Towards personalised medicine for cancer

*From initial therapy to follow-up*

Molenaar, R.J.

Citation for published version (APA):
Molenaar, R. J. (2017). Towards personalised medicine for cancer: From initial therapy to follow-up.
Chapter 9: The therapy-sensitizing effects of IDH1 and IDH2 mutations in cancer

Based on:

Abstract
Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are key enzymes in cellular metabolism, redox states and DNA repair. IDH1/2 mutations are early and causal in the development of various types of cancer due to supraphysiological production of D-2-hydroxyglutarate (D2HG). In various tumour types, IDH1/2-mutated cancers predict for improved responses to treatment with irradiation or chemotherapy. This chapter reviews the molecular basis of the sensitivity of IDH1/2 mutations with respect to their function in cellular processes and their interactions with novel IDH1/2-mutant inhibitors. Finally, lessons learned from IDH1/2 mutations for future clinical applications in IDH1/2 wild-type cancers are discussed.

Introduction
IDH1 and IDH2 are key enzymes that function at a crossroads of cellular metabolism, redox states and DNA repair. Mutations in the genes encoding for these enzymes occur in various types of malignancies, including >80% of low-grade gliomas and secondary glioblastomas,12,17,289 ~60% of chondrosarcomas,71 ~20% of intrahepatic cholangiocarcinoma19 and ~10% of acute myeloid leukaemias (AML).18,32,280 These mutations occur in a hotspot fashion in the catalytically active sites of these enzymes and the main driver of oncogenesis is the neomorphic production of D2HG (Figure 1).13 The resulting D2HG accumulation competitively inhibits αKG-dependent enzymes, causing cellular alterations in the abovementioned trias of cellular metabolism, redox states, and DNA repair, all of which may contribute to carcinogenesis as has been extensively reviewed elsewhere.15,33,324 The neomorphic production of D2HG is essentially a gain of function that is exclusive to mutant IDH1/2 enzymes and it was quickly realised that these frequently-occurring genetic alterations were promising targets for personalised anti-cancer therapy.14 Within five years after the initial development of these compounds, the IDH2-mutant inhibitor enasidenib was approved by the FDA as a first-in-class inhibitor for the treatment of relapsed or refractory IDH2-mutated AML.325,326

Soon after their discovery, it was appreciated that IDH1/2 mutations were associated with a relatively prolonged patient survival for glioma,17 glioblastoma12 and intrahepatic cholangiocarcinoma19 but not for AML280,327 or chondrosarcoma.71 The assumption that IDH1/2 mutations are causal for the improved clinical outcome was supported by clinical evidence in glioma, where IDH1/2 mutations predicted for improved tumour responses to chemotherapy and/or irradiation in clinical trials284,328 and retrospective analyses.329-333 Furthermore, cancer cells are sensitised to radiation and chemotherapy by the introduction of mutant IDH1/2 enzymes and it was quickly realised that these frequently-occurring genetic alterations were promising targets for personalised anti-cancer therapy.14 Within five years after the initial development of these compounds, the IDH2-mutant inhibitor enasidenib was approved by the FDA as a first-in-class inhibitor for the treatment of relapsed or refractory IDH2-mutated AML.325,326

IDH1/2 mutations or the absence of IDH1/2 wild-type enzymes create downstream vulnerabilities in cancer that can be therapeutically targeted with small-molecule inhibitors, such as poly(ADP-ribose) polymerase (PARP) inhibitors, nicotinamide phosphoribosyltransferase (NAMPT) inhibitors, BCL-2 inhibitors and biguanides. A better understanding of the mechanisms of these vulnerabilities (Figure 1) may aid to improve personalised therapy for patients with IDH1/2-mutated and IDH1/2 wild-type cancers. Prolonged survival can have two possible causes: an instrinsic effect (i.e. slower tumour growth due to disturbed cellular metabolism), or an extrinsic effect (i.e. sensitisation to therapy). Both possibilities are discussed.
The therapy-sensitizing effects of IDH1/2 mutations

**Figure 1. Effects of IDH1/2 mutations and D2HG accumulation on cellular metabolism, redox states and DNA damage repair.**

**Abbreviations:** ALKBH, alkylation repair homologue; ATM, ataxia-telangiectasia mutated; ATP5, adenosine triphosphate synthase; CoA, coenzyme A; COX, cytochrome C oxidase; D-2HG, D-2-hydroxyglutarate; ETC, electron transport chain; FOXO, forkhead box proteins; HuR, human antigen R; IDH, isocitrate dehydrogenase; KDM, lysine histone demethylase; NAD(P), nicotinamide dinucleotide (phosphate); NAD(P)H, nicotinamide dinucleotide (phosphate), reduced; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NRF2, nuclear factor (erythroid-derived 2)-like; ROS, reactive oxygen species.

**IDH1/2 enzymes in metabolism**

IDH1 and IDH2 catalyse the reversible oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG) in the cytoplasm and mitochondria, respectively, with concomitant reduction of NADP+ to NADPH. Although IDH1/2 do not generate NADH, the canonical product of the TCA cycle, IDH1/2 perform the same isocitrate-to-αKG conversion as the traditional TCA cycle enzyme IDH3. IDH1/2 mutations are neomorphic, but also confer a loss of function of wild-type IDH1/2 kinetics and redirect carbon metabolites away from the TCA cycle and oxidative phosphorylation towards D2HG production. This is evidenced by decreased expression of TCA cycle enzymes downstream of IDH1/2 and decreased oxidative metabolism in Seahorse metabolic assays using IDH1/2-mutated cancer cells. IDH1 mutant-induced mitochondrial dysfunction is also compensated by an increase in the number of mitochondria in IDH1/2-mutated cells. As a result, IDH1/2-mutated cancer cells are vulnerable to inhibition of the residual oxidative metabolism with inhibitors of the electron transport chain (ETC), such as the biguanides metformin and phenformin that inhibit NADH dehydrogenase (complex I) of the ETC. Metformin is currently investigated for safety and efficacy in a clinical trial of patients with IDH1/2-mutated solid tumours.

A second explanation of increased vulnerability of IDH1/2-mutated cancers to ETC inhibition may be that inhibition by D2HG of cytochrome c oxidase (complex IV) and prevention of cytochrome c release into the mitochondrial matrix. Besides the restriction of oxidative mitochondrial metabolism, cytochrome c release puts IDH1/2-mutated cells on the brink of apoptosis through BAX/BAK-mediated permeabilization of the outer mitochondrial membrane. Under steady-state conditions, this is prevented by BCL-2 binding to the proapoptotic proteins BAX and BAK, but disruption of this binding by the BH3 mimic ABT-199 (registered as venetoclax for the treatment of chronic lymphocytic leukaemia) results in apoptosis of IDH1/2-mutated cells while wild-type cells are relatively insensitive to ABT-199.

A consequence of rewired metabolism in IDH1/2-mutated cells is the dependence on the glutaminolysis pathway, which provides anaplerosis to the TCA cycle at the level of αKG. IDH1/2-mutated cells need αKG to produce D2HG but at the same time they restrict αKG production by impairing glycolytic influx and TCA cycle metabolism. αKG production from glutamine provides an alternative source of fuel to satisfy IDH1/2-mutated cells in their D2HG production, but also render...
these cells vulnerable to pharmacological inhibition of glutaminolysis with the use of aminooxyacetic acid, BPTES, zaprinast or chloroquine.\textsuperscript{129,134,290,351,352} It has been hypothesised that \textit{IDH1}-mutated glioma depend on glutamate rather than glutamine for TCA cycle anaplerosis.\textsuperscript{16,244}

\textit{D2HG} downregulates NAMPT, an enzyme in the NAD\textsuperscript{+} salvage pathway, which causes sensitivity towards depletion of NAD\textsuperscript{+} by pharmacological inhibition of NAMPT with the preclinical compounds FK866 and GMX1778 leading to AMP kinase-initiated autophagy and cell death in \textit{IDH1}-mutated cancer cells.\textsuperscript{353}

Finally, \textit{IDH1/2} mutations or \textit{IDH1} knockdown disable the oxidative decarboxylation reaction that converts \(\alpha\)-KG to isocitrate.\textsuperscript{48} This reaction occurs predominantly in hypoxia, when glycolytic influx of pyruvate in the TCA cycle is compromised and cells use the reverse \textit{IDH1/2} reaction to generate citrate and acetyl-CoA from glutamine and glutamate in order to preserve the capacity to synthesise lipids in hypoxic contexts.\textsuperscript{45-48} When a glioma-initiating cell had an \textit{EGFR} amplification present, knockdown of \textit{IDH1} sensitised these cells to treatment with erlotinib through decreased fatty acid and cholesterol biosynthesis, and this vulnerable phenotype was rescued by treatment with cell membrane-permeable \(\alpha\)-KG or the fatty acid palmitate plus the cholesterol precursor mevalonate.\textsuperscript{342}

## \textit{IDH1/2} enzymes in redox states

Since the reductive carboxylation of isocitrate to \(\alpha\)-KG by \textit{IDH1/2} is redundant besides the function of \textit{IDH3} in the TCA cycle, \textit{IDH1} and \textit{IDH2} presumably arose in evolution for the purpose of NADPH production in the cytoplasm and mitochondria, respectively.\textsuperscript{15} NADPH is an important source of cellular reducing power and is required to recharge, activate or generate reduced glutathione (GSH),\textsuperscript{6} thioredoxin,\textsuperscript{37} catalase tetramers\textsuperscript{38} and cytochrome P450,\textsuperscript{6,39} all of which are involved in the reduction of reactive oxygen species (ROS). Moreover, NADPH is necessary for the synthesis of deoxynucleotides and thus for DNA damage repair.\textsuperscript{354} \textit{IDH1/2} mutations cause loss of \textit{IDH1/2} wild-type functions,\textsuperscript{43,143} resulting in decreased NADPH and GSH levels and increased ROS levels, both in steady-state conditions and after induction of ROS production (Figure 2).\textsuperscript{143,290,341,342,353,355} In addition, \textit{D2HG} accumulation induces oxidative stress independently of \textit{IDH1/2} mutations.\textsuperscript{122-124} Oxidative stress is possibly induced via inhibition of wild-type \textit{IDH1/2} activity due to \(\alpha\)-KG mimicry of \textit{D2HG}, which results in a pseudo-product inhibition of wild-type \textit{IDH1/2} or via increased mitochondrial transmembrane proton leakage due to cytochrome c retention in the mitochondrial intermembrane space as described above.\textsuperscript{162} Further evidence of increased ROS levels as mediator of increased therapy sensitivity of \textit{IDH1/2}-mutated cells is shown by the almost complete reversal of this sensitivity in various cell models by the antioxidant and GSH surrogate \textit{N}-acetyl cystein. This reversal of sensitivity has been shown in the presence of carmustine (BCNU),\textsuperscript{290} irradiation,\textsuperscript{143,212,350} cisplatin, temozolomide\textsuperscript{211} and erlotinib in glioma-initiating cells with \textit{EGFR} amplification, where increased ROS levels increase erlotinib-induced apoptosis after \textit{IDH1} knockdown.\textsuperscript{342} While some \textit{in vitro} studies showed depleted GSH levels and increased ROS levels in cancer cells with \textit{IDH1} mutations\textsuperscript{143,211,334,356}, other studies have shown that \textit{IDH1/2} mutations do not alter ROS levels in brains and haematopoietic cells of \textit{IDH1\textsubscript{R132H}} knock-in mice\textsuperscript{34,41,17} or immortalised human astrocytes.\textsuperscript{31} However, these studies only interrogated steady-state conditions and used a ROS marker (CM-\textit{H}_{2}\textit{DCFDA}) that is insensitive to \textit{H}_{2}\textit{O}_{2},\textsuperscript{130} the oxidant that is most probably elevated in cells that are depleted of NADPH and have limited peroxidase and peroxiredoxin activity.

\textit{IDH1} and 2 are the most important NADPH producers in most human organs, including the brain,\textsuperscript{43,76} and \textit{IDH1} mutations lead to depleted NADPH levels in colorectal\textsuperscript{143} and glioma cells.\textsuperscript{211,334} In myeloid cells, glucose-6-phosphate dehydrogenase (G6PDH) of the pentose phosphate pathway is the major NADPH provider.\textsuperscript{15} \textit{IDH1} is the highest upregulated NADPH-producing enzyme when glioblastoma \textit{IDH1} and 2 are the most important NADPH producers in most human organs, including the brain,\textsuperscript{43,76} and \textit{IDH1} mutations lead to depleted NADPH levels in colorectal\textsuperscript{143} and glioma cells.\textsuperscript{211,334} In myeloid cells, glucose-6-phosphate dehydrogenase (G6PDH) of the pentose phosphate pathway is the major
The therapy-sensitizing effects of IDH1/2 mutations

<table>
<thead>
<tr>
<th>Agent</th>
<th>IDH1/IDH2</th>
<th>Model or patient population</th>
<th>Protection by IDH1/2(^{MT}) inhibitor?</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiation</td>
<td>IDH1(^{WT/R132H}) isogenic</td>
<td>HCT116 colorectal cancer cells; U251 glioblastoma cells; HeLa cells, murine HSCs</td>
<td>Yes</td>
<td>143, 338, 340</td>
</tr>
<tr>
<td></td>
<td>IDH1(^{R132H}), IDH2(^{R172K}) overexpression</td>
<td>U87 and U373 glioblastoma cells</td>
<td>No</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td>IDH1(^{R132H}) and IDH2(^{R140Q}) endogenous</td>
<td>Primary human AML cells</td>
<td>No</td>
<td>340</td>
</tr>
<tr>
<td>IDH1 knockdown</td>
<td>U87 glioblastoma cells \textit{in vivo}, U138 and A172 glioblastoma cells</td>
<td>N/A</td>
<td>358</td>
<td></td>
</tr>
</tbody>
</table>

**Chemotherapy**

|                              | IDH1\(^{R132H}\) overexpression | U87 glioblastoma cells | No | 334  |
| Busulfan                     | IDH1\(^{R132H}\) overexpression | U87 glioblastoma cells | No | 337  |
| Carmustine (BCNU)            | IDH1\(^{R132C/R140L}\) overexpression and IDH1 knockdown | LN229 glioblastoma cells and HEK293 cells | No | 290  |
| Cisplatin (CDDP)             | IDH1\(^{R132H}\) overexpression | U87 and U251 glioblastoma cells | No | 211  |
|                              | IDH1\(^{WT/R132H}\) isogenic | HeLa cells | No | 340  |
| Daunorubicin                 | IDH1\(^{WT/R132H}\) | Murine HSCs | No | 338  |
| Gemcitabine                  | IDH1 knockdown | Mia pancreatic cancer cells | N/A | 357  |
| Lomustine (CCNU)             | IDH1\(^{R132H}\) overexpression | U87 glioblastoma cells | No | 337  |
| Procarbazine, lomustine (CCNU) and vincristine (PCV) | IDH1\(^{MUT}\) endogenous | WHO grade II and III glioma patients | No | 284, 328 |
| Temozolomide                 | IDH1\(^{R132H}\) overexpression | U87 and U251 glioblastoma cells \textit{in vivo}, UACC257 melanoma cells | No | 339, 341 |
|                              | IDH1\(^{MUT}\) endogenous | Primary glioma neuropheres, HT1080 chondrosarcoma cells \textit{in vivo}, low-grade glioma patients | No | 332, 336 |

**Targeted therapy**

|                              | IDH1\(^{WT/R132H}\) isogenic | HeLa cells | No | 340  |
| Berzosertib                  | IDH1 knockdown | Glioma stem cells | N/A | 342  |
| Erlotinib                    | IDH1\(^{WT/R132H}\) isogenic | Primary glioma neuropheres, SW1353 chondrosarcoma cells, HT1080 chondrosarcoma cells \textit{in vivo} | No | 336, 353 |
|FK866 and GMX1778            | IDH1\(^{MUT}\) endogenous | Primary glioma neuropheres, SW1353 chondrosarcoma cells, HT1080 chondrosarcoma cells \textit{in vivo} | No | 336, 353 |
| Niraparib (MK-4827)          | IDH1\(^{WT/R132H}\) isogenic | HeLa cells | No | 340  |
| Rucaparib                    | IDH1\(^{WT/R132H}\) isogenic | HeLa cells | No | 340  |
| Olaparib                     | IDH1\(^{WT/R132H}\) isogenic | HCT116 colorectal cancer and HeLa cells \textit{in vivo}, THP-1 AML cells, HT1080 | Yes | 336, 340 |
Talazoparib (BMN-673)  
| IDH1<sup>WT/R132H</sup> isogenic | HCT16 colorectal cancer, THP-1 AML cells, HeLa cells | Yes | 340  
| IDH1<sup>WT/R132H</sup> endogenous | primary human glioma cells | No | 340  

Venetoclax (ABT-199)  
| IDH1<sup>R132H</sup>, IDH2<sup>R140Q, R172K</sup> overexpression | THP-1 acute myeloid leukaemia cells | Yes | 162  
| IDH1<sup>MUT</sup> endogenous | Primary human AML cells | No | 162  

| Metabolic therapy |  
| Aminooxyacetic acid | IDH1<sup>R132H</sup> overexpression and knockdown | LN229 glioblastoma cells | No | 290  
| BPTES | IDH1<sup>R132H</sup> overexpression | D54 glioblastoma cells | No | 134  
| | IDH1<sup>R132C/G/H, R140Q</sup> endogenous | Primary AML cells cells | No | 351  
| Metformin | IDH1<sup>WT/R132H</sup> isogenic | HCT 116 colorectal cancer cells | Yes | 143,144  
| Phenformin | IDH1<sup>WT/R132H</sup> isogenic | HCT 116 colorectal cancer cells | No | 48  
| Zaprinast | IDH1<sup>R132H</sup> overexpression | Normal human astrocytes | No | 352  

Berzosertib is an ATR/ATM inhibitor; FK866 and GMX1778 are NAMPT inhibitors; niraparib, rucaparib, olaparib and talazoparib are PARP inhibitors; venetoclax is a BCL-2 inhibitor. Abbreviations: HSC, haematopoietic stem cell.

NADPH provider.<sup>15</sup> IDH1 is the highest upregulated NADPH-producing enzyme when glioblastoma and normal brain tissue are compared and IDH1 mRNA and protein expression is upregulated following radiation, suggesting a role for IDH1 in cellular responses to radiation.<sup>342,350</sup> Possibly by induction of IDH1 expression via Forkhead box O (FOXO) transcription factors.<sup>358</sup> In pancreatic cancer cells, IDH1 expression is induced by HuR (<i>ELAVL1</i>) after treatment with gemcitabine and the HuR-IDH1 regulatory axis is essential for adaptive pancreatic cancer cell survival under acute stress.<sup>357</sup> Furthermore, radiosensitization of glioblastoma cells and other cancerous and noncancerous cells can be realised by the introduction of a mutant IDH1/2 protein.<sup>143,338,340</sup> Radiosensitization also occurs after knockdown of IDH1,<sup>346,349,350</sup> whereas overexpression of IDH1 protects cancer cells against chemotherapy.<sup>135,357</sup> These latter two findings are important, because it isolates the role of IDH1 loss-of-function in cellular radiosensitization and rules out D2HG from being solely responsible for this phenomenon. Radiosensitivity of <i>IDH1</i>-/- cells was related to increased cellular senescence due to depletion of antioxidants and deoxynucleotides in <i>IDH1</i>-/- cells following irradiation, whereas apoptosis, necrosis, autophagy, unrepaired DNA double-strand breaks and homologous recombination repair remained unchanged.<sup>350</sup> This partly corroborates and partly contrasts the situation in <i>IDH1</i>/2-mutated cells, where radiosensitivity is caused by depletion of antioxidants,<sup>143</sup> and by reduced DNA damage responses and double-strand break repair.<sup>143,340</sup> Since D2HG accumulation is strongly linked to perturbed DNA damage repair (see below), it seems plausible that ROS-associated therapy sensitivity of <i>IDH1</i>-2-mutated cells mainly occurs via increased cellular senescence due to depleted levels of antioxidants and deoxynucleotides, whereas D2HG-associated therapy sensitivity of <i>IDH1</i>-2-mutated cells is predominantly associated with inhibition of DNA damage response proteins. Finally, <i>IDH1</i> mutations are implicated in the downregulation and aberrant subcellular localization of nuclear factor-erythroid 2-related factor 2 (NRF2) and NAD(P)H quinine oxidoreductase 1 (NQO1), which are important cellular defense proteins against oxidative stress. Their downregulation and dysfunction is associated with increased...
The therapy-sensitizing effects of IDH1/2 mutations

sensitivity to chemotherapy with temozolomide, but a regulatory relationship between IDH1 and NRF2 remains unclear.

IDH1/2 enzymes in DNA repair

Besides the indirect effects of IDH1/2 mutations on DNA repair via redox state perturbations, IDH1/2 and DNA repair are interwoven via direct inhibition by D2HG of αKG-dependent dioxygenases involved in DNA repair. For example, D2HG inhibits the DNA repair enzyme alkB homolog (ALKBH)337,359 and the DNA damage response proteins lysine-specific demethylase 4A/B (KDM4A/B)340,360,361 and suppresses the expression of the DNA damage response protein ATM.338 These findings are linked with increased DNA damage in IDH1/2-mutated cells as compared to IDH1/2 wild-type cells, either in steady-state conditions or after treatment with cytotoxic or targeted agents.143,338-340 Perturbed steady-state DNA repair may contribute to oncogenesis of IDH1/2-mutated cancers, but a perturbed DNA damage response is even more likely to be related to the increased susceptibility of IDH1/2-mutated cancers to DNA damage-inducing cytotoxic agents.

Inhibition of ALKBH by D2HG results in sensitization of IDH1/2-mutated cancers cells to alkylating agents such as busulfan and CCNU.337 These findings provide a molecular basis for the sensitivity of IDH1/2-mutated glioma towards a regimen of radiotherapy in the presence or absence of procarbazine, CCNU and vincristine (PCV).284,328 of which the first two are DNA alkylators. Moreover, temozolomide is another DNA-alkylating agent that is the standard of care first-line therapy for glioblastoma and IDH1/2 mutations predict for glioblastoma responses to temozolomide.332

IDH1/2-mutated cancers are known to confer defects in homologous recombination, whereas the other major DNA double-strand break repair pathway, non-homologous end-joining, remains intact.340 The result is increased levels of DNA damage,143,339,340 which prompted researchers to perform a focused high-throughput screen of DNA repair inhibitors.340 This resulted in the observation that IDH1/2-mutated cancers are sensitive to PARP inhibitors in vivo and this sensitivity for PARP inhibitors synergises with temozolomide or cisplatin treatment in vitro, but there is disagreement on the underlying mechanisms.339,340 One study implicated NAD+ deficiency in IDH1/2-mutated cells in PARP dysfunction, because NAD+ is an essential cofactor for PARP-mediated single-strand DNA repair.339 However, this conclusion was based on associative evidence obtained from findings that PARP DNA machinery was intact in IDH1/2-mutated cells whereas NAD+ levels were more depleted in IDH1/2-mutated cells than in wild-type cells after DNA-damaging temozolomide treatment in vitro. However, mechanistic experiments to pinpoint NAD+ levels as the critical factor for IDH1/2 mutation-mediated PARP dysfunction were not performed.339 Another study showed that NAD+ levels have no role in IDH1/2 mutation-induced PARP sensitivity but instead convincingly showed that D2HG inhibits the αKG-dependent dioxygenases KDM4A/B. Inhibition of KDM4A/B induces a homologous recombination defect that creates a "BRCAness" phenotype in IDH1/2-mutant cells, which results in PARP inhibitor sensitivity in vivo.340 Of note, IDH1/2 mutations and D2HG accumulation were already linked to KDM4 inhibition several years earlier but thus far this observation was only related to global histone hypermethylation and not to decreased DNA damage responses.35 The mechanism behind the latter phenomenon is that histone methylation, such as demethylation of histone H3 lysine 20 (H3K20) and trimethylation of H3K9, are barriers to DNA double-strand break repair that can be relieved by KDM4A360 and KDM4B,361 respectively. Moreover, these demethylases cooperate with or sometimes orchestrate the activity of canonical DNA damage response proteins, such as 53BP1 in the case of KDM4A360 and PARP1 in the case of KDM4B.361 TET2 is a major downstream target of D2HG accumulation and is considered to be a major mediator of IDH1/2 mutant-mediated oncogenesis.15,30,33 However, it is unlikely that TET2 inhibition contributes to sensitization of IDH1/2-mutated cells to PARP inhibitors, because restoration of TET2 function sensitises rather than protects TET2 haploinsufficient AML cells to PARP inhibitors.362 This is an intriguing finding that also questions how IDH1/2-mutant inhibitors reverse the PARP inhibitor sensitization of IDH1/2-mutated cells, as IDH1/2-mutant inhibitors reduce D2HG levels and should
restore TET2 function.\textsuperscript{363} One study speculated that perturbed DNA damage repair and increased temozolomide sensitivity of \textit{IDH1/2}-mutated cells are caused by impaired oxidative metabolism because administration of αKG protected \textit{IDH1/2}-mutated cells against temozolomide treatment. However, it now seems more plausible that αKG administration reduces the competitive inhibition of \textit{D2HG} of ALKBH and/or KDM4A/B and restore the activity of these DNA damage repair enzymes.\textsuperscript{339} It was also demonstrated that temozolomide treatment made a bigger dent in NAD\(^+\) levels in \textit{IDH1/2}-mutated than in wild-type cells which was driven by NAD\(^+\) consumption by PARP.\textsuperscript{336} As a result, combined treatment with temozolomide and NAMPT inhibitors had a synergistic effect in \textit{IDH1/2}-mutated cancers \textit{in vivo} and may represent a promising therapeutic avenue for \textit{IDH1/2}-mutated cancer patients.

Whole-proteome analyses of \textit{IDH1} wild-type and \textit{IDH1}-mutated murine haematopoietic stem cells revealed that the latter had lower levels of the (phosphorylated) DNA damage response proteins phospho-Atm, phospho-Chek2 and yH2ax. Mutant IDH1 downregulates the DNA damage response protein ATM via an epigenetic mechanism that involves chromatin modifications via histone lysine demethylation. Mechanistic experiments involving inhibitors of epigenetic modifiers affecting H3K9 and H3K27 showed a direct link between repressive trimethylation at these histone marks and downregulation of ATM. The authors speculated that KDM4 inhibition by \textit{D2HG} is responsible for ATM suppression and excluded TET2 as mechanistic link between \textit{D2HG} accumulation and epigenetic suppression of ATM expression, because \textit{TET2}\textsuperscript{-/-} mice had normal ATM levels. At the therapeutic level, reduced ATM activity was associated with increased sensitivity to irradiation and the DNA-damaging chemotherapeutic agent daunorubicin.\textsuperscript{338} Reduced ATM expression has also been observed in human primary \textit{IDH1/2}-mutated AML cells as compared to their wild-type counterparts and was rescued by using an \textit{IDH1/2}-mutant inhibitor. In these human cell models, \textit{IDH1/2} mutations and ATM suppression caused sensitivity to irradiation and daunorubicin (see Chapter 14).

**IDH1/2-mutant inhibitors and therapy responses**

\textit{IDH1/2} mutations are inaugural or at least early events in the formation of glioma,\textsuperscript{364} chondrosarcoma,\textsuperscript{71} intrahepatic cholangiocarcinoma\textsuperscript{365} and AML\textsuperscript{366} (although in that case the data are conflicting\textsuperscript{280}) and are thus present in the large majority, if not all, cancer cells. This makes \textit{IDH1/2} mutations very attractive therapeutic targets, because such tumour homogeneity decreases the risk of therapy resistance since targeting \textit{IDH1/2} mutations affects all cancer cells. The appreciation of the role of \textit{IDH1/2} mutations in oncogenesis and their early occurrence prompted the development of \textit{IDH1/2}-mutant inhibitors.\textsuperscript{14} Enasidenib (AG-221/CC-90007) is now registered for the treatment of refractory/relapsed \textit{IDH2}-mutated AML and the \textit{IDH1}-mutant inhibitor ivosidenib (AG-120) is in clinical trials. Enasidenib suppresses \textit{D2HG} production, reverses epigenetic dysregulation and induces cellular differentiation in \textit{IDH2}-mutated AML patients in a single-arm clinical trial, where it achieved an overall response rate of 40% and a complete remission rate of 19%.\textsuperscript{325,367,368} These results with enasidenib monotherapy are promising for the difficult-to-treat population of patients with refractory/relapsed AML, but even before the first clinical trials with \textit{IDH1/2}-mutant inhibitors started it was doubted whether combination regimens of \textit{IDH1/2}-mutant inhibitors and conventional chemotherapy or targeted DNA damage-inducing agents would be safe and efficacious.\textsuperscript{15} In the context of our increasing understanding of the therapy response-modulating effects of \textit{IDH1/2} mutations, which almost exclusively point at an increased sensitization to cytotoxic agents of most types of cancer, it is plausible that concomitant administration of such cytotoxic agents and \textit{IDH1/2}-mutant inhibitors counteract each other. For example, \textit{IDH1/2}-mutant inhibitors protect \textit{IDH1/2}-mutated glioma, AML, chondrosarcoma and colorectal carcinoma cells against irradiation, daunorubicin and PARP inhibitors.\textsuperscript{143,340} In all cases, the mechanism of therapy protection by \textit{IDH1/2}-mutant inhibitors was based on the reversal of the mechanism that rendered \textit{IDH1/2}-mutated cancer cells sensitive to the therapeutic agent. For example, pretreatment with the \textit{IDH1}-mutant inhibitor AGI-5198 (the preclinical version of ivosidenib/AG-120) decreased \textit{D2HG} levels, restored NADPH production and decreased ROS levels and these phenomena collectively or ultimately resulted in less...
**The therapy-sensitizing effects of IDH1/2 mutations**

*IDH1/2*-mutated cell death after irradiation. In the case of protection of *IDH1*-mutated cells against pharmacological PARP inhibition, pretreatment with AGI-5198 decreased D2HG levels and the number of DNA double strand breaks and reverted the PARP inhibitor sensitivity of *IDH1*-mutated cells to levels observed in *IDH1* wild-type cells. In both instances, the therapy-protective effects of AGI-5198 was overcome by administration of exogenous D2HG to increase D2HG levels independently of the inhibited mutant IDH1 enzyme.

**Intrinsic effects of IDH1/2-mutations as a possible explanation for improved survival?**

In all, there is extensive evidence that *IDH1/2* mutations improve responses to DNA damage-inducing cytotoxic therapy and this seems to be the most likely mechanism how *IDH1/2* mutations may contribute to prolonged patient survival. In the case that *IDH1/2* mutations cause a prolonged survival, wholly or partially, because *IDH1/2*-mutated tumours are less aggressive than their wild-type counterparts, there is evidence that this can be attributed to either 1) slowly proliferating cancer cells or 2) prognostically favourable histological and radiological features. Both options are reviewed.

**Slow cellular proliferation.** In vitro investigations have not yet unequivocally shown whether or not *IDH1/2* mutations intrinsically inhibit tumour growth. Both a diminished and increased proliferation after transfection of U87 glioblastoma cells with *IDH1* have been reported. The relation between *IDH1* mutations and tumour growth was investigated in glioblastoma patients as well. MRIs of treatment-naïve patients with glioma-like symptoms at the time of clinical diagnosis and shortly before surgery showed that *IDH1* mutations are not associated with differences in spontaneous tumour growth. In the case that *IDH1/2* mutations intrinsically inhibit spontaneous tumour growth, the underlying mechanisms are unclear. It may be attributed to suppressed expression of branched-chain amino acid transporter 1 (BCAT1) in *IDH1/2*-mutated glioma. BCAT1 protein expression is mutually exclusive with *IDH1/2* mutations and promotes the catabolism of branched-chain amino acids, which is associated with increased proliferation in other types of cancer, such as mamma carcinoma. Alternatively, the impairment of *IDH1*-mutated cancer cells to generate citrate from glutamine may hinder lipid formation and thus cellular proliferation in certain (i.e. hypoxic) contexts.

**Favourable histological and radiological features.** When *IDH1* mutations were discovered in glioblastoma, it was immediately recognised that they occurred in younger patients, as compared with *IDH1* wild-type glioblastoma (33 versus 63 years). Although age is an established favourable prognostic factor in glioblastoma, *IDH1* mutations are independent prognostic factors in multivariate analysis.

---

**Figure 2.**

**NADPH generation and consumption in wild-type and mutant IDH1/2 cells.**

In most human cells, such as glioma cells, *IDH1/2* is the most important NADPH producer. NADPH is consumed for detoxification, biosynthetic purposes and D2HG production by *IDH1/2* mutants. Although *IDH1/2* mutations do not result in lower cellular NADPH levels under physiological conditions, they cause ~50% decrease in total NADPH production capacity that results in higher levels of ROS in treated *IDH1/2*-mutated cells (orange) compared with *IDH1/2* wild-type cells (blue).
analyses that control for age. \cite{43, 289} Histological comparison of IDH1 wild-type and mutated primary glioblastoma showed that IDH1-mutated glioblastoma contain less necrosis and vascular abnormalities occur less frequently. Preoperative MRIs revealed that IDH1-mutated glioblastoma are larger and show less oedema and necrosis, and more frequently a non-enhancing tumour component, indicating less blood-brain barrier destruction. \cite{375} The last three features are known to be prognostically favourable and one study even suggested that IDH1 mutations are not independent prognostic factors when controlled for the extent of oedema. \cite{376} These radiological features can be explained by the molecular effects of IDH1/2 mutations. Oedema and tumour enhancement are induced by high vascular permeability, which is regulated by vascular endothelial growth factor (VEGF). VEGF expression is dependent on HIF1α, which is degraded in IDH1/2-mutated cells. \cite{31} Indeed, VEGF levels are lower in secondary glioblastoma compared with primary glioblastoma. \cite{77} IDH1/2-mutated glioma may have less necrosis because the lower HIF1α levels suppress hypoxia-mediated responses to coagulation, a cascade that ultimately leads to necrosis. \cite{377} The larger preoperative tumour size suggests that IDH1/2-mutated glioblastoma can grow larger until they become clinically manifest. Most glioblastomas are diagnosed when patients experience neurological symptoms. The lesser extent of oedema may explain why IDH1/2-mutated glioma can grow larger without provoking symptoms. Patients with IDH1-mutated glioma have been reported to have an improved survival after maximal safe tumour resection, as compared with wild-type IDH1 glioma patients. \cite{378} In contrast, other studies described that the association between IDH1/2 mutations and prolonged glioma patient survival is independent of the extent of resection. \cite{43, 289, 379} Overall, the literature on effects of IDH1/2 mutations on tumour aggressiveness is inconclusive at this moment.

**Concluding remarks and future perspectives**

IDH1/2 mutations are attractive therapeutic targets for various reasons, but most prominently because they are early events in oncogenesis. This tumour homogeneity ensures that the chance of relapse of IDH1/2-mutated cancers is theoretically small after a complete response/remission obtained by the application of targeted therapeutic agents. Another prediction on the basis of the early occurrence of IDH1/2 mutations in oncogenesis and the plethora of downstream cellular effects of IDH1/2 mutations is the profoundly altered tumour biology during oncogenesis. Consequently, many research efforts have been devoted to the discovery of specific vulnerabilities, especially in the domains of metabolism and DNA damage responses. These research efforts have resulted in an increasing understanding of the sensitization of cancer cells by IDH1/2 mutations to conventional chemo/radiotherapy, but also of the susceptibility to targeted agents that have maximal efficacy and minimal side effects. We expect that pharmacological inhibition of BCL-2, NAMPT and PARP are the most promising therapeutic avenues in this category, \cite{162, 340, 353} especially in combination with each other or with chemo/radiotherapy. Because of the inherent counteracting nature of IDH1/2-mutant inhibitors on one hand and personalised targeted therapy of IDH1/2-mutated cancers on the other hand, it is doubtful whether there will ever be clinically safe and effective combinations of IDH1/2-mutant inhibitors and cytotoxic agents in cancers where IDH1/2 mutations sensitise that cancer towards DNA damage-inducing therapies. \cite{143, 340} The future looks bright for preclinical and clinical research on IDH1/2-mutated cancers and its advances may eventually trickle down to the much larger populations of patients with IDH1/2 wild-type cancers. It is becoming increasingly clear that inhibition of wild-type IDH1/2 to mimic therapy responses of IDH1/2-mutant cancer biology may have therapeutic potential. This was recently demonstrated in three preclinical models. \cite{342, 350, 357} Another example of the trickle-down effect of the increased research on IDH1/2 mutations is that reasonably potent pharmacological inhibitors of wild-type IDH1 such as GSK864 (IC$_{50}$: ~470 nM) have become available whereas we previously only had RNA interference, genetic modification or the unspecific and impotent oxalomalate \cite{349} to inhibit wild-type IDH1. GSK864 was originally developed as IDH1-mutant inhibitor but also showed activity against wild-type IDH1. \cite{380} In this way, we learn from lessons from nature so that ultimately IDH1/2 wild-type patients may benefit as well from our understanding of the increased therapy sensitivity of IDH1/2-mutated cancers.