Protein Methyltransferase Inhibitors as Personalized Cancer Therapeutics

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- Grant/Research support from: LLS, MMRF, GSK, Eisai & Celgene
- Stockholder in and Employee of: Epizyme, Inc.
- Scientific Advisory Board Member of: Mersana
- Ad hoc Consultant for: NEA

- and –

- I will not discuss off label use and/or investigational use in my presentation.
Posttranslational Modifications of Histones

PMTs
- Arginine (RMTs)
- Lysine (KMTs)

PRMT (44)
KDM (26)
HAT (28)
PKMT (52)
DNMT (18)
HDAC (17)
Kinases (5)
RDI (5)

Copeland et al. (2012) Oncogene
The human protein methyltransferases

Methyltransferases are enzymes that facilitate the transfer of a methyl (CH$_3$) group to specific nucleophile sites on proteins, nucleic acids or other biomolecules. They share a reaction mechanism in which the nucleophile acceptor site attacks the electrophilic carbon of S-adenosyl-L-methionine (SAM) in an S$_\text{N}_2$ displacement reaction that produces a methylated biomolecule and S-adenosyl-L-homocysteine (SAH) as a byproduct. Methyltransfer reactions are essential transformations in small-molecule metabolism, and methylations are a common modification of DNA and RNA. The recent discovery of dynamic and reversible methylation of amino acid side chains of chromatin proteins, particularly within the N-terminal tail of histone proteins, has revealed the importance of methyl ‘marks’ as regulators of gene expression. Human protein methyltransferases (PMTs) fall into two major families – protein lysine methyltransferases (PKMTs) and protein arginine methyltransferases (PRMTs) – that are distinguishable by the amino acid that accepts the methyl group and by the conserved sequences of their respective catalytic domains. Given their involvement in many cellular processes, PMTs have attracted attention as potential drug targets, sparking the search for small-molecule PMT inhibitors. Several classes of inhibitors have been identified, but new specific chemical probes that are active in cells will be required to elucidate the biological roles of PMTs and serve as potent leads for PMT-focused drug development.
A Spectrum of Genetic Alterations Confer Dependence on PMT Activity to Cancer Cells

- Point mutations in PMT (e.g., EZH2)
- Amplification/increased expression (e.g., several KMTs)
- LoF mutations in corresponding demethylase (e.g., UTX)
- Ectopic recruitment to aberrant gene locations (e.g., DOT1L)
- Indirect chromosomal translocations (e.g., DOT1L)
- Direct chromosomal translocations (e.g., MMSET)
- Synthetic lethal relationships (e.g., SWI/SNF with PRC2)

### DOT1L and PRC2 Alterations Drive Distinct Human Cancers

<table>
<thead>
<tr>
<th>Type of Alteration</th>
<th>Genetic Alteration</th>
<th>Relationship to HMT</th>
<th>Oncogene (HMT) Addiction</th>
<th>Clinical Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 11q23 translocation</td>
<td>MLL-fusion proteins</td>
<td>Indirect. Ectopic recruitment</td>
<td>DOT1L</td>
<td>MLL-r leukemias</td>
</tr>
<tr>
<td>Partial gene duplication</td>
<td>MLL-PTD</td>
<td>Unknown</td>
<td>DOT1L</td>
<td>MLL-PTD leukemia</td>
</tr>
<tr>
<td>Mutation</td>
<td>EZH2 Point Mutations</td>
<td>Target-directed gain of function</td>
<td>PRC2</td>
<td>Mutant-bearing NHL</td>
</tr>
<tr>
<td>Gene amplification</td>
<td>EZH2 amplification</td>
<td>Gain of function</td>
<td>PRC2</td>
<td>Multiple solid tumors</td>
</tr>
<tr>
<td>Chromosome 22 deletion/mutation</td>
<td>INI1-deficiency</td>
<td>Synthetic Lethal</td>
<td>PRC2</td>
<td>MRT, synovial sarcoma</td>
</tr>
<tr>
<td>Mutation</td>
<td>LoF of UTX</td>
<td>Repression of demethylation</td>
<td>PRC2</td>
<td>Multiple myeloma</td>
</tr>
</tbody>
</table>
DOT1Li for MLL-r Leukemia

- **Oncogenic target** with strong validation in acute leukemias involving MLL gene translocation (MLL-r leukemia)
  - AML and ALL
  - Adult and pediatric populations

- **Poor prognosis** in adult and pediatric populations despite intensive chemotherapy
  - Adult MLL-r AML patients with 5-24% expected 5 year survival
  - Pediatric MLL-r ALL patients with 27% 5 year event free survival rate

- High existing **physician awareness**, currently diagnosed as standard of care
MLL Translocation Partner Gene Distribution in Clinical Subgroups

MLL (11q23) translocations

<table>
<thead>
<tr>
<th>Partner Protein</th>
<th>Chromosomal Translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF4</td>
<td>t(4;11)(q21;q23)</td>
</tr>
<tr>
<td>AF6</td>
<td>t(6;11)(q27;q23)</td>
</tr>
<tr>
<td>AF9</td>
<td>t(9;11)(p22;q23)</td>
</tr>
<tr>
<td>AF10</td>
<td>t(10;11)(p12;q23)</td>
</tr>
<tr>
<td>ENL</td>
<td>t(11;19)(q23;p13.3)</td>
</tr>
<tr>
<td>ELL</td>
<td>t(11;19)(q23;p13.1)</td>
</tr>
<tr>
<td>EPS15</td>
<td>t(1;11)(p32;q23)</td>
</tr>
</tbody>
</table>
EPZ-5676 is a Potent and Highly Selective DOT1L Inhibitor

<table>
<thead>
<tr>
<th>Property</th>
<th>EPZ-5676</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOT1L inhibition $K_i$ (nM)</td>
<td>$\leq 0.08 \pm 0.03$</td>
</tr>
<tr>
<td>Residence time (hr)</td>
<td>$&gt; 24$</td>
</tr>
<tr>
<td>Fold selectivity</td>
<td>$&gt; 37,000$</td>
</tr>
<tr>
<td>MV4-11 proliferation IC$_{50}$ (nM)</td>
<td>$3.5 \pm 0.7$</td>
</tr>
<tr>
<td>H3K79me2 inhibition IC$_{50}$ (nM)</td>
<td>$2.7 \pm 0.9$</td>
</tr>
<tr>
<td>HOXA9 inhibition IC$_{50}$ (nM)</td>
<td>$67 \pm 27$</td>
</tr>
<tr>
<td>MEIS1 inhibition IC$_{50}$ (nM)</td>
<td>$53 \pm 17$</td>
</tr>
</tbody>
</table>

Methylation selectivity

H3K79me2
H3
DOT1Li EPZ-5676 Selective Killing of MLL-r Cells In Vitro

Target methyl mark inhibition leads to specific killing of genetically defined cancer cells

MLL-Rearranged Cells

Non-MLL-Rearranged Cells

Daigle et al. (2013) Blood 122: 1017-1025
MLL Translocation Partner Gene Distribution in Clinical Subgroups

Infants
n = 558

Pediatric
n = 416

Adult
n = 616

Meyer C et al. Leukemia (2013), 1-12
**MLL Translocation Partner Genes Sensitive to DOT1L Inhibition**

- **Infants**
  - n = 558

  - AF9/MLLT3: 18
  - ENL/MLLT1: 40
  - AF4/AFF1: 18
  - AF10/MLLT10: 3
  - ELL: 11
  - EPS15: 13
  - other: 9

- **Pediatric**
  - n = 416

  - AF9/MLLT3: 13
  - ENL/MLLT1: 24
  - AF4/AFF1: 27
  - AF10/MLLT10: 11
  - ELL: 8
  - EPS15: 13
  - other: 11

- **Adult**
  - n = 616

  - AF9/MLLT3: 13
  - ENL/MLLT1: 45
  - AF4/AFF1: 8
  - AF10/MLLT10: 6
  - ELL: 5
  - EPS15: 13
  - other: 11

**Demonstrated sensitivity to DOT1L inhibitor in human MLLr leukemia cell lines or MLL-fusion transformed murine hematopoietic progenitors**

**Not tested**
EPZ-5676 Efficacy Study in Nude Rats

Median Tumor Volume

Dose Dependent H3K79 Reduction

Effect on Gene Expression

Tumor

Bone Marrow

Daigle et al. (2013) *Blood* 122: 1017-1025
**EPZ-5676 Phase 1 Protocol Summary**

**Dose Escalation**

- **Initiated September 2012**
  - Patients with advanced hematologic malignancies (including MLL-r patients)
  - 6 sites currently, additional sites being added for expansion cohort
  - Outcome measures
    - MTD
    - PK (dose and exposure)
    - PD (methyl mark inhibition)

**Expansion Cohort**

- **Expected initiation 4Q 2013**
  - Only MLL-r patients
  - Up to 12 sites (US + Europe)
  - Outcome measures
    - Safety
    - Early assessment of therapeutic effect in MLL-r patients
EPZ-5676 Phase 1 Patient Data from May 2013

4 patients completed dosing as of May 2013
• 1 patient in 1st dose cohort at 12 mg/m2/day
• 3 patients in 2nd dose cohort at 24 mg/m2/day
• No dose limiting toxicities, no adverse events greater than Grade 2
• No responses in 3 non-MLL patients
• 1 patient with ALL MLL-r
  − Partial ~60% DOT1L methyl mark inhibition (below anticipated therapeutic level)
  − 90% reduction in circulating blasts
  − Resolution of fever by day 5
  − Therapy ended on day 10 due to CNS disease progression (late-stage ALL patient)
EOL1 (MLL-PTD⁺) cells are sensitive to DOT1Li in vitro and in vivo.

Inhibition of EOL-1 proliferation correlates with DOT1L inhibitor biochemical potency.

**MLL-PTD**
- 5 – 8% of AML, predominantly assoc. with normal karyotype or trisomy 11.
EZH2 Inhibitor for Multiple Genetically-Defined Cancers

**SWI/SNF**
- Loss of Function Mutation
  - Rhabdoid Tumors

**PRC2 Complex**
- Change of Function Mutation
  - Non-Hodgkins Lymphoma
- Amplification of PRC2 Subunits
  - Multiple Solid Tumors

**Methylation**
- K27

**Demethylation**
- K27(me)_3

**UTX**
- Loss of Function Mutation
  - Myeloma
  - Renal
  - Esophageal

AACR-NIH-EOTRC
October 21, 2013
EPZ6438: A Potent, SAM-Competitive Inhibitor of EZH2
EPZ6436 Shows Strong Antitumor Activity in Several EZH2 Mutant Xenograft Models (NHL)

A  **WSU-DLCL2 (Y641F)**

- **Vehicle**
- **40 mg/kg TID x 28**
- **80 mg/kg TID x 28**
- **160 mg/kg TID x 28 (**)**
- **No treatment**
- **CPA 100 mg/kg QD X 5**

(**) $P < 0.01$ vs. vehicle
Repeated measures ANOVA
Dunnet's post test

B  **Pfeiffer (A677G)**

C  **KARPAS-422 (Y641N) – BID schedule**

No tumor re-growth for 63 days after dosing stop on Day 28 in two highest dose groups
EPZ6438 Inhibits H3K27 Methylation in Non-Tumor Tissues

A. PBMCs

B. Bone marrow

C. Skin

D. Baseline H3K27Me3 in human PBMCs

Knutson 2013, submitted
EPZ-6438 Phase 1/2 Protocol Summary

Initiated June 2013

- Patients with advanced solid or hematologic malignancies (including EZH2-mutated NHL)
- Two Phase 1 locations currently active
- Outcome measures
  - MTD
  - PK (dose and exposure)
  - PD (methyl mark inhibition)

Expected 2014

- Restricted to patients with relapsed or refractory EZH2-mutated NHL (DLBCL and Grade 3 FL)
- Planned multinational expansion of sites
- Outcome measures
  - Safety
  - Early assessment of therapeutic effect in EZH2-mutated NHL (n~25)
Regulation of Gene Expression by Modifying Chromatin

- Covalent histone modification
  - Methylation
  - Acetylation
  - Phosphorylation
  - Ubiquitination

- ATP-dependent chromatin remodeling
  - e.g. SWI/SNF complex
The SWI/SNF5 Chromatin Remodeling Complex Antagonizes with PRC2 and Is Genetically Altered in Cancer

SWI/SNF subunits are mutated in 19.6% of all human tumors (Kadoch 2013), for instance:
- SMARCB1: rhabdoid tumors, soft tissue sarcomas
- ARID1A: ovarian, breast, endometrial cancers
- BRG/BRM: lung cancer
- PBRM1: renal cell carcinoma

Normal Cells

SWI/SNF

Polycomb Targets

Stem Cell Programs

Cancer Cells
e.g. >90% of Rhabdoid Tumors

SWI/SNF

Polycomb Targets

Stem Cell Programs

Oncogenic Transformation

Wilson Cancer Cell 2010
EZH2 Inhibition is Effective in Malignant Rhabdoid Tumors

SS18-SSX Translocation in Synovial Sarcoma Impacts SWI/SNF Function

Proteolytic degradation of evicted SMARCB1 (=BAF47)
INI1-Deficiency May Confer Sensitivity to EZH2 Inhibition in a Spectrum of Soft Tissue Sarcomas

Epithelioid Sarcoma (81%)

Synovial Sarcoma (SS18-SSX Fusion; 100%)

Extraskeletal Mixoid Chondrosarcoma (18%)

Atypical Chordoma (100%)
Summary
Genetic Alterations Result in Unique Dependency on PMTs in Cancers

- **AML with MLL-r**
- **AML with wt MLL**
- **DLBCL with Y641F EZH2**
- **DLBCL with WT EZH2**
- **Cells with mutant SMARCB1 (MRT)**
- **Cells with WT SMARCB1**

**MLL-r**

**NHL w/EZH2 Mutation**

**MRT**