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AG-881, a brain-penetrant, potent, pan-mutant IDH (mIDH) inhibitor for use in mIDH solid and hematologic malignancies

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BACKGROUND

- Somatic point mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 and 2 occur in multiple solid and hematologic tumors, including acute myeloid leukemia (AML) and gliomas.[1-3]
- The mutant IDH (mIDH) enzymes have a gain-of-function activity, catalyzing the reduction of alpha-ketoglutarate (α-KG) to produce the oncometabolite D-2-hydroxyglutarate (2-HG).[4,5]
- *In vitro* studies suggest that accumulation of 2-HG leads to epigenetic alterations that block cellular differentiation, thereby promoting oncogenesis.[6-8]
- Small molecule inhibition of the mIDH protein represents a targeted approach to cancer treatment for patients with tumors that harbor an IDH1 and/or an IDH2 mutation.
- Direct inhibition of the gain-of-function activity of the mIDH protein is intended to inhibit 2-HG production and induce tumor cell differentiation.
- Here, we present AG-881, an orally available, potent, small molecule inhibitor of the IDH1 **and** IDH2 mutant proteins that can penetrate the blood-brain barrier.

OBJECTIVE

- To disclose the binding site of AG-881 along with the drug properties, including biochemical half-maximal inhibitory concentration (IC_{50}) values, cell-based IC_{50} values, dual mIDH1/2 inhibition, brain penetration, and pharmacokinetic/pharmacodynamic (PK/PD) profile.

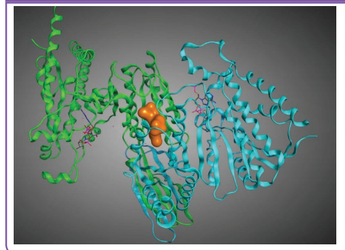
METHODS

- High-resolution X-ray crystal structures of AG-881 in complex with mIDH1-R132H and mIDH2-R140Q were generated and overlaid.
- *In vitro* enzyme activity assays were performed as previously described.[9]
  - mIDH activity (conversion of α-KG and NAPDH to 2-HG and $NADP^+$) was measured in an end-point assay of NADPH depletion.
  - NADPH consumption was coupled to diaphorase-catalyzed conversion of resazurin to fluorescent resorufin (excitation 544 nm, emission 590 nm).
- Cell line assays: cells were seeded into 96-well plates and AG-881 added to generate a 7-point dose-response assay in duplicate. Doses were usually started at 3 μM or 100 nM with 1:3 or 1:10 dilutions in dimethyl sulfoxide (DMSO). Control was 0.1% DMSO.
  - 2-HG inhibition was assessed by assaying 2-HG in medium after incubation for 48 hr (72 hr for TF-1 cells).
  - For cell growth assays, cells were incubated as above for a further 24 hr and total cellular ATP measured.
- Primary human patient samples: blood- or bone marrow-derived AML blasts were sorted and cultured in serum-free medium for 6 days in the presence of 0.5 or 1 μM AG-881 or vehicle (0.1% v/v DMSO).
  - 2-HG levels in cell supernatants were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).
  - Fluorescence-activated cell sorting (FACS) using anti-CD15 (H198 clone), anti-CD24 (ML5 clone), and anti-CD11b (ICRF44 clone) antibodies was performed to assess effects on differentiation.
- Brain and cerebrospinal fluid penetration of AG-881 was assessed in mouse, rat, and cynomolgus monkey after oral doses.
- *In vivo* inhibition of 2-HG production was assessed in a TS603 IDH1-R132H orthotopic xenograft mouse glioma model after repeated oral doses of AG-881.

RESULTS

- Co-crystal structures of AG-881 with IDH1-R132H and IDH2-R140Q showed that AG-881 binds to mIDH enzymes at an allosteric site (**Figure 1**).

Figure 1. Co-crystal structure of AG-881 with IDH1-R132H



Co-crystal structures of AG-881 with IDH1-R132H and IDH2-R140Q were obtained with 2.1 Å and 1.99 Å resolution, respectively. Both co-crystal structures overlay well and AG-881 binds at an allosteric site bounded by the central four helices, as depicted for IDH1-R132H. Similar results were obtained with IDH2-R140Q.

The two monomers of the IDH1-R132H dimer are shown in green and blue. AG-881 is shown in a space-filling representation in orange.

The AG-881*IDH2-R140Q structure is not shown for clarity.

Biochemical profiling

- Biochemical studies demonstrated that AG-881 has low nanomolar potency (IC_{50}) against multiple mIDH enzymes (**Tables 1** and **2**).
  - It is a rapid-equilibrium inhibitor of the mIDH1-R132H, mIDH1-R132C, and mIDH2-R172K homodimers.
  - It is a slow-binding inhibitor of the mIDH2-R140Q homodimer and wtIDH1/mIDH1-R132H, wtIDH2/mIDH2-R140Q, and wtIDH2/mIDH2-R172K heterodimers.

Table 1. Potency of AG-881 against mIDH1 enzymes

IDH1 enzyme	Preincubation time	IC_{50} (μM), mean ± SD	Max inhibition (%), mean ± SD	Repeats (n)
mIDH1-R132H homodimer	1 hr	0.006 ± 0.002	92.6 ± 5.3	20
	16 hr	0.008	76.4	1
mIDH1-R132C homodimer	1 hr	0.019 ± 0.004	67.0 ± 4.4	10
mIDH1-R132G homodimer	1 hr	0.017 ± 0.002	115.0 ± 4.9	9
mIDH1-R132L homodimer	1 hr	0.034 ± 0.005	80.0 ± 1.0	9
mIDH1-R132S homodimer	1 hr	0.006 ± 0.003	96.7 ± 8.8	9
wtIDH1/mIDH1-R132H heterodimer, reverse (mutant) direction	1 hr	0.004 ± 0.001	110.3 ± 3.8	8
	16 hr	0.0006 ± 0.0001	115.4 ± 6.4	9

Table 2. Potency of AG-881 against mIDH2 enzymes

IDH2 enzyme	Preincubation time	IC_{50} (μM), mean ± SD	Max inhibition (%), mean ± SD	Repeats (n)
mIDH2-R140Q homodimer	1 hr	0.118 ± 0.014	112.8 ± 5.8	9
	16 hr	0.012 ± 0.002	102.7 ± 3.2	9
mIDH2-R172K homodimer	1 hr	0.032 ± 0.004	88.6 ± 3.4	9
	16 hr	0.094 ± 0.016	61.6 ± 3.7	9
wtIDH2/mIDH2-R140Q heterodimer, reverse (mutant) direction	1 hr	0.251 ± 0.037	81.8 ± 1.0	9
	16 hr	0.032 ± 0.005	105.3 ± 2.8	9
wtIDH2/mIDH2-R172K heterodimer, reverse (mutant) direction	1 hr	0.049 ± 0.005	98.8 ± 1.2	9
	16 hr	0.008 ± 0.002	87.8 ± 3.3	9

Cell-based assays

- The potency of AG-881 against mIDH1 and mIDH2 enzymes was also shown in cell lines.
- The IC_{50} range for 2-HG inhibition by AG-881 was 0.04–22 nM in cells expressing mIDH1-R132C, mIDH1-R132G, mIDH1-R132H, or mIDH1-R132S mutations and was 7–14 nM and 130 nM in cells expressing mIDH2-R140Q and mIDH2-R172K mutations, respectively (**Table 3**).

Table 3. 2-HG inhibition in cells expressing mIDH1 and mIDH2

Cell line	2-HG IC_{50} (nM), mean ± SD	Max inhibition (%)	Replicates (n)	GI_{50} 3 μM top dose
Neurospheres TS603 (mIDH1-R132H)[a]	0.250 ± 0.16	97	43	No fit
Neurospheres HK213 (mIDH1-R132H)[a]	0.043	92	1	ND
Neurospheres HK252 (mIDH1-R132H)[a]	0.059	91	1	ND
Neurospheres 522 (mIDH1-R132H)[a]	0.292	96	1	ND
Neurospheres BT142 (mIDH1-R132H/-)[b]	2 ± 0.9	88	6	No fit
HCT-116 KI mIDH1-R132C[c]	22 ± 12	87	2	ND
HCT-116 KI mIDH1-R132H[c]	3 ± 1	90	6	ND
HCT-116 KI mIDH2-R172K[c]	130 ± 59	87	10	ND
COR-L105 (mIDH1-R132C)[d]	3.8 ± 5	91	5	No fit
HCCC-9810 (mIDH1-R132S)[e]	0.845 ± 0.3	91	13	No fit
HT1080 (mIDH1-R132C)[f]	4.0 ± 0.3	91	5	No fit
JJ012 (mIDH1-R132G)[f]	6.6 ± 2.4	93	12	No fit
TF-1 pLVX mIDH1-R132H[g]	3.2 ± 0.8	85	3	No fit
TF-1 pLVX mIDH2-R140Q[g]	14 ± 1.1	89	3	No fit
U87MG pLVX mIDH2-R140Q[g]	7.1 ± 3	95	7	No fit

[a]Patient-derived mIDH1-R132H gliomasphere lines. [b]Patient-derived IDH1-R132H glioma brain tumor stem-cell line that has lost the wtIDH1 allele *in vitro*, leading to mIDH1-R132H homozygosity. [c]Cell lines engineered to express the mIDH protein through KI at the endogenous locus. [d]Patient-derived mIDH1-R132C lung adenocarcinoma cell line. [e]Patient-derived mIDH1-R132S cholangiocarcinoma cell line. [f]Patient-derived mIDH1 chondrosarcoma cell lines. [g]Cell lines were engineered to express the mIDH protein using a lentiviral expression system

GI_{50} = the AG-881 concentration that causes 50% growth inhibition; HCT-116 = a human colon cancer cell line; KI = knock in; ND = not determined; pLVX = lentiviral expression vector; SD = standard deviation; TF-1 = human erythroleukemic cell line; U87MG = human glioma cell line

Primary human samples

- mIDH1 or mIDH2 primary human AML samples are described in **Table 4**.
- *Ex vivo* treatment of blasts with AG-881 suppressed levels of 2-HG by 76–99% (**Figure 2A**).
- AG-881 treatment also readily restored the ability of blasts to differentiate along the myelogranulocytic lineage, as shown by increased surface expression of one or more differentiation markers (**Figure 2B**).
  - Of note, treatment of wtIDH blast cells with AG-881 did not affect the surface levels of the assessed differentiation markers, indicating that the ability of AG-881 to restore blast-cell differentiation is specific to mIDH cells.

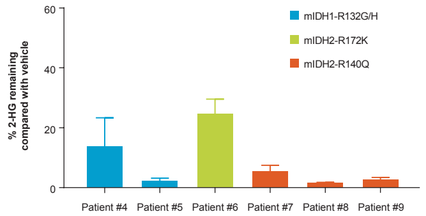
Table 4. Clinical description of primary human AML samples

Patient	Disease status	IDH mutation	FAB	Karyotype	Other mutations	Sample source
#1	Diagnosis	wtIDH	AML M1	CN	FLT3-ITD	PB
#2	Diagnosis	IDH1-R132C	AML M1	ND	NPM1	PB
#3	Relapse	IDH2-R172K	AML M2	CN	DNMT3A	PB
#4	Relapse	IDH1-R132G	ND	ND	DNMT3A, NRAS	BM
#5	Relapse	IDH1-R132H	AML M5	CN	NPM1	BM
#6	Relapse	IDH2-R172K	AML M4	Del(7q)	DNMT3A	BM
#7	Relapse	IDH2-R140Q	AML M5	CN	FLT3, WT1	BM
#8	Relapse	IDH2-R140Q	AML M2	Del(7q)	RUNX1, SRSF2, JAK2, FLT3	BM
#9	Relapse	IDH2-R140Q	AML M1	CN	DNMT3A, SRSF2, FLT3	PB

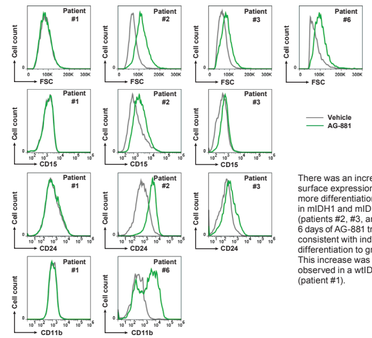
BM = bone marrow; CN = cytogenetically normal; FAB = French-American-British classification of AML (M1 = AML with minimal maturation; M2 = AML with maturation; M4 = acute myelomonocytic leukemia; M5 = acute monocytic leukemia); ND = not determined; PB = peripheral blood

Figure 2. Effects of *ex vivo* AG-881 treatment of mIDH1 and mIDH2 primary human AML blasts

A. Reduction in 2-HG levels following AG-881 treatment (1 μM)



B. AG-881 (0.5 μM) alleviates the cell differentiation block conferred by mIDH



There was an increase in the surface expression of one or more differentiation markers in mIDH1 and mIDH2 samples (patients #2, #3, and #6) after 6 days of AG-881 treatment, consistent with induction of differentiation to granulocytes. This increase was not observed in a wtIDH sample (patient #1).

In vivo systems

- AG-881 exhibited excellent brain penetration in rodents (**Figure 3**).
- The PK of AG-881 are characterized by rapid oral absorption and low total body plasma clearance in mice (0.406 L/hr/kg) and rats (0.289 L/hr/kg).
- In the TS603 mIDH1-R132H orthotopic xenograft mouse glioma model, AG-881 demonstrated the following PK/PD attributes across the oral dose range of 0.03–10 mg/kg twice daily:
  - Dose-linear PK
  - Brain-to-plasma AUC_{0-12hr} ratios ranging from 0.9 to 2.0
  - Dose-dependent tumor 2-HG inhibition (**Figure 4**), with levels reduced by 75.2–99.98%.
- In addition, twice-daily dosing of AG-881 in HT1080 (mIDH1-R132C) and U87 (mIDH2-R140Q) mouse models reduced tumor 2-HG levels by >96% at doses ≥30 mg/kg (data not shown).
- Based on *in vivo* exposure-response analyses, plasma AG-881 AUC_{0-24hr} values of 402 hr•ng/mL and 45,200 hr•ng/mL are projected to result in a sustained 97% reduction in tumor 2-HG levels in the glioma indication and the non-glioma solid and liquid tumor indications, respectively.
- AG-881 had an acceptable preclinical safety profile, supporting clinical testing.

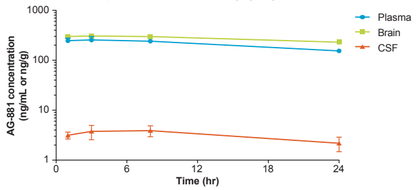
Figure 3. AG-881 readily crosses the blood-brain barrier and is also detected in the CSF

A. Brain-to-plasma and CSF-to-plasma AG-881 AUC_{0-24hr} ratios across species

Species	Brain-to-plasma AUC ratio	CSF-to-plasma AUC ratio
Balb/C mouse (50–150 mg/kg, single dose; n=3)	0.62–0.72	
SCID mouse (0.03–10 mg/kg, 6 days BID; n=3)	0.95–1.96	
Sprague Dawley rat (single dose; n=3)	1.1–1.4	0.0147–0.0152
Sprague Dawley rat (5 days BID; n=3)	1.2–1.5	0.0148–0.0199
Cynomolgus monkey (3–40 mg/kg, 28 days QD)	1.25–2.43*	

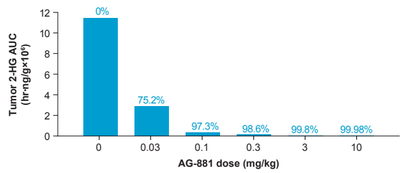
The CSF-to-plasma ratios are in good agreement with the fraction of AG-881 unbound in plasma (0.0257)
*Concentration at 24 hr post last dose, not AUC

B. AG-881 concentrations in plasma, brain, and CSF (3 mg/kg single dose in rat; n=3)



AUC = area under the curve; BID = twice daily; CSF = cerebrospinal fluid; QD = once daily; SCID = severe combined immunodeficiency

Figure 4. Reduction in brain tumor 2-HG after dosing with AG-881 in mice bearing orthotopic human mIDH1-R132H gliomas*



*Reduction in mean 2-HG tumor concentration in male ICR SCID mice bearing orthotopic TS603 IDH1-R132H gliomas after six oral doses of AG-881 administered in 12-hour intervals

CONCLUSIONS

- These data show that AG-881 is a potent, brain-penetrant, pan-mIDH inhibitor that can suppress 2-HG production by both IDH1 and IDH2 mutant proteins in biochemical, cell-based, and *in vivo* systems.
- Pharmacology studies support that the suppression of 2-HG levels by AG-881 in mIDH tumor cells results in a release of the differentiation block.
- AG-881 has acceptable drug properties and an acceptable preclinical safety profile for clinical testing.
- AG-881 is currently in phase 1 clinical development in patients with an IDH1 and/or IDH2 mutation who have advanced solid tumors, including gliomas (ClinicalTrials.gov NCT02481154) and advanced hematologic malignancies (ClinicalTrials.gov NCT02492737; enrollment complete).

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Acknowledgments We would like to thank the volunteers taking part in this study.
Disclosures This work was funded by Agios Pharmaceuticals, Inc.
KY, ZK, LD, HY, RN, YC, ZS, HK, CG, BN, LS, and SB: Agios Pharmaceuticals – employment and stockholder. KS and AO: Agios Pharmaceuticals – stockholder. EA, ET, and JPM: Agios Pharmaceuticals – stockholder; KSQ Therapeutics – employment. CC: No relevant financial relationship(s). SMS: Agios Pharmaceuticals – advisor/board member, consultant/independent contractor and stockholder. IM: Agios Pharmaceuticals – consultant. MD, CQ, and VPL: no conflict of interest to disclose. SdB: Agios Pharmaceuticals, Celgene, Novartis, Pfizer, and Servier – honorarium recipient.
Editorial assistance was provided by Susanne Vidot, PhD, CMPP, Excel Scientific Solutions, Horsham, UK, and supported by Agios.

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Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 26–30, 2017, Philadelphia, PA, USA