Epigenetic Modifiers for Combination with Cancer Immunotherapy

June 2017, WPC Cancer Immunotherapies and Combinations
Epigenetic regulation of gene expression

Yan et al. 2010, Journal of applied physiology
Epigenetic therapy of cancer

- Cancer cells acquire pathological epigenetic modifications resulting in gene expression patterns that facilitate and sustain tumorigenesis

- HDAC inhibitors
  - Affect cancer cell biology, inhibit proliferation and induce apoptosis of cancer cells
  - In monotherapy have demonstrated clinical efficacy in various hematological indications and in selected solid cancers
  - Reported to have immunological effects which are less well understood
  - In-depth evaluation of immunological effects to allow rational design of clinical trials
HDACi in clinical development

- **Resminostat**
  - Small molecule inhibitor of class I, IIb and IV HDAC isoforms
  - Clinically evaluated in HL, HCC, CRC, NSLC; pivotal Phase II in CTCL ongoing

- **4SC-202**
  - HDAC class I specific small molecule
  - Evaluated in hematological indications; clinical studies in combination with PD-1 blockade in melanoma and MSS gastrointestinal tumors are in preparation

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class IIa</th>
<th>Class IIb</th>
<th>Class III</th>
<th>Class IV</th>
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<td>3</td>
<td>8</td>
<td>4</td>
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<td>5</td>
<td>6</td>
<td>10</td>
<td>sirtuins</td>
<td>11</td>
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</table>

- **localization**
  - nucleus
  - nucleus/cytoplasm
  - cytoplasm
  - nucl./cytopl.

- **resminostat**
- **4SC-202**
Broad-spectrum HDACi resminostat for combination with NK cell based therapy
Resminostat enhances sensitivity of tumor cells to NK cell mediated lysis
Resminostat increases expression of NKG2D ligands on tumor cells of various origins

### Graphs

#### ULBP2

<table>
<thead>
<tr>
<th>Resminostat</th>
<th>0 µM</th>
<th>0.5 µM</th>
<th>1 µM</th>
<th>2 µM</th>
<th>4 µM</th>
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<tbody>
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#### ULBP3

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<tr>
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### Table

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<tr>
<th>NKG2DL</th>
<th>A549 lung</th>
<th>HepG2 liver</th>
<th>K562 CML</th>
<th>U2OS sarcoma</th>
<th>Huh7 liver</th>
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Blocking of NKG2D abrogates resminostat mediated increase of sensitivity to NK cells

![NKG2D blockade chart]

<table>
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<tr>
<th>PBMC</th>
<th>-</th>
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<th>+</th>
<th>+</th>
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<tr>
<td>resminostat</td>
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<td>-</td>
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HDAC inhibitors seem to negatively affect the viability of NK cells
Resminostat treatment results in increased killing of tumor cells in whole blood/target cell co-culture.
Treatment with Resminostat doesn’t affect NK cells number *in vivo* in blood
Resminostat increases NK cells in Renca tumors

**Renca tumor weight**

- **Renca tumor weight**
  - **Vehicle**
  - **Resminostat**

**NK cells in Renca TME**

- **% of TILs**
  - **Vehicle**
  - **Resminostat**

**Statistics:** Mann-Whitney test

**Notes:**

- Resminostat increases NK cells in Renca tumors.
- Statistics: Mann-Whitney test.
If resminostat is toxic to NK cells, why do NK cells work \textit{in vivo} and in blood incubated with resminostat?

- Activation-induced cell death?
Resminostat directly activates NK cells and enhances their anti-tumoral activity.

NK cells, 24h

- CD69
- Count
- resminostat
- DMSO

K562, pre-treated PBMC as effector

- %PI K562
- Ratio 25:1

RPMI8226

- %PI RPMI8226
- PBMC treated with
- DMSO
- resminostat
Resminostat treatment of effector and target cells resulted in higher cytolysis of target cells.

NK cell cytotoxicity assay

<table>
<thead>
<tr>
<th>Target</th>
<th>Effector</th>
<th>Resminostat Treatment</th>
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<tbody>
<tr>
<td>-</td>
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<tr>
<td>+</td>
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Pl^+ K562 cells (%)

0 10 20 30 40 50 60 70 80

- - - + - + + +
Resminostat beneficially affects anti-tumoral NK cell response

• Implication for clinics?
  ➢ Combination with opsonizing antibodies
Resminostat does not affect CD20 expression on B cell lymphoma cell lines
Combination of resminostat and rituximab results in enhanced killing of B cell lymphoma

According to BLISS independency test synergistic for all ratios
Resminostat increasing tumor cells’ sensitivity to NK cells and activation of NK cells

- Increasing tumor cell sensitivity to NK cells
- Directly activating NK cell function

Combination with Rituximab results in synergistic lysis of tumor cells
Class I HDACi 4SC-202 for combination with T cell based therapy
Controversial effects of HDACi on anti-tumoral T cell response

• HDAC inhibitors were reported
  o To enhance immunogenicity of tumor cells by upregulation of tumor-associated antigens, presentation machinery, MHC and co-stimulatory molecules

  But:
  o To have inhibitory effects on CD4 and CD8 T cell viability and function
  o To enhance the function or number or $T_{\text{reg}}$

• Class-specificity or even compound-specific effects may play a role
HDACi differently affect viability of T cells

<table>
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<tr>
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<th>CD4⁺ T cells</th>
<th>CD8⁺ T cells</th>
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<tr>
<td></td>
<td>naive</td>
<td>memory</td>
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<tr>
<td>vorinostat</td>
<td>1.5</td>
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<td>mocetinostat</td>
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<td>entinostat</td>
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<tr>
<td>4SC-202</td>
<td>&gt;10</td>
<td>&gt;10</td>
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</table>

Viability of activated T cell subpopulations (IC₅₀ in µM)
In vivo Model Systems: „hot“ C38 versus „cold“ CT26 tumors

C38 tumors:
high in vivo infiltration with CTLs

CT26 tumors:
low in vivo infiltration of CTLs
4SC-202 increases the number of CTLs in both tumor systems

C38 tumor, CD8+ Tcells

CT26 tumor, CD8+ T cells

% of tumor

% in blood

**

vehicle

4SC-202 20 mg/kg SID

4SC-202 20 mg/kg BID

4SC-202 60 mg/kg SID

vehicle

4SC-202
Increase of CTLs is specific for 4SC-202

CT26 tumor, CD8⁺ T cells

CT26 tumor size (d20)

% of tumor

vehicle  bs-HDACi  4SC-202

CT26 tumor size (d20)
4SC-202 increases the number of CTLs in tumor microenvironment

- Implication for clinics?
- Combination with CIT therapies targeting anti-tumoral T cell response initiation and maintenance
4SC-202 synergizes with PD-1 blockade in C38 tumors

- PD-1 blockade: 2/20 CR
- 4SC-202 controlled tumor growth, 2/20 CR
- Combination of 4SC-202 and PD-1 blockade: regression of tumors, 11/20 CR
Combination of 4SC-202 with PD-1 blockade increases survival rate

**C38 survival**

- In the “hot” C38 tumors combination of 4SC-202 and anti-PD-1 resulted in 55% tumor-free animals

<table>
<thead>
<tr>
<th></th>
<th>Median (d)</th>
<th>OS (%)</th>
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<tbody>
<tr>
<td>vehicle</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Anti-PD-1</td>
<td>41</td>
<td>10</td>
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<tr>
<td>4SC-202</td>
<td>58</td>
<td>10</td>
</tr>
<tr>
<td>Combo</td>
<td>&gt;70</td>
<td>55</td>
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55% long term CR
Combination of 4SC-202 with PD-L1 blockade significantly prolongs survival in CT26

- In the “cold” CT26 tumors combination of 4SC-202 and anti-PD-L1 significantly reduces growth and prolonged survival of tumor-bearing animals.
4SC-202 increases CTL infiltration and synergizes with PD-1/PD-L1 blockade

- 4SC-202 enhances tumor cell immunogenicity by upregulation of TAA, MHC and co-stimulatory molecules
- 4SC-202 increases infiltration of TME with CTLs
- 4SC-202 is not toxic on T cells

Combination with PD-1/PD-L1 blockade results in synergistic effect on tumor growth and survival
Combination strategies

resminostat

NK cell based therapy
- Opsonizing ab
- Adaptive NK cell transfer
- NKG2D-CAR T cells
- NK cell engaging bispecific ab

4SC-202

T cell based therapy
- Vaccination
- Agonistic CD40, OX-40, CD137, GITR ab
- Checkpoint blockade
- Adoptive T cell transfer
- CAR T cells
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