The implications of *IDH* mutations for cancer development and therapy

Christopher J. Pirozzi[1,2] ✉ *and Hai Yan*[1,2] ✉

Abstract | Mutations in the genes encoding the cytoplasmic and mitochondrial forms of isocitrate dehydrogenase (*IDH1* and *IDH2*, respectively; collectively referred to as *IDH*) are frequently detected in cancers of various origins, including but not limited to acute myeloid leukaemia (20%), cholangiocarcinoma (20%), chondrosarcoma (80%) and glioma (80%). In all cases, neomorphic activity of the mutated enzyme leads to production of the oncometabolite D-2-hydroxyglutarate, which has profound cell-autonomous and non-cell-autonomous effects. The broad effects of *IDH* mutations on epigenetic, differentiation and metabolic programmes, together with their high prevalence across a variety of cancer types, early presence in tumorigenesis and uniform expression in tumour cells, make mutant IDH an ideal therapeutic target. Herein, we describe the current biological understanding of *IDH* mutations and the roles of mutant IDH in the various associated cancers. We also present the available preclinical and clinical data on various methods of targeting *IDH*-mutant cancers and discuss, based on the underlying pathogenesis of different *IDH*-mutated cancer types, whether the treatment approaches will converge or be context dependent.

Recurrent mutations in the isocitrate dehydrogenase gene *IDH1* were first identified in a whole-exome sequencing study of 22 glioblastomas[1]. Subsequent studies revealed that mutations in *IDH1* or its paralogue *IDH2* (collectively referred to as *IDH*) are prevalent in various types of cancer, including low-grade glioma and secondary glioblastoma (80%)[2,3], acute myeloid leukaemia (AML; 20%)[4–6], cholangiocarcinoma (20%)[7,8], chondrosarcoma (80%)[9], sinonasal undifferentiated carcinoma (49–82%)[10–12] and angioimmunoblastic T cell lymphoma (32%)[13,14], among others (FIG. 1a), thereby solidifying a key pathogenetic role for such mutations. *IDH* mutations result in single amino acid substitutions predominantly affecting the arginine 132 residue (R132) in IDH1, the analogous residue arginine 172 (R172) of IDH2 or arginine 140 (R140) in IDH2, making these mutational hotspots.

Herein, we describe the contributions and effects of *IDH* mutations in AML, cholangiocarcinoma, chondrosarcoma and low-grade glioma. As hotspot mutations that occur early in tumorigenesis with uniform and specific expression in tumour cells[15,16], *IDH* mutations constitute appealing therapeutic targets. To this end, small-molecule inhibitors of mutant IDH, mutant IDH-directed immunotherapies and agents targeting mutant IDH-induced metabolic liabilities are active areas of research and the focus of clinical trials in patients with *IDH*-mutant cancers (TABLE 1; Supplementary Tables 1, 2).

[1]*Department of Pathology, Duke University Medical Center, Durham, NC, USA.*

[2]*Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, NC, USA.*

✉*e-mail: christopher.pirozzi@duke.edu; hai.yan@duke.edu*

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The biology of *IDH* mutations in cancer

IDH1 is localized in the cytosol and IDH2 in the mitochondria, although both isozymes catalyse the reversible oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) while reducing $NADP^+$ to NADPH. Biochemical analyses have identified a neomorphic activity of the mutant IDH enzymes, specifically, conversion of α-KG into the oncometabolite D-2-hydroxyglutarate (D-2HG) in a process that consumes rather than produces NADPH and instead generates $NADP^+$ (REFS[17–19]). A series of in vitro genetic and crystallographic studies revealed that maintenance of the heterozygous state is required for D-2HG production[18,20–22]. Indeed, *IDH* mutations are almost always heterozygous, although rare cases of homozygosity have been reported[23,24]. Crystallographic analyses have also revealed that wild-type IDH proteins form homodimers that can transition between an inactive open state, an inactive semi-open state and a catalytically active closed conformation. The presence of a mutant IDH subunit in the enzymatic complex favours the closed conformation and confers a high affinity for NADPH, with subsequent reduction of α-KG to D-2HG[19,25]. *IDH*-mutant cancers with loss of heterozygosity (LOH) during disease progression contain mutant IDH homodimers in the inactive open conformation[25], leading to decreased D-2HG levels and highlighting the importance of a balanced ratio of wild-type and mutant alleles for D-2HG production[22,26,27].

Key points

- Mutations in *IDH1* or *IDH2* are frequent among several cancer types with various tissues of origin; the resultant mutated enzymes have neomorphic activity that leads to production of the oncometabolite D-2-hydroxyglutarate (D-2HG), which has profound effects on cellular epigenetic programmes, differentiation patterns and metabolic profiles.
- The high prevalence of the *IDH* hotspot mutations, their occurrence early in tumorigenesis and the resulting uniform expression of the mutated protein in tumour cells make mutant isocitrate dehydrogenase (IDH) an appealing therapeutic target.
- The roles of mutant IDH1 and IDH2 in cancer development and progression are probably transient or dynamic and context dependent.
- *IDH* mutation status at disease recurrence can provide insights into their overall pathogenic role. In acute myeloid leukaemia, resistance mutations that restore the generation of D-2HG arise in response to inhibition of mutant IDH1 or IDH2, whereas recurrent gliomas often have a loss of heterozygosity of the affected *IDH* gene and decreased D-2HG production.
- The greater efficacy of mutant IDH inhibitors against non-enhancing gliomas suggests that the timing of treatment with such agents is of crucial importance.

The precise mechanism underlying the pathogenic role of *IDH* mutations in cancer remains unclear, although much has been learned regarding their biological effects. Many of these effects are thought to reflect structural similarities between D-2HG and α-KG, with the sole difference being the oxidation state of the carbon-2 position (FIG. 1b). Consequently, this structural similarity results in competitive inhibition, especially among the large family of α-KG-dependent dioxygenases, which number upwards of 70 (REFS[28–32]). Thus, pathways that utilize α-KG as a substrate are perturbed in *IDH*-mutant cancers, leading to epigenetic dysregulation with aberrant histone and DNA methylation, chromatin restructuring, blocking of cellular differentiation and other transformative effects[33–38].

A hypermethylated state is a consistently observed phenotype among many *IDH*-mutant cancers[38–40]. This epigenetic state can be phenocopied upon expression of mutant IDH in a variety of cell types, including primary human astrocytes[33] and mouse bone marrow cells[38]. In an analysis of 272 glioblastoma samples from The Cancer Genome Atlas, a distinct subset was identified that had a DNA hypermethylation phenotype, referred to as the glioma CpG island methylator phenotype (G-CIMP)[40]. One well-studied mechanism contributing to the hypermethylated state involves a class of α-KG-dependent dioxygenases, the TET family of methylcytosine hydroxylases, which promote DNA demethylation via conversion of 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC)[38]. Specifically, the competitive inhibition of TET enzymes by D-2HG induces a hypermethylated state with low levels of 5hmC[33].

DNA methylation, which predominantly occurs at CpG islands, has variable effects on gene expression depending on the balance between activating histone H3 lysine 4 trimethylation (H3K4me3) and repressive histone H3 lysine 27 trimethylation (H3K27me3). Indeed, paired RNA sequencing and methylation analyses have revealed the major contribution of changes in H3K27me3 dynamics, which probably reflects the D-2HG-mediated inhibition of α-KG-dependent histone demethylases, to the transcriptional alterations of glioma cells[41]. In this study, the genes found to be most commonly dysregulated in glioma cells were the same genes that had bivalent H3K27me3 and H3K4me3 modifications in embryonic stem cells and neural progenitor cells[41]. In the glioma cells, CpG hypermethylation was found to be present in genes also repressed in non-malignant brain cells. This finding suggests that further repression induced by mutant IDH via increased H3K27me3 limits epigenetic plasticity, thereby reinforcing the epigenetic state of glioma cells, restricting their ability to differentiate and contributing to pathogenesis.

Mutant IDH-mediated epigenetic dysregulation with subsequent effects on differentiation states can be observed in several cell types. For example, *IDH*-mutant mouse hepatoblasts fail to differentiate into hepatocytes owing to D-2HG-mediated silencing of the master transcriptional regulator HNF4α, which correlates with reduced H3K4me3 at the hepatocyte-specific promoter region of *Hnf4a*[42]. In the mouse pre-adipocyte 3T3-L1 cell line, expression of mutant IDH causes a defect in the adipogenesis programme via downregulation of several transcription factors, including those encoded by *Cebpa, Pparg* and *Adipoq*[34]. Interestingly, mutant IDH does not affect DNA methylation at the promoters of these genes but does increase H3K9me3 and H3K27me3 levels[34]. Notably, short interfering RNA-mediated knockdown of the α-KG-dependent, H3K9me3-specific demethylase KDM4C recapitulates this block to adipocyte differentiation[34]. Similarly, expression of IDH1-R132C in human mesenchymal stem cells results in increased levels of H3K9me3 and H3K27me3 as well as H3K4me3, in association with upregulation of several early and late markers of chondrogenic differentiation and downregulation of osteogenic markers[43]. The differential effects on these markers reflect gene-specific histone modifications (activating H3K4me3 versus repressive H3K9me3)[43]. These findings might explain why IDH mutations are prevalent in chondrosarcomas but not in osteosarcomas. Together, the results of these studies implicate histone methylation defects in mutant *IDH*-mediated impairments in cellular differentiation.

The additional cellular effects of mutant IDH result from disrupted NADPH production. By consuming rather than generating NADPH, mutant IDH causes metabolic reprogramming that results in dysregulation of gene expression, DNA damage repair, inflammation, intracellular trafficking, ageing and cell death[44–46]. In particular, evidence from several studies indicates that a low basal level of NAD^+ in *IDH1*-mutated cells confers a potential therapeutic liability that can be exploited using various drugs, including temozolomide and poly(ADP-ribose) polymerase (PARP) inhibitors[17,44–49]. Indeed, the greater chemosensitivity and radiosensitivity of *IDH*-mutant tumours relative to their *IDH*-wild-type counterparts confers a better patient prognosis[48,50,51]. The reduction in NAD^+ levels in *IDH1*-mutant cells is attributed to decreased expression of the NAD^+ salvage pathway enzyme nicotinate phosphoribosyltransferase (NAPRT1)[46]. In *IDH1*-mutant tumour xenograft models, this vulnerability is further compounded by inhibition of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD^+ synthesis pathway,

resulting in decreased tumour growth and prolonged survival[46]. These findings contributed to the basis for targeting NAD^+-dependent pathways in *IDH*-mutant tumours. The DNA damage response is one such pathway, given that PARP utilizes NAD^+ to generate poly(ADP-ribose) (PAR) chains that coordinate this process. This pathway is further implicated by the observation of an increased abundance of unrepaired DNA double-strand breaks following ionizing radiation specifically in cell lines expressing mutant IDH[52]. This effect was found to result from D-2HG-mediated suppression of homologous recombination[52] via inhibition of the histone demethylase KDM4B, which leads to increased H3K9me3 and thereby disrupts DNA damage signalling[53]. Moreover, *IDH*-mutant cells can also have increased H3K9me3 and downregulation of the gene encoding the DNA damage sensor ATM, resulting in an impaired DNA damage response[54]. The discovery of the homologous recombination deficiency associated with *IDH* mutations led to the screening of a panel of DNA repair pathway inhibitors; *IDH1*-mutant cells were found to have a 45-fold increase in sensitivity to the

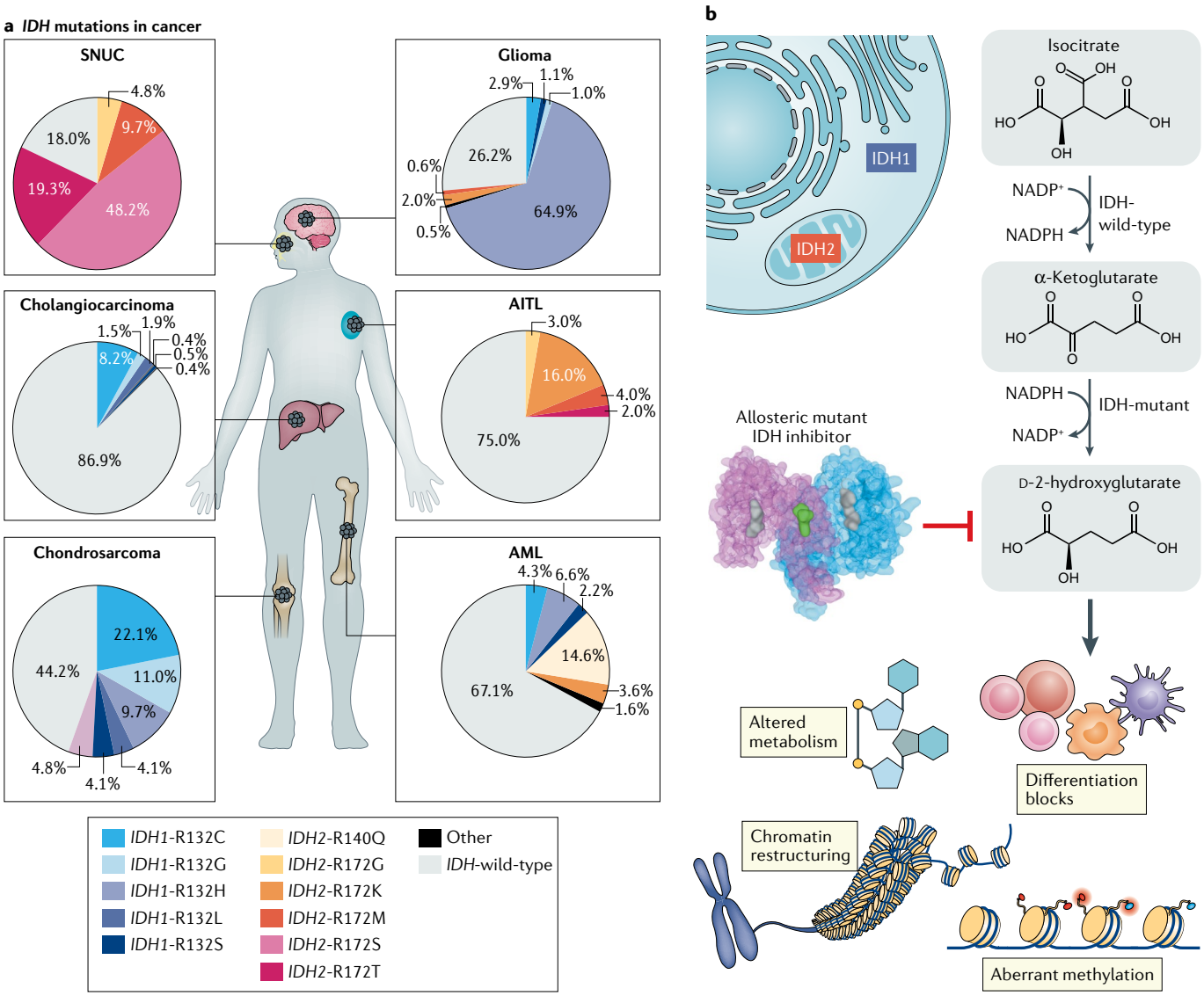

Fig. 1 | **Prevalence and function of *IDH* mutations in cancers. a** | The isocitrate dehydrogenase genes (*IDH1* and *IDH2*) are mutated at varying frequencies in several cancers, including angioimmunoblastic T cell lymphoma (AITL)[13,14], acute myeloid leukaemia (AML)[4–6], cholangiocarcinoma[7,8], chondrosarcoma[9], low-grade glioma[2,3] and sinonasal undifferentiated carcinoma (SNUC)[10–12], among others. The prevalence of various *IDH* mutations in each of these cancer types is indicated in the pie charts. **b** | The *IDH* genes encode the metabolic enzymes IDH1 (which is localized to the cytosol) and IDH2 (which is localized to mitochondria). Both IDH1 and IDH2 normally catalyse the oxidative decarboxylation of isocitrate to α-ketoglutarate; however, the hotspot mutations at R132 in IDH1 and R140 or R172 in IDH2 lead to neomorphic enzymatic activity that results in overproduction of D-2-hydroxyglutarate. This oncometabolite has broad effects on cellular biology, including altered metabolism, aberrant DNA and histone methylation, chromatin restructuring and blocks to normal differentiation patterns. Various approaches to therapeutically target *IDH*-mutant cancer cells are currently being investigated in clinical trials (TABLE 1; Supplementary Tables 1, 2), including the use of direct allosteric inhibitors of mutant IDH1 and/or IDH2. The crystal structure of IDH1 bound to the dual IDH1 and IDH2 inhibitor vorasidenib (also known as AG-881) was generated using RCSB PDB 6ADG[57,58].

Table 1 | **Results of trials of IDH-targeted therapies for *IDH*-mutant cancers reported to date**

Population	Treatment	Study phase	Efficacy results	Most common grade ≥3 TRAEs	Ref.
27 patients with *IDH1*-mutant AML	BAY-1436032	I	ORR 14.8%; median OS 6.6 months	Fatigue (3.7%); differentiation syndrome (3.7%)	96
258 patients with *IDH1*-mutant haematological malignancies, including 242 with AML, of whom 179 had R/R AML	Ivosidenib	I	ORR 41.6%; CR rate 21.6%; median OS 8.8 months[a]	QT prolongation (7.8%); differentiation syndrome (3.9%); anaemia (2.2%); thrombocytopenia (3.4%); leukocytosis (1.7%)[b]	92
23 patients with newly diagnosed *IDH1*-mutant AML ineligible for intensive induction chemotherapy	Ivosidenib + azacitidine	Ib/II	ORR 78.3%; CR rate 60.9%; estimated 12-month OS 82.0%	Neutropenia (21.7%); anaemia (13.0%); QT prolongation (13.0%); leukocytosis (8.7%); differentiation syndrome (8.7%)	105
17 patients with *IDH1*-mutant AML and 2 patients with *IDH1*-mutant high-risk MDS	Ivosidenib and venetoclax ± azacitidine	Ib/II	ORR 88.9%; CR rate 38.9%[c] Among 9 patients with R/R AML: median OS 9.7 months	Differentiation syndrome (5.3%); tumour lysis syndrome (5.3%)	104
Patients with newly diagnosed *IDH1*-mutant AML ($n = 60$) or *IDH2*-mutant AML ($n = 91$)	Ivosidenib or enasidenib with induction and consolidation therapy	I	CR rate 55.0% (ivosidenib) and 47.3% (enasidenib)	TRAEs not defined; however, grade ≥3 differentiation syndrome occurred in 2.0% of patients overall; grade ≥3 QT prolongation occurred in 2.9–10.0%	169
345 patients with *IDH2*-mutant haematological malignancies, predominantly R/R AML ($n = 280$) or high-risk MDS ($n = 17$)	Enasidenib	I/II	ORR 38.8%; CR rate 19.6%; median EFS 4.7 months; median OS 8.8 months[d]	Hyperbilirubinaemia (10.0%); thrombocytopenia (7.0%); differentiation syndrome (6.0%)	86
21 patients with *IDH1*-mutant R/R AML and 3 patients with *IDH1*-mutant high-risk MDS	IDH305	I	In patients with AML: ORR 33.3%; CR rate 9.5%	Increased serum bilirubin (4.2%)	97
35 patients with *IDH1*-mutant R/R AML or MDS	Olutasidenib ± azacitidine	I/II	Olutasidenib monotherapy ($n = 16$): CR rate 12.5%. Combination therapy ($n = 11$): CR rate 18.2%	Across monotherapy and combination groups: febrile neutropenia (22.9%); anaemia (20.0%); pneumonia (17.1%); differentiation syndrome (14.3%)	170
73 patients with previously treated advanced-stage *IDH1*-mutant cholangiocarcinoma	Ivosidenib	I	ORR 5.5%; SD rate 56.2%; median PFS 3.8 months; 6-month PFS 40.1%; 12-month PFS 21.8%; median OS 13.8 months	Fatigue (2.7%); decreased serum phosphorus (1.4%); increased serum alkaline phosphatase (1.4%)	117
185 patients with advanced-stage *IDH1*-mutant cholangiocarcinoma after ≤2 prior lines of treatment	Ivosidenib ($n = 124$) vs placebo ($n = 61$)	III	ORR 2.4% vs 0%; SD rate 50.8% vs 27.9%; median PFS 2.7 months vs 1.4 months (HR 0.37, 95% CI 0.25–0.54; $P < 0.0001$); 6-month PFS 32% vs 0%; 12-month PFS 22% vs 0%; median OS 10.8 months vs 9.7 months (HR 0.69, 95% CI 0.44–1.10; $P = 0.06$)	Hypophosphataemia (1.7%); fatigue (1.7%); anaemia (0.8%)	118
21 patients with advanced-stage *IDH1*-mutant chondrosarcoma	Ivosidenib	I	SD rate 52.4%; median PFS 5.6 months; 6-month PFS 39.5%	Hypophosphataemia (4.8%)	130
66 patients with *IDH1*-mutant glioma that had recurred after or not responded to initial surgery, radiation or chemotherapy	Ivosidenib	I	ORR 2.9% among 35 patients with non-enhancing gliomas and 0% in those with enhancing lesions; SD rate 66.7% overall; median PFS 13.6 months and 1.4 months in patients with non-enhancing and those with enhancing gliomas, respectively	2 patients had grade ≥3 TRAEs (neutropenia, decreased weight, hyponatraemia and/or arthralgia); grade ≥3 treatment-emergent events in the dose-expansion cohort included seizure (4.0%), hypophosphataemia (4.0%), headache (2.0%) and hyperglycaemia (2.0%)	156
33 patients with *IDH1*-R132H-mutant newly diagnosed WHO grade III or IV astrocytoma	20-mer IDH1-R132H peptide vaccine	I	ORR (stable disease) 84.4%; 63% free from progression at 3 years; 84% alive at 3 years	None	164

AML, acute myeloid leukaemia; CR, complete remission/response; EFS, event-free survival; MDS, myelodysplastic syndrome; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; R/R relapsed and/or refractory; SD, stable disease; TRAEs, treatment-related adverse events. [a]In the primary efficacy population comprising 125 patients with R/R disease receiving 500 mg of ivosidenib daily with at least 6 months of follow-up data. [b]Among 179 patients with R/R disease. [c]In 18 evaluable patients across three treatment groups. [d]Among 214 patients with R/R AML receiving 100 mg of enasidenib daily.

PARP inhibitor olaparib relative to *IDH*-wild-type cells[52]. Several studies have sought alternative mechanisms to further deplete NAD^+ and exploit this vulnerability of *IDH*-mutant cells. These approaches include activation of the NAD^+-consumer sirtuin 1 using sirtuin-activating compounds[55] as well as inhibition of PAR glycohydrolase (PARG), which leads to NAD^+ sequestration in non-hydrolysed PAR chains[44]. While the mechanism underlying the sensitivity of *IDH*-mutant cells to genotoxic treatments is debated[52,56], the NAD^+ dependency and PARP inhibitor sensitivity of such cells form the foundations of several ongoing trials in patients with *IDH*-mutant cancers (Supplementary Table 2).

In summation, epigenetic disarray, aberrant gene expression, blocks to differentiation and altered metabolism all contribute to the transformed and tumorigenic state of *IDH*-mutant cells. Additionally, the effects of *IDH* mutations are probably dependent on the cell type and genetic context, and the overall prognostic and therapeutic implications of such mutations are similarly tumour context dependent, as will be discussed below.

Development of mutant IDH inhibitors

The unique structural and functional features of mutant IDH has facilitated the discovery of small-molecule inhibitors[57,58] (FIG. 1b). Compound 35, also known as AGI-5198, was the first molecule identified as a potent inhibitor of mutant IDH1, with a 90% reduction in D-2HG production observed in an *IDH1*-mutant tumour xenograft model[59–61]. Despite the promising activity in inducing gliogenic differentiation and slowing the growth of *IDH1*-mutant glioma cells in vitro and in vivo[61,62], the overall pharmacokinetic and pharmacodynamic properties of AGI-5198 prohibited its clinical use. A series of chemical optimizations resulted in more favourable pharmacological properties and led to the development of inhibitors specific to mutant IDH1 (ivosidenib, also known as AG-120)[62] and mutant IDH2 (enasidenib; AG-221)[63] as well as of a dual inhibitor of mutant IDH1 and IDH2 (vorasidenib; AG-881)[64]. These inhibitors stabilize the mutant enzyme in an open inactive conformation by binding at an allosteric site and preventing the conformational change required for catalysis[58]. Specifically, the regulatory segment of IDH, comprising an α-helix, is destabilized as a result of *IDH* mutations, which increases accessibility of the inhibitors to the allosteric site[65]. When this helix is destabilized through other means (for example, Mg^{2+} depletion), the inhibitors are additionally capable of binding to the wild-type protein. In either case, multiple hydrogen bonds and hydrophobic interactions anchor the inhibitor within the allosteric pocket, resulting in tight binding and slow-on/slow-off kinetics[63].

BAY-1436032 is another compound that, like ivosidenib, has been shown to inhibit D-2HG production by the R132H, R132C, R132G, R132S and R132L variants of IDH1 (REF.[66]). Similar to the aforementioned inhibitors, BAY-1436032 acts via an allosteric mechanism of inhibition and reduces the proliferation and induced differentiation of primary glioma and AML cells in vitro[66,67].

With a particular focus on ensuring penetration of the blood–brain barrier (BBB) as well as achieving maximal inhibition of D-2HG production, high-throughput screening using an NADPH fluorescence-based biochemical assay led to the identification of IDH125 as an inhibitor of mutant IDH1 (REF.[65]). The continued optimization of this agent to improve potency against the IDH1 wild-type–mutant heterodimer resulted in the development of IDH305 (REF.[68]). IDH305 has favourable solubility, clearance kinetics and inhibitory potency, although this agent does not reduce D-2HG production in *IDH2*-mutant cells[68]. A clinical trial of this agent in patients with various advanced-stage *IDH1*-mutant malignancies is currently ongoing (NCT02381886; Supplementary Table 1).

Similarly, DS-1001b was designed to have a high level of BBB permeability as well as to robustly inhibit D-2HG production by IDH1-R132H and IDH1-R132C. This agent has a lower potency against IDH1-R132G, IDH1-R132L and IDH1-R132S (with a half-maximal inhibitory concentration of ~200 nM versus ~30 nM for IDH1-R132H and IDH1-R132C in in vitro cell assays), with no inhibition of IDH2 variants[69]. DS-1001b is currently being investigated in two clinical trials involving patients with *IDH1*-mutant gliomas (NCT03030066 and NCT04458272; Supplementary Table 1).

A high-throughput biochemical screen for agents targeting the IDH1 wild-type–R132H heterodimer led to the discovery of a tetrahydro-pyrazolopyridine class of inhibitors[70]. Through a series of optimizations, GSK321 was generated as a highly potent inhibitor of IDH1-R132 variants but also a modest inhibitor of wild-type IDH1 (REF.[70]). Further refinements yielded GSK864, which has superior pharmacokinetic properties to GSK321 despite being structurally similar[70]. Several preclinical investigations of GSK321 and GSK864 have been performed in models of AML[70], although no clinical trials have been announced to date.

Several other inhibitors of mutant IDH are in early stages of development, with minimal preclinical and clinical data available. For example, the mutant IDH1-specific inhibitor olutasidenib (also known as FT-2102)[71] is being investigated in a phase I/II study involving patients with *IDH1*-mutant AML or myelodysplastic syndrome (NCT02719574; TABLE 1; Supplementary Table 1). Additionally, a unique first-in-class covalent inhibitor of mutant IDH1 has been developed. This novel compound, known as LY3410738, is regarded as a 'second-generation' inhibitor owing not only to its covalent mode of action but also to its potency against second-site *IDH1* mutations that confer resistance to other inhibitors. Phase I studies evaluating the safety, tolerability and preliminary efficacy of LY3410738 in patients with advanced-stage *IDH1*-mutant solid tumours (NCT04521686) or *IDH*-mutant haematological malignancies are under way (NCT04603001)[72,73].

IDH-mutant AML

Prognosis and biology

AML, the most common acute leukaemia in adults, is characterized by the uncontrolled proliferation of poorly differentiated cells of the myeloid lineage, leading to the accumulation of immature myeloid cells or blasts[74]. This disease is associated with a 5-year overall survival

(OS) of approximately 40–50%, although the prognosis worsens with increasing age and with the emergence of relapsed and/or refractory (R/R) disease, at which point the 5-year OS decreases to 5–10%[75]. Approximately 20% of patients with AML harbour somatic mutations in *IDH1* (R132C and R132H) or, more frequently, *IDH2* (R172 and R140)[5,6,28,76] (FIG. 1a). Interestingly, AML is one of the only cancers in which *IDH2*-R140 mutations can be found[77]. The overall relevance of this finding is unclear, although patients with such mutations have higher complete response (CR) rates, longer OS, greater 5-year OS and a reduced risk of relapse compared with patients with AML harbouring *IDH2*-R172 (REFS[6,78]). The reason for these differences is also unclear, although IDH2-R140Q has been shown to produce lower levels of D-2HG compared with other IDH variants, including IDH2-R172K[22]; considering the dose-dependent effects of D-2HG on cell differentiation[22], these reduced levels might also confer differential disease phenotypes and outcomes.

A meta-analysis of data from 33 studies involving 12,747 patients with AML has been performed to clarify the prognostic value of *IDH* mutations[79]. A broad categorization according to *IDH* mutation status did not yield prognostic implications; however, the subgroup of patients with *IDH1*-mutant AML had worse OS (HR 1.17; 95% CI 1.05–1.31) and event-free survival (HR 1.29; 95% CI 1.07–1.56) rates than those with *IDH*-wild-type disease, as well as a reduced CR rate (RR 1.21; 95% CI 1.02–1.44)[79]. Interestingly, favourable OS (HR 0.78; 95% CI 0.66–0.93) was observed among patients with mutations in *IDH2* (both R172 and R140), although those with *IDH2*-R172 mutations had reduced CR rates (RR 2.14; 95% CI 1.61–2.85)[79]. While this analysis was comprehensive and acknowledged heterogeneity between the studies, other studies have found no prognostic implications for *IDH* mutations in AML[80]. These discrepancies might reflect variations in patient cohorts, contributions of co-occurring mutations or differences in the genetic subsets chosen for analysis.

On the basis of *IDH* variant allele frequencies (VAFs) in patients with AML, *IDH1* mutations have been determined to be ancestral (clonal) in 19% of patients and subclonal in 55%, whereas *IDH2* mutations were clonal in 34% and subclonal in 45% (the remaining patients had indeterminate VAFs)[81]. Patients with clonal *IDH1* mutations have a worse prognosis than those with subclonal mutations, whereas no difference in outcome has been observed between clonal and subclonal *IDH2* mutations[81]. The clonal heterogeneity of AML has been characterized through single-cell DNA sequencing of 154 bone marrow mononuclear cells (BMMCs) from 123 patients[82]. Both linear and branching patterns of evolution were observed in several patients, as was convergent evolution, with *IDH* mutations being present in each pattern[82].

IDH mutations contribute to a hypermethylated state in AML[38], which is similar to that of other *IDH*-mutant cancers[38,83]. The D-2HG-mediated inhibition of TET methylcytosine hydroxylases is a proposed mechanism for this phenotype associated with low levels of 5hmC[38]. Interestingly, *IDH*-mutant AML and *TET2*-mutant AML have similar methylation and gene-expression profiles, suggesting a common pathogenic pathway[38]. Indeed, mutations in *IDH* and *TET2* tend to be mutually exclusive in AML[38,80]. By contrast, co-occurring *TET2* mutations have been reported in up to 68% of *IDH*-mutated angioimmunoblastic T cell lymphomas[14]; however, this study involved bulk tumour sequencing and, therefore, whether the *IDH* and *TET2* mutations were present in the same or independent clones is unknown, although this issue can be addressed through single-cell sequencing.

Treatment with mutant IDH inhibitors

Enasidenib. Enasidenib, which was optimized from the initial lead compound AGI-6780 (REF.[61]), is an orally available, selective and potent inhibitor of mutant IDH2 that is capable of reducing D-2HG levels, reversing histone methylation patterns and inducing cell differentiation in both in vitro and in vivo models of AML[63]. In mice xenografted with primary human *IDH2*-R140Q-mutant AML cells, this agent was well tolerated and reduced intracellular D-2HG levels to below detectable limits in the transplanted cells, which began to express differentiation markers, including CD11b, CD14, CD15 and CD24 (REF.[63]). By day 38, >60% of the total human cells had differentiated[63]. Correspondingly, a decrease in immature cells expressing human KIT and a 2–35-fold reduction in the percentage of AML blasts were observed[63]. These effects translated into prolonged survival in this model[63]. However, apoptosis was not observed, suggesting that the therapeutic activity of enasidenib lies in induction of differentiation rather than in cytotoxicity. Interestingly, expression of CD15 was absent in non-responders, further indicating that induction of differentiation is needed for a survival benefit.

Several clinical trials of enasidenib are under way (TABLE 1; Supplementary Table 1). The first-in-human phase I/II trial of this agent involved 345 patients with advanced-stage *IDH2*-mutant haematological malignancies (predominantly AML or high-risk myelodysplastic syndrome)[84–86], 25% of whom had *IDH2*-R172 mutations and 75% had *IDH2*-R140 mutations (NCT01915498). Enasidenib reduced plasma D-2HG levels by 93–99% in patients with the R140Q mutation and by 28–94% in patients with the R172K mutation[84]. Overall, the treatment was well tolerated, with the most common grade ≥3 treatment-related adverse events (TRAEs) being hyperbilirubinaemia (in 10% of patients) and thrombocytopenia (7%) as well as mutant IDH inhibitor-associated differentiation syndrome (6%)[86]. In this disease setting, differentiation syndrome occurs when the blocks to leukaemia cell proliferation and differentiation are released, resulting in imbalanced cytokine production, inflammation and potential tissue damage, and has recognizable signs and symptoms that include dyspnoea, unexplained fever, pulmonary infiltrates and hypoxia[87]. This TRAE can be fatal, although early detection and treatment with corticosteroids enable its effective management[87].

Among 214 patients with R/R AML treated with enasidenib at the target dose of 100 mg daily, the objective response rate (ORR) was 38.8% and the CR rate was 19.6%. When stratified by mutation status, patients with

R172 mutations had an ORR of 47.1% compared with 35.8% in those with R140 mutations[86]. Myeloid differentiation and trilineage haematopoietic recovery were identified through morphological assessments and immunophenotyping[86]. Follow-up analyses of *IDH2* VAFs throughout treatment revealed that patients with a CR had significantly greater reductions in VAF than non-responders: 98% versus 16% among those with *IDH2*-R140 mutations ($P<0.0001$) and 62% versus 7% in those with *IDH2*-R172 mutations ($P=0.013$)[86]. Notably, however, an earlier biomarker analysis using samples from this trial produced slightly different results, with only insignificant correlations between *IDH2* VAF and clinical responses[88]. The median OS duration was 8.8 months, in both the entire group with R/R AML and the subgroup treated at the target dose[86]. These results are encouraging when compared with data from other studies showing a median OS of 3.3 months in cohorts treated with other therapies[89] and have contributed to the FDA approval of enasidenib for the treatment of patients with *IDH2*-mutant R/R AML.

The potential survival benefit of enasidenib in patients with AML is curious, given that the *IDH2* mutations are subclonal in 45% of these individuals[81]. Flow cytometry-based immunophenotyping analyses of clonal evolution in response to enasidenib have revealed that the proportions of haematopoietic stem, progenitor, precursor and mature cells in bone marrow samples from patients with a CR are similar to those of non-malignant control samples[90]. Additionally, the number of mature cells increased from baseline following treatment, suggesting that the responses largely reflect differentiation of leukaemia cells and result from an effect on both *IDH2*-mutant and *IDH2*-wild-type cells. D-2HG can be released from cells and is able to be taken up by surrounding cells via the dicarboxylate transporter SLC13A3 (REF.[91]); therefore, the possibility exists that enasidenib can affect *IDH2*-mutant cells directly and *IDH2*-wild-type cells indirectly, thereby enhancing the differentiation-inducing capacity of enasidenib and resulting in greater therapeutic benefit.

Ivosidenib. Ivosidenib is under active investigation in patients with *IDH1*-mutant AML (TABLE 1; Supplementary Table 1). Data from a phase I trial of this agent in 258 patients with *IDH1*-mutated haematological malignancies, 179 of whom had R/R AML, have been reported[92]. Ivosidenib was generally well tolerated, although grade ≥3 TRAEs occurred in 20.7% of patients, most commonly prolongation of the QT interval (in 7.8%), differentiation syndrome (3.9%) and decreased platelet counts (3.4%)[92]. In 125 patients with R/R AML who received 500 mg of ivosidenib daily and had at least 6 months of follow-up data, the ORR was 41.6%, the CR plus CR with partial haematological recovery (CR/CRh) rate was 30.4% and the CR rate was 21.6%[92]. Among these 125 patients, the median OS duration was 8.8 months overall, not reached in those with a CR or CRh (at a median follow-up duration of 14.3 months), 9.3 months in those with an objective response other than CR or CRh, and only 3.9 months among non-responders[92]. Moreover, 21% of patients with a CR or CRh had clearance of the *IDH1* mutation in BMMCs at one or more time points[92]. Indeed, this feature was associated with a better response: 28% of 25 patients with a CR had *IDH1*-mutation clearance versus none of those without a CR[92]. These findings formed the foundations for the FDA approval of ivosidenib for the treatment of patients with *IDH1*-mutant R/R AML.

More recently, data from 34 patients with newly diagnosed *IDH1*-mutant AML who received ivosidenib 500 mg daily as part of this phase I trial have been reported[93]. Among this subgroup of patients who were ineligible for standard intensive induction chemotherapy, the rate of grade ≥3 TRAEs was 38%, with differentiation syndrome in 9% and QT prolongation in 6%[93]. The CR/CRh rate was 42.4% (CR rate of 30.3%), the ORR was 54.5% and the median OS duration was 12.6 months[93]. *IDH1* VAFs were assessed longitudinally in 30 patients, and mutation clearance from BMMCs was observed in 64.3% of those with versus 0% of those without a CR or CRh ($P<0.001$)[93]. These data supported the FDA approval of ivosidenib as a frontline treatment for this subset of patients.

Despite the small cohort size, these data indicate that patients with newly diagnosed AML have better responses to ivosidenib than those with R/R disease, suggesting that the timing of this treatment in the course of the disease is important. In this regard, the combined use of ivosidenib or enasidenib with induction and consolidation chemotherapy for patients with newly diagnosed *IDH*-mutant AML has been investigated in a phase I trial[94]. TRAEs were similar to those observed with the mutant IDH inhibitors as monotherapy[94]. In 60 patients receiving ivosidenib and 91 patients receiving enasidenib, the CR rates at the end of induction therapy were 55% and 47%, respectively, with CR/CRh rates of 72% and 63%[94]. With both agents, patients with de novo AML had better overall responses than those with secondary AML[94]. Among patients with a CR or CRh, 39% had *IDH1*-mutation clearance and 23% had *IDH2*-mutation clearance from BMMCs[94]. The 12-month OS probabilities exceeded 75% in both treatment groups[94], surpassing the low probability observed in other studies, especially among elderly patients[75,93,95]. Thus, mutant IDH inhibition in combination with intensive induction and consolidation chemotherapy is a promising therapeutic approach for patients with *IDH*-mutant AML.

BAY-1436032. The oral pan-mutant IDH1 inhibitor BAY-1436032 reduces D-2HG levels, clears leukaemia blast cells, induces myeloid differentiation at the expense of leukaemia stem cells and thereby confers prolonged survival in patient-derived xenograft (PDX) models of *IDH1*-mutant AML[67]. In a phase I trial of this agent in 27 patients with AML harbouring various *IDH1* mutations[96] (TABLE 1), the median maximal decrease in plasma D-2HG levels was 66%; however, only 5 (19%) of 26 patients had a reduction of D-2HG to normal physiological levels[96]. Plasma D-2HG levels prior to and during treatment were highly variable and were not associated with specific *IDH1* mutations. Blast counts were reduced in most patients during treatment, although the ORR was only 15%, with a median OS duration of 6.6 months[96].

IDH305. IDH305 is an orally available, selective allosteric inhibitor of mutant IDH1 that has been shown to have promising antitumour activity in patients with *IDH1*-mutant AML[97]. In a phase I study involving 81 patients with advanced-stage *IDH1*-R132-mutant malignancies (NCT02381886; TABLE 1; Supplementary Table 1), 21 patients with AML received IDH305 at various twice-daily doses (range 75–900 mg). Objective responses were observed in 7 (33%) of these patients, including 2 (9.5%) with a CR, 1 (4.8%) with a CR with incomplete haematological recovery and 4 (19%) with partial remission[97]. Dose-limiting toxicities, predominantly increased serum bilirubin levels, occurred in patients treated with the highest doses (550–900 mg), although all were considered reversible[97]. Nevertheless, subsequent clinical trials of this agent have been withdrawn prior to patient enrolment owing to continued safety evaluations (NCT02977689).

GSK321 and GSK864. In preclinical models of *IDH1*-mutated AML, GSK321 and GSK864 reduce D-2HG levels by up to 78% in vitro[70]. An initial 2–15-fold increase in the number of *IDH1*-R132H-mutant cells has been observed in these models; however, this effect is transient, with a stabilization of cell numbers and decreased viability by day 15 of treatment[70]. In mouse PDX models, GSK864 decreases the percentage of blast cells in parallel with increases in the proportion of mature lymphoid and granulocytic/monocytic cells, reflecting an effective release from the mutant IDH1-induced differentiation block[70]. These compounds have not yet entered clinical testing.

Insights into resistance mechanisms. Several case studies have revealed mechanisms of resistance to mutant IDH inhibitors that are dependent on the restoration of D-2HG production, through either isoform switching or acquisition of additional mutations in *IDH1* or *IDH2* (REFS[94,98,99]). In two patients with *IDH2*-R140Q-mutant AML, increases in D-2HG levels and disease progression following an initial clinical response to enasidenib were associated with the emergence of second-site *IDH2* mutations, which can occur in cis or trans[99]. Specifically, *IDH2*-Q316E in one patient and *IDH2*-I319M in the other were identified in the *IDH2* allele lacking the original R140Q mutation[99]. These resistance mutations affect residues located at the enasidenib interface in the IDH2 dimer and prevent the binding of this drug[99]. Interestingly, when expressed singly, these second-site variants fail to generate D-2HG; however, D-2HG production is restored upon co-expression with IDH2-R140Q, resulting in the re-establishment of the self-renewal capacity of leukaemia cells in vitro that translates into an in vivo fitness advantage in xenograft models[99].

Similar observations have been made in patients receiving ivosidenib. In this setting, the original *IDH1*-R132C mutation was followed by an *IDH1*-S280F mutation, which is paralogous to the I319 residue in *IDH2* (REF.[94]). Other acquired second-site mutations in *IDH1* include R199P, G131A, G289D and H315D[94]. Notably, no second-site mutations were identified in pretreatment samples[94], suggesting that these mutations were present in a rare pre-existing subclone below the limit of detection or arose during the course of treatment. However, additional elegant investigations using single-cell sequencing enabled nine patients with disease recurrence and the emergence of a new *IDH* mutation to be grouped according to three mechanisms of resistance[94]: six patients had no detectable second-site mutation at the onset of treatment, and the new mutation arose in the same clone as the original *IDH* mutation; one patient had no detectable second-site mutation at the onset of treatment, and the new mutation was present in a different subclone at emergence; and two had the second-site mutation present at the onset of treatment but in a separate subclone. These findings show that there is parallel expansion of multiple subclones with patterns of branching and linear clonal evolution.

The restoration of D-2HG production and acquired resistance to mutant IDH inhibitors through isoform switching has been detailed in several case reports[98]. This mechanism of resistance to either an IDH1-mutant or IDH2-mutant inhibitor is associated with the emergence of mutations in the opposite IDH isoform.

The emergence of resistant subclones that continue to be driven by D-2HG despite treatment with a mutant IDH inhibitor (via multiple mechanisms) is intriguing. A clinical trial of the dual IDH1 and IDH2 inhibitor vorasidenib has been completed (NCT02492737), although the results have not yet been reported, and whether patients have recurrence with emergent D-2HG-producing resistant subclones in this context remains to be determined. Additionally, LY3410738 is currently being evaluated in clinical trials (Supplementary Table 1) and is speculated to be active in the setting of known second-site *IDH1* mutations owing to its unique mode of binding, although whether this drug can prevent isoform switching remains to be determined[72]. D-2HG-restorative second-site *IDH* mutations are rare; however, mutant IDH-independent elevations in D-2HG levels have been observed in *IDH*-wild-type breast carcinoma cells[100,101] and glioblastoma cells[102]. Together, these findings suggest that certain cancers have a predilection for D-2HG, thus warranting further investigations of these pathways and modes of resistance.

Other therapeutic approaches to targeting *IDH*-mutant AML are being investigated as an alternative to, or in combination with, inhibitors of mutant IDH (Supplementary Tables 1, 2). Notably, an in vitro screen using 27,500 unique short-hairpin RNAs targeting 5,043 genes led to the identification of a synthetic lethal relationship between *BCL2* and *IDH* aberrations in AML cells[103]. Validation studies with multiple short-hairpin RNAs as well as the BCL-2 inhibitor venetoclax confirmed this vulnerability, both in vitro and in vivo[103]. These findings formed the basis for a phase I/II trial designed to investigate the safety and efficacy of enasidenib in combination with venetoclax in patients with *IDH2*-mutant R/R AML (NCT04092179). A phase I/II trial of ivosidenib plus venetoclax in patients with *IDH1*-mutant haematological malignancies is also ongoing (NCT03471260), with promising preliminary activity and safety observed in those with R/R AML[104] (TABLE 1).

The proposed mechanism underlying the sensitivity of *IDH*-mutant cells to BCL-2 inhibition involves a D-2HG-mediated reduction in cytochrome *c* oxidase activity[103], which decreases the mitochondrial threshold for triggering of apoptosis upon BCL-2 inhibition. Therefore, the effect of enasidenib, which reduces D-2HG levels, on cytochrome *c* oxidase activity and the subsequent effect on venetoclax sensitivity should be thoroughly investigated to ensure that the sensitivity to BCL-2 inhibition remains intact.

Other trials are evaluating strategies to improve on the outcomes observed with monotherapies by combining agents involved in the same pathways, for example, mutant IDH inhibitors and hypomethylating agents such as azacitidine (NCT02677922; Supplementary Table 1). In contrast to treatment with ivosidenib alone, the combination of this agent with azacitidine induces both leukaemia cell differentiation and apoptosis, contributing to an ORR of 78.3% and a CR rate of 60.9% in a phase I/II trial involving patients with newly diagnosed *IDH1*-mutant AML (TABLE 1)[105]. These promising results led to the initiation of the placebo-controlled phase III AGILE trial of this combination in the same setting (NCT03173248). Indeed, as more data accrue from preclinical and clinical studies, the onus will be on the development of therapies that exploit the vulnerabilities conferred by *IDH* mutations.

IDH-mutant cholangiocarcinoma

Prognosis and pathogenesis

Cholangiocarcinoma is an aggressive malignancy of the biliary epithelium; these tumours are usually of an advanced stage at the time of diagnosis and are typically chemotherapy refractory, conferring a dismal prognosis with a 5-year OS of 7–20%[106]. Although rare, cholangiocarcinoma is increasing in incidence globally and currently accounts for ~15% of all primary liver cancers[106]. Intrahepatic cholangiocarcinoma comprises 10–20% of all cholangiocarcinomas and is further subgrouped into bile ductular, small duct or large duct subtypes[106]. The bile ductular and small duct subtypes most frequently harbour *IDH1* mutations, specifically *IDH1*-R132C, with an additional small fraction having *IDH2*-R172 mutations[7,8,107–109] (FIG. 1a). Several studies have assessed the prognostic implications of *IDH* mutations in cholangiocarcinoma and have revealed conflicting survival outcomes, with one study reporting a reduced probability of recurrence (a 10.5% recurrence rate at 1 year versus 41.7% for *IDH*-wild-type tumours) and longer OS[108], whereas other studies found no statistically significant differences in survival according to *IDH* mutation status[8].

In mouse models, expression of mutant IDH2 under the control of the transcription factor SOX9 (an early marker of intrahepatic biliary cells) leads to downregulation of HNF4α, which results in impaired hepatocyte differentiation and increased cell proliferation in the liver[42]. Similar to observations made in glioma models[110,111], *IDH* mutations alone do not generate hepatic or biliary lesions in vivo[42]. However, when combined with the oncogene *Kras*-G12D, *IDH* mutations confer a more aggressive tumour phenotype, an observation that contrasts with findings in models of glioma[110,112,113]. In another mouse model with somatic integration of *IDH1*-R132C, loss of p53 expression and activation of Notch signalling in the liver, intrahepatic cholangiocarcinoma arises as early as 12 weeks after injection of the sleeping beauty transposase expression vector and displays several hallmarks of human intrahepatic cholangiocarcinoma, including CD19 expression, the presence of collagen fibres and active cell proliferation[114].

Similar to their effects in other cancers, *IDH* mutations in cholangiocarcinoma disrupt the normal differentiation patterns of hepatocytes and lead to aberrant hypermethylation profiles elicited through D-2HG[115]. An analysis integrating whole-genome, transcriptomic and epigenomic data from 489 cholangiocarcinomas revealed four clusters of tumours; *IDH* mutations were mostly present in cluster 4, which was associated with a hypermethylation phenotype[115]. Notably, cluster 1 tumours often had downregulation of TET2 function that also induced a hypermethylation signature, suggesting phenotypic overlap similar to that observed between *IDH* and *TET2* mutations in AML[39,115]. A comparison between the gene expression and methylation profiles of 19 *IDH*-mutant and 31 *IDH*-wild-type cholangiocarcinomas resulted in the identification of 5,758 CpG sites associated with 2,309 hypermethylated genes in *IDH*-mutant tumours[108]. Interestingly, nearly half of these genes (1,149 in total) in cholangiocarcinomas were also found to be hypermethylated in *IDH*-mutant glioblastomas, and reduced expression of 16 hypermethylated genes was observed in both tumour types[108]. These shared phenotypes with a comparable genetic basis among cancers suggest a common mechanism of pathogenesis, with the potential for overlapping therapeutics.

Therapeutic implications

Data from studies designed to identify synthetic lethal relationships in *IDH*-mutant cholangiocarcinomas have presented interesting and cholangiocarcinoma-specific therapeutic strategies. In an initial screen of 17 biliary tract cancer cell lines using 122 FDA-approved drugs, the *IDH*-mutant cell lines were specifically sensitive to the multi-target tyrosine kinase inhibitor dasatinib[116]. Indeed, dasatinib-induced cleavage of caspase 3 and rapid apoptosis were specific to the *IDH*-mutant intrahepatic cholangiocarcinoma cells, with no such effects observed in cell lines derived from other cancers[116]. Moreover, the intrahepatic cholangiocarcinoma cell lines were resistant to drugs that are active against other *IDH*-mutant cancers[116], including BCL-2 inhibitors[103] and NAMPT inhibitors[46]. Dasatinib also resulted in widespread necrosis in PDX models harbouring the *IDH1*-R132C mutation[116]. Given that this drug inhibits >40 kinases, further mechanistic analyses were performed using a multiplexed inhibitor bead column strategy, with the results implicating six targets: SRC, YES1, LYN, DDR1, ABL1 and ABL2. Through the introduction of 'gatekeeper' mutations that confer resistance to dasatinib into each of these kinases using the CRISPR–Cas9 system, SRC was ultimately identified as being the crucial target[116]. This finding provided a basis for testing dasatinib in a clinical trial involving patients

with *IDH*-mutated cholangiocarcinoma (NCT02428855; Supplementary Table 1).

Mutant IDH inhibitors are also being investigated in patients with *IDH*-mutant cholangiocarcinoma, including in a phase I study of ivosidenib monotherapy[117] (NCT02073994; TABLE 1). Among the 73 patients enrolled in this trial, ivosidenib was well tolerated, with fatigue and nausea being the most common adverse events (AEs)[117]. Four patients had an objective response, all partial responses (PRs), and 41 had stable disease. Plasma D-2HG levels were reduced by up to 98.4% relative to baseline levels and, for the majority of patients ($n = 69$), they were maintained at concentrations similar to those of volunteers without cancer; however, two patients developed emergent *IDH* mutations during treatment (*IDH1*-R132F and *IDH2*-R172V), resulting in treatment resistance[117].

Additional data on this treatment approach come from the phase III ClarIDHy trial (NCT02989857; TABLE 1), in which patients with chemotherapy-refractory *IDH1*-mutant cholangiocarcinoma (70% with the *IDH1*-R132C mutation) received either ivosidenib ($n = 124$) or placebo ($n = 61$)[118]. Ivosidenib was well tolerated and improved progression-free survival (PFS; median 2.7 months versus 1.4 months with placebo; HR 0.37, 95% CI 0.25–0.54; $P < 0.0001$); however, the median OS was not significantly different (10.8 months versus 9.7 months; HR 0.69, 95% CI 0.44–1.10; $P = 0.06$).

IDH mutations in chondrosarcoma

Prevalence and pathogenetic effects

Chondrosarcoma is a heterogeneous primary bone cartilage malignancy with 'conventional' tumours comprising the most common subtype (~75%); rarer subtypes include mesenchymal (~2%), clear cell (~2%) and dedifferentiated chondrosarcomas (~10%)[119]. Additionally, the conventional subset encompasses a spectrum of tumours, from benign (enchondromas) to high-grade malignant lesions[120]. In a genetic screen of 1,200 mesenchymal cancers, *IDH* mutations were identified in 56% of 137 central and periosteal cartilaginous tumours but not in other tumour types[9]. The *IDH* mutations were predominantly *IDH1*-R132C (~40%), followed by *IDH1*-R132G and *IDH1*-R132H mutations[9] (FIG. 1a). Chondrosarcomas can be further classified into three molecular subgroups, namely M1, M2 and M3, based on their DNA methylation profiles[121]. *IDH* mutations are most often found in high-grade lesions (M2) as well as in dedifferentiated tumours (M3)[121]. Mutations in both *IDH1* and *IDH2* occur in the M2 subset, most frequently *IDH1*-R132C, whereas only *IDH2*-R172S, *IDH2*-R172W and *IDH2*-R172T mutations are detected in M3 tumours[121]. No prognostic implications of these *IDH* mutations have been reported[121].

Mice conditionally expressing mutant IDH1 (R132Q) under the control of the cartilage-specific *Col2a1* promoter have proliferative chondrocytes that form aberrant columnar structures, leading to cartilaginous dysplasia of the long bones, ribs and tracheal cartilage[122]. Spatiotemporally controlled expression of mutant IDH1 starting at 4 weeks of age induced multiple enchondroma-like lesions in the knees, which was associated with the perturbed expression of collagen 10a1, suggesting a dysregulation of chondrocyte differentiation[122].

The effects of *IDH* mutations on the general biology of chondrosarcoma cells remain unclear. Treatment of human *IDH1*-mutant chondrosarcoma cell lines with AGI-5198 causes a marked decrease in colony formation with minimal effects on viability[123]. Despite using similar cell lines, another study produced contradictory results, with AGI-5198 having no effect on colony formation or cell migration[124]. CRISPR–Cas9-mediated knockout of the *IDH* mutation from chondrosarcoma cell lines has a minimal effect on overall cell proliferation but substantially impairs anchorage-independent cell growth and cell migration[125]. These defects have been attributed to a downregulation of integrins, which implicates mutant IDH1 in the epithelial-to-mesenchymal transition in chondrosarcoma[125]. The CRISPR–Cas9 system was not specific for a particular *IDH* allele, thus leading to knockout of both alleles in most instances; however, restoration experiments revealed that the attenuated tumorigenicity is the result of loss of the mutant and not the wild-type allele[125]. In vivo, subcutaneously implanted *IDH*-knockout chondrosarcoma lines had a reduced growth rate and produced smaller tumours than the *IDH*-mutant parental lines[125].

Further studies have shown that DS-1001b impairs the proliferation of both the conventional chondrosarcoma cell line L835 and the dedifferentiated chondrosarcoma cell line JJ012 but does not induce apoptosis[126]. RNA sequencing analyses of DS-1001b-treated L835 cells revealed upregulation of mesodermal and chondrocyte differentiation pathways, which correlated with increased expression of both *SOX9* and *RUNX2*, two genes involved in chondrocyte differentiation, as well as of *COL2A1*, *COL10A1* and *ACAN*[126]. *IDH*-mutant cell lines had greater levels of H3K4me3, H3K9me3 and H3K27me3 than *IDH*-wild-type cells, and H3K4me3 and H3K9me3 levels were reduced following DS-1001b treatment, with chromatin immunoprecipitation revealing decreased repressive H3K9me3 at the *SOX9* locus[126]. Interestingly, different effects were observed in the JJ012 cell line, with DS-1001b inducing minimal differentiation but rather an upregulation of *CDKN1C* expression, with induction of G1 phase cell cycle arrest[126]. Similarly to *SOX9*, upregulation of *CDKN1C* reflected a reduction in H3K9me3 at this locus[126]. In mouse xenograft models, DS-1001b reduced tumour volume and intratumoural D-2HG levels in association with increased *CDKN1C* expression in the JJ012 cells[126]. Together, these data underscore the importance of cell type and differentiation state when assessing the activity of mutant IDH inhibitors.

The histone methylation states and 5mC and 5hmC levels of 9 enchondromas, 11 osteochondromas, 92 central chondrosarcomas and 45 peripheral chondrosarcomas have been assessed using immunohistochemistry[127]. Minimal differences were observed between *IDH*-wild-type and *IDH*-mutant tumours in terms of H3K4me3, H3K9me3 or H3K27me3, levels of 5mC or 5hmC, or OS. Lower 5hmC levels were observed in some tumours but in association with exclusion of TET1 from the nucleus rather than with *IDH* mutations.

Overall, many of the findings relating to *IDH* mutations in chondrosarcoma are in contrast to observations in other *IDH*-mutated cancers and this disparity also applies to prognostic implications. In a study involving 89 patients with central chondrosarcomas, *IDH* mutations were not associated with OS — unlike their effects in glioma[2,128] and AML[6,78] — but were correlated with longer relapse-free and metastasis-free survival durations[129].

Potential therapeutic targeting

A phase I study of ivosidenib monotherapy for *IDH1*-mutant solid tumours included 21 patients with advanced-stage chondrosarcoma[130] (NCT02073994; TABLE 1). Ivosidenib reduced D-2HG production in all of these patients, resulting in plasma levels similar to those observed in patients without cancer[130]. Two patients also underwent biopsy sampling during treatment and intratumoural D-2HG levels were found to be reduced by 85.4% and 98.6% compared with baseline levels[130]. The safety profile of ivosidenib among patients with chondrosarcoma was similar to that seen in patients with other cancers, with fatigue, diarrhoea and nausea being the most common AEs[130]. None of the 21 patients had an objective response, although 11 had stable disease[130]. A number of other trials are investigating various mutant IDH inhibitors in patients with *IDH*-mutant chondrosarcoma (Supplementary Table 1).

IDH mutations in low-grade glioma

Pathogenetic roles of IDH mutations

Gliomas are the most common malignant tumours of the central nervous system (CNS), accounting for 82% of all brain cancers, the most aggressive of which is glioblastoma[131]. The current standard-of-care treatment for patients with gliomas is resection followed by chemoradiotherapy; however, owing to their highly infiltrative nature, complete tumour resection is nearly impossible, resulting in a high rate of recurrence[128]. *IDH1* is the most frequently mutated gene in low-grade gliomas, with the R132H mutation being most common[2,3,128] (FIG. 1a). Patients with *IDH*-mutant gliomas tend to have a better prognosis than those with *IDH*-wild-type tumours; the median OS duration has been reported to be 51 months versus 22 months among patients with grade III astrocytomas and 31 months versus 13 months among those with grade IV glioblastomas[2,132]. In addition to a favourable OS, *IDH* mutations confer the G-CIMP hypermethylated state and a proneural gene-expression profile[40,133,134].

Numerous studies have investigated the metabolic ramifications of *IDH1* mutations in glioma, including whether they engender treatment sensitivities. The neurotransmitter precursor glutamine is abundant in the CNS and is converted by glutaminase into glutamate, which can be further metabolized to α-KG[135]. In a metabolomic profiling study of 33 gliomas, levels of glutamine, glutamate and α-KG were substantially lower in *IDH*-mutant versus *IDH*-wild-type tumours[136]. Ongoing studies are investigating the efficacy of glutaminase inhibitors in the context of *IDH*-mutant tumours based on the hypothesis that skewing the carbon source yielding α-KG can subsequently affect interconversion to D-2HG[137]. In one study[138], short interfering RNA-mediated or pharmacological inhibition of glutaminase specifically reduced the growth of D54 glioblastoma cells exogenously expressing mutant IDH1 but did not induce apoptosis. Additionally, although the levels of α-KG, glutamate and other tricarboxylic acid (TCA) cycle intermediates were reduced, D-2HG levels remained unchanged[137]. This finding can be partially explained by a contribution of glucose to D-2HG production as opposed to just glutamine as was previously assumed[137]. Follow-up studies using a panel of patient-derived *IDH*-mutant cell lines revealed variable responses to glutaminase inhibition, which the authors concluded was a reflection of heterogeneity in metabolic plasticity among these cells[137].

Given the invariable tendency of gliomas to recur, investigations into their molecular evolution over time and in response to therapy are under way. In an analysis of longitudinal samples from 222 patients, a minimal change in mutational profiles was observed between initial and recurrent tumour specimens, with many of the driver genes being retained at disease recurrence[139]. These 222 gliomas were classified into three groups based on whether they were *IDH* mutated, *IDH* mutated and 1p/19q co-deleted, or *IDH* wild-type. The initial tumours had comparable mutational burdens (2.20 to 2.85 mutations per megabase); however, the *IDH*-mutant group tended to have a higher frequency of treatment-induced hypermutation, defined as >10 mutations per megabase (47% compared with 25% in the *IDH*-mutated and 1p/19q co-deleted group and 16% in the *IDH*-wild-type group)[139]. Considering the implications for tumour mutational burden and immunotherapy response, subsequent studies have investigated the mechanisms underlying this hypermutation phenotype associated with temozolomide and other alkylating agents. In a study of 10,294 gliomas, 558 were found to have hypermutation signatures associated with either de novo mismatch repair deficiency (dMMR) or, more commonly, MMR defects induced by temozolomide[140]. To better understand the relationship between these two hypermutation signatures, glioma cells with isogenic defects in MMR were generated and exposed to temozolomide in vitro. Notably, these dMMR cells but not MMR-proficient control cells developed the treatment-induced hypermutated signature[140,141], suggesting that temozolomide selects for subclones that are MMR deficient and, therefore, temozolomide resistant. Additional analyses revealed an association between *IDH* mutations and post-treatment dMMR, with a loss of MMR protein expression observed in 20% of *IDH*-mutant samples compared with 2% of *IDH*-wild-type samples[140]. In other cancer types, such as colorectal cancer, the role of dMMR as an indicator of response to PD-1 inhibitors is hypothesized to reflect a high neoantigen burden and subsequent immune cell infiltration into the tumour[142]. Despite generating neoantigens and in contrast to other hypermutated cancers, gliomas lack tumour-infiltrating lymphocytes regardless of MMR status[113,143]. This observation brings into question the effectiveness of inhibitors of the PD-1 immune

checkpoint in patients with glioma, with no improvement in PFS or OS identified in retrospective analyses[140].

The associations between PD-1, its ligand PD-L1 and *IDH* mutations are being actively investigated to gain insights into the immunosuppressive phenotype of gliomas. Several studies indicate that *IDH*-wild-type gliomas express higher levels of PD-L1 than *IDH*-mutant gliomas and that *PDL1* is hypermethylated and downregulated in *IDH*-mutant tumours[144–147]. This phenotype was reversible with AGI-5198 in mouse models of *IDH1*-mutant glioma[148]. Moreover, complete tumour regression was observed in 60% of mice when this inhibitor was combined with standard-of-care therapy and an anti-PD-L1 antibody, thus providing a foundation for trials combining ivosidenib and nivolumab in patients with *IDH1*-mutant gliomas (NCT04056910; Supplementary Table 1). Further studies are warranted to ascertain the contribution of standard-of-care therapy, which includes ionizing radiation and temozolomide, to the responses observed in preclinical models; such therapy might need to be incorporated into the treatment regimen for efficacy in clinical trials.

Although the immunosuppressive nature of gliomas has been well documented[149], the precise roles of mutant IDH and D-2HG production in immunosuppression are just beginning to be understood. The overall abundance of tumour-infiltrating lymphocytes is consistently lower in *IDH*-mutant versus *IDH*-wild-type gliomas[91,113,143]; further analyses of particular immune cell subsets have revealed global reductions spanning microglia, macrophages, dendritic cells, B cells and T cells[113]. Correspondingly, analyses of The Cancer Genome Atlas data have demonstrated downregulation of immune-related signalling pathways as well as chemotactic proteins in *IDH*-mutant gliomas[113,143,150]. *IDH*-mutant gliomas also have epigenetic silencing of genes encoding NKG2D ligands and are therefore resistant to natural killer cell-mediated lysis, which is usually the first line of defence against neoplasia[151]. This finding might offer insights into how *IDH* mutations can persist following the initial oncogenic hit.

A further component of the immunosuppressive activity of mutant IDH1 and D-2HG lies in the finding that T cells express SLC13A3 and can therefore take up D-2HG[91]. In mouse models, D-2HG uptake suppresses T cell proliferation and cytokine secretion[91], which might preclude effective antitumour responses against immunogenic neoepitopes that are known to be generated from mutant IDH1 (REF.[152]). These observations warrant further investigations of the paracrine effects of D-2HG, including how this oncometabolite modulates T cell activity and whether these effects can be counteracted using mutant IDH inhibitors (or other agents) in order to enhance antitumour immune responses.

Evidence indicates that consideration of the cell context is important in understanding the biological effects of *IDH* mutations. For example, transduction of a glioblastoma cell line with *IDH1*-R132H led to depletion of NADPH and NAD^+, whereas compensatory upregulation of NAMPT prevented this effect in immortalized astrocytes[153]. These findings suggest differential effects of the mutation on redox state, metabolism and energy homeostasis in neoplastic versus non-neoplastic cells in the context of the CNS. This variability is likely to be particularly important considering that *IDH* mutations occur early in tumorigenesis and might have different roles as the tumour evolves[16].

Several reports have described the loss of the wild-type *IDH1* allele in vitro and in vivo, which confers a more aggressive phenotype resembling that of the mesenchymal subtype of glioblastoma[26,133,154]. Thus, mutant IDH, which is not functional in the absence of the wild-type protein, might not be required for tumour maintenance and progression. Indeed, AGI-5198 enhances the 3D growth of *IDH1*-mutant glioma cells in vitro[154], an effect that might be glioma specific. In a longitudinal analysis of *IDH1*-mutant low-grade glioma samples from 50 patients[26], 2 recurrent tumours had an *IDH1* VAF below the detection threshold and 4 additional recurrent tumours had *IDH1* LOH, typically resulting in reduced levels of D-2HG relative to those of paired initial samples (exceptions might be explained by subclonal alterations). The LOH and reduction of D-2HG levels were found to correlate with an increased proliferative index[26]. Interestingly, a retrospective analysis revealed that brain tumour-initiating cell lines could be successfully generated from 63% of *IDH*-wild-type gliomas but only from 8% of *IDH1*-mutant glioma samples; moreover, the mutant cell lines systematically lost either the wild-type or mutant *IDH1* allele[26]. This LOH was not observed in the *IDH1*-wild-type cell lines, which further supports the concept that *IDH* mutations are necessary for tumour initiation but that their continued presence might not be necessary for tumour cell survival and, indeed, their loss might contribute to a more aggressive phenotype. Importantly, however, these instances of allelic loss are rare and *IDH* mutations are usually retained upon glioma recurrence, indicating that mutant IDH might be positively selected for in most tumours.

Therapeutic vulnerabilities

Gliomas present several fundamental challenges to the delivery of systemic therapies, including difficulties relating to the selective permeability of the BBB[155]. Additionally, the expression of ATP-binding cassette transporters in tumour cells often results in the efflux of drugs that do penetrate the BBB. Furthermore, the tumour vasculature changes during brain tumour development, leading to a tortuous architecture that can prevent adequate drug perfusion. These hurdles must be overcome during drug development to ensure effective glioma targeting.

Inhibition of mutant IDH. Patients with glioma were included in the previously mentioned phase I study of ivosidenib in patients with *IDH1*-mutant solid tumours (NCT02073994). Overall, the drug was well tolerated, with 13 (19.7%) of 66 patients with advanced-stage gliomas having grade ≥3 AEs, only 2 of which were considered to be treatment related (TABLE 1)[156]. Among the 35 patients with non-enhancing lesions, the ORR was 2.9%, with 1 PR[156]. Stable disease was observed in 30 (85.7%) of these patients compared with 14 (45.2%) of

those with enhancing gliomas. The median PFS duration was 13.6 months for those with non-enhancing gliomas and 1.4 months for those with enhancing lesions. A reduced tumour volume was observed in 22 (66.7%) of 33 and 9 (33.3%) of 27 evaluable patients with non-enhancing and enhancing lesions, respectively. In those with non-enhancing lesions, the estimated tumour growth rate per 6 months was reduced from 26% in the pretreatment period to 9% with ivosidenib. Notably, a case study has described a patient with recurrent *IDH1*-mutant glioblastoma who had improved seizure control and radiographic stable disease for >4 years following treatment with ivosidenib[157]. These observations underline the importance of identifying the patient populations that are most responsive to mutant IDH inhibitors.

IDH305 was optimized from the brain-penetrant compound IDH125 and, accordingly, reduced intratumoural D-2HG levels by 97% and induced tumour regression by up to 32% in PDX models of *IDH1*-mutant glioma with no effect on animal weight after 21 days of continuous treatment[65,68]. The pharmacodynamic effects of IDH305 have been evaluated in eight patients with glioma enrolled in the phase I trial of this agent discussed above (NCT02381886)[97]. In these patients, 3D MRI was used to non-invasively assess the intratumoural levels of D-2HG and other metabolites prior to treatment and after 1 week of IDH305 treatment (500 mg twice daily)[158]. IDH305 reduced D-2HG levels by up to 70% relative to creatine levels and D-2HG levels remained suppressed in three patients who had additional imaging performed after 1 month of treatment. The fluid-attenuated inversion recovery volume tended to increase post-treatment, although simultaneous increases in the apparent diffusion coefficient suggest that this finding constitutes pseudoprogression; the researchers cautiously concluded that the increases in apparent diffusion coefficient and decreases in D-2HG levels might be interpreted as objective responses.

DS-1001b is another mutant IDH1 inhibitor with a high level of BBB permeability[69]. In PDX models of glioblastoma, continuous treatment with DS-1001b substantially impaired tumour growth and improved event-free survival in subcutaneous models and reduced tumour area and volume in orthotopic models. Additionally, an increase in intratumoural levels of glial fibrillary acidic protein (GFAP), a marker of astrocytes, was observed. This finding suggests that DS-1001b is able to release the differentiation block conferred by *IDH* mutations in glioblastoma cells. Two clinical trials of DS-1001b in patients with *IDH1*-mutant gliomas are under way (NCT03030066 and NCT04458272; Supplementary Table 1).

Epigenetic therapy. The contributions of mutant IDH to DNA methylation, histone methylation and the G-CIMP phenotype coalesce to maintain glioma cells in a self-renewing dedifferentiated state; thus, *IDH*-mutant gliomas are ideal candidates for epigenetic therapies. The notion of reversing the hypermethylation phenotype and thereby re-activating silenced genes to release the differentiation block underscores the potential of DNA methyltransferase (DNMT) inhibitors as glioma therapeutics. Azacitidine and decitabine are cytosine analogues that are incorporated into DNA and subsequently trigger the proteasomal degradation of DNMTs by covalently trapping these enzymes, thereby promoting DNA hypomethylation[159]. Indeed, exposure of a *IDH1*-R132H-mutant and 1p/19q co-deleted grade III anaplastic oligodendroglioma cell line to decitabine reduces global DNA methylation, decreases colony formation by 90%, suppresses cell growth and induces differentiation (as indicated by both morphological changes and expression of GFAP)[160]. Similarly, azacitidine reverses DNA methylation of promoter loci, induces glial differentiation, reduces cell proliferation and induces durable tumour regression in a PDX model (JHH-273) of *IDH1*-R132H grade III anaplastic astrocytoma[161]. The combined effects of azacitidine and temozolomide have been investigated in the same model. Individually, both agents reduced tumour growth; however, the combination resulted in increased efficacy and a 53% prolongation of survival compared with temozolomide monotherapy[162]. These data have provided a basis for investigating azacitidine in patients with recurrent *IDH*-mutant gliomas (NCT03666559; Supplementary Table 2).

Evaluations of the combined effects of DNMT inhibitors and mutant IDH inhibitors are also of interest considering the promising activity of both classes of agent against *IDH*-mutant gliomas and their roles in promoting cell differentiation. Although preliminary preclinical data suggest a lack of activity of azacitidine in combination with AGI-5198 (REF.[162]), further studies with optimized inhibitors in improved models are warranted.

IDH *neoepitope vaccines.* Treatment modalities beyond small-molecule inhibitors are being investigated in patients with *IDH*-mutant gliomas, including immunotherapies (Supplementary Tables 1, 2). Several preclinical studies have revealed that the *IDH1*-R132H mutation produces a neoepitope that can be presented on major histocompatibility complex (MHC) class II molecules and is capable of inducing spontaneous mutation-specific $CD4^+$ T helper cell and antibody responses in patients[152,163]. These findings led to the development of an IDH1-R132H peptide vaccine, which has been shown to slow the growth of syngeneic IDH1-R132H-expressing sarcomas in MHC-humanized A2.DR1 mice[152]. Several trials investigating the safety and efficacy of IDH1-R132H-specific peptide vaccines in patients with grade II–IV gliomas have since been initiated (NCT02193347, NCT02454634 and NCT03898903; Supplementary Table 1), and preliminary safety and efficacy data from one of these trials have been reported[164]. In this trial[164], 32 patients with WHO grade III or IV glioma were vaccinated with a 20-mer peptide vaccine. All patients had received radiotherapy, chemotherapy or combined chemoradiotherapy prior to enrolment. TRAEs occurred in 90.6% of patients, although all were of grade 1. The majority of patients had vaccine-induced immune responses, including T cell responses (26 out of 30 evaluable patients) and B cell responses (in 28 of 30 patients). These responses occurred regardless of HLA haplotype, suggesting that this treatment does not have to be restricted to patients with specific HLA

alleles. Among patients with vaccine-induced immune responses, 82% were free of progression at 2 years, whereas the 2 patients without an immune response had disease progression within 2 years. Interestingly, 12 of 32 patients had pseudoprogression, compared with 10 of 60 patients in a molecularly matched control cohort; this feature was not observed in patients with a vaccine-induced immune response. Further analysis of a biopsy sample from a single pseudo-progressive lesion revealed IDH1-R132H-reactive T cells, which comprised $CD4^+$ regulatory T cells as well as $CD40L^+CD4^+$ T cells and $CXCL13^+CD4^+$ T cells; the latter two populations were dominated by a single clonotype with a specific T cell receptor (TCR), designated as TCR14. The expression of TCR14 in a TCR-deficient T cell line conferred reactivity to IDH1-R132H[164]. Follow-up studies are needed to better define the overall efficacy of this peptide vaccine yet the promising responses warrant further investigation of this treatment strategy. Similarly, the identification of TCR14 as a dominant IDH1-R132H-specific TCR is also encouraging and additional analysis of the prevalence of TCR14 among the other vaccinated patients would be informative. Determining whether cells expressing this or other IDH1-R132H-specific TCRs have functional activity in vivo following adoptive transfer is also of interest given that such a cell therapy approach could present another exciting potential route forward. Moreover, additional vaccines comprising autologous dendritic cells pulsed with tumour lysate are also being investigated in trials involving patients with *IDH1*-mutant glioma (NCT02771301 and NCT01635283).

Considerations for future research

Many of the biological effects of *IDH* mutations are independent of the cell or cancer type, including overproduction of D-2HG, hypermethylation and blocks to normal differentiation patterns. Despite these commonalities, major differences exist in the effects on metabolism and response to therapy, suggesting cancer-dependent, tissue-dependent and even differentiation state-dependent phenotypes. These points are exemplified by data from a study comparing the epigenetic effects of *IDH* mutations in several cancer types, including AML, glioma, cholangiocarcinoma and melanoma[150]. All cancers had a hypermethylated phenotype, although the hypermethylation was more extensive in gliomas, with 19% of CpG sites being hypermethylated compared with 2–4% in the other cancer types. The consequences of ectopic expression of mutant IDH on cells with various differentiation states, including non-malignant human astrocytes and neural progenitor cells, was also investigated; the neural progenitor cells had a more robust induction of hypermethylation, particularly at CpG islands and surrounding regions, suggesting that the effects of *IDH* mutations depend on the cell lineage and differentiation state. Other studies have revealed a relationship between the bivalent H3K27me3 and H3K4me3 chromatin signature and CpG island hypermethylation among cells of various differentiation states, including embryonic stem cells, neural progenitor cells and glioma samples[41], with a dysregulation of this signature observed in gliomas. These findings corroborate those of other studies in which chromatin immunoprecipitation with H3K27me3 antibodies, methyl-DNA immunoprecipitation and microarray hybridization were used to investigate the epigenetic landscapes of a variety of cell types, including mesenchymal progenitor cells, embryonic stem cells and colorectal cancer cell lines[165–167]. Together, these findings indicate that the pattern of bivalent histone methylation in progenitor cells confers susceptibility to DNA methylation and repression of lineage-restricted gene expression. These alterations lead to differentiation blocks and promote the self-renewal programme of progenitor cells, contributing to cancer development and forming the pathogenic basis of *IDH* mutations[167,168].

Discrepancies in the literature regarding the precise role of mutant IDH in tumorigenesis can be partially explained by the different model systems used. The ratio between wild-type and mutant *IDH* alleles is a crucial factor in recapitulating D-2HG production and human disease phenotypes. Therefore, caution is warranted when choosing models with overexpression rather than endogenous expression of mutant IDH. Another crucial consideration regarding the choice of model relates to interactions between the cell type of interest and the tumour microenvironment. With cell lineage and differentiation state being so important in the overall effect of *IDH* mutations, all potential contributions and interactions of surrounding stromal cells on the biology of mutant IDH must be clarified. Furthermore, establishing models with expression of *IDH* mutations in the representative cell of origin and that spontaneously generate tumours is imperative. The use of cell lines that encompass the full spectrum of genetic mutations is key, given that different combinations of co-occurring mutations can yield substantially different results and therefore affect interpretations and conclusions.

Several important lessons from the studies discussed herein will inform future clinical trials. First, the emergence of novel *IDH* mutations that restore D-2HG production in patients with AML receiving mutant IDH inhibitors indicates a continued reliance of the cancer cells on D-2HG[94,98,99]. Studies of second-generation mutant IDH inhibitors such as LY3410738, or of combinations with other therapies, are warranted to overcome or prevent such mechanisms of resistance. Second, the loss of wild-type or mutant *IDH* alleles leading to reduced D-2HG production during glioma progression[26], coupled with an overall better response to mutant IDH inhibitors in patients with non-enhancing gliomas[156], suggests that targeting gliomas at an early stage, when they are perhaps most dependent on *IDH* mutations, must be considered for maximum therapeutic efficacy. Third, several studies have revealed that treatment with mutant IDH inhibitors can remove mutant IDH-induced sensitivities[44,48,103], making their use in combination with other agents overall less effective and possibly counter-effective. Understanding the functional spectrum of *IDH* mutations in a cancer context-dependent manner will facilitate the development of successful therapeutic strategies. Fourth, transcriptomic data and gene set enrichment analysis have revealed that immune response pathways are suppressed

in all *IDH*-mutant cancer types[150]. Modulating the tumour microenvironment using mutant IDH inhibitors or other therapeutics, with the goal of converting these immunologically cold tumours into immunologically hot tumours, might prove beneficial for immunotherapy. Fifth, as discussed, the epigenetic and biological effects of *IDH* mutations differ depending on the cell lineage and differentiation state, underscoring the importance of selecting relevant cells of origin for study. Sixth, single-cell sequencing studies are elucidating the evolution of cancer cells during tumorigenesis and therapy. The integration of a multi-omics approach to further understand tumour cell trajectories and the emergence of subclones will help identify the most effective therapies and administration timelines for patients with *IDH*-mutant cancers. Seventh, differentiation blocks are a consistent feature of *IDH*-mutant cancers and mutant IDH inhibitors are effective in inducing differentiation; however, apoptosis and cell death are seldom observed with these agents, highlighting the importance of combinatorial treatments for maximum therapeutic benefit. Finally, *IDH* mutations are known to occur early in tumorigenesis but most of the current trials are enrolling patients with advanced-stage disease (Supplementary Tables 1, 2). Given that the reliance on D-2HG varies between cancers and with tumour evolution, the timing of IDH-targeted therapy and the inclusion of additional agents are imperative to enhance the outcomes of patients with *IDH*-mutated cancers.

Conclusions

Overall, great progress has been made in understanding the biology of *IDH* mutations in a variety of cancers and their pathogenic roles are beginning to be elucidated. These hotspot mutations remain a promising and provocative therapeutic target; however, understanding the nuances of their effects in particular cell and cancer types is imperative to their successful clinical translation.

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Author contributions
C.J.P. researched data for the article and wrote the manuscript. Both authors made substantial contributions to the discussion of content and reviewed/edited the manuscript before submission.

Competing interests
H.Y. is the chief scientific officer and has ownership interest in Genetron Holdings and receives royalties from Agios, Genetron and Personal Genome Diagnostics (PGDX). H.Y. holds a patent related to genetic alterations in *IDH* and other genes in malignant glioma (US Patent 8,685,660B2) issued, licensed and with royalties paid by Agios; a patent for genetic alterations in *IDH* and other genes in malignant glioma issued, licensed and with royalties paid by PGDX; a patent on methods for the rapid and sensitive detection of hotspot mutations (US 10,633,711B2) issued, licensed and with royalties paid by Genetron Holdings; a patent on homozygous and heterozygous *IDH1* gene-defective human astrocytoma cell lines (US 9,695,400B2); and a patent on homozygous and heterozygous *IDH1* gene-defective cell lines derived from human colorectal cells (US 9,074,221B2). C.J.P. declares no competing interests.

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