Targeting Epigenetics in Hematological Malignancies
Heritable change in pattern of gene expression mediated by mechanisms other than alterations in primary nucleotide sequence

“The difference between genetics and epigenetics can probably be compared to the difference between writing and reading a book. Once a book is written, the text (the genes or DNA: stored information) will be the same. However, each individual reader of a given book may interpret the story slightly differently. In a very similar manner, epigenetics would allow different interpretations of a fixed template (the book or genetic code) and result in different read-outs, dependent upon the variable conditions under which this template is interrogated.”

Thomas Jenuwein (Vienna, Austria)
Epigenetics

Epigenetic differences arise during the lifetime of monozygotic twins

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Dysregulation of epigenetic processes - Disease development

Environmental exposure
Maternal Factors
Diet and Life style

Germline epimutation
Genome-wide Demethylation
Developmental epigenetic reprogramming
Somatic epimutation

Gametes → Zygote → Embryo → Fetus → Baby → Adolescent → Adult → Elderly

Dysregulation of epigenetic processes - Disease development
Epigenome and Epigenetic Mechanisms

The epigenome regulates gene expression patterns, in other words, what genes need to be silenced or expressed in a cell.

- DNA Methylation
- Histone Modifications
- Nucleosome remodelling
- Non coding RNAs
DNA Methylation

- Best characterized epigenetic event
- Methylation occurs in a cytosine-containing nucleotides that are immediately followed by guanine-containing nucleotides (CpG regions)
- CpG regions in 60% of promoters
- Usually associated to gene silencing
- More frequent that tumor suppressor gene mutations
DNA Methylation and Normal Cell

- X chromosome inactivation
- Silencing of repetitive sequences
- Chromatin organization
- Tissue specific methylation
- Imprinting
DNA Methylation and Cancer

Cancer cell

- CpG-island hypermethylation
- ‘Closed’ chromatin conformation
- DNA hypomethylation
- ‘Open’ or ‘relaxed’ chromatin conformation

- Entry into cell cycle
  - Avoidance of apoptosis
  - Defects in DNA repair
  - Angiogenesis
  - Loss of cell adhesion

- Loss of imprinting and overgrowth
  - Inappropriate cell-type expression
  - Genome fragility
  - Activation of endoparasitic sequences

Unmethylated CpG  Methylated CpG

Tumorigenesis

Regulation of DNA Methylation

DNMT3L and DNMT2
Enzymes involved in DNA modification

Cytosine (C, ~20%) → 5-methylcytosine (5mC, ~1%)

Passive demethylation

SAM → SAH

DMNTs

TDG, BER

Passive demethylation

5-carboxycytosine (5caC)
5-formylcytosine (5fC)
5-hydroxymethylcytosine (5hmC, ~0.1%)

Succinate/CO₂

2-OG/Fe²⁺/O₂

ATP

TET

Trends in Genetics 2014; 30:464-474
## Mutations in enzymes involved in DNA modification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer</th>
<th>Frequency or stage of cancer</th>
<th>Frequency of mutation (N)</th>
<th>Effect</th>
<th>Refs</th>
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<tbody>
<tr>
<td><strong>DNA methyltransferases</strong></td>
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<tr>
<td>DNMT1</td>
<td>Colorectal cancer</td>
<td></td>
<td>2% (29)</td>
<td>Mutation</td>
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<tr>
<td>DNMT3A</td>
<td>AML</td>
<td>Stage M4</td>
<td>13.6% (66)</td>
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<td>87</td>
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<tr>
<td></td>
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<td>Stage M5</td>
<td>20.5% (112)</td>
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<td>87</td>
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<tr>
<td></td>
<td>AML</td>
<td>Common</td>
<td>22.1% (281)</td>
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<td>88</td>
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<td><strong>DNA demethylases</strong></td>
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<tr>
<td>TET2</td>
<td>BCR-ABL-negative myeloproliferative neoplasms</td>
<td>Rare form</td>
<td>13% (239)</td>
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<td>152</td>
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<td></td>
<td>CMML</td>
<td>Common form</td>
<td>50% (88)</td>
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<td>90</td>
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<td></td>
<td>MDS</td>
<td>Rare</td>
<td>26% (102)</td>
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<td>153</td>
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<tr>
<td>IDH1</td>
<td>Anaplastic astrocytoma</td>
<td>Rare</td>
<td>73% (52)</td>
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<td>154</td>
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<tr>
<td></td>
<td>Diffuse astrocytoma</td>
<td>Rare</td>
<td>90% (30)</td>
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<td></td>
<td>AML</td>
<td>Common</td>
<td>6.2% (385)</td>
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<td>89</td>
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<tr>
<td>IDH2</td>
<td>AML</td>
<td>Common</td>
<td>8.6% (385)</td>
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</table>

Histone Modifications

**Des/Acethylation**

**Methylation**

**Phosphorylation**

**Ubiquitination**

**ADP-Ribosilation**

**Isomerization**

Normal Cell

Gene promoters

Tumor cell

- Acetylation
- Methylation
### Mutations in regulators of the epigenome identified in cancer

<table>
<thead>
<tr>
<th>Histone modification</th>
<th>Writers</th>
<th>Acetylation</th>
<th>Methylation</th>
<th>Phosphorylation</th>
<th>Readers</th>
<th>Acetylation, methylation and phosphorylation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CDYL, CLOCK, CREBBP, EP3, EP300, GTF3C4, HAT1, KAI3, NAC1, NCOA5</td>
<td>ASH1L, CARM1, DOT1L, EHMT2, EZH2, MLL1, NSD1, PRDM9, PRMT5, SETDM, SMYD3, SULF2, SETMAR</td>
<td>ANKRD5, DUSP1, EYA1, EYA5, PPP5, SMEK1</td>
<td>TAF5, CHD5, MGA, ZMYMs, PHF5, ZNF5, ADNP, ATXN7, DHX39s, EP400, FAM5, GABRG1, GATAD2s, HCF5, NIPBL, POGZ, RAI1, SMC1A, SMCHD1, TRIM6, TRRAP, ZMYND8</td>
<td></td>
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</tbody>
</table>

Role of epigenetics

- Epigenetic changes as prognostic factors and biomarkers
- Development of therapeutic approaches
DNA methylation as epigenetic marker: AML

DNA Methylation Captures Clinically Significant Differences among AML Patients

344 pacientes LAM

A
inv(16)  t(8;21)  t(15;17)  t(v;11q23)

Cluster 1  Cluster 3  Cluster 6  Cluster 11

B
CEBPA-dm  CEBPA-mut  CEBPA-sil

Cluster 4  Cluster 9  Cluster 10

C
NPM1 mutations

Cluster 12  Cluster 13  Cluster 14  Cluster 16

D
Epigenetically defined clusters

Cluster 2  Cluster 5  Cluster 7  Cluster 8  Cluster 15

Overall survival – Novel clusters
Log rank p<0.0001

Overall survival – Novel clusters
Log rank p<0.04

Cancer Cell. 2010; 17:13-27
DNA methylation as epigenetic marker: ALL

DNA methylation as epigenetic marker: CLL

Kulis M. Nature Genetics 2012
Hypomethylating Agents: Mechanism of Action (MoA)

- Decitabine and 5-Azacytidine are S-phase specific
- Higher potency of Decitabine versus 5-Azacytidine (Decitabine only binds to DNA)
- The majority (80–90%) of 5-azacitidine is incorporated into RNA
- High-dose causes DNA damage and DNA synthesis arrest, leading to cytotoxicity
- Low-dose induces DNMT inhibition with minimal cytotoxicity

New Hypomethylating Agents

A

Azacytidine (AZA)  Decitabine (DAC)
NPEOC-DAC  SGI-110
CP-4200

5,6-Dihydroazacytidine  5-Fluoro-2'-deoxycytidine  Zebularine

B

Hydralazine  Procainamide  Procaine  (-)-Epigallocatechin-3-gallate

Mythramycin A  Nanaomycin A  RG-108

SGI-1027  NSC-14778  NSC-106084
Hypomethylating Agents (Caveats)

- MoA: evidence for hypomethylation of specific genes is limited
- Biomarkers to predict response
- Combinations with other chemotherapy
HAT and HDACs

Panobinostat/Vorinostat

Trichostatin A (TSA)

Deacetylation

Inhibition

Histone

DNA

HDAC

Acetylation

HAT

Activation of gene expression

Acetyl group

Trapoxin (TPX)
HDAC inhibitors (Panobinostat) in Cancer

Multiple myeloma

Mithraprabhu S, Br J Haematol, 2013

Chuang MJ, Plos One, 2013

Lymphoma

Lemoine M, Blood, 2012

ALL

Rag2 + LAL humana

Mithraprabhu S, Br J Haematol, 2013

Chuang MJ, Plos One, 2013

Agirre X, Vilas-Zornoza A. Leukemia 2012
Protein Methyltransferase and Demethylase Inhibitors

### Selected novel drugs in preclinical or clinical development targeting components of the epigenetic machinery

<table>
<thead>
<tr>
<th>Substance</th>
<th>Target structure</th>
<th>Clinical trial</th>
<th>Disease</th>
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</thead>
<tbody>
<tr>
<td>SGI110</td>
<td>DNMT</td>
<td>Phase I/II</td>
<td>MDS, AML, ovarian and hepatocellular cancer</td>
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<tr>
<td>AGI-5198</td>
<td>Mutant IDH</td>
<td>Preclinical</td>
<td>Glioma</td>
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<tr>
<td>Pivanex (also known as AN-9)</td>
<td>HDAC</td>
<td>Phase I/II</td>
<td>CLL, small lymphocytic lymphoma, malignant melanoma and NSCLC</td>
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<tr>
<td>ACY-1215</td>
<td>HDAC6</td>
<td>Phase I/II</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Resveratrol (SRT501)</td>
<td>SIRT1 and SIRT5 activation</td>
<td>Phase I/II</td>
<td>Colorectal cancer, melanoma, multiple myeloma</td>
</tr>
<tr>
<td></td>
<td>SIRT3 inhibition</td>
<td>Phases I–III</td>
<td>Metabolic and cardiovascular diseases</td>
</tr>
<tr>
<td>Curcumin</td>
<td>HAT</td>
<td>Phase I/II</td>
<td>Breast cancer, colorectal cancer and multiple myeloma</td>
</tr>
<tr>
<td>Tranilcypromine</td>
<td>KDM1A</td>
<td>Phase II</td>
<td>AML</td>
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<tr>
<td>EPZ-5676</td>
<td>DOT1L</td>
<td>Phase I</td>
<td>Advanced haematological malignancies and acute leukaemia with 11q23 or MLL abnormalities</td>
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<tr>
<td>EPZ-6438</td>
<td>EZH2</td>
<td>Phase I</td>
<td>NHL and breast cancer</td>
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<tr>
<td>GSK126</td>
<td>EZH2</td>
<td>Preclinical</td>
<td>Haematological malignancies, including NHL</td>
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<td>GSK525762</td>
<td>BET bromodomain</td>
<td>Phase I</td>
<td>NMC</td>
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<tr>
<td>RVX-208</td>
<td>BET bromodomain</td>
<td>Phase II</td>
<td>Atherosclerosis</td>
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<td></td>
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<td>Preclinical</td>
<td>Haematological malignancies</td>
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<td>JQ1</td>
<td>BET bromodomain</td>
<td>Preclinical</td>
<td>NMC, AML and multiple myeloma</td>
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<tr>
<td>PFI-1</td>
<td>BET bromodomain</td>
<td>Preclinical</td>
<td>B cell acute lymphoblastic leukaemia</td>
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</tbody>
</table>

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