Transcriptional impact of resminostat and other HDAC inhibitors on disease-related gene expression in CTCL

Dr. Gundula Streubel, 4SC AG
EORTC CLTF 2019, Athens
HDAC inhibitors as treatment opportunity in CTCL

• Vorinostat and romidepsin are FDA-approved for systemic therapy in CTCL

• In Europe, HDAC inhibitors are not yet approved for the treatment of CTCL*
  o Resminostat is investigated in a placebo-controlled trial in CTCL, maintenance setting (RESMAIN)

• HDACi differ in class specificity, chemical structure, clinical setting, administration and dosing, pharmacology ➔ MoA?

<table>
<thead>
<tr>
<th>HDACs in CTCL</th>
<th>Resminostat*</th>
<th>Vorinostat</th>
<th>Romidepsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDAC inhibition</td>
<td>Class I, IIb, IV</td>
<td>Class I, II, IV</td>
<td>Class I</td>
</tr>
<tr>
<td>Chemical class</td>
<td>Hydroxamic acid</td>
<td>Hydroxamic acid</td>
<td>Bicyclic depsipeptide</td>
</tr>
<tr>
<td>Administration</td>
<td>Oral (5 d treat./9 break)</td>
<td>Oral (continuously)</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Pharmacokinetics (at dosing)</td>
<td>(C_{\text{max}} \approx 6 , \mu\text{M},)</td>
<td>(C_{\text{max}} \approx 1.2 , \mu\text{M},)</td>
<td>(C_{\text{max}} \approx 0.7 , \mu\text{M})</td>
</tr>
</tbody>
</table>

Vorinostat data taken from SmPC, status 12/2018
Resminostat data from FIM study, 600mg single dose
Comparative RNA-Seq gene expression analysis for HDAC inhibitors in CTCL cells

- Gene expression analysis (RNA-Seq)
  - 24 h compound treatment of 3 CTCL cell lines with non-toxic concentrations

- Cell lines
  - My-La CD4+ ➔ Mycosis fungoides (MF), plaque biopsy
  - HH ➔ Mycosis fungoides (MF), peripheral blood
  - HuT78 ➔ Sézary syndrome (SzS), peripheral blood

<table>
<thead>
<tr>
<th>Cell line</th>
<th>My-La CD4+</th>
<th>HH</th>
<th>HuT78</th>
<th>Common</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>HDAC inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resminostat [3 µM]</td>
<td>1882</td>
<td>342</td>
<td>2877</td>
<td>1507</td>
</tr>
<tr>
<td>Vorinostat [3 µM]</td>
<td>3525</td>
<td>2485</td>
<td>2843</td>
<td>2041</td>
</tr>
<tr>
<td>Romidepsin [30 nM]</td>
<td>5933</td>
<td>4335</td>
<td>5016</td>
<td>4459</td>
</tr>
</tbody>
</table>

Cutoff DEG: log2 FC > 1, adj p-value < 0.1

- Low number of common genes per compound → Cell line dependent gene expression alterations

De-regulation of > 1000 genes by HDAC inhibitors
HDAC inhibitor dependent differences and similarities in gene expression

- PCA from differential gene expression analysis ➔ similarity (proximity) and variance (distance)
  - Romidepsin different from Resminostat and Vorinostat
  - Resminostat and Vorinostat differ more in My-La cells, cluster in HH and HuT78

➔ Focus on disease relevant genes

Resminostat modulates the expression of disease-associated genes

↓ Skin homing T cell receptors

<table>
<thead>
<tr>
<th>TPM (RNA-Seq)</th>
<th>My-La CD4+</th>
<th>HH</th>
<th>HuT78</th>
</tr>
</thead>
<tbody>
<tr>
<td>row min</td>
<td>ctrl</td>
<td>Resm</td>
<td>ctrl</td>
</tr>
<tr>
<td>row max</td>
<td>not expressed</td>
<td>not expressed</td>
<td>not expressed</td>
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↓ Th2 T cell phenotype linked with pathogenesis

<table>
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<tr>
<th>STAT4, Th1</th>
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<tr>
<td>HH</td>
</tr>
<tr>
<td>rel. mRNA (qPCR)</td>
</tr>
<tr>
<td>ctrl</td>
</tr>
<tr>
<td>0</td>
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</table>

<table>
<thead>
<tr>
<th>STAT6, Th2</th>
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</thead>
<tbody>
<tr>
<td>HH</td>
</tr>
<tr>
<td>rel. mRNA (qPCR)</td>
</tr>
<tr>
<td>ctrl</td>
</tr>
<tr>
<td>0.0</td>
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↓ Advanced disease progression

Litvinov et al. 2017, Up-regulated in advanced (≥IIB) stage versus early (≤IIA) stage CTCL

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<tbody>
<tr>
<td>row min</td>
<td>ctrl</td>
<td>Resm</td>
<td>ctrl</td>
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<tr>
<td>row max</td>
<td>not expressed</td>
<td>not expressed</td>
<td>not expressed</td>
</tr>
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Resminostat may be beneficial to prevent/delay disease progression and skin infiltration
De-regulation of skin-homing T cell receptors gene expression by HDAC inhibitors

- Vorinostat and resminostat reduce skin-homing T cell receptor expression (MF cells)
- Cell line dependent up- or down-regulation of skin-homing T cell receptors by romidepsin

Cell-line dependent reduction of skin-homing T cell receptors by HDAC inhibition
HDAC inhibitors modulate CTCL progression genes

**A**

TPM (RNA-Seq) row max

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<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>GNSL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GTSF1</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>CCL5</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>TIMP1</strong></td>
<td></td>
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</table>

- **Romidepsin up**
- **Resminostat, Vorinostat down**
- **All HDACi down**

**B**

- **HH Resminostat**
- **HH Vorinostat**
- **HH Romidepsin**

* adj p-value < 0.01

**CD70**

**IL21R**

**TRIP13**

**PTPN7**

**GNSL**

**GTSF1**

**CCL5**

**TIMP1**

Heatmap of mean-centered gene expression (TPM) per cell line, hierarchical clustering of genes (https://software.broadinstitute.org/morpheus/)


TPM data/DeSEQ2 analysis

Resminostat/vorinostat down-regulate disease progression genes
HDACi mediated up-regulation of the Th1 lineage transcription factor TBX21/TBET

- Th1 / Th2 imbalance in CTCL
- Phenotypic Th1 to Th2 shift linked with pathogenesis ➔ possible mechanism of pathogenesis:

HDACi mediated expression change of Th1 lineage genes

Resminostat and vorinostat effectively upregulate the Th1 lineage associated genes

- Romidepsin has less potential to upregulate Th1 gene expression
Summary

• The data confirm that resminostat beneficially affects disease-related gene expression
  o ↓ Skin homing T cell receptors
  o ↓ Disease progression genes
  o Th1↑/Th2↓ ➔ may favor the Th1 T cell phenotype

• Vorinostat had comparable effects on disease-related gene expression (equimolar concentration)
  o PK data: exposure of resminostat ≈ 5-fold higher than of vorinostat

• Romidepsin
  o Stronger effects on gene expression
  o Importantly, it down- but also up-regulates the expression of disease-related genes

• Resminostat and vorinostat were more potent to upregulate Th1 lineage transcription factors compared to romidepsin

Resminostat has the potential to delay or prevent CTCL disease progression
Mode of action of resminostat in CTCL cells

Resminostat’s mode of action in CTCL cells

Debulking effects ↔ Modulation of cell phenotype

Cell death / Apoptosis

Cell Proliferation

Hyperacetylation of histone and non-histone proteins

Immune Modulation
NK cell response

Gene Regulation
Th1/Th2 Skin homing receptors

IL-31 cytokine reduction

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4SC AG Team

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