Combined use of the pan-IDH mutant inhibitor AG-881 with radiation therapy shows added benefit in an orthotopic IDH1 mutant glioma model in vivo
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**BACKGROUND**

- Somatic mutations in isocitrate dehydrogenase (IDH) 1 or 2 confer neomorphic enzyme activity, producing the oncometabolite 2-hydroxystermitrate (2-HG).\(^\text{1,2}\)
- Accumulation of 2-HG facilitates tumorigenesis through DNA hypermethylation, increased repressive histone methylation, and inhibition of differentiation processes.\(^\text{3,4}\)
- Over 70% of diffuse low-grade gliomas (LGG) harbor IDH1/2 mutations.\(^\text{5}\)
- Standard of care treatment for patients with diffuse LGG involves combined modality approaches, including surgery, radiation, and chemotherapy.\(^\text{5}\)
- Studies performed with an early tool compound, AGI-5198, which is active against the mutant IDH1 protein (mIDH1) but not mIDH2, have demonstrated that mIDH1 inhibition can repress growth of mIDH1-driven gliomas in some models but not others.\(^\text{6,7}\)
- Furthermore, recent in vitro studies using AGI-5198 in mIDH1 glioma models have suggested that 2-HG production by mIDH1 may radiosensitize glioma cells\(^\text{8}\) and that mIDH1 inhibition may result in a loss of radiosensitivity.\(^\text{9}\)
- mIDH1-driven LGGs have been found to be particularly sensitive to temozolomide (TMZ) treatment, presumably due to hypermethylation of the MGMT promoter.\(^\text{10}\)
  - There is potential for antagonism between mIDH1 inhibition and TMZ treatment, owing to the possibility of high levels of 2-HG leading to or enforcing hypermethylation of the MGMT promoter.
- AG-881 is a novel, potent inhibitor of both the mIDH1 and mIDH2 proteins that is currently in phase 1 clinical development in patients with advanced mIDH1 or mIDH2 solid tumors, including gliomas (ClinicalTrials.gov NCT02481154) and hematologic malignancies (ClinicalTrials.gov NCT02492737).
  - As demonstrated previously, AG-881 is highly brain penetrant.\(^\text{11}\)

**OBJECTIVES**

- In a mouse xenograft model of a human mIDH1-R132H grade 3 oligodendroglioma, we assessed the potential for antagonism between 2-HG suppression using the pan-IDH mutant inhibitor AG-881 and:
  - Radiation therapy (RT)\(^\text{12}\)
  - TMZ treatment.

**METHODS**

**Orthotopic mouse xenograft model of human grade 3 mIDH1-R132H glioma**

- T9306 glioma cells with an endogenous heterozygous IDH1-R132H mutation\(^\text{5}\) (5×10⁵ cells) were intracranially implanted into female CB17 SCID mice.\(^\text{7}\)
- The T9306 cell line was derived from a patient with grade 3 anaplastic oligodendrogliomas, and also harbors a co-deletion of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q).\(^\text{7}\)
- Following tumor engraftment (12 days), animals were treated with vehicle, AG-881, RT, or AG-881 in combination with RT (AG-881 + RT), and pharmacokinetics (PK), pharmacodynamics (PD), tumor volume, and survival were assessed, as shown in Figure 1A.

**Subcutaneous mouse xenograft model of human grade 3 mIDH1-R132H glioma**

- Male ICR SCID mice were injected subcutaneously with 10⁴ T9306 glioma cells.\(^\text{7}\)
- Following tumor engraftment for 34–44 days, animals were treated with vehicle, AG-881, TMZ, or AG-881 + TMZ and assessed for tumor volume as shown in Figure 1B.

**RESULTS**

**Orthotopic grade 3 mIDH1 glioma model**

- AG-881 readily crossed the blood-brain barrier and strongly inhibited 2-HG production in mIDH1 brain tumor xenografts (Figure 2).
- mIDH1 inhibition with AG-881 modestly impeded glioma growth in vivo. The combination of AG-881 + RT produced significantly greater inhibition of tumor growth than either of the monotherapies (Figure 3).
  - AG-881 did not antagonize the survival benefit conferred by RT (Figure 4).

**Subcutaneous grade 3 mIDH1 glioma model**

- AG-881 treatment impeded in vivo tumor growth (Figure 5).
- AG-881 treatment did not antagonize TMZ efficacy (Figure 6).
- 2-HG concentrations were reduced to baseline levels in terminal tumor samples from animals treated with AG-881 alone or in combination with TMZ, but remained elevated in mice treated with TMZ alone (Figure 7).

**CONCLUSIONS**

- In an orthotopic human grade 3 mIDH1-R132H glioma mouse model:
  - Treatment with the highly brain penetrant mIDH1 inhibitor AG-881 resulted in >98% inhibition of 2-HG levels in brain tumor xenografts.
  - mIDH1 inhibition by AG-881 impeded glioma growth in vivo.
  - The combination of AG-881 + RT produced a significantly greater inhibitory effect on tumor growth than each single-modality treatment.
- In a subcutaneous human grade 3 mIDH1-R132H glioma model:
  - AG-881 treatment resulted in inhibition of 2-HG to baseline levels in tumors.
  - AG-881 had an inhibitory effect on glioma growth.
- Our findings do not support previous in vitro nonclinical work\(^\text{12}\) that suggested a potential antagonism between mIDH1 inhibition and RT.

**DISCUSSION**

This work was funded by Agios Pharmaceuticals, Inc., Boehringer Ingelheim, USA, and Viasat Inc. – intellectual property and regulatory support. The authors declare no conflicts of interest.