EXTH-34

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-hydroxyglutarate (2-HG).[1,2]
- Accumulation of 2-HG facilitates tumorigenesis through DNA hypermethylation, increased repressive histone methylation, and inhibition of differentiation processes.[3,4]
- Over 70% of diffuse low-grade gliomas (LGG) harbor IDH1/2 mutations.[5]
- Standard of care treatment for patients with diffuse LGG involves combined modality approaches, including surgery, radiation, and chemotherapy.[6]
- 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.[7,8]
- Furthermore, recent *in vitro* studies using AGI-5198 in mIDH1 glioma models have suggested that 2-HG production by mIDH1 may radiosensitize glioma cells[9-12] and that mIDH1 inhibition may result in a loss of radiosensitivity.[12]
- mIDH1-driven LGGs have been found to be particularly sensitive to temozolomide (TMZ) treatment, presumably due to hypermethylation of the MGMT promoter.[13-15]
  - There is potential for antagonism between mIDH inhibition and TMZ treatment, owing to the possibility of high levels of 2-HG leading to and/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.[16]

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)
  - TMZ treatment.

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.[7]
- 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).[7]
- Following tumor engraftment (tumor size 5–20 mm^3), 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 10^6 TS603 glioma cells.
- 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

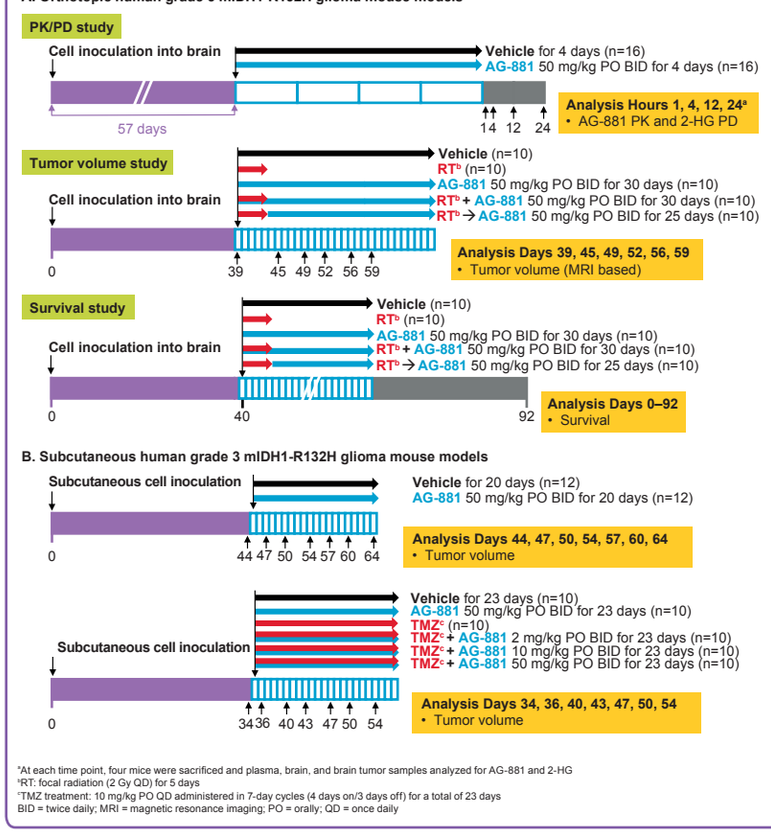
Orthotopic grade 3 mIDH1 glioma model

- 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

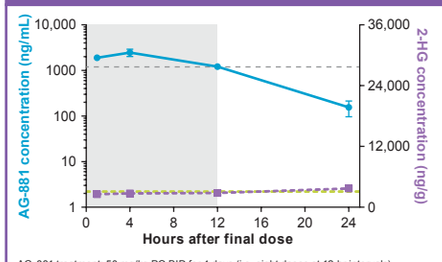
- AG-881 treatment impeded *in vivo* tumor growth (**Figure 5**).
- AG-881 treatment did not antagonize TMZ efficacy (**Figure 6**).
- 2-HG concentrations were reduced to baseline levels in terminal tumor samples from animals treated with AG-881 alone or in combination with TMZ, but remained elevated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

Figure 1. Study designs



[a]At each time point, four mice were sacrificed and plasma, brain, and brain tumor samples analyzed for AG-881 and 2-HG
[b]RT: focal radiation (2 Gy QD) for 5 days
[c]TMZ treatment: 10 mg/kg PO QD administered in 7-day cycles (4 days on/3 days off) for a total of 23 days
BID = twice daily; MRI = magnetic resonance imaging; PO = orally; QD = once daily

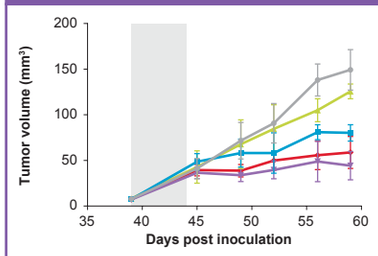
Figure 2. AG-881 inhibited 2-HG in an orthotopic grade 3 mIDH1 glioma model



Parameter	Value
Brain $AUC_{0\text{-}12hr}$ (hr•ng/g)	30,300
Plasma $AUC_{0\text{-}12hr}$ (hr•ng/mL)	22,800
% 2-HG inhibition ($AUC_{0\text{-}12hr}$)[b]	97.8%
Whole brain-to-plasma ratio	1.33
Brain tumor-to-plasma ratio	1.25

AG-881 treatment: 50 mg/kg PO BID for 4 days (i.e. eight doses at 12-hr intervals)
[a]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
[b]Established after subtracting 2-HG $AUC_{0\text{-}12hr}$ of "normal" brain observed in left hemisphere of brains in all animals from brain tumor $AUC_{0\text{-}12hr}$
$AUC_{0\text{-}12hr}$ = area under the curve from 0 to 12 hr

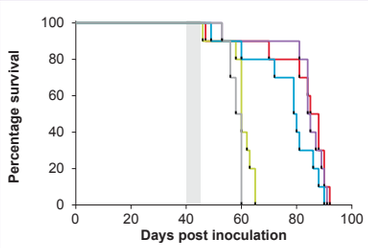
Figure 3. No antagonistic effect of AG-881 on RT efficacy in an orthotopic grade 3 mIDH1 glioma model



Group	p-value compared with vehicle	p-value compared with RT
AG-881	0.011	–
RT	0.0001	–
RT + AG-881	<0.0001	0.052
RT → AG-881	<0.0001	0.002

Median tumor volume (MRI based) ± median absolute distribution
RT: focal radiation (2 Gy QD) for 5 days
AG-881 treatment: 50 mg/kg PO BID for 25 or 30 days

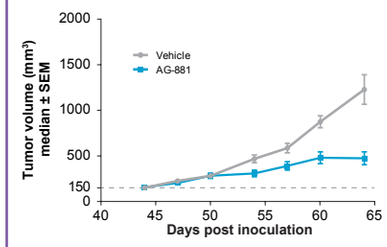
Figure 4. No antagonistic effect of AG-881 on RT survival in an orthotopic grade 3 mIDH1 glioma model



Group	Median survival (days)	p-value compared with vehicle[a]
Vehicle	59	–
AG-881	60	ns
RT	79.5	<0.001
RT + AG-881	84.5	<0.0003
RT → AG-881	86.5	<0.0004

RT: focal radiation (2 Gy QD) for 5 days
AG-881 treatment: 50 mg/kg PO BID for 25 or 30 days
[a]p-values from Mantel-Cox tests. No significant difference observed between RT and RT + AG-881 (p=0.13) or RT → AG-881 (p=0.12)
ns = not significant

Figure 5. AG-881 impeded *in vivo* tumor growth in a subcutaneous grade 3 mIDH1 glioma model

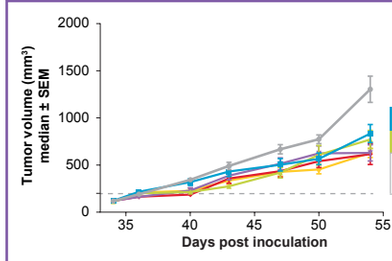


	2-HG tumor AUC (hr•ng/mg)	% 2-HG inhibition
Vehicle	671,000	–
AG-881	28,200	95.8

TGI terminal AG-881/vehicle = 71% (p<0.002)

AG-881 treatment: 50 mg/kg PO BID for 20 days
SEM = standard error of the mean; TGI = tumor growth inhibition

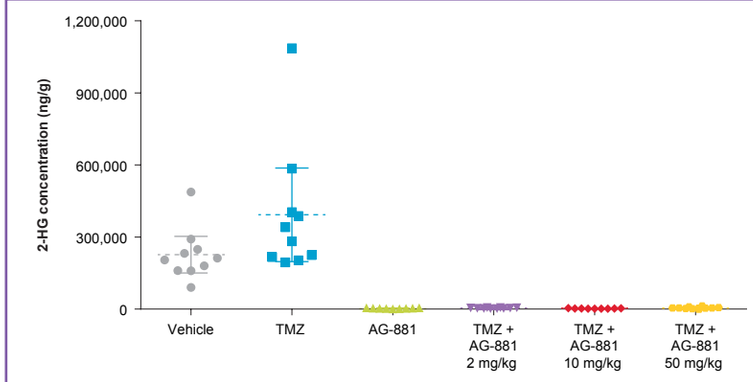
Figure 6. No antagonistic effect of AG-881 on TMZ efficacy in a subcutaneous grade 3 mIDH1 glioma model



TGI terminal TMZ/vehicle = 34% (p<0.02)
TGI terminal AG-881/vehicle = 41% (p<0.02)
No statistical difference between single agents and combination

AG-881 treatment: 2, 10, or 50 mg/kg PO BID for 23 days
TMZ treatment: 10 mg/kg PO QD administered in 7-day cycles (4 days on/3 days off) for a total of 23 days

Figure 7. AG-881 reduced 2-HG concentrations to baseline level in a subcutaneous grade 3 mIDH1 glioma model



AG-881 treatment: 2, 10, or 50 mg/kg PO BID for 23 days
TMZ treatment: 10 mg/kg PO QD administered in 7-day cycles (4 days on/3 days off) for a total of 23 days

CONCLUSIONS

- In an orthotopic human grade 3 mIDH1-R132H glioma mouse model:
  - Treatment with the highly brain penetrant mIDH1/2 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 mouse model:
  - AG-881 treatment resulted in inhibition of 2-HG to baseline levels in tumors.
  - AG-881 had an inhibitory effect on glioma growth.
  - No antagonism with TMZ was observed.
- These observations support the clinical investigation of AG-881 in patients with mIDH1-driven gliomas.
- Our findings do not support previous *in vitro* nonclinical work[12] that suggested a potential antagonism between mIDH1 inhibition and RT.

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