Clinical Translation of Epigenetics in Cancer Therapy

Targeting the Menin-MLL1 Interaction Site as a Treatment for Mixed Lineage Leukemia-rearranged (MLL-r) and NPM1c+ AML

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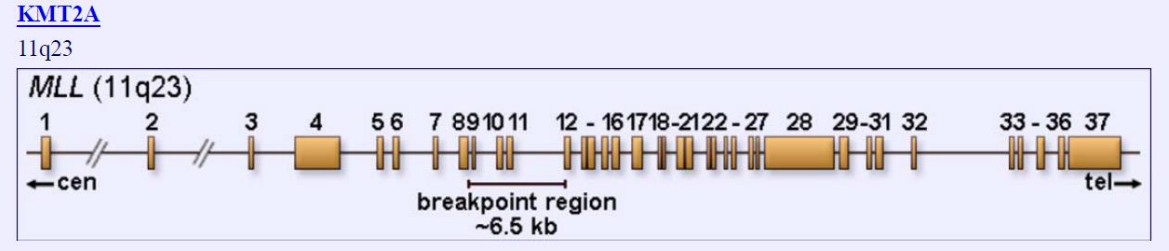
CANCER AND BLOOD DISORDERS CENTER

NCI Pediatric Preclinical Testing Consortium
A program funded by the National Cancer Institute of the National Institutes of Health

Children's Cancer Institute

Syndax

Translocations at MLL1 Locus Create Fusion Proteins with a Common N-terminal Sequence



NPM1c

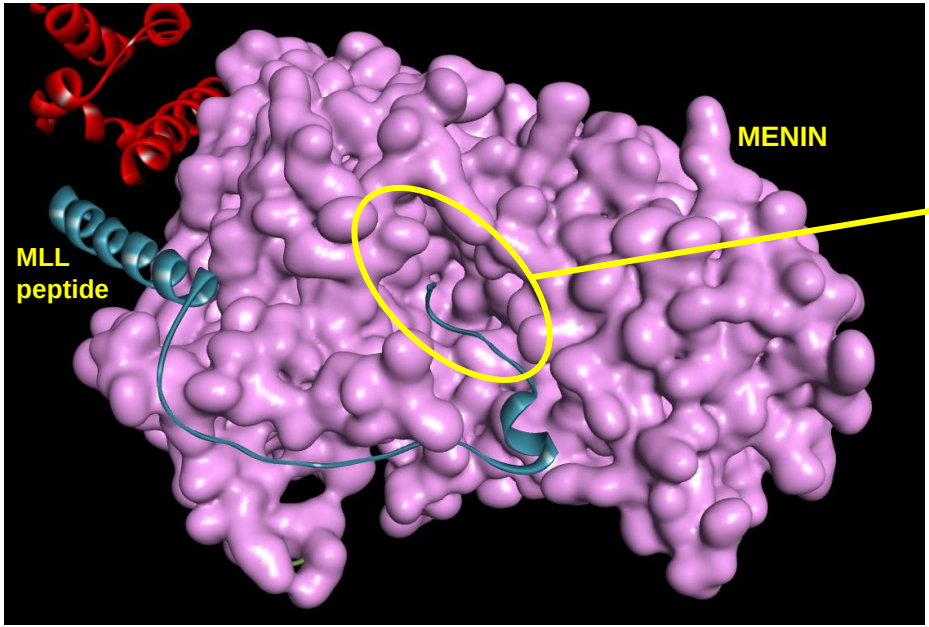
MLLr

>90 MLL fusions known

- Menin-MLL interaction inhibitors should block the binding of all fusions to menin

Nilson, et al. Br J Haematol. 1996 Jun;93(4):966-72

Menin-MLL Inhibitors Target the High Affinity Binding Site of MLL1 (aa 9-13) on Menin

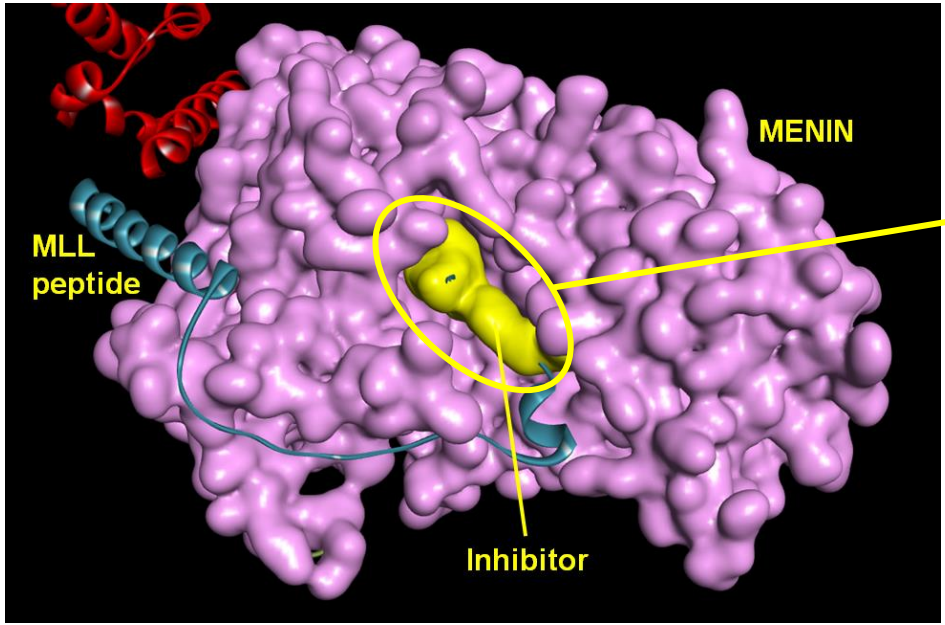


9------13

M-A-H-S-C-R-W-R-***F-P-A-R-P***-G-T-T-G-G-G-

- Menin-MLLr interaction inhibitors derived through structure-based drug design in the high affinity MLL1 binding pocket

Menin-MLL Inhibitors Target the High Affinity Binding Site of MLL1 (aa 9-13) on Menin



MLL Binding Pocket

9------13

M-A-H-S-C-R-W-R-***F-P-A-R-P***-G-T-T-G-G-G-

- Inhibitors bind with high affinity in MLL1 pocket in menin

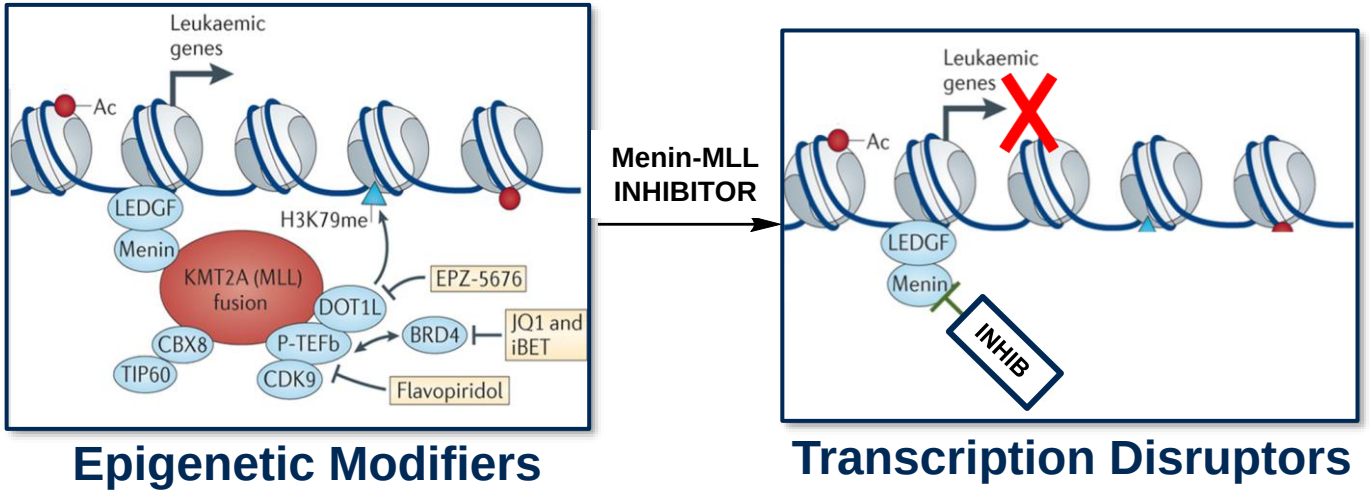
Potent, Orally Active Menin Inhibitor (VTP-50469) Used to Interrogate Menin-MLL Biology

Parameter	VTP-50469
Binding K_i (nM)	0.104 **
Dissociation $t_{1/2}$ (min)	198
MV4;11 Cellular IC_{50} (nM)	17 $\pm$ 4
$t_{1/2}$ (h):Rat, Dog	4.1, 4.8
%F: Rat, Dog	61, >100

- Used in vitro and in vivo to define the scope of Menin-MLL inhibitor biology

** Equipotent on mouse menin

MLLr enables leukemia development by promoting an aberrant transcription program

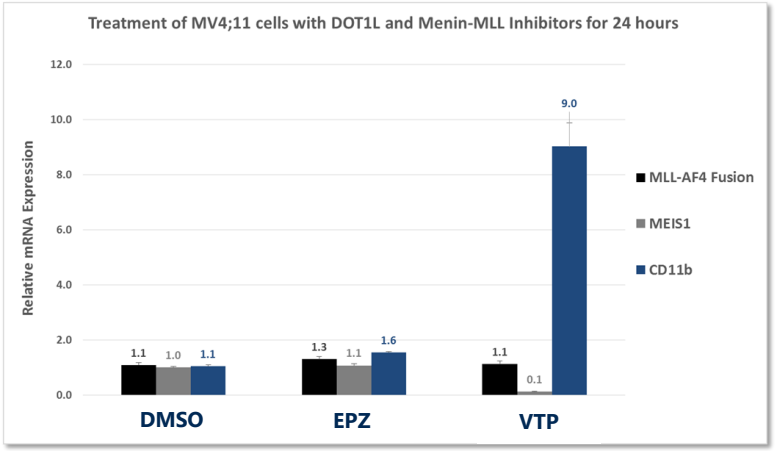


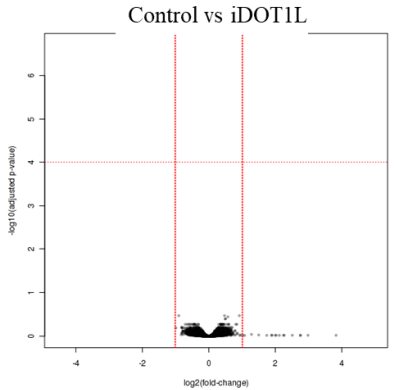
Epigenetic Modifiers **Transcription Disruptors**

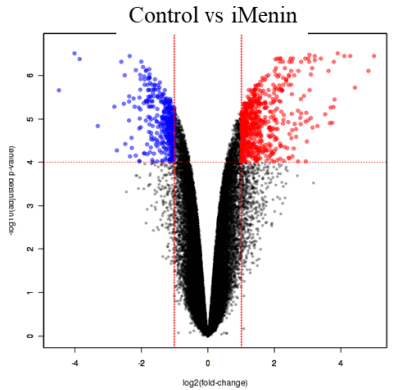
- Disruption of the Menin-MLLr interaction should have a direct, rapid global effect on the transcription profile

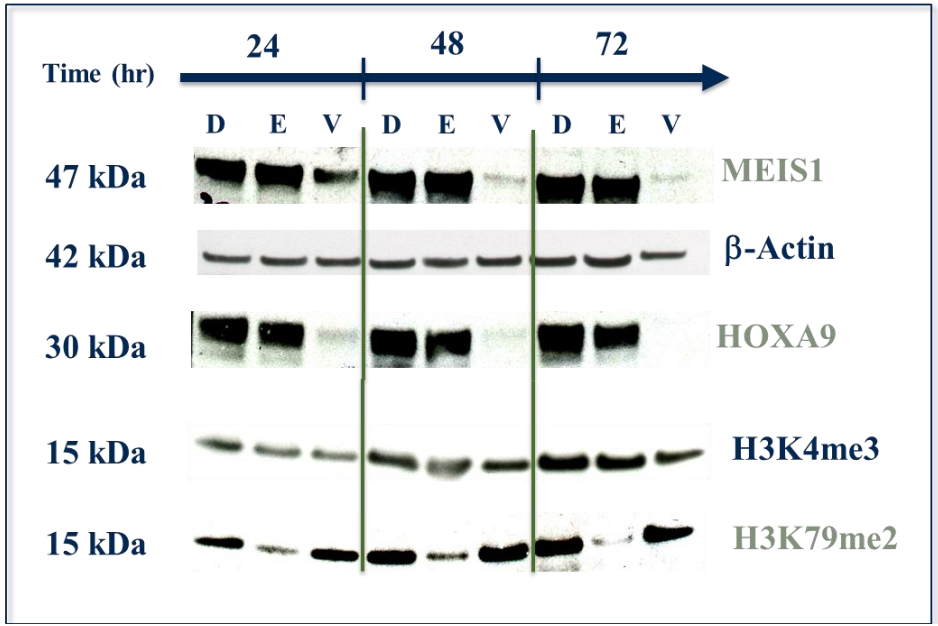
Nature Reviews | Cancer

Menin inhibition in MV4;11 cells has acute treatment effects that are differentiated from DOT1L inhibition



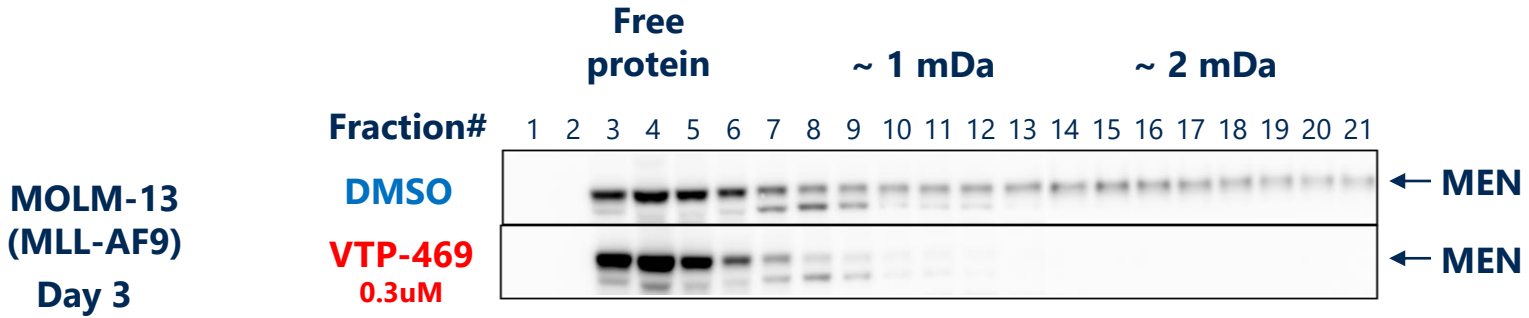






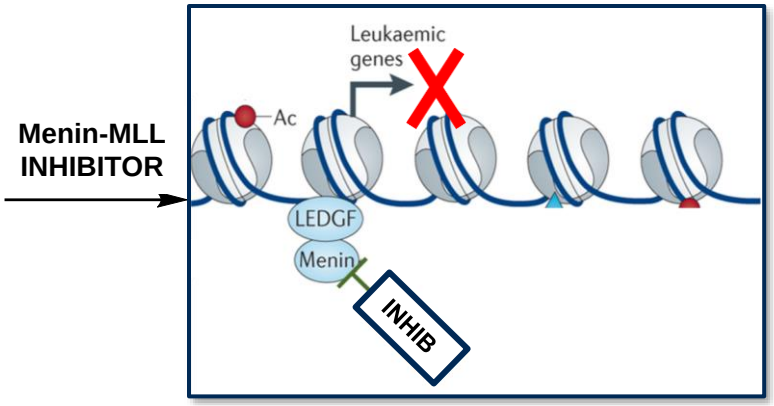
VTP-50469 dissociates MEN from nuclear complexes

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

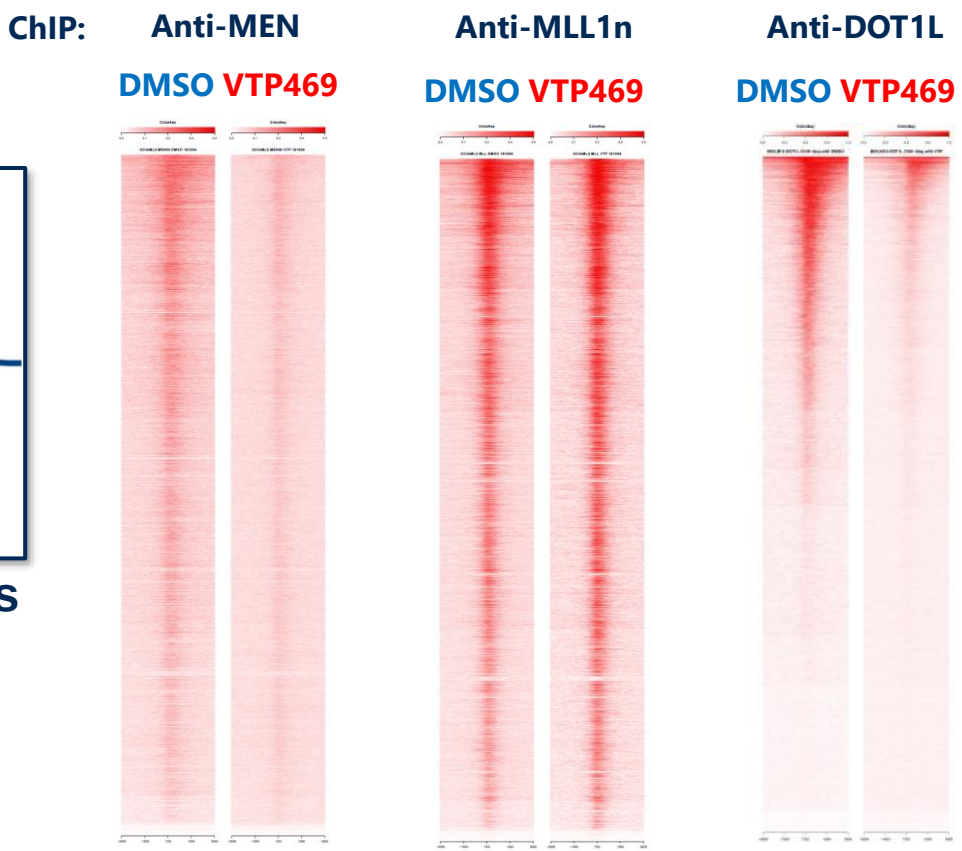


- Identical fractionation results obtained from RS4;11 (MLL-AF4), ML-2 (MLL-AF6) and OCI-AML3 (NPM1c+)

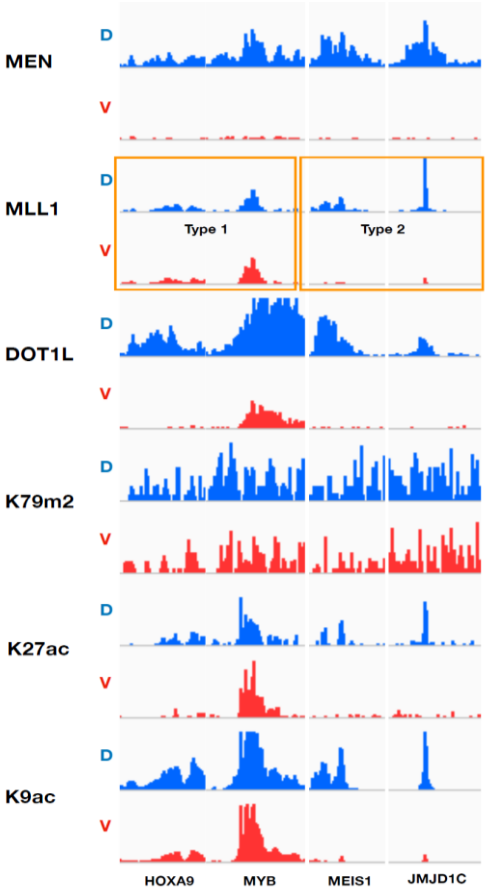
VTP-50469 treatment evicts Menin and DOT1L from chromatin…but not MLL1

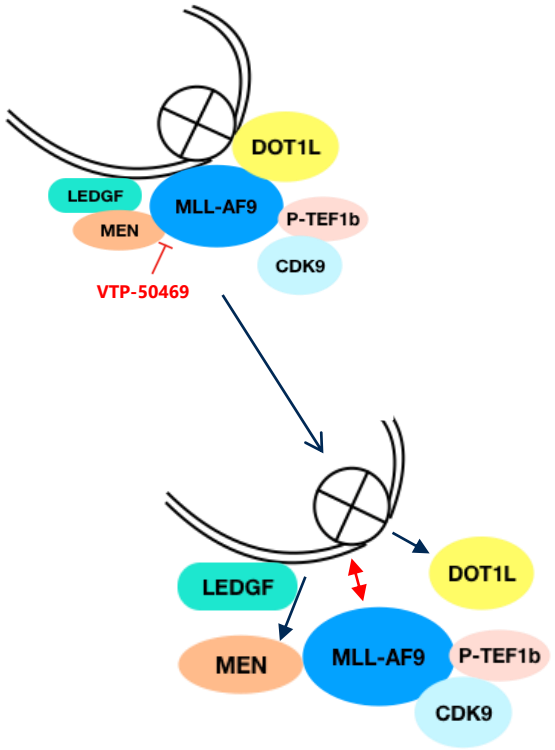


Transcription Disruptors



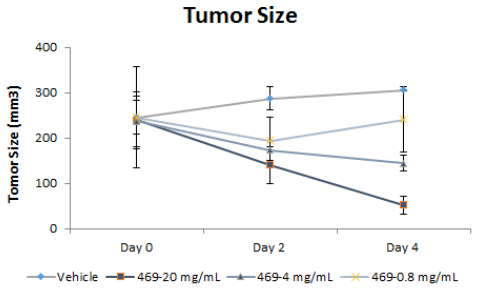
Menin-MLL blockade has differential effects at chromosomal loci

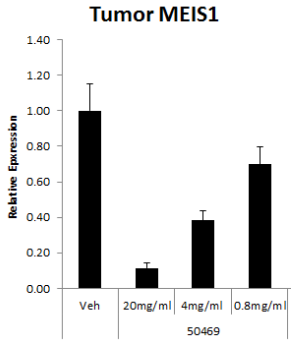


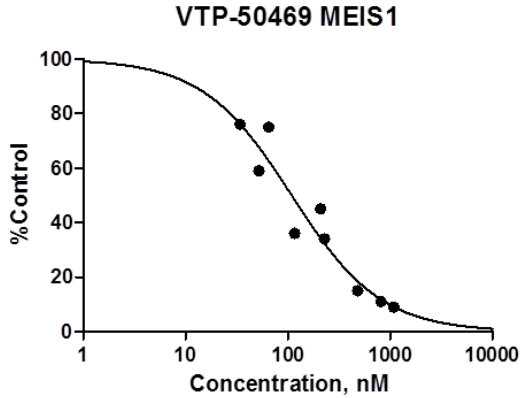


Steady-state infusion of VTP-50469 can be used to define PK/PD in vivo using MV4;11 s.c. tumors in nu Rats

- Implant MV4;11 cells s.c. and grow to 200-300 mm^3
- Implant Alzet 7-day mini-pumps containing VTP-50469 (0.8, 4, 20 mg/ml) contralaterally
- Measure changes in tumor size over 3-4 days and in target transcripts at sacrifice
- Measure blood levels to establish PK/PD relationship



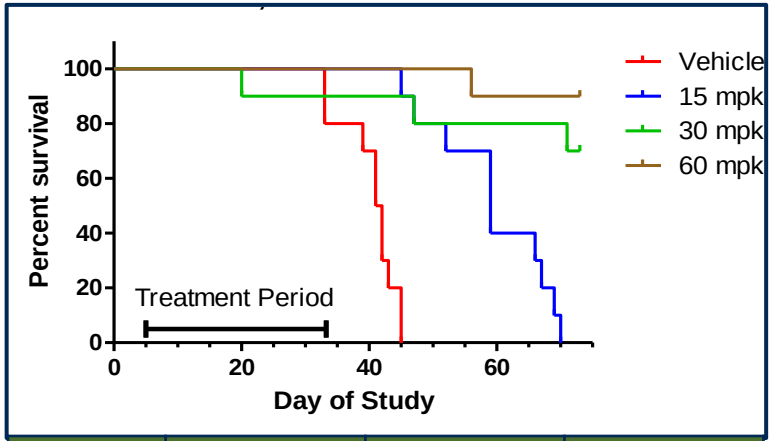




Plasma IC_{50} = 109 ± 16 nm

VTP-50469 Provides a Significant Dose-Dependent Survival Benefit (K-M) in Disseminated MV4;11 Tumors

- Engraft MV4;11-luc+ cells given i.v.
- Animals randomized at d5 by BLI
- VTP-50469 administered orally twice a day (15, 30, 60 mpk bid) for 28 day
- Survival monitored until d74
- For high dose group, 7 of 9 survivors had <0.1% of MV4;11 cells in BM



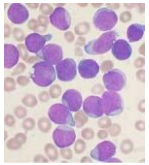
Plasma Collection Time	15 mg/kg bid	30 mg/kg bid	60 mg/kg bid
	Mean (nM)	Mean (nM)	Mean (nM)
4hr	234	635	1621
pre-dose	8	18	37

PDX Efficacy studies conducted with VTP-50469

DFCI (0.1% in Chow) NCI/CCI (120 mg/kg bid MTD)

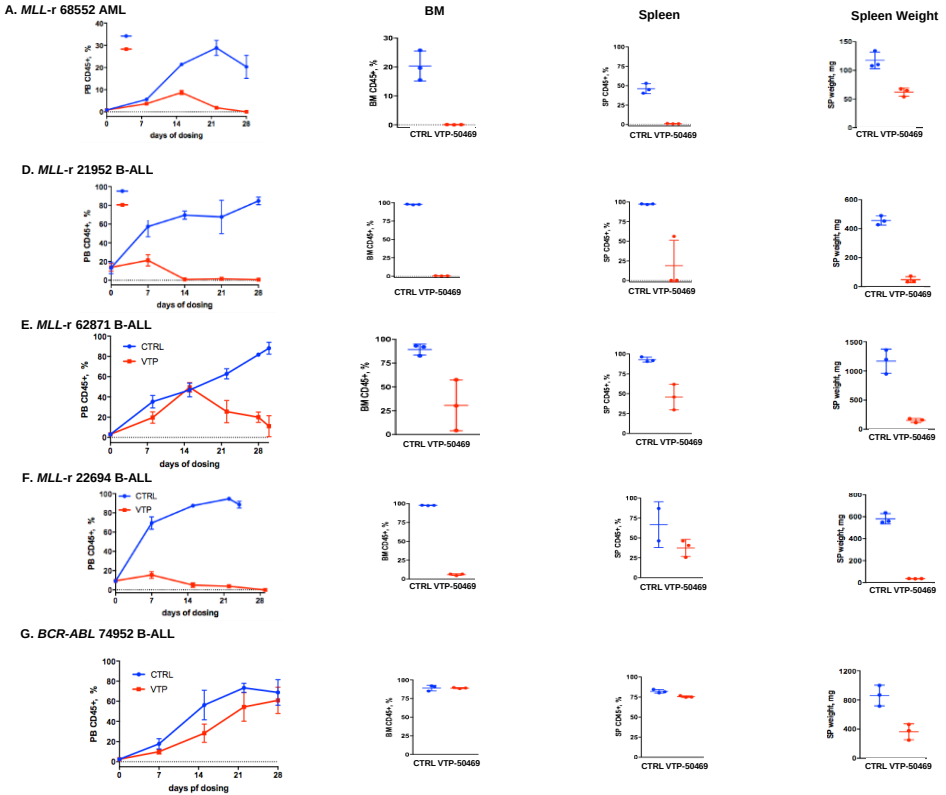
PDX NUMBER	DISEASE	AGE (yr)	TX NAÏVE	MUTATIONS	TREATMENT DAYS	TREATMENT
CBAB-21952	• MLLR B-ALL	• 2.7	• NO	• t(4;11)(q21;q23) MLL-AF4	28	0.1% CHOW
CBAB-22694	• MLLR B-ALL	• 1.3	• YES	• MLL-split by FISH	30	0.1% CHOW
CBAB-62871	• MLLR B-ALL	• 2.3	• YES	• t(4;11)(q21;q23) MLL-AF4	30	0.1% CHOW
CBAM-68552	• MLLR AML	• 1.25	• NO	• MLL-split by FISH	28	0.1% CHOW
DFAM-40315	• MLLR AML	• 65	• NO	• t(9;11) MLLT3-MLL	109	0.1% CHOW
MLL-1	• MLLR B-ALL		• YES	• t(1;11), MLL-EPS15	28	120 mg/kg bid
MLL-2	• MLLR B-ALL		• YES	• t(4;11) MLL-AF4	28	120 mg/kg bid
MLL-3	• MLLR B-ALL		• YES	• t(11;17) MLL-GAS7	28	120 mg/kg bid
MLL-5	• MLLR B-ALL		• YES	• t(10;11) MLL-MLLt10 (AF10)	28	120 mg/kg bid
MLL-6	• MLLR B-ALL		• YES	• t(11;19) MLL-ENL	28	120 mg/kg bid
MLL-7	• MLLR B-ALL		• YES	• t(4;11) MLL-AF4	28	120 mg/kg bid
MLL-8	• MLLR B-ALL		• YES	• t(11;19) MLL-ENL	28	120 mg/kg bid
MLL-14	• MLLR B-ALL	• 2.5	• YES	• t(11;19) MLL-ENL	28	120 mg/kg bid
DFAB-74952	• Ph+ B-ALL	• 44	• YES	• BCR-ABL |MLL1-WT | NPM1-wt	28	0.1% CHOW
DFAM-16835	• NPM1c AML	• 61	• NO	• FLT3 ITD | FLT3 TKD | NPM1 |	63	0.1% CHOW
DFAM-22359	• NPM1c AML	• 44	• NO	• NPM1c+ | FLT3	30	0.1% CHOW
DFAM-61345	• NPM1c AML	• 55	• NO	• NPM1c+ | FLT3-ITD	37	0.1% CHOW
MDAM-12313	• NPM1c AML	• 54	• YES	• NPM1c+ | FLT3-TKD	43	0.1% CHOW

Responses of MLLr AML and ALL PDXs to VTP-50469 (DFCI)

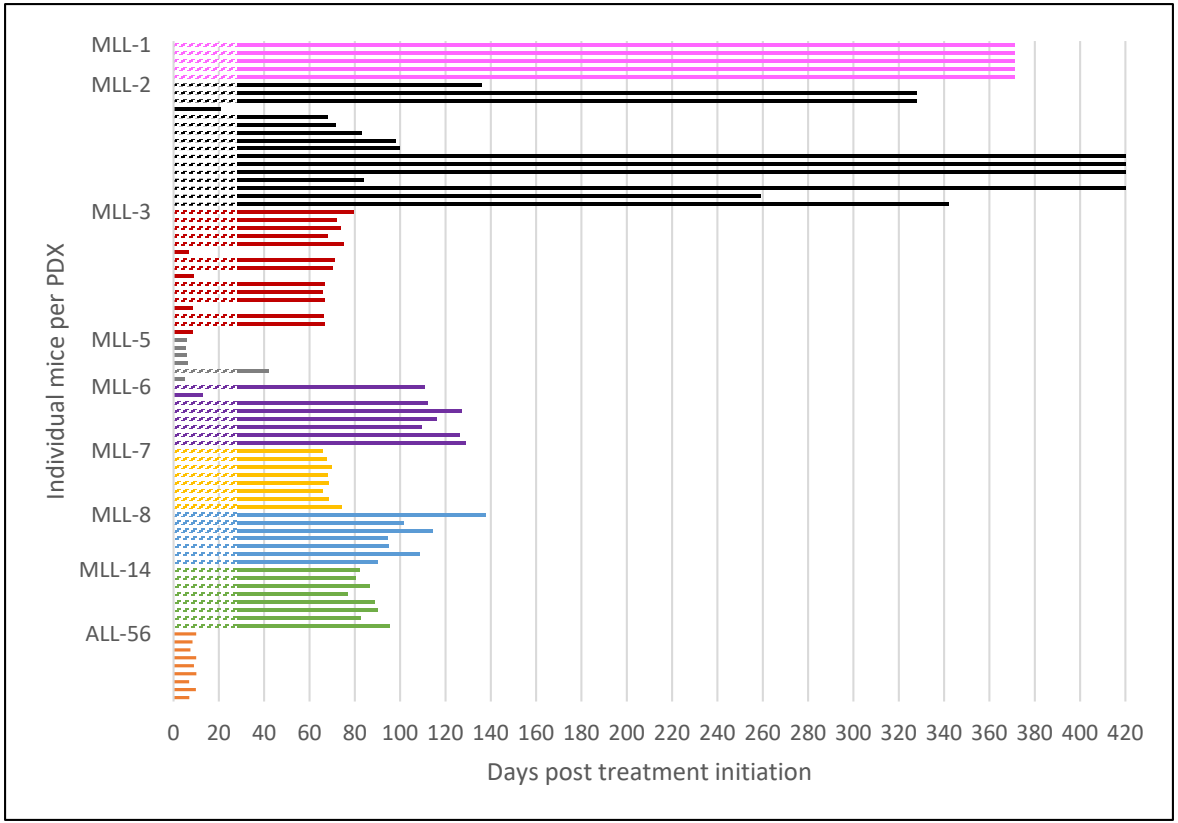


1-10% PB Leukemia

0.1% Chow
0.6 µM – 2 µM

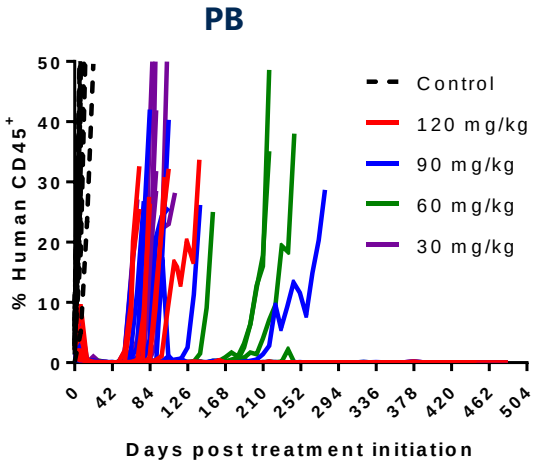


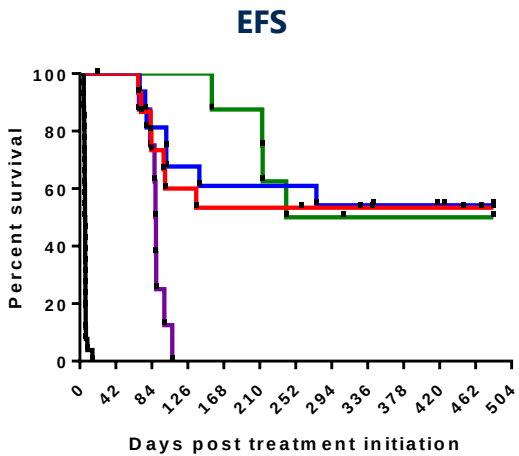
Swimmer plots of ALL PDXs tested at 120 mg/kg (CCI)

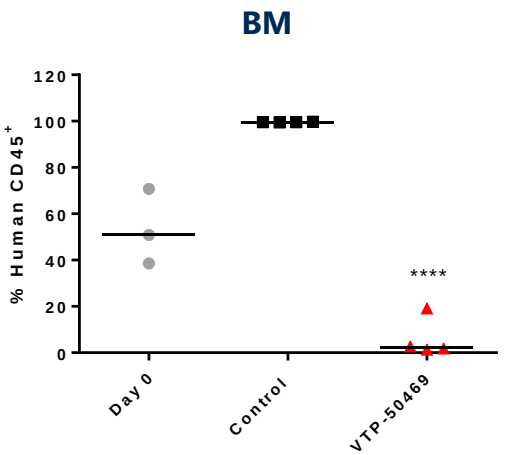


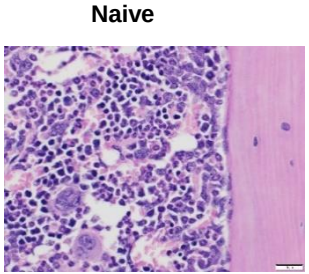
- Robust single agent effect in multiple pediatric MLLr PDXs
- Potential cures with certain MLLr subtypes observed
- Interrogate dose-dependence of the drug response

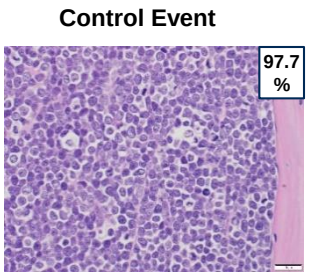
Dose-dependent responses of MLL-2 ALL PDX to VTP-50469

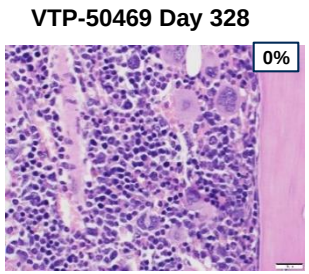




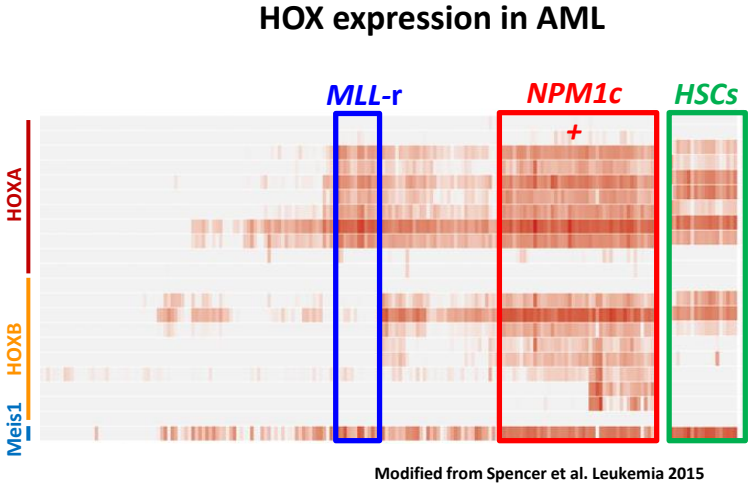






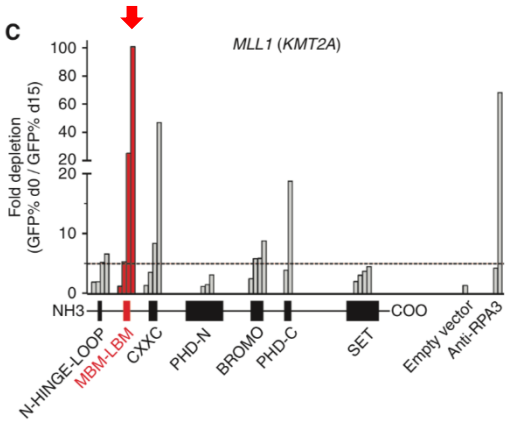


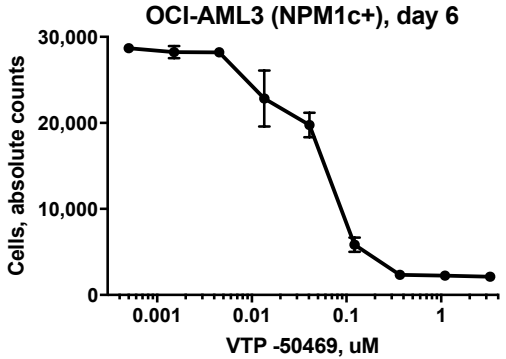
MLLr and NPM1c AML share common HOX signature and dependencies



Can MLL-Menin inhibition reverse NPM1c induced self-renewal program?

Menin interaction domain

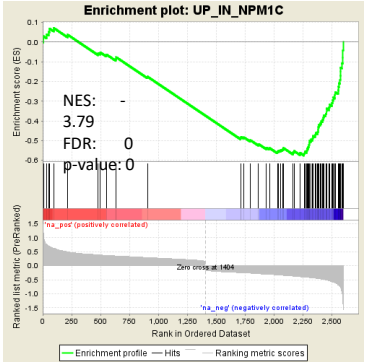




Meis1 is repressed by VTP-50469 in NPM1c mutant cells

NPM1c regulated genes

A



RNAseq

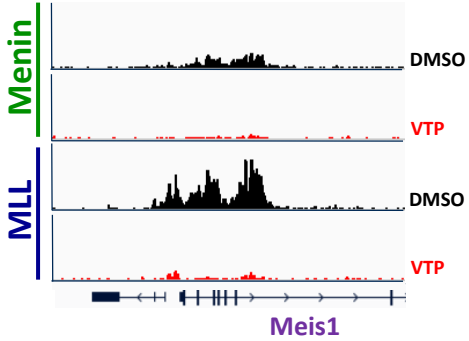
B

Top Ten DOWN VTP:

Symbol	FoldChange (Log2)	p-value
Mecom	-1.57	9.02e-19
Dmrta2	-1.45	3.25e-16
Meis1	-1.36	7.86e-15
Hnf4a	-1.13	7.74e-11
Pcp4l1	-1.12	1.07e-11
Ddx4	-1.10	9.13e-11
Pbx3	-1.07	1.05e-22
Spns2	-1.03	2.75e-09
Kdm5b	-1.00	3.37e-21
Angpt1	-0.99	4.65e-11

C

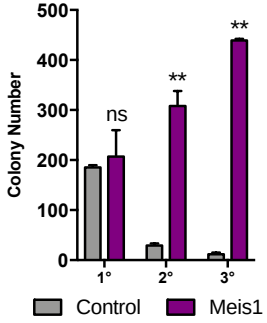
ChIPseq



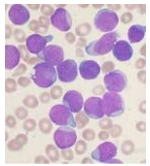
D

***In vitro* Rescue**

10nM VTP-50469

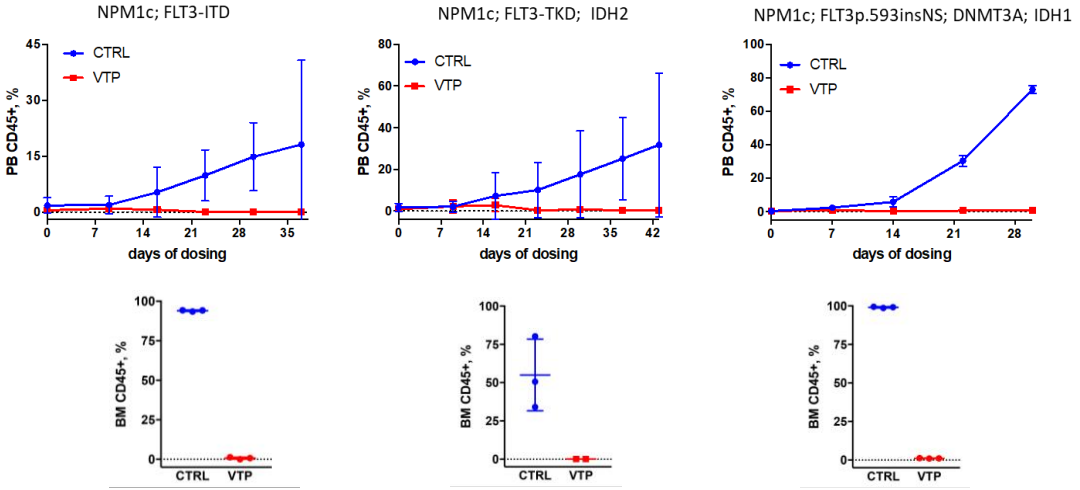


Treatment with VTP50469 reduces leukemia burden in NPM1c+ AML PDX models



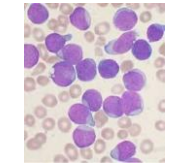
1-10% PB Leukemia

0.1% Chow
0.6 µM – 2 µM



- Compound administered in feed significantly reduced leukemic burden in whole blood and BM at sacrifice

VTP-50469 treatment has durable effect in NPM1c AML



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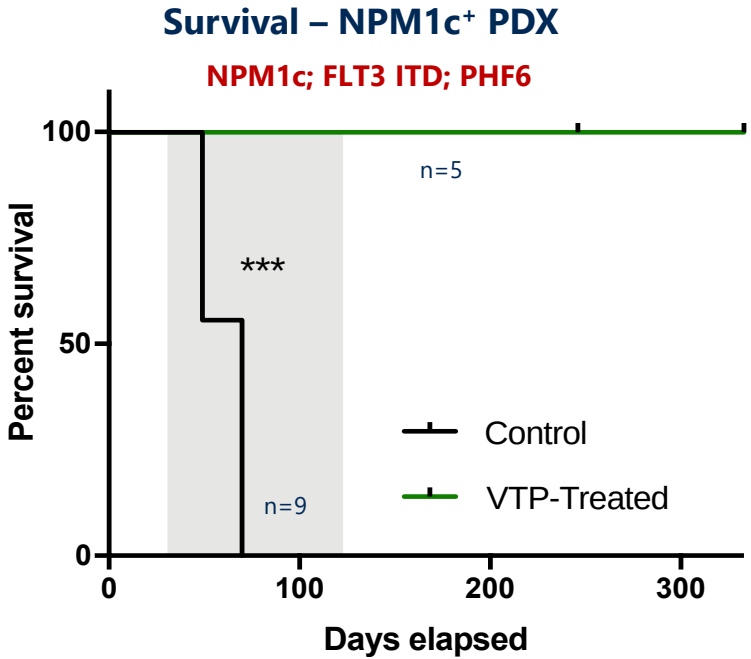
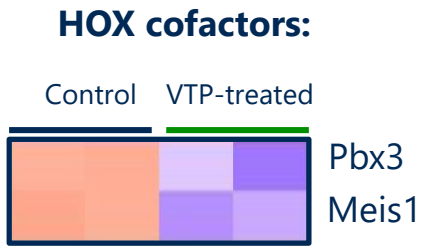
0.6 µM – 2 µM





Summary

- Menin inhibitors demonstrate potent anti-proliferative activity in both MLLr and NPM1c+ AML
- Menin-MLL inhibition exerts broad effects on the transcriptional landscape in
- VTP-50469 has profound single agent activity and confers a significant survival advantage in PDX models of MLLr (AML, ALL) and NPM1c+ AML

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