Targeting chromatin remodelling for haematological malignancies – lessons we can learn for the treatment of latent HIV

Professor Miles Prince
Director, Centre for Blood Cell Therapies

Peter MacCallum Cancer Centre, Melbourne
Same genes........different expression
Same genes........different expression

The “kick and kill” approach to cure HIV

Reactivate latent viral expression
- HDAC inhibitors, PKC activators, BET bromodomain inhibitors, etc.
Epigenetic targets in Oncology: DNA methylation and histone modification

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity

Histone modification
A combination of different molecules can attach to the “tails” of proteins called histones. These alter the activity of the DNA wrapped around them.
Epigenetic alterations in Tumour Progression

A multistage model of carcinogenesis in skin is shown. In conjunction with phenotypic cellular changes and the accumulation of genetic defects, there is a progressive loss of total DNA methylation content, an increased frequency of hypermethylated CpG islands, and an increased histone-modification imbalance in the development of the disease. H-ras denotes Harvey-ras oncogene, and 5mC 5-methylcytosine.
# Epigenetic aberrations amongst tumours

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Epigenetic Disruption</th>
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<tbody>
<tr>
<td>Colon cancer</td>
<td>CpG-island hypermethylation (hMLH1, p16^{INK4a}, p14^{ARF}, RARB2, SFRP1, and WRN), hypermethylation of miRNAs (miR-124a), global genomic hypomethylation, loss of imprinting of IGF2, mutations of histone modifiers (EP300 and HDAC2), diminished monoacetylated and trimethylated forms of histone H4</td>
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<tr>
<td>Breast cancer</td>
<td>CpG-island hypermethylation (BRCA1, E-cadherin, TMS1, and estrogen receptor), global genomic hypomethylation</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>CpG-island hypermethylation (p16^{INK4a}, DAPK, and RASSF1A), global genomic hypomethylation, genomic deletions of CBP and the chromatin-remodeling factor BRG1</td>
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<tr>
<td>Glioma</td>
<td>CpG-island hypermethylation (DNA-repair enzyme MGMT, EMP3, and THBS1)</td>
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<tr>
<td>Leukemia</td>
<td>CpG-island hypermethylation (p16^{INK4a}, EXT1, and ID4), translocations of histone modifiers (CBP, MOZ, MORF, MLL1, MLL3, and NSD1)</td>
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<tr>
<td>Lymphoma</td>
<td>CpG-island hypermethylation (p16^{INK4a}, p73, and DNA-repair enzyme MGMT), diminished monoacetylated and trimethylated forms of histone H4</td>
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<td>Bladder cancer</td>
<td>CpG-island hypermethylation (p16^{INK4a} and TPEF/HPP1), hypermethylation of miRNAs (miR-127), global genomic hypomethylation</td>
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<td>Kidney cancer</td>
<td>CpG-island hypermethylation (VHL), loss of imprinting of IGF2, global genomic hypomethylation</td>
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<tr>
<td>Prostate cancer</td>
<td>CpG-island hypermethylation (GSTP1), gene amplification of polycomb histone methyltransferase EZH2, aberrant modification pattern of histones H3 and H4</td>
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<tr>
<td>Esophageal cancer</td>
<td>CpG-island hypermethylation (p16^{INK4a} and p14^{ARF}), gene amplification of histone demethylase JMJD2C/GASC1</td>
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<td>Stomach cancer</td>
<td>CpG-island hypermethylation (hMLH1 and p14^{ARF})</td>
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<tr>
<td>Liver cancer</td>
<td>CpG-island hypermethylation (SOCS1 and GSTP1), global genomic hypomethylation</td>
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<tr>
<td>Ovarian cancer</td>
<td>CpG-island hypermethylation (BRCA1)</td>
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</table>
Demethylating agents

5-aza-2' deoxycytidine (decitabine, DAC)
5-azacytidine (azacitidine, AZA)
Zebularine
5-Fluoro-2'-deoxycytidine

S110

Diagram showing the interaction of 5-Azacytidine with DNMT to convert cytosine to 5-Methyl Cytosine.
Effects of demethylating agents

- Myelodysplasia
- Acute myeloid leukemia (myeloma)
- Lymphoma
The basic functional unit of chromatin is the nucleosome (Panel A), which is composed of a histone octamer around which DNA is wrapped. Octamers are separated by linker DNA. The histone octamer is assembled from a histone H3/H4 tetramer and two H2A:H2B dimers. The histone tails of all four core histones are subject to a variety of post-translational modifications (Panel B). These include methylation (Me), acetylation (Ac), phosphorylation (Ph), ubiquitylation (Ub), and proline isomerization (Iso), all of which occur at the site of a specific amino acid, such as K4 and K9 on the histone H3 tail. The same histone amino acid may be subject to different post-translational modifications, which may facilitate different biologic outcomes.
Chromatin epigenetic regulation
Cancer mutations in Epigenetic Regulators

DNA Methylation

Methyltransferases

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<tr>
<th>Enzyme</th>
<th>Mutation</th>
<th>Tumor</th>
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<tr>
<td>DNMT3A^*</td>
<td>M, F, N, S</td>
<td>AML, MDS, MPD</td>
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Histone modification

‘writers’

Histone modification

Methyltransferases

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<tr>
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<th>Tumor</th>
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<td>NSD3^</td>
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<td>KMT6 (EZH2)</td>
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<td>DLBCL, MPD, MDS</td>
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‘readers’

DNA Methylation

Histone modification

‘writers’

Hydroxymethylation and derivatives

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<td>TET2</td>
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‘erasers’

DNA Methylation

Histone modification

Methyltransferases

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<td>KDM5C (JARID1C^*)</td>
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<td>KDM6A (UTX)</td>
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Readers

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<td>MSH6^*</td>
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Demethylases

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| * = Bromodomain

* = Bromodomain

* = PHD Finger

^ = PWPP domain

Dawson and Kouzarides - Cell (2012)
Anti-cancer activities of HDACi

HDACi

Histone targeting

HDACs

Non-histone substrates

Transcription factors (p53, E2F1, STAT1, NF-κB)

α-tubulin, Hsp90, Ku70

Histone hyperacetylation

Transcription activation/repression

Senescence

Cell-cycle arrest

Angiogenesis

Differentiation

Immune modulation

Apoptosis

Autophagy

HDAC = eraser

HAT = writer

There are 2 Classes of HDACs which Act on Different Target Proteins

**Class I DACs**
- act on **HISTONES and TRANSCRIPTION FACTORS**
- located in the nucleus

**Class II DACs**
- act on **NON-HISTONE proteins**
- located in the cytoplasm (e.g. HDAC6)
Table 1 Classes of DAC inhibitors, their HDAC targets and HDAC cellular distribution

<table>
<thead>
<tr>
<th>HDACi Class</th>
<th>HDAC Class</th>
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</table>

Table 1: Classes of DAC inhibitors, their HDAC targets and HDAC cellular distribution.

**HDAC specificity**

Nuclear, Nuclear, Cytoplasmic, Cytoplasmic, Nuclear

Cutaneous T cell lymphoma

Myeloma, Hodgkins Lymphoma, T cell lymphoma, myeloid leukemia

T cell lymphoma – peripheral and cutaneous
Summary of clinical studies to date

- HDACi have activity in
  - T cell lymphomas – CTCL and PTCL
  - Hodgkin Lymphoma
  - Myeloid Malignancies – combination studies
  - B-cell neoplasms – follicular NHL
  - Myeloma – combination studies

  - Scheduling likely important
  - ? Difference b/w isotype-specific or panHDACi

- Toxicity profile - dose/scheduling/duration dependent
  - Fatigue
  - Diarrhoea
  - Nausea
  - Transient Thrombocytopenia (and other cytopenias)
  - Q-T prolongation (dose and schedule)
  - Electrolyte disturbance (K+, Mg+, PO4)
  - Any differences between Class I specific vs. panHDACi ??
Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma


blood
2011 117: 5827-5834
Prepublished online February 25, 2011;
doi:10.1182/blood-2010-10-312603

Results From a Pivotal, Open-Label, Phase II Study of Romidepsin in Relapsed or Refractory Peripheral T-Cell Lymphoma After Prior Systemic Therapy

Bertrand Coiffier, Barbara Pro, H. Miles Prince, Francine Foss, Lubomir Sokol, Matthew Greenwood, Dolores Caballero, Peter Borchmann, Franck Morschhauser, Martin Wilhelm, Lauren Pinter-Brown, Swaminathan Padmanabhan, Andrei Shustov, Jean Nichols, Susan Carroll, John Balser, Barbara Balser, and Steven Horwitz

RR = 38%
Phase II Multi-Institutional Trial of the Histone Deacetylase Inhibitor Romidepsin As Monotherapy for Patients With Cutaneous T-Cell Lymphoma


Final Results From a Multicenter, International, Pivotal Study of Romidepsin in Refractory Cutaneous T-Cell Lymphoma

Sean J. Whittaker, Marie-France Demierre, Ellen J. Kim, Alain H. Rook, Adam Lerner, Madeleine Duvic, Julia Scarisbrick, Sunil Reddy, Tadeusz Robak, Jürgen C. Becker, Alexey Samtsov, William McCulloch, and Yoon H. Kim
Romidepsin in CTCL

Jan 04

Feb 04

Piekarz et al. ASCO 2007
Differential Gene Expression Changes In Response To Panobinostat

- LBH589 induces rapid (by 4 hours) and robust changes in tumor cell gene expression
- 1-4% of genes were significantly altered with the majority of genes **down regulated**
- Persisted for at least 8 hours for most genes
- Consistent with cell line data
- Combined data identified 23 genes that were altered in all patients
Delayed response to panobinostat

Discontinued therapy due to toxicity

HDACs Modulate Histone and **Non-Histone Proteins** Involved in Oncogenesis

Histone proteins are implicated in epigenetic modifications that could cause cancer.

Non-histone proteins are implicated in multiple oncogenic pathways.
AML is a malignant disorder of progenitor cells in myeloid haematopoiesis

Genetically heterogeneous cancer (>100 genetic aberrations have been implicated)

Frequent chromosomal translocation observed is t(8;21), also known as AML1-ETO (present in ~15% of all cases of AML with different subtypes)

AML1-ETO binds HDACs (and corepressors) via the C terminus of ETO, leading to repression of the AML1 target genes
Transcription alteration NOT just via chromatin modification

HDACi

Histone targeting

Histone hyperacetylation

Transcription activation/repression

Non-histone substrates

Transcription factors (p53, E2F1, STAT1, NF-κB)

α-tubulin, Hsp90, Ku70

HDACs

Hsp90

AML1-ETO

Senescence

Cell-cycle arrest

Angiogenesis

Immune modulation

Apoptosis

Autophagy

Differentiation
Chromatin epigenetic regulation

Histone acetyl transferase
Histone methyltrasferase

Histone deacetyylase
Histone demethylase
Chromatin epigenetic regulation

Epigenetic “writer”
Epigenetic “eraser”
Epigenetic “reader”

Addition of chemical modification
Removal of modification
Recruitment
Alteration of DNA-templated process

Histone acetyl transferase
Histone methyltransferase
Histone deacetylase
Histone demethylase
EZH2i for Multiple Genetically-Defined Cancers

**PRC2 Complex**

- Over expression of PHF19
  - Multiple Solid Tumors
- Amplification of PRC2 Subunits
  - Multiple Solid Tumors
- Change of Function Mutation
  - Non-Hodgkins Lymphoma

**Methylation**

- Demethylation

**K27**

**UTX**

**K27(me)₃**

**Loss of Function Mutation**

- Myeloma
- Renal
- Esophageal

21 June 2013
KARPAS422 EZH2 Y646N Mutant Xenografts are Sensitive to Orally Dosed EPZ-6438 in a Dose Dependent Manner

28 day anti-tumor study

7 day PK/PD study
Target inhibition in tumor (ELISA)

- All doses were BID in efficacy study, no significant body weight loss during study
- Mice were kept alive and remain tumor free 63 days after cessation of dosing
- More sensitive than WSU-DLCL2 model in vivo, despite similar potency in vitro

21 June 2013
EPZ-6438 Phase 1/2 Protocol Summary

**Phase 1**
- **Initiation 2Q 2013**
  - Patients with advanced solid or hematologic malignancies (including EZH2-mutated NHL)
  - Phase 1 initiated at IGR-Paris
  - Outcome measures
    - MTD
    - PK (dose and exposure)
    - PD (methyl mark inhibition)

**Phase 2**
- **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)

21 June 2013
Chromatin epigenetic regulation

Proteins recognize post-translational modifications on histones
Epigenetic “reader” domains

Proteins recognize post-translational modifications on histones
Epigenetic “reader” domains

Proteins recognize post-translational modifications on histones.

Bromodomain
“BET” proteins contain bromodomain that bind acetyl groups

BET ‘reads’ the acetylation
“BET” can no longer bind to acetyl group

BET (bromodomain) inhibitors

BET ‘reads’ the acetylation
# Bromodomain containing proteins

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<thead>
<tr>
<th>Protein</th>
<th>Name</th>
<th>Function</th>
<th>BRDs</th>
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<td>Peregrin</td>
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<td>BRPF3A</td>
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<td>JAK/STAT signalling</td>
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<td>CECR2</td>
<td>Cat eye syndrome critical region 2</td>
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<td>CREBBP</td>
<td>CREB-binding protein</td>
<td>Histone acetyltransferase</td>
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<td>HAT p300</td>
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<td>Mixed lineage leukaemia</td>
<td>SWI/SNF PBAF subunit</td>
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<td>PH-interacting protein</td>
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<td>Protein kinase C-binding protein 1</td>
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<td>SWI/SNF ATPase</td>
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<td>TAF1/TAF1L</td>
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<td>ZMYND11</td>
<td>Zinc finger MYND-domain-containing protein 11</td>
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BRD, bromodomain; HAT, histone acetyltransferase; MOZ, monocytic leukaemia zinc finger protein; PHD, plant homology domain; SNF, sucrose nonfermenting.
Mixed lineage leukemia (MLL) a model for epigenetic therapy

MLL fusions present in approximately 10% of ALL and AML
MLL is Found in 73 different translocations and 54 partner genes have been cloned
LETTER TO THE EDITOR

MLL-aberrant leukemia: complete cytogenetic remission following treatment with a histone deacetylase inhibitor (HDACi)

Kate L. Burbury · Mark J. Bishton · Ricky W. Johnstone · Michael J. Dickinson · Jeffrey Szer · H. Miles Prince
Mixed lineage leukemia (MLL) a model for epigenetic therapy

MLL fusions present in approximately 10% of ALL and AML

MLL is Found in 73 different translocations and 54 partner genes have been cloned

EAP = ENL associated protein complex
pTEFb = positive transcription elongation factor B
MLL-fusion Partners Are In Transcriptional Elongation Complexes

SUPER ELONGATION COMPLEX

MLL-fusion partners
AF4
AF9
ENL

pTEFb
CycT1
CDK9

PAF Complex

ELL

BET

Ac
Ac

K
K

Pol II

ELONGATION

pTEFb = positive transcription elongation factor B
• Targeting epigenetic readers is an exciting new therapeutic avenue

• BET inhibition has therapeutic promise in AML & Multiple Myeloma Zuber et al; Nature 2011, Delmore et al; Cell 2011, Mertz et al; PNAS 2011
Transcription co-factors in MLL and HIV can be similar

MLL - Mixed lineage leukemia - MLL/AF9

Tat recruitment of super elongation complex (SEC)
Transcription co-factors in MLL and HIV can be similar
Transcription co-factors in MLL and HIV can be similar
Transcription co-factors in MLL and HIV can be similar
Transcription co-factors in MLL and HIV can be similar
AML driven by MLL Fusion Proteins

Recruit transcription/epigenetic regulatory proteins

- pTEFb - CycT1/CDK9 – phosphorylates RNA Pol II
- HDACs
- BET(Brd4)
- Dot1L (histone methyltransferase)
Mouse model of t(9;11) AML (AF9)

- MLL-AF9
- IRES
- GFP
- Luciferase
- IRES
- NRAS^{G12D}

Pregnant mouse → Fetal liver day 13.5-15 → Hematopoietic stem cells → Lethally irradiated recipient → Tumour progression
Sensitivity of MLL-AF9 tumors

Vehicle
JQ1
EPZ4777
Etoposide
Dinaciclib
Panobinostat

Annexin/PI +ve %

nM concentration

ppRNA Pol II CTD

Total RNA Pol II
Response of MLL-AF9 leukemias to dinaciclib in vivo

MLL-AF9 AML (i.v.) → Dinaciclib (every 4 days i.p.) → determine response and monitor survival

Percent survival

MLL-AF9 NRAS+ve

Dinaciclib

Dinaciclib

Vehicle
Differences and similarities

The “kick and kill” approach to cure HIV

Reactivate latent viral expression
- HDAC inhibitors, PKC activators, BET bromodomain inhibitors, etc.

JQ1 (Brd4)
Dinaciclib (CDK9i)
Panobinostat (HDACi)
EPZ4777 (DOT1Li)
Differences and similarities

Panobinostat (HDACi)

Brd4 Competes with HIV Tat for P-TEFb Binding

- CDK9
- PID
- BRD4
- CycT1

Basal HIV Transcription
Cellular Genes

Tat-mediated HIV Transcription

JQ1 (Brd4)

HDAC

Brd4

EAP
- AF4
- AF5
- LAF4
- pTEFb
- RNA Pol II
- CTD kinase

MLL N
- ENL
- AF9
- DOT1L

RNA Pol II

Ac

H3

H4

H3K79 methylation

EPZ4777 (DOT1Li)

Dinaciclib (CDK9i)

Prostratin
Bryostatin
Ingénol

HEXIM-1

HMBA

75K snRNA

NF-kB sites

nuc-1

P-TEFb

Tat

5'

Cellular genes

CREB

Rab8a

nucleus

HIV-1
Differences and similarities

Panobinostat (HDACi)

JQ1 (Brd4)

Brd4

HDAC

EPZ4777 (DOT1Li)

Dinaciclib (CDK9i)
Differences and similarities

Panobinostat (HDACi)

JQ1 (Brd4)

Dinaciclib (CDK9i)

HDAC

Brd4

EPZ4777 (DOT1Li)

PKC stim

Brd4 Competes with HIV Tat for P-TEFb Binding

Basal HIV Transcription Cellular Genes

Tat-mediated HIV Transcription

Ac Histones

TF (such as NF-KB)

HEXIM-1

HMBA

75K snRNA

P-TEFb

P-TEFb

Brd4

Cellular genes

NF-kB sites

nuc-1

Ac

H4

H3

H3K79 methylation

RNA Pol II CTD kinase

RNA Pol II

DOT1L

DOT1L

AF4

AF5

LAF4

pTEFb

EAP

AF4

AF5

LAF4

MLL

ENL

AF9

Ac

LEDGF

Menin

AT

CxxC
A LOT MORE WORK TO DO!
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