Discovery of novel menin-MLL small molecule inhibitors that display high potency and selectivity *in vitro* and *in vivo*

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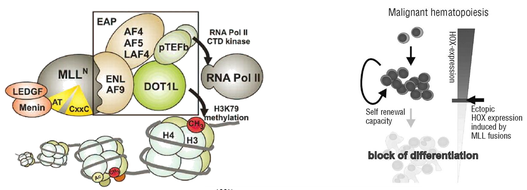
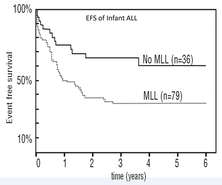
Abstract

Chromosomal translocations that affect the Mixed Lineage Leukemia (MLL) gene result in aggressive acute myeloid and lymphoid leukemias that are resistant to standard chemotherapy. MLL leukemias constitute approximately 5-10% of acute leukemias and 70% of acute leukemias in adults and infants, respectively. MLL fusion proteins require menin for leukemogenic activity, and selective disruption of the menin-MLL interaction represents a potential therapeutic approach for the treatment of acute leukemias with MLL rearrangements. Here we describe the characterization of KO-382, a small molecule inhibitor of the menin-MLL interaction, in biochemical, cellular and *in vivo* activity assays.

KO-382 is a potent inhibitor of menin-MLL interaction that binds to menin with a Kd of ~30 nM. It potently inhibits the proliferation of MLL-AF9 transformed leukemic cells with GI_{50} of 30 nM and MLL-fusion cell line MV4:11 with GI_{50} of 35 nM. KO-381, an enantiomer of KO-382, is approximately 25-fold less potent, which underscores the specificity of the interaction. KO-382 displays a greater than 50-fold reduction in potency in non-MLL-fusion leukemia cell lines and induces regression in a MV4:11 mouse xenograft model while KO-381 displays limited activity in the same settings. The anti-tumor activity of KO-382 correlates with target engagement in the tumors. KO-382 demonstrates potent efficacy in an aggressive disseminated leukemia model as measured by significant extension of survival, inhibition of HOXA9 and MEIS1 gene expression and induction of differentiation, as measured by CD11b expression.

KO-382 is a highly potent and selective inhibitor of the menin-MLL interaction with robust efficacy in subcutaneous and disseminated models of MLL-fusion leukemias. These results demonstrate the potential clinical utility of menin inhibitors in MLL leukemias.





Background

MLL-r leukemias are an aggressive type of blood cancer.

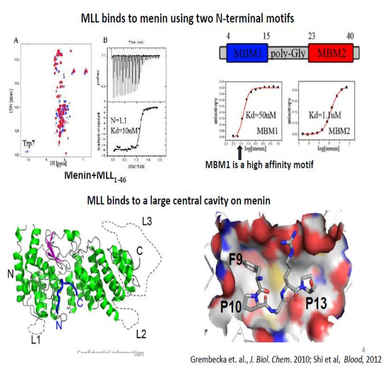
Predominantly in children and therapy-related leukemia in adults

Chromosomal translocations in the *KMT2A* (MLL) gene at 11q23 result in MLL fusions with any of > 50 partner genes.

MLL fusion proteins drive leukemogenesis through deregulation of *HOX* genes.

The leukemogenic activity of MLL fusion proteins is critically dependent on menin binding.

Characterization of the menin-MLL complex

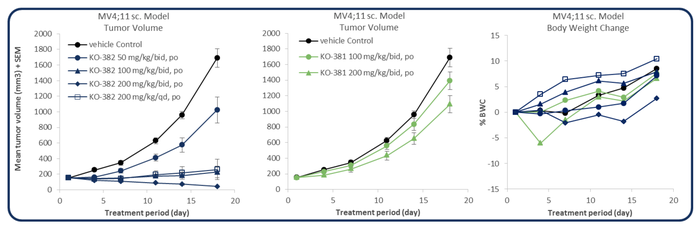


KO-382 is a potent and selective menin inhibitor

Compound	KO-382	KO-381
Biochemical Assay: Menin/MLL FP Assay		
MLL 4-43 IC_{50}	38 nM	1,067 nM
MLL Fusion Leukemia Cell Line Proliferation Assays		
MLL-AF9 GI_{50}	30 nM	600 nM
MV4;11 (MLL-AF4) GI_{50}	35 nM	500 nM
MOLM13 (MLL-AF9) GI_{50}	75 nM	-
Non-MLL Cell Line Proliferation Assays		
U937 GI_{50}	> 2 µM	> 2 µM
HL-60 GI_{50}	> 2 µM	> 2 µM

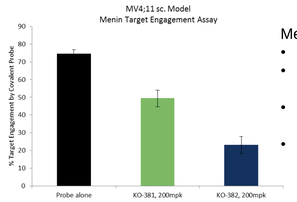
- KO-382 is a potent inhibitor of menin-MLL binding and potently inhibits growth of cell lines containing MLL fusions
- KO-382 is selective: >50 fold more potent in MLL-r leukemia cell lines
- KO-381 is a less potent enantiomer of KO-382

KO-382 demonstrates robust efficacy *in vivo* in MLL-r leukemia model (MV4;11 xenograft)



- Significant efficacy at well-tolerated doses for KO-382 (oral dosing)
  - Regression at 200 mg/kg BID
  - Similar efficacy with QD (200 mg/kg) vs. BID (100 mg/kg/dose = 200mg/kg/day)
- KO-382 displays greater activity than the less active enantiomer KO-381
  - Demonstrates that the antitumor activity is result of target inhibition
  - KO-381 and KO-382 have similar *in vivo* exposure

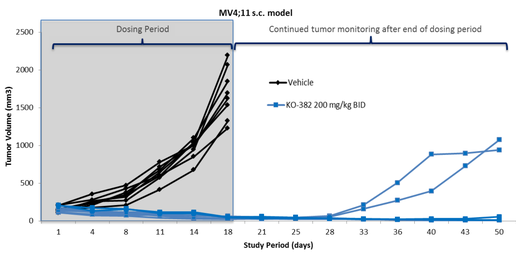
Efficacy *in vivo* correlates with target engagement in tumors



Menin target engagement assay in MV4;11 sc. tumor model

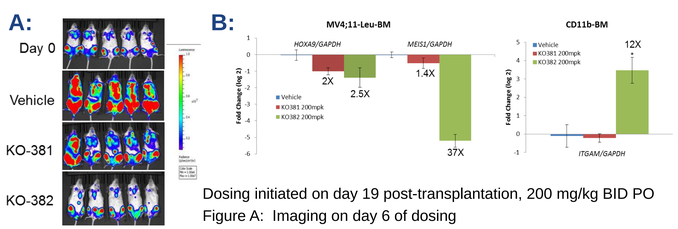
- Probe covalently engages menin cysteine-329
- Inhibition of covalent probe engagement as a measure of menin target occupancy
- Treatment with single dose for 1 hour followed by administration of probe for 1 hour
- Demonstrates greater target occupancy (> 80% using kinetic modelling) by KO-382 than by less active enantiomer KO-381

Durable *in vivo* response



- Durable tumor growth inhibition in MV;411 xenograft model
- Most tumors (6/8) were still of undetectable size (< 50 mm^3) one month after cessation of treatment

Correlation between efficacy and PD biomarkers in MV4;11 disseminated leukemia model



Dosing initiated on day 19 post-transplantation, 200 mg/kg BID PO

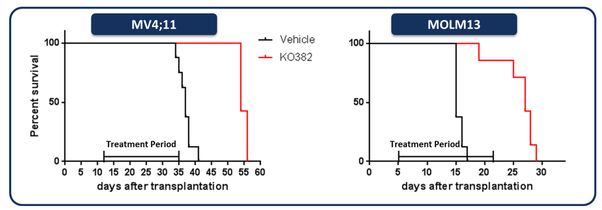
Figure A: Imaging on day 6 of dosing

Figure B: Gene expression samples collected day 7 of dosing

Figure C: Quantitation of tumor response induced by KO-382 measured by luminescence

- KO-382 inhibits expression of MLL fusion target genes *HOXA9* and *MEIS1*
- KO-382 induces expression of *ITGAM* (CD11b), a marker of differentiation

KO-382 demonstrates robust survival benefit in disseminated models of leukemia



- Dosing at 120 mg/kg BID PO
  - MV4;11(MLL-AF4): 46% survival benefit (n = 8)
  - MOLM13(MLL-AF9): 80% survival benefit (n = 7)
- Overall survival endpoints consistent with efficacy in subcutaneous model, and with gene expression in disseminated model

Conclusions

- KO-382 is a potent and selective inhibitor of the menin-MLL interaction.
- KO-382 demonstrates robust, sustained tumor inhibition in subcutaneous and disseminated models of MLL-r leukemias that correlates with target engagement and inhibition of target gene expression.
- Additional efforts underway to assess potential utility of menin-MLL inhibitors in additional hematological malignancies and solid tumor indications.

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