VTP-50469 is a novel, orally-available Menin-MLL1 inhibitor effective against *MLL*-rearranged and *NPM1c*+ leukemia

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Introduction:

• MLL-rearrangements are found approximately 5-10% of AML and B-ALL cases, also >70% of infant leukemias (Krivtsov and Armstrong 2007). NPM1c+ mutations are found in about 30% of all adult AML (Ley T et al., 2013).

• First generation MLL:MEN inhibitors show that targeting of the MEN:MLL1 interaction inhibits cell proliferation in MLL-rearranged and NPM1c+ AML. (Yokoyama et al 2005; Borkin et al., 2015; Kuhn et al., 2015)

• Currently available MEN:MLL interaction inhibitors have modest drug like properties. Therefore, VTP-50469 was developed as a novel orally available MEN:MLL1 inhibitor.
VTP-50469 selectively inhibits proliferation of cell lines with \textit{MLL}-rearrangements and \textit{NPM1c}⁺ mutations.

\begin{itemize}
    \item Colony forming assay in semi-solid media
    \item CellTiter-Glo assay
\end{itemize}

\begin{table}
\begin{tabular}{|c|c|c|}
\hline
CELL LINE & FUSION & IC\textsubscript{50} nM \\
\hline
MV4;11 & MLL-AF4 & 17 \\
SEM-K2 & MLL-AF4, AF4-MLL & 27 \\
RS4;11 & MLL-AF4, AF4-MLL & 25 \\
MOLM-13 & MLL-AF9 & 13 \\
KOPN-8 & MLL-ENL & 15 \\
IBI1;19 & MLL-ENL & 36 \\
REH & NONE & >>2000 \\
HL-60 & NONE & >>2000 \\
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\end{tabular}
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VTP-50469 dissociates MEN from nuclear complexes

Glycerol gradient (10%-20%) fractionation of nuclear extracts, 300mM NaCl

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<th>Fraction#</th>
<th>Free protein</th>
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MOLM13 (MLL-AF9)

Day 3

VTP-469 0.3uM

Identical fractionation results obtained from RS4;11 (MLL-AF4), ML-2 (MLL-AF6) and OCI-AML3 (NPM1c+) Cells
VTP-50469 treatment leads to MEN loss from TSS in *MLL*-rearranged cell lines
VTP-50469 treatment evicts DOT1L from Chromatin
VTP-50469 treatment evicts DOT1L from MLL-fusion target genes

Identical ChIP-seq results obtained from RS4;11 (MLL-AF4) cells
Treatment with VTP-50469 suppresses MLL-fusion target and DOT1L inhibitor sensitive genes

RS4;11, 3 days

VTP,469 vs DMSO

243

335

Gene lists:
Tags > 10
p-val < 0.05

MLL-AF4 targets

HOXA5
MEIS1
HOXA9
MEF2C
HOXA10

Fold change (log2)

VTP-50469 treatment changes expression of MLL-target and DOT1L inhibitor sensitive genes faster as compared to EPZ4777

May be in part due to eviction of DOT1L from chromatin as opposed to enzyme inhibition
VTP-50469 treatment reduces leukemia burden in PDX models of MLL-r and NPM1 mutant leukemia

1-10% Leukemia in PB

0.1% VTP, 100 mpk (IC₉₀)

Plasma conc. 1-2 uM

R²=0.93

MLL-r B-ALL (n=3) and AML (n=2); NPM1c⁺ AML (n=4)

No response in AML without NPM1 mutations or MLL-Rearrangements
Combined DOT1L and Menin Inhibitors are Active Against MLL-Rearranged NPM1 Mutant AML Cells

MLL-r-AML

Npm1-mutant AML

![Diagram showing molecular interactions and experimental results]
Conclusions:

• VTP-50469 specifically inhibits proliferation of cell lines carrying *MLL*-rearrangements or *NPM1c*+ mutations with an IC$_{50}<$40 nM.

• VTP-50469 facilitates dissociation of MEN from high molecular weight complexes and leads to eviction of MEN, MLL-fusions (at some loci) and DOT1L from chromatin and reverses MLL-fusion driven gene expression.

• Treatment of *MLL*-r and *NPM1c*+ PDX models with VTP-50469 leads to differentiation, significant reduction of leukemia burden and prolonged survival.
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