Targeting CDK9 in KMT2A-rearranged infant leukemia: Evidence for activity and drug synergy with enitociclib

Ritul Sharma[1], Melanie M. Frigault[2], Amy J. Johnson[2], Raquel Izumi[2], Ahmed Hamdy[2], Joseph Birkett[3], Beatrix Stelte-Ludwig[3], Luke Maese[4], Norman Lacayo[5] and Aru Narendran[1]

[1]Department of Oncology, University of Calgary, Calgary, AB, Canada; [2]Vincerx Pharma, Inc. Palo Alto, CA, USA; [3]VIncerx Pharma GmbH, Monheim, Germany; [4]Department of Pediatrics and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA; [5]Division of Hematology/Oncology, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA.

UNIVERSITY OF CALGARY

INTRODUCTION

- Chromosomal translocations at chromosome 11q23 with KMT2A (MLL) gene rearrangement (KMT2A-r) occur in approximately 5% of pediatric ALL overall, but in a significantly increased proportion in infant ALL (70 – 80%).
- In pediatric AML, it is found in approximately 20% of cases but is found in about half of the patients in the infant age group.
- Critical interactions in the KMT2A-r complex drive the transforming effects of KMT2A-r leukemia by upregulating the HOXA9 transcription factor.
- P-TEFb is a complex of CDK9 and cyclin T1 that acts as a regulator of transcriptional elongation in the KMT2A-r complex, thereby contributing to the development of this disease.
- Enitociclib (Vincerx Pharma), a potent and selective CDK9/P-TEFb inhibitor, has demonstrated effective anti-tumor activity in various tumor models.
- Since leukemic cells with KMT2A-r are generally dependent on P-TEFb for regulating transcriptional elongation, our studies evaluated the potential of enitociclib for KMT2A-r leukemia.

METHODS

- A panel of pediatric leukemia cell lines with and without KMT2A-r was used to evaluate the cytotoxicity of enitociclib. Cell viabilities after 72 hours were measured using Alamar blue assay. Apoptosis was measured by Annexin / Propidium Iodide assay.
- Modulation of targets by enitociclib was demonstrated using immunoblotting. The effect of CDK9/P-TEFb inhibition on cell viabilities of leukemic cells supported by bone marrow (BM) derived stroma was demonstrated by direct and indirect co-culture experiments.
- Group comparisons were calculated using Student's t-test. For group comparisons, a p-value < 0.05 was considered statistically significant. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001)
- Drug synergy between enitociclib and traditional chemotherapeutic agents/ small molecule inhibitors was quantified using CompuSyn software based on Chao Talalay software. Combination Index (CI) of less than, equal to or more than one between two drugs indicates synergistic, additive or antagonistic effect, respectively.
- *In vivo* experiments using enitociclib were done using MV4-11, a KMT2A-r cell line, in female nude rats. Enitociclib (4.5mg/kg) was administered intravenously once per week for three weeks.

RESULTS

Enitociclib leads to decreased cell viability in KMT2A-r leukemia and induces apoptosis

Cell Line	Age / Sex	Leukemia	IC_{50} (nM)
MV4-11	10Y / M	KMT2A-r AML	30
KOPN-8	3M / F	KMT2A-r B-ALL	77
THP-1	1Y / M	KMT2A-r AML	135
INL2	3M / M	KMT2A-r B-ALL	168
SUP-B15	9Y / M	BCR-ABL+ B-ALL	143
MOLT-4	19Y / M	T-ALL	158
K562	53 Y / F	BCR-ABL+ CML	406
Jurkat	14Y / M	T-ALL	190
PBMCs	35Y / M	-	883
CD34+	Normal donor	-	306

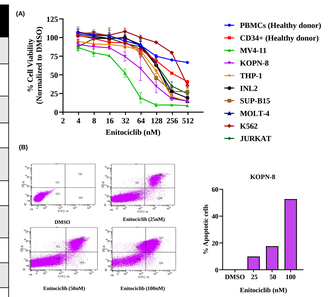


Figure 1 Enitociclib demonstrates cytotoxic activity in KMT2A-r pediatric leukemia cells (A) Cell viability of various leukemia cell lines and healthy controls after 72-hour treatment with enitociclib were measured by alamar blue assay. Data represented as percent viability normalized to respective DMSO controls **(B)** Enitociclib treatment increased apoptosis in a dose dependent manner in KOPN-8 cells as measured by Annexin V/PI staining.

Enitociclib disrupts transcriptional elongation and leads to decreased c-MYC and MCL-1 protein levels

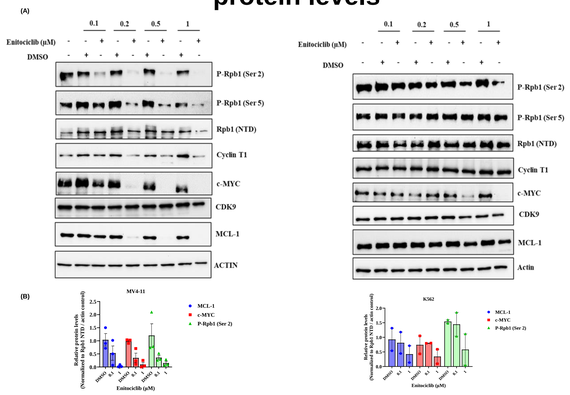


Figure 2 Enitociclib has effective on-target activity in KMT2A-r pediatric leukemia cells (A) MV4-11 (KMT2A-r) and K562 (KMT2A-wt) cells were treated with varying concentrations of enitociclib for 6 hours. Western blot was performed to measure changes in Ser 2 phosphorylation of RNA polymerase II (Rpb1) as well as changes in levels of MCL-1 and c-MYC. Blots are representative images of 2-3 independent biological repeats. **(B)** Densitometry analysis show changes in protein levels of MCL-1 and c-MYC as well as phosphorylation levels of Ser 2 of Rpb1.

Enitociclib overcomes bone marrow derived stroma mediated survival advantage

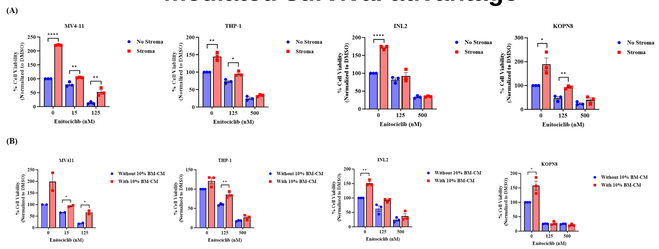
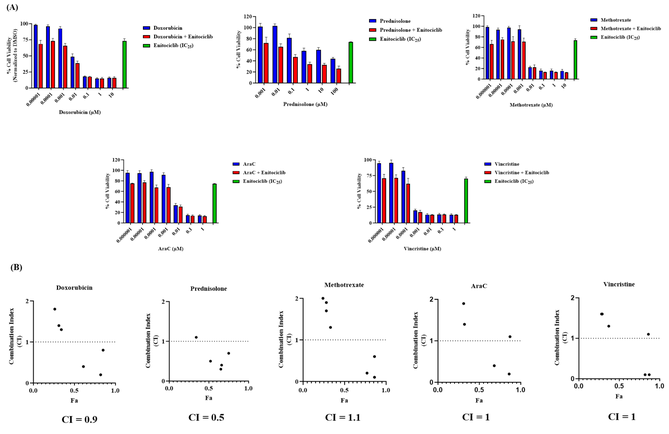


Figure 3 Enitociclib decreases the bone marrow (BM) derived stromal mediated survival support in KMT2A-r leukemic cells (A) Four KMT2A-r leukemic cell lines were cultured with and without BM derived stromal cells and treated with enitociclib for 72 hours. **(B)** Four KMT2A-r leukemic cell lines were cultured with and without 10% BM-CM and treated with enitociclib for 72 hours. Data represented as means ± SEM from 2-3 independent biological experimental repeats.

Enitociclib potentiates the cytotoxic effect of doxorubicin and prednisolone in KMT2A-r pediatric leukemia cells



	Doxorubicin	Prednisolone	Methotrexate	AraC	Vincristine
Single Agent	0.01	25	0.005	0.006	0.0004
Single Agent + Enitociclib	0.002	0.1	0.005	0.008	0.0004

Figure 4 Enitociclib demonstrated effective synergistic activity with doxorubicin and prednisolone in KMT2A-r pediatric leukemia cells (A) MV4-11 cells were treated with various concentrations of different chemotherapeutic agents either alone or in combination with low dose (IC_{25} value) of enitociclib for 72 hours. (B) Fa-CI plots overall combination index values are given for each combination.

Enitociclib in combination with the menin inhibitor MI-463 decreases HOXA9 levels in KMT2A-r leukemia

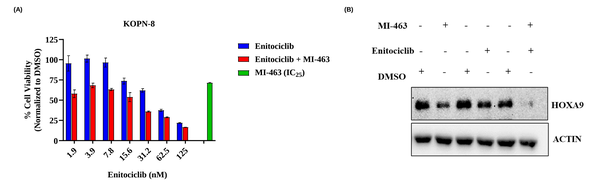


Figure 4 Synergistic interaction between enitociclib and MI-463 in KMT2A-r cells. (A) KMT2Ar leukemic cells were treated with enitociclib either alone or in combination with MI-463 for 72 hours**. (B)** HOXA9 protein levels were significantly diminished after treatment with the combination. KOPN8 cells treated with a combination of MI-463 and enitociclib for 24 hours.

Enitociclib achieved complete remission in a subcutaneous MV4-11 rat model

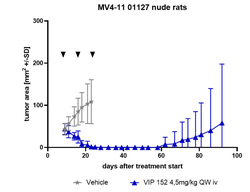


Figure 6 Enitociclib achieved durable tumor inhibition in subcutaneous MV4-11 rat model Cells were suspended in 0.2mL of 100% matrigel and were implanted into the flank of female nude rats. Randomization occurred after a tumor size of 35-65mm^2 was achieved (14-18 days after inoculation). 4.5mg/kg i.v. of enitociclib was administered once per week for three weeks.

CONCLUSION

- The CDK9/P-TEFb targeting agent enitociclib shows effective cytotoxicity against KMT2A-r cells *in vitro,* with a decease in relevant transcriptional elongation.
- In addition, our data showed that enitociclib may also inhibit BM stroma supported cell growth advantage in KMT2A-r cells.
- Simultaneous targeting of two critical interactions in the KMT2A-r complex with enitociclib and menin inhibition demonstrated effective synergistic activity. This combination strategy also leads to a reduction of HOXA9 (60% reduction in protein levels) in these cells.
- Overall, this study provides the initial proof-of-concept evidence to include targeted cytotoxicity with enitociclib in future clinical trials for KMT2A-r leukemia, in particular for the treatment of infant leukemia.

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Corresponding author: Dr. Aru Narendran (a.narendran@ucalgary.ca)