Resminostat’s action in CTCL – hints from a genome-wide study

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Introduction

Cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin lymphoma characterized by proliferation of skin-homing T cells. In malignant T cells of CTCL epigenetic alterations are known to play a key role in pathogenesis. An imbalance between Th1/Th2 cells with deregulated STAT4/STAT6 levels is discussed as a possible mechanism of pathogenesis. Furthermore, pruritus is one of the major symptoms affecting HRQoL of CTCL patients and is associated with high levels of IL31 expression.

Resminostat is a potent, orally available inhibitor of class I, IIb and IV HDAC enzymes in Phase II clinical development. Resminostat inhibits HDACs, modifies histone acetylation and induces changes in gene expression in tumor cells. This results in cell growth inhibition, modified cell differentiation and enhanced tumor immunogenicity.

We investigated the mode of action of resminostat more extensively in vitro using a genome-wide approach evaluating global histone acetylation and gene expression by ChIP-seq and RNA-seq. Here we show first results from the analysis of the CTCL cell line My-La CD4+.

Resminostat affects the acetylation landscape

- **Resminostat induces genome-wide increase of histone H3K27 acetylation**
- **Resminostat regulates gene expression in a dose-dependent manner**
- **Majority of differentially regulated genes is up-regulated**

**Resminostat modulates RNA expression**

- RNA-seq differential analysis of H3K27ac enriched regions in the CTCL cell line My-La CD4+ after resminostat treatment
- My-La CD4+ cells were incubated for 24h with resminostat [1µM, 4µM] or DMSO [0.1%] as control. H3K27ac was enriched by chromatin immunoprecipitation and DNA was analyzed by NGS. As reference, Drosophila chromatin was used as spike-in.

**Resminostat regulates biomarker expression**

- **Resminostat shifts gene expression from unfavorable Th2 to favorable Th1**
- **Resminostat modulates advanced / active disease markers**

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**RNA expression of CTCL biomarkers regulated by resminostat**

- Normalized RNA expression of RNA-seq is shown as log2 fold change of normalized reads to control (ctrl, DMSO 0.1%).
- -log10 (p-value)

**Validation of IL31 as a target gene of resminostat**

- Left: RNA-seq result of IL31. Normalized RNA expression as log2 fold change (FC) to control (ctrl, DMSO 0.1%).
- Middle: Validation by qPCR. RNA expression shown as log2 fold change to control (ctrl, DMSO 0.1%).
- Right: Advanced disease marker RAD23B and active disease marker KIR3DL2 are down-regulated by resminostat.

**ITCHING mediator IL31 is a target gene of resminostat**

- **Resminostat reduces IL31 expression (related to itching in CTCL)**

**Resminostat – promising drug for CTCL therapy**

- **Results from genome-wide in vitro study of resminostat in CTCL:**
  - Effects are translated genome-wide in a dose-dependent manner
  - Genome-wide increase of histone H3K27 acetylation levels
  - Significant modulation of gene expression
  - Regulation of both gene induction and repression
- **Resminostat affects CTCL-relevant genes**
  - Modulation of immune, stress response and adhesion-related pathways
  - Switch from unfavorable Th2 to favorable Th1 gene expression
  - Regulation of advanced and active disease markers
  - Repression of the itching-related factor IL31

**Data support the clinical development of resminostat in CTCL in a maintenance setting (REMAIN study)**

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