EZH2 Inhibitors as Novel Cancer Therapeutics

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Disclosure Information
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I have the following financial relationships to disclose:

- 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.
Three Main Mechanisms of Chromatin Remodeling

Posttranslational Modification of Histones

- PMTs
  - Arginine (RMTs)
  - Lysine (KMTs)

DNA Methylation at CpG Islands

ATPase-Driven Topological Changes to Chromatin
The human protein methyltransferases

Methyltransferases are enzymes that facilitate the transfer of a methyl (-CH₃) group to specific nucleophilic sites on proteins, nucleic acids, or other biomolecules. They share a reaction mechanism in which the nucleophilic acceptor site attacks the electrophilic carbon of S-adenosyl-L-methionine (SAM) in an SN₂ displacement reaction that produces a methylated biomolecule and S-adenosyl-L-homocysteine (SAH) as a byproduct. Methyltransferase reactions are essential transformations in small-molecule metabolism and 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.
EZH2 Catalyzed Chromatin Remodeling

- **EZH2** is the **catalytic subunit** of the multiprotein **PRC2** (polycomb repressive complex 2)
- PRC2 is the **only PMT** in humans that can methylate H3K27
- H3K27 is the only significant substrate for PRC2
- PRC2 catalyzes mono-, di- and tri-methylation of H3K27
- H3K27me3 is a transcriptionally repressive histone mark
- Hyper-trimethylation of H3K27 is **tumorigenic** in a broad spectrum of human cancers, including GC NHL

![Diagram of EZH2 catalyzed chromatin remodeling](image)

Combinatorial Control of H3K27me3 and Downstream Gene Transcription

- MLL family (methyltransferases)
- KDMs (demethylation)
- HATs (acetylation)
- PRC2
- SWI/SNF

H3

K4me3

K27me3

Transcriptional Activation

Transcriptional Repression

SAM

SAH
Dysregulation of H3K27me3 in Cancers

MLL family (methyltransferases)
K4me3
K27me3
H3
Transcriptional Activation
Transcriptional Repression

LoF Muts in NHL, etc.
KDMs (demethylation)
HATs (acetylation)
PRC2
LoF Muts in NHL
Hot Spot Muts EZH2 Amp PRC2 subunit Amp Overexpression
LoF Muts in T-ALL & MDS
LoF Muts in MRT & Synovial Sarcoma
LoF Muts in NHL, etc.

◆ Proximal and distal genetic lesions can result in cancer cell reliance on PRC2 activity and thus confer sensitivity to EZH2 inhibition
Gene Regulation in B-Cell Maturation and Lymphoma

Kuppers 2005 Nat Rev Cancer
*Source: Clarion Report (2014), incorporating GLOBOCAN epidemiology, and recent literature from ASH, NCI, NIH, and academic investigators
Gene Regulation in B-Cell Maturation and Lymphoma

Genetic Alterations Affecting H3K27me3

- Point mutations of EZH2
- Overexpression of EZH2
- Overexpression of other PRC2 subunits
- LoF of HATs
- LoF of MLL2

Kuppers 2005 Nat Rev Cancer
*Source: Clarion Report (2014), incorporating GLOBOCAN epidemiology, and recent literature from ASH, NCI, NIH, and academic investigators
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NHL-Associated EZH2 Mutations: Substrate Preference and Coupled Gain of Activity


Sneeringer et al. (2010) PNAS
EPZ-6438 (E7438) – EZH2 Inhibitor Clinical Candidate

- **Potent** against intended target in **wild type and mutant** form – 2.5 nM biochemical assay
- Highly **selective** vs. HMTs and other targets
  - Biochemical – >20,000-fold by $K_i$ (except EZH1)
  - Cellular – only inhibits target associated methyl mark
- **Orally bioavailable**
- Target **methyl mark inhibition** that leads to **specific killing** of genetically defined cancer cells **in vitro**
- Profound and sustained **in vivo activity** in animal models following inhibition of target methyl mark

Knutson et al. 2013 *PNAS*, Knutson et al. 2014 *MCT*
EPZ-6438 Specifically Inhibits Cellular H3K27 Methylation in a Time- and Dose-Dependent Manner

**WSU-DLCL2 cells (EZH2 Y646F) in vitro**
Methylation by ELISA

- **IC$_{50}$ = 9 nM**

**WSU-DLCL2 cells (EZH2 Y646F) in vitro**
Time Course at 1 µM

- **t$_{1/2}$ = 1 day**

**OCI-LY19 cells (EZH2 WT) in vitro**
4-Day Treatment

- IC$_{50} =$ 9 nM
- $t_{1/2} =$ 1 day

EZH2 Products

- H3K27Me1
- H3K27Me2
- H3K27Me3
- H3K27acetyl
- H3K4Me3
- H3K9Me3
- H3K36Me2
- H3K79 Me2
- Total H3
KARPAS422 (EZH2 Y646N Mutant) Xenografts Are Highly Sensitive to Orally Dosed EPZ-6438

- All doses were BID in efficacy study, no significant body weight loss during study
- In a 2nd study, mice were kept alive and remained tumor free 63 days after cessation of dosing

Knutson et al. 2014 MCT
# EPZ-6438 Synergy with CHOP Components: Driven by GR Agonists and Extends to GC DLBCL with WT EZH2

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>EZH2 Mutant GCB</th>
<th>EZH2 WT GCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSU-DLCL2</td>
<td>SU-DHL10</td>
<td>SU-DHL6</td>
</tr>
<tr>
<td>C Mafosfamide</td>
<td>Additive</td>
<td>Additive</td>
</tr>
<tr>
<td>H Doxorubicin</td>
<td>Synergy</td>
<td>Additive</td>
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<tr>
<td>O Vincristine</td>
<td>Additive</td>
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<tr>
<td>P Prednisolone</td>
<td>Synergy</td>
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<td>Dexamethasone</td>
<td>Synergy</td>
<td>Synergy</td>
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</tbody>
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**WSU-DLCL2 (EZH2 Y646F)**

**30-fold shift**

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**Graphs**

- **Graph 1:** EPZ-6438 IC\(_{50}\) (nM) with [Dexamethasone], nM
  - WSU-DLCL2 (EZH2 Y646F)
  - 30-fold shift

- **Graph 2:** EPZ-6438 IC\(_{50}\) (nM) Day 4
  - EPZ-6438
  - EPZ-6438 + prednisolone
  - Sensitivity to single agent EZH2i

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Knutson et al (2014) PLOS One
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:
- INI1: rhabdoid tumors, soft tissue sarcomas
- ARID1A: ovarian, breast, endometrial cancers
- BRG/BRM: lung cancer
- PBRM1: renal cell carcinoma
EZH2 Inhibition is Effective in Malignant Rhabdoid Tumors

Cells with mutant INI1

Cells with WT INI1

Mean tumor volume ± SEM (mm³)

Days

0 10 20 30

0 400 800 1200 1600 2000 2400 2800

INHIBITOR

Vehicle

Knutson et al. PNAS (2013) 20: 7922-7927
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 (17%)

Atypical Chordoma (100%)

Nature Reviews Cancer
EPZ-6438 Shows Activity in Preclinical Models of Synovial Sarcoma

In Vitro Cell Growth Inhibition Specifically in SS18-SSX Fusion Positive Cells

EPZ-6438 (µM)

- 10
- 2.5
- 0.63
- 0.16
- 0.039
- 0.010
- 0.0024
- 0.00061
- 0

Activity in Synovial Sarcoma PDX Model (SS18-SSX2)

Tumor Growth Inhibition

Tumor Growth Delay (Endpoint at 1200 mm³)

Keilhack et al. CTOS Berlin, 2014
Summary: EZH2 & H3K27me3 in Cancer

- Corticosteroids
- BCL2 & 6 Inhibitors
- B-Cell Signaling Inhibitors
- H3K27me3
- MLL LoF Muts
- HAT LoF Muts
- KDM6A LoF Muts
- EZH2 Hot Spot Muts
- SWI/SNF LoF Muts & Translocations
- PRC2 Subunit Amp
Summary: EPZ-6438

Continuing to explore a broad spectrum of cancer indications

Potent & Selective For WT & Mut EZH2

Robust Efficacy in GCB DLBCL models

Strong Synergy with Corticosteroids

Robust Efficacy in Synovial Sarcoma models

Robust Efficacy in MRT models

TRANSITIONED TO PHASE 1 CLINICAL STUDIES: NTC-1897571, SPONSORED BY EISAI

Continued to explore a broad spectrum of cancer indications

EPZ-6438 (E7438)

Transitioned to Phase 1 Clinical Studies: NTC-1897571, sponsored by Eisai

Strong Synergy with Corticosteroids

Robust Efficacy in Synovial Sarcoma models

Robust Efficacy in MRT models
We would like to thank the principal investigators and their institutions, the employees of Epizyme and Eisai, and most importantly, the patients participating in the Phase 1 study.