemigatinib, and derazantinib) and the non-ATP-competitive, covalent inhibitor, futibatinib, also called TAS-120. Mechanisms of FGFR tyrosine kinase inhibitor (TKI) resistance are acquired by the tumor cell, such as FGFR kinase mutations, being more frequent than gatekeeper mutations.[59] These new agents have shown high response rates in early data from phase I and II clinical trials. Reversible inhibitors reported an ORR of 15%-35%, with a median PFS of 5.7-6.9 months.[60-63] The covalent inhibitor, futibatinib, reported an ORR of 41.7% and median PFS of 9 months, and can also overcome acquired resistance to ATP-competitive inhibitors.[57,64] Infigratinib or BGJ398, a reversible inhibitor, was tested in a multicenter, open-label, phase II study which included CCA containing *FGFR2* fusions, mutations, or amplifications and its primary objective was ORR by investigator assessment. The ORR was 14.8% and estimated PFS was 5.8 months. The final analysis reported an ORR of 23%, including one complete response and 24 partial responses (PRs). The most common adverse events included hyperphosphatemia (77% any grade), stomatitis in 55%, and fatigue in 40% of patients. Grade $\geq$3 adverse events occurred in 41% of patients.[61,65,66] Pemigatinib, another reversible inhibitor, was tested in a multicenter, open-label, single-arm, phase II study (FIGHT-202), which included three cohorts: *FGFR2* fusions or rearrangements, patients with other *FGFR* alterations, or patients with no *FGFR* alterations. The primary endpoint was ORR among those with *FGFR2* fusions or rearrangements who received at least one dose of pemigatinib. Around 35% of patients with *FGFR2* fusions or rearrangements achieved an ORR; 3 of them included a complete response. Adverse events resembled those reported for infigratinib, with hyperphosphatemia being the most common adverse event (60% of patients).[62] Pemigatinib has been approved by both the Food and Drug Administration (FDA) and the European Medicines Agency, and infigratinib has FDA approval. After these promising results, FGFR2 inhibitors are being tested in patients harboring *FGFR2* rearrangements as first-line setting in the phase III FIGHT-302 and PROOF trials, in which both drugs are compared with CisGem.[67,68]

The most common adverse event is characteristically hyperphosphatemia, with ~55%-81% of patients developing it. Hyperphosphatemia can be treated by reducing doses of the FGFR2 inhibitor or by adding phosphate-lowering therapy using phosphate binding agents, and a low phosphate diet should also be considered.[69] Other common adverse events are fatigue, stomatitis, alopecia, and palmar-plantar erythrodysesthesia. Grade 3/4 adverse events occur in >60% of patients, requiring a dose delay or dose reduction on many occasions, but with a low discontinuation rate of ~4%-6%.[60-64]

Isocitrate dehydrogenase 1 and 2 mutations. Isocitrate dehydrogenase 1 (*IDH1*) mutations are only found in gliomas and acute myeloid leukemias, but are rarely found in other tumors.[70] *IDH1* mutations are present in 9%-25% of

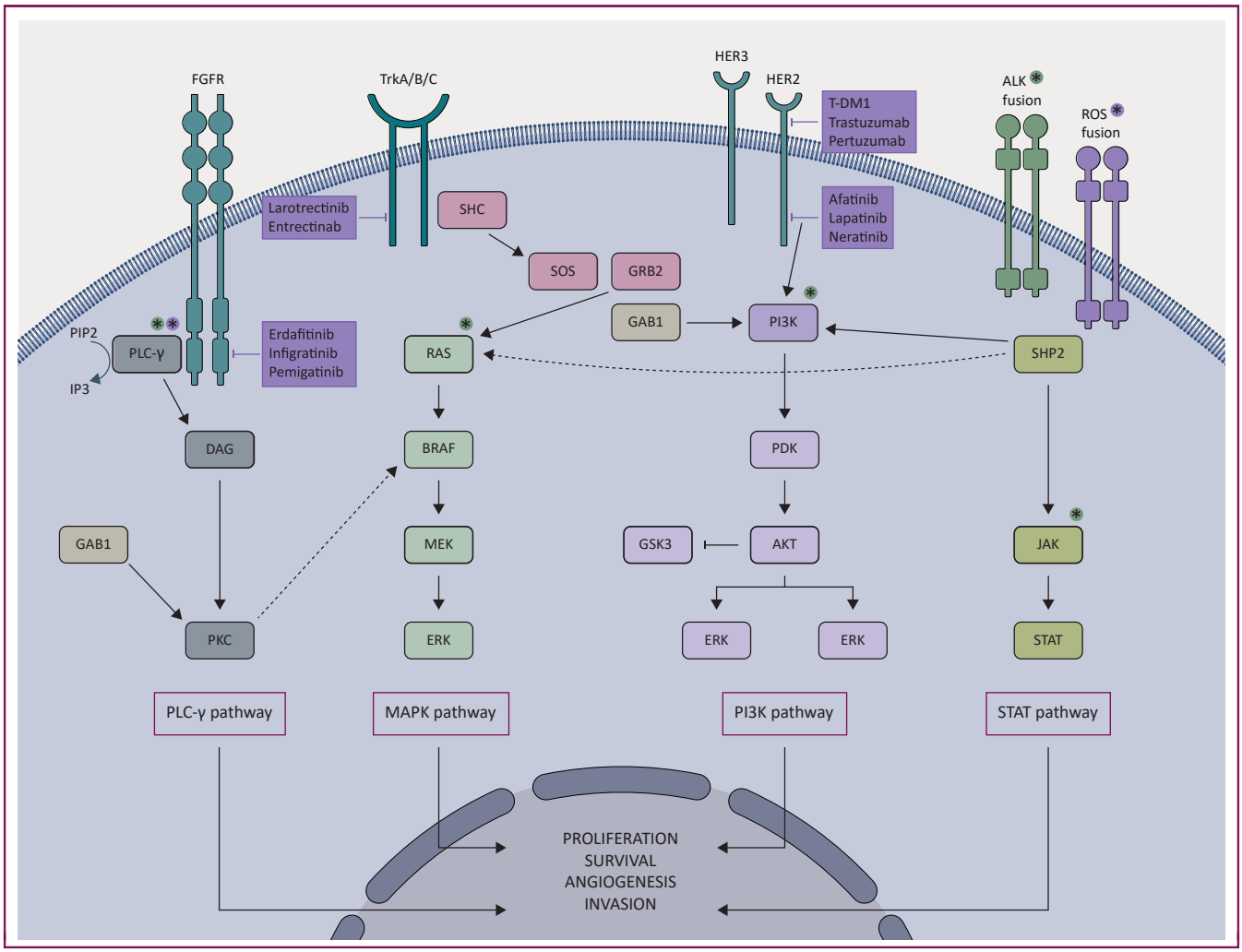

Figure 4. FGFR structure, signaling, and its alterations in cancer. FGFR is a transmembrane receptor tyrosine kinase, activating downstream signaling through three different pathways: via intracellular receptor substrates STAT, FRS2, and phospholipase C-γ1 (PLC-γ), leading ultimately to up-regulation of the RAS-dependent MAPK and Ras-independent PI3K-Akt signaling pathways (Adapted from Brooks et al., 2012).[51] **Trk receptors, ALK and ROS fusions; signaling pathway.** TrkA, B, and C upon neurotrophin binding, activate downstream signaling cascades of the MAPK, PI3K, and PLC-γ pathways. ALK and ROS fusions, through the activation of the intracellular substrate SHP2, activate the same pathways mentioned before; PLC-γ, MAPK, PI3K, and the STAT pathway through the activation of JAK (Adapted from Kheder and Hong, 2018,[52] Della Corte et al., 2018,[53] and Davies and Doebele, 2013[54]). **HER2 receptor: signaling pathway and alterations.** The HER2 has tyrosine activity similar to EGFR. The HER2 activation leads to tumorigenesis through the activation of MAPK and PI3K pathways, increasing proliferation, cell cycle progression through the activation of cyclin D, and inhibition of p27, and ultimately leading to cell survival (Adapted from Pollock and Grandis, 2015[55] and LV et al., 2016[56]).
AKT, protein kinase B; ALK,anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; GAB1, GRB2-associated-binding protein 1; GRB2, Growth factor receptor-bound protein 2; GSK3, Glycogen synthase kinase-3; HER, human epidermal growth factor receptor; IP3, inositol triphosphate; JAK, Janus tyrosine kinase; MAPK, mitogen-activated protein kinase; MDM2, Mouse double minute 2 homolog; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian/mechanistic target of rapamycin; PDK, phosphoinositide-dependent protein kinase; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; RAS, rat sarcoma virus; ROS, c-ros oncogene 1; SHC, src homology/collagen; SHP2, tyrosine phosphatase 2; SOS, son of sevenless; STAT, signal transducers and activators of transcription; T-DM1, trastuzumab emtansine; TrK, tropomyosin receptor kinase.

patients with CCA, varying depending on the location, more commonly found in iCCA and on the cohort analyzed.[71-73] *IDH1* (R132H) and *IDH2* (R172, R140) mutations within the isocitrate binding site result in a decreased enzymatic activity for oxidative decarboxylation of isocitrate to α-ketoglutarate.[71,73] As a result of this molecular alteration, tumors gain the ability to catalyze the reduction of alpha-ketoglutarate to R(-)-2-hydroxyglutarate (2HG) (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2022.100503). The accumulation of 2HG is almost pathognomonic of the presence of a tumor with *IDH* mutation, and the measurement of the circulating oncometabolite in a cohort of iCCAs correlated to tumor burden and may be an indicator of response to IDH inhibitors.[72,74,75] Ivosidenib (AG-120) is an oral targeted inhibitor of *IDH1* which showed activity in early clinical trials. The pivotal trial that led to the approval of ivosidenib was the ClarIDHy phase III trial, a multicenter, randomized, double-blind, placebo-controlled trial which included patients with advanced, *IDH1*-mutant CCA who had progressed on up to two previous treatment regimens. A total of 780 patients were prescreened for *IDH1* mutations and 187 were randomized 2 : 1 to 500 mg ivosidenib once a day or placebo. Most of

them had iCCA and presented metastatic disease at randomization. There was a statistically significant improvement in median PFS of 2.7 months in the ivosidenib group versus 1.4 months in the placebo group (HR = 0.37; $P < 0.0001$). Median OS was 10.3 months for ivosidenib and 7.5 months in the placebo group (HR = 0.79; $P = 0.093$). The non-significant difference in OS could be explained by the 57% crossover from placebo to ivosidenib, which was permitted on radiological progression. Common adverse events were nausea in 41%, diarrhea in 35%, and fatigue in 31% of patients.[76] Ivosidenib was well tolerated and resulted in an improved PFS and a trend towards favorable OS despite crossover, demonstrating clinical benefit. Patients with advanced *IDH1*-mutated CCA benefit from this treatment and it has been recently approved by the FDA. Enasidenib, a selective *IDH2* inhibitor, has been recently approved by the FDA for acute myeloid leukemia and is currently under investigation for CCA (NCT02273739).[77]

Neurotrophic tyrosine receptor kinase fusions. The neurotrophic tyrosine receptor kinase genes (*NTRK1-3*), which occur in ∼0.3%-1% of all solid tumors and are extremely rare in BTC (0.67%), encode three membrane-bound receptors called tropomyosin receptor kinases (TrkA, B, and C).[78-80] Neurotrophin binding triggers activation of the cytoplasmic kinase, activating downstream signaling cascades of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and phospholipase C-γ1 pathways (Figure 4).[81] Oncogenic fusions occur when one of the three NTRK genes fuses with a wide variety of partners and constitutively activates the Trk pathway, driving tumorigenesis.[82] Two highly selective small molecules, larotrectinib and entrectinib, inhibitors of all three *TRK* proteins, have been developed and showed activity in early clinical trials.[79,83] An analysis of the first 55 patients included into a phase I clinical trial of larotrectinib (LOXO-101) showed an ORR of 75% and the median PFS was not reached at 9.9 months. Only two patients with CCA were included; one patient had a progressive disease and the other showed a PR of −80%.[83] A grouped analysis of the three ongoing phase I or II clinical trials of entrectinib (STARTRK-1, STARTRK-2, and ALKA-372-001) was carried out. All trials together included 54 patients with 10 different *NTRK* fusion-positive tumor types, demonstrating a median PFS of 12.9 months and an ORR of 57%. Only one patient with CCA was included, who showed a PR with a reduction of 40%.[79] The most common grade ≥3 adverse events in patients treated with larotrectinib were anemia (11%), increased body weight (7%), and increased alanine aminotransferase or aspartate aminotransferase levels (7%).[83] The most common grade ≥3 adverse events in patients treated with entrectinib were anemia (12%), increased body weight (10%), and fatigue (7%).[79] An ongoing trial in China, called VISIONARY, is evaluating targeted therapies with FGFR2, IDH1, NTRK, and BRAF inhibitors in refractory gastrointestinal tumors, which also includes patients with BTC (NCT04584008).

BRAF mutations. Mutation of the *BRAF* gene is one mechanism of constitutive activation of the MAPK pathway, which regulates cellular proliferation, migration, and is aberrantly activated in many human cancers including melanoma, non-small-cell lung cancer, and papillary thyroid carcinoma.[84] The most common *BRAF* mutation is the single amino acid change of valine to glutamic acid at residue 600 (V600E). The frequency of *BRAF* V600E in BTC is very low, ∼1%-4%, and most commonly found in iCCA.[85] Vemurafenib was the first *BRAF* V600E inhibitor tested in eight patients with CCA in a phase II basket study. One PR and five SDs were reported in the CCA cohort.[86] Subbiah et al.[87] carried out the Rare Oncology Agnostic Research (ROAR) trial, a phase II, open-label, single-arm trial evaluating the efficacy and safety of dabrafenib and trametinib. A total of 43 patients were included, 91% had iCCA, 2% pCCA, 2% GBC, and 2% were of unknown origin. An ORR of 47% was reported by independent assessment, a median PFS of 9 months, and a median OS of 14 months. These results suggested that BRAF inhibition is canonical in BTC, at variance from metastatic colorectal cancer in which epidermal growth factor receptor (EGFR) inhibition is mandatory. The most common grade 3 or 4 adverse events in patients treated with dabrafenib and trametinib were increased gamma-glutamyl transferase (12%), decreased white blood cell count (7%), and hypertension (7%).[87] We encourage the development of phase III trials, but the low proportion of *BRAF*-mutated patients impairs solid research of *BRAF* inhibitors in BTC.

Human EGFR 2. Human EGFR2 (HER2) is an oncogenic growth factor receptor. There are four members of the same family which are named as EGFR, HER1 (ERBB1), HER2 (ERBB2), HER3 (ERBB3), and HER4 (ERBB4)[88] (Figure 4). These transmembrane growth factor receptors, upon phosphorylation of their intracellular domains, activate downstream secondary messengers, leading to diverse biological effects. The HER2 activation leads to tumorigenesis through the activation of MAPK and PI3K pathways, loss of cell polarity and cell adhesion, and deregulated cell cycle through the activation of cyclin D and inhibition of p27. HER2 has the strongest catalytic kinase activity, especially when partnering with HER3.[88] Overexpression of the HER2 protein has been reported in 25%-30% of breast and ovarian cancers, but has also recently been reported in ∼13% of GBCs and up to 18% of eCCAs.[89,90] HER2 overexpression has been associated with worse prognosis and increased propensity to metastasize, but also has increased sensitivity to cytotoxic and targeted agents. In a retrospective review published by Javle et al.,[90] eight patients with advanced GBC and five patients with CCA with *HER2/neu* gene overexpression or amplification were treated with HER2/neu-directed therapy. In the GBC group treated with trastuzumab, four patients had a PR, one had a complete response, and three presented SD. No responses, however, were seen in five patients with CCA who received trastuzumab. The phase II SUMMIT trial, which analyzed the efficacy of neratinib in *HER2*-mutant all-solid malignancies,

included 25 patients with BTC. In this specific population, an ORR of 12%, a median PFS of 2.8 months, and a median OS of 5.4 months were reported.[91] The MyPathway trial, a phase II trial, multicenter, open-label, which included 39 patients with *HER2*-positive BTC evaluating the combination of trastuzumab and pertuzumab, has recently been published. Authors reported an ORR of 23% (95% CI 11% to 39%), a PFS of 4 months (95% CI 1.8-5.7 months), and an OS of 10.9 months (95% CI 5.2-15.6 months).[92] There are currently multiple clinical trials testing HER2/neu-directed therapy for solid tumors, such as the MATCH trial (NCT02465060), which tests multiples TKIs matched to the tumor's molecular alteration, including trastuzumab alone or in combination with pembrolizumab in *HER2*-positive tumors. The TAPUR trial (NCT02693535) is also testing multiple TKIs with the same approach, including trastuzumab and pembrolizumab or the new molecule PESGHO, composed of pertuzumab, trastuzumab, and recombinant human hyaluronidase, in *HER2*-positive tumors.

Immunotherapy. The mismatch repair (MMR) system recognizes and repairs erroneous insertions or deletions that arise during DNA replication. Cancers deficient in the MMR (dMMR) system contain many somatic mutations, and make them especially sensitive to immune checkpoint inhibitors (ICIs), such as anti-programmed cell death protein 1 antibodies.[93] More than 5% of adenocarcinomas of the gastrointestinal tract, endometrium, cervix, and liver are dMMR. They are more commonly found in localized stages (8%) than in metastatic stages (4%).[93] In BTC, dMMR accounts for 2%-18% of tumors, depending on the location and series published, being more common in iCCA (10%) and AC (6%-20%) than in eCCA or GBC (5%-8%).[94-96]

The phase II KEYNOTE-158 study evaluated the antitumor activity of pembrolizumab against non-colorectal dMMR cancer.[97] A total of 233 patients were included, 22 (9.4%) of whom had BTC. An ORR of 41%, median PFS of 4.2 months, and a median OS of 24.3 months were reported for patients with CCA tumors. Overall, 14.6% reported any grade 3 or 4 adverse event, the most common adverse events being severe skin reactions (1.3%), pneumonitis (1.3%), colitis (0.9%), and hepatitis (0.9%).[97]

Regardless of the MMR status, many clinical trials tested ICIs for BTC all-comers. A phase II study tested nivolumab 240 mg i.v. every 2 weeks for 16 weeks and then 480 mg i.v. every 4 weeks, until disease progression or unacceptable toxicity, in 54 patients with refractory BTC. The study reports an ORR of 11% by central review with a DCR of 50%, a median PFS of 3.7 months, and a median OS of 14 months. When patients were stratified by anti-programmed death-ligand 1 (PD-L1) expression status ($\geq$1% cut-off), both median PFS and OS improved compared with patients with PD-L1-negative expression.[98] The TOPAZ-1 phase III clinical trial evaluated the combination of CisGem with 1500 mg every 3 weeks durvalumab versus CisGem. After 685 patients were randomized, the experimental arm showed an improvement of median OS and PFS (HR 0.80 and 0.75, respectively, both $P < 0.05$). These new data may bring a new first-line treatment to clinical practice.[38] The LEAP-005 phase II clinical trial evaluated the efficacy and safety of the combination of lenvatinib and pembrolizumab for pretreated tumors that are not microsatellite instability-high or dMMR. Out of 187 patients enrolled, 31 (16.5%) were patients with BTC, in whom an ORR of 9.7% with a DCR of 68% was reported. In this subset of patients, the duration of response was 5.3 months, and grade 3 or higher adverse events were reported in 48% of patients.[99,100] Pembrolizumab was tested at a second-line setting or beyond in a single Chinese center, single-arm study, which recruited 40 patients, 50% with iCCA. An ORR of 10% with four PRs and 15 SDs, and a median duration of response of 2.1 months were reported.[101] The role of bintrafusp alfa, a novel anti-PD-L1/transforming growth factor-β receptor II fusion protein, in combination with CisGem, was being tested in the first-line setting (NCT04066491) until 2021, when the trial was discontinued due to inefficacy after an analysis by the independent monitoring committee. Another phase III trial, KEYNOTE-966,[37] is testing the efficacy of pembrolizumab in combination with CisGem in the first-line setting in a worldwide population and an extension with only Chinese adults with advanced BTC (NCT04003636 and NCT04924062, respectively).

FUTURE PERSPECTIVES AND CONCLUSIONS

Many patients with BTC are diagnosed at an advanced stage, making the disease incurable in most cases. Surgery of the primary tumor detected in early stages of selected patients is the best available potentially curative treatment, but the field of advanced disease is open.

The current understanding of the biology is opening doors for new strategies, as BTCs have multiple molecular targetable alterations. Thus, it is highly recommended that all patients with BTC have a comprehensive molecular panel before the initiation of systemic treatment, since many genetic alterations may be encountered and will change the outcome of our patients. There are currently multiple clinical trials ongoing testing the efficacy and safety of many drugs in the first- and second-line setting with different strategies summarized in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2022.100503.

The first-line approved treatment remains cisplatin and gemcitabine after the positive results of the phase III ABC-02 which demonstrated an increase of median OS compared with gemcitabine alone. However, there is a new direction to push forward on patients who do not harbor driver mutations set by the TOPAZ-1 clinical trial, showing for the first time in years an improvement in OS, PFS, and ORR with immunotherapy in combination with chemotherapy.

In the second-line setting, FOLFOX has shown a modest but statistically significant benefit in median OS compared with active symptom control. As shown before, targeted therapies such as FGFR2, IDH1, and BRAF inhibitors have shown promising results in clinical trials for BTC patients with specific molecular alterations. Other strategies in

development are targeted therapies, chemotherapy combinations, immunotherapy, and antiobdy-drug conjugates which will impact the future of BTC treatment.

ACKNOWLEDGEMENTS

We would like to thank all the patients and families who allow research to move forward with their willingness to participate. The corresponding author would like to thank graphic designer Mario Silva for the collaboration with the illustrations (*OMMS*©). The corresponding author would also like to thank all of the authors who have contributed to this article.

FUNDING

None declared.

DISCLOSURE

JT reports consulting fees from Array Biopharma, AstraZeneca, Avvinity, Bayer, Boehringer Ingelheim, Chugai, Daiichi Sankyo, F. Hoffmann-La Roche Ltd, Genentech Inc, HalioDX SAS, Hutchison MediPharma International, Ikena Oncology, Inspirna Inc, IQVIA, Lilly, Menarini, Merck Serono, Merus, MSD, Mirati, Neophore, Novartis, Ona Therapeutics, Orion Biotechnology, Peptomyc, Pfizer, Pierre Fabre, Samsung Bioepis, Sanofi, Seattle Genetics, Scandion Oncology, Servier, Sotio Biotech, Taiho, Tessa Therapeutics and TheraMyc; and honoraria for lectures from Imedex, Medscape Education, MJH Life Sciences, PeerView Institute for Medical Education and Physicians Education Resource (PER). TM reports grants from Abbvie Farmaceútica, Ability Pharmaceuticals, Agios Pharmaceuticals, Amc Medical Research, Amgen, Armo Biosciences, Aslan Pharmaceuticals, Astrazeneca, Basilea Pharmaceutica International, Bayer, Beigene, Biokeralty Research Institute, Biolinerx, Blueprint Medicines, Boston Biomedical, Bristol-Myers Squibb (BMS), Cantargia, Celgene, Eisai, Erytech Pharma, F. Hoffmann-la Roche, Fibrogen, Halozyme, Incyte, Ipsen Bioscience, Ipsen Pharma, Lilly, Loxo Oncology, Medimmune, Merck Sharp & Dohme, Nelum, Novartis, Novocure, Oncomed Pharmaceuticals, QED Therapeutics, VCN Biosciences and Zymeworks; consulting fees from Swedish Orpahn Biovitrum AB, Ability Pharmaceuticals SL, Aptitude Health, AstraZeneca, Basilea Pharma, Baxter, BioLineRX Ltd, Celgene, Eisai, Ellipses, Genzyme, Got It Consulting SL, Hirslanden/GITZ, Imedex, Incyte, Ipsen Bioscience, Inc, Janssen, Lilly. Marketing Farmacéutico & Investigación Clínica, S.L, MDS, Medscape, Novocure, Paraxel, PPD Development, Polaris, QED Therapeutics, Roche Farma, Sanofi-Aventis, Servier, Scilink Comunicación Científica SC, Surface Oncology, TRANSWORLD EDITORS, SL and Zymeworks; support for attending meetings from Servier, AstraZeneca, Sanofi and Incyte; and advisory board at Swedish Orpahn Biovitrum AB, Ability Pharmaceuticals SL, Aptitude Health, AstraZeneca, Basilea Pharma, Baxter, BioLineRX Ltd, Celgene, Eisai, Ellipses, Genzyme, Got It Consulting SL, Hirslanden/GITZ, Imedex, Incyte, Ipsen Bioscience, Inc, Janssen, Lilly. Marketing Farmacéutico & Investigación Clínica, S.L, MDS, Medscape, Novocure, Paraxel, PPD Development, Polaris, QED Therapeutics, Roche Farma, Sanofi-Aventis, Servier, Scilink Comunicación Científica SC, Surface Oncology, TRANSWORLD EDITORS, SL and Zymeworks. All the other authors have declared no conflicts of interest.

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