Preclinical characterization of 4SC-202, a novel isotype specific HDAC inhibitor

S.W.Henning, R.Dobilhofer, H.Kohlhoft, R.Jankowski, T.Maier, T.Beckers, M.Schmidt and B.Hentsch

1 4SC AG, Martinsried, Germany, 2 Nycomed GmbH, Konstanz, Germany

Background

- Histone deacetylases (HDACs) are Zn²⁺ containing enzymes that control the deacetylation of nuclear histones and other proteins. They are involved in the re-modeling of chromatin and have a key role in the epigenetic regulation of gene expression
- Inhibition of HDACs has emerged as a potential strategy to reverse aberrant epigenetic changes associated with cancer. Various HDAC inhibitors (HDACi) are currently under clinical investigation in a broad range of tumour entities including both haematological malignancies and solid tumours

- The novel HDACi 4SC-202 is an orally available potent HDACi specific for class I HDAC isoenzymes. It has shown substantial anti-tumour activity in a broad panel of cancer cell lines as well as in various cancer xenograft models. A Phase I study with 4SC-202 in haematological malignancies is currently prepared and about to start soon

Molecular Characterization

Structure

- 4SC-202 is the tetracylate salt of a benzamide type HDAC inhibitor containing a N-sulfonfylpyrrole scaffold with a MW of 447.5 g/mol and a pKa of 3.3

HDAC Isoenzyme Inhibition

- 4SC-202 shows selective inhibition of recombinant class I HDAC isoenzymes with IC₅₀ values of about 1 µM

<table>
<thead>
<tr>
<th>HDAC class</th>
<th>Isoenzyme</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>HDAC-1</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>HDAC-2</td>
<td>1.12</td>
</tr>
<tr>
<td>Class IIa</td>
<td>HDAC-3</td>
<td>0.57</td>
</tr>
<tr>
<td>Class IIb</td>
<td>HDAC-4</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Class i</td>
<td>HDAC-5</td>
<td>11.3</td>
</tr>
<tr>
<td>Class II</td>
<td>HDAC-6</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Class II</td>
<td>HDAC-7</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Class I</td>
<td>HDAC-8</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Class II</td>
<td>HDAC-9</td>
<td>50.0</td>
</tr>
<tr>
<td>Class III</td>
<td>HDAC-10</td>
<td>21.0</td>
</tr>
<tr>
<td>Class IV</td>
<td>HDAC-11</td>
<td>9.7</td>
</tr>
</tbody>
</table>

In vitro Studies

Histone Hyperacetylation

- 4SC-202 induces hyperacetylation of histone H3 in a dose dependent manner with a cellular potency of IC₅₀ = 1.1µM

In contrast to hydroxamate based HDACi SAHA (vorinostat), the benzamide 4SC-202 induces a G2/M cell cycle arrest with a concomitant increase in sub G1 (apoptotic) cells

Cell Cycle Arrest

- 4SC-202 shows a broad anti-proliferative/cytostatic activity towards human cancer cell lines from various indications with a mean inhibitory potency of IC₅₀ = 0.7±µM after 72 hrs

Xenograft Tumour Models

- Xenograft animal studies with 4SC-202 were performed on animal model systems for various cancer indications such as colorectal, lung and prostate

Pharmacokinetics & Toxicology

Toxicology

- In animal studies 4SC-202 shows a very favorable safety pharmacology profile. Cardiovascular effects are limited to slight reductions in heart rate with no relevant prolongations of QTc intervals
- The toxicity profile of 4SC-202 after oral application is characteristic for HDAC inhibitors with the main target organ being the haemolymphopoietic system
- Toxicological effects are reversible after 2 weeks discontinuation of treatment

Summary & Outlook

- 4SC-202 is a novel benzamide type histone deacetylase inhibitor with selectivity for class I HDACs
- Molecular pharmacodynamic activity of 4SC-202 was demonstrated by histone hyperacetylation, G2/M cell cycle arrest and interference with normal mitotic spindle development
- 4SC-202 showed pronounced and robust anti-tumour activity in various cancer cell lines and xenograft animal models
- Good bioavailability and low clearance led to high plasma exposure of 4SC-202 after oral application
- 4SC-202 was well tolerated and showed a favorable safety pharmacology profile
- A first-in-man phase I clinical study with an oral formulation of 4SC-202 in haematological malignancies is in preparation

22nd EORTC-NCI-AACR symposium on "Molecular Targets and Cancer Therapeutics" Berlin, Germany, 16 - 19 November 2010 Abstract # 178, Poster Board # 155