Preclinical *In Vivo* Activity of the Menin Inhibitor Ziftomenib (KO-539) in Pediatric *KMT2A*-Rearranged Acute Lymphoblastic Leukemia

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Introduction

- Chemotherapy resistance and subsequent relapse remain a major cause of childhood cancer mortality, particularly for infants with *KMT2A*-rearranged B-acute lymphoblastic leukemia (ALL).
- KMT2A is a histone lysine N-methyltransferase involved in HOX-family and *MEIS1* gene regulation.
- Recent preclinical and early clinical studies have reported successful targeting of the menin scaffold protein within KMT2A fusion complexes in adults with *KMT2A*-rearranged acute leukemias.

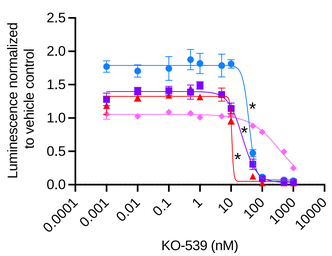
Hypothesis

The selective menin inhibitor ziftomenib (KO-539) will have potent activity in preclinical patient-derived xenograft models of infant and non-infant pediatric *KMT2A*-rearranged ALL and could enhance chemosensitivity in these often-chemoresistant leukemias.

Methods

- We measured the ability of ziftomenib to inhibit *in vitro* viability in *KMT2A*-rearranged and *KMT2A* wild-type ALL cell lines using Cell Titer Glo assays.
- *In vivo* activity of ziftomenib as monotherapy or in combination with chemotherapy was assessed in luciferase+ ALL cell line xenograft models and in 8 patient-derived xenograft (PDX) models of *de novo* or relapsed *KMT2A*-R ALL harboring various 5' fusion partners.
- NSG mice engrafted with human *KMT2A*-rearranged ALL cells were randomized to treatment with vehicle, ziftomenib, vincristine, dexamethasone, ziftomenib + vincristine, or ziftomenib + dexamethasone.
- Human CD45+ CD19+ cell counts were measured weekly via retro-orbital venous bleeding and in end-study murine spleens via quantitative flow cytometry.

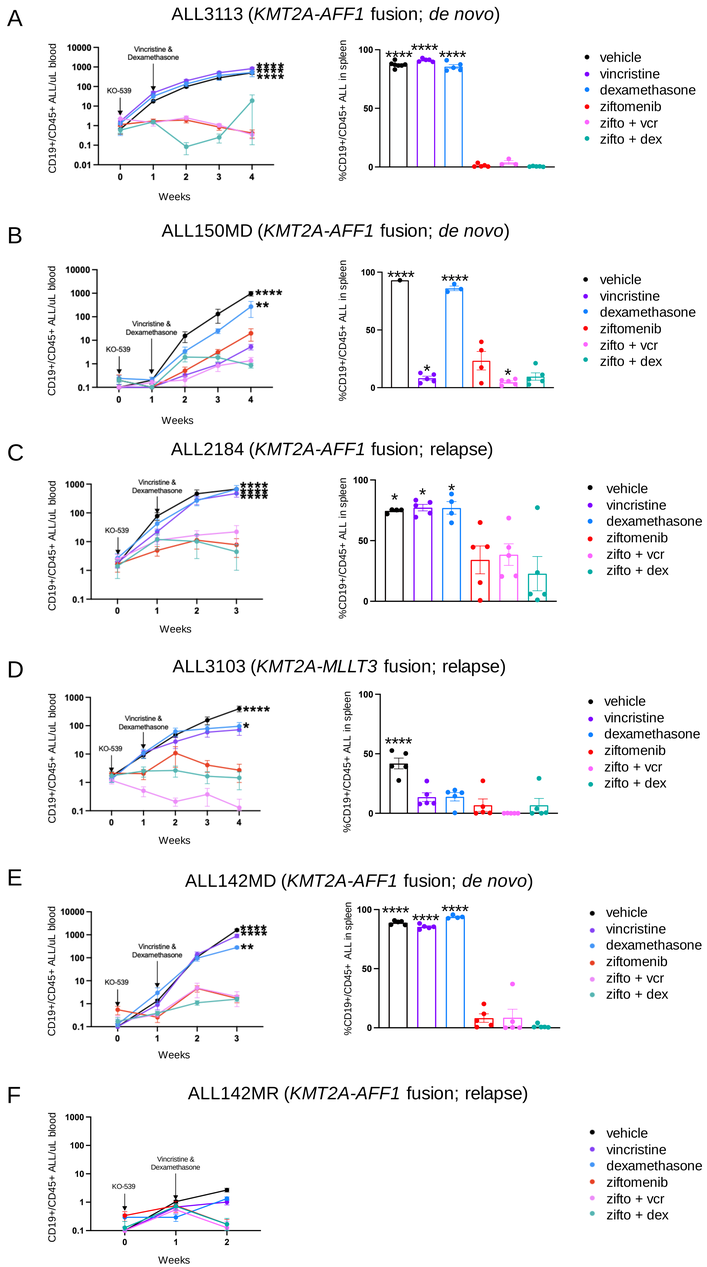
Results



Cell line	Ziftomenib IC_{50}
SEM	32.6 nM
KOPN-8	49.1 nM
HB11;19	11.1 nM
NALM-6	400.8 nM

Figure 1. Ziftomenib preferentially inhibits *in vitro* viability of *KMT2A*-rearranged ALL. *KMT2A*-rearranged ALL (SEM, KOPN8, HB11;19) and *KMT2A* wild-type (NALM-6) cell lines were treated with escalating doses of ziftomenib or vehicle control *in vitro* for 7 days. Cell Titer Glo analysis was performed to assess cell viability. Data were normalized to vehicle-treated cells as mean with standard deviation. Half-maximal inhibitory concentrations (IC_{50}) of ziftomenib were calculated. Statistical analysis was performed with two-way ANOVA with Tukey's post-test for multiple comparisons. *p<0.05.

Results



Results

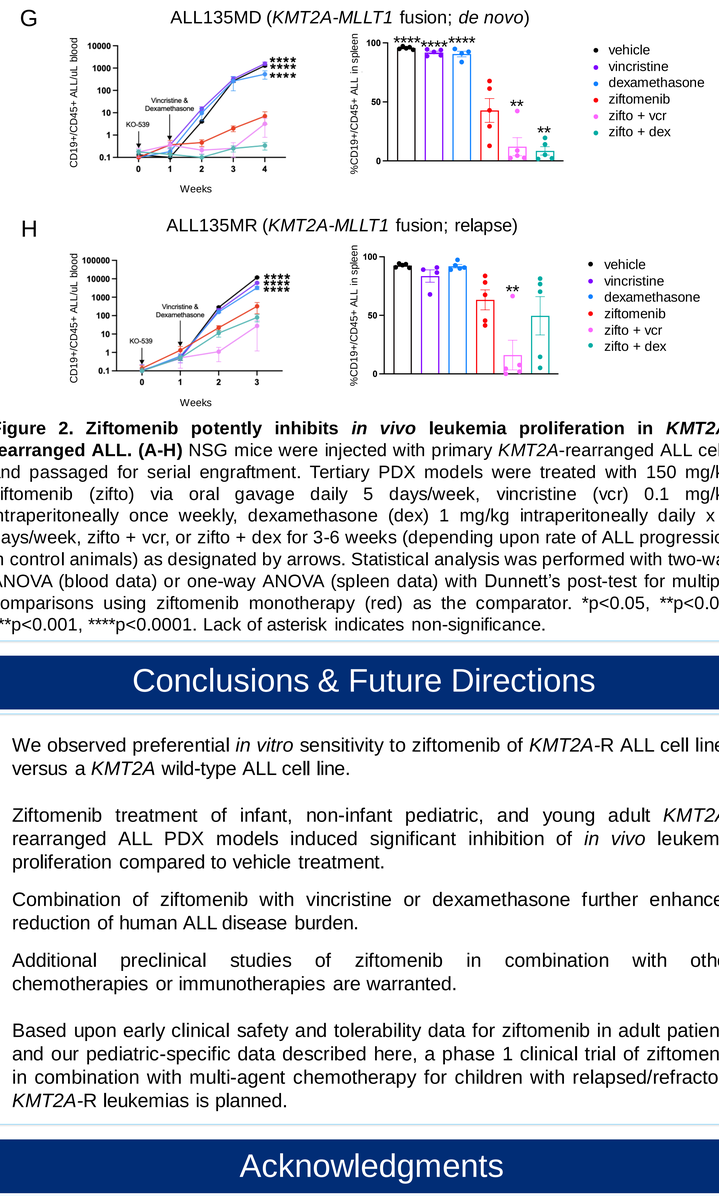


Figure 2. Ziftomenib potently inhibits *in vivo* leukemia proliferation in *KMT2A*-rearranged ALL. (A-H) NSG mice were injected with primary *KMT2A*-rearranged ALL cells and passaged for serial engraftment. Tertiary PDX models were treated with 150 mg/kg ziftomenib (zifto) via oral gavage daily 5 days/week, vincristine (vcr) 0.1 mg/kg intraperitoneally once weekly, dexamethasone (dex) 1 mg/kg intraperitoneally daily x 5 days/week, zifto + vcr, or zifto + dex for 3-6 weeks (depending upon rate of ALL progression in control animals) as designated by arrows. Statistical analysis was performed with two-way ANOVA (blood data) or one-way ANOVA (spleen data) with Dunnett's post-test for multiple comparisons using ziftomenib monotherapy (red) as the comparator. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Lack of asterisk indicates non-significance.

Conclusions & Future Directions

- We observed preferential *in vitro* sensitivity to ziftomenib of *KMT2A*-R ALL cell lines versus a *KMT2A* wild-type ALL cell line.
- Ziftomenib treatment of infant, non-infant pediatric, and young adult *KMT2A*-rearranged ALL PDX models induced significant inhibition of *in vivo* leukemia proliferation compared to vehicle treatment.
- Combination of ziftomenib with vincristine or dexamethasone further enhanced reduction of human ALL disease burden.
- Additional preclinical studies of ziftomenib in combination with other chemotherapies or immunotherapies are warranted.
- Based upon early clinical safety and tolerability data for ziftomenib in adult patients and our pediatric-specific data described here, a phase 1 clinical trial of ziftomenib in combination with multi-agent chemotherapy for children with relapsed/refractory *KMT2A*-R leukemias is planned.

Acknowledgments

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