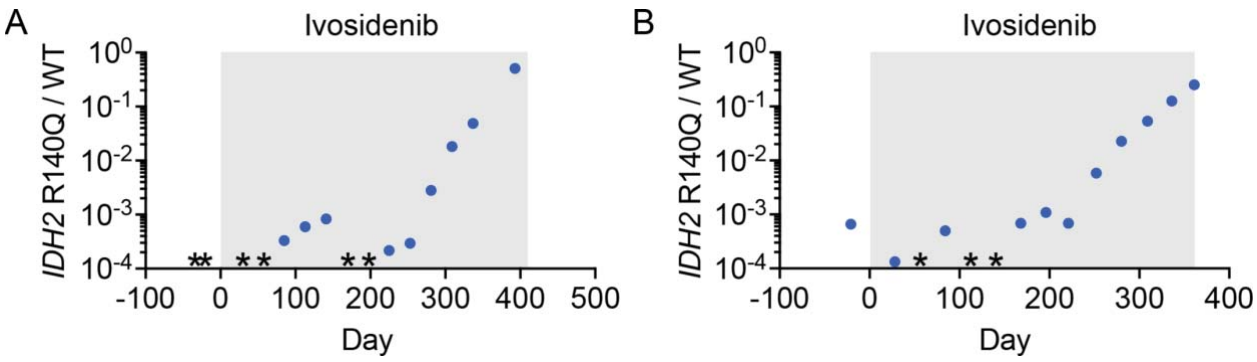
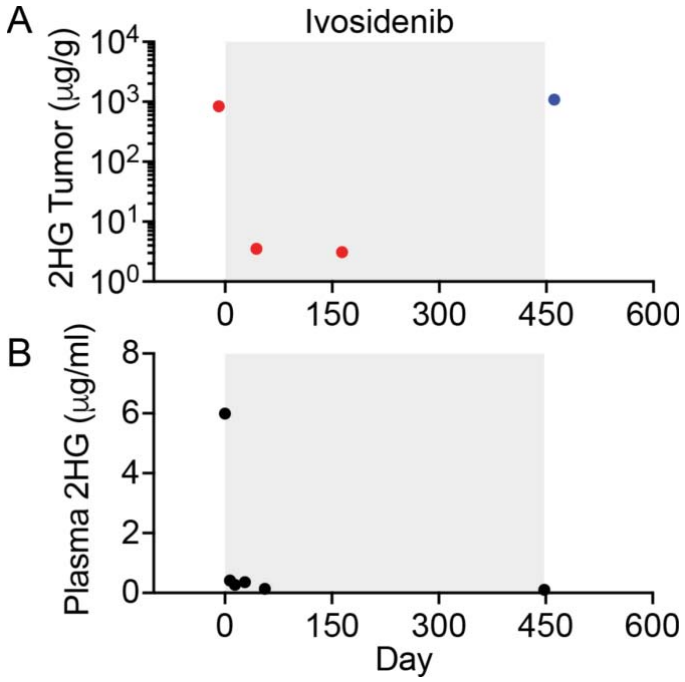
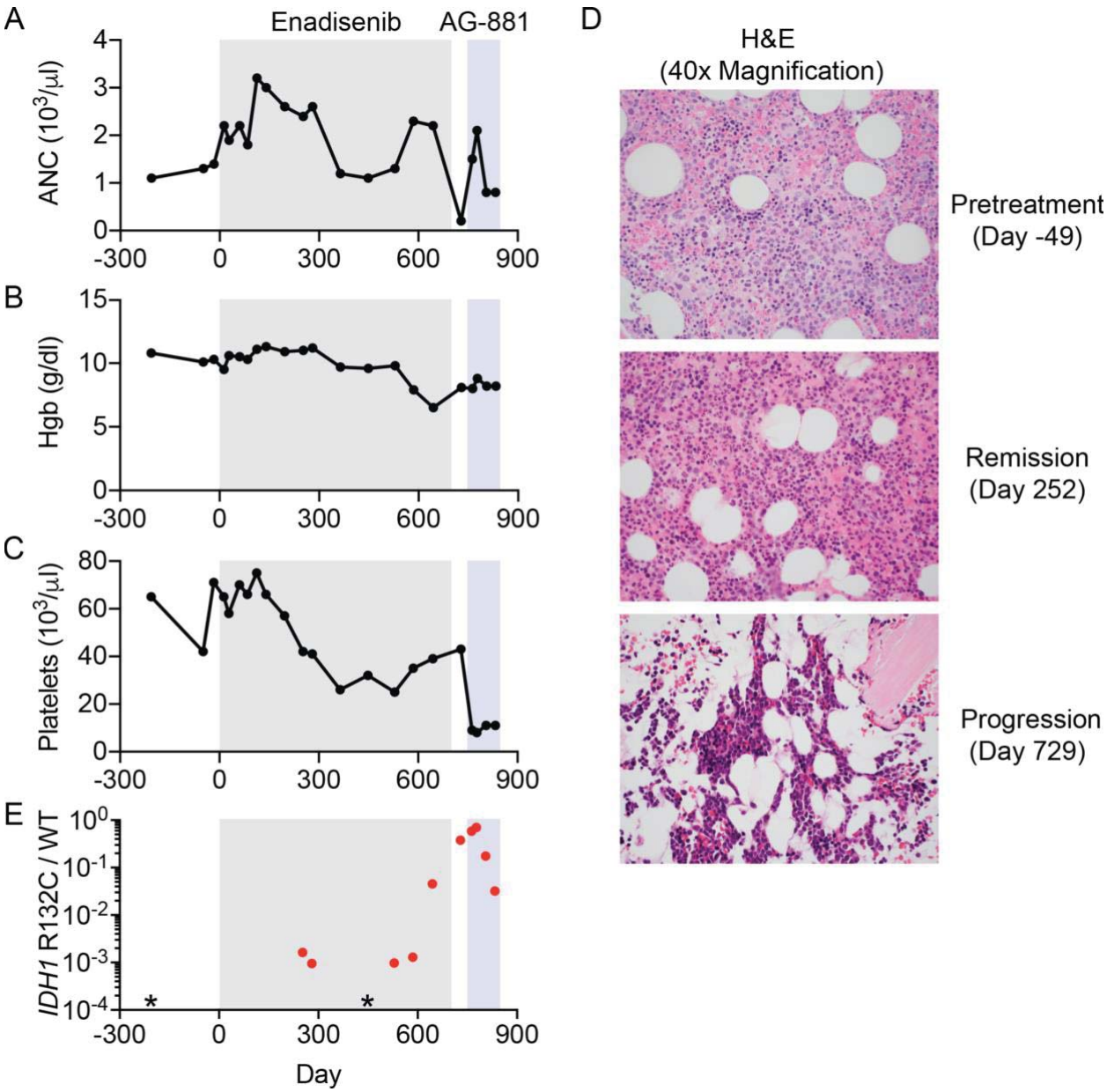


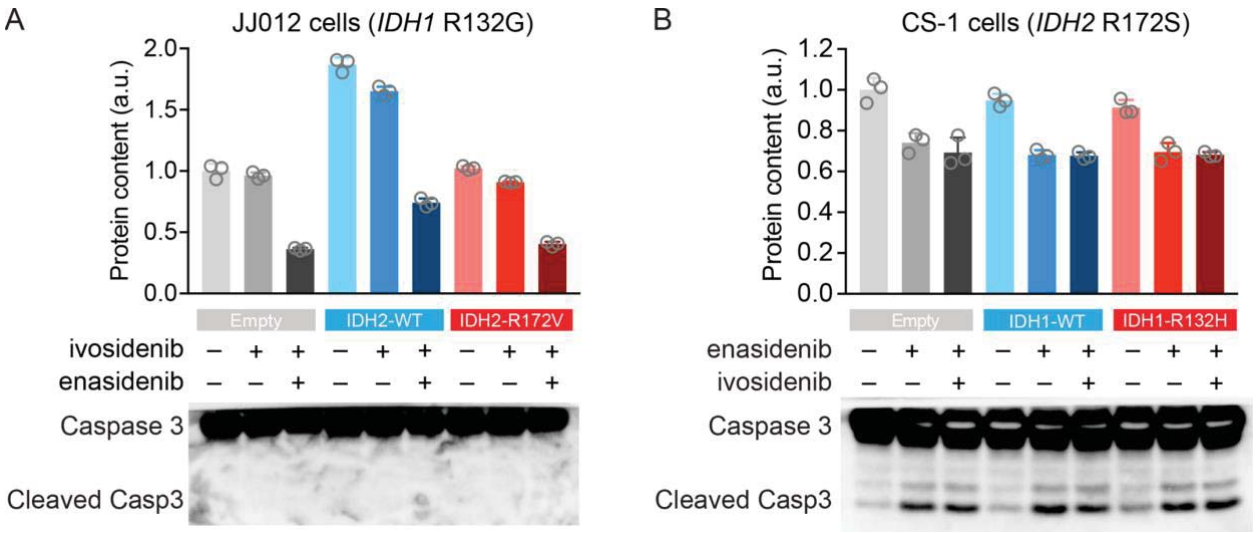
Supplemental Figure 1. *IDH2* R140Q mutations precede overt clinical resistance to IDH1 inhibition. Ratios of sequencing reads for *IDH2* R140Q/WT from the two patients with *IDH2*-mutant AML (Case 1 = A; Case 2 = B) as assessed by droplet digital PCR of DNA isolated from bone marrow cells. Asterisks indicate timepoints with no detectable mutant sequencing reads.



Supplemental Figure 2. 2HG levels in primary specimens from Case 3. (A) Tumor and (B) plasma 2-hydroxyglutarate (2HG) levels as measured by liquid chromatography–mass spectrometry (LC-MS) for the patient with *IDH1* R132C-mutant intrahepatic cholangiocarcinoma (Case 3) treated with the mutant IDH1 inhibitor ivosidenib (gray box). For (A), red dots indicate biopsies from the primary tumor on days –9, 44, and 164 of ivosidenib therapy and the blue dot indicates a biopsy from the escape lesion (see Figure 2) on day 13 post-discontinuation of ivosidenib. For (B) plasma 2HG was measured on days 0, 7, 14, 28, 56, and 448 of ivosidenib therapy.



Supplemental Figure 3. Additional clinical and laboratory features for the patient described in Case 4. (A) Absolute neutrophil count (ANC), (B) hemoglobin (Hgb) concentration, (C) platelet count, and (D) hematoxylin and eosin (H&E) staining of bone marrow at indicated timepoints in relation to treatment with the mutant IDH2 inhibitor enasidenib (gray box) and the dual IDH1/2 inhibitor AG-881 (blue box). Images show 40x magnification and are representative fields of a single bone marrow core biopsy performed at each time point. (E) Ratios of sequencing reads for *IDH1* R132C/WT as assessed by droplet digital PCR of DNA isolated from bone marrow cells. Asterisks indicate timepoints with no detectable mutant sequencing reads.



Supplemental Figure 4. Assessment of biomass and apoptosis in response to IDH inhibition in cells expressing both mutant IDH isoforms. (A) *IDH1* R132G-mutant chondrosarcoma cells (JJ012) were transfected with empty vector (Empty), IDH2 wildtype (IDH2-WT), or IDH2-R172V. Cells were treated for 24 hr with vehicle, ivosidenib (50 μM), or ivosidenib (50 μM) plus enasidenib (50 μM). (B) *IDH2* R172S-mutant chondrosarcoma cells (CS-1) were stably transduced with retrovirus expressing doxycycline-inducible empty vector (Empty), IDH1 wildtype (IDH1-WT), or IDH1-R132H. Cells in doxycycline were treated for 24 hr with vehicle, enasidenib (50 μM), or enasidenib (50 μM) plus ivosidenib (50 μM). For both (A) and (B), total protein content was determined by the bicinchoninic acid assay. Error bars depict mean ±SEM for triplicate cultures. Western blot showing intact (upper bands) and cleaved (lower bands) caspase 3. Results are representative of ≥2 independent experiments.