



# Isocitrate dehydrogenase variants in cancer — Cellular consequences and therapeutic opportunities

Shuang Liu<sup>a</sup>, Tom Cadoux-Hudson and Christopher J. Schofield

## Abstract

Abnormal metabolism is common in cancer cells and often correlates with mutations in genes encoding for enzymes involved in small-molecule metabolism. Isocitrate dehydrogenase 1 (*IDH1*) is the most frequently mutated metabolic gene in cancer. Cancer-associated substitutions in *IDH1* and *IDH2* impair wild-type production of 2-oxoglutarate and reduced nicotinamide adenine dinucleotide phosphate (NADPH) from isocitrate and oxidised nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>), and substantially promote the *IDH* variant catalysed conversion of 2-oxoglutarate to D-2-hydroxyglutarate (D-2HG). Elevated D-2HG is a biomarker for some cancers, and inhibition of *IDH1* and *IDH2* variants is being pursued as a medicinal chemistry target. We provide an overview of the types of cancer-associated *IDH* variants, discuss some of the proposed consequences of altered metabolism as a result of elevated D-2HG, summarise therapeutic efforts targeting *IDH* variants and identify areas for future research.

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## Keywords

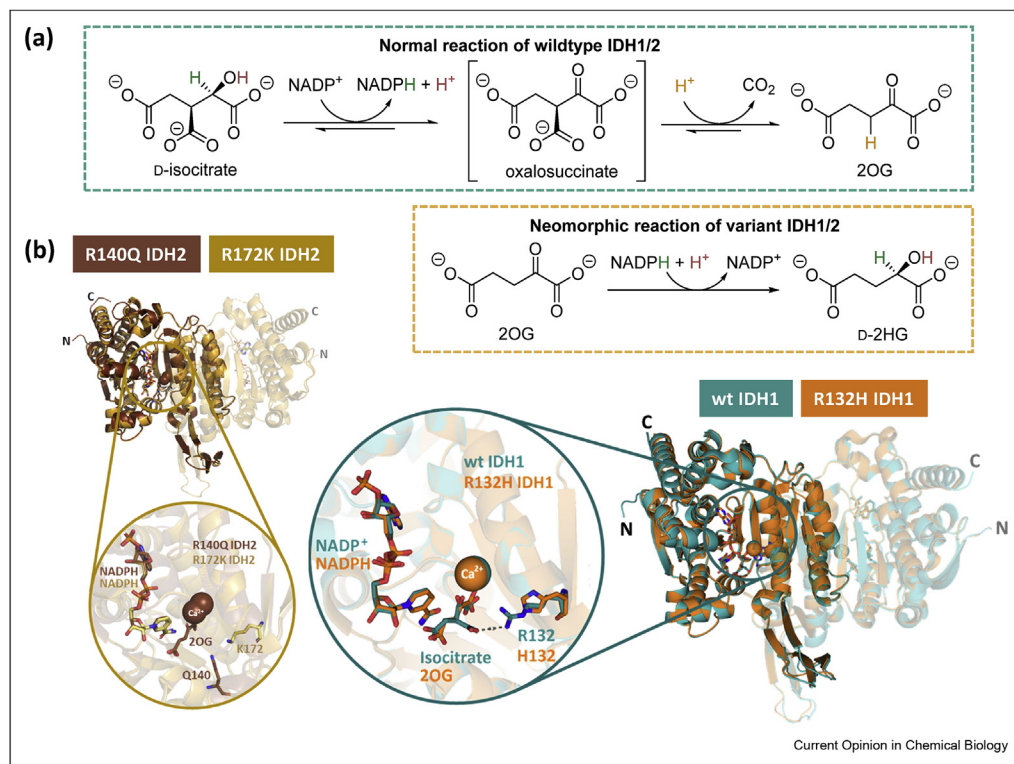
2-Hydroxyglutarate, Isocitrate dehydrogenase, Cancer metabolism, 2-oxoglutarate, Alpha-ketoglutarate, JmJc demethylase, Epigenetics, Hypoxia-inducible factor, Glioma, Acute myeloid leukaemia.

## Introduction to *IDH* mutations and cancer

Abnormal metabolism of cancer cells often correlates with mutations in genes encoding for metabolic enzymes, including those involved in the tricarboxylic acid (TCA) cycle and related metabolism, such as succinate dehydrogenase and fumarate hydratase [1]. The isocitrate dehydrogenase 1 (*IDH1*) gene is the most frequently identified mutated metabolic gene in cancer; *IDH1* and *IDH2* mutations cause active site substitutions with consequent profound effects on *IDH* activity, cellular metabolism and cancer development [2–5]. There are three human *IDH* isoforms, that is, the closely related homodimeric *IDH1* and *IDH2* (~70% identity) and the more distantly related heterotetrameric (2 $\alpha$ ,1 $\beta$ ,1 $\gamma$ ) *IDH3*. *IDH1* localises to the cytoplasm and peroxisomes; *IDH2* and *IDH3* localise to mitochondria. *IDH1* and *IDH2* undergo mutations correlating with >80% of low-grade glioma (LGG) [6] and ~20% of acute myeloid leukaemia (AML) cases [7]. By contrast, no tumour-associated *IDH3* mutations are reported [8]. *IDH3* catalyses the NAD<sup>+</sup>-dependent oxidative decarboxylation of D-isocitrate giving 2-oxoglutarate (2OG) in the TCA cycle, a reaction reported to be irreversible under physiological conditions [9]. *IDH1* and *IDH2* catalyse the reversible oxidised nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>)-dependent oxidative decarboxylation of D-isocitrate to 2OG [10], in a manner regulating isocitrate and 2OG levels and which provides reduced nicotinamide adenine dinucleotide phosphate (NADPH) [10]. Cancer-associated substitutions in *IDH1* and *IDH2* impair wild-type (wt) activity—producing 2OG by promoting a ‘neomorphic’ reaction that converts 2OG to D-2-hydroxyglutarate (D-2HG), using NADPH as a cosubstrate [11] (Figure 1a).

The nature of *IDH* substitutions varies with the cancer type; in many cancers *IDH* mutations are rare or not observed; the reasons for these differences are unclear [4,5]. In AML, for example, *IDH* substitutions are common, whereas with multiple myeloma, another blood cancer, they are rare. In LGG, the majority (>80%) of *IDH* mutations occur in the *IDH1* gene, being dominated by R132H *IDH1* [15]. Less frequently, substitutions occur at *IDH2* R172 [6,16], which is located at a structurally

Figure 1



**Reactions catalysed by wild-type (wt) and variant isocitrate dehydrogenases.** (a) Oxidative and reductive reactions catalysed by wt and variant IDH1/2, respectively. The reversible conversion of isocitrate to 2OG and CO<sub>2</sub> by wt IDH1/2 proceeds via NADP<sup>+</sup>-mediated oxidation of isocitrate giving unstable oxalosuccinate, which undergoes β-keto decarboxylation giving 2OG. IDH1/2 variants catalyse reduction of 2OG to D-2HG using NADPH. IDH reactions require Mg<sup>2+</sup>/Mn<sup>2+</sup> [11]. (b) Overall and expanded active site views from crystal structures of wt IDH1 (teal, PDB 1TOL) [12], R132H IDH1 (orange, PDB 3INM) [11], R140Q IDH2 (brown, PDB 5I95) [13] and R172K IDH2 (gold, PDB 5SVN) [14]. One monomer in the homodimer is differentiated by a different transparency level. Each active site is bound to a cofactor (NADP<sup>+</sup> for wt IDH1; NADPH for R132H IDH1, R140Q IDH2 and R172K IDH2) and a substrate (isocitrate for wt IDH1; 2OG for R132H IDH1 and R140Q IDH2) and an inhibitory Ca<sup>2+</sup> (positioned to coordinate to the substrate). 2OG, 2-oxoglutarate; IDH, isocitrate dehydrogenase; wt, wild-type.

analogous position to IDH1 R132 (Figure 1b). This contrasts with AML where *IDH2* mutations occur at a similar or higher frequency compared with *IDH1* mutations [15]. The most common IDH substitution in AML is IDH2 R140Q. The analogous IDH1 R100Q variant is rarer, being only found in grade II/III gliomas [17,18]. Interestingly, *IDH1* and *IDH2* mutations appear to be mutually exclusive [19]. All the substituted arginine residues (IDH1 R132/R100 and IDH2 R172/R140) are likely directly or indirectly involved in binding isocitrate and 2OG at the IDH1/2 active sites [12] (Figure 1b). The precise details of how substitutions impact on the individual steps of the complex Mg<sup>2+</sup>-using IDH mechanisms are unclear.

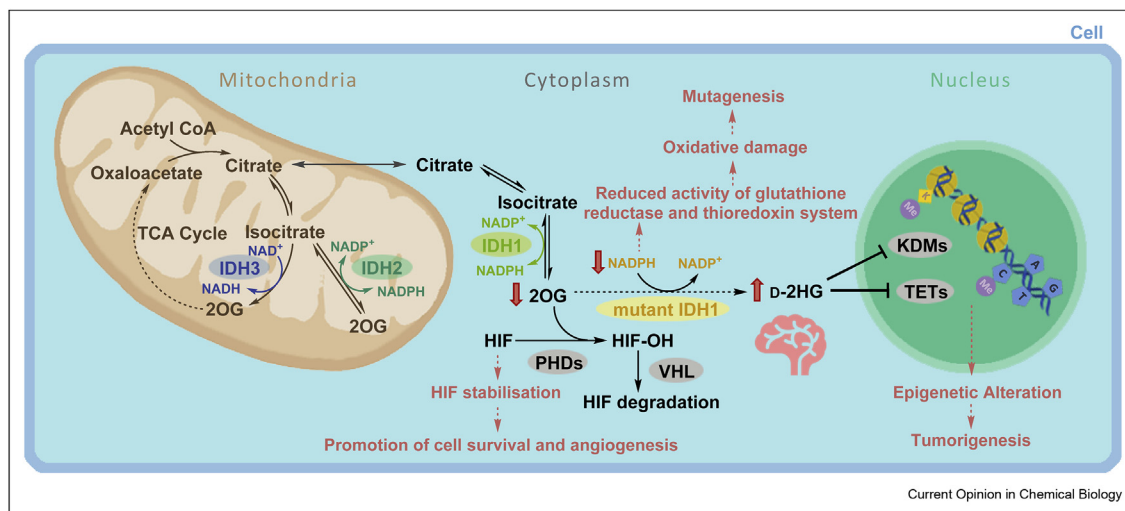
## The metabolic consequences of IDH mutations

### Elevated D-2HG levels

Amongst the multifaceted cellular impacts of *IDH* mutations in malignancies (Figure 2), the substantially increased levels of D-2HG stand out, leading to its

description as an ‘oncometabolite’ and the proposal that elevated D-2HG levels promote tumorigenesis [20]. Studies using metabolomics mass spectrometry analyses demonstrated that the D-isomer of 2HG ((*R*)-2HG) accumulates in >100-fold excess relative to normal levels in cells/tissues of patients with LGG and AML, harbouring *IDH1/2* mutations [11,21,22]. Most, but not all, studies report a less substantial 2OG reduction, with other TCA cycle intermediate levels being relatively unchanged [23]. Although variant IDHs consume 2OG, cellular 2OG stocks can be replenished from other sources, including glutamine [24]. On the other hand, whilst D-2HG produced in normal cells (where its roles are unclear) can be cleared by D-2HG dehydrogenase (D2HGDH) catalysed conversion to 2OG, it seems the normal clearance rate of D2HGDH is insufficient to suppress the high levels of D-2HG produced in IDH variant-bearing cells [11,25]. The mitochondrial localisation of D2HGDH might further contribute to its ineffectiveness in clearing cytosolic D-2HG produced by IDH1 variants [25].

Figure 2



**Roles of wt IDH1/2/3 and some of the potential multiple effects of IDH mutation in cells (exemplified for IDH1).** Different subcellular localisations (IDH1: cytoplasm; IDH2/3: mitochondria) and cosubstrate usage (IDH1/2: NADP<sup>+</sup>; IDH3: NAD<sup>+</sup>) distinguish the 3 human IDHs. The effects of IDH1 variants, including promotion of tumorigenesis, are proposed to manifest because of metabolic changes including D-2HG accumulation, depletion of NADPH and/or reduced 2OG. Changes in D-2HG/2OG levels are proposed to inhibit 2OG oxygenases involved in regulation of expression, for example, PHD, JmJc KDM, or TET enzymes. 2OG, 2-oxoglutarate; D-2HG, D-2-hydroxyglutarate; IDH, isocitrate dehydrogenase; HIF, hypoxia-inducible factor; HIF-OH, hydroxylated HIF; PHD, HIF prolyl hydroxylase domain enzyme; KDM, histone lysine demethylase; NADP<sup>+</sup>, oxidised nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; TCA, tricarboxylic acid; TET, ten-eleven translocation oxygenase; VHL, Von Hippel-Lindau; wt, wild-type.

Elevated D-2HG levels serve as a robust biomarker for *IDH1/2* mutations in gliomas [26]. D-2HG levels in plasma/serum can be analysed by liquid chromatography-mass spectrometry (LC-MS) and in the case of gliomas, D-2HG can be imaged by magnetic resonance spectroscopy [27,28]. Antibodies for IDH variants, in particular for IDH1 R132H, are also potentially useful for diagnosis. Aside from diagnosis and medicinal chemistry opportunities, the discovery of the elevated D-2HG levels was exciting from a cancer biochemistry perspective, because it opened opportunities to link readily quantifiable (*in vitro* and *in vivo*) changes in the levels of a specific metabolite (D-2HG), with cellular processes relevant to cancer, such as tumorigenesis and epigenetic regulation.

### 2-oxoglutarate-dependent oxygenases

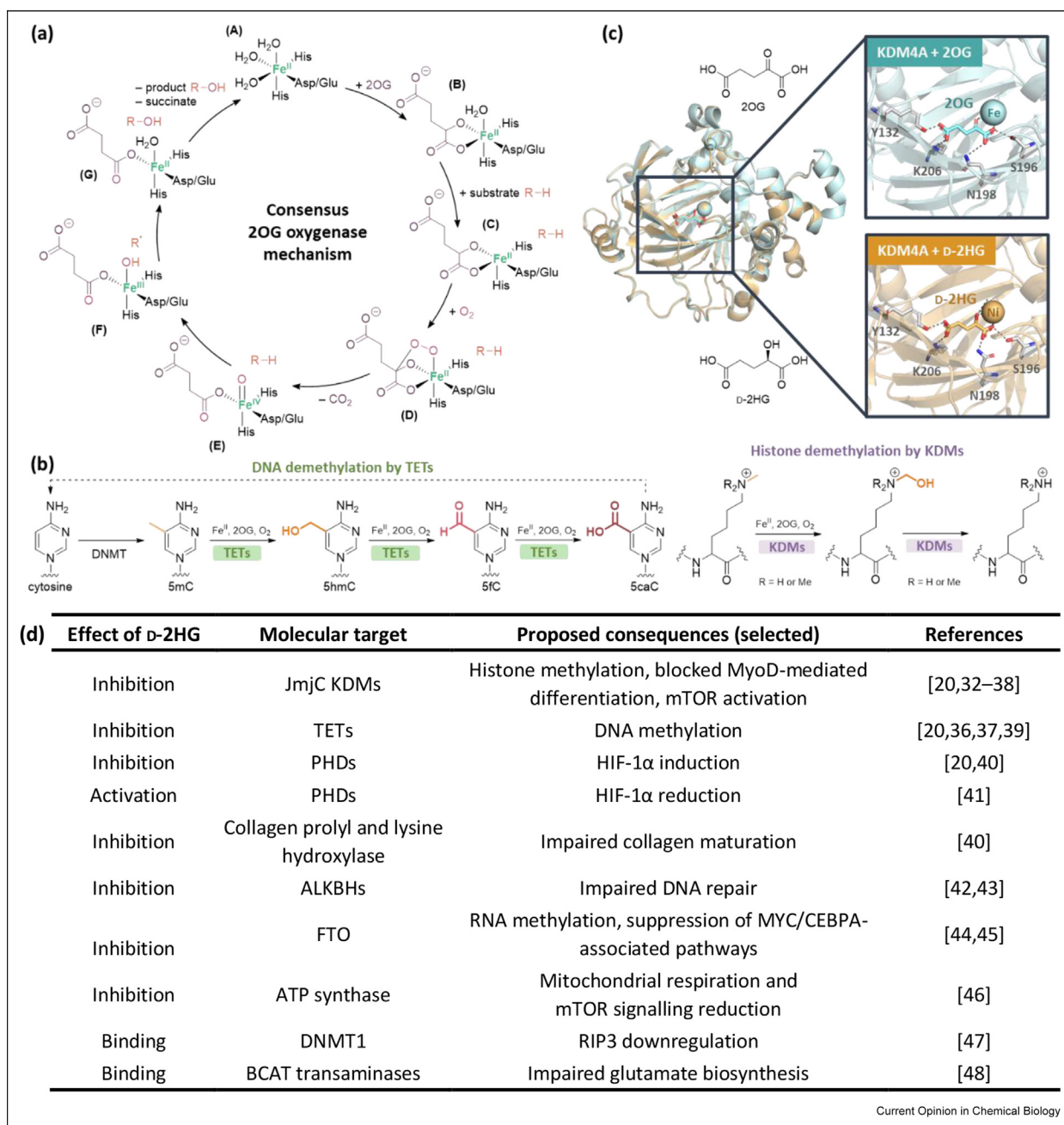
Before the work on IDH variant neomorphic activity, cancer-linked loss-of-function mutations to TCA cycle enzymes other than IDH, succinate dehydrogenase and fumarate hydratase, were identified [29]. The consequently elevated levels of succinate and/or fumarate are proposed to inhibit human 2OG and Fe(II) dependent oxygenases [30]. Given the structural similarity between D-2HG and 2OG, it was also proposed that elevated D-2HG levels competitively inhibit 2OG oxygenases in manner relevant to cancer (Figure 3c). 2OG oxygenases couple two electron substrate

oxidations, typically hydroxylation or demethylation via hydroxylation, to the conversion of 2OG and dioxygen to succinate and carbon dioxide (Figure 3a). Human 2OG oxygenases have roles in collagen biosynthesis, lipid metabolism, DNA/RNA damage repair/modification, ribosomal/translation machinery modification, the hypoxic response, and epigenetics/chromatin biology [31].

It is proposed that elevated levels of D-2HG competitively inhibit 2OG oxygenases involved in epigenetic regulation, including the Jumonji C domain-containing histone lysine demethylases (JmJc KDMs) and the ten-eleven translocation (TET) oxygenases, which regulate expression by catalysing histone demethylation and oxidation of *N*-methylcytosine in DNA, respectively (Figure 3b,d) [20,32–39]. Competitive inhibition of the JmJc KDMs and the TETs by D-2HG could contribute to the histone and DNA hypermethylation states manifested in IDH variant gliomas and AML [20,32–35,39,50]. Such chromatin states are proposed to block differentiation of (potential) cancer cells, enabling them to grow and proliferate, thereby promoting tumorigenesis [51]. Figure 3d summarises the diverse cellular consequences proposed to result from elevated levels of D-2HG.

The combined studies indicate that the effects of D-2HG on chromatin are potentially complex, though in

Figure 3



**2-hydroxyglutarate may inhibit 2-oxoglutarate oxygenases in a cancer-relevant manner.** (a) Consensus mechanism for the 2OG oxygenases. The substrate is hydroxylated in the presence of Fe(II), 2OG and O<sub>2</sub>, giving succinate and CO<sub>2</sub> as by-products. (b) Examples of reactions catalysed by 2OG oxygenases involved in chromatin regulation, as catalysed by TETs and JmjC KDM methyl-group modifying enzymes; DNA cytosine demethylation is catalysed by TETs and histone lysine demethylation catalysed by the JmjC KDMs. (c) Views from crystal structures of the JmjC KDM4A in complex with 2OG or D-2HG showing their analogous binding modes. Both 2OG (pale teal, PDB 2YBK [32]) and D-2HG (pale orange, PDB 2GP5, half maximal inhibitory concentration [IC<sub>50</sub>] against KDM4A = 24 μM [49]) occupy the same binding site and interact with KDM4A Y132, S196, N198 and K206. (d) Some of the multiple cellular targets and pathways potentially affected by D-2HG accumulation. There is mixed evidence on whether the mammalian target of rapamycin (mTOR) [35,46] and hypoxia-inducible factor-1α (HIF-1α) [20,40,41] are activated by D-2HG [42–45,47]. ALKBH, 2OG-dependent ALKB homologue; 2OG, 2-oxoglutarate; BCAT, branched-chain amino acid transferase; CEBPA, CCAAT enhancer binding protein alpha; D-2HG, D-2-hydroxyglutarate; DNMT1, DNA methyltransferase 1; FTO, fat mass and obesity-associated protein; HIF, hypoxia-inducible factor; HIF-OH, hydroxylated HIF; IDH, isocitrate dehydrogenase; JmjC KDM, Jumonji C domain-containing histone lysine demethylase; KDM, histone lysine demethylase; mTOR, the mammalian target of rapamycin; PHD, HIF prolyl hydroxylase domain enzyme; RIP3, receptor-interacting protein 3; TET, ten-eleven translocation oxygenase [42–45,47,83–89].

some contexts dysregulation of specific enzymes may be particularly important, leading to candidate medicinal chemistry targets. It should be noted that D-2HG is not a potent inhibitor of, at least many, 2OG-dependent oxygenases, even though its high concentrations in (specific regions of) tumour cells may compensate for its weak inhibition against isolated enzymes.

#### IDH variants and regulation of hypoxia-inducible factors

Hypoxia-inducible factor (HIF) is an  $\alpha,\beta$ -heterodimeric transcription factor that is a central regulator of the chronic human hypoxic response. HIF is reported to be elevated in multiple types of cancer, in a manner often, but not always, associated with hypoxia and/or mutations to TCA cycle enzymes, including succinate dehydrogenase and IDHs [52]. Both the levels and transcriptional activity of HIFs (human HIF-1 $\alpha$ , -2 $\alpha$  and -3 $\alpha$ ) are directly regulated by 2OG oxygenases. Catalysis by the HIF $\alpha$  prolyl hydroxylase domain enzymes (human HIF prolyl hydroxylase domain enzyme [PHD] 1–3) signals for HIF $\alpha$  degradation in an oxygen availability–limited manner, a property enabling them to act as hypoxia sensors (Figure 2). The factor inhibiting HIF, a JmjC HIF $\alpha$  asparaginyl hydroxylase, regulates HIF transcriptional activity by limiting its interactions with transcriptional coactivators [53].

There is mixed evidence on the effects of D-2HG on HIF activity in IDH variant gliomas. Some studies show no correlation between HIF levels and IDH variant gliomas [54,55]; others show either upregulation [40,56] or downregulation [41] of HIF-1 $\alpha$  in cells expressing mutant *IDH1*, possibly reflecting variations in O<sub>2</sub> availability in different studies. It is proposed that microvascular proliferation in glioblastoma multiforme is in part due to vascular endothelial growth factor upregulation due to increased HIF [57]. In a perhaps counterintuitive mechanism for HIF downregulation, PHD catalysis is proposed to be coupled to D-2HG oxidation, that is, D-2HG behaves as a PHD agonist rather than an inhibitor; however, the mechanism(s) underlying the cellular observations is unclear because other studies do not find D-2HG to be a PHD substrate [41,58]. Note that the reported half maximal inhibitory concentration (IC<sub>50</sub>) of D-2HG for PHD2 is high ( $\sim$ 7.3 mM [32]), and D-2HG concentration in *IDH* mutant glioma is 5–35 mM [11]. Thus, pathophysiologically relevant PHD inhibition by D-2HG is possible, but if this level of inhibition is biologically relevant one might expect multiple enzymes to be inhibited causing cytotoxicity. One possible reason for the cellular observations concerning the agonist activity of D-2HG is its nonenzymatic or enzymatic conversion to 2OG [58], although whether this can occur at sufficient levels is unclear. HIF is often

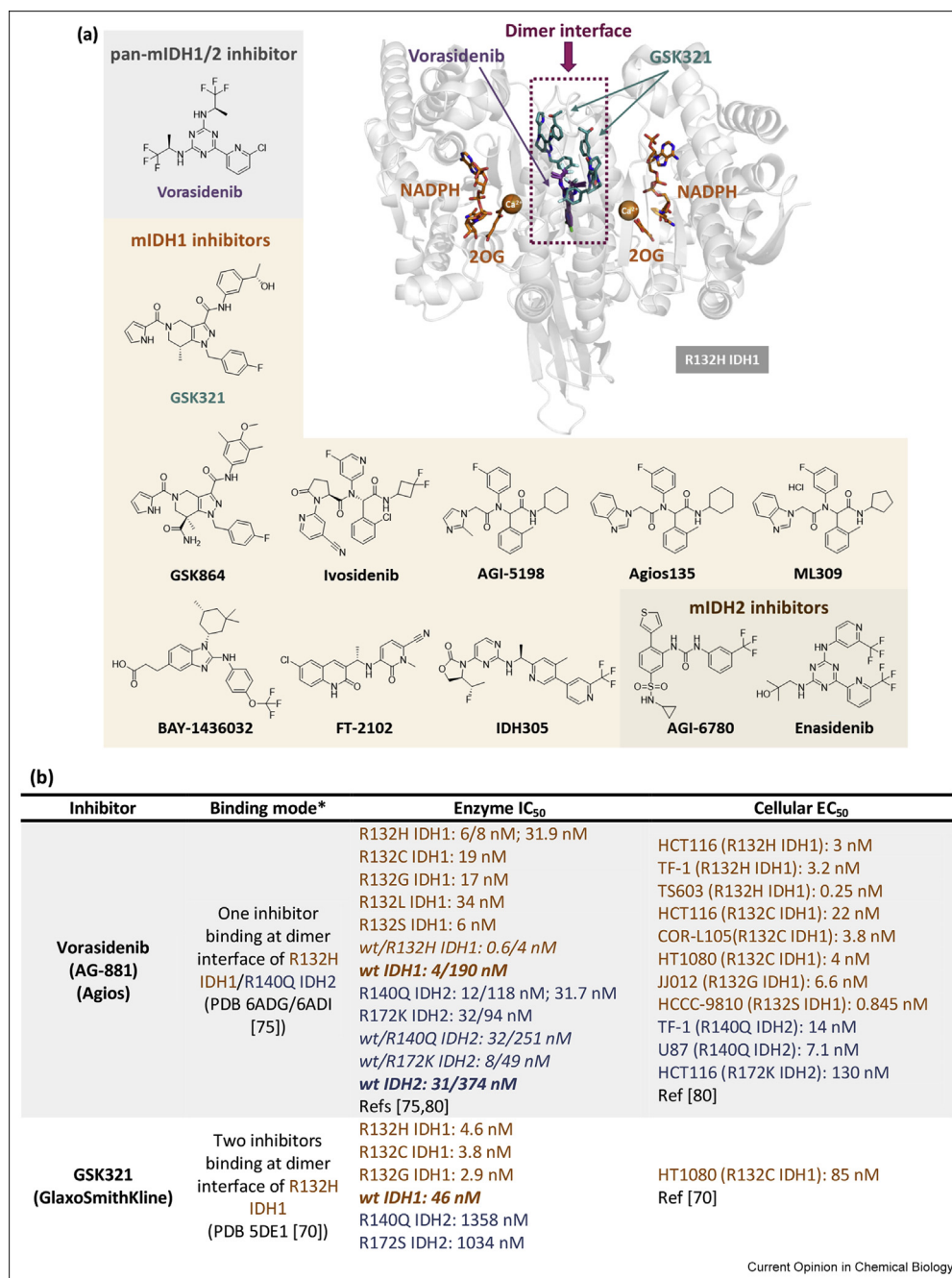
viewed as an oncogene (or proto-oncogene) that is predicted to be activated in cancers and HIF-1 $\alpha$ – and HIF-2 $\alpha$ – suppressing drugs are being pursued [59]. However, some studies suggest that, at least in particular contexts, HIF- $\alpha$  has a tumour suppressor role, including in glioma [60] and leukaemic [61] cells. For example, inhibition of HIF-2 $\alpha$  reduces angiogenesis but enhances tumour growth in glioma cells, partly because of a reduction in tumour cell apoptosis [60]. The expression levels of many HIF target genes are also decreased in *IDH* mutant gliomas [62]. Collectively, these findings raise the possibility that HIF activation via PHD inhibition, by direct or indirect means, may impair mutant *IDH* tumour growth [41].

#### IDH variants and alterations in the NADPH/NADP<sup>+</sup> ratio

Mutations in *IDH* genes result in a reduction in the cellular NADPH/NADP<sup>+</sup> ratio. IDH1 and IDH2 are important sources of NADPH in the cytoplasm and mitochondria, respectively [63]. The overall neomorphic conversion of isocitrate to D-2HG does not necessarily directly change the NADP<sup>+</sup>/NADPH ratio, whereas the normal forward IDH reactions produce NADPH for use in fatty acid biosynthesis and protection against oxidative damage; hence *IDH* mutant–bearing cells may be unusually sensitive to redox-related damage [40,64,65]. One study suggests that the R132H substitution in IDH1 results in  $\sim$ 38% reduction of its NADPH production capacity in glioblastoma tissue samples [64]. Consumption of NADPH for D-2HG synthesis is reported to decrease NADPH-dependent fatty acid synthesis, thereby increasing pentose phosphate pathways to support the NADPH demands and sensitising *IDH1* mutant cells to oxidative stress [66].

Depletion of NADPH also impairs regeneration of the thiol form of glutathione (GSH) ( $\gamma$ -glutamyl-cysteinylglycine) from its disulfide form; GSH is a thiol-containing reducing agent which protects cells from oxidative stress by neutralising reactive oxygen species (ROS). GSH is present at lower levels in both R132H IDH1 overexpressing glioma cells [65] and knock-in mice [40]. Reduction in NADPH and GSH levels can result in oxidative damage, which may contribute to mutations, ultimately promoting tumorigenesis [67]. The reduction in GSH may be exacerbated by depletion of glutamate (a GSH component) in *IDH* mutant–bearing cells [24], where glutamine is converted to glutamate and then to 2OG to replenish its role in the TCA cycle. In support of this, isotope-labelling experiments indicate that D-2HG produced by variant IDH1/2 is derived not only from glucose, but also from glutamine via glutamate [11,66].

Figure 4



**Summary of selected variant IDH1 and IDH2 inhibitors.** (a) Structures of variant IDH (mIDH) inhibitors and a crystallography-derived representation of how two allosteric inhibitors (superimposed) bind at the dimer interface of R132H IDH1. Vorasicenib (purple, PDB 6ADG [75]) and GSK321 (teal, PDB 5DE1 [70]) have binding stoichiometries of 1 and 2 inhibitors per R132H IDH1 dimer, respectively. The dimer interface where most reported variant IDH inhibitors bind is indicated by the dotted magenta region and is proximate to the active site ( $\text{Ca}^{2+}$  is inhibitory). (b) Binding modes, biochemical half maximal inhibitory concentration ( $\text{IC}_{50}$ ) and cellular half maximal effective concentration ( $\text{EC}_{50}$ ) values of variant IDH inhibitors. Note: Different  $\text{IC}_{50}$  values may be (partly) attributed to the different enzyme concentrations/assay conditions. “/” in  $\text{IC}_{50}$  values refers to measurements from different incubations times from the same report; “;” separates values from different reports. “wt/mutant” refers to a heterodimer, with other entries representing a homodimer. Data for IDH1 and IDH2 are shown in brown or navy respectively [83-89]. NR: not reported. \*As observed by crystallography except where “cryo-EM” is stated. 2OG, 2-oxoglutarate; IDH, isocitrate dehydrogenase; wt, wild-type [42–45,47,83–89].

		<b>wt IDH2: 496 nM</b> Ref [70]	
<b>GSK864</b> (GlaxoSmithKline)	NR	R132H IDH1: 15.2 nM; 0.16 $\mu$ M R132C IDH1 : 8.8 nM; 0.10 $\mu$ M R132G IDH1 : 16.6 nM <b>wt IDH1: 466.5 nM; 2.74 <math>\mu</math>M</b> R140Q IDH2: 1916 nM R172Q IDH2: 22 nM R172S IDH2: 997 nM <b>wt IDH2: 1360 nM; &gt;30 <math>\mu</math>M</b> Refs [70,83]	U87 (R132H IDH1): 191 nM THP1 (R132H IDH1): 120 nM HT1080 (R132C IDH1): 320 nM; 299 nM SNU1079 (R132C IDH1): 341 nM JJ012 (R132G IDH1): 519 nM RBE (R132S IDH1): 532 nM Refs [70,83]
<b>Ivosidenib</b> (AG-120) (Agios)	NR	R132H IDH1: 12 nM; 0.04 $\mu$ M R132C IDH1 : 13 nM; 0.05 $\mu$ M R132G IDH1: 8 nM R132L IDH1: 13 nM R132S IDH1: 12 nM <i>wt/R132H IDH1: 5/12 nM</i> <b>wt IDH1: 24/71 nM; 4.26 <math>\mu</math>M</b> R172Q IDH2: >30 $\mu$ M <i>wt/R172K IDH2: 72 <math>\mu</math>M</i> <b>wt IDH2: &gt;30 <math>\mu</math>M</b> Refs [82,83]	U87 (R132H IDH1): 19 nM; 50 nM THP1 (R132H IDH1): 19 nM HT1080 (R132C IDH1): 8 nM; 36 nM COR-L105 (R132C IDH1): 15 nM SNU1079 (R132C IDH1): 46 nM JJ012 (R132G IDH1): 16 nM HCCC-9810 (R132S IDH1): 12 nM RBE (R132S IDH1): 220 nM Refs [82,83]
<b>AGI-5198</b> (Agios)	NR	R132H IDH1: 0.07 $\mu$ M; 0.39 $\mu$ M R132C IDH1: 0.16 $\mu$ M; 1.28 $\mu$ M <b>wt IDH1: &gt;100 <math>\mu</math>M; &gt;30 <math>\mu</math>M</b> R140Q IDH2: >100 $\mu$ M R172K IDH2: >100 $\mu$ M R172Q IDH2: >30 $\mu$ M <b>wt IDH2: &gt;100 <math>\mu</math>M; &gt;30 <math>\mu</math>M</b> Refs [83–85]	U87 (R132H IDH1): 0.07 $\mu$ M; 43 nM THP1 (R132H IDH1): 52 nM HT1080 (R132C IDH1): 0.48 $\mu$ M; 1.2 $\mu$ M SNU1079 (R132C IDH1): 1.5 $\mu$ M JJ012 (R132G IDH1): 1.6 $\mu$ M RBE (R132S IDH1): 2.0 $\mu$ M Refs [83,85]
<b>Agios135</b> (Agios)	NR	R132H IDH1: 42 nM; 0.38 $\mu$ M R132C IDH1: 4 nM; 0.09 $\mu$ M <i>wt/R132H IDH1: 80 nM</i> <b>wt IDH1: 2.00 <math>\mu</math>M; 15.6 <math>\mu</math>M</b> R140Q IDH2: >10 $\mu$ M R172K IDH2: >10 $\mu$ M R172Q IDH2: >30 $\mu$ M <b>wt IDH2: &gt;10 <math>\mu</math>M; &gt;30 <math>\mu</math>M</b> Refs [74,83]	HEK293 (R132H IDH1): 81.5 nM U87 (R132H IDH1): 217 nM THP1 (R132H IDH1): 212 nM SNU1079 (R132C IDH1): 480 nM HT1080 (R132C IDH1): 530 nM JJ012 (R132G IDH1): 681 nM RBE (R132S IDH1): 810 nM Refs [74,83]
<b>ML309</b> (NCATS/Agios)	Cryo-EM: One inhibitor binding at the dimer interface of R132C IDH1 (PDB 5K11 [72])	R132H IDH1: 96 nM; 0.34 $\mu$ M R132C IDH1: 62 nM; 0.09 $\mu$ M <b>wt IDH1: 36 <math>\mu</math>M; 20.9 <math>\mu</math>M</b> R172Q IDH2: >30 $\mu$ M <b>wt IDH2: &gt;30 <math>\mu</math>M</b> Refs [83,86]	U87 (R132H IDH1): 150 nM; 248 nM THP1 (R132H IDH1): 238 nM SNU1079 (R132C IDH1): 541 nM HT1080 (R132C IDH1): 623 nM JJ012 (R132G IDH1): 711 nM RBE (R132S IDH1): 970 nM Refs [83,86]
<b>BAY-1436032</b> (Bayer)	Compound 1 (analogue of BAY-1436032): One inhibitor binding at dimer interface of R132H IDH1 (PDB 5LGE [71])	R132H IDH1: 15 nM R132C IDH1: 15 nM <b>wt IDH1: 20 <math>\mu</math>M</b> <b>wt IDH2: &gt;100 <math>\mu</math>M</b> Ref [71]	LN229 (R132H IDH1): 73 nM HCT116 (R132H IDH1): 47 nM NCH551b (R132H IDH1): 13 nM HT1080 (R132C IDH1): 135 nM Mouse hematopoietic: R132H IDH1: 60 nM R132C IDH1: 45 nM Human AML patient-derived: R132H IDH1: 5 nM R132C IDH1: 5 nM R132G IDH1: 4 nM R132L IDH1: 3 nM

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Figure 4

			R132S IDH1: 16 nM Refs [71,87]
<b>IDH305</b> (Novartis)	Three inhibitors binding at dimer interface of R132H IDH1 (PDB 6B0Z [73])  IDH889 (analogue of IDH305): two inhibitors binding at dimer interface of R132H IDH1 (PDB 5TQH [88])	R132H IDH1: 18/27 nM R132C IDH1: 28 nM <b>wt IDH1: 6.14 μM</b> Ref [73]	HCT116 (R132H IDH1): 24 nM HCT116 (R140Q IDH2): 3.8 μM HCT116 (R172K IDH2): 10 μM Ref [73]
<b>FT-2102</b> (Olutasidenib) (Forma)	Two inhibitors binding at dimer interface of R132H IDH1 (PDB 6U4J [76])	R132H IDH1: 21.2 nM R132C IDH1: 114 nM <b>wt IDH1: 22.4 μM</b> R140Q IDH2: >100 μM R172K IDH2: 27.3 μM Ref [76]	HCT116 (R132H IDH1): 21 nM U87 (R132H IDH1): 9 nM HCT116 (R132C IDH1): 94 nM U87 (R132C IDH1): 39 nM U87 (R132G IDH1): 6 nM U87 (R132L IDH1): 42 nM U87 (R132S IDH1): 9 nM Ref [76]
<b>AGI-6780</b> (Agiros)	One inhibitor binding at dimer interface of R140Q IDH2 (PDB 4JA8 [77])	R132H IDH1: 11 μM; 14 μM R132C IDH1: 9.7 μM <b>wt IDH1: &gt;100 μM; &gt;30 μM</b> R140Q IDH2: 23/170 nM R172Q IDH2: 9 nM <b>wt/R140Q IDH2: 4/120 nM</b> <b>wt IDH2: 190/2700 nM; 12 μM</b> Refs [77,83]	U87 (R132H IDH1): >100 μM TF-1 (R140Q IDH2): 18 nM U87 (R140Q IDH2): 11 nM Ref [77]
<b>Enasidenib</b> (AG-221) (Agiros)	One inhibitor binding at dimer interface of R140Q IDH2 (PDB 5I96 [13])	R132H IDH1: 48/78 μM; 4.95 μM; 16 μM R132C IDH1: 16 μM <b>wt/R132H IDH1: 0.677 μM</b> <b>wt IDH1: 0.45/1.12 μM; 15 μM</b> R140Q IDH2: 0.1/0.32 μM; 0.77 μM; 0.009 μM R172K IDH2: 0.2/0.4 μM; 0.21 μM R172Q IDH2: 44 nM R172S IDH2: 0.155 μM <b>wt/R140Q IDH2: 0.03/0.31 μM</b> <b>wt/R172K IDH2: 0.01/0.11 μM</b> <b>wt IDH2: 1.8/40 μM; 34 μM; &gt;30 μM</b> Refs [13,80,83,89]	U87 (R132H IDH1): >3 μM HT1080 (R132C IDH1): >3 μM TF-1 (R140Q IDH2): 0.02 μM; 0.012 μM U87 (R140Q IDH2): 0.01 μM; 0.012 μM HCT116 (R172K IDH2): 0.53 μM TF-1 (R172K IDH2): 0.98 μM U87 (R172K IDH2): 1.59 μM; 1.4 μM SW1353 (R172S IDH2): 2.1 μM Refs [13,89]

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(Continued).

### IDH variants and branched-chain amino acid transferase-1

A related link between *IDH1* mutant cells and amino acids, concerns branched-chain amino acid transferase-1 (BCAT1) which is a 2OG-dependent enzyme catalysing the transamination of branched-chain amino acids (valine, leucine and isoleucine) with 2OG giving glutamate and branched-chain  $\alpha$ -ketoacids. In glioma, *IDH1* mutations correlate with lower levels of BCAT1 [68]; D-2HG is also reported to directly inhibit BCAT1, although only weakly [48]. The impairment of BCAT1 catalysis, however, has an impact on cellular metabolism,

in particular, an increase in branched-chain amino acid levels and a decrease in glutamate levels [48]. Levels of other amino acids and other metabolites, including lipids, are reported to be changed in *IDH* mutant-bearing cells, although results are sometimes conflicting and the disease relevance of these changes are unclear.

### Therapeutic advances with variant IDH1/2 inhibitors

Following the identification of *IDH* mutations in gliomas and AML, multiple drug discovery campaigns targeting variant IDH1/2 were initiated. The inhibitors developed



can successfully reduce D-2HG levels as shown by studies in cells and animals [69]. The majority of potent ( $IC_{50} \leq 100$  nM) R132H IDH1 inhibitors for which crystal structures are available inhibit via an allosteric mechanism, involving binding at the dimer interface, instead of the more typical active-site binding mode. This is interesting given the structural diversity in the allosteric inhibitors [14,70–73] (Figure 4a). The allosteric inhibition is proposed to involve disruption of the binding of catalytically required metal ion ( $Mg^{2+}$  or  $Mn^{2+}$ ) at the active site [74]. Crystallographic data for ivosidenib and analogues (AGI-5198, Agios135, ML309) is lacking, although cryogenic electron microscopy (cryo-EM) data for ML309 [72] suggest it binds at the dimer interface like other allosteric inhibitors (vorasidenib [75], GSK321 [70], BAY-1436032 [71], FT-2102 [76], Novartis 305 [73], AGI-6780 [77], enasidenib [13]) (Figure 4b).

Ivosidenib [78] and enasidenib [79], which target variant IDH1 and IDH2, respectively, received FDA approval for AML treatment. Ivosidenib is in ongoing clinical trials for glioma treatment among other malignancies, in some cases as a combination therapy, for example, with vorasidenib (NCT03343197) and nivolumab (NCT04056910). Enasidenib is in clinical trials mostly for AML and haematological malignancies, including in combination therapy with azacitidine (NCT03683433). Vorasidenib is the only reported inhibitor that targets both variants of IDH1 and IDH2 [80]. Given its blood–brain barrier penetrating ability [80], vorasidenib is promising for glioma treatment, and it is currently in a phase 3 clinical trial for residual and recurrent grade 2 glioma (NCT04164901) and a phase 1 clinical trial for advanced solid tumours including gliomas (NCT02481154). BAY-1436032 has completed a phase 1 clinical trial for advanced AML (NCT03127735) and is currently in a phase 1 clinical trial for advanced solid tumours (NCT02746081). FT-2102 is in phase 1/2 clinical trials for advanced solid tumours and gliomas (NCT03684811), AML and myelodysplastic syndrome (MDS) (NCT02719574). Similarly, IDH305 is in a phase 1 clinical trial for advanced malignancies including gliomas, AML/MDS (NCT02381886); unfortunately, dose-limiting toxicities appear to have halted its clinical development [81]. One series developed by GlaxoSmithKline (e.g. GSK321 and the more bioavailable analogue GSK864) shows low selectivity between wt and variant IDH1 [70], potentially hindering its clinical development—although low wt/variant selectivity is also observed for vorasidenib and ivosidenib (Figure 4b). Preclinical compounds including AGI-5198 and AGI-6780 serve as useful tool compounds but lack clinical applications because of poor metabolic stability [82] and lack of an *in vivo* response, respectively.

## Conclusions

The breakthrough discovery that cancer-linked mutations to *IDHs* cause major metabolic changes, notably increased D-2HG, has opened up exciting new therapeutic and diagnostic possibilities. It also provided an opportunity for research to connect *in vitro* and *in vivo* small-molecule biochemistry with the pathophysiology of cancer. From an IDH-variant drug development perspective, work has progressed rapidly with compounds approved for use in AML. Resistance to ivosidenib and enasidenib in the form of a second mutation at the IDH1/2 dimer interface has emerged [90], potentially compromising the long-term efficacy of similar IDH variant inhibitors. It is, however, important to state that the optimal patient populations for deployment of mutant IDH inhibitors have likely not yet been identified. There is also scope for developing new types of IDH inhibitor, including molecules that target the active site, which might manifest reduced resistance compared with the current allosteric type inhibitors.

At least in model systems, it is also of interest to explore inhibition of wt IDH, for which no (selective) inhibitors has been developed. In part, this is because nearly all reported cancer-linked *IDH* gene mutations are heterozygous [6,91], and both the variant homodimer and the wt/variant heterodimer of IDH1 can generate D-2HG. For heterozygous *IDH1* mutant tumours, a significantly lower D-2HG level is found in gliomas that undergo loss of the wt *IDH1* allele [91]. Thus, at least in some circumstances, inhibiting the remaining wt *IDH1* allele to reduce local 2OG availability may alleviate protumour effects of *IDH1* mutations. Wild-type IDH inhibitors are also of interest because there is evidence TCA cycle disruption holds promise for cancer treatment, for example, via inhibition of the 2OG dehydrogenase complex, which converts 2OG to succinyl CoA [92].

Despite the rapid progress in the *IDH* mutant field important basic questions remain. These include the definition of exactly how *IDH* mutations promote tumorigenesis. Studies to date have highlighted the potential of D-2HG to compete with 2OG in its role as a cosubstrate for enzymes involved in epigenetic/transcriptional regulation and metabolism. However, small molecules other than D-2HG may well be involved, and the role of altered metabolism in tumorigenesis and cancer progression is likely context dependent. Although it has not shown to be relevant in humans, the recent discovery that the lysine metabolite 2-oxoadipate can be converted into D-2HG via oxygenase catalysis in bacteria reveals the potential for discovery of new metabolic processes relating to 2OG/2HG and cancer metabolism [93].

One interesting observation is that glioma patients with *IDH1/2* mutations are associated with an increase in overall survival compared with those with wt *IDH* genes [6,64,94]. Consistent with the observations for human gliomas, mouse models expressing R132H *IDH1* manifest increased median survival [95]; in addition to increased D-2HG production, they manifest increased DNA cytosine methylation and reduced infiltration of immune cells [96]. By contrast, wt *IDH1* gliomas correlate with high levels of chemokines and interleukins that stimulate infiltration of immune cells, consistent with poor prognosis [96,97]. Other studies attribute improved prognosis of *IDH* mutant gliomas to their higher sensitivity to chemotherapy [65] and radiotherapy [98]. This may be due to the R132H *IDH1*–induced depletion of NADPH and GSH and/or increased reactive oxygen species generation. These observations highlight the need for detailed context-dependent studies on the biochemistry of tumorigenesis and subsequent events in cancer progression.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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