Svetlana Hamm

Regulation of tumor microenvironment by HDAC class I selective inhibitor 4SC-202
Disclosure

• Employee of 4SC AG developing 4SC-202 for anti-cancer therapy
Immunomodulatory effects of HDAC inhibitors in cancer

• Balance of immunomodulatory effects of 4SC-202 *in vitro* and *in vivo*?
  • Clinically relevant concentration and dosage
4SC-202 is a clinical stage epigenetic modulator

<table>
<thead>
<tr>
<th>4SC-202</th>
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<tbody>
<tr>
<td><strong>Class I HDACi</strong></td>
</tr>
<tr>
<td>Completed clinical phase I in hematological indications</td>
</tr>
<tr>
<td>Safe and well tolerated</td>
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<tr>
<td>Single agent activity demonstrated in a Phase I trial, including two objective responses: one CR and one good PR, DCR 83%</td>
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<tr>
<td>Oral</td>
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<tr>
<td>RP2D: 200-400 mg tdd</td>
</tr>
<tr>
<td>14+7</td>
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<tr>
<td>Steady state 2 μM</td>
</tr>
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</table>
4SC-202 enhances immunogenicity of tumor cells
4SC-202 induces long-lasting expression of TAA
4SC-202 enhances expression of MHC molecules
4SC-202 enhances expression of co-stimulatory molecules

**Table:**

<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
4SC-202 is less toxic to immune cells than comparator HDAC inhibitors

Effect of HDAC inhibitors on viability and function of T cell subsets ($IC_{50}$ in µM)

<table>
<thead>
<tr>
<th>HDAC inhibitor</th>
<th>CD4$^+$ T cells*</th>
<th>CD8$^+$ T cells*</th>
<th>CD8$^+$ memory T cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>naive</td>
<td>memory</td>
<td>naive</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>1.5</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Mocetinostat</td>
<td>1.3</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Entinostat</td>
<td>2.1</td>
<td>2.7</td>
<td>2.2</td>
</tr>
<tr>
<td>4SC-202</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>8.2</td>
</tr>
</tbody>
</table>

* T cells were stimulated with CD3/28 beads; different subpopulations were isolated using appropriate Miltenyi kits according to manufacturers instructions; cells were treated with indicated compounds for 48 h; viability was determined using Vialight Kit (Lonza); ** up to 10 µM
Immunomodulatory effects of 4SC-202 *in vivo*
The anti-tumoral effect of 4SC-202 in CT26 depends on intact immune system.
4SC-202 increases T cell and reduces myeloid cell infiltration into TME of CT26 tumors
4SC-202 increases T cell infiltration into TME

**CD4⁺ T cells**

- % of tumor
- Vehicle
- 4SC-202

**CD8⁺ T cells**

- % of tumor
- Vehicle
- 4SC-202
4SC-202 increases T cell infiltration into TME

**CD8+ T cells**

- Vehicle
- 4SC-202

**CD8 in blood**

- Vehicle
- 4SC-202
4SC-202 increases T cell infiltration into TME

IHC analysis of CT26 tumors – CD3⁺/CD8⁺

Vehicle

4SC-202

% of tumor

CD8⁺ T cells

**

Vehicle

4SC-202
4SC-202 enhances CD8/Treg ratio in TME

**Tregs**

**CD8/Treg ratio**
4SC-202 beneficially modifies TME

- **4SC-202**
  - Increased expression of chemokines
  - Decreased expression of pro-inflammatory cytokines

- **4SC-202**
  - Increased IFN-γ
  - IFN-γ production correlated with anti-tumoral response
Broad-spectrum HDACi does not enhance CTL number in tumor microenvironment
4SC-202: Combination with cancer immunotherapies
4SC-202 as immunomodulatory agent for combination with cancer immunotherapies

• 4SC-202 in vivo:
  • Enhances cytotoxic T cells in TME
  • Increases expression of chemokines in TME
  • Increases IFN-γ in TME

➢ 4SC-202 increases tumor-specific T cell responses and infiltration of tumor with cytotoxic T cells

➢ This qualifies 4SC-202 for combination with various cancer immunotherapy approaches

1. Immune response initiation ➔ generation of CTLs
   - Vaccination
   - TLR/RIG-I agonists
   - Agonistic CD40
   - CTLA-4 blockade
   - Cytokines (IFNs, IL-12)

2. Co-stimulation of T cells
   - OX-40 agonists
   - CD137 agonists
   - GITR agonists

3. Blockade of inhibitory T cell checkpoints
   - PD-1/PD-L1 blockade
   - LAG-3 blockade
   - TIM3 blockade

Adoptive cell transfer
Existence of CTL correlates with response rate to PD-1/PD-L1 blockade

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

4SC-202 synergizes with PD-1 blockade in C38 tumors

- 4SC-202 reduced growth of C38 tumors
- Combination of 4SC-202 with PD-1 blockade synergized to reduce tumor growth
4SC-202 synergizes with PD-1 blockade in C38 tumors

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- Combination of 4SC-202 with PD-1 blockade synergized to reduce tumor growth
4SC-202 synergizes with PD-1 blockade in C38 tumors

- PD-1 blockade: 2 responder, 18 non-responding
- 4SC-202 controlled tumor growth
- Combination of 4SC-202 and PD-1 blockade ➔ regression of tumors
Combination of 4SC-202 with PD-1 blockade increases survival rate

- In the C38 tumors combination of 60 mg/kg 4SC-202 and anti-PD-1 antibody resulted in 55% tumor-free animals
Combination of 4SC-202 with PD-1 blockade results in durable responses

- In the C38 tumors combination of 4SC-202 and anti-PD-1 resulted in 83% durable complete responses
4SC-202 as immunomodulatory agent

• **4SC-202 in vitro:**
  - Enhances expression of tumor associated antigens
    - Effect increases over time and is long-lasting
  - Enhances expression of MHC and co-stimulatory molecules
  - In contrast to comparator molecules, is not toxic to activated T cells

• **4SC-202 in vivo:**
  - Enhances infiltration of tumor microenvironment (TME) with cytotoxic T cells
  - Increases expression of chemokines in TME
  - Increases IFN-γ in TME
  - As mono-therapy controls tumor growth
  - In combination with PD-1/PD-L1 blockade induces durable responses, prolongs survival, and increases survival rate
4SC-202 in combination with I-O therapies

- Anti-PD1/PD-L1 antibodies are the new standard of care in many solid tumor indications
  - But a high proportion of patients still do not respond to therapy
  - Lack of T-cell response and tumor infiltration, other immunosuppressive mechanisms, and tumor escape

- 4SC-202 enhances immunogenicity of tumor cells, increases cytotoxic T cell infiltration into the tumor and synergize with PD-1/PD-L1 blockade

- 4SC-202/Pembrolizumab combination trial is ongoing: SENSITIZE, NCT03278665

<table>
<thead>
<tr>
<th>Checkpoint inhibitors- overall response rates remain low*</th>
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<tbody>
<tr>
<td>Non-small cell lung cancer</td>
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<tr>
<td>Head &amp; neck squamous cell</td>
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<td>Gastric cancer</td>
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<tr>
<td>Colorectal cancer (MSS)</td>
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<tr>
<td>Merkel cell carcinoma</td>
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<tr>
<td>Melanoma</td>
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* Source: Based on Curie Institute, Bryan, Garnier & Co research; MSS: microsatellite stable
Many thank

• 4SC research group

• Patients and their families