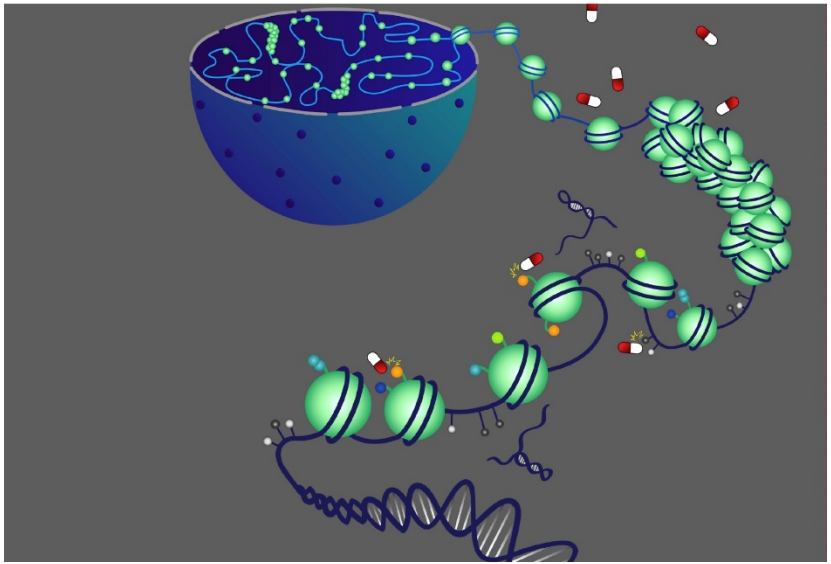
Targeting Epigenetic Mechanisms to Reverse Stem Cell Programs in Cancer

Scott A. Armstrong MD, Ph.D.



DANA-FARBER Boston Children's
CANCER AND BLOOD DISORDERS CENTER

HARVARD
MEDICAL SCHOOL

Disclosures

Scientific advisory boards (Compensation or equity stake)

1. C4 Therapeutics
2. Syros Pharma
3. Accent Pharma
4. Imago Biosciences
5. OxStem Pharma
6. Cyteir Therapeutics
7. Mana Therapeutics

Sponsored Research

1. Janssen Pharma
2. AstraZeneca
3. Novartis

MLL1 Translocations: An Oncogenic Chromatin Regulator

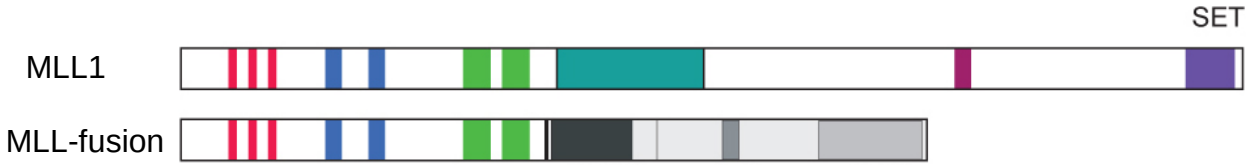
-Located at 11q23 and consistently rearranged in infant acute leukemia resulting in AML or B-ALL. MLL-ALL has a particularly poor prognosis

1992: Rowley, Cleary, Korsmeyer, Croce
-11q23 Breakpoint Cloned and Identified as MLL gene

1995: Korsmeyer
-MLL1 controls development and *HOX* gene expression

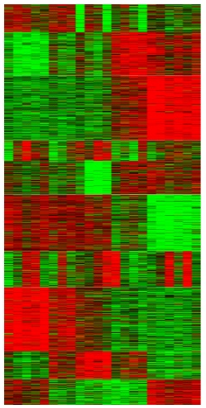
1996: Cleary, Rabbitts
-MLL1-fusion proteins oncogenic

2002: Allis/Hess, Canaani
-MLL1 is an H3K4 Methyltransferase

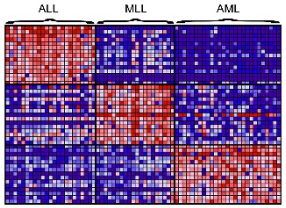


Progression from Stem or Progenitor to Leukemia Stem Cell

Mouse Models

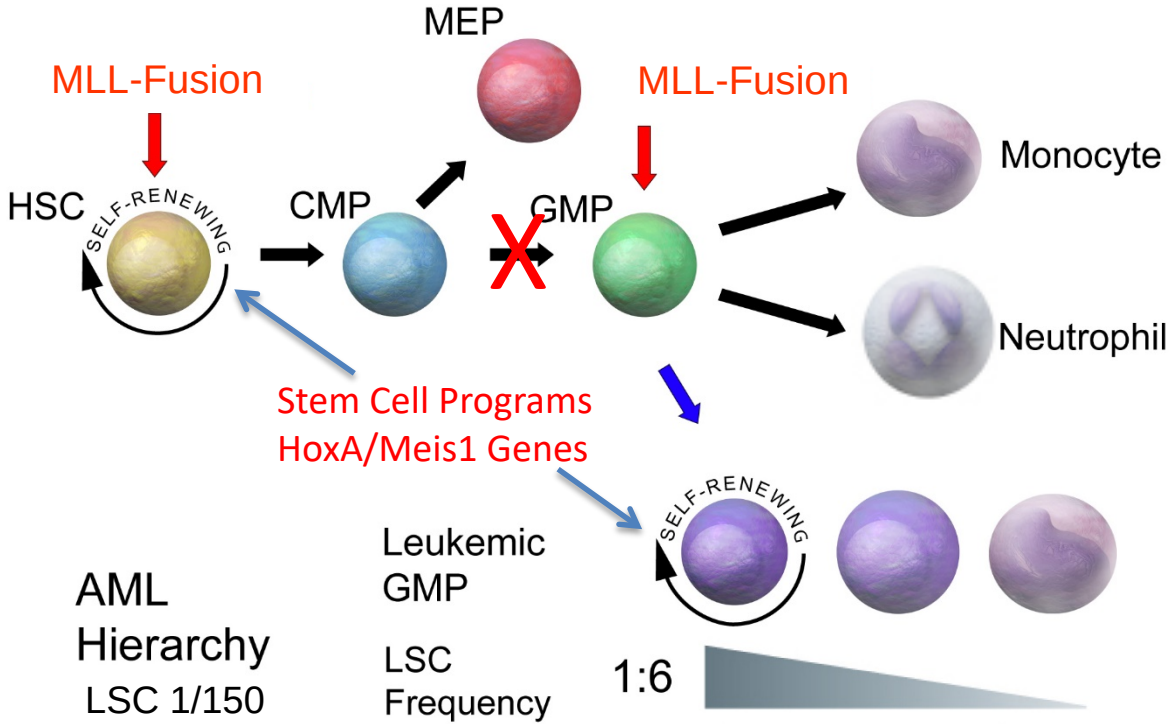


Human Leukemias

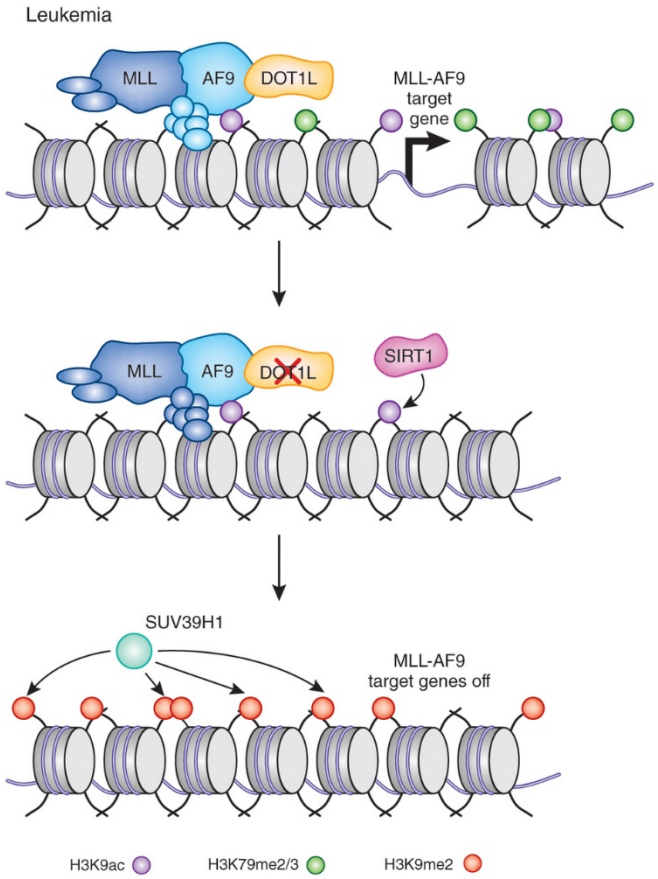


HOXA
MEIS1
MYB
MYC
MEF2C

Krivtsov et al., *Nature* 2006
Wang et al., *Science* 2010
Yi et al., *Cell Stem Cell* 2015



DOT1L Is Required for *MLL*-Rearranged Leukemia



-DOT1L deficient MLL-fusion cells do not induce leukemia

-MLL-fusion driven gene expression program decreased

-Cells Transformed by HoxA9/Meis1 Proliferate Normally

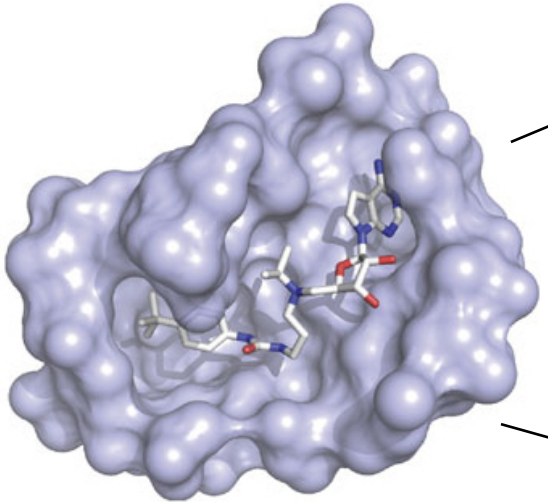
-DOT1L inhibitor inhibits proliferation of MLL-fusion human Leukemia

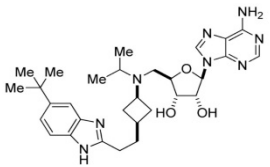
-Minimal effect on normal CD34+ BM cells

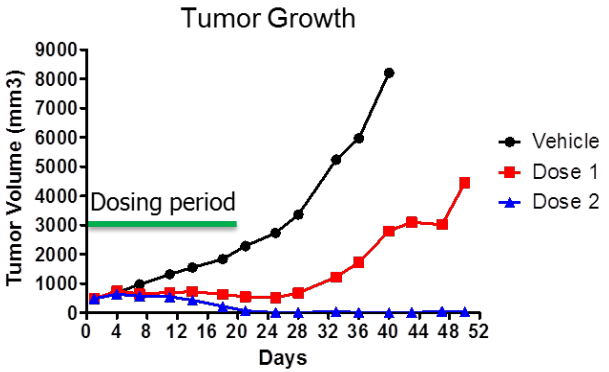
-H3K79me inhibits repressive mechanisms (H3K27/H3K9me)

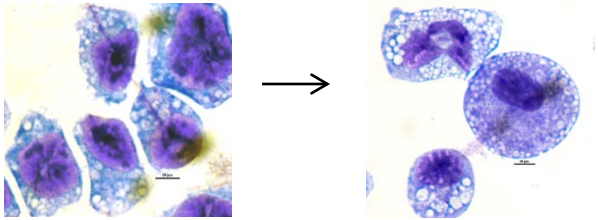
Daigle et al., *Cancer Cell* 2011
Bernt, et al., *Cancer Cell* 2011
Chen et. al., *Nature Medicine*, 2015

DOT1L Inhibitors Induce Differentiation of *MLL*-Rearranged Leukemia Cells

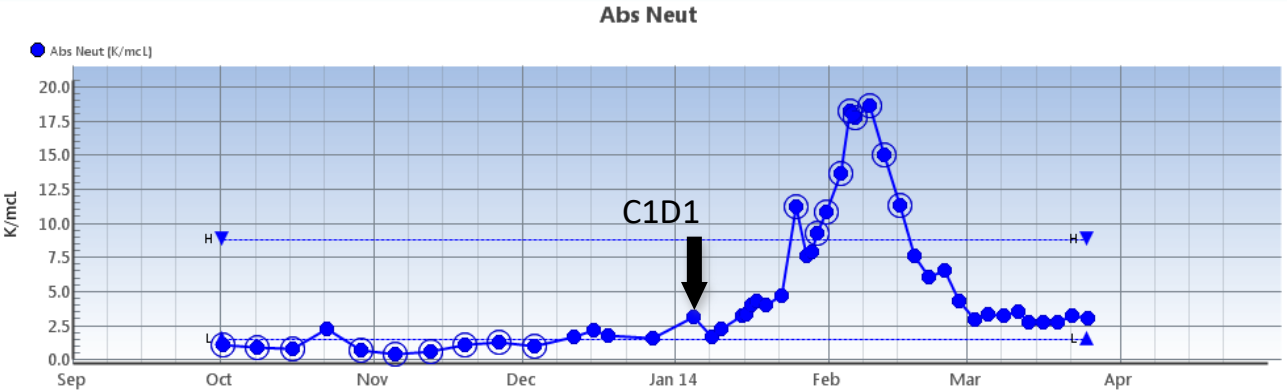


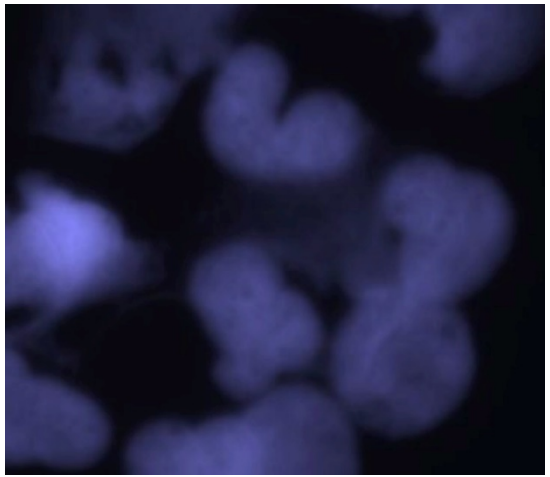


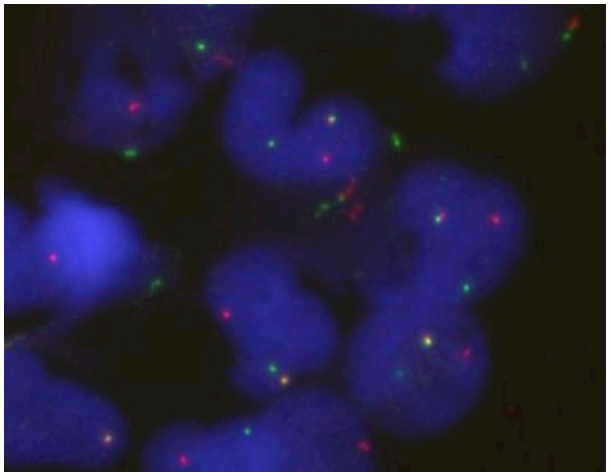




EPZ5676 Induces Differentiation-like Effects in Patients



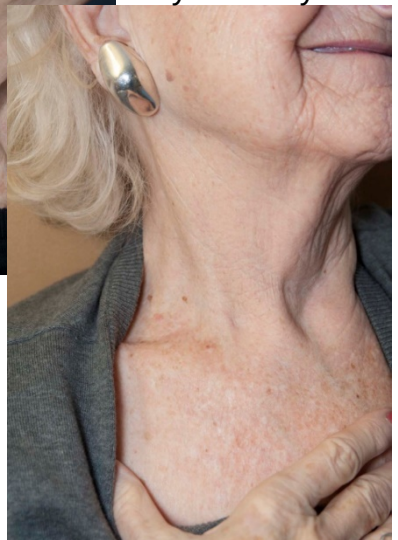




Phase 1 trial of DOT1L Inhibitor in Patients with MLL-Rearranged Leukemia

Cycle 2 day 1

Cycle 3 day 1



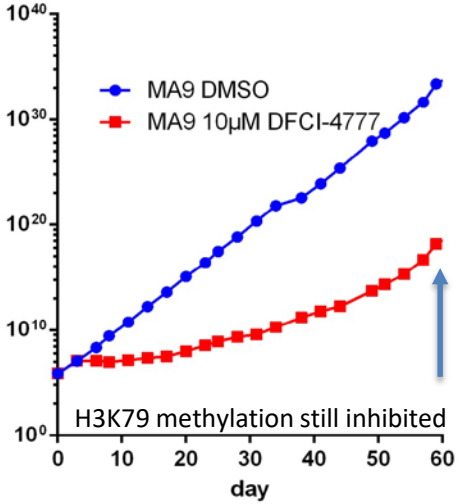
Cycle 4 day 1

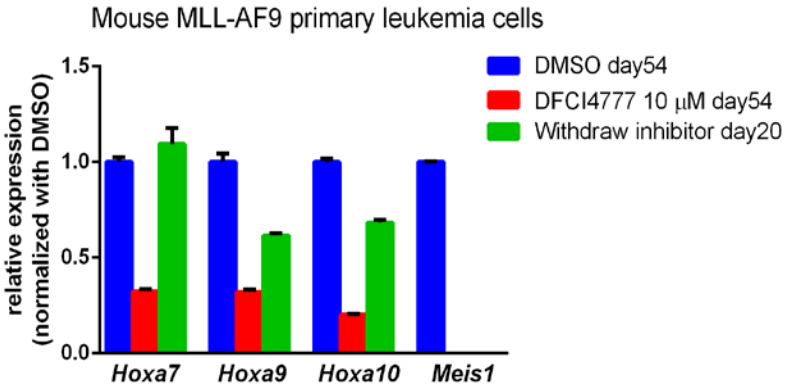
Phase 1:
-No MTD reached

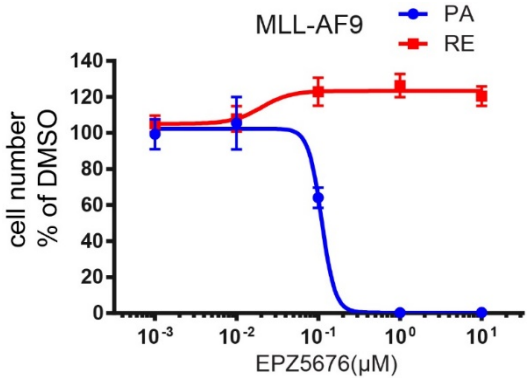
*~25% of patients in phase 1 had some evidence of biological activity (2CRs)

1. Responses take time
2. Need deep and consistent inhibition
3. Need Combinations
4. Minimal Toxicity

Mechanisms of Resistance to Constant DOT1L inhibition

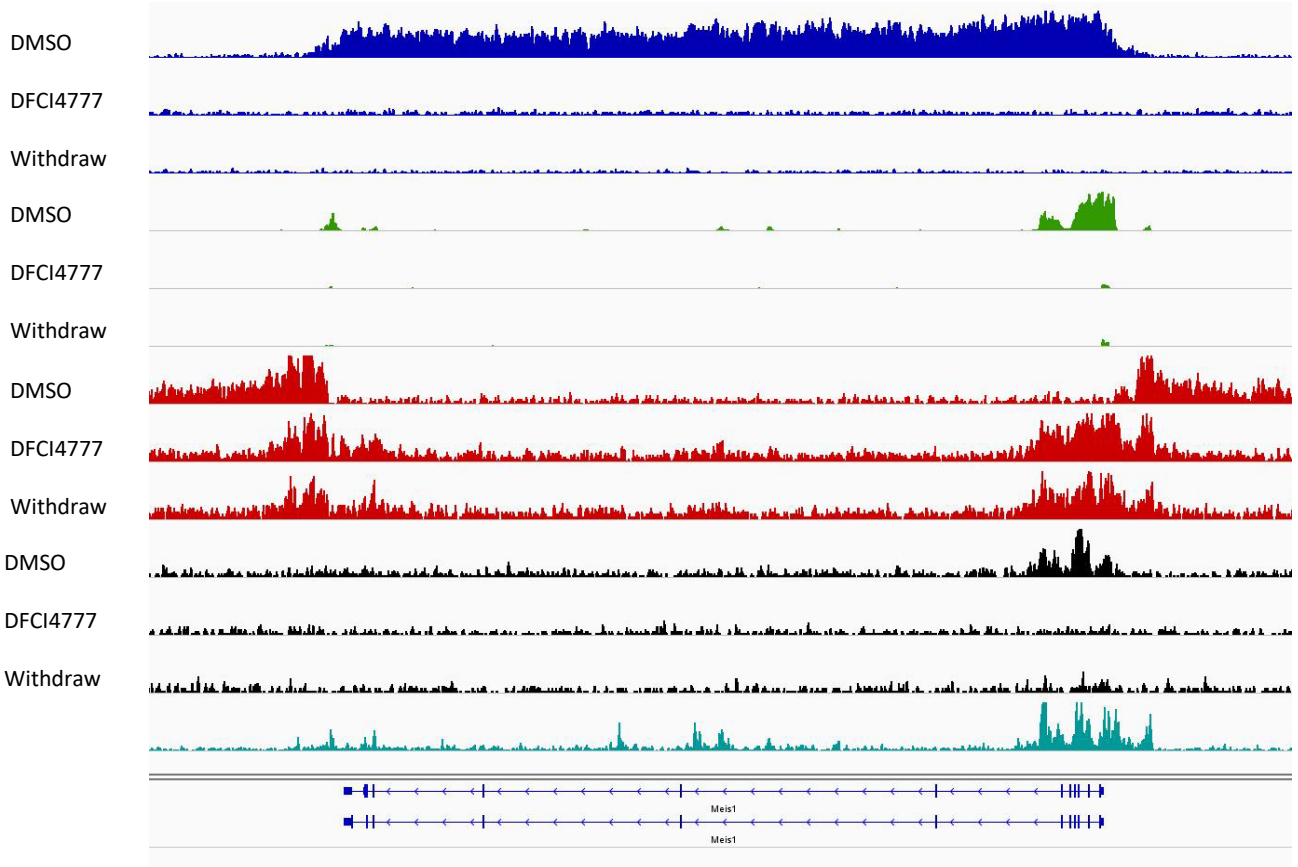






-Inhibition of a histone methyltransferase leads to irreversible gene expression changes
-Same changes occur in human MLL-fusion AML cells

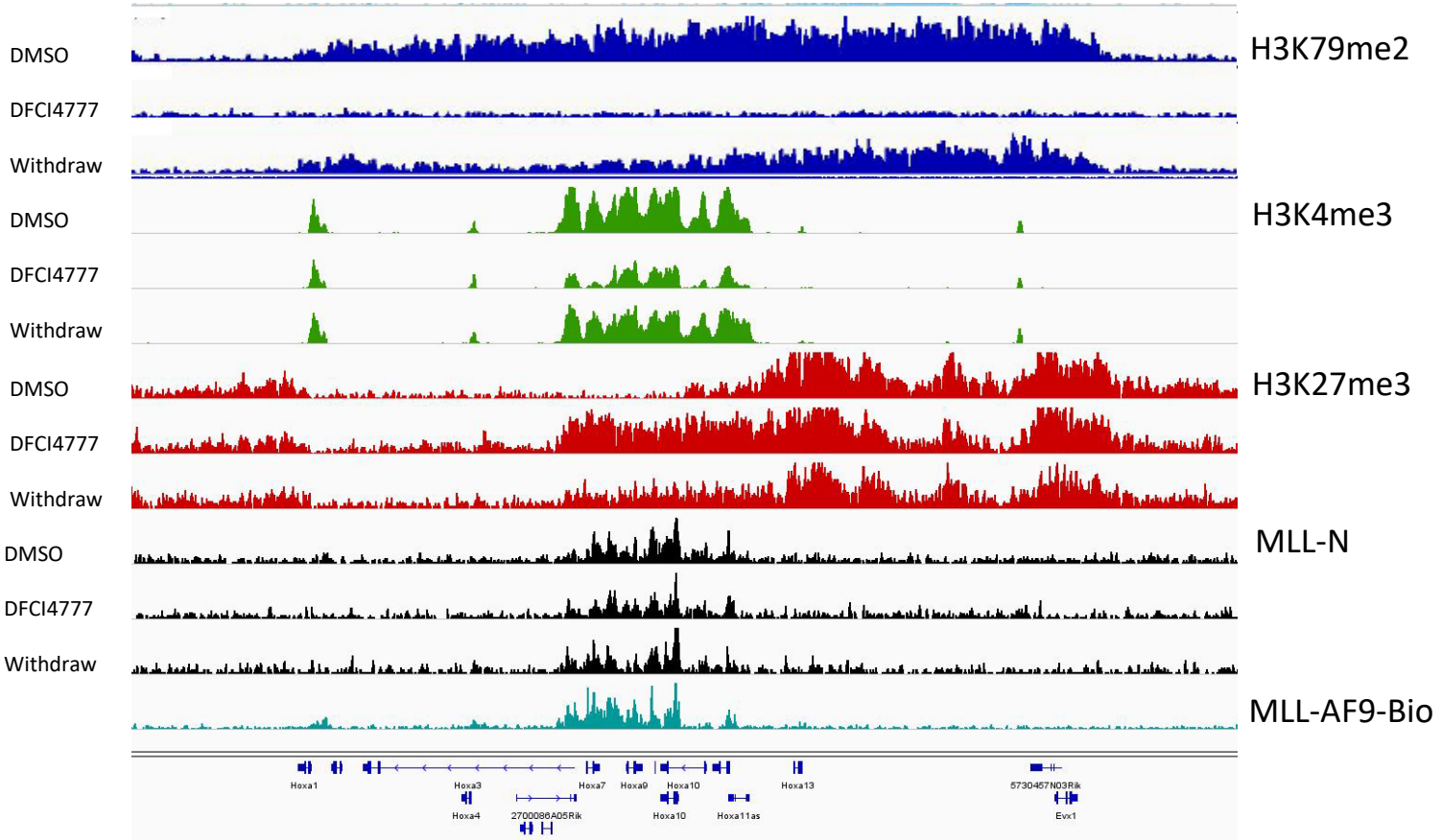
Histone Methylation on the Meis1 locus is Irreversibly Influenced by Dot1L inhibition



Meis1

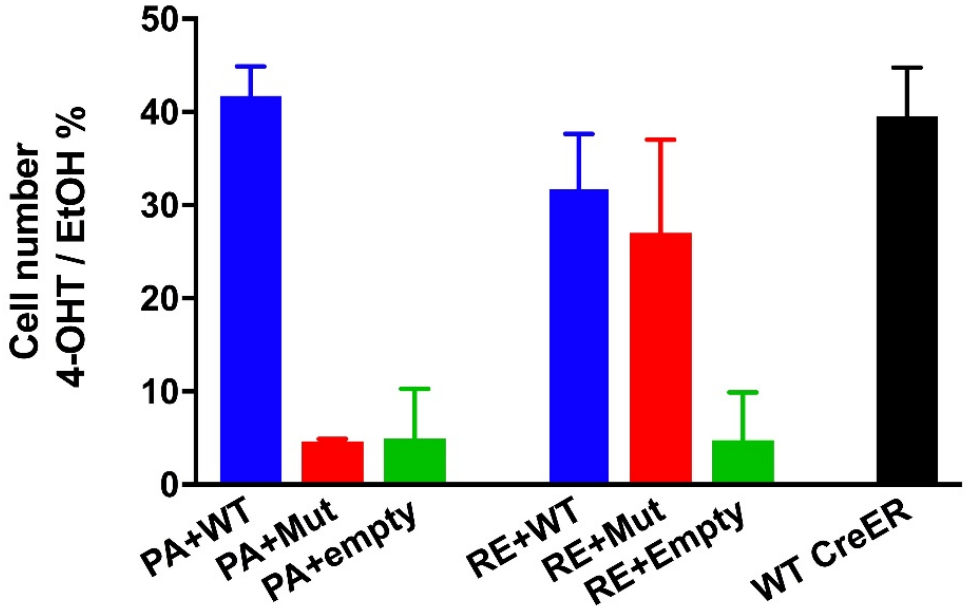
MLL-AF9 is lost from TSS after DOT1L inhibition

Histone Methylation on the HOXA loci is not Irreversibly Influenced by Dot1L inhibition

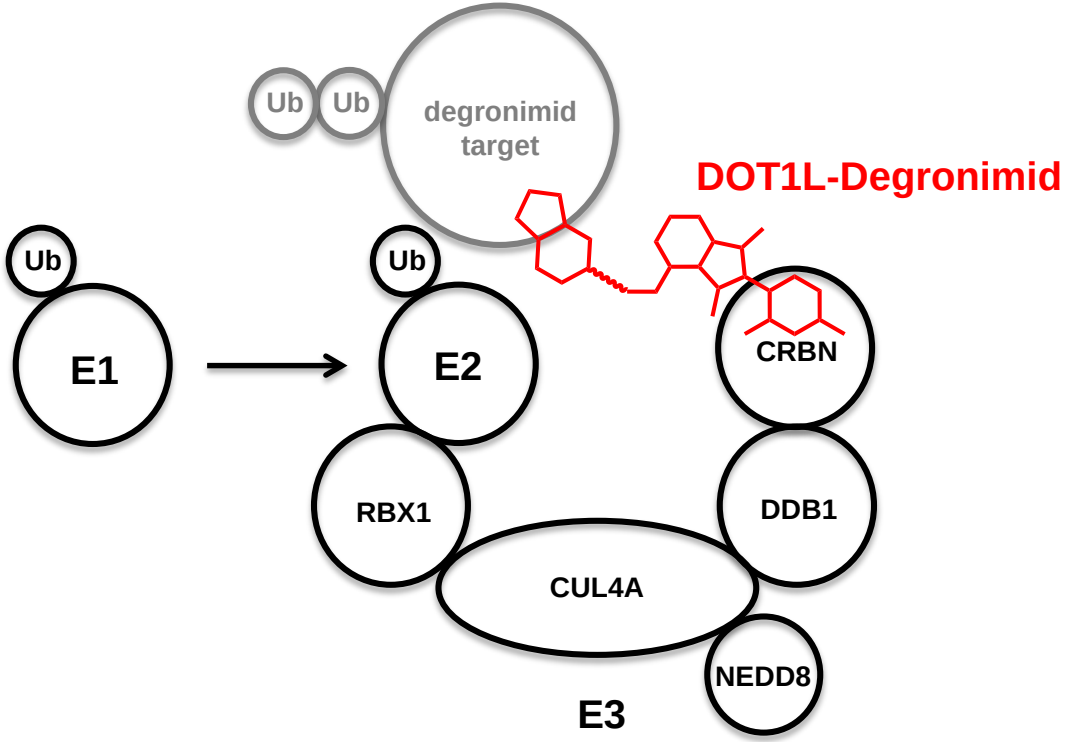


HoxA cluster

Both WT and mutant DOT1L rescue resistant cells upon genetic deletion of Dot1L



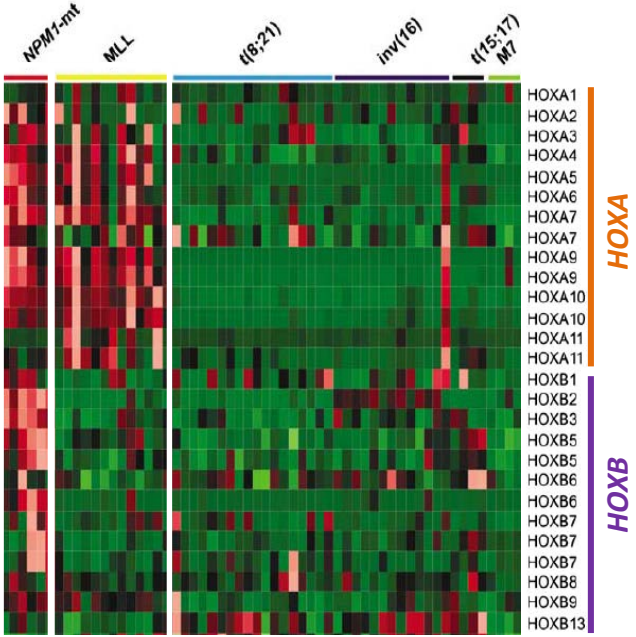
Targeted Protein Degradation with Degronimids



All-Chemical Targeted Protein Degradation with Degronimids

Bradner Lab, *Science* 2015

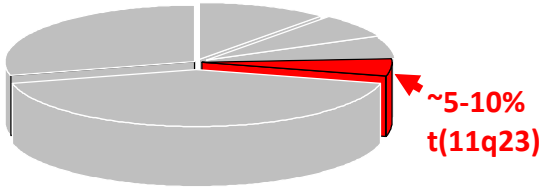
HOX Gene Expression in AML with Mutated Nucleophosmin (*NPM1*c+)



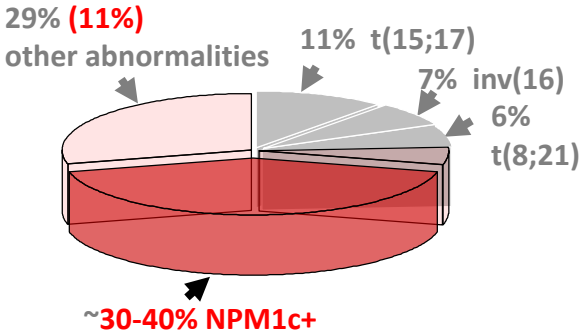
Mullighan et al. Leukemia 2007

Acute Myeloid Leukemia

***HOXA*: *MLL*-Rearranged AML**



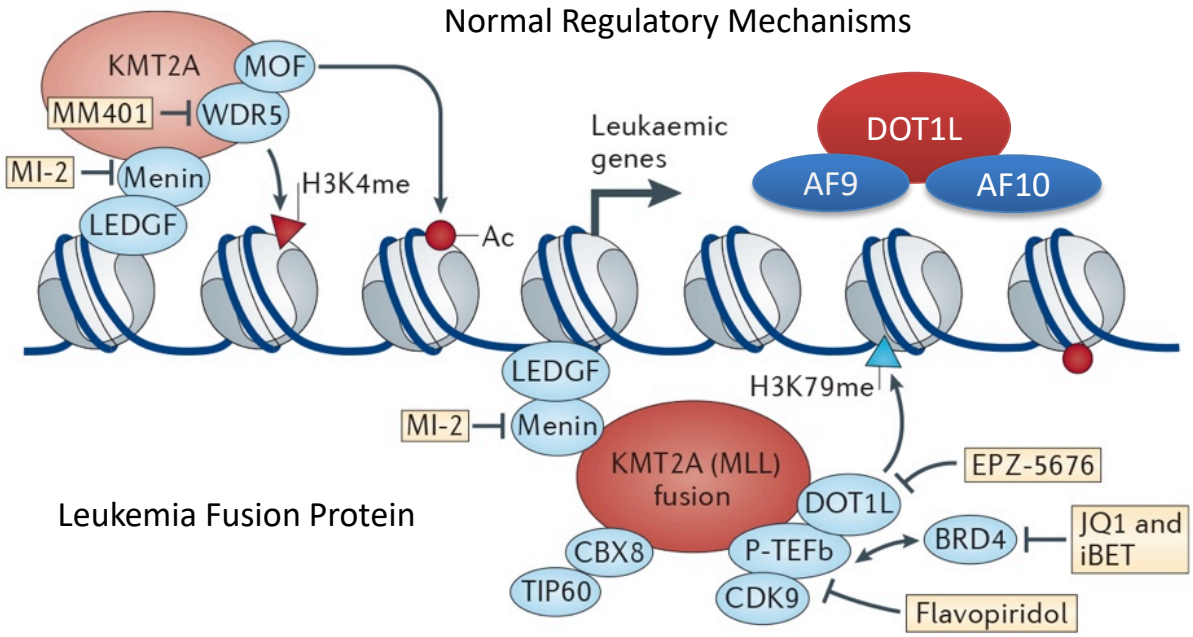
***HOXA* / *HOXB*: *NPM1*c+ AML (~40% of all AML cases)**



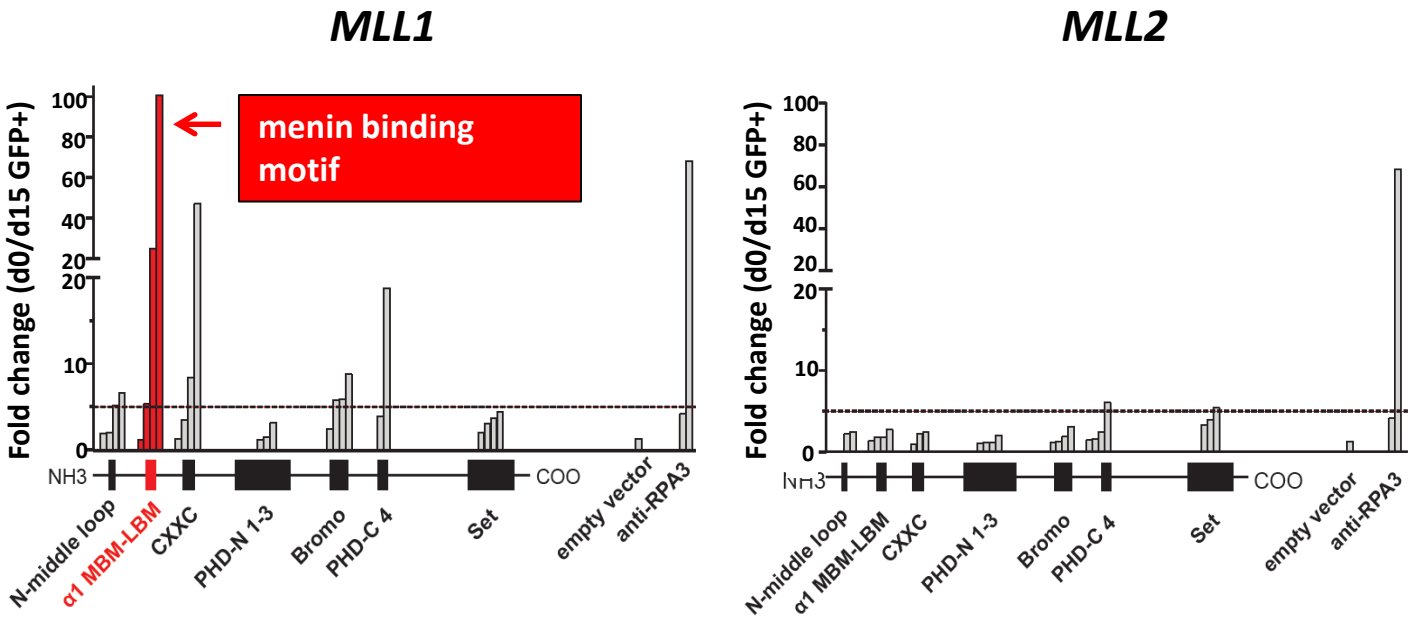
***HOX* gene activation in *NPM1*c+ AML: Mechanism unkown**
DOT1L involved in *NPM1*c+ leukemogenesis?

Adapted from: Döhner & Estey Lancet 2006 and *Verhaak et al. Blood 2005*

Multiple Complexes Influence MLL-Leukemia Gene Expression



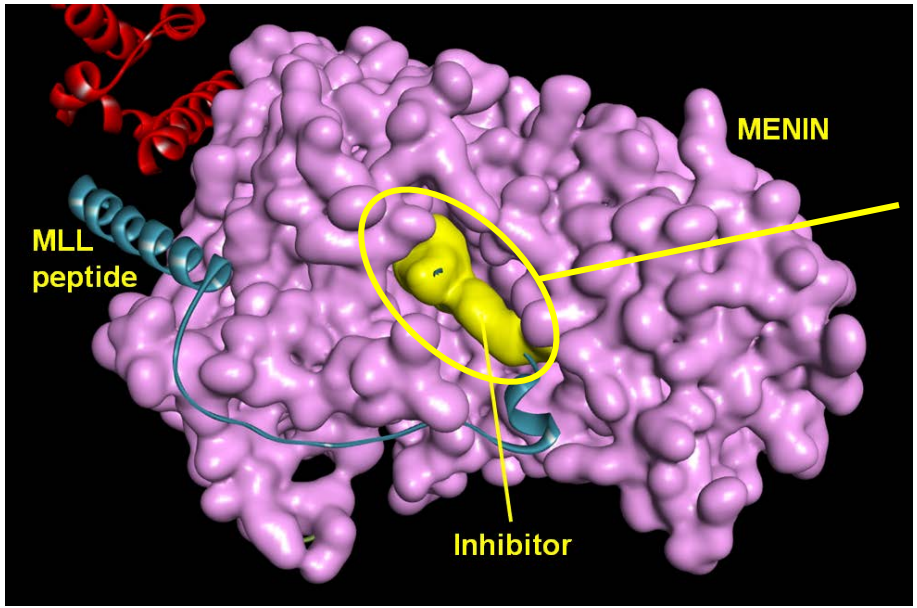
CRISPR-Cas9 Mutagenesis: Requirement for MLL1 in NPM1^{mut} AML



--NPM1c+ AML cell lines also sensitive to DOT1L inhibition

Kuehn et al., *Cancer Discovery* 2016

Menin Blockers Compete with the High Affinity Binding Motif of MLL1 (aa 9-13) for Menin Binding



MLL Binding Pocket

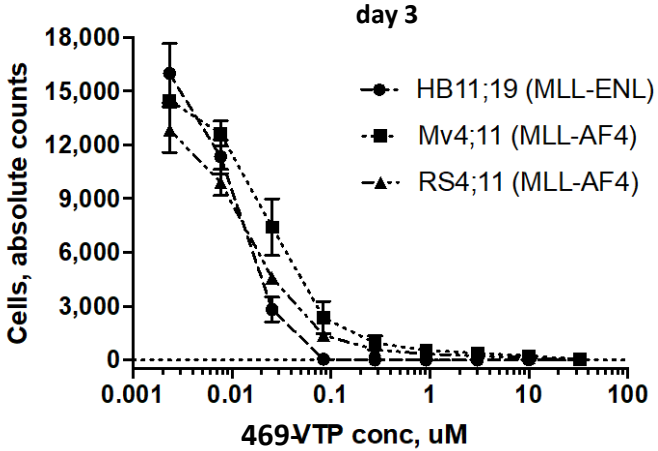
VTP-50469 is a potent and selective orally bioavailable Small molecule inhibitor developed by Vitae/Syndax

9------13

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

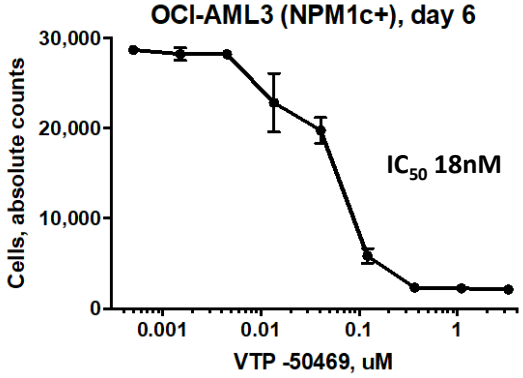
- Inhibitors bind to a highly conserved binding pocket in menin

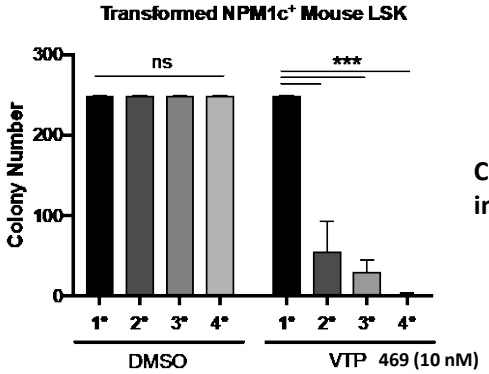
VTP-50469 selectively inhibits proliferation of cell lines with *MLL*-rearrangements and *NPM1c*$^{+}$ mutations



CellTiter-Glo assay

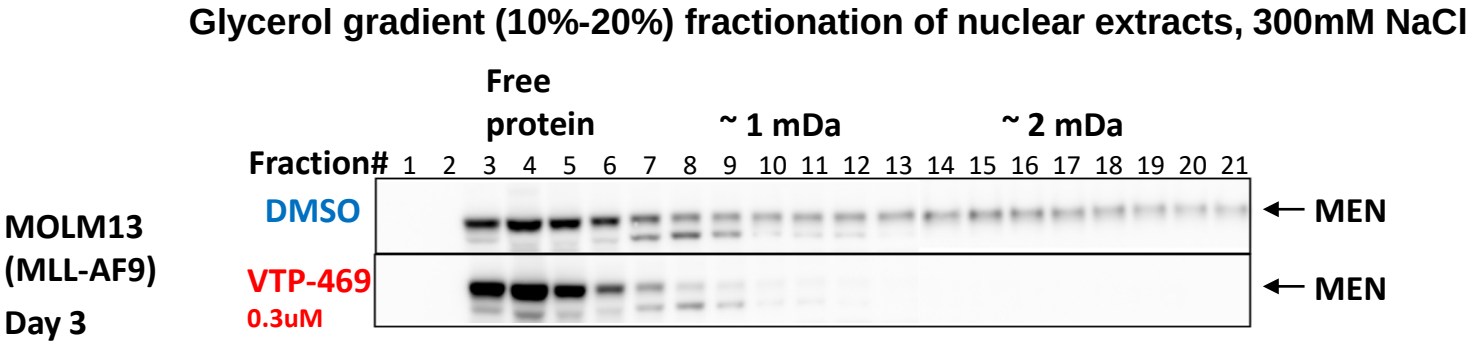
CELL LINE	FUSION	IC_{50} nM
MV4;11	MLL-AF4	17
SEM-K2	MLL-AF4, AF4-MLL	27
RS4;11	MLL-AF4, AF4-MLL	25
MOLM-13	MLL-AF9	13
KOPN-8	MLL-ENL	15
HB11;19	MLL-ENL	36
REH	NONE	>>2000
HL-60	NONE	>>2000





Colony forming assay in semi-solid media

VTP-50469 dissociates MEN from nuclear complexes

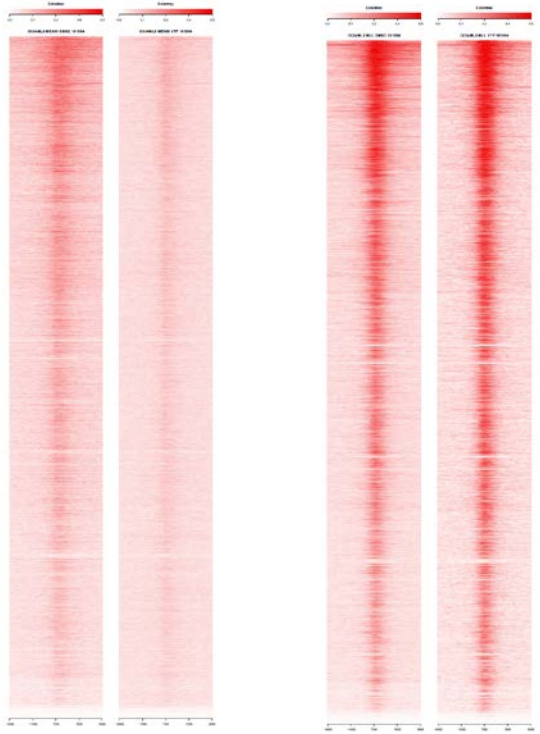


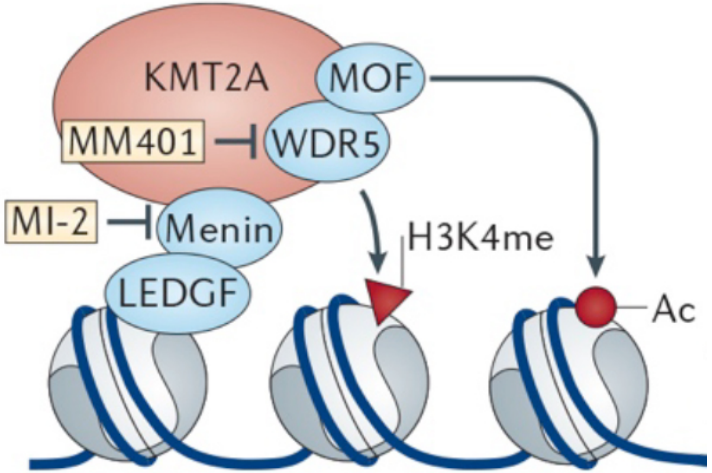
Identical fractionation results obtained from RS4;11 (MLL-AF4), ML-2 (MLL-AF6) and OCI-AML3 (NPM1c+) Cells

VTP-50469 treatment evicts Menin from chromatin but not MLL1

ChIP: Anti-MEN Anti-MLL1n

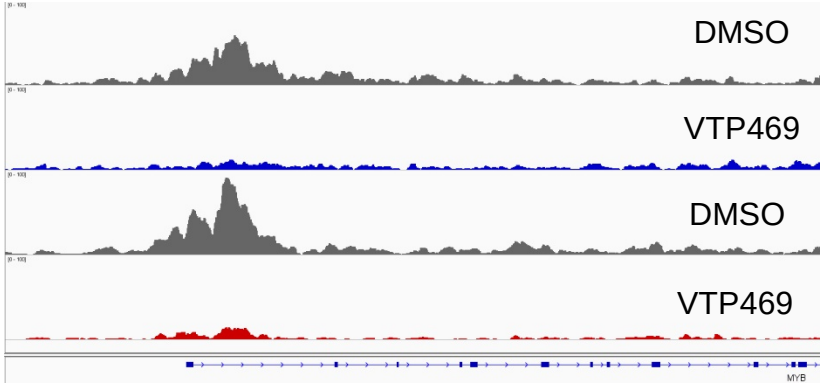
DMSO VTP469 DMSO VTP469





MLL1 is Lost at Selected Loci After Menin-MLL inhibition

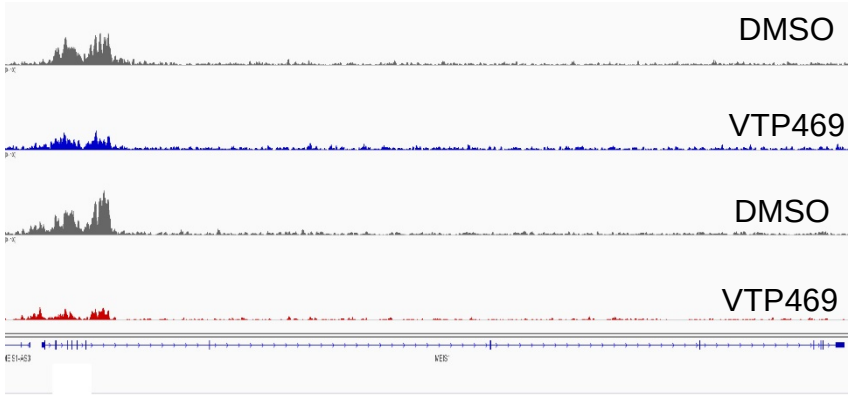
MYB

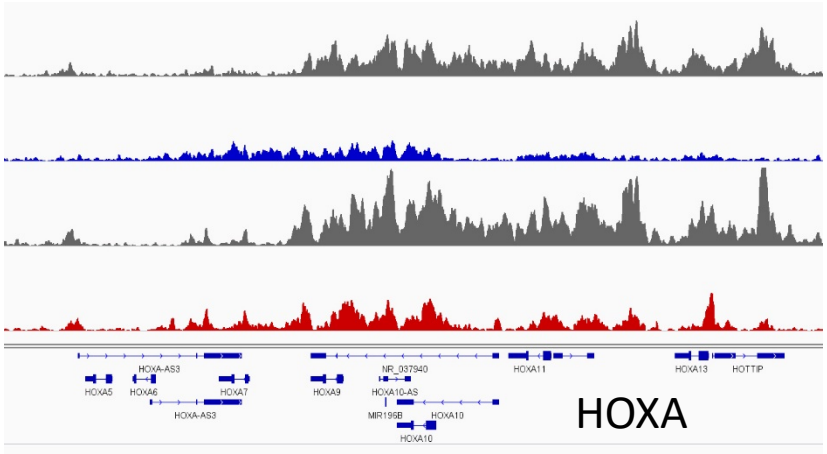


Menin

MLL1

MEIS1





Menin

MLL1

HOXA

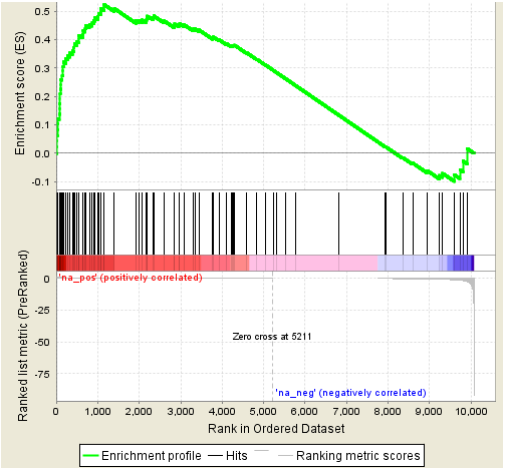
Decreased Expression for Genes that Lose MLL1 Occupancy

MEIS1
SATB1
EVI1
RUNX2
MYB
IGF2BP2
MYB
CDK6
MBNL1
BAZ1A
PBX3
JMJD1C

Ranked by MLL loss

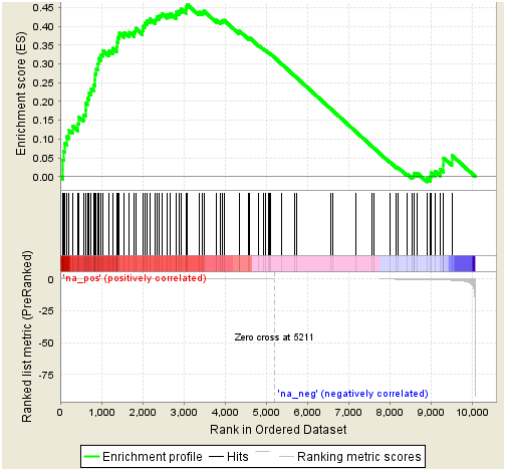
1-100

(NES 1.83; FWER pVal 0.000)



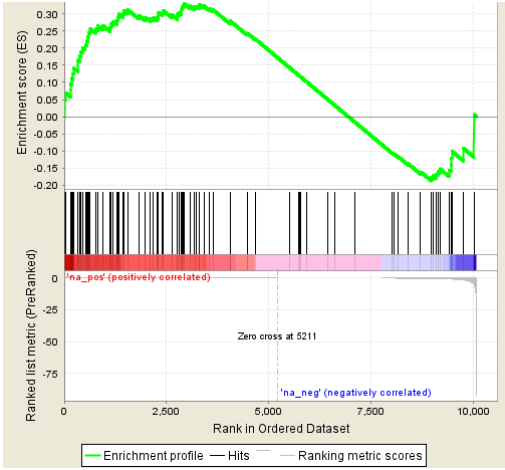
100-200

(NES 1.60; FWER pVal 0.003)



200-300

(NES 1.13; FWER pVal 0.203)



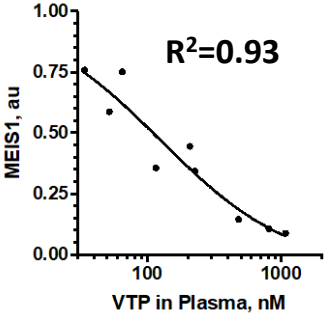
***VTP-50469 treatment changes expression of MLL-target and DOT1L inhibitor sensitive genes faster as compared to EPZ4777**

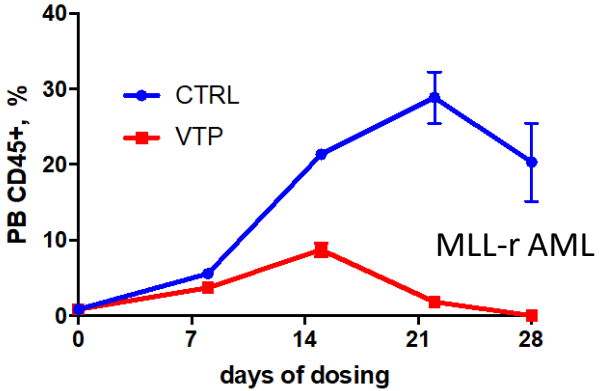
VTP-50469 treatment reduces leukemia burden in PDX models of MLL-r mutant leukemia

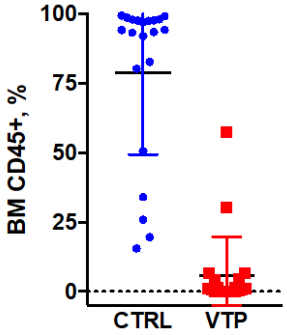
PDX

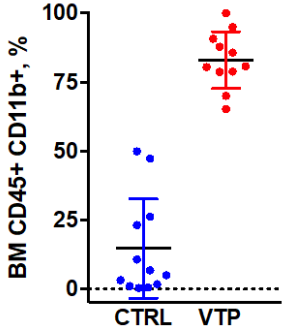
1-10% Leukemia in PB

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

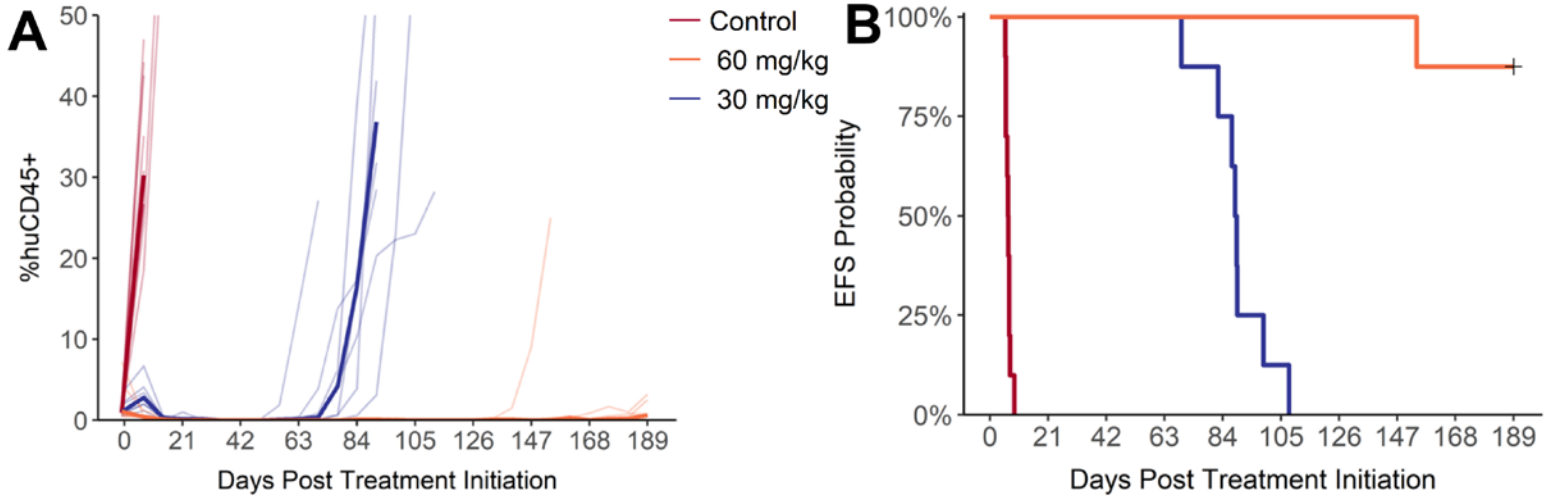








VTP-50469 increases survival in MLL-rearranged ALL PDX models



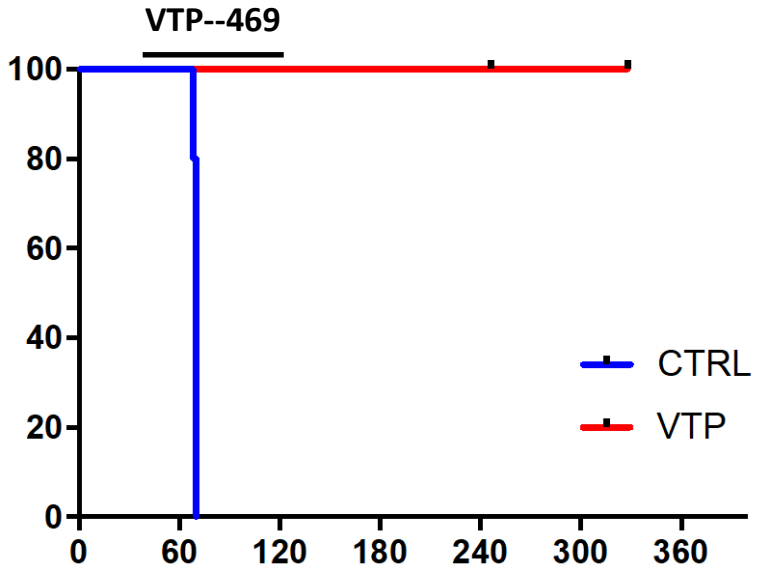
Malcolm Smith (NCI) and Richard Lock (CCI)

VTP-50469 treatment Prolongs Survival in NPM1 mutant PDX models

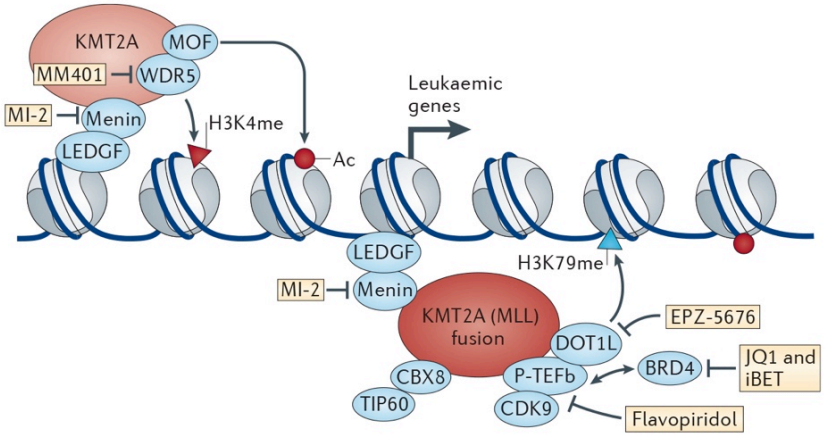
PDX

1-10% Leukemia in PB

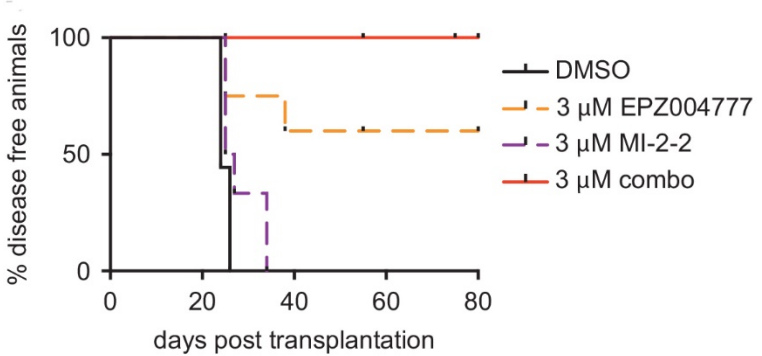
0.1% VTP, 100 mpk (IC_{90}) Plasma conc. 1-2 uM



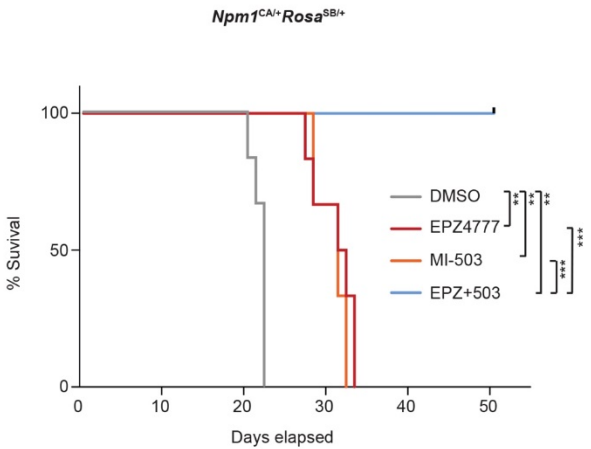
Combined DOT1L and Menin Inhibitors are Active Against MLL-Rearranged NPM1 Mutant AML Cells



MLL-r-AML



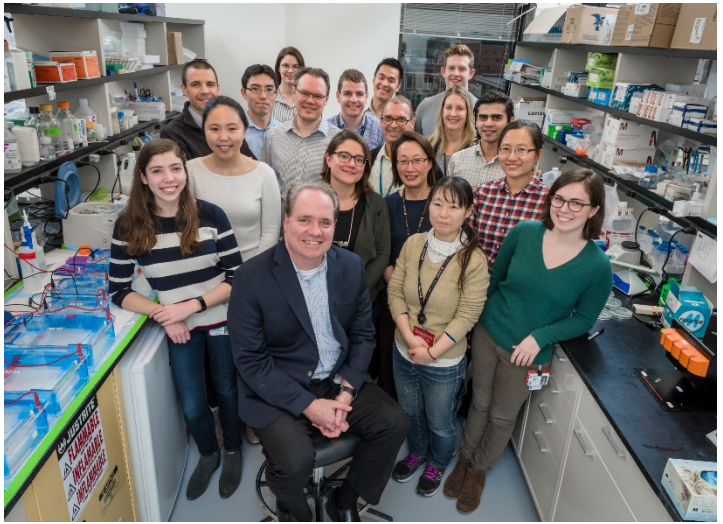
Npm1-mutant AML



Acknowledgments

Armstrong Lab
Zhaohui Feng
Andrei Krivtsov
Xi Wang
Haihua Chu
Takayuki Hoshii
Gerry Brien
Haiming Xu
Matt Hemming
Chris Delaney
Jonathan Chabon
Hannah Uckelmann
Sarah Perlay
Stephanie Kim
Sayuri Kitajima
Brandon Aubrey
Naomi Olsen

DFCI/Childrens
James Bradner
Nathaneal Gray
Cigall Kadoch
Michael Erb
David Remillard
Jun Qi
Stuart Orkin

CSHL
Chris Vakoc
Junwei Shi

MSKCC
Ross Levine
Sheng Cai
Richard Koche