Targeting Epigenetic Mechanisms to Reverse Stem Cell Programs in Cancer

Scott A. Armstrong MD, Ph.D.
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

AML
Hierarchy
LSC 1/150

Leukemic
GMP

LSC
Frequency

1:6

Stem Cell Programs
HoxA/Meis1 Genes

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

Tumor Growth

Dosing period

Vehicle
Dose 1
Dose 2

Days
0 4 8 12 16 20 24 28 32 36 40 44 48 52

Tumor Volume (mm³)
0 1000 2000 3000 4000 5000 6000 7000 8000 9000

Chemical structure of DOT1L inhibitor

Images of differentiated leukemia cells
EPZ5676 Induces Differentiation-like Effects in Patients
Phase 1 trial of DOT1L Inhibitor in Patients with MLL-Rearranged Leukemia

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H3K79me2</th>
<th>H3K4me3</th>
<th>H3K27me3</th>
<th>MLL-N</th>
<th>MLL-AF9-Bio</th>
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MLL-AF9 is lost from TSS after DOT1L inhibition
Histone Methylation on the HOXA loci is not Irreversibly Influenced by Dot1L inhibition
Both WT and mutant DOT1L rescue resistant cells upon genetic deletion of Dot1L
Targeted Protein Degradation with Degronimids

E1 → E2 → E3

Ub → Ub → Ub

degronimid target

DOT1L-Degronimid

CRBN

CUL4A

DDB1

NEDD8

Bradner Lab, Science 2015
HOX Gene Expression in AML with Mutated Nucleophosmin (NPM1c+)

*HOXA* and *HOXB* gene activation in NPM1c+ AML: Mechanism unknown

DOT1L involved in NPM1c+ leukemogenesis?

\[\text{~30-40% NPM1c+}\]

- 29% (11%) other abnormalities
- 11% t(15;17)
- 7% inv(16)
- 6% t(8;21)

\[\text{~5-10% t(11q23)}\]

**Adapted from:** Döhner & Estey Lancet 2006 and Verhaak et al. Blood 2005
Multiple Complexes Influence MLL-Leukemia Gene Expression

Normal Regulatory Mechanisms

Leukemia Fusion Protein

DOT1L
AF9
AF10

KMT2A (MLL) fusion

EPZ-5676
JQ1 and iBET

H3K79me

BRD4

DOT1L

P-TEFb

CBX8

TIP60

CDK9

Ac

H3K4me

KMT2A

MM401

MOF

WDR5

LEDGF

Menin

MI-2

MEN1

Normal Regulatory Mechanisms

Leukemic genes
CRISPR-Cas9 Mutagenesis: Requirement for MLL1 in NPM1mut AML

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

Kuehn et al., Cancer Discovery 2016
Inhibitors bind to a highly conserved binding pocket in menin.

VTP-50469 is a potent and selective orally bioavailable Small molecule inhibitor developed by Vitae/Syndax.
VTP-50469 selectively inhibits proliferation of cell lines with MLL-rearrangements and NPM1c+ mutations

CellTiter-Glo assay

<table>
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<tr>
<th>CELL LINE</th>
<th>FUSION</th>
<th>IC50 nM</th>
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<tbody>
<tr>
<td>MV4;11</td>
<td>MLL-AF4</td>
<td>17</td>
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<tr>
<td>SEM-K2</td>
<td>MLL-AF4, AF4-MLL</td>
<td>27</td>
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<td>RS4;11</td>
<td>MLL-AF4, AF4-MLL</td>
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<tr>
<td>MOLM-13</td>
<td>MLL-AF9</td>
<td>13</td>
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<td>KOPN-8</td>
<td>MLL-ENL</td>
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<td>HB11;19</td>
<td>MLL-ENL</td>
<td>36</td>
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<tr>
<td>REH</td>
<td>NONE</td>
<td>&gt;&gt;2000</td>
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<tr>
<td>HL-60</td>
<td>NONE</td>
<td>&gt;&gt;2000</td>
</tr>
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Colony forming assay in semi-solid media
VTP-50469 dissociates MEN from nuclear complexes

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

<table>
<thead>
<tr>
<th>Fraction#</th>
<th>Free protein</th>
<th>~ 1 mDa</th>
<th>~ 2 mDa</th>
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<tr>
<td>1-5</td>
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<td>6-10</td>
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<td>11-15</td>
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<td>16-21</td>
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MOLM13 (MLL-AF9) Day 3

DMSO

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 evicts Menin from chromatin but not MLL1
MLL1 is Lost at Selected Loci After Menin-MLL inhibition

Menin

MLL1

MYB

DMSO

VTP469

DMSO

VTP469

MEIS1

DMSO

VTP469

DMSO

VTP469

HOXA

Menin

MLL1
Decreased Expression for Genes that Lose MLL1 Occupancy

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

**Ranked by MLL loss**

- **1-100**
  - MEIS1
  - SATB1
  - EVI1
  - RUNX2
  - MYB
  - IGF2BP2
  - CDK6
  - MBNL1
  - BAZ1A
  - PBX3
  - JMJD1C
  - (NES 1.83; FWER pVal 0.000)

- **100-200**
  - MEIS1
  - SATB1
  - EVI1
  - RUNX2
  - MYB
  - IGF2BP2
  - CDK6
  - MBNL1
  - BAZ1A
  - PBX3
  - JMJD1C
  - (NES 1.60; FWER pVal 0.003)

- **200-300**
  - MEIS1
  - SATB1
  - EVI1
  - RUNX2
  - MYB
  - IGF2BP2
  - CDK6
  - MBNL1
  - BAZ1A
  - PBX3
  - JMJD1C
  - (NES 1.13; FWER pVal 0.203)
VTP-50469 treatment reduces leukemia burden in PDX models of MLL-r mutant leukemia.
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

1-10% Leukemia in PB
0.1% VTP, 100 mpk (IC90)
Plasma conc. 1-2 uM

Days post transplant

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

- **MLL-r-AML**
- **Npm1-mutant AML**
Acknowledgments

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