Current development and future perspective of IDH1 inhibitors in cholangiocarcinoma

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Abstract

Background and Aims: Biliary tract cancer (BTC) represents a major public health problem due to its increasing rates of incidence and mortality, especially the intrahepatic cholangiocarcinoma (IHCCA) subtype. First line palliative systemic treatment with cisplatin and gemcitabine has been the unique level IA evidence option until last few years when a deeper understanding of its molecular landscape has unveiled CCA as a very rich targetable disease. This has revolutionised the patient’s scenario and has brought new targeted therapies guided by molecular aberrations. Isocitrate dehydrogenase (IDH)1 mutations are the most prevalent targetable alteration in CCA (13% of IHCCA). Ivosidenib has been very recently approved by FDA for IDH1 mutated CCA patients based on a randomised clinical trial (ClarIDHy).

Methods: We review evidences related to IDH1 mutations in general oncogenesis focusing on CCA and summarise the present and potential future clinical options for the diagnosis and treatment of IDH1m CCA patients.

Results: Although the knowledge of the pathogenesis of IDH mutation is still a work in progress, epigenetic and metabolic dysregulation due to the oncometabolite 2-hydroxyglutarate (2HG) are key in this process. Blocking mIDH1 with ivosidenib has shown significant and clinical benefit for this subgroup of patients. Primary and secondary resistance mechanisms to IDH inhibitors are now being described leading to the investigation of novel treatments such as more potent IDH inhibitors or other drugs that indirectly targets mIDH, as well as looking for novel biomarkers.

Conclusions: Although ivosidenib is already available for treating advanced refractory IDH1 mutated CCA, there is still much space to improve outcomes. Several new treatment and diagnostic options in development could hopefully do it for this important subgroup of patients.

KEYWORDS
bile tract cancer, cholangiocarcinoma, IDH1, IDH inhibitors
Biliary tract cancer represents 3% of the gastrointestinal tract cancers. Although it is considered a rare disease in western countries (incidence of 0.3-3.5 cases/100,000), its high morbidity and mortality and trending ascend incidence (specially the intrahepatic CCA [IHCCA] subtype possibly related to the increase of obesity and metabolic syndrome) makes of it a major public health problem.

BTC is a very heterogeneous disease regarding multiple aspects such as anatomic location, aetiology, natural history, demography, clinical presentation or surgical management. Only around 30% of patients are resectable at diagnosis (the unique potential curative option). The high recurrence rate after surgery even with adjuvant therapy (around 50%) and the frequent advanced stage already at initial diagnosis (70%) make usually palliative systemic treatment the only potentially active option.

Chemotherapy based on cisplatin and gemcitabine is still the standard of care in the first line setting and has represented the only 1A level of evidence option for a decade achieving a median overall survival of 11.7 months in the ABC-02 landmark trial. Last few years have brought new systemic treatment options both in first and second line settings such as new chemotherapy combinations (FOLFOX, Xeliri, gemcitabine-nabpaclitaxel-cisplatine) and targeted therapies non-biomarker-guided (regorafenib) (Figure 1).

However, it has been the deeper molecular understanding which has opened a new era for this orphan disease. As many as 40% of the IHCCA harbour molecular alterations which can be triggered. Neurotrophic tyrosine Receptor kinase (NTRK) rearrangement and microsatellite instability-high (MSI-H) are agnostic tumour molecular biomarkers for the treatment with NTRK inhibitors (entrectinib or larotrectinib) and immune checkpoint inhibitors respectively.

Key Points
- BTC, especially IHCCA, has been shown to be a very targetable rich disease.
- IDH1 mutation, previously described in other malignancies, is the most prevalent clinically targetable alteration in IHCCA patients.
- Pathogenic mechanism of IDH mutation is still not well understood. 2HG is the oncometabolite product of the IDH mutation and competes with alpha-ketoglutarate (aKG) causing epigenetic (blocks cellular differentiation) and metabolic dysregulation.
- Ivosidenib, a first-in-class inhibitor of mIDH1, has been approved by FDA in mIDH1 CCA as a biomarker-guided targeted therapy, being the first one with an evidence-based on a randomised clinical trial.
- Primary and secondary resistance mechanisms to IDH inhibitors are now being described.
- New second class more potent IDH inhibitors and novel therapeutically approach looking for potential vulnerabilities related directed or undirected to the IDH mutation, such as targeting DDR or immunotherapy, are in clinical development.

**FIGURE 1** Traditional backgrounf of BTC (left side). Novel targeted treatment options after NGS including IDH1 mutation and IDH inhibitors
Although these alterations are relatively rare in CCA (less than 1%), other targetable alterations such as Fibroblastic Growth Factor Receptor 2 (FGFR2) (rearrangement/fusions or mutations) (10%-15%), BRAF V600E (3%), Human Epidermal Growth Factor Receptor 2 (HER2) amplification/mutations (15%) and Isocitrate dehydrogenase 1 (IDH1) mutations (13%) are not uncommon, especially in IHCCA.10

FGFR2 inhibitors in CCA with fusion or other rearrangements in FGFR2 (FDA/EMA approval of pemigatinib and FDA approval of infgratinib),11,12 BRAF and MEK inhibitors combo (dabrafenib and trametinib) in BRAF V600E13 and, very recently, double HER2 inhibition (trastuzumab-pertuzumab)14 have shown clear efficacy data in terms of response rate in phase 1/2 non-randomised clinical trials.

Nonetheless, ClarIDHy has been the first positive randomised clinical trial for a molecular guided- targeted therapy, showing a PFS benefit for ivosidenib (a first-in-class oral IDH inhibitor) vs placebo in the second/third line treatment of CCA patients with IDH1 mutation.15 This has led to the very recent approval by FDA. The lower response rate (3%-5%) and frequent primary or secondary resistance events to IDH1 inhibitors merits to keep looking for new treatment options for this population.

In this article, we review the biological basis of the IDH pathway and the oncogenic effects of its mutation in cancer, specifically in CCA. We also review the clinical implications of the IDH1 mutation in terms of diagnosis, prognosis and the current treatment options in CCA. Finally, we explore the potential future treatment options still in progress, attending monitoring of response and resistance mechanism for the IDH mutated CCA patients.

2 | IDH AND CANCER

2.1 | IDH physiological role

Isocitrate dehydrogenase (IDH) is an essential metabolic enzyme for cellular respiration that participates in the Krebs cycle. IDH has three isoforms called IDH1, IDH2 and IDH3 (the last one has not been described as relevant in cancer). IDH1 is located in the cytosol and the peroxisomes while IDH2 and IDH3 are located in the mitochondria.

The catalytic function of the three enzyme is the conversion of isocitrate into alpha-ketoglutarate (aKG) by an oxidative decarboxylation reaction. IDH1 and IDH2 are Nicotinamide adenine dinucleotide phosphate (NADP) dependent enzymes and use NADP+ in the reaction that turns into NADPH and CO2. IDH3 is NAD dependent and use NADP+ in the reaction that turns into NADP and CO216 (Figure 2).

IDH1 and IDH2 are homodimers while IDH3 is a heterotetramere. Focusing on IDH1, each unit is composed of 414 amino acids forming a three regions structure (large, small and claps regions).17 The conformation can adopt two possible and reversible states; open (deactivated form) and closed (activated form). Transition between these two states depends on aKG concentration and on the NADP+/NADPH ratio.

By the mentioned enzymatic reaction, IDH is implicated in multiple cellular processes not only metabolic but also epigenetic. It also participates in redox equilibrium and DNA repair mechanisms. aKG, one of the reaction products, has pleotropic functions. It is cofactor of multiple enzymes called aKG-dependent dioxygenases, such as TET (Ten eleven translocation hydroxylases), KDMs (Jumonji c-domain containing lysine demethylase), PHD (prolyl-hydroxylases domain containing), FIH (Factor inhibitor of HIF) or ALKBH (aKG-dependent dioxygenase homologs) (Table 1).

TET family (5-methyl cytosine hydroxylase) and KDMs are enzymes implicated in DNA and Histone demethylation respectively. This is a crucial step in cell differentiation since gen transcriptional regulation processes will or not allow a cell to express certain genes depending on the context. For instance, maturation of hepatic progenitor cells (cellular differentiation) requires of the expression of HNF4a.
TABLE 1 Pathogenic mechanisms of mIDH1

| IDH1                        | mIDH1                        | 2HG                      |
|-----------------------------|------------------------------|--------------------------|
| aKG DNA/Hys demethylation   | TET KDM                      | Hypermethylator phenotype |
| Regulation HIF1a            | PHD FIH                      | High HIF1a               |
| Regulation mTOR             | KDM4A                        | High mTOR                |
| Collagen folding            | P4HA PLOD                    | Collagen disfunction      |
| DDR mechanism               | ALKBH KDM4                   | Genome instability        |
| NADPH REDOX control         | Oxidative stress             | NADP+                    |
| Lipid, aminoacids, deoxyrybonucleotids sintesis | Metabolic impairment |

Note: aKG (alpha-keto glutarate); Hys (hystone); HIF1a (Hypoxia inducible factor 1*); TET (Ten eleven traslocases); KDM (jumonji c-domain containing lysine ddemthylase); PHD (Prolyl-hidroxilase domain containing); P4HA (prolyl -4- hydroxilase); REDOX (reduction – oxidation); NADPH (Nicotaminade adenosine dinucleotide phosphate).

DEPTOR, the endogenous inhibitory regulator of mTOR, is stabilised by KDM4A (by inhibiting its polyubiquitylation). By this action, DEPTOR decreases mTORC1 and mTORC2 activation (avoiding signals related to cell growth, cell survival, angiogenesis and metabolism).

Regulation of hypoxia inducible factor 1a (HIF1a) pathway depends on PHD and FIH by the hydroxylation of proline and asparagine residues respectively. PHD mediates degradation of HIF1a (by its ubiquitination) while FIH interferes with its catalytic function by avoiding its association with transcription factors such as CBP (CREB binding protein) and p300. This finally controls the hypoxia response.18

Collagen maturation also depends on prolyl and lysine hydroxylases such as P4HA (prolyl -4-hydroxilase) and PLOD (procollagen lysine 2 oxoglutarate 5 dioxygenase) which collaborate in its correct folding.

ALKBH and also KDM4B belongs to the DNA damage response (DDR) pathway participating in homologous recombination (HR). In such a way aKG also contributes to the genomic stability maintenance.19

Importantly, IDH1 is the main source of non-mitochondrial NADPH, the other physiological catalytic product. This metabolite participates in different functions. REDOX reactions (such as reduction of glutathione and activation of catalase and citP450) require NADPH to attenuate oxidation damage in cells.20 Also, NADPH, as an electron donor, participates in the synthesis of many molecules such as lipids (steroids, phospholipids, triacylglycerol), amino acids (proline and glutamate) and deoxyribonucleotides.

2.2 | IDH1 mutations and cancer

IDH1 is an oncogene located in 2q34 while IDH2 is in 15q26.1. Hotspot heterozygous missense mutations (no truncation nor frameshift) of IDH1 were first described in low grade glioma (LGG) and secondary glioblastoma followed by acute myeloid leukaemia (AML). Soon after the IDH1 mutation was related to many other solid tumours. LGG and secondary glioblastoma (23%),21 CCA (14%)22 and AML (7%),23 are the three most frequent mIDH1 tumours.

IDH1 mutations are more frequent than IDH2 and both mutually exclusive. IDH1 mutations affect substitution of arginine residue in 132 position (R132) where the enzyme binds isocitrate.24 Hotspot mutations in IDH2, out of the scope, affect R172 and R140.21 These mutations occur mainly in heterozygosis so homodimers turn into heterodimers.25

Although the exact pathogenesis of the IDH1 mutation in cancer is still a matter of debate, it has been clearly demonstrated that mutations confer a gain of function (neomorphic activity) so that the new heterodimer (R132/WT) has more affinity for NADPH than for isocitrate. mIDH acquires a constant closed (activated) conformation leading to a change in the original reaction with the final generation of an oncometabolite called 2hydroxylglutarate (2HG) (now converting NADP+ to NADPH).26 Levels of 2HG in these tumours reach 50-100 fold compared to the wild type. Potency for producing 2HG depends on the allelic substitution.27 Maintenance of the heterozygous state has been reported necessary to maintain closed and activated conformation.16

Pathogenic consequences of IDH1 mutation are leaded by the increase of 2HG and the reduction of aKG and NADPH (Figure 2). 2HG has two enantiomers, D2HG and L2HG, being the first one more potent as an epigenetic deregulator. It competes with aKG for the already mentioned aKG dioxygenases, due to its structural similarity (oxidation of C-2 position is the only difference) but with higher affinity.28

Regarding TET and KDMs, due to the DNA / histone demethylation inhibition, mIDH1 generates a hypermethylator phenotype which causes a gene expression dysregulation (epigenetic "rewiring"). DNA hypermethylation will occur mainly in the CpG islands (low levels of 5-OH-metilcytosine)29 while histone hypermethylation will generate an increase of methylation markers such as H3K27me3,
H3K9me3 and H3K4me3. H3K27me3 and H3K9me3 are transcription repressors and this will affect genes of differentiation such as the previously mentioned HNF4a for hepatoblasts, blocking them in the progenitor state and avoiding the cellular differentiation.\textsuperscript{29,30} This probably creates a pathological self-renewal of stem-like progenitor cells (unchecked cell proliferation) that is more permissive to malignant transformation. It has been reported in glioma that IDH1 mutations are related to a colder TME with less CD8 infiltration because of down-regulation of STAT1.\textsuperscript{31} Moreover, genes related to the immune response, such as NKG2D, are also silenced in IDH mutant glioma cells, generating an immunosuppressive environment.\textsuperscript{32}

Since KDM4A stabilises DEPTOR, its inhibition by 2HG increases the level of mTOR by a PTEN-independent mechanism.\textsuperscript{33} Also, alterations in the maturation of collagen such as defects in the glycosylation of the OH-lysine of collagen 4 (due to impairment of the already mentioned P4HA and PLOD activity) generate instability and more solubility. This means fragility of the basal membrane of blood vessel facilitating epithelial invasion.\textsuperscript{34} Moreover, defects in endostatin gene (an antiangiogenic protein that is produced from the collagen XIII and that downregulates HIF1a) generate neoangiogenesis as a consequence.\textsuperscript{28} The negative interaction with PHD and HIF leads to an increase of the HIF1a signalling which also activates angiogenesis.\textsuperscript{35}

Low levels of NADPH, the other consequence of the mutation in IDH, affect the REDOX reactions leading to a higher sensitivity to oxidative stress and to DNA damage agents. Also, NADPH deficiency impairs lipogenesis and the synthesis of amino acids. It has been reported in mIDH1 glioma that decrease in NADPH mitochondrial pool decreases sensitivity to apoptoses\textsuperscript{36} and also induces the contribution of glutamine to lipogenesis under conditions of hypoxia.\textsuperscript{37}

Interestingly, low basal levels of NAD+ in IDH mutated cells, consequence of a decreased expression of the Nicotinate phosphoribosyltransferase (NAPRT1) (enzyme of the NAD+ salvage pathway), disrupts the DDR pathway since PARP utilise NAD+ to generate poly-ADP-ribose (PAR) chains.\textsuperscript{37} Also, increase level of H3K9me3 downregulates ATM (damage sensor of DNA).\textsuperscript{38} This mechanism is implicated also in genome instability which confers in glioma more radiosensitivity, enhancing agents and PARP inhibitors.\textsuperscript{39} Moreover, D2HG also disrupts enzymes related to the repairation of DNA damage such as previously mentioned ALKBH and KDM4B leading a genomic instability and mutagenesis.\textsuperscript{39}

The reported biological consequences of the IDH mutations until now do not meet exactly between different tumours suggesting that each cell and its specific genetic context is relevant to it. For instance, the level of the classic methylated phenotype described above is higher in glioma than other mIDH tumours (19% of CpG islands methylated vs 2%-4% respectively).\textsuperscript{40}

## 3 IDH1 MUTATIONS IN CHOLANGIOCARCINOMA

Improvement in molecular technology has revealed that BTC is a very heterogeneous disease not only at clinical level which classically depends on its anatomic origin but also at a molecular level. In the last few years several papers have described many different molecular alterations such as mutations, fusions or amplifications between other. Importantly, some of them such as FGFR2, IDH1, HER2, MSI or BRAF are potentially targetable.

IDH mutations in IHCCA were first described in 2012 by Borger. Most of the IDH mutations in BTC occur in the intrahepatic cholangiocarcinoma subtype which is originated above second degree biliary radical.\textsuperscript{22} The incidence of IDH1 and IDH2 mutations in IHCCA is about 14% and 4% respectively being both mutually exclusive\textsuperscript{41} (Table 2). Some authors classify IHCCA in large duct (ductal or mucin-adenocarcinoma) and small duct (ductular or cholangiocarcino-type without mucin) being the latter the typical subtype associated with IDH mutations. The classical histology here harbours abundant intratumoral stroma and the tumor cells arranged in nests or glands predominating of poorly or moderately differentiation.\textsuperscript{42}

Seminal papers have tried to correlate this molecular landscape with other factors such as the aetiology or the anatomical location leading to different classifications based on clusters. IDH1 and IDH2 mutations were predominantly described in fluke negative CCA (such as FGFR and Pi3Kca mutations).\textsuperscript{10} This could explain why IDH mutation are more frequent in non-Asian-centres than in Asian-centres (16.5% vs 8.8%).\textsuperscript{43} Chan-On et al analysed the molecular differences regarding of the presence of a specific class of fluke infestation (Opisthorchis viverrini positive or negative) showing that IDH mutations, among others (such as PD1/PDL1, BAP1, FGFR, PRKA), are more prevalent in the O. viverrini negative cohort (22.2% vs 32%).\textsuperscript{44} A negative correlation for the mIDH was also shown for viral hepatitis infection (2% vs 20%).\textsuperscript{45}

Missense mutation R132C is the most prevalent IDH1 mutation (44%) over the rest (R132G, R132H, R132S, R132L).\textsuperscript{46} The association of IDH mutation with other molecular alterations has been published by different authors.\textsuperscript{43,47} In a big retrospective NGS study of more than 27 000 GI cancers, mIDH CCA showed lower frequency of some mutations respecting to WT tumours such

| Chromosome | Ubicacion | Typical mutation | Targeted drug (approved) | Frequency mutated in CCA |
|------------|-----------|------------------|--------------------------|--------------------------|
| IDH1       | 2q34      | Cytosol Microsomes | R132 | Ivosidenib (AG-120) | 14% |
| IDH2       | 15q26.1   | Mitochondria      | R172, R140 | Enasidenib (AG-221) | 4% |
as TP53 (13% vs 43%), KRAS (8% vs 19%), CDKN2A (1% vs 9%) and SMAD4 (0% vs 9% respectively). However, for PBRM1 (14% vs 5%) and ARID1A and BAP1 (only a trend) mutations were higher than in the IDH wild type subgroup. It was also described lesser proportion of HER2 expression / amplification (0.5% vs 3%), FGFR2 fusions (2% vs 7%), a lower TMB (0.7 vs 3.7) and lesser MSI-H (only a trend).48 This has been reproduced similarly by other authors, also showing lesser PDL1 (CPS 1%-49%) but without differences in immune expression markers (CD3, CD4 and CD8) neither in high loss of heterozygosity (LOH).51 Some authors have stated some genes to be mutually exclusive of IDH1 mutation such as TP53, KRAS and FGFR2,47 but this has not been reproduced in others. Heterogeneity of the cohorts and samples issues may explain different results between authors.

The majority of knowledge about the cancer pathogenesis of IDH mutations comes from other tumours such as glioma and LMA as we have mentioned before. The overproduction of 2HG both in tissue and blood has also been described in CCA.49 In a molecular clustering reported by the International Cancer Genome Consortium (ICGC) based on the multiplatform analyses of 489 CCA, IDH mutated tumours were allocated mostly to cluster 4. This cluster showed a hypermethylator phenotype. Interestingly, cluster 1 also showed hypermethylation but in this case this was directed by down-regulation of TET2 in a similar way to AML.10 Moreover, gene expression profile and methylome studies of CCA samples from mutated and wild type IDH patients, showed that half of the hypermethylated genes in CCA mIDH samples met with the ones found in glioblastoma which suggests a similar pathogenesis.52 The epigenetic consequences of hypermethylation in CpG islands (DNA) and methylation alterations in histones are the blocking of the cellular differentiation (since maturation of hepatic progenitor cells requires the expression of HNF4a) and stimulates stem cell proliferation.30 This could explain that poor grade of histological differentiation is a frequent event in the pathological reports of CCA samples with IDH mutations. In the same direction, in the TCGA classification, cluster 1 (IDH1 enriched) shows a hypermetlhylator phenotype in a similar way that AML does.50 This cluster presents a high mitochondrial gene expression (MGE) (electron chain transport and Krebs cycle), a high mitochondrial copy number and a low expression of the chromatin remodelling genes (such as ARID1A silencing). This anticorrelation between mitochondrial and chromatin remodelling pathways is a biological link along the TCGA and normal tissue. However, the association of IDH mutation and MGE seen in CCA does not occur other cancer (glioma, melanoma or AML).

In a recent publication comparing molecular profiles of HCC, CCA and PDAC tumour samples, it was described a new molecular subtype with DNA hypermethylation present among the three subtypes. In fact, some HCC with mutation in IDH1 show an atypical histology with common molecular signatures to CCA confirming the role of the IDH in the hepatic programme of differentiation.

The role of IDH mutation as a prognostic factor in CCA is still a matter of debate with retrospective studies showing opposite results.23,43 In a metaanalyses of 104 CCA patients, although there was a trend for longer OS and lower levels of Ca 19.9 in mIDH patients, this was not statistically significant.51 There was neither an association with other factors such as sex, histologic grade, age nor pattern of metastases.

Regarding diagnosis, although classical polymerase chain reaction (PCR) or immunohistochemistry (IHC) methods are possible, next generation sequencing (NGS) is the gold standard. FDA has very recently approved the Oncomine Dx Target Test (Thermo Fisher Scientific) to identify patients with IDH1-mutated cholangiocarcinoma. This NGS test can detect 23 genes in 1 potentially limited sample, which can minimise the risk of depleting tissues and requiring additional biospies. These results can be produced within 4 days. Getting enough and adequate tissue can be challenging in the clinics. Lamarca et al reported that up to one in four archived tissue samples were insufficient for genomic-analyses in the real life setting.52

Magnetic resonance spectroscopy has demonstrated utility for diagnosis mIDH gliomas. However, there are scarce data in CCA patients for this imaging approach. It has been reported that texture features on CT image in portal vein could predict IDH mutation in CCA with 72% and 88% of sensitivity and specificity respectively.53 Attending to the histology, mIDH tumours are histologically similar to wild type (wt) IDH tumours with the exception that mIDH tumours preferentially show cytoplasmic characteristics that may overlap with HCC.

Some authors have reported little impact in repeating a new biopsy at progression disease time after standard of care treatment. In a retrospective non-selected tumour study, this procedure identified new targetable biomarker for clinical trials in just 9% of patients. This suggests a limited evolution of the actionable genome.54

Following diagnosis of IDH mutations, circulating tumour (ct) DNA has been reported such an optimal tool for diagnosis with a very high correlation between tissue and plasma was 84%-92%.55-57 Elevation of 2HG was shown a valuable sensitive and specific marker (levels of 2HG over 170 ng/mL showed 83% and 90% respectively) for diagnosis of mIDH and also correlated with a high tumoral burden.22

Some authors have reported IDH mutation as a truncal step in the carcinogenesis process. However, others have reported that only in 20% of mutated IDH1 CCA patients, this mutation was already present in the primary tumor suggesting that it could represent a subclonal event instead of a truncal one.58 In a recent report of a retrospective analysis comparing CGP from primary biospies of IHCCA to the metastatic ones in 1268 samples, although many findings were common, some of them such as IDH1 mutations and FGFR2 fusion were significantly more frequent in the primary site.59 The authors concluded that this was suggestive of a wrong primary diagnosis in the metastatic samples.
4 | MEDICAL TREATMENT OF mIDH1 CCA PATIENTS

Publication of the results of the ClarIDHy, the first positive randomised trial for a targeted therapy (using the IDH1 inhibitor IVOSIDENIB in CCA patients with an IDH1 mutation), has opened the pathway not only for using this targeted therapy in a selected group of patients (very recently approved by FDA) but also for doing molecular testing in every advanced CCA patient candidate to systemic treatment, especially in intrahepatic CCA.

4.1 | IDH inhibitors: Ivosidenib

Direct IDH inhibition is the most exploited treatment option for mIDH CCA patients. The mechanism of action of IDH inhibitors consists of stabilising the mutated enzyme in its open conformation (inactivated) by binding tight to its allosteric site (hydrophobic interaction and hydrogen bridges) avoiding the transformation to its closed conformation (activated).39 Blocking the 2HG production, IDH inhibitors may restore appropriate methylation state, releasing again the cellular differentiation process.23 Most of these drugs are nonspecific for an isoform.

The first publications about preclinical and clinical data of IDH inhibitors were about glioma and AML tumours. Main biological effects in vitro consist of inducing the differentiation of progenitor cells and decreasing the stem cell markers and proliferation.60

AG5198 showed in preclinical models of glioma a reduction in 2HG,61 in histone methylation markers and in the cellular proliferation but an increase in differentiation markers expression.62 Issues with its pharmacodynamics led to modify the compound resulting in new compound called AG120 or ivosidenib.

AG120 is a first-in-class oral, once daily, oral, potent, selective, reversible, small-molecule inhibitor of the 5 R132 isoforms of IDH1. It shows a higher polarity, solubility, permeability and stability inside the liver microsomes. Interestingly, AG120 also inhibits the IDH wild type enzyme. AG120 has lower PXR (human pregnane X Receptor) activation and a less ratio of efflux.63 Induction of differentiation in AML cells and inhibition of migration/invasion in chondrosarcoma cells by AG120 was demonstrated in vitro.64

Based on the results of a phase 1 trial, ivosidenib was approved by FDA in relapse/refractory AML after demonstrating a CR of 25% with median duration of response (mDOR) of 8.2 months.65 Interestingly, after QTc prolongation (7.8%), the second most frequent treatment-related adverse effect (TRAEs) G3-4 was a differentiation syndrome. Furthermore, in a phase 1 (NCT02073994) of 168 mLIDH solid tumours (including CCA, chondrosarcoma and non-enhancing glioma patients), AG120 demonstrated a 98% reduction of 2HG levels leading to the ones of healthy people selecting 500 mg QD for the expansion phase. The drug also demonstrated a rapid oral absorption and a long half-life (40-120 hours).66 In the CCA subgroup patients (N 73), ivosidenib showed a good tolerability without any DLT and a TRAEs G3-4 rate of 5%. Regarding all grade TRAEs, asthenia (25%), nausea (19%) and diarrhoea (12%) were the three most common. In terms of efficacy the ORR was 5% and the DCR 56%. Median progression free survival (mPFS) and median overall survival (mOS) were 3.8 months and 13.8 months respectively. Interestingly, the rates of PFS at 6 and 12 months were 38.5% and 20.9% respectively.67

These results conducted to the Ph3, a pivotal international randomised, double-blind, placebo-control, ClarIDHy trial.15 In this landmark trial, 186 CCA patients with IDH1 mutation (centrally confirmed) and refractory to one or two previous lines of treatment, ECOG 0-1, and measurable disease, were 2:1 randomised to ivosidenib (500 mg OD) vs placebo in 28 days’ cycles.15 The primary endpoint was achieved with a mPFS (Independent central Radiology review) of 2.7 months in the ivosidenib arm vs 1.4 months in the placebo (HR, 0.37; 95% CI, 0.25-0.54; P < .0001). Although ORR was 2.4% for ivosidenib (0% for placebo), the SD rate was 51% vs 28% respectively. Interestingly, the PFS at 6 and 12 months in the ivosidenib arm were 32% and 21.9% respectively while there were no patients free of survival at these points in the placebo arm. This benefit was consistent in the subgroup analyses. The study design allowed the cross over at PD from the placebo arm to ivosidenib and this occurred in 70.5% of patients. This could explain why although the mOS was numerically longer in favour of ivosidenib arm (10.3 months vs 7.5 months respectively), the difference was not statistically significant (HR, 0.79; 95% CI, 0.56-1.12; P = 0.93). However, in a preplanned analysis called RPFST (Rank Preserving Failure Structural Test) which estimates what would have been the mOS in the placebo arm in case cross over had not occur, the mOS for the placebo was 5.1 months. In this case, the difference in OS was statistically significant (HR, 0.49; 95% CI 0.34–0.7, 0.001). Moreover, it has a nice tolerability profile. The most common G3-4 TEAEs (placebo vs ivosidenib) were ascites (6.8% vs 9%), anaemia (0% vs 7.2%), blood bilirubin increased (1.7% vs 5.4%). Interestingly, although TEAEs leading to dose reduction or interruption were more common in the ivosidenib arm (0% vs 3% and 18.6% vs 30.1% respectively), TEAS leading to discontinuation were more common in the placebo arm (6.6% vs 8.5%). In a postdoc analysis, ivosidenib has found to preserve physical, cognitive and emotional functioning and also to improve pain from baseline (QLC-Q30 questionnaire). These results have led to the very recent approval by FDA of ivosidenib for CCA patients with IDH1 mutation in PD to standard chemotherapy (Figure 3).

5 | NEW STRATEGIES FOR IDH PATIENTS

5.1 | Biomarkers

In parallel with the development of new treatments, the validation of novel biomarkers for predicting and monitoring response as well as for understanding the mechanisms of resistance are in progress too (Table 3).
5.1.1 Predicting response

Besides its value as a diagnostic tool as previously mentioned, ctDNA has been reported such a tool for predicting response. In a retrospective study of 31 patients treated with ivosidenib, lower basal variant allele frequency (VAF) respecting median VAF showed a trend in longer TTP with IDH inhibitors (HR 0.49, P 0.1). In a ClarIDHy postdoc subanalyses, VAF was also longitudinal analysed (using Beaming dPCR) and clearance was considered when VAF was <0.02% (R132C / L / S / G) and <0.04% (R132H). There were 27% of patients with clearance achievement between that whit PFS >2.7 months. However, there were no patients in the subgroup with PFS<2.7 months neither in the placebo subgroup. Other posthoc analyses have elucidated ivosidenib as a differentiation-based therapy. Paired biopsies acquired both baseline and on-treatment showed that in patients with prolonged PFS, mIDH1 inhibition leads to decreased cytoplasm and expression of hepatocyte lineage markers along with down-regulation of biliary fate, cell cycle progression and AKT pathway activity. Monitoring of HG levels has also been described as a pharmacodynamic marker of target inhibition in other tumours. However, this has not been yet clearly related.

5.1.2 Monitoring response

Monitoring of response to IDH inhibitors is feasible. In the mentioned study of Lapin et al, it was shown that a decreasing of VAF respecting to non-decreasing had a trend for longer overall survival (p0.06) but not for TTP (P 0.4). In a ClarIDHy postdoc subanalyses, VAF was also longitudinal analysed (using Beaming dPCR) and clearance was considered when VAF was <0.02% (R132C / L / S / G) and <0.04% (R132H). There were 27% of patients with clearance achievement between that whit PFS>2.7 months. However, there were no patients in the subgroup with PFS<2.7 months neither in the placebo subgroup. Other posthoc analyses have elucidated ivosidenib as a differentiation-based therapy. Paired biopsies acquired both baseline and on-treatment showed that in patients with prolonged PFS, mIDH1 inhibition leads to decreased cytoplasm and expression of hepatocyte lineage markers along with down-regulation of biliary fate, cell cycle progression and AKT pathway activity. Monitoring of HG levels has also been described as a pharmacodynamic marker of target inhibition in other tumours. However, this has not been yet clearly related.
to antitumor activity in CCA. As we mentioned before, magnetic resonance spectroscopy for glioma spectroscopy can also show monitor 2HG levels.\(^5\)

### 5.1.3 | Mechanisms of resistance

Resistance mechanisms to IDH1 inhibitors have been first described in AML. In a retrospective molecular profiling for AML patients treated with ivosidenib, 14% developed a 2nd site mutation in IDH1 (S280F the most frequent). Interestingly, 12% developed a first site mutation in IDH2 (R140Q).\(^6\) These mutations avoid the binding of ivosidenib and increase 2HG.\(^7\)

Regarding to CCA, in the Ph1 trial, 59% of patients underwent paired pretreatment and post-progression tumor sequencing and new mutation were found in 6 of them (IDH2-R172V, IDH-R132F, and in other gens (TP53, ARID1A, POLE, PIK3R and TXB3)). Harding et reported a case of a CCA IDH1m patient who after achieving a partial response to ivosidenib (decrease of tumoral mutation burden of 50%), developed an IDH2 mutation (R172V).\(^8\) In the study reported by Lapin, it was found a median of 1-5 new alterations by each patient, being TP53 and ARID1 the newest frequent found but no new isoforms of IDH were present.\(^9\) Primary resistance mechanism is not known.

## 5.2 | New IDH Inhibitors

In an attempt to improve results of ivosidenib, there are new IDH inhibitors in development in CCA (Table 4).

### 5.2.1 | IDH305

This drug inhibits two isoforms of IDH1 (R132H and R132C) and has demonstrated a high penetrance in HEB and a low clearance by the liver microsomes.\(^10\) A Ph1 trial is ongoing that includes mIDH1 CCA patients (NCT02381866).

### 5.2.2 | LY3410738

LY3410738 is a novel first-in-class inhibitor that achieves a covalent and irreversible IDH inhibition and is considered a second generation IDH inhibitor. It does not only inhibit R132H and R132C with high potency (IC50 4.16 nM and 2.35 nM respectively) but also inhibits its resistance mutations, such as IDH1m new isoforms (like S280F) or new primary IDH2 mutation (R140Q and R172K).\(^11\) LY3410738 is now being tested in a phase 1/2 clinical trial for mIDH solid tumours including CCA, both in monotherapy (refractory patients) and in combination with cisplatin-gemcitabine (first line setting) (NCT04521686).

### 5.2.3 | FT 2102 (Olutasidenib)

This drug inhibits 2 isoforms of IDH1 (R132H and R132C) by its binding to the isocitrate pocket avoiding the conformational change to the activated state. It has shown in xenografts a good oral bioavailability and high penetrance of HEB achieving a decrease in 2HG.\(^8\) Olutasidenib is being tested in a Ph1/2 clinical trial that includes CCA patients both in monotherapy or in combination with cisplatin-gemcitabine or with nivolumab (NCT03684811).

### 5.2.4 | BAY1436032

This pan-mutant IDH1 inhibitor inhibits the 5 R132 isoforms of IDH1 and was selected after screening of more than 30 million compounds. BAY143602 demonstrated activity both in preclinical models of glioma and AML by reducing 2HG and increasing overall survival although without decreasing tumour size.\(^8\) This efficacy was also clinically in a phase 1/2 of AML patients and it is now being tested in a phase 1 trial of solid mIDH1 tumours (NCT02746081).

### 5.2.5 | AG-881 (Vorasidenib)

This is a first-in-class pan inhibitor of IDH (IDH1 and IDH2). In preclinical models of AML, AG881 is able to induce myeloid differentiation of blasts.\(^8\) This dual inhibition could represent an opportunity to face the mentioned switch of isoform mutation at resistance. A very recent publication of the LGG cohort of a phase 1 (NCT02492737) for mutant IDH1/2 solid tumours (including CCA), vorasidenib was well tolerated (DLT of elevated transaminases occurred at doses ≥100 mg) and showed preliminary antitumor activity (response rate by RANO of 18%).\(^8\) HMPL-306 is another pan inhibitor of IDH and it is also being tested in a phase 1 trial that includes mIDH CCA patients (NCT04762602).

## 5.3 | Other vulnerabilities for IDH mutated CCA

As we have previously mentioned, IDH1 mutant CCA presents other potential vulnerabilities that could be exploited besides IDH1 inhibitors. We summarise some of the strategies in development (Table 4).

### 5.3.1 | IDH2 inhibitors

Although IDH2 inhibitors have been developed in AML, we already have described that around 4% of IHCCA patient harbour an IDH2 mutation. Also, new IDH2 mutations are a secondary resistance mechanism in IDH1m patients treated with IDH inhibitors.
| Drug                        | Mechanism of action | Phase | N | Population                                                                 | Prior IDH inhibitors allowed | Endpoint       | Identification   | Stimulated study completion date |
|-----------------------------|---------------------|-------|---|-----------------------------------------------------------------------------|------------------------------|----------------|------------------|-----------------------------|
| HMPL-306                    | iIDH1/IDH2          | 1 (Esc/Exp) | 90 | mIDH solid refractory tumours (CCA included)                              | Yes                          | MTD/             | NCT04762602      | Jan 23                      |
| Olaparib + Durvalumab       | iPARP+ iPD1         | 2     | 78 | mIDH solid tumours (Cohort B: CCA, maximum 2 prior lines of chemotherapy)  | Yes                          | ORR/             | NCT03991831      | Sep 22                      |
| IDH-305                     | iIDH1               | 1     | 166| mIDH1 (R132) solid tumours (CCA included)                                  | No                           | MTD/             | NCT02381886      | Oct 24                      |
| FT2102 ± Nivolumab ± Cis-gem| iIDH1 iPDL1 QT     | 1b/2  | 200| mIDH1 (R132) solid tumours Cohort 2a/2b (CCA) Cohort 4a/4b (CCA) FT2102 (Monotherapy): Refractory FT2102 + Nivolumab and FT2102+ cis-Gem (received <2 cycles of cis-gem) | No (monotherapy cohort) Yes (in combination) | Ph1b: DLT Ph2: ORR | NCT0368411       | Sep 21                      |
| AG881                       | iIDH1/2             | 1 (Esc/Exp) | 95 | mIDH1/2 solid tumours (CCA included)                                       | Yes                          | MTD/             | NCT02481154 “Mellinghoff” | Dec 21                      |
| Ivosidenib + Nivolumab      | iIDH1 + iPD1        | 2     | 35 | mIDH1 solid tumours (CCA included)                                         | No                           | ORR/ PFS6m       | NCT04056910      | Dec 22                      |
| Olaparib                    | iPARP               | 2     | 145| mIDH1/2 solid tumours (CCA included)                                       | Yes                          | ORR              | NCT03212274      | Jul 22                      |
| LY3410738 ± Cis-Gem         | iIDH1 QT            | 1 (Esc/Exp) | 180| mIDH1 (R132) solid tumours (CCA included) LY3410738 (Monotherapy): refractory CCA LY3410738 + Cis-Gem (CCA 1st L) | Yes (just in the escalation cohort) | DLT/             | NCT04521686      | Feb 23                      |
AG221 (Enasidenib), an oral reversible inhibitor of IDH2, demonstrated in AML xerograph a reduction in 2HG (plasma, urine and bone marrow). In a phase 1 trial of refractory/relapsed AML with IDH2 mutation, patients were treated with enasidenib (100 mg QED) achieving an ORR of 43% (complete response in 19%) and a mod of 9.3 months. This led to the FDA approval in 2017. A phase 1 (NCT02273739) is testing AG221 in R140 and R172 IDH2 mutated T angioimmunoblastic lymphomas and solid tumours, including CCA patients (4 of the 21 patients). Enrollment was closed before entry into the expansion cohort due to non-clinical issues; there were no safety concerns.

5.3.2 | Dasatinib

In an in vitro study using multiple cell lines (17 of CCA) that tested the efficacy of 122 FDA approved drugs, the multikinase inhibitor dasatinib was the most potent inhibitor of mIDH CCA cell lines. Rapid activation of apoptosis, by cleavage of Caspase-3 by this drug, only occurred in IDH mutant cells. Although dasatinib inhibits more than 40 kinases, a mechanistic analysis revealed that SRC was the key target showing that mIDH has a critical dependence of SCR for survival and survivorship. In a phase 2 clinical trial (NCT02428855) of 8 mIDH CCA patients treated with dasatinib were no PR and the mPFS was 8.7 weeks and a mOS of 37.9 weeks. Lymphopenia was the most frequent SAE (50%).

5.3.3 | Chemotherapy association

Due to the mainly cytostatic mechanism of action of IDH inhibitors and aiming to increase the ORR there is phase 1 trial on going combining ivosidenib with systemic chemotherapy (cisplatin – gemcitabine) in the first line setting in CCA IDH1m patients (NCT04088188).

5.3.4 | Immunotherapy

As we have mentioned, mIDH CCA seems to be an immune cold tumour. In theory, inhibition of mIDH could reverse this immune state and make the tumour more sensible to immune checkpoint inhibitors. In fact, in a posthoc analyses of the phase 1 trial previously mentioned of ivosidenib, it was shown that in the subgroup of patients with a cytoplasm decrease after treatment there was also upregulation of several immune responses –related genes such as CTLA4, CXCL10 and CD3G. Moreover, in a mouse model of IHCCA with IDH1 mutation treated with ivosidenib it was detected an enhanced immune response to ivosidenib with an increase of recruitment of CD8-positive T-cells to the tumours and induction of immune stimulatory interferon signalling (PD-L1 expression was also upregulated). In such a way, there is a strong preclinical rationale for combination treatment strategies with ivosidenib. A ph1 trial is evaluating the combination of ivosidenib with a PD1 inhibitor (nivolumab) in IDH1m solid tumours (NCT04056910).

5.3.5 | DNA damage response (DDR)

As we mentioned before, DDR alterations have been described as associated with IDH mutations due to their effect on aKG dioxygenases. This rational has led to several trials with PARP inhibitor such as olaparib in monotherapy (NCT03212274). A phase 2 ongoing study combining olaparib (PARP inhibitor) with durvalumab (PD1 inhibitor) includes one cohort of patients with mIDH CCA (NCT03991832). Other phase 2 trial is combining olaparib with the ceralasertib (ATR inhibitor) in mIDH solid tumours (NCT038780950). Recent findings, showing mIDH CCA do not have higher LOH, do not suggest that this way is being very successful.

5.3.6 | Bromodomain and extraterminal domain (BET) inhibitors

BET proteins are considered essential “chromatin readers”. It has been described in preclinical models of CCA cell lines that only mIDH1 (RBE) is sensible to JQ1 (a BET inhibitor) by blocking growth tumour and apoptosis induction. These effects do not occur in IDH WT cells (HuCCT1). Upregulation of BAX and BIM (pro-apoptotic genes) by H3K4me3 of their respective promoter regions following treatment with JQ1 seem to be the mechanism.

5.3.7 | Hypomethylator agents

Reversion of the hypermethylator phenotype is a slow process. This has opened a rational for combining IDH inhibitors with epigenetic therapy such as hypomethylating agents (HMA). A phase ½ trial is testing a combination of FT-2102 with the HMA azacitadine (NCT03212274). A phase 2 ongoing study combining olaparib (PARP inhibitor) with durvalumab (PD1 inhibitor) includes one cohort of patients with mIDH CCA (NCT03991832). Other phase 2 trial is combining olaparib with the ceralasertib (ATR inhibitor) in mIDH solid tumours (NCT038780950). Recent findings, showing mIDH CCA do not have higher LOH, do not suggest that this way is being very successful.

5.3.8 | Targeting wild type IDH

Although we have focused this review on targeting mutated IDH1, it has been described that aberrant expression of the wild type of IDH generates cellular proliferation, invasion and migration. In fact, this was previously reported for different types of tumour (SCLC, NSCLC, PDAC, primary GBM) linked to a worse prognosis and resistance to treatment. This makes IDH wild type a potential target. In fact, some previously mentioned drugs such as ivosidenib and a new class of inhibitors called tetrahydro-pyrazolopyridine (GSK321 and GSK864) also inhibit IDH1 wild type. This could open a new option treatment to explore in the future.
5.3.9 | IDH-like tumours

High intratumoral heterogeneity (ITH) has been described in many tumours including CCA patients. In a recent study of 73 tumour samples obtained from 14 CCA patients, it was shown high concordance of heterogeneity across genomic, transcriptomic and immune level for each patient. Remarkably, it was found that high intratumoral heterogeneity CCAs overlapped with the IDH subgroup of the TCGA. This subgroup included IDH mutated tumours but also IDH-like tumours, which are those IDH WT but with a similar profile to the mutated. In these cases, IDH WT patients but with high ITH were reclassified as IDH-like tumours. Regarding prognosis, it is interesting that mutations in IDH did not impact OS. However, it did the molecular subtype attending ITH; IDH subgroup patients had worse prognosis than the non-IDH subgroup. Furthermore, regarding immune status, the IDH subgroup (and in parallel high ITH tumours) showed a colder tumour microenvironment (TME) with fewer CD8+ and more neutrophils ($P < .01$). These results suggest that single region sequencing could not be enough to evaluate the molecular profile of a tumour.85

5.3.10 | Radiotherapy

It has been reported in preclinical models that the REDOX misbalance due to IDH mutation could potentially make these cells more sensible to ionizing agents like radiation. More research in this field for CCA could bring new options.

6 | CONCLUSIONS

Molecular profiling by NGS for advanced BTC patients, specifically IHCCA, has become a new standard of care. Chemotherapy is still the reference treatment for the first line setting. However, given that almost half of the IHCCA harbour a potentially targetable molecular alteration, new treatment agents are looking for its role in specific subtypes of patients. Although there is a chance to find BRAF V600E, NTRK fusions, HER2 amplification or dMMR / MSI-H with the consequent specific treatment option, IDH1 mutations and FGFR2 are the two most important target because of its higher frequency and grade of clinical development. FGFR2 fusions or rearrangements are present in about 10% of IHCCA patients and FGFR inhibitors have shown an impressing clinical activity (response rate about 30% and mPFS of 7 months). Pemigatinib and infigratinib have received accelerated approval by FDA in the second line setting for CCA patients harbouring FGFR2 rearrangement or fusions based on phase 2 non-randomised clinical trials. There is now a plethora of FGFR inhibitors trying to show their efficacy in different phases of investigation event in the first line setting with phase III trials against the SOC with cisplatin-gemcitabine. For IDH1 mutations, although present in a higher percentage of IHCCA patients (13%), IDH inhibitors have not found so far the same grade of efficacy in terms of response rate (around 2%-3%). Although Ivosidenib has been FDA approved very recently for mIDH1 CCA patients as a result of a small absolute benefit in terms of PFS (4.4 months), this has been statistically significant in a very first randomised clinical trial with an increment of 22% of patients free of disease progression at one year (0% in the placebo arm). On the other hand, new standard of care with FOLFOX in the second line setting in a non-selective biomarker population achieved similar results in terms of efficacy. These results together with financial toxicity, have make some authors argue against ivosidenib. However, comparison is biased because of the still not well-known prognostic value for the IDH patients. Also, toxicity profile looks better for ivosidenib than for chemotherapy in a population that usually needs a good tolerability profile. Moreover, chemotherapy could be still a reasonable option.
after progression to ivosidenib in patients with an acceptable ECOG although ABC06 was in the second line setting. The still in progress knowledge about IDH mutation pathogenesis is probably one of the reasons to explain the slower progress in drug development for this subgroup of patients. Doubtless, there is much space to improve outcomes of CCA with IDH1 mutation. Rapid advances in molecular technology and translational and international collaborative work are making possible that new IDH inhibitors, new combination strategies and new vulnerability opportunities are already ongoing and hopefully will be soon incorporated in the armamentarium to improve quantity and quality of life of this high need group of patients (Figure 4).

DATA AVAILABILITY STATEMENT
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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