Resminostat – an epigenetic approach for CTCL maintenance treatment

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Introduction & Objectives

Advanced stage CTCL is characterized by a phenotypic plasticity with regard to T helper cell status, switching from Th1 to Th2 status at progression. This switch is associated with epigenetic induced changes in the expression of STAT4/STAT6 (Litvinov et al. 2014). Resminostat is a potent, orally available inhibitor of HDACs, already in Phase II clinical development. Resminostat 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-tumoral 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. A. Representative histogram of HuT78 cells. B. Total acetylation in all CTCL cell lines.

- Resminostat induces significant increase in protein acetylation in 3 different CTCL cell lines (HH, HuT78 and MyLa CD4+)

Resminostat affects growth of CTCL cell lines

In vitro potency on CTCL cell lines (IC50)

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>HH</th>
<th>HuT78</th>
<th>MyLa CD4+</th>
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<tbody>
<tr>
<td>Resminostat IC50 [µM]</td>
<td>0.58 (+/− 0.18)</td>
<td>0.98 (+/− 0.28)</td>
<td>0.89 (+/− 0.35)</td>
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Cell cycle analysis

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

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. Staining: viable, non-apoptotic cells: Annexin V-PI-, early apoptotic cells: Annexin V+PI-, late apoptotic or dead cells: Annexin V+PI+. The different populations are represented as percentages.

- 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
  - Increasing fraction of apoptotic cells

Resminostat affects cell differentiation

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 influences STAT4 / STAT6 expression

HH and MyLa CD4+ cells were treated with resminostat at indicated concentrations and expression of STAT4 and STAT6 was measured by means of qPCR after 24 h.

- Resminostat treatment results in:
  - Induction of STAT4 expression (STAT4 high correlates with Th1)
  - Reduction of STAT6 expression (STAT6 high correlates with Th2)

Conclusions

Resminostat displayed conclusive in vitro anti-tumor activities both in mycosis fungoides and Sézary syndrome cells. The regulation of the aberrant STAT signaling on transcription level suggests a stabilization of the less advanced CTCL stage (Th1) or even a reconversion 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. A clinical Phase II trial to evaluate resminostat for maintenance treatment of patients with advanced stage (IIB-IV) mycosis fungoides or Sézary syndrome that have achieved disease control with systemic therapy is currently in preparation.

RESMAIN: Phase II Maintenance Trial

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