Epigenetic Therapeutics for Combination with Cancer Immunotherapy

June 2016
Epigenetic regulation of gene expression

Yan et al. 2010, Journal of applied physiology
Resminostat and 4SC-202 clinical stage epigenetic modulators

<table>
<thead>
<tr>
<th>Resminostat</th>
<th>4SC-202</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Broad-spectrum HDACi</strong> (class I, IIb, IV)</td>
<td><strong>LSD1 + Class I HDACi</strong></td>
</tr>
<tr>
<td>Clinical phase II, pivotal</td>
<td>Completed Clinical phase I</td>
</tr>
<tr>
<td>CTCL, HL, HCC, CRC, NSCLC</td>
<td>Various hematological indications</td>
</tr>
<tr>
<td>Oral</td>
<td>Oral</td>
</tr>
<tr>
<td>200-600 mg daily, 5+9 (14+7)</td>
<td>RP2D: 200 mg daily (QD + BID), 14+7</td>
</tr>
<tr>
<td>Up to 10 µM</td>
<td>Steady state 2 µM</td>
</tr>
</tbody>
</table>
# Immunomodulatory effects of epigenetic modulators in cancer

## Anti-tumoral NK cell response vs. Anti-tumoral T cell response

<table>
<thead>
<tr>
<th>Anti-tumoral NK cell response</th>
<th>Anti-tumoral T cell response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NKG2D ligands up ↔ NKG2D</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>B7H6 down ↔ NKp30</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>MHC class I up</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>NK cell viability down</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>TAA up</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>MHC class I and II up</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>Co-stimulatory molecules up</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>T cell viability and function up/down</strong></td>
<td>+/-</td>
</tr>
</tbody>
</table>

- Effects vary between tumor types and HDACi used
- Effect on NK cell viability → functional impact?
- Increased immunogenicity and recognition of tumor cells by T cells
- Effect on T cell viability → functional impact?
Resminostat and 4SC-202: Immunomodulatory effects in cancer?

- Resminostat (broad-spectrum HDACi)
- 4SC-202 (LSD1 + class I HDACi)

Best suitable cancer immunotherapy approach?
Best suitable cancer immunotherapy approach?

Clinically relevant dosage regimen

NK cell based CIT
T-cell based CIT

Increase anti-tumoral response (synergistic/potentiating effects)
Combination of resminostat and 4SC-202 with NK cells based therapy
Resminostat and 4SC-202 upregulate NKG2D ligands

- **ULBP2**
  - DMSO: 0 x fold induction
  - Resminostat: 5 x fold induction
  - 4SC-202: 20 x fold induction

- **ULBP3**
  - DMSO: 0 x fold induction
  - Resminostat: 25 x fold induction
  - 4SC-202: 140 x fold induction

**Resminostat**

<table>
<thead>
<tr>
<th>NKG2D ligands</th>
<th>A549 lung</th>
<th>HepG2 liver</th>
<th>K562 CML</th>
<th>U2OS sarcoma</th>
<th>Huh7 liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICA</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>MICB</td>
<td>→</td>
<td>↑</td>
<td>→</td>
<td>n.d.</td>
<td>↑</td>
</tr>
<tr>
<td>ULBP1</td>
<td>→</td>
<td>↑</td>
<td>→</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>ULBP2</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>ULBP3</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>
Resminostat strongly boosts NK cell activity
HDAC inhibition decreases viability of isolated NK cells *in vitro*

![Graph showing NK cell viability](image_url)
Resminostat does not affect viability of NK cells under physiological conditions.

NK cell number in BALB/C animals treated with resminostat for 14 d

% NK cell in blood

vehicle 30 mg/kg 60 mg/kg 120 mg/kg

Resminostat does not affect viability of NK cells under physiological conditions.
Resminostat does not affect viability of NK cells under physiological conditions

NK cells in blood, 48 h

DMSO | 1.5µM | 3µM | 6µM

0% | 10% | 8% | 6%

IC50 of resminostat on NK cells

medium | TLR agonist | cytokines

0 | 5 | 4
Resminostat treatment leads to tumor cell lysis in whole blood

**Cytolytic assay in whole blood**

<table>
<thead>
<tr>
<th></th>
<th>K562 cells (normalized to counting beads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>5</td>
</tr>
<tr>
<td>6 µM</td>
<td>4</td>
</tr>
<tr>
<td>3 µM</td>
<td>3</td>
</tr>
<tr>
<td>1.5 µM</td>
<td>2</td>
</tr>
</tbody>
</table>

**K562 number in blood plasma**

<table>
<thead>
<tr>
<th></th>
<th>K562 cells (normalized to counting beads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>120</td>
</tr>
<tr>
<td>6 µM</td>
<td>130</td>
</tr>
<tr>
<td>3 µM</td>
<td>120</td>
</tr>
<tr>
<td>1.5 µM</td>
<td>110</td>
</tr>
</tbody>
</table>
Resminostat increases rituximab mediated ADCC

* BLISS independency model
Combination of Resminostat and 4SC-202 with T cell based therapy
Resminostat and 4SC-202 upregulate expression of TAA

**Resminostat**

- 0 µM
- 0.5 µM
- 1 µM
- 2 µM

CTp11 (fold induction)

0
20
40
60
80
100
1 d

**4SC-202**

- 0 µM
- 0.3 µM
- 1.0 µM

CTp11 (fold induction)

0
20
40
60
80
100
1d
5d

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4SC-202 induced expression of TAA is long-lasting

5 d 4SC-202 treatment

2 d wash-out

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Resminostat enhances expression of MHC and co-stimulatory molecules

- **Huh7**
  - HLA-B, HLA-C, HLA-E, HLA-F
  - Fold induction

- **K562**
  - HLA-ABC (MFI)
  - DMSO, 2 µM, 1 µM

- **HepG2**
  - HLA-DMA, HLA-DMB, HLA-DOA, HLA-DPA1, HLA-DPB1
  - Fold induction

- **HepG2**
  - 4-1BBL, CD70
  - Fold induction

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4SC-202 enhances expression of MHC and co-stimulatory molecules

<table>
<thead>
<tr>
<th>gene</th>
<th>4SC-202’s effect</th>
<th>Receptor (T cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL1A</td>
<td>6.4x ↑</td>
<td>DR3</td>
</tr>
<tr>
<td>LIGHT</td>
<td>3.1x ↑</td>
<td>HVEM</td>
</tr>
<tr>
<td>CD70</td>
<td>2.5x ↑</td>
<td>CD27</td>
</tr>
<tr>
<td>4-1BB-L</td>
<td>1.5x ↑</td>
<td>4-1BB</td>
</tr>
<tr>
<td>CD86</td>
<td>2-10x ↑</td>
<td>CD28</td>
</tr>
</tbody>
</table>

* THP-1, HL-60, and MOLM13 cells: FACS

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# Epigenetic Effect on T cell viability

## Viability of immune cells (IC50 in µM)

<table>
<thead>
<tr>
<th></th>
<th>CD4⁺ T cells</th>
<th>CD4⁺ memory T cells</th>
<th>CD8⁺ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>resminostat</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>SAHA</td>
<td>0.6</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>mocetinostat</td>
<td>0.6</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>entinostat</td>
<td>4.0</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>4SC-202</td>
<td>Not toxic</td>
<td>Not toxic</td>
<td>Not toxic</td>
</tr>
</tbody>
</table>
Resminostat and 4SC-202 enhance immunogenicity of tumor cells

RESMINOSTAT/4SC-202

MHC CLASS I

TAA

MHC CLASS II
CO-STIMULATORY MOLECULES

CD8 T cell

CD4 T cell

recognition

enhancement

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Effects of Resminostat and 4SC-202 on immunosuppression
Resminostat and 4SC-202 reduce immunosuppression

**Resminostat**

IDO (% expression) vs Resminostat concentration for SKOV-3 and MDA-MB231 cell lines.

**4SC-202**

IDO and ARG expression

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IDO</th>
<th>ARG</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549 lung</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>K562 CML</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>MDA-MB231 breast</td>
<td>↓</td>
<td>n.d.</td>
</tr>
<tr>
<td>SKOV3 ovarian</td>
<td>↓</td>
<td>n.d.</td>
</tr>
<tr>
<td>HepG2 liver</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Huh7 liver</td>
<td></td>
<td>↓</td>
</tr>
</tbody>
</table>

**Gene expression**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A549 rel. Copies (normalised to RPS18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO unstim.</td>
<td>0.3µM 4SC-202 3µM 1µM 0.3µM IFN-g</td>
</tr>
</tbody>
</table>

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HDACi reduces immunosuppression

Resminostat

4SC-202
Effect on tumor-microenvironment
Immunomodulatory features of Resminostat and 4SC-202 in vivo

CT26, d20

- Median tumor volume (mm$^3$)
- Vehicle
- Resminostat 30 mg/kg, QD
- 4SC-202 20 mg/kg, BID

p=0.0003

p=0.011
Resminostat and 4SC-202 reduce MDSC in tumor microenvironment

![Graph showing the reduction of MDSC with different treatments.](image)
4SC-202 strongly enhances invasion of CTLs in tumor microenvironment

![Graph showing CD8+ T cells levels for vehicle, resminostat (30 mg/kg, QD), and 4SC-202 (20 mg/kg, BID). The graph indicates a significant increase in CD8+ T cells with 4SC-202 treatment compared to vehicle and resminostat. The median is marked with a horizontal line at p=0.00002.](image)
4SC-202 increases the number of T cells in tumormicroenvironment

**T cells**

- CD3+ T cells (% of tumor cells)
- **p=0.00007**

**CD4+ T cells**

- CD4+ T cells (% of tumor cells)
- **p=0.0004**

**CD8+ T cells**

- CD8+ T cells (% of tumor cells)
- **p=0.00002**

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4SC-202 increases the number of T cells in tumormicroenvironment

**regulatory T cells**

- CD4\(^+\)Foxp3\(^+\) T cells (% of tumor) for 4SC-202 vs. vehicle, with a significant difference (p=0.0005).

**CD8/Treg ratio**

- CD8/Treg ratio for 4SC-202 vs. vehicle, with a significant difference (p=0.001).
Existence of anti-tumoral immune response correlates with response rate to PD-1/PD-L1 blockade

- Infiltration of tumor core or invasive matrix with cytotoxic T cells correlates better with response to pembrolizumab therapy than PD-1/PD-L1 expression

4SC-202 synergizes with PD-1 therapy

- **INCREASE IMMUNOCITICITY**
- **ENHANCE IMMUNE CELL INFILTRATION**
- **REDUCE UNSPECIFIC IMMUNOSUPPRESSION**

**PD1/PD-L1 BLOCKADE**

**Renca tumor burden (mg)**

- Vehicle
- Anti-PD-1
- 4SC-202
- Combo

Significance levels:
- p=0.018
- p=0.00013
- p=0.017

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Summary, Conclusions, and Plans
Resminostat and 4SC-202: common features

Vaccination:
- Peptide-
- RNA-
- DC-based

CD8 T cell

CD4 T cell

recognition

enhancement

IMMUNO-SUPPRESSION

IMMUNO-GENICITY

CO-STIMULATION

TUMOR CELL

RESMINOSTAT/4SC-202
Resminostat’s specific effects

NK cell based:
• Opsonizing ab
• NKG2D-CAR Tcells
• Adoptive NK cell transfer

Recognition and killing

NK cell sensitivity

RESMINOSTAT

TUMOR CELL
4SC-202’s specific effects

T cell based:
• Immune checkpoint blockers
• CAR therapy
• Vaccination
Study Concept: Combination of 4SC-202 with immune Checkpoint Blockade

Patients
- Patients with solid cancers amenable to serial biopsies
- Indications: e.g. Melanoma, BCC, SCC, MCC

Design

A: Immune cell infiltration (mono)

Histological Assessment baseline → 4SC-202 N=2/3x10 → Histological Assessment (2w) → ORR

B: Safety and efficacy + Immune cell infiltration (combi)

PD1/PD-L1 blockade + 4SC-202 → Histological Assessment at Response → ORR

Endpoints: Immune infiltration, safety, ORR

La/m BCC: locally advanced and metastatic basal cell carcinoma, SCC: squamous cell carcinoma, MCC: merkel cell carcinoma, BC: breast cancer, ORR: overall response rate
Thank you!
Contact

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