These data show that AG-881 is a potent, brain-penetrant, pan-mIDH inhibitor. The PK of AG-881 are characterized by rapid oral absorption and low total exposure. Relapse AML M1 patients were treated with 3 µM AG-881 or vehicle (0.1% v/v DMSO). These results in cell viability assays confirmed that AG-881 reduced cell viability in a dose-dependent manner and was more effective in inhibiting mIDH-R140Q homodimer and mIDH1/mIDH2 heterodimers compared to mIDH2-R172K homodimers. Direct inhibition of the gain-of-function activity of the mIDH protein is intended to inhibit 2-HG production and induce tumor cell differentiation. In addition, twice-daily dosing of AG-881 in HT1080 (mIDH1-R132C) and U87 MG (mIDH2-R140Q) glioma cell line was associated with increased surface expression of one or more differentiation markers (Figure 2B). The CSF-to-plasma ratios are in good agreement with the fraction of AG-881 unbound in plasma (0.0257). AG-881 demonstrated the following PK/PD attributes across the oral dose range of 0.03–10 mg/kg twice daily: the PK/PD profile.

**RESULTS**

**Biochemical profiling**

- Co-crystal structures of AG-881 with IDH1-R132H and IDH2-R140Q showed that AG-881 forms a bridging interaction between the two IDH monomers through a non-conserved N-terminal region of the enzyme.
- In vitro biochemical assays demonstrated that AG-881 has low nanomolar potency (IC50) against multiple mIDH enzymes (Tables 1 and 2).
- AG-881 readily crosses the blood-brain barrier and is also detected in cerebrospinal fluid (CSF) (Figure 3).

**Cell-based assay**

- The potency of AG-881 against mIDH and mIDH2 enzymes was also shown to have a strong correlation with the IC50 values for the inhibition of 2-HG production in mammalian cell line assays (Figure 4). This shows that AG-881 has an acceptable drug properties and an acceptable preclinical safety profile, supporting clinical testing.

**Primary human samples**

- mIDH1 or mIDH2 primary human AML samples are described in Table 4. In vitro treatment of blasts with AG-881 suppressed levels of 2-HG by 76–99% (Figure 4A).
- AG-881 treatment also restored the ability of blasts to differentiate along the myelomonocytic lineage, consistent with induction of cell cycle arrest by increased levels of p21 and increased expression of one or more differentiation markers (Figure 2B).
- For cell line assays, cell viability was assessed by Trypan blue exclusion for 1-3 days. Data were statistically analyzed using a two-tailed Student’s t-test or one-tailed Mann-Whitney U test. Data are expressed as mean ± SEM and p-values were calculated by Student’s t-test or one-tailed Mann-Whitney U test. Data are expressed as mean ± SEM and p-values were calculated by Student’s t-test or one-tailed Mann-Whitney U test. Data are expressed as mean ± SEM and p-values were calculated by Student’s t-test or one-tailed Mann-Whitney U test. Data are expressed as mean ± SEM and p-values were calculated by Student’s t-test or one-tailed Mann-Whitney U test. Data are expressed as mean ± SEM and p-values were calculated by Student’s t-test or one-tailed Mann-Whitney U test.