Targeting isocitrate dehydrogenase mutations in cancer: emerging evidence and diverging strategies.

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Abstract

Isocitrate dehydrogenase active-site mutations cause a neomorphic enzyme activity that results in the formation of supraphysiological concentrations of D-2-hydroxyglutarate (D-2HG). D-2HG is thought to be an oncometabolite that drives the formation of cancers in a variety of tissue types by altering the epigenetic state of progenitor cells by inhibiting enzymes involved in histone and DNA demethylation. This model has led to the development of pharmacological inhibitors of mutant IDH activity for anti-cancer therapy, which are now being tested in several clinical trials. Emerging evidence in preclinical glioma models suggests that the epigenetic changes induced by D-2HG may persist even after mutant IDH activity is inhibited and D-2HG has returned to basal levels. Therefore, these results have raised questions as to whether the exploitation of downstream synthetic lethal vulnerabilities, rather than direct inhibition of mutant IDH1, will prove to be a superior therapeutic strategy. In this review, we summarize the preclinical evidence in gliomas and other models on the induction and persistence of D-2HG-induced hypermethylation of DNA and histones, and we examine emerging lines of evidence related to altered DNA repair mechanisms in mutant IDH tumors and their potential for therapeutic exploitation.

Introduction

Mutations in the isocitrate dehydrogenase genes (*IDH1 and IDH2*) occur in the vast majority of grade II/III gliomas and secondary glioblastomas (grade IV gliomas) (1–6). *IDH1/2* mutations also occur in chondrosarcoma, hepatocellular carcinoma, acute myeloid leukemia, intrahepatic cholangiocarcinoma, and thyroid cancers (7–10) and occur with relatively rare frequency in prostate cancers, melanomas, non-small cell lung cancers, and solid papillary carcinoma with reverse polarity (SPCRP) breast cancers (9,11–14). *IDH1/2* mutations almost always occur as heterozygous missense substitutions in which a conserved arginine residue, located within the active site of IDH1/2 enzymes, is substituted to a different amino acid, with $IDH1^{R132H}$ being the most frequent mutation in glioma (1,2,15). Regardless of the specific amino acid substitution, mutations in Arg^{132} of IDH1 and Arg^{140} or Arg^{172} of IDH2 (IDH^{mut}) cause a neomorphic enzyme activity in which α-ketoglutarate (αKG) is converted into the putative oncometabolite D-2-hydroxyglutrate (D-2HG) (16,17). In cells expressing IDH^{mut}, D-2HG accumulates to supraphysiological concentrations (i.e. millimolar levels) and inhibits a variety of α-ketoglutarate-dependent chromatin remodeling enzymes, including TET hydroxylases and lysine demethylases involved in histone demethylation (16,18–22). Based on this evidence, *IDH1/2* mutations are thought be initiating oncogenic events that cause epigenetic remodeling in neural progenitor cells, thus leading to an inhibition of normal cellular differentiation processes in a manner that promotes gliomagenesis. However, efforts to establish $IDH1^{mut}$ glioma cell lines have been challenging, and genetically engineered mouse models of IDH1 mutations have generally failed to promote gliomagenesis without concurrent expression of oncogenic drivers (23–27).

Genetic sequencing studies indicate that *IDH1/2* mutations are early, truncal genetic events in the pathogenesis of malignant gliomas, and expression of $IDH1^{R132H}$ in neural progenitor cells induces DNA and histone hypermethylation that coincides with an inhibition of normal cellular differentiation (4–6). It is clear that a portion of grade II/III diffuse gliomas with *IDH1/2* mutations can arise in the absence of known mitogenic driver alterations that are characteristic of higher-grade anaplastic gliomas and glioblastoma (e.g. *EGFR* mutations, PI3K pathway-activating mutations, *PDGFRA* amplification), suggesting that *IDH1* mutations are a driver of gliomagenesis in these grade II/III tumors and are therapeutically targetable (4,6,15). The vast majority of IDH^{mut} grade II/III glioma also harbor secondary genetic alterations in either *ATRX* and *TP53* (characteristic of astrocytoma) or CIC, FUBP1, chromosome 1p/19q co-deletion, and the *TERT* promoter (characteristic of oligodendroglioma) (4,6,15,28). In addition, tertiary driver alterations ultimately arise as IDH^{mut} lower-grade gliomas progress to anaplastic gliomas and secondary GBM (4,5,15,28–30).

In this article, we review the preclinical evidence in gliomas and cancer cell types on the induction of epigenetic dysregulation in response to D-2HG and examine a number of studies that have investigated the extent to which these processes are reversible, with an emphasis on glioma models. In addition, we examine emerging lines of evidence related to altered DNA damage and repair mechanisms in IDH mutant gliomas, particularly the use of PARP inhibitors. We summarize recent preclinical studies that have tested PARP inhibitors with or without alkylating agents and radiation, and discuss ongoing clinical trials testing these approaches. In depth discussions of the biology and metabolism of IDH mutations (31–33), their relevance to non-invasive diagnostics (34), their role in glioma classification (35), and pharmacological efforts to target these mutations have been reviewed in detail elsewhere (36).

Reversibility and Persistence of IDH1-induced epigenetic effects

The first direct preclinical investigation of $IDH1^{mut}$ inhibition was published in 2013, when Rohle et al. reported that treatment with AGI-5198, an inhibitor of $IDH1^{R132H}$, slowed growth of a subcutaneous $IDH1^{mut}$ glioma xenograft in mice, but did not inhibit growth of an $IDH1^{WT}$ xenograft (37). Following this initial report of preclinical efficacy by Rohle et al., the efficacy of $IDH1^{R132H}$ inhibition in preclinical glioma models has been at

best inconsistent and in some cases clearly ineffective, suggesting that the results presented by Rohle et al. in 2013 may not be robustly reproducible across a wide range of model systems and conditions (38–40). Hypothetically, one reason for the observed lack of efficacy in preclinical glioma models is that virtually all established IDH1^{mut} glioma cell lines are derived from grade III or grade IV tumors that have acquired tertiary genetic alterations in mitogenic signaling pathways (29,41). Such tertiary driver alterations may predominate over *IDH1* mutations as drivers of high-grade gliomas (29), thus rendering the continued D-2HG production non-essential during disease progression.

On the other hand, multiple lines of evidence suggest that supraphysiological D-2HG levels produced by *IDH1/2* mutations are sufficient to inhibit αKG-dependent lysine demethylases and TET dioxygenases involved in DNA demethylation, which results in hypermethylation of histones and DNA and an inhibition of cellular differentiation (19,21,22,42,43). Therefore, the extent to which epigenetic dysregulation induced by IDH1/2 mutations is reversible remains a key question, particularly for gliomas, where preclinical results with IDH^{mut} inhibitors have been less promising.

In their initial study, Rohle et al. showed that treatment of mice with established TS603 xenografts with AGI-5198 slowed growth over a 21-day timecourse. The investigators observed that xenografts treated with AGI-5198 contained fewer nuclei that stained positive for histone H3 Lys^{9} trimethylation (H3K9^{me3}) compared to untreated xenografts. However, there were no significant differences in DNA methylation after AGI-5198 treatment (although the TS603 cell line was confirmed to exhibit the glioma CpG island methylator phenotype, G-CIMP) (37). Shortly thereafter, Turcan et al. reported that the DNA methyltransferase inhibitor decitabine, but not AGI-5198, inhibited anchorage-independent growth of TS603 colonies and induced significant DNA demethylation in this cell line (44). In 2015, Tateishi et al. found that treatment of mice bearing MGG152 orthotopic xenografts with AGI-5198 did not result in any significant changes in animal survival, orthotopic xenograft size, or expression of the proliferative marker Ki-67 (38). Further, inhibition of mutant IDH1 did not cause any detectable changes in H3K9^{me2} or H3K9^{me3} (38). Long-term incubation of IDH1^{mut} gliomas cells with AGI-5198 (12-months) did not result in observable differences in H3K9^{me3} or H3K27^{me3} and did not alter genome-wide patterns of DNA methylation. In fact, rather than inhibiting growth of IDH1^{mut} cell lines, Tateishi et al. reported a slight acceleration of proliferation after long-term incubation and significantly shorter survival of animals after transplantation of MGG152 cells (38). The results from these targeting studies collectively suggest that suppression of D-2HG via IDH^{mut} inhibition is unlikely to induce major changes in DNA and histone methylation, in spite of the well documented efficacy in suppressing D-2HG levels.

Several recent studies have used spontaneous or genetically-engineered loss of IDH^{mut} expression to investigate the reversibility of D-2HG-induced hypermethylation. In 2017, Turcan et al. used constitutive and doxycycline-inducible expression of IDH1^{R132H} in immortalized human astrocytes (IHA) to investigate the induction and reversibility of DNA hypermethylation, gene expression changes, and the histone methylation landscape (22). Using this approach, they compared the dynamic changes in the epigenetic landscape of their model system to the epigenetic state of gliomas and patient derived cell lines, including TS603 (22). The investigators found that expression of IDH1^{R132H} generated a small subpopulation (~2-6%) of IDH1^{R132H} CD24+ cells, which exhibited a stem-like gene expression profile and formed significantly more colonies in soft agar relative to IDH1^{R132H} CD24- IHAs (22). IDH1^{R132H} expression induced progressive increases in H3K4^{me2}, H3K4^{me3}, H3K4^{me3}, H3K9^{me2}, H3K9^{me3}, and H3K36^{me3}, as well as significant increases in hypermethylated DNA loci at similar regions to hypermethylated loci in the IDH1^{R132H} cell line TS603. Importantly, Turcan et al. found that the vast majority of methylated loci ultimately returned to basal levels following withdrawal of doxycycline (loss of IDH1^{R132H} and D-2HG). However, unsupervised hierarchical clustering of DNA methylation in IHAs that had previously expressed IDH1^{R132H} for 40 passages showed that it took approximately 20 passages after loss of IDH1^{R132H} expression for these cells to cluster with IHAs that had never expressed IDH1^{R132H}. Notably, a

subset of IDH1^{R132H}-induced methylated loci persisted even after long-term withdrawal of doxycycline for 40 passages (22).

In a longitudinal study of lower-grade gliomas, Mazor et al. reported recurrent copy number alterations at the *IDH1* locus at recurrence, which were associated with lower D-2HG production, maintenance of G-CIMP phenotypes, and clonal expansion of *IDH1* CNA (45). While the G-CIMP phenotype was retained in this study following loss of D-2HG production, the authors noted that CpG sites outside of CpG islands (CGI), were associated with decreased methylation and hypomethylated CpG sites in samples with *IDH1* CNAs. Although *IDH1* mutations are generally retained at recurrence, the investigators noted that their data may indicate that loss of IDH^{mut} is associated with an adaptive advantage, as those cells with *IDH1* CNAs, and therefore reduced D-2HG production, were observed to clonally expand in recurrent gliomas and patient-derived xenografts after serial passaging (45). These data may indicate that continued D-2HG production is not required for progression of IDH^{mut} gliomas.

Moure et al. recently used CRISPR/Cas9 gene editing to systematically delete individual *IDH1* alleles in patient-derived glioma cells (46). In this study, the IDH1^{R132H} allele was specifically deleted in 3 individual clones from a patient derived IDH1$^{R132H/WT}$ astrocytoma cell line, and the IDH1^{WT} allele was deleted from an IDH1$^{R132H/WT}$ secondary GBM cell line. In both cell lines, deletion of an individual *IDH1* allele was sufficient to ablate D-2HG production without inhibiting cellular proliferation. Further, *MGMT* promoter methylation and G-CIMP status was maintained in all clones, although there was a trend toward decreased methylation in clones that had lost D-2HG production, which was enriched in open sea genomic loci and CpG-island shores of transcription start sites (46).

Collectively, results from studies using pharmacological inhibition of IDH^{mut} or genetic deletion of IDH^{mut} alleles indicates that glioma recurrence and patient-derived cell line proliferation can occur in the absence of continued D-2HG production (38,44–46). Furthermore, many of the hypermethylated features of IDH^{mut} gliomas are maintained in the absence of D-2HG and may require many cell divisions to revert to baseline levels (22). Given these observations, it is tempting to conclude that pharmacological inhibition of IDH^{mut} alone may not be an effective way of preventing tumor recurrence or slowing tumor progression. However, one important caveat to that interpretation is that essentially all available glioma cell lines and xenografts used to study IDH^{mut} to-date are derived from anaplastic gliomas or secondary GBMs that have acquired tertiary driver alterations (29,41). Therefore, it is critically important to note that because we do not have patient-derived pre-clinical models of IDH^{mut} lower-grade gliomas that do not also harbor tertiary driver alterations, *there is an absence of preclinical evidence over whether patients with corresponding lower-grade diffuse gliomas could benefit from mutant IDH1/2 inhibition*. In other words, we cannot rule out the possibility that there remains a therapeutic opportunity for IDH^{mut} small molecule inhibitors for the treatment of IDH^{mut} grade II/III gliomas that have not yet acquired driver alterations in mitogenic-signaling oncogenic driver genes. Additionally, several studies have now reported that D-2HG produced by IDH^{mut} may elicit immunosuppressive effects (47–50). Such effects may be mediated by epigenetic regulation of glioma gene expression and effects on tumor-infiltrating immune cells within the glioma microenvironment. Therefore, it remains to be determined whether the combination of IDH^{mut} inhibition and anti-glioma immunotherapy may be a viable therapeutic strategy.

Exploiting altered DNA damage and repair responses in IDH1^{mut} gliomas.

Owing in large part to the limited promise of directly targeting IDH1^{mut} in preclinical glioma models (25,38–40,44), numerous studies published over the past several years have investigated synthetic lethal/sick approaches as alternatives for treating *IDH1/2* mutant gliomas (38,51,52). In particular, several recent studies have identified dysregulated DNA damage and repair processes in IDH^{mut} gliomas, and these findings have prompted the design of novel strategies to target these tumor-specific properties for therapeutic purposes,

including the commencement of several clinical trials to test the use of PARP inhibitors in combination with TMZ or radiation (27,52–54).

The initial study by Sulkowski et al. used HCT116 colorectal cancer cells, HeLa cells, and HEL (human erythroid leukemia) cells expressing $IDH1^{WT}$ or $IDH1^{R132H}$ and found that D-2HG produced by $IDH1^{R132H}$ impaired homologous recombination (HR) efficiency in these cell lines, thus conferring sensitivity to PARP1/2 inhibitors (52). The investigators also reported that IDH^{mut} patient-derived glioma cells exhibit elevated basal levels of DNA damage, and that IDH^{mut} patient-derived glioma cells, as well as $IDH1^{WT}$ cells treated with cell-permeable D-2HG, are more sensitive to the PARP1/2 inhibitor BMN-673 (talazoparib). Subsequently, Molenaar et al. reported that IDH^{mut} was similarly associated with sensitivity to PARP inhibitors in AML cells and that this sensitivity could be reversed by pharmacological inhibition of IDH^{mut} (54). A separate study, using a panel of IDH^{WT} and IDH^{mut} chondrosarcoma cell lines, found that sensitivity to talazoparib varied between cell lines and was independent of IDH^{mut} status (55). Notably, this study showed that talazoparib treatment acted synergistically with either TMZ or irradiation, but also noted that the observed talazoparib-mediated radiosensitizing effect was partially reversed when IDH^{mut} was inhibited in the JJ012 cell line (55). Using a panel of glioma cell lines, Tateishi et al. found that TMZ treatment induced activation of PARP and decreased levels of NAD+, which could then be further exploited by inhibiting NAD+ biosynthesis via NAMPT inhibition (53). In this study, addition of PARP inhibitors reversed the TMZ-induced sensitizing effect to NAMPT inhibition.

More recently, Higuchi et al. used isogenic pairs of glioma cells with or without RNAi-mediated MSH6 deficiency to model the emergence of mismatch repair (MMR) defects that is associated with resistance to alkylating agents in GBM (56). In this study, the investigators found that PARP inhibition alone did not significantly alter cell viability and this occurred independent of IDH^{mut} and MSH6 status. However, PARP inhibition was sufficient to reverse TMZ resistance that was acquired via MSH6 depletion (MMR deficiency) (56). Most recently, Wang et al. used a combination of genetically engineered astrocytes, glioma cell lines, and a cholangiocarcinoma cell line to investigate the relationship between IDH^{mut} status, PARP inhibitor sensitivity, and the response to radiotherapy. The investigators found that PARP inhibitor treatment enhanced the efficacy of radiotherapy in mice bearing $IDH1^{R132H}$ TS603 xenografts, as well as IDH^{mut} murine gliomas generated using the RCAS-TVA system (57). The PARP inhibitor-mediated radiosensitizing effect was not observed in IDH^{WT} xenografts (58).

Collectively, the findings described above have moved rapidly from initial reports to translation into the clinic, with at least two clinical trials are underway to investigate the role of PARP inhibitors in combination with TMZ for the treatment of IDH^{mut} gliomas (NCT03914742, NCT03749187) (**Table 1**). It is important to note, however, that the preclinical results have been somewhat varied with respect to IDH^{mut} status and PARP inhibitor sensitivity across different model systems and cell types. Further, the benefit of PARP inhibition for glioma therapy appears to be most promising in combination with either TMZ or radiotherapy and available evidence to date suggests that this may not necessarily be specific to IDH^{mut} gliomas. (56,58) Further, the use of PARP inhibitors in IDH^{mut} astroctyomas based on a putative HR-mediated DNA repair defect is somewhat unclear for this glioma subtype(52), as the vast majority of $IDH1^{mut}$ astrocytomas produce high levels of D-2HG while maintaining cellular immortality via alternative lengthening of telomeres (**ALT**), a mechanism of telomere maintenance that utilizes HR to maintain telomere length (15,59,60). Therefore, it is unclear whether D-2HG produced in $IDH1^{mut}$ astrocytomas impairs HR efficiency in this glioma subtype.

Conclusion

The studies described above serve to highlight the emerging strategies in which the activity of $IDH1^{mut}$ is intentionally maintained in order to exploit and target downstream synthetic lethal / sick sensitivities for glioma

therapy. In light of these findings, how should the role of IDH1 mutations in glioma be considered? Given the genetic evidence of IDH1 mutations as early, truncal mutations in gliomas, coupled with the epigenetic impact of D-2HG and inhibition of differentiation, it is relatively clear that D-2HG plays a central role in driving gliomagenesis in grade II/III gliomas (3,4,6,15,61). Given this conclusion, coupled with evidence from AML clinical trials showing mutant IDH inhibition induces therapeutic responses, it seems clear that these mutations function as early oncogenic drivers of grade II/III gliomas. However, as discussed above, emerging evidence also indicates that D-2HG produced by mutant IDH enzymes becomes non-essential for at least a subset of gliomas as they progress to higher-grade tumors, and this transition likely coincides with acquisition of tertiary driver alterations (29,41,45,46,62). Therefore, the role of *IDH1/2* mutations in gliomas appears to involve early essentiality followed by a transition to a passenger function during disease progression. Although the mechanism for such a transition requires further investigation, studies of spontaneous loss of D-2HG production or specific CRISPR/Cas9-mediated deletion of the $IDH1^{R132H}$ allele in patient-derived cells demonstrate that hypomethylation occurs predominantly at CpG island shores/shelves (45,46). An intriguing hypothesis stemming from this observation is that once D-2HG-mediated hypermethylation is established via TET hydroxylase inhibition, glioma cells are capable of maintaining G-CIMP independent of D-2HG through the actions of DNA methyltransferases (e.g. DNMT1).This may be consistent with loss of methylation at CpG island shores/shelves, which are susceptible to passive demethylation and more rapid turnover of methylation during cell division (63,64).

A better understanding of the role of $IDH1^{R132H}$ in gliomas, including the evolution of $IDH1^{R132H}$ function over the course of disease progression, is critically important given that emerging evidence from the field is beginning to inform divergent strategies for the treatment of IDH^{mut} gliomas (**Table 1**). On one hand, at least two clinical trials are underway to investigate the role of PARP inhibitors in combination with temozolomide for the treatment of IDH^{mut} gliomas (NCT03914742, NCT03749187) and both of these trials list prior use of small molecule mutant IDH1/2 inhibitors as an exclusion criterion. In contrast, numerous clinical trials investigating the efficacy of mutant IDH1/2 inhibitors in lower-grade gliomas (and other cancers) are ongoing (**Table 1**). Notably, the available clinical evidence to-date comes from a previous Phase I/II clinical trial that tested the combination of TMZ and veliparib in bevacizumab-naïve and bevacizumab-refractory cohorts. In this trial, there was no observed benefit in overall or progression-free survival with veliparib plus TMZ therapy, although IDH^{mut} status was not reported (65). To prioritize pre-clinical research for the benefit of glioma patients, an emphasis should be placed on investigating the robustness of PARP inhibitor sensitivity across various patient-derived cell lines and pre-clinical model systems. This should include testing of $IDH1^{R132H}$ inhibitors for inducing radiosensitivity or radioresistance, as well as testing HR-mediated DNA damage repair in patient-derived glioma models in the presence or absence of IDH^{mut} inhibitors. This approach will help to better understand the relationship between IDH^{mut} status, PARP inhibitor sensitivity, and the role that each of these plays in response to standard of care therapies. Finally, special attention should be paid to the glioma subtype in these studies to identify whether underlying genetic factors may play a role in IDH^{mut} inhibitor or PARP inhibitor response.

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NCT Number	Status	Conditions	Interventions	Phases	Strategy
NCT03666559	Not yet recruiting	Recurrent IDH1/2 Mutated Glioma	Azacitidine	Phase 2	Chemotherapy*
NCT03030066	Active, not recruiting	Glioma with IDH1-R132 mutation	DS-1001b	Not Applicable	IDH^{mut} inhibition
NCT02481154	Active, not recruiting	Solid tumor with IDH1 or IDH2 mutation	AG881	Phase 1	IDH^{mut} inhibition
NCT02073994	Active, not recruiting	Cholangiocarcinoma, Chondrosarcoma, Glioma, Other Advanced Solid Tumors	AG-120	Phase 1	IDH^{mut} inhibition
NCT03914742	Not yet recruiting	Recurrent glioma and glioblastoma with IDH1/2 mutations	PARP Inhibitor BGB-290; Temozolomide; Conventional Surgery	Phase 1/ Phase 2	Synthetic lethal/sick
NCT02333513	Unknown status	Recurrent High-grade Glioma	Lomustine/Vincristine/Procarbazine	Not Applicable	Chemotherapy
NCT03684811	Recruiting	Glioma/Glioblastoma; Hepatobiliary Tumors; Chondrosarcoma; Intrahepatic Cholangiocarcinoma; Other Solid Tumors with IDH1 Mutations	FT-2102; Azacitidine; Nivolumab; Gemcitabine and Cisplatin	Phase 1|Phase 2	IDH^{mut} inhibition
NCT03749187	Recruiting	Newly diagnosed or recurrent grade I-IV gliomas with IDH1/2 mutations in adolescents and young adults (ages 13-25)	Drug: PARP Inhibitor BGB-290|Drug: Temozolomide	Phase 1	Synthetic lethal/sick
NCT03343197	Active, not recruiting	Glioma	AG-120; AG881	Phase 1	
NCT04056910	Not yet recruiting	Advanced Solid Tumor; Contrast-enhancing IDH mutant glioma	Ivosidenib; nivolumab	Phase 2	IDH^{mut} inhibition; Immunotherapy
NCT02496741	Unknown status	Glioma, Cholangiocarcinoma,Chondrosarcoma	Metformin and chloroquine combination	Phase 1|Phase 2	Synthetic lethal/sick
NCT03718767	Recruiting	Recurrent glioma with IDH mutation	Nivolumab	Phase 2	Immunotherapy
NCT02746081	Active, not recruiting	Solid Tumors	BAY1436032	Phase 1	IDH^{mut} inhibition
NCT01358058	Active, not recruiting	Low Grade Glioma, WHO Grade 3 Glioma IDH1/2 Mutation and/or 1p/19q Codeletion	Proton radiation	Not Applicable	Radiation
NCT01534845	Unknown status	Anaplastic Glioma of Brain|Loss of Chromosomes 1p/19q	Temozolomide (Temodal)	Phase 2	Chemotherapy
NCT03180502	Recruiting	WHO grade II/III glioma with IDH mutation	Intensity-Modulated Radiation Therapy; Proton Beam Radiation Therapy; Temozolomide	Phase 2	Radiation
NCT02381886	Active, not recruiting	Advanced Malignancies with IDH1-R132 Mutations	IDH305	Phase 1	IDH^{mut} inhibition

Table 1. Ongoing clinical trials for the treatment of IDH^{mut} gliomas.

A number of clinical trials investigating therapies for the treatment of IDH^{mut} gliomas are focused on testing the direct inhibition of IDH^{mut} enzymes via small molecule inhibition. In contrast, several trials, including the use of PARP inhibitors, are being investigated to evaluate the potential for synthetic lethal/sick strategies that allow IDH^{mut} activity to continue while targeting a downstream vulnerability created by elevated levels of D-2HG or compensatory adaptations to IDH^{mut} activity.
*Azacytidine is a hypomethylation chemotherapeutic agent that is used to target (and potentially reverse) the downstream DNA hypermethylation caused by D-2HG-mediated inhibition of TET hydroxylases.