Regulation of tumor microenvironment by the HDAC/LSD1 inhibitor 4SC-202

September 2016
Cancer-immunity cycle

1. Release of cancer cell antigens (cancer cell death)
2. Cancer antigen presentation (dendritic cells/APCs)
3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumors (CTLs)
5. Infiltration of T cells into tumors (CTLs, endothelial cells)
6. Recognition of cancer cells by T cells (CTLs, cancer cells)
7. Killing of cancer cells (Immune and cancer cells)

Chen & Mellman, 2013, Immunity
Cancer-immunity cycle: What are the problems?

1. Low-grade inflammation
   - MDSC↑
   - Immunosuppression↑
   - T cell response is not efficient enough

2. Low immunogenicity of tumors:
   - Immune response initiation↓
   - Tumor cell recognition↓

3. Infiltration of tumoricidal immune cells impaired

Chen&Mellman, 2013, Immunity
Cancer-immunity cycle: How to overcome the problems?

1. Reduce immunosuppression
   - Unspecific: MDSCs, TAMs
   - Specific: Tregs, inhibitory checkpoint receptors on effector T cells

2. Enhance immunogenecity and recognizability:
   - ICD
   - TAA, presentation, co-stimulation

3. Enhance infiltration of tumor microenvironment with tumoricidal immune cells

Chen&Mellman, 2013, Immunity
Immunomodulatory effects of epigenetic modulators in cancer

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

- Effects vary between tumor types and HDACi used
- Effect on immune cell viability vary between tumor types and HDACi used
4SC-202 is a clinical stage epigenetic modulator

<table>
<thead>
<tr>
<th>4SC-202</th>
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</thead>
<tbody>
<tr>
<td>LSD1 + Class I HDACi</td>
</tr>
<tr>
<td>Completed clinical phase I in hematological indications</td>
</tr>
<tr>
<td>Safe and well tolerated</td>
</tr>
<tr>
<td>Single agent activity demonstrated in a Phase I trial, including two objective responses: one CR and one good PR</td>
</tr>
<tr>
<td>Oral</td>
</tr>
<tr>
<td>RP2D: 200-400 mg tdd</td>
</tr>
<tr>
<td>14+7</td>
</tr>
<tr>
<td>Steady state 2 µM</td>
</tr>
</tbody>
</table>

Clinically relevant conc./dosage regimen for preclinical experiments
4SC-202 enhances immunogenicity of tumor cells
4SC-202 enhances expression of tumor associated antigens in tumor cells
4SC-202 induced expression of TAA is long-lasting

CTp111 (fold Induction)

DMSO
1 µM 4SC-202

4SC-202 treatment
wash-out

5 d 4SC-202 treatment
wash-out

5d + 0d
5d + 1d
5d + 2d
5d + 3d

CTp111 (fold Induction)

DMSO
1 µM 4SC-202

0
5
10
15
20
25
30

5d + 0d
5d + 1d
5d + 2d
5d + 3d

4SC-202 induced expression of TAA is long-lasting
4SC-202 enhances expression of MHC and co-stimulatory molecules

**4SC-202**

<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>

*MiaPaca2*

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

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4SC-202 enhances immunogenicity of tumor cells
4SC-202 does not affect T cell viability

<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>vorinostat</td>
<td>0.6</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>mocetinostat</td>
<td>n.d.</td>
<td>n.d.</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>

Viability of immune cells (IC50 in µM)
4SC-202 enhances immunogenicity of tumor cells: Translation *in vivo*
4SC-202 enhances MHC class II *in vivo*
Tumor microenvironment

- Tumor cell
- Fibroblasts
- MDSC
- TAM
- T reg
- DC, M1 -MΦ
- Cytotoxic T or NK cell

immuno-suppressive and tumor-promoting
tumoricidal
The anti-tumoral effect of 4SC-202 in CT26 depends on intact immune system.
4SC-202 increases leukocyte number in tumor microenvironment
4SC-202 increases T cell and reduces myeloid cell infiltration into tumor microenvironment

**CD3⁺ T cells**

**MDSC**
4SC-202 increases T cell infiltration into tumor microenvironment.

**CD4⁺ T cells**

**CD8⁺ T cells**

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4SC-202 increases T cell infiltration into tumor microenvironment
4SC-202 enhances CD8/Treg ratio in tumor microenvironment
Broad-spectrum HDACi does not affect tumor microenvironment

**CD8⁺ T cells**

- Vehicle
- bs-HDACi
- 4SC-202

**MDSC**

- Vehicle
- bs-HDACi
- 4SC-202

**CT26 tumor size (d20)**

- Vehicle
- bs-HDACi
- 4SC-202
4SC-202: Combination with cancer immunotherapies
4SC-202: combination strategies

1. Reduce immunosuppression
   - MDSCs
   - Tregs
   - Inhibitory checkpoint receptors
   - PD-1/PD-L1 antibodies

2. Enhance immunogenicity and recognition
   - Cytokines (IL-2, IL-12, IFN-α, IFN-γ)
   - TLR agonists
   - Vaccination
   - CTLA4 antibodies
   - Agonistic antibodies

3. Enhance infiltration with tumoricidal immune cells

Chen&Mellman, 2013, Immunity
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 anti-PD-L1 blockade
Combination of 4SC-202 with PD-L1 blockade results in a beneficial CD8/Treg ratio
Combination of 4SC-202 with anti-PD-L1 significantly prolongs survival.

CT26, survival

- Vehicle
- PD-L1
- 4SC-202
- Combo
4SC-202: Epigenetic drug for combination with cancer immunotherapy

• 4SC-202 enhances immunogenicity of tumor cells
• 4SC-202 increases infiltration of tumor microenvironment with CD8+ cytotoxic T cells: cold tumors $\rightarrow$ hot tumors
• Safe and well tolerated as mono-therapy; clinical schedule suitable for combination with PD1/PD-L1 blockade
Study Concept: Combination of 4SC-202 with immune Checkpoint Blockade

**Patients**
- Advanced melanoma patients refractory to PD1/PD-L1 blockade
- Eligible to serial biopsies

**Design**

**Phase I: dose evaluation**
- 4SC-202 [14 d]
- PD1-CPI q3w
- Well-tolerated dose
- Histological assessment

**Phase II: expansion**
- 4SC-202 [14 d]
- PD1-CPI q3w
- Clinical efficacy / biomarker
- Histological assessment
Thank you!
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