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

28 September 2017, Discovery on Target 2017
Cancer-immunity cycle

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**

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

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

3. **Enhance infiltration of tumor microenvironment with tumoricidal immune cells**

Immunomodulatory effects of HDAC inhibitors in cancer

- Balance of immunomodulatory effects of 4SC-202 in vitro and in vivo?
  - Clinically relevant concentration and dosage

- Enhancing Immune Response
  - Viability and function
    - CD4+ T cells
    - CD8+ T cells
    - Cytokine induction

- Inhibiting Immune Response
  - Immunogenicity
    - MHC molecules
    - Co-stimulatory molecules
    - Tumor associated antigen

- Immunogenicity
  - MHC molecules
  - Co-stimulatory molecules
  - Tumor associated antigen

- Viability and function
  - CD4+ T cells
  - CD8+ T cells
  - Cytokine induction
4SC-202 is a clinical stage epigenetic modulator

<table>
<thead>
<tr>
<th>Class I HDACi</th>
</tr>
</thead>
<tbody>
<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>
4SC-202 enhances immunogenicity of tumor cells
4SC-202 enhances expression of tumor associated antigens
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>

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

**MOLM-13**

### fold induction

- HLA-DPA
- HLA-DPB
- HLA-DR
- HLA-DQ
- HLA-DMA
- HLA-DMB
- HLA-DOA
- HLA-DOB

**THP-1**

- CD86

**MiaPaca2**

- TL1A 6.4x ↑ DR3
- LIGHT 3.1x ↑ HVEM
- CD70 2.5x ↑ CD27
- 4-1BB-L 1.5x ↑ 4-1BB
- CD86 2-10x ↑ CD28

**CD86**

**HLA-DR**

- isotype
- DMSO
- 4SC-202
4SC-202 enhances immunogenicity of tumor cells

4SC-202

TUMOR CELL

MHC CLASS II
CO-STIMULATORY MOLECULES

TAA

CD8 T cell

CD4 T cell

recognition

enhancement
4SC-202 has a beneficial profile on T cell viability and function.

### 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

*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 tumor microenvironment of CT26 tumors
4SC-202 increases T cell infiltration into tumor microenvironment

**CD4^+ T cells**

**CD8^+ T cells**
4SC-202 increases T cell infiltration into tumor microenvironment
4SC-202 enhances CD8/Treg ratio in tumor microenvironment
4SC-202 enhances expression of MHC molecules in vivo

<table>
<thead>
<tr>
<th>gene</th>
<th>class</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2-M2</td>
<td>lb</td>
</tr>
<tr>
<td>H2-M11</td>
<td>lb</td>
</tr>
<tr>
<td>H2-Aa</td>
<td>IIA</td>
</tr>
<tr>
<td>H2-Eb1</td>
<td>IIA</td>
</tr>
<tr>
<td>H2-Ob</td>
<td>IIB</td>
</tr>
</tbody>
</table>
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
1. Reduce immunosuppression
   • Unspecific: MDSCs, TAMs
   • Specific: Tregs, inhibitory checkpoint receptors on effector T cells
   + Enhance immune cell function

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

3. Enhance infiltration of tumor microenvironment with tumoricidal immune cells

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

Evaluation systems: „CTL inflamed“ C38 versus „non-CTL-inflamed“ CT26 tumors

- C38 tumors: high infiltration with T cells, especially with CD8$^+$ CTLs
- CT26 low number of CTLs

Mean volume in both systems ~ 1000 mm$^3$
4SC-202 increases the number of CTLs in both tumor systems

**C38 tumor, CD8⁺ T cells**
- Vehicle
- 4SC-202, 20 mg/kg SID
- 4SC-202, 20 mg/kg BID
- 4SC-202, 60 mg/kg SID

**CT26 tumor, CD8⁺ T cells**
- Vehicle
- 4SC-202

**% in blood**
- Vehicle
- 4SC-202

IHC analysis of CT26 tumors – CD3⁺/CD8⁺
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 achieve partial and complete responses
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 achieve partial and complete responses
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

**C38 survival**

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

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

In the “non-inflamed” CT26 tumors combination of 4SC-202 and anti-PD-L1 significantly reduces growth and prolonged survival of tumor-bearing animals.
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 prolongs survival and increases survival rate

> **4SC-202 increases tumor-specific T cell responses and CTL infiltration into the tumor**
Checkpoint inhibitors* 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 infiltration, effective tumor escape mechanisms (suppression of the adaptive immune response)

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Non-responding tumor (%)</th>
<th>Tumor responsive to checkpoint inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell lung</td>
<td>15-20%</td>
<td>~5%</td>
</tr>
<tr>
<td>Head &amp; neck squamous cell</td>
<td>15-25%</td>
<td>30-50%</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>~20%</td>
<td>~40%</td>
</tr>
<tr>
<td>Colorectal cancer (MSS)</td>
<td></td>
<td>~5%</td>
</tr>
<tr>
<td>Merkel Cell Carcinoma</td>
<td></td>
<td>30-50%</td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td>~40%</td>
</tr>
</tbody>
</table>

* Anti-PD-(L)1 antibodies; ** Source: Based on Curie Institute, Bryan, Garnier & Co research; MSS: microsatellite stable; MHC: Major Histocompatibility Complex
# SENSITIZE, NCT03278665

## Patients

Unresectable stage III / metastatic stage IV cutaneous melanoma primary refractory/non-responding to prior anti-PD-1 therapy

## Design

<table>
<thead>
<tr>
<th>Dose finding</th>
<th>Expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=10 per cohort</td>
<td>4SC-202</td>
</tr>
<tr>
<td>4SC-202</td>
<td>Recommended Phase II dose</td>
</tr>
<tr>
<td>200 mg BID</td>
<td>+ Pembrolizumab</td>
</tr>
<tr>
<td>200 mg OD</td>
<td>+ Pembrolizumab</td>
</tr>
<tr>
<td>100 mg OD</td>
<td>4SC-202</td>
</tr>
</tbody>
</table>

- 100 mg OD
- 200 mg BID
- 4SC-202
- + Pembrolizumab
Thank you!
Disclaimer

The information contained in this presentation is for background purposes only and is subject to amendment, revision and updating. Certain statements and information contained in this presentation may relate to future expectations and other forward-looking statements that are based on management’s current views and assumptions and involve known and unknown risks and uncertainties. In addition to statements which are forward-looking by reason of context, including without limitation, statements referring to risk limitations, operational profitability, financial strength, performance targets, profitable growth opportunities, and risk adequate pricing, other words such as "may, will, should, expects, plans, intends, anticipates, believes, estimates, predicts, or continue", "potential, future, or further", and similar expressions identify forward-looking statements. By their nature, forward-looking statements involve a number of risks, uncertainties and assumptions which could cause actual results or events to differ materially from those expressed or implied by the forward-looking statements. These include, among other factors, changing business or other market conditions and the prospects for growth anticipated by 4SC's management. These and other factors could adversely affect the outcome and financial effects of the plans and events described herein. Statements contained in this presentation regarding past trends or activities should not be taken as a representation that such trends or activities will continue in the future. 4SC does not undertake any obligation to update or revise any statements contained in this presentation, whether as a result of new information, future events or otherwise. In particular, you should not place undue reliance on forward-looking statements, which speak only as of the date of this presentation.
The EMTherapy project is conducted in the framework of the European Eurostars program and has received funding from the Federal Ministry of Education and Research.