EXTH-59

The IDH1 mutant inhibitor AG-120 shows strong inhibition of 2-HG production in an orthotopic IDH1 mutant glioma model *in vivo*

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BACKGROUND

- Somatic mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 and 2 confer neomorphic enzymatic activity, which results in the accumulation of the oncometabolite 2-hydroxyglutarate (2-HG).[1,2]
- 2-HG drives multiple oncogenic processes, including increased histone and DNA methylation, leading to a block in cellular differentiation.[3,4]
- The threshold of inhibition of 2-HG production required for antitumor activity remains to be defined.
- IDH1/2 mutations occur in >70% of diffuse low-grade gliomas (LGG).[5]
- Standard of care treatment for patients with diffuse LGG involves combined modality approaches, including surgery, radiation, and chemotherapy.[6]
- Previously reported data suggested that 2-HG production by the mutant IDH1 (mIDH1) protein radiosensitizes glioma cells[7-10] and that inhibition of mIDH1 resulted in a loss of radiosensitivity *in vitro*.[10]
- AG-120 (ivosidenib) is an orally available, potent, targeted inhibitor of the mIDH1 protein that is currently being assessed in two clinical trials in solid tumors: ClinicalTrials.gov NCT02073994 and NCT02989857.
  - Clinical data from the subset of patients with nonenhancing glioma were reported in oral presentation ACTR-46.

OBJECTIVES

- Validate that AG-120 crosses the blood-brain barrier and inhibits 2-HG production in an orthotopic mouse xenograft model of a human mIDH1-R132H glioma.
- Determine whether AG-120 treatment antagonizes the efficacy of radiation therapy (RT) *in vivo*.

METHODS

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

- TS603 glioma cells with an endogenous heterozygous IDH1-R132H mutation (5×10^4 cells) were intracranially implanted into female CB17 SCID mice on Day 0.[11]
- The TS603 cell line was derived from a patient with grade 3 anaplastic oligodendroglioma, and also harbors a co-deletion of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q).[11]
- Assessment of pharmacokinetics (PK) and pharmacodynamics (PD) (**Figure 1A**):
  - Following tumor engraftment for 40 days, mice were randomized to receive either vehicle (n=12) or AG-120 50, 150, or 450 mg/kg orally (PO) twice daily (BID) (n=16 each) for 4 days.
  - At 1, 4, 12, and 24 hr after the last dose of AG-120, three mice in the vehicle group and four mice in each AG-120 dose group were sacrificed, and plasma, brain, and brain tumor samples were analyzed for AG-120 and 2-HG.
- Evaluation of AG-120 in combination with RT (**Figure 1B**):
  - Following tumor engraftment for 36 days, 60 mice were randomized into five treatment groups (n=12 each) and treated with either vehicle, RT (2 Gy focal radiation once daily [QD]), and/or AG-120 (150 mg/kg PO BID) as indicated for 21 days (Days 37–57).
  - Tumor volume was measured by magnetic resonance imaging (MRI) on Day 36 and every 3–5 days during dosing, and survival was assessed from Day 0 to Day 85.

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

- Male ICR SCID mice were injected subcutaneously with 10^6 TS603 glioma cells (**Figure 1C**).
- Following tumor engraftment for 43 days, 24 mice were randomized to receive either vehicle (n=12) or AG-120 (150 mg/kg PO BID, n=12) for 21 days.
- Tumor volume was assessed every 3–4 days during dosing.

RESULTS

Validation of 2-HG inhibition by AG-120 in orthotopic grade 3 mIDH1 glioma

- AG-120 strongly inhibited 2-HG production in human mIDH1 brain tumor xenografts in mice dosed at 50, 150, or 450 mg/kg PO BID (**Figure 2**).
- AG-120 was detectable in the brain and brain tumor tissues of the mice, although at much lower exposures than in the plasma (**Table 1**).

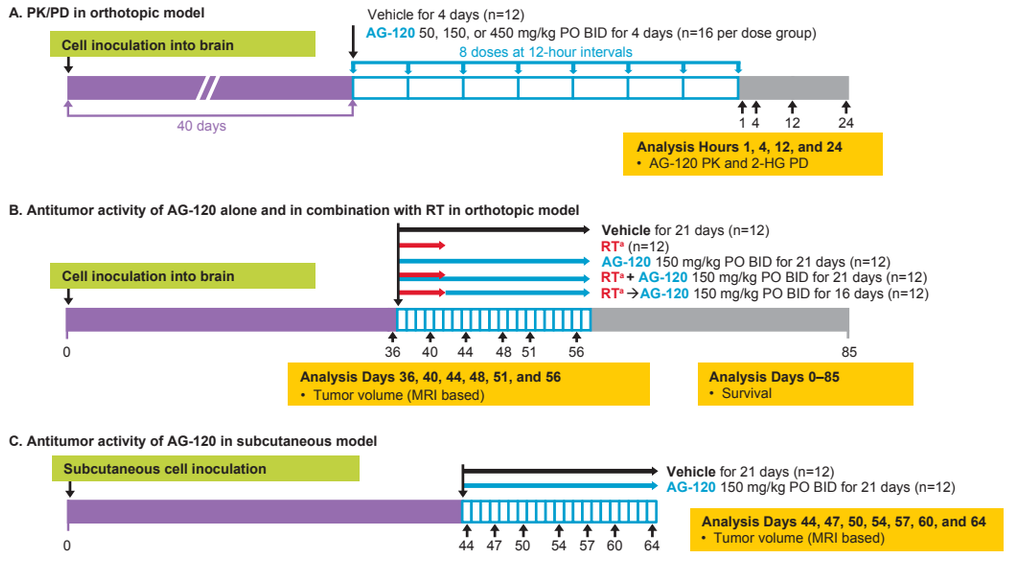
AG-120 treatment did not antagonize RT efficacy in orthotopic grade 3 mIDH1 glioma

- AG-120 dosed at 150 mg/kg PO BID, which inhibits tumor 2-HG production by 77–79% (**Figure 2**), did not have an antagonistic effect on the antitumor activity of RT, but did not confer monotherapy benefit in this model (**Figure 3**).
- Likewise, AG-120 (150 mg/kg PO BID) did not antagonize the RT survival benefit, but AG-120 alone did not confer a survival benefit in this model (**Figure 4**).

AG-120 treatment reduced *in vivo* growth of a subcutaneous grade 3 mIDH1 glioma

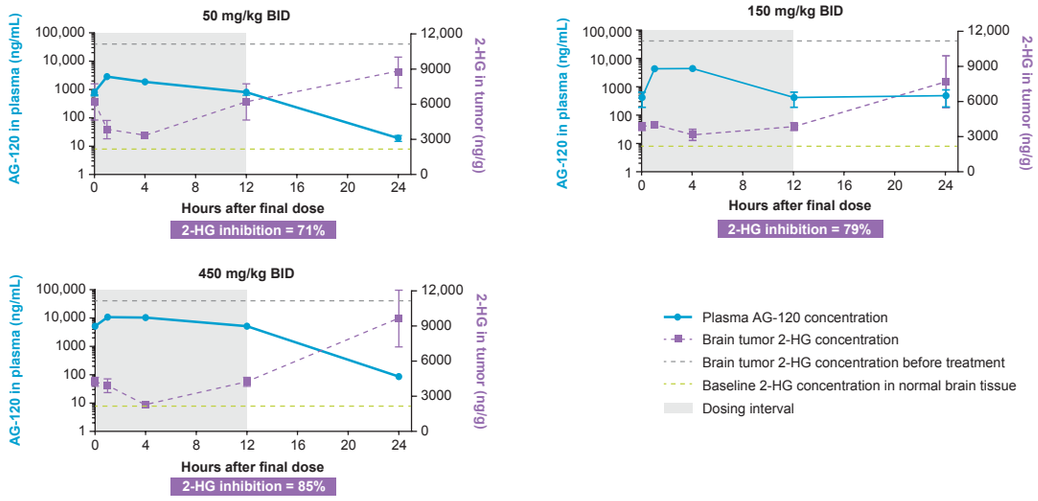
- AG-120 (150 mg/kg PO BID) inhibited 2-HG production by >80% and reduced *in vivo* growth of a subcutaneous grade 3 mIDH1 glioma by 52% (**Figure 5**).

Figure 1. Human grade 3 mIDH1-R132H glioma mouse xenograft model development and *in vivo* study designs



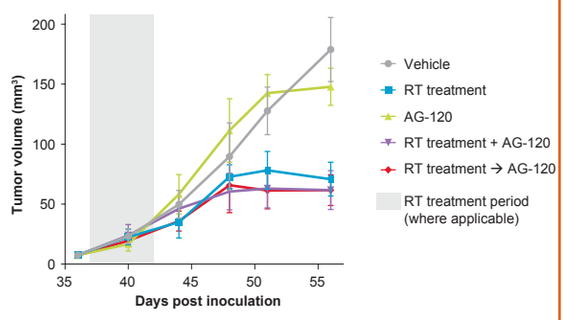
[a]RT: focal radiation (2 Gy QD) for 5 days

Figure 2. AG-120 concentration in plasma and 2-HG concentration in orthotopic grade 3 mIDH1 glioma after eight oral doses



AG-120 treatment: eight oral doses at 12-hr intervals. The grayed area represents the dose interval; the 24-hr time point was included to assess how long 2-HG inhibition was sustained in the brain tumor. The percentage inhibition of 2-HG is based on the area under the curve from 0 to 12 hr ($AUC_{0\text{-}12hr}$)

Figure 3. No antagonistic effect of AG-120 treatment on RT efficacy in orthotopic grade 3 mIDH1 glioma

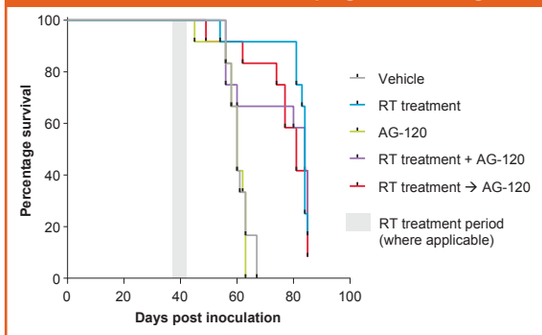


Median tumor volume (MRI based) ± median absolute distribution
RT: focal radiation (2 Gy QD) for 5 days
AG-120 treatment: 150 mg/kg PO BID for 16 or 21 days

Table 1. Brain penetrance of AG-120

	50 mg/kg BID	150 mg/kg BID	450 mg/kg BID
Brain $AUC_{0\text{-}12hr}$ (hr•ng/g)	727	1480	3550
Plasma $AUC_{0\text{-}12hr}$ (hr•ng/mL)	19,200	37,400	99,400
Brain-to-plasma ratio	0.037	0.039	0.035
% 2-HG inhibition ($AUC_{0\text{-}12hr}$)	71	79	85

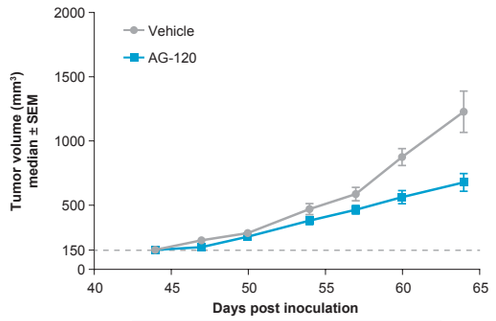
Figure 4. No antagonistic effect of AG-120 treatment on RT survival benefit in orthotopic grade 3 mIDH1 glioma



Group	Median survival (days)	p-value compared with vehicle[a]
Vehicle	60	–
AG-120	60	ns
RT	84	<0.0001
RT + AG-120	84	<0.0001
RT → AG-120	81	<0.0045

RT: focal radiation (2 Gy QD) for 5 days
AG-120 treatment: 150 mg/kg PO BID for 16 or 21 days
[a]p-values from Mantel-Cox tests
ns = not significant

Figure 5. Inhibition of 2-HG production and tumor growth by AG-120 in a subcutaneous grade 3 mIDH1 glioma



	2-HG tumor AUC (hr•ng/g)	% 2-HG inhibition
Vehicle	671,000	–
AG-120	105,000	84.4

AG-120 150 mg/kg PO BID for 21 days
SEM = standard error of the mean; TGI = tumor growth inhibition

CONCLUSIONS

- In mice engrafted with orthotopic human grade 3 mIDH1-R132H gliomas:
  - AG-120 had very low brain penetrance following oral administration, but sufficient AG-120 brain exposure was achieved to confer a dose-dependent reduction in 2-HG levels in brain tumors, with 85% maximal inhibition achieved.
  - Inhibition of 2-HG by 79% did not confer an antitumor effect in this model.
  - The combination of AG-120 + RT demonstrated no antagonism of RT efficacy.
- In a subcutaneous human grade 3 mIDH1-R132H glioma mouse model, mIDH1 inhibition by AG-120 impeded tumor growth *in vivo* after achieving >84% 2-HG production inhibition.
- These observations support the clinical investigation of AG-120 in patients with mIDH1-driven gliomas.
- Our findings do not support previous *in vitro* nonclinical work[10] that suggested a potential antagonism between mIDH1 inhibition and RT.

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