Resminostat – an epigenetic approach for CTCL maintenance treatment

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Introduction & Objective

CTCL (cutaneous T-cell lymphoma) is characterized by the presence of a clonal T-cell population in the skin and/or blood, lymph nodes or visceral organs. Patients with early disease can be treated effectively with topical treatments. However, a key challenge is to achieve durable remissions in patients with advanced disease who require systemic treatment. In malignant T cells of CTCL patients many oncogenic alterations have been described and epigenetic alterations are known to play a key role in pathogenesis. Disease progression is associated, among others, with a switch in the T-helper cell status from Th1 to Th2 phenotype. This switch is associated with epigenetic induced changes in the expression of STAT4/STAT6 (Litvinov et al. 2014).

Resminostat is an orally available HDAC-inhibitor, which induces changes in gene expression resulting in growth inhibition, modified cell differentiation and enhanced tumor immunogenicity.

The purpose of this in vitro study is to investigate resminostat’s anti-tumor efficacy against CTCL-derived cell lines and its impact on STAT4/STAT6 expression to support resminostat’s clinical development in CTCL.

Primary Mode of Action

Analysis of protein acetylation

Upon treating three different CTCL cell lines (HH, HuT78 and MyLa CD4+) with vehicle control or 3 μM resminostat for 3 h, cells were fixed, stained with anti-acetylated lysine antibody and subsequently analyzed via flow cytometry.

- Resminostat induces significant increase in protein acetylation in three different CTCL cell lines (HH, HuT78 and MyLa CD4+).

Resminostat affects growth of CTCL cell lines

In vitro potency in CTCL cell lines (IC50)

<table>
<thead>
<tr>
<th>Resminostat IC50 [μM]</th>
<th>HH</th>
<th>HuT78</th>
<th>MyLa CD4+</th>
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<tbody>
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<td></td>
<td>0.58 (+/− 0.18)</td>
<td>0.98 (+/− 0.20)</td>
<td>0.89 (+/− 0.35)</td>
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Cell cycle analysis

MyLa CD4+ cells were treated with vehicle control (DMSO) or with 4 μM resminostat for 72 h. Cell cycle distribution was determined by Propidium Iodide (PI) staining. Cell cycle phases are depicted in %.

Analysis of apoptotic processes

MyLa CD4+ cells were treated with vehicle control (DMSO) or with 4 μM resminostat for 72 h. Apoptosis was determined via Annexin V and Propidium Iodide (PI) staining using flow cytometry analysis. The different populations are represented as %.

At clinical relevant concentration (4 μM) resminostat shows in vitro
- Growth inhibitory potency in 3 different CTCL cell lines
- Only a moderate effect on cell cycle distribution
- An increasing fraction of apoptotic cells

Resminostat might affect differentiation of CTCL cells

Resminostat influences STAT4 / STAT6 expression

Early stages of CTCL are associated with an overexpression of STAT4, which favors the T-helper (Th) 1 differentiation. Late disease stages are associated with a predominantly Th2 phenotype and loss of the STAT4 expression (Litvinov et al. 2014).

Resminostat treatment results in
- Induction of STAT4 expression (STAT4 high correlates with Th1)
- Reduction of STAT6 expression (STAT6 high correlates with Th2)
- Increase of STAT4 promoter histone acetylation

Conclusions

Resminostat displayed conclusive in vitro anti-tumor activities in CTCL cells. The regulation of the aberrant STAT signaling on transcription level suggests a stabilization of the less advanced CTCL stage (Th1) or even a reversion of the advanced Th2 to the Th1 phenotype. Normalizing the epigenetic dysregulation which drives CTCL to progression provides a biological rationale for a maintenance therapy.

RESMAIN: Phase II Maintenance Trial

A clinical Phase II trial to evaluate resminostat as maintenance treatment for patients with advanced stage (IIb-IV) mycosis fungoides or Sézary syndrome is now open for recruitment (ClinicalTrials.gov Identifier: NCT02953301).

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The ChIP experiments are part of the EMTherapy project, which is conducted within the framework of the European Framework program and has received funding from the Federal Ministry of Education and Research.

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