KME-0584, a Highly Potent IRAK1/IRAK4/panFLT3 Inhibitor, Is a Promising Clinical Candidate for Hypomethylating Agent Plus Venetoclax Resistant AML/MDS Patients

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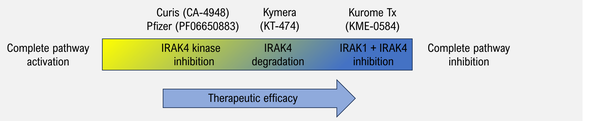
INTRODUCTION

- Leukemic cells exhibit dysregulation of innate immune signaling pathways upon diagnosis, and these pathways become further activated in drug resistance.
- IRAK1/IRAK4 kinase complex is part of a critical signaling node that becomes activated in these dysregulated pathways [1].
- IRAK4 inhibitors are currently under investigation for the treatment of AML and MDS and have shown encouraging, though modest responses in clinical studies.
- We have recently demonstrated that limited responses to IRAK4 inhibitors in the leukemic setting can be explained by a compensated activation of IRAK1 and the need to inhibit both IRAK1 and IRAK4 to achieve maximal therapeutic efficacy [2].
- **KME-0584 is an IND ready**, highly potent **IRAK1/IRAK4/panFLT3** inhibitor that exhibits superior potency and therapeutic efficacy compared to IRAK4 inhibitors in the FLT3 WT as well as the FLT3 mutant setting.



METHODS

NF-κB Reporter assay. Performed according to THP-1-Blue NF-κB cells and QUANTI-Blue reagent manufacturing protocol (InvivoGen).

Colony forming units (CFU). Primary AML patient samples were obtained from Discovery life Sciences (DLS) and tested at ReachBio in methylcellulose assay.

Biochemical and Cell-based Kinase inhibitory assays. Kinase inhibitory data and cell-based kinase assays were obtained using the RBC HotSpot® Kinase and NANOBRET® Assay, respectively performed at ReactionBio (Malvern, PA).

Binding kinetics assay. Performed at Enzymlogic using KINETICfinder® TR-FRET assay.

Xenograft. Survival analysis was performed in NSG-SGM3 mice xenografted with MOLM14 FLT3-ITD (D835Y) cells.

Pharmacokinetics screening and Ion Channel Panel. Testing was performed at Pharmaron.

RNASeq. THP-1 cells were treated in liquid culture for 24hr. Total RNA was extracted and RNAseq was performed at 30M reads per sample.

Statistical analysis. All data were plotted using Graphpad Prism, and statistical significance was determined using T-test.

CONTACT INFORMATION

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ACKNOWLEDGMENTS

This work was supported in part by NCATS grant #1ZIATR000044-08

KME-0584 is a highly potent and selective IRAK1/IRAK4/panFLT3 inhibitor

A

Kinase	Biochemical IC_{50} (nM)	Cell-Based IC_{50} (nM)	Residence Time t (min)
IRAK1	24.8	Not tested	0.1
IRAK4	<1.31	<0.5	22
FLT3	<0.5	<0.5	73
PHKg1	5	<0.5	Not tested
PHKg2	82	Not tested	Not tested
PDGFRα	19	Not tested	Not tested
PDGFRβ	56	18	0.27
RET	25	84	1.6
CLK1	55	33	1.5
CLK4	70	1	3.7

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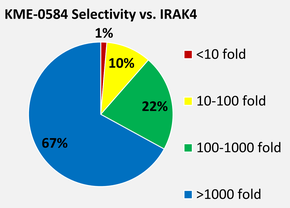
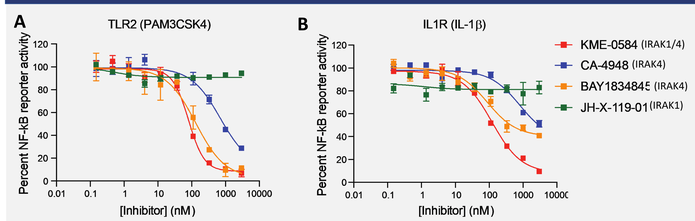


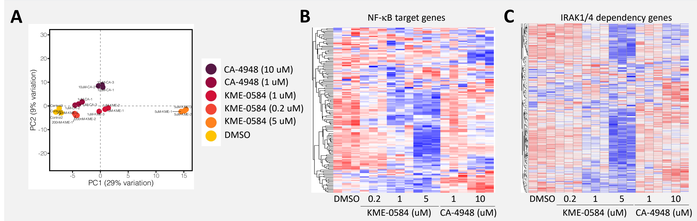
Selectivity of KME-0584 in biochemical, kinetic, and cell-based assays. A- High on-target activity of KME-0584 and only 7 off-targets with <100nM **cell-based activity** or high residence time **B-** Selectivity of KME-0584 vs off-target kinases demonstrating it is >100-fold selective relative to IRAK4 IC_{50} vs. 89% of a **panel of 370 kinases** in the HotSpot® assay.

Combined IRAK1 and IRAK4 inhibition is required to suppress NF-κB signaling



Complete antagonism of NF-κB activity through multiple receptors requires inhibition of both IRAK1 and IRAK4. A- Antagonism of the NF-κB response of PAM3CSK4 through the TLR2 receptor. **B-** Antagonism of the NF-κB response of IL-1β through the IL-1 receptor. **Error bars represent SEM from triplicate replicates. The experiment was replicated two additional times with similar results.

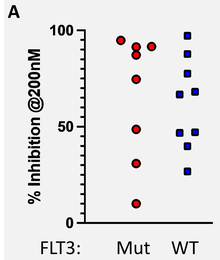
KME-0584 suppresses NF-κB and IRAK1/4-dependent signaling in AML cells



Gene expression resulting from IRAK1/4 inhibition differs from IRAK4 inhibition in FLT3 WT cells. A- Principal component analysis (PCA) plot showing the variation between samples in THP-1 cells. **B-** Relative expression of NF-κB target genes **C-** Relative expression of IRAK1/4 dependency genes. Z-score (blue downregulated genes, red upregulated genes)

KME-0584 potently inhibits leukemic stem cell progenitor function regardless of mutational status

A

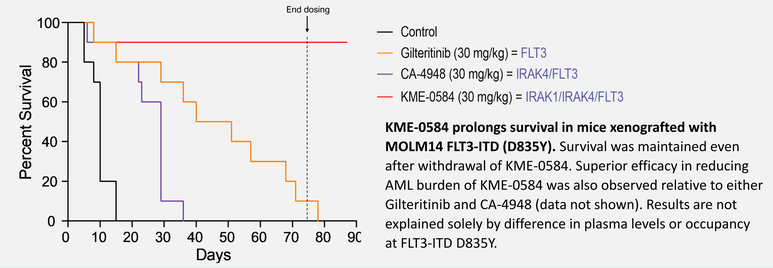


B

Patient ID	Mutational Status*
AML(507)	ABL1, ASXL1, BCORL1, BRAF, CDKN2A, CEBPA, DNMT3A, ETV6, EZH2, IDH2, KDM6A, KIT, KRAS, PTEN, RUNX1, SF3B1, TET2, TP53, ZRSR2
AML (276)	ABL1, ASXL1, ATRX, BCOR, BCORL1, CALR, CDKN2A, DNMT3A, IDH1, KIT, KRAS, PDGFRA, PTEN, SF3B1, TET2, TP53
AML(562)	ASXL1, ATRX, BCORL1, BRAF, DNMT3A, EZH2, FBXW7, GATA1, KDM6A, KIT, KRAS, NOTCH1, PTEN, RUNX1, STAG2, TET2, TP53
AML(509)	ASXL1, BCORL1, BRAF, CDKN2A, DNMT3A, EZH2, HRAS, KIT, KRAS, MPL, PHF6, PTEN, RUNX1 TET2, TP53, WT1
AML(029)	ABL1, ASXL1, ATRX, BCORL1, CBL, CDKN2A, DNMT3A, GATA1, JAK2, KDM6A, KIT, KRAS, PTEN, PTPN11, SRSF2, TET2, TP53
AML(126)	ABL1, ASXL1, BCORL1, CDKN2A, CEBPA, HRAS, IDH1, KDM6A, KRAS, NOTCH1, PTEN, SRSF2, TP53
AML(395)	ABL1, ASXL1, BCORL1, CBL, CDKN2A, FBXW7, JAK2, KDM6A, KIT, KRAS, PTEN, SRSF2, TET2, TP53
AML(487)	ATRX, BCOR, BCORL1, BRAF, CDKN2A, FBXW7, KIT, NRAS, PTEN, RUNX1, STAG2, TET2, TP53
AML(870)	ABL1, ASXL1, BCOR, BCORL1, BRAF, CDKN2A, ETV6, FBXW7, KDM6A, KRAS, NRAS, PTEN, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, WT1

KME-0584 suppresses colony formation in primary AML patient samples. A- Comparison between % inhibition of colony formation at 200nM of KME-0584 across primary patient samples that are either wildtype (WT) or mutant for FLT3. No significant difference in response to inhibitor between WT and FLT3 mutant samples. **B-** Mutational status of the corresponding FLT3 WT patient AML primary bone marrow blasts. *Similar mutational profiles were seen in FLT3 mutant AML patient samples (Data not shown).

KME-0584 shows superior activity *in vivo* compared to benchmark compounds



KME-0584 prolongs survival in mice xenografted with MOLM14 FLT3-ITD (D835Y). Survival was maintained even after withdrawal of KME-0584. Superior efficacy in reducing AML burden of KME-0584 was also observed relative to either Gilteritinib and CA-4948 (data not shown). Results are not explained solely by difference in plasma levels or occupancy at FLT3-ITD D835Y.

KME-0584 exhibits high oral bio-availability across multiple species

Species	Dose (IV/PO) (mg/kg)	Clearance (mL/min/kg)	AUC (PO) (nM*h)	Cmax (nM)	Half-life (h)	Oral bio-availability (F%)*
Rat	1 / 3	44	1861	319	2	76%
Dog	1 / 3	19	3477	467	5.4	64%
Mouse	1 / 10	177	3901	405	2.2	100%
Mouse	N.D. / 30	NA	22621	1973	NA	NA

Pharmacokinetics of KME-0584 in multiple species. KME-0584 shows high oral bioavailability, moderate clearance, and good oral exposure in mouse, rat, and dog. **F -** Fraction

KME-0584: optimized preclinical safety profile

Metabolism
No active metabolites
No CYP3A4 interactions

Cardiovascular (CV) safety

- KME-0584: No effect on beat rate, FPDc (≈QTc) and no EADs in **human iPSC-CMs** to 10 μM
- No effect on QTc in **guinea pig cardiovascular function** (ECG) to high dose ~25x mouse AUC_{eff}
- No changes in function or structure in **rat echocardiography** to high dose ~44x mouse AUC_{eff}
- No ECG (QT, QTc, HR) or BP findings in single dose **GLP Dog CV study** to high dose ~11x mouse AUC_{eff}

BP – blood pressure, **EADs** – early after depolarizations (torsadogenic potential); **FPDc** – heart rate-corrected field potential duration; **iPSC-CMs** – human induced-pluripotent stem cell cardiomyocytes; **QTc** – heart rate-corrected QT interval

CONCLUSIONS

- **KME-0584 is a potent and selective IRAK1/4 and pan FLT3 inhibitor with superior potency and efficacy** to IRAK4 and FLT3 inhibitors in preclinical settings
- **IND ready;** acceptable safety and tolerability in IND-enabling toxicology studies
- Studies in primary patient samples suggest **similar drug doses can be used in both FLT3 WT and FLT3 mutant patient population**
- **Phase one** study is projected to initiate in QI/II '24

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