TARGETING CANCER STEM CELLS WITH 4SC-202 – AN EPIGENETIC WNT AND HH INHIBITOR

CSC SYMPOSIUM, MMTC 2014
Epigenetics:
inhibition of LSD1 and HDAC1,2 and 3

Cancer stem cells:
inhibition of Wnt and Hh pathways
IMPACT OF CSC CONCEPT ON CLINICAL OUTCOME

Conventional Chemotherapy/Radiation → Relapse → Progression

- Clonogenic cell “Cancer Stem Cell”
- Tumor progenitor
- Differentiated tumor cell
IMPACT OF CSC CONCEPT ON CLINICAL OUTCOME
4SC-202 APPROACH

Classical treatment

1st line therapy (or surgery)  
Time to Recurrence  
BSC

Adding CSC treatment

1st line therapy (or surgery)  
Time to Recurrence  
BSC

4SC-202 – adjuvant treatment

Conventional Chemotherapy/Radiation

4SC-202 treatment

Long term Clinical benefit and improved OS

Response  
Tumor Relapse  
Death  
Diagnosis
PHASE I STUDY „TOPAS“ SHOWS CLINICAL ACTIVITY AND SAFETY
### 4SC-202 FIRST-IN-MAN – TOPAS STUDY DESIGN

- First in Man, open-label, dose escalation clinical trial

<table>
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<tr>
<th>INCLUSION CRITERIA</th>
<th>MAIN TREATMENT</th>
<th>FOLLOW-UP PHASE</th>
<th>END POINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced hematological malignancies</td>
<td>Monotherapy</td>
<td>For patients with clinical benefit</td>
<td>MTD, DLT</td>
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<tr>
<td>No previous HDAC therapy</td>
<td>25 mg start dose</td>
<td></td>
<td>Optimal dosing schedule</td>
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<td>Dose escalation</td>
<td></td>
<td>Safety, PK</td>
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<tr>
<td></td>
<td>6 weeks (2 cycles)</td>
<td></td>
<td>Tumor response</td>
</tr>
</tbody>
</table>

**6**
- Complete response (not confirmed) in patient with T-NHL (AITL)
- Patient on study medication for more than a year, still on treatment
Partial response in patient with classical HL for > 6 month
MODE OF ACTION

⇒ KEY PHENOTYPES
4SC-202 blocks clonogenic capacity of HepG2 cells

- **4SC-202** inhibits clonogenic growth of HepG2 cells.

<table>
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<tr>
<th>Treatment</th>
<th>No further treatment, growth medium only</th>
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<td>2 d</td>
<td>17 d</td>
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</table>

Wash, trypsinize, plate 500 viable cells

Stain colonies

- Medium
- 0.1% DMSO
- 4SC-202 1μM
- Sorafenib 2μM
- SAHA 0.8μM
• Tumor Initiating Cell phenotype assay – Anchorage independent growth assay
  • 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 inhibits colony formation of melanoma (stem) cells and NCCIT cells.
Induction of neutral lipid synthesis is a marker of MDA-MB-468 breast cancer cell differentiation

- Cells were treated for 24h and 48h with 0.8μM and 10μM 4SC-202
- Read out: neutral lipids stained with Nile red

At submicromolar concentrations, 4SC-202 induces differentiation in breast cancer cells.
4SC-202 inhibits primary tumor and metastasis

- **Orthotopic Renca metastasis model, kidney injection**
- **Determination of lung metastasis**

**4SC-202 exhibits strong anti-tumor and anti-metastasis efficacy.**
4SC-202 in MDA-MB-468 orthotopic xenograft model – slow growing model

- Anti-tumor efficacy of 4SC-202 might be due to induction of differentiation of tumor cells \(\rightarrow\) inhibition of stemness
4SC-202 inhibits Hedgehog signaling

- GLI1/2 reporter assay in murine 3T3 cells
  - Smo dependent
  - Stimulation with sHh
  - Result: strong inhibition, EC50 of 60nM

![Graph of Gli1/2 Reporter Assay](image)

**Graph Details:**
- **X-axis:** -log(c) (concentration) from 8.80 to 5.30
- **Y-axis:** Relative Light Units (RLU) from 0 to 60,000
- **EC50:** 0.06µM
Human Medulloblastoma cell line

- Strong inhibition of primary HH target genes

Murine Basal Cell Carcinoma cell lines ASZ, BSZ, CSZ

- BCC cells are very sensitive to 4SC-202 treatment \( \uparrow >300 \text{ nM} \)
4SC-202 INHIBITS SMOOTHENED (SMO) INDEPENDENT MEDULLOBLASTOMA SIGNALING

- 4SC-202 inhibits SAG mediated activation of HH target genes HHIP and PTCH

- SMO independent GLI activation via knockdown of suppressor of Fused (SUFU)
MODE OF ACTION
→ MOLECULAR TARGETS
→ PATHWAY REGULATION
Unique pattern of epigenetic regulation of WNT and HH pathways

- Efficient transcriptional regulation of Wnt associated genes
- Repression of GLI activity by posttranslational modifications

Underlying primary pharmacological mechanisms

- 4SC-202 → Specific inhibition
  - LSD1(KDM1A) – a protein demethylase
  - HDAC1, HDAC2 and HDAC3
- LSD1 and HDAC1/2 are essential components of CoREST and NuRD repressor complexes

A model of the LSD1–CoREST complex docked with the nucleosom [1]

Treatment of Colon cancer RKO xenograft model with 4SC-202, Entinostat and Mocetinostat uncovers huge differences in gene regulation within tumor and whole blood samples.
Positive regulators of WNT pathway are repressed by 4SC-202 treatment.
Down regulated WNT related genes are positively enriched in tumor and blood samples of 4SC-202 treated animals

up regulated genes are negatively enriched → leads to coordinated inhibition of pathway activity

Enrichment of WNT associated genes in Tumor samples

<table>
<thead>
<tr>
<th>Enrichment (regulated genes/expected genes)-1</th>
<th>4SC-202</th>
<th>Entinostat</th>
<th>Mocetinostat</th>
</tr>
</thead>
<tbody>
<tr>
<td>up</td>
<td>-0.4</td>
<td>0.0</td>
<td>-0.4</td>
</tr>
<tr>
<td>down</td>
<td>0.4</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05
- Overlap of genes regulated in tumor and blood
  → Potential blood based biomarker
- This pattern is unique for 4SC-202
Wnt target gene regulation in 4SC-202 treated spheroids on protein level

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<tr>
<th>Wnt target</th>
<th>Level of protein repression</th>
<th>Function</th>
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<tbody>
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<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>
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4SC-202 inhibits Wnt target genes on protein level
POTENTIAL:
HEMATOLOGIC TUMORS WITH ACTIVITY TARGETING CANCER STEM CELLS BY WNT AND HH INHIBITION
Responses seen in 2 Patients – 1 CR and on PR

- Exposure in patients is high enough to see anti-cancer effects
- Safety and tolerability of the 14+7d schedule is very good; continuous dosing is under evaluation currently

Long term stabilization

- 50% of patients (12/24) were SD > 100 days
- 3/24 treated >1 year (2 of them still on treatment)
- 1/24 SD for ~ 2 years, no new lesions occurred

Link to regulated pathways?
TOPAS BIOMARKER (BLOOD) - PATIENT 108

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<tr>
<th>Code</th>
<th>Time point</th>
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<tr>
<td>1</td>
<td>C1D1H5</td>
</tr>
<tr>
<td>2</td>
<td>C1D1H24</td>
</tr>
<tr>
<td>3</td>
<td>C1D14H24</td>
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[1] Metacore analysis

**Gene** | **Regulation**
---|---
TCF7L1 (TCF3) | - 5 x
WNT6 | - 1.2 x
FZD1 | - 1.5 x
SLUG | - 2.5 x
LMX1A | - 1.7 x

**Diagram:**
- WNT1 and WNT6-induced epithelial-to-mesenchymal transition
- WNT4 and WNT10b-induced initial phase of mesenchymal-to-epithelial transition
- Frizzled
- Casein kinase II, alpha chains
- Casein kinase I epsilon
- Pygo1
- Bel-56
- Lrp5
- Ppp2c alpha Dsh
- Gsk3 beta
- Axin
- Apc protein
- Beta-catenin
- Snail1
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TWO TRACK DEVELOPMENT

Fast Track

2019/20

Phase II hematology

- Rapid demonstration of activity e.g. in HL or NHL patients
- Randomized, placebo controlled
- Inclusion of WNT/HH biomarker analysis

Treating CSCs

Phase I/II in solid tumors

- Patients with solid tumors and proven WNT/HH involvement
- Biomarker and histology guided
- Sequencing of tumor samples at inclusion and during therapy
- Prove Cancer Stem Cell hypothesis
4SC-202 offers unique new approach for future cancer therapies

- Treatment of cancer stem cells offer opportunities for various kinds of
  - Combination therapies
  - Adjuvant therapy
  - Neo-adjuvant therapy

- Targeting long-term survival and quality of life of cancer patients
The team at 4SC: Hella Kohlhof, Roland Baumgartner, Tanja Prenzel, Thomas Herz, Sabine Schrepfer, Anna Mais, Bernhard Hauns, Babett Krauss, Rolf Krauss, Manfred Gröppel, Brita Schulze, Bernd Hentsch

- Fritz Aberger
- Wolfgang Huber

- Yvonne Heubach
- Markus Templin
- Michael Pawlak

- Christian Regenbrecht
- Yvonne Welte
THANK YOU VERY MUCH!

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