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

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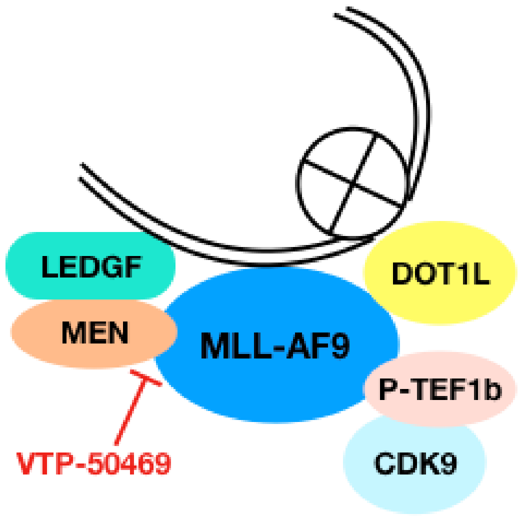
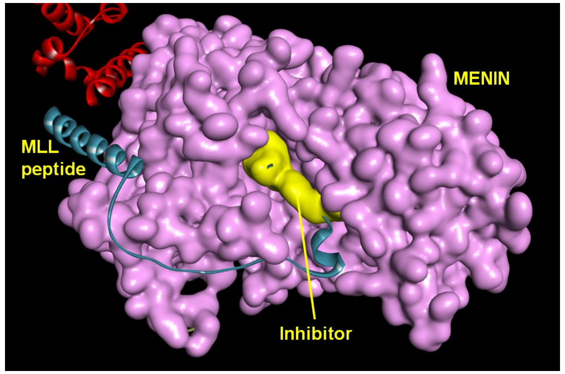
Introduction:

- *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).

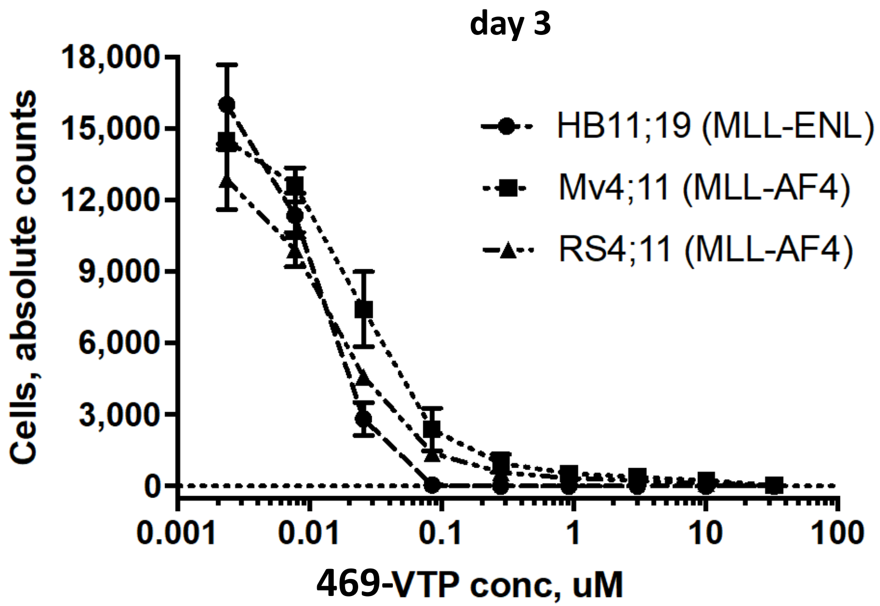


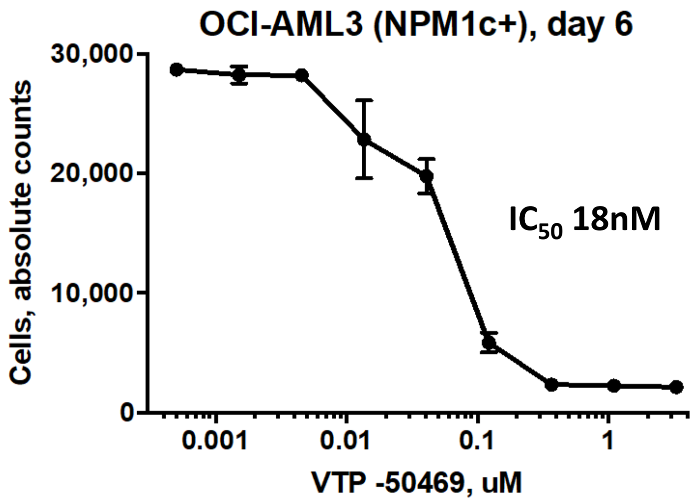
- First generation MLL:MEN inhibitors show that targeting of the MEN:MLL1 interaction inhibits cell proliferation in *MLL*-rearranged and *NPM1c*$^{+}$ AML. (Yokoyama et al 2005; Borkin et al., 2015; Kuhn et al., 2015)



- Currently available MEN:MLL interaction inhibitors have modest drug like properties. Therefore, VTP-50469 was developed as a novel orally available MEN:MLL1 inhibitor.

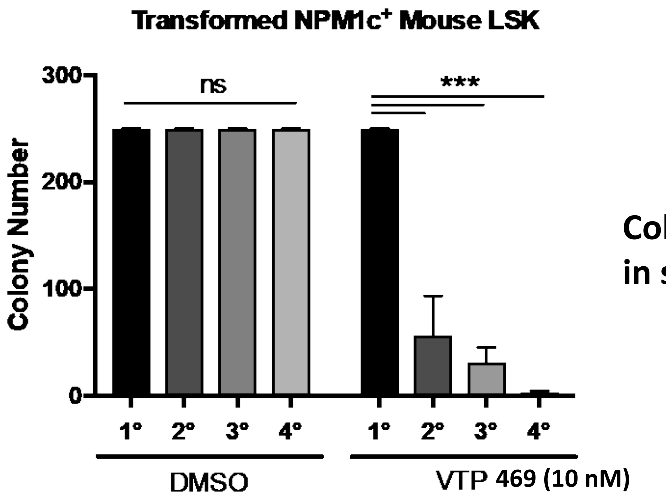
VTP-50469 selectively inhibits proliferation of cell lines with *MLL*-rearrangements and *NPM1c*$^{+}$ mutations





CellTiter-Glo assay

CELL LINE	FUSION	IC$_{50}$ nM
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
HB11;19	MLL-ENL	36
REH	NONE	>>2000
HL-60	NONE	>>2000



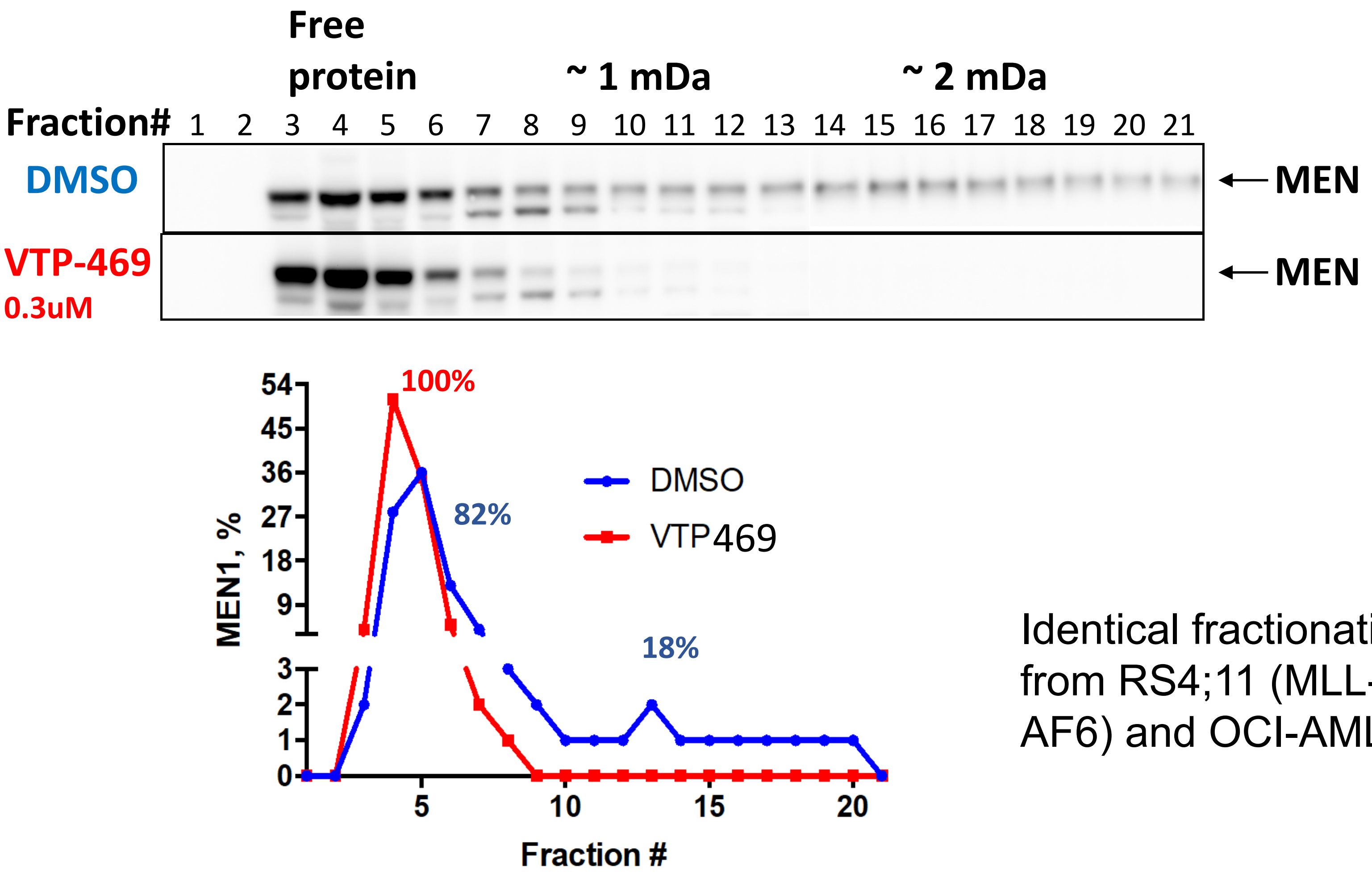
Colony forming assay in semi-solid media

VTP-50469 dissociates MEN from nuclear complexes

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

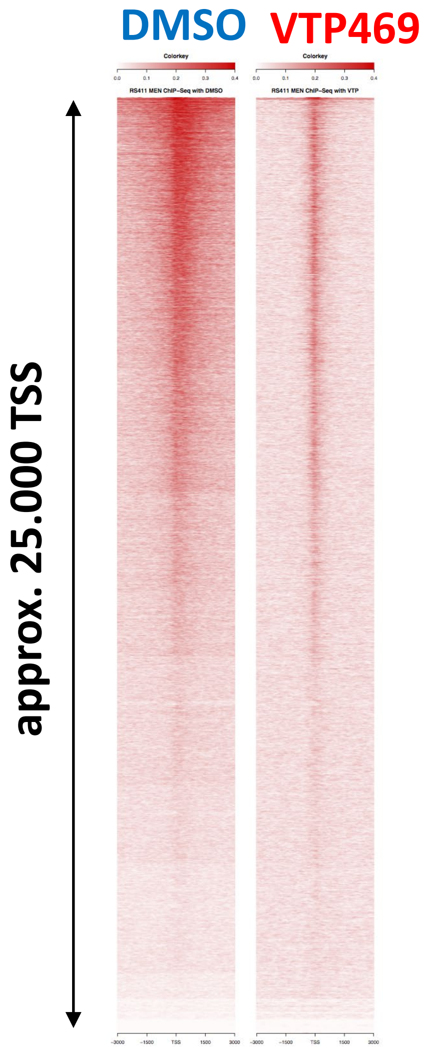
MOLM13 (MLL-AF9)

Day 3

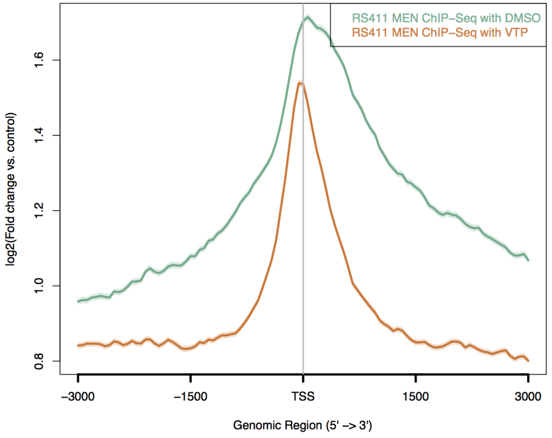


Identical fractionation results obtained from RS4;11 (MLL-AF4), ML-2 (MLL-AF6) and OCI-AML3 (NPM1c+) Cells

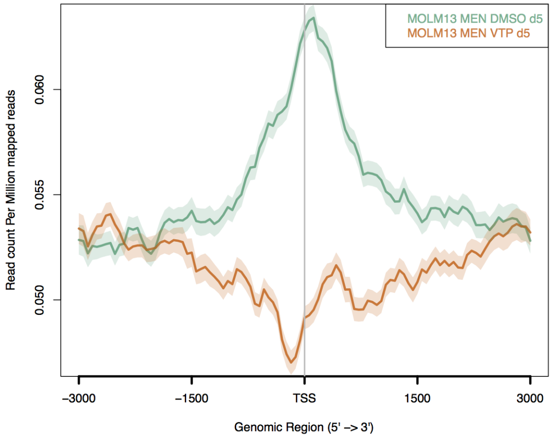
VTP-50469 treatment leads to MEN loss from TSS in *MLL*-rearranged cell lines

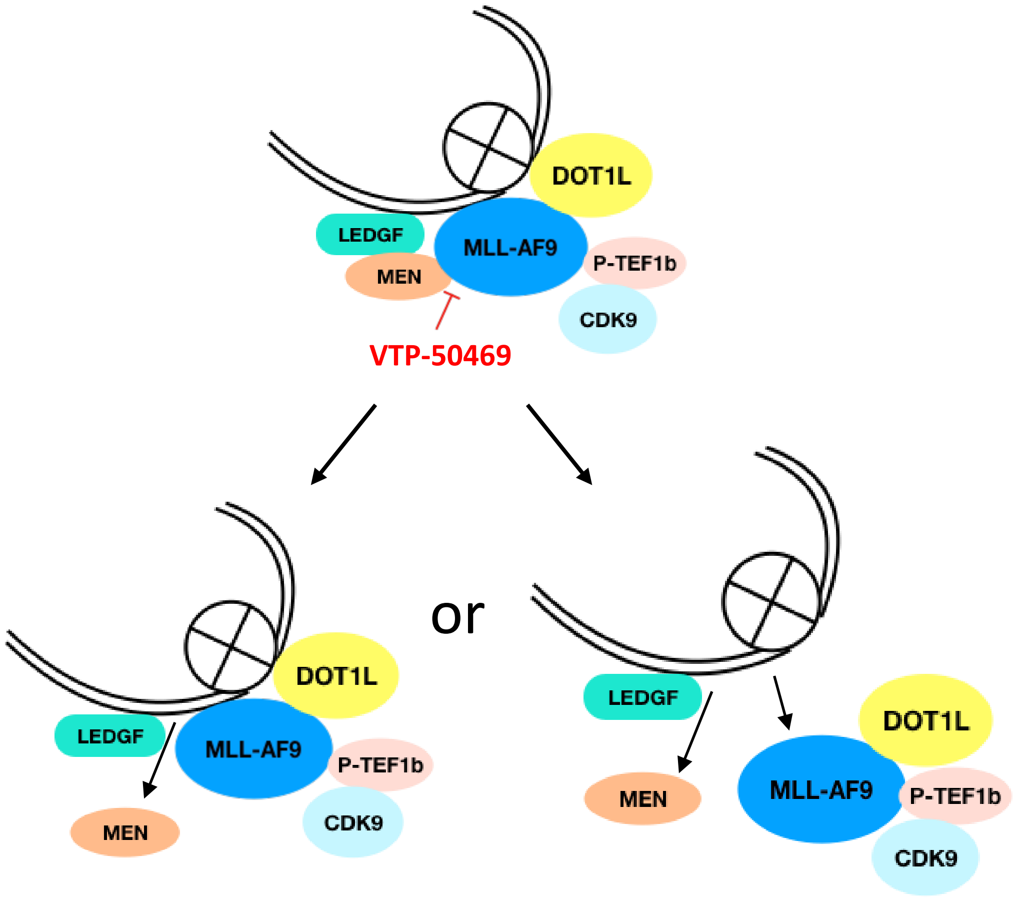


RS4;11, 2 days



MOLM13, 5 days





VTP-50469 treatment evicts DOT1L from Chromatin

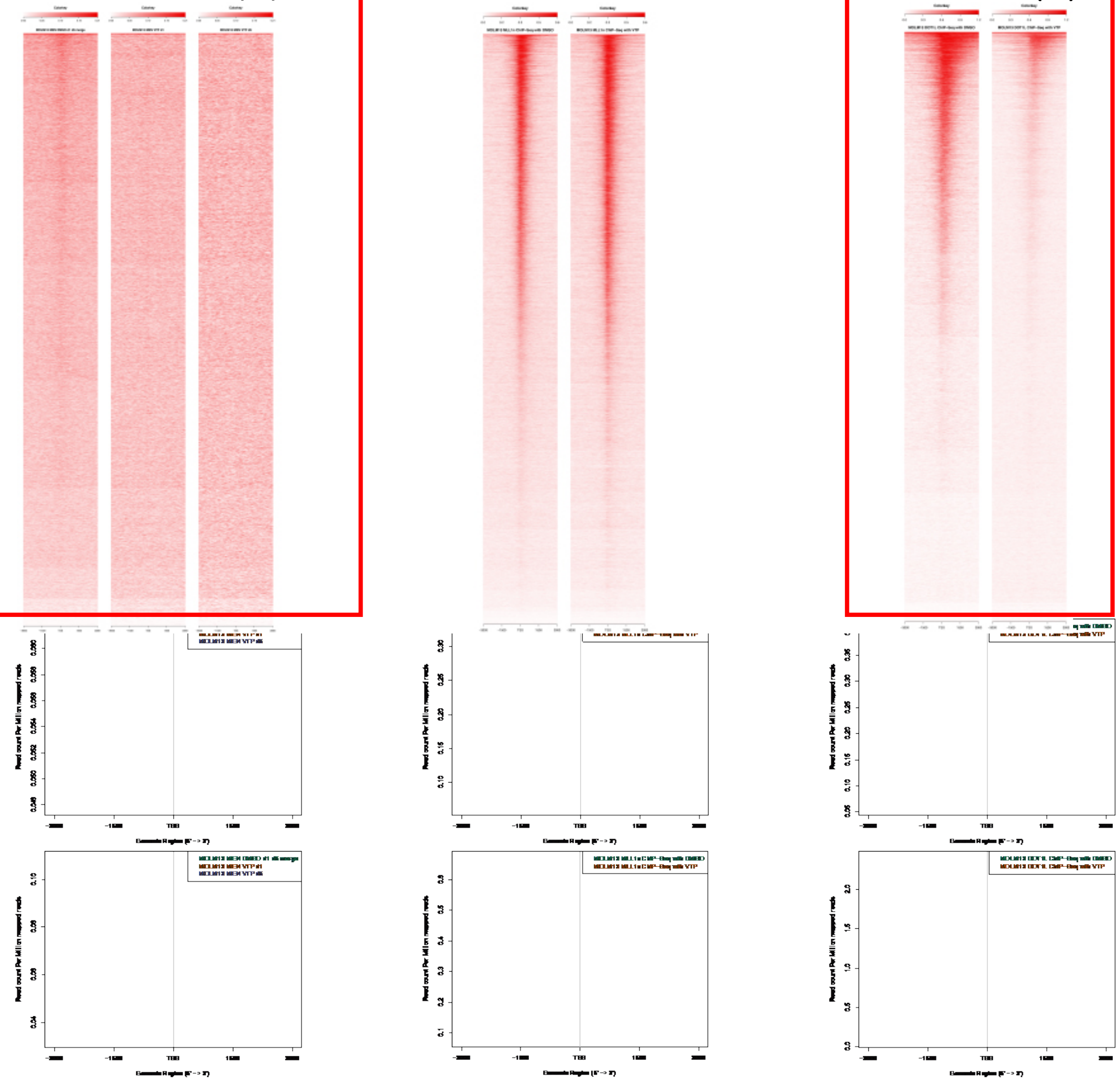
ChIP: Anti-MEN | Anti-MLL1n | Anti-DOT1L

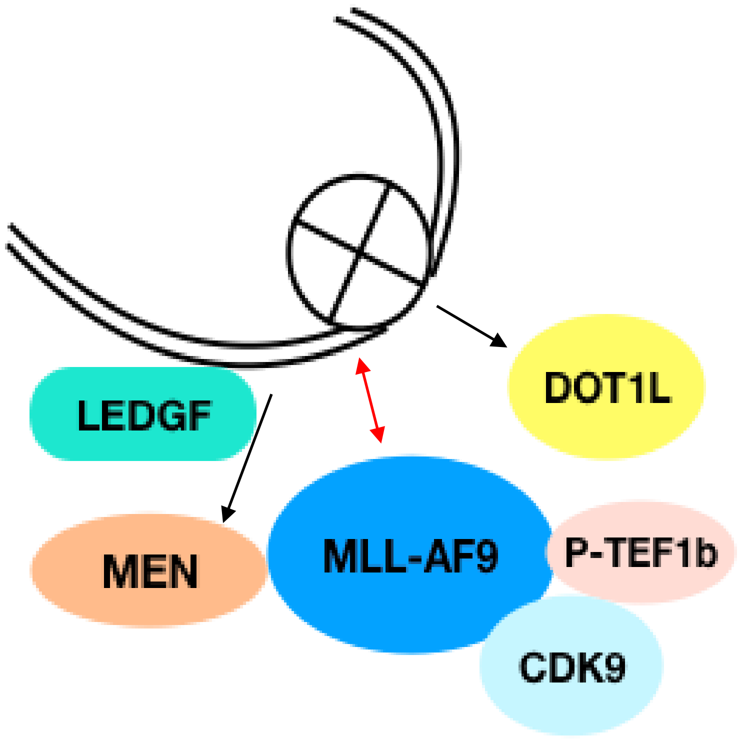
DMSO 469(1d) 469(5d) | DMSO 469(3d) | DMSO 469(3d)

MOLM13

TSS

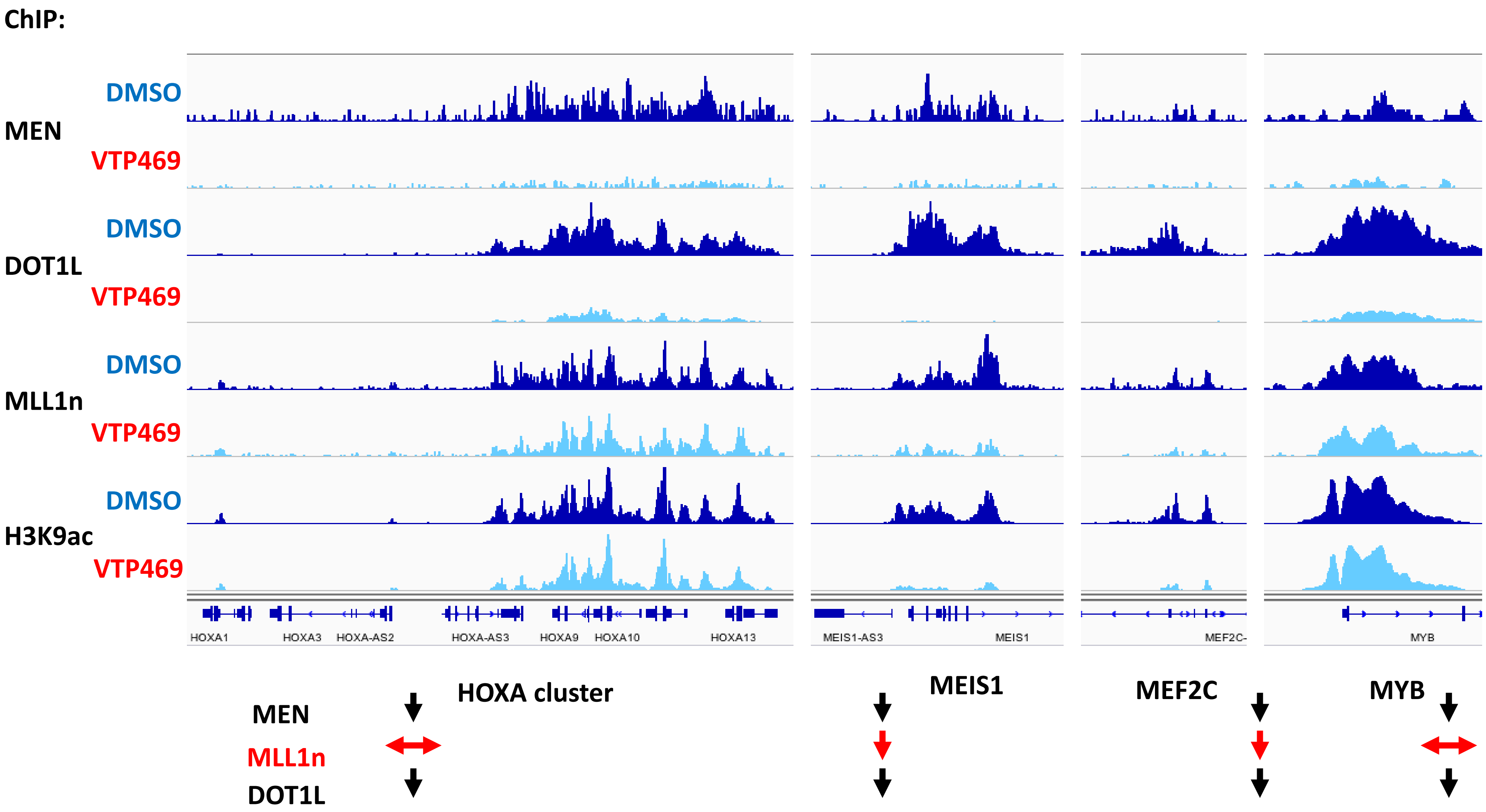
MLL-AF9 (Bernt)





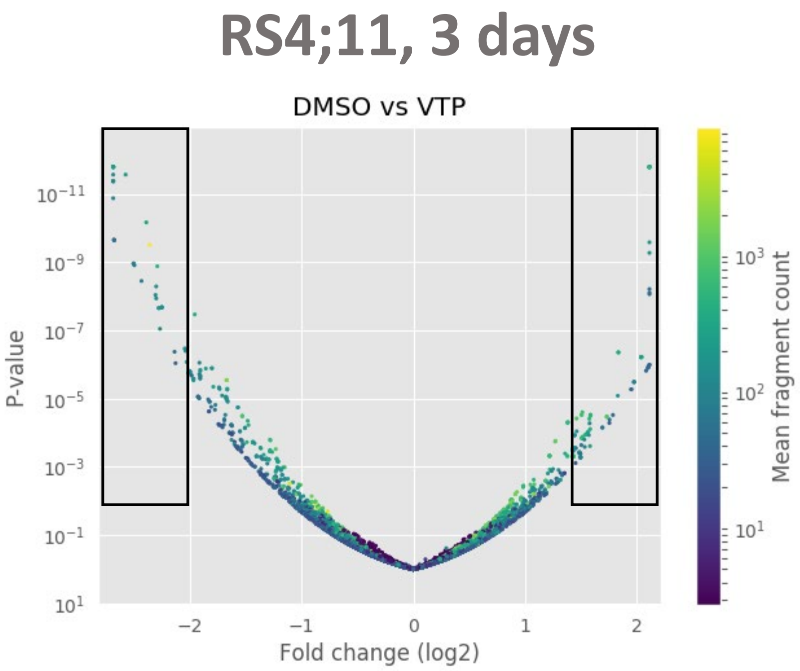
VTP-50469 treatment evicts DOT1L from MLL-fusion target genes

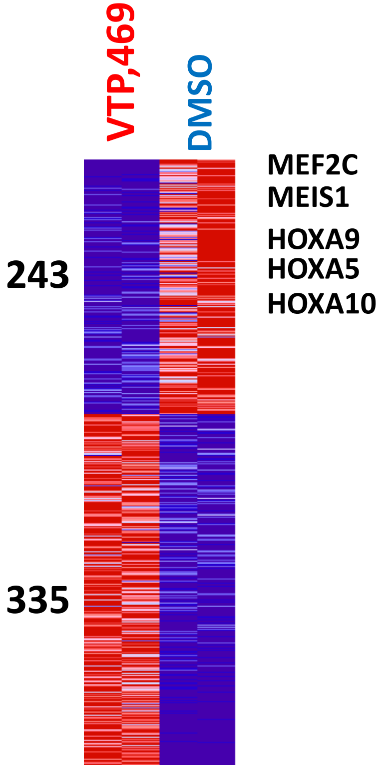
Molm 13 Cells



Identical ChIP-seq results obtained from RS4;11 (MLL-AF4) cells

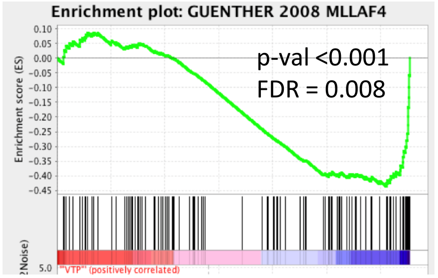
Treatment with VTP-50469 suppresses MLL-fusion target and DOT1L inhibitor sensitive genes



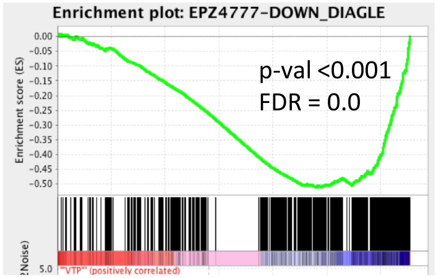


Gene lists:
Tags > 10
p-val < 0.05

MLL-AF4 targets

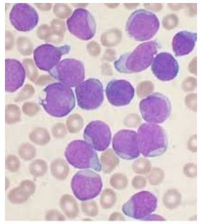


DOT1L inhibitor (EPZ4777)



***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**

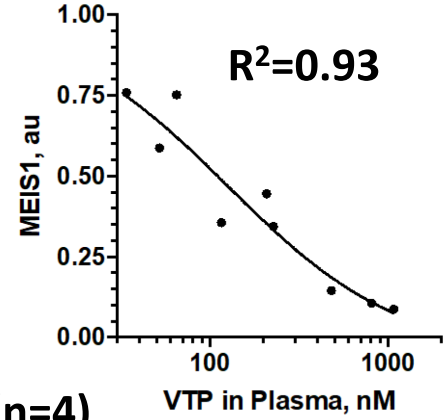
VTP-50469 treatment reduces leukemia burden in PDX models of MLL-r and NPM1 mutant leukemia



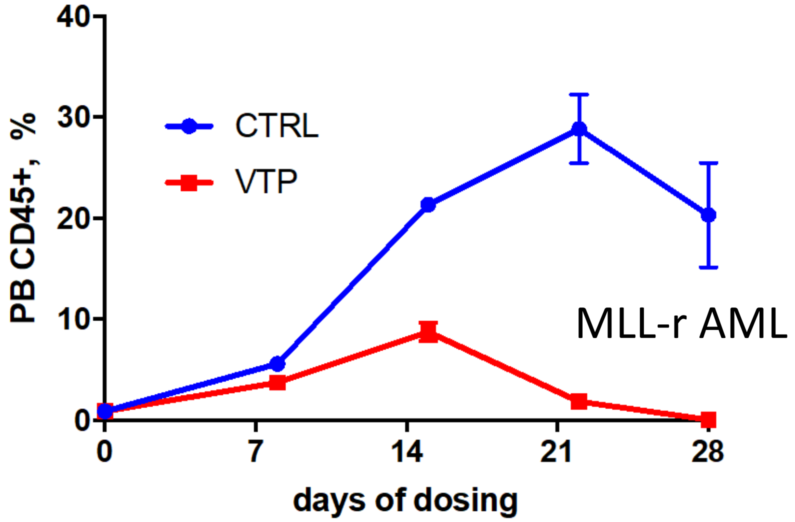
PDX (n=9)

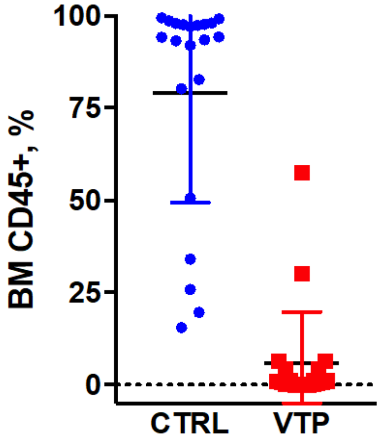
1-10% Leukemia in PB

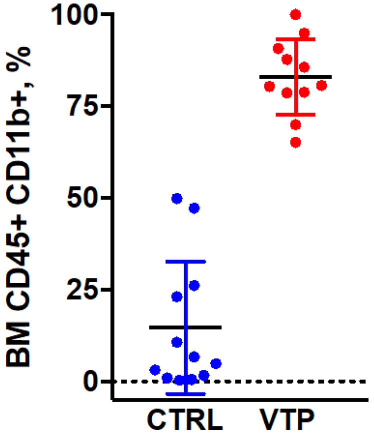
0.1% VTP, 100 mpk (IC_{90})
Plasma conc. 1-2 uM

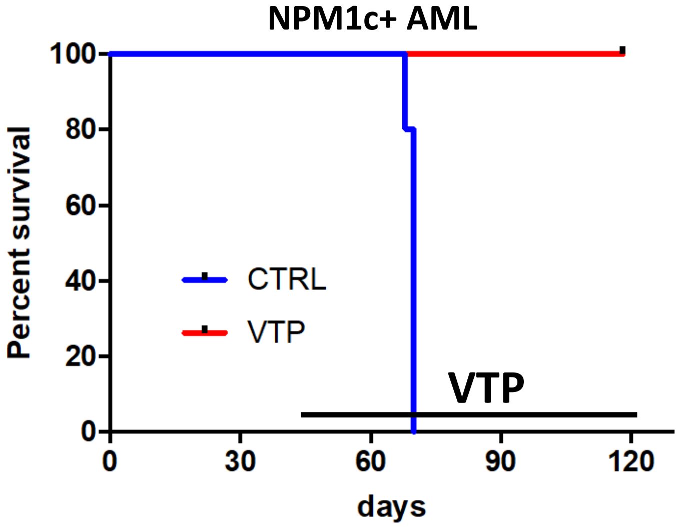


MLL-r B-ALL (n=3) and AML (n=2); NPM1c[+] AML (n=4)



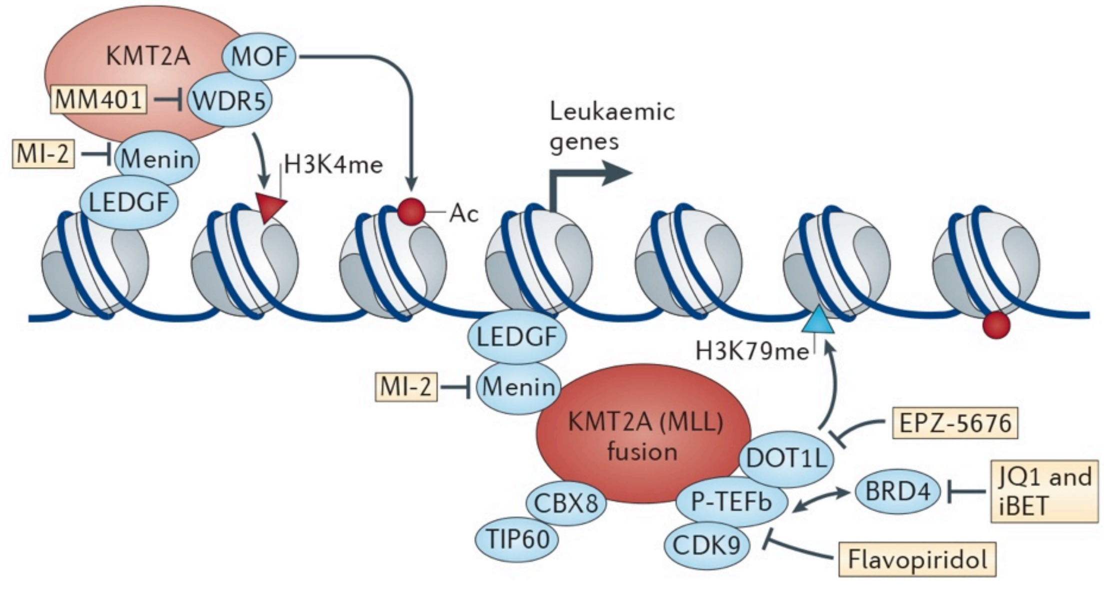




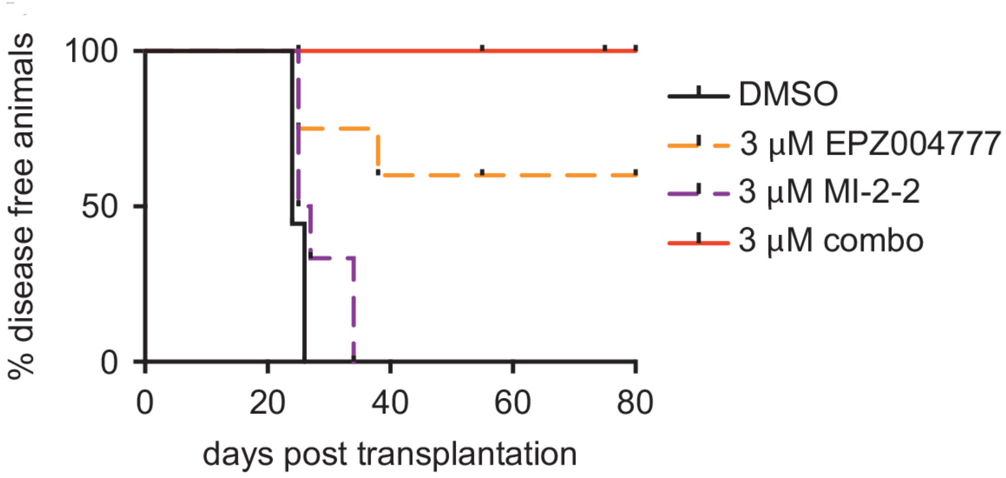


No response in AML without NPM1 mutations or MLL-Rearrangements

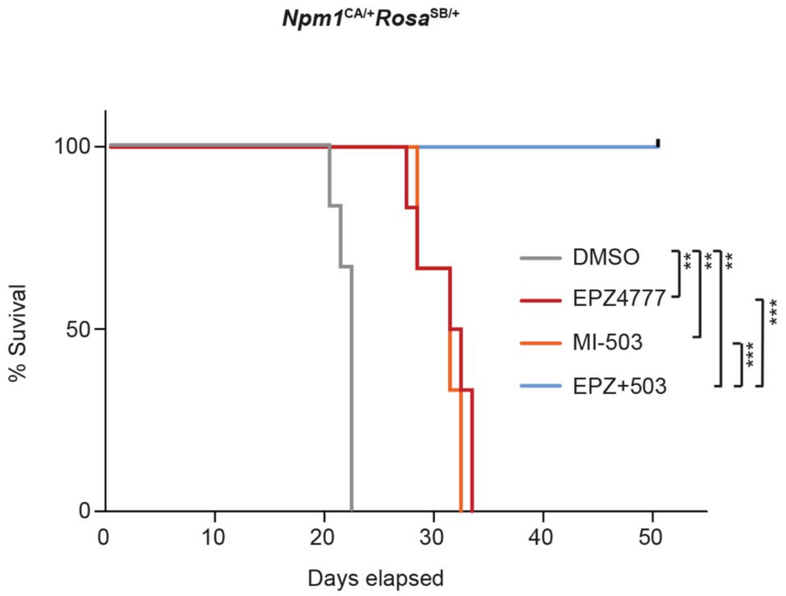
Combined DOT1L and Menin Inhibitors are Active Against MLL-Rearranged NPM1 Mutant AML Cells



MLL-r-AML



Npm1-mutant AML

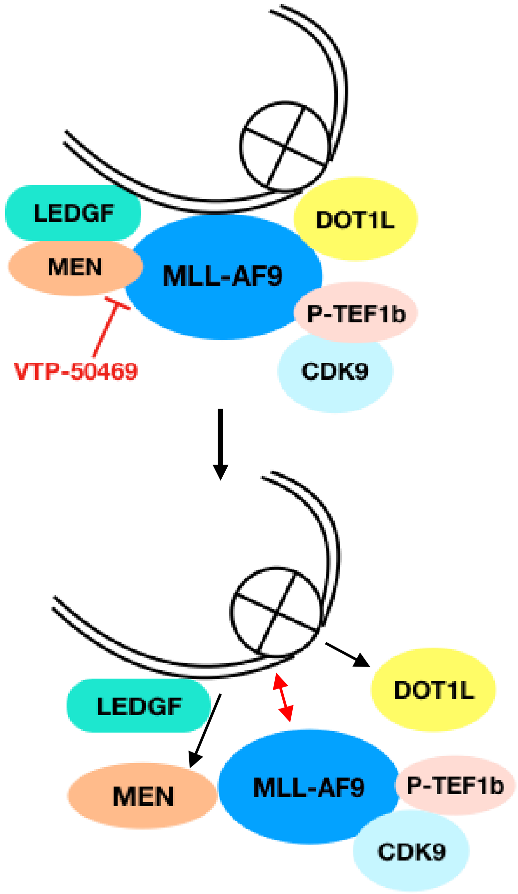


Conclusions:

- 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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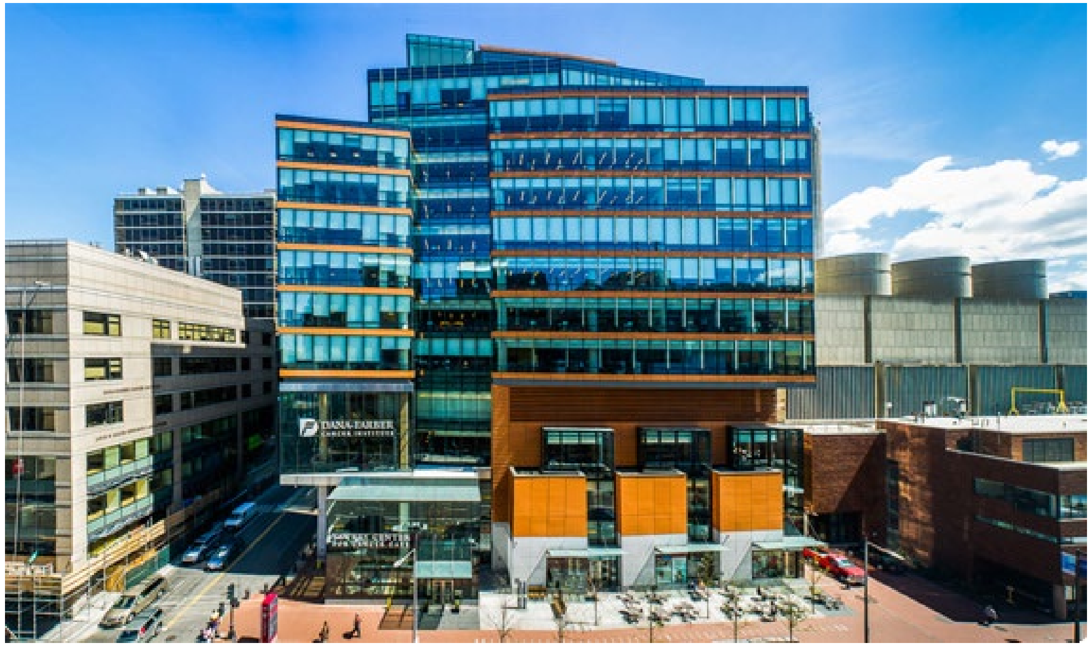

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