Inhibiting IDH mutations in low-grade glioma alters cellular function and the immune environment

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BACKGROUND

Isocitrate dehydrogenase (*IDH*) mutations in cancer

- Somatic mutations in the metabolic enzyme IDH occur in many cancers, with mutations in *IDH1* and *IDH2* occurring in approximately 80% and 4% of lower-grade gliomas (LGG; WHO grade 2/3), respectively[1-3]
- Mutant IDH (mIDH) proteins have a gain-of-function enzyme activity, catalyzing the reduction of alpha-ketoglutarate to the oncometabolite D-2-hydroxyglutarate (2-HG)[3,4]
  - 2-HG inhibits alpha-ketoglutarate–dependent enzymes, resulting in epigenetic dysregulation, impaired cellular differentiation, and oncogenesis[5-7]
- In preclinical models of leukemia, glioma, and sarcoma, inhibitors of mIDH enzymes blocked 2-HG production and showed antitumor activity[8,9]
- *IDH* mutations are associated with immune evasion in gliomas
  - m*IDH* gliomas exhibit fewer tumor-infiltrating lymphocytes and reduced protein expression of programmed death ligand 1 (PD-L1) than wild-type (WT) counterparts[10-15]
  - Mechanistically, 2-HG is immunosuppressive and plays a key role in modulating the tumor immune microenvironment in m*IDH* gliomas[10-13]
  - mIDH inhibition in combination with vaccine therapy or PD-L1 blockade increased tumor-infiltrating lymphocytes and improved survival in preclinical glioma models[10,12]

Inhibitors of mIDH

- **Ivosidenib** (IVO; AG-120) is a first-in-class, oral, small-molecule inhibitor of **mIDH1**
  - Approved by the US FDA for m*IDH1* relapsed/refractory acute myeloid leukemia and in a subset of patients with m*IDH1* newly diagnosed acute myeloid leukemia
  - In an ongoing phase 1 study including 66 patients with glioma, IVO was associated with a favorable safety profile and clinical activity at 500 mg once daily (QD)
    - Objective response rate of 5.7% (including 1 minor response) and 83% stable disease rate, with a median progression-free survival of 13 months in non-enhancing glioma (n = 35)[16]
- **Vorasidenib** (VOR; AG-881) is an oral, potent, reversible, brain-penetrant, pan-inhibitor of **mIDH1 and mIDH2**
  - In an ongoing phase 1 study including 52 patients with glioma, VOR was associated with a favorable safety profile and clinical activity at doses < 100 mg QD
    - Objective response rate of 18.2% (including 1 partial response and 3 minor responses) and 73% stable disease rate, with a median treatment duration of 25.8 months and a median progression-free survival of 31.4 months in non-enhancing glioma (n = 22)[17]
- In an ongoing perioperative study (ClinicalTrials.gov NCT03343197), IVO and VOR demonstrated brain penetrance and > 90% suppression of 2-HG in resected m*IDH1* gliomas after preoperative treatment for approximately 4 weeks[18]

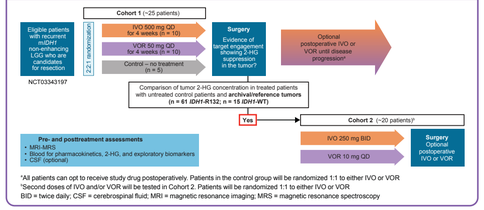
OBJECTIVES

- To understand the molecular and cellular mechanisms underlying mIDH inhibition by IVO or VOR in LGG using resected tumor tissues from patients with glioma enrolled in a phase 1 perioperative study (ClinicalTrials.gov NCT03343197)

METHODS

- The study schema for the phase 1 glioma perioperative study is shown in **Figure 1**

Figure 1. Study design



*All patients can opt to receive study drug postoperatively. Patients in the control group will be randomized 1:1 to either IVO or VOR
†Second doses of IVO and/or VOR will be tested in Cohort 2. Patients will be randomized 1:1 to either IVO or VOR
BID = twice daily; CSF = cerebrospinal fluid; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy

Sample collection

- Fresh frozen tissue samples included a set of banked reference *IDH* WT and m*IDH* LGG samples, as well as m*IDH* samples collected from enrolled patients who underwent surgery
- Formalin-fixed paraffin-embedded samples included paired archival (pretreatment) and surgical (posttreatment) samples from enrolled patients
- The actual number of patients with available data is noted in each figure

METHODS (CONTINUED)

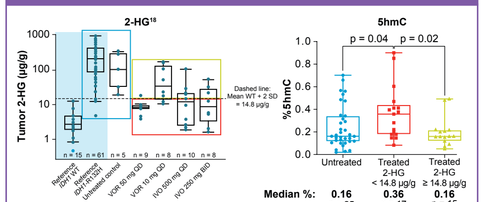
Biomarker assessment

- Next-generation sequencing: DNA and RNA samples were extracted from fresh frozen tumor tissues, and DNA sequencing and RNA sequencing were conducted at Personalis (Menlo Park, CA)
- 5-hydroxymethylcytosine (5hmC) analysis: DNA samples were digested with DNA degradase (Zymo Research, Irvine, CA) to generate single nucleosides; 5hmC, 5-methylcytosine, and cytosine were later quantified using liquid chromatography-tandem mass spectrometry
- RNA sequencing analysis: mRNA expression levels in transcripts per million (TPM; raw counts corrected for gene length and sequencing depth) were compared between untreated samples and samples treated with IDH inhibitors, and the significance of the difference was assessed using the Wald test
- T-cell receptor (TCR) sequencing was performed at Life Technologies Clinical Services Lab (Sacramento, CA) using Oncomine TCR Beta-SR Assay with RNA samples extracted from fresh frozen tissue
- Immunohistochemistry (IHC) for Ki-67, CD3, and CD8 was performed by Mosaic Laboratories (Lake Forest, CA), and quantification derived from an annotation including all tumor and intervening stroma within the tumor nest
- Multiplex IHC for CD68, CD163, and HLA-DR was carried out at Akoya Biosciences (Marlborough, MA), and image analysis performed using Phenochart software

RESULTS

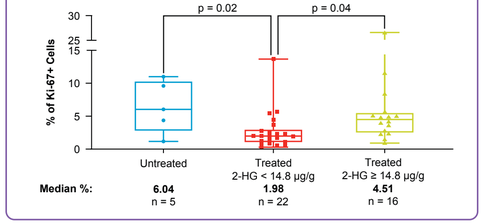
- mIDH inhibition increased 5hmC, a marker of DNA demethylation, suggesting reversal of DNA hypermethylation (**Figure 2**)
  - Optimal 2-HG suppression (defined as posttreatment 2-HG levels < 14.8 µg/g, which is the upper range of *IDH* WT tumor 2-HG levels) in IVO- or VOR-treated samples (n = 17) led to a ~2-fold increase in 5hmC compared with untreated controls and banked reference samples (n = 35; p = 0.04)

Figure 2. 2-HG and 5hmC levels following mIDH inhibition with IVO or VOR



- IHC analysis of the proliferation marker Ki-67 showed a ~3-fold decrease in the percentage of Ki-67–positive cells in IVO- or VOR-treated samples with optimal 2-HG suppression (n = 22) compared with untreated controls (n = 5; p = 0.02) (**Figure 3**)

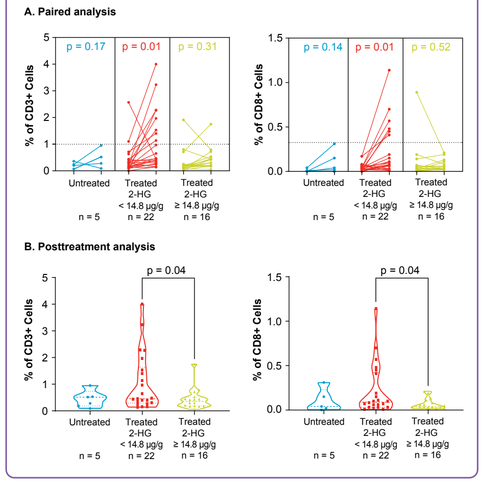
Figure 3. Tumor cell proliferation assessment by IHC following mIDH inhibition with IVO or VOR



RESULTS (CONTINUED)

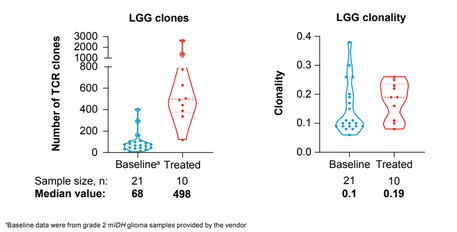
- IHC analysis from paired pretreatment (archival) and posttreatment samples showed an increase of CD3+ T-cell infiltration (p = 0.01) and CD8+ T-cell infiltration (p = 0.01) in IVO- or VOR-treated samples with optimal 2-HG suppression (n = 22) (**Figure 4A**)
- IHC analysis from posttreatment samples also showed an increase of CD3+ and CD8+ T-cell infiltration in IVO- or VOR-treated samples with optimal 2-HG suppression compared with untreated controls (**Figure 4B**)

Figure 4. T-cell infiltration assessment by CD3 IHC and CD8 IHC following mIDH inhibition with IVO or VOR



- mIDH inhibition increased the number of unique TCR clones as well as clonality assessed by TCRB sequencing (**Figure 5**)

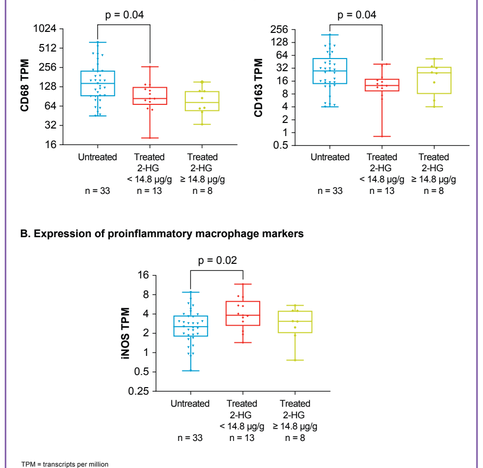
Figure 5. TCR clones and clonality assessment by TCRB sequencing following mIDH inhibition with IVO or VOR



[a]Baseline data were from grade 2 m*IDH* glioma samples provided by the vendor

- IDH inhibition decreased the expression of tumor-associated macrophage marker genes and increased the expression of proinflammatory macrophage marker genes in m*IDH* oligodendroglioma samples with optimal 2-HG suppression (**Figure 6**)

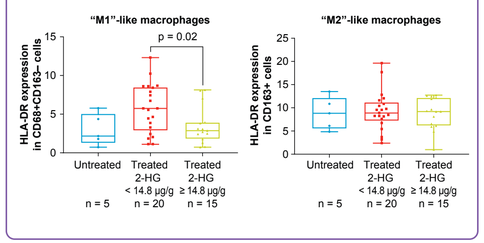
Figure 6. Expression of macrophage markers following mIDH inhibition with IVO or VOR



TPM = transcripts per million

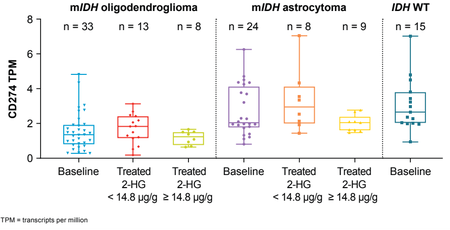
- mIDH inhibition increased the expression of HLA-DR in "M1"-like macrophages (p = 0.02) but not in "M2"-like macrophages in samples with optimal 2-HG suppression assessed by IHC (**Figure 7**)

Figure 7. Expression of HLA-DR following mIDH inhibition with IVO or VOR



- mIDH inhibition upregulated PD-L1 mRNA expression in samples with optimal 2-HG suppression (**Figure 8**)

Figure 8. Expression of PD-L1 mRNA following mIDH inhibition with IVO or VOR



TPM = transcripts per million

CONCLUSIONS

- **Overall, these findings suggest that both tumor-intrinsic and tumor-extrinsic mechanisms underlie mIDH inhibition by IVO or VOR treatment**

Tumor-intrinsic mechanisms	+	Tumor-extrinsic mechanisms
• Reversal of DNA hypermethylation • Inhibition of tumor cell proliferation		• Increase of T-cell infiltration, TCR clones, and clonality • Decrease of tumor-associated macrophage gene expression • Increase of proinflammatory macrophage gene expression • Increase of PD-L1 mRNA expression

- **These data support further development of mIDH inhibitors in m*IDH* LGG either as a monotherapy or in combination with immunotherapy**

Questions?
Please contact Min Lu, Min.Lu@agios.com.

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Disclosures
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