4SC-202: A NOVEL EPIGENETIC MODULATOR TO TARGET CANCER STEM CELLS

BOSTON, NOV 8TH, 2012

HELLA KOHLHOF  4SC AG
This presentation may contain projections or estimates relating to plans and objectives relating to our future operations, products, or services; future financial results; or assumptions underlying or relating to any such statements; each of which constitutes a forward-looking statement subject to risks and uncertainties, many of which are beyond our control. Actual results could differ materially, depending on a number of factors.
4SC-202: HDAC INHIBITION

- **4SC-202**
  - HDAC Inhibitor of the class I specific „benzamide type“
  - Potent inhibition of HDAC 1, 2, 3 (8 not affected)
  - Induction of Histone H3 and whole lysine acetylation

**Graph:**

*Lysine Acetylation*

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>10µM 4SC-202</th>
<th>10µM Entinostat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fold induction of acetylation</strong></td>
<td>1.0</td>
<td>3.4 ± 0.2</td>
<td>4.8 ± 0.3</td>
</tr>
</tbody>
</table>

Jurkat cells, 16h treatment, ELISA

4SC-202 inhibits selectively specific HDACs and induces protein acetylation
Colon cancer RKO orthotopic xenograft model
RKO Wnt pathway wildtype
Treatment:
• 4SC-202: 120 mg/kg d1-12 SID → T/C 0.36
• Entinostat: 25 mg/kg d1-12 SID → T/C 0.32
• Mocetinostat: 90 mg/kg d1-12 SID → T/C 0.42

Gene expression analysis was performed in the tumor tissue
Gene expression analysis demonstrates strong differences between 4SC-202, Entinostat and Mocetinostat.
**4SC-202 IN VIVO PD – EPIGENETIC REGULATORS**

### Writers

- **Histone acetyltransferases**
  - MYST3, 4
  - K → K

- **Protein methyltransferases**
  - DOT1L, EHMT1
  - K → K
  - CARM1
  - R → R

### Erasers

- **Histone deacetylases**
  - K → K
  - KDM2A, KDM4B, KDM5C
  - KDM6B, JMJD1C, SMARCA4
  - SMARCB1, SMARCC2

- **Lysine spec. demethylases**
  - K → K

### Readers

- **Bromodomain containing proteins**
  - BAZ2A, BPTF
  - BRD3, 4, 8
  - BRPF1, BRWD2

- **Chromodomain etc. containing proteins**
  - CHD1L, CHD3, 7, 9

- **PHD containing proteins**
  - K → K
  - R → R

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All genes mentioned were repressed in the tumor samples of the 4SC-202 treated RKO xenograft models.
The Wnt pathway is differentially expressed by 4SC-202 treatment

Possible levels of regulation

- Ligand – Receptor
- Signaling molecules
- Transcription factors
- Chromatin modifiers
- Wnt target genes

Adapted from Hans Clevers, Cell, 2006
Positive regulators were repressed by 4SC-202 treatment

Ligand – Receptor

Signaling molecules

Transcription factors
Chromatin modifiers
Wnt target genes

Frizzled 5&7
LRP5/6

Dvl
FRAT/GBP

TCF4, LEF1

CARM1, DOT1L

CD44, FOSL1

Adapted from Hans Clevers, Cell, 2006
Wnt target gene regulation in 4SC-202 treated spheroids

<table>
<thead>
<tr>
<th>Wnt target gene</th>
<th>Level of repression</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD133</td>
<td>-</td>
<td>Stem cell marker</td>
</tr>
<tr>
<td>Nanog</td>
<td>-</td>
<td>Stemness transcription factor</td>
</tr>
<tr>
<td>BMP4</td>
<td>-</td>
<td>Stemness transcription factor</td>
</tr>
<tr>
<td>Sox9</td>
<td>-</td>
<td>Stemness transcription factor</td>
</tr>
<tr>
<td>CD44</td>
<td>- -</td>
<td>Stem cell marker</td>
</tr>
</tbody>
</table>

4SC-202 inhibits Wnt target genes on RNA and protein level
CLINICAL IMPLICATIONS OF CSC THEORY AND THERAPEUTIC INTERVENTION OPTIONS

Adapted from B.B.S. Zhou et.al, Nature Rev. Drug Disc., 2009, 806-823, doi:10.1038/nrd2137

Tumor-initiating cell „TIC“
Tumor progenitor
Differentiated tumor cell
Therapeutic intervention

TIC destruction
TIC differentiation

Conventional Chemotherapy/Radiation
Clinical Response
Relapse
Progression

Adapted from B.B.S. Zhou et.al, Nature Rev. Drug Disc., 2009, 806-823, doi:10.1038/nrd2137
- Formation of spheroids is phenotypic for cancer stem cells

- NCCIT cells are embryonal carcinoma cells with a strong cancer stem cell character
  - NCCIT cells express core stemness transcription factors like Oct4, Nanog and Sox2

4SC-202 inhibits spheroid formation at low µM concentrations
A hallmark of cancer stem cells is their potency to induce colony growth in an anchorage independent set-up.

4SC-202 was tested to inhibit anchorage independent growth in an HCT116 soft agar assay.

Cells were seeded into soft agar, different doses of compound were added once and the number of colonies were calculated after one week.

4SC-202 shows a potent inhibition of anchorage independent growth with an EC\textsubscript{50} of 220 nM.
Tumor Initiating Cell phenotype test

- 2D layer of patient derived melanoma (ChaMel) and NCCIT cells were treated for 48h
- Remaining cells were seeded into soft agar
- Read out: Number of colonies after 4-6 weeks

At micromolar concentration, 4SC-202 completely inhibits colony formation of melanoma (stem) cells and NCCIT cells.
Migration and invasion of tumor cells is described for stem-like tumor cells

- Human glioblastoma stem-like cells (GS-9) were treated with different concentrations of 4SC-202
- Inhibition of the migratory capacity of GS-9 cells was tested in a Boyden chamber assay
- Inhibition of the invasive capacity of GS-9 cells was tested in a Matrigel assay


**Migration**

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>10</th>
<th>4SC-202 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

**Invasion**

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>10</th>
<th>4SC-202 [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40</td>
<td></td>
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</tbody>
</table>

4SC-202 inhibits migration and invasion of glioblastoma stem-like cells.
**4SC-202 IN VITRO PD – PROLIFERATION**

A2780 (ovarial CA) cells were treated for 24h with 10 µM of drug or 0.1 % DMSO as control.

**Table:**

<table>
<thead>
<tr>
<th>Drug</th>
<th>G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.8 %</td>
<td>37.7 %</td>
<td>16.5 %</td>
</tr>
<tr>
<td>4SC-202</td>
<td>4.4 %</td>
<td>17.7 %</td>
<td>77.9 %</td>
</tr>
<tr>
<td>Entinostat</td>
<td>82.7 %</td>
<td>5.9 %</td>
<td>11.4 %</td>
</tr>
<tr>
<td>Mocetinostat</td>
<td>37.7 %</td>
<td>51.2 %</td>
<td>11.1 %</td>
</tr>
</tbody>
</table>

4SC-202 is unique amongst HDAC class I inhibitors by inducing a strong G2/M arrest.
4SC-202 is a novel protein deacetylase inhibitor combining three key features for cancer treatment.

- Stemness
- Epigenetics
- Proliferation
4SC-202 in PC3 xenograft model

4SC-202 shows complete tumor suppression in a prostate xenograft model.
Human Granta 519 NHL in SCID

4SC-202 shows dose-dependent efficacy in a NHL animal model
Potent inhibitor of protein deacetylation and of Wnt signaling pathway

4SC-202 effectively suppresses CSC markers and phenotypes in cell based assays

Induction of G2/M arrest, mitotic figures and apoptosis in a broad range of cell lines and cellular assays

Excellent effectiveness in preclinical *in vivo* models
BY PEOPLE. WITH PEOPLE. FOR PEOPLE

PHASE I STUDY „TOPAS“
First in Man, open-label, dose escalation clinical trial

- **Study goals**
  - Determination of MTD, Dose Limiting Toxicities (DLTs) and optimal dosing schedule of 4SC-202 investigating safety, tolerability and pharmacokinetics
  - Assessment of potential anticancer activity of 4SC-202 (Tumor response, PFS, DOR)

- **Dosing**
  - 21 day cycle consisting of 14 day QD oral treatment followed by 7 days pause
  - Starting dose 25 mg 4SC-202

**INCLUSION CRITERIA**
- Advanced hematological malignancies
- No previous HDAC therapy

**MAIN TREATMENT**
- Monotherapy
- 25mg start dose
- Dose escalation
- 6 weeks
- Approx. 36 patients

**FOLLOW-UP PHASE**
- For patients with clinical benefit

**END POINTS**
- MTD, DLT
- Optimal dosing schedule
- Safety, PK
- Tumor response
Determination of HDAC activity in peripheral blood
- Reduction of HDAC activity (Class I specific inhibitor)

Gene expression in PBMCs
- Chip analysis ongoing

Determination of whole lysine acetylation
- Potent induction of lysine acetylation from pre-dose to steady state
  - HL patient, 4SC-202 OD 100 mg
  - Determination of acetylation in Cycle 1
  - Patient now treated in cycle 13
Dose escalation is still ongoing

5th dose group: finished, BID opened

Patients included with HL, NHL, CLL, MDS, MM, AML

14/15 patients went into Follow Up

4SC-202 has shown

- Good PK properties with half life of 10-12 hours in patients
- Good tolerability in dose levels tested so far
- Stabilization of heavily pre-treated patients
THANK YOU VERY MUCH!

University Konstanz, Germany
Thomas U. Mayer
Elena Bausch
Lucia Sironi

NMI Reutlingen, Germany
Yvonne Heubach
Markus Templin
Michael Pawlak

Charité Berlin, Germany
Christian Regenbrecht
Yvonne Welte

Colleagues from 4SC AG

Patients from TOPAS Study

:: CONTACT

Dr. Hella Kohlhof
Manager Translational Pharmacology
4SC AG
hella.kohlhof@4sc.com