HDACi resminostat causes a sustained reduction of the pruritus mediator IL-31 in CTCL cells.

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IL-31 is a Th2 cytokine and itch mediator

• Pruritus (itching) has a severe negative impact on HrQoL in CTCL patients

• The Th2 cytokine IL-31 has been described as an itch-mediator in pruritic disorders [1].

• CTCL-related pruritus correlates with elevated levels of IL-31 in the skin [2], [3]

• HDAC inhibitors reduce itch severity in CTCL patients [4]
  o Reduction of IL-31 levels after HDAC inhibitor treatment [5]

HDACi resminostat downregulates IL-31

Resminostat reduces the IL-31 on protein level in My-La cells
Pharmacokinetics of resminostat – clinical dosing scheme

• CTCL RESMAIN trial:
  o Resminostat is given 600 mg/day for 5 days, 9 days treatment break

• Key questions regarding dosing scheme:
  o Are resminostat-mediated effects sustained during treatment break?
  o Impact of consecutive treatment for 5 days (5 h)?

![Graph showing individual PK profiles (1 day, 800 mg)]

- C<sub>max</sub> at 600 mg ≈ 6 µM
- Clearance ≈ 5 hours
Experimental layout in CTCL cells adapted to the dosing schedule of resminostat

Addresses:
- Durable and sustained effects by resminostat?
- Impact of consecutive treatment for 5 days (5 h) in CTCL cell lines?

Treatment of CTCL cells with resminostat

Time after compound washout

Treatment
- 24 h treatment
- 5 days x 5 hours

NO treatment
- ELISA for secreted IL-31
- No toxicity in cell viability assays
Sustained reduction of secreted IL-31 protein level following compound withdrawal

% Reduction of IL-31 protein level

<table>
<thead>
<tr>
<th>Compound</th>
<th>1 day Treatment</th>
<th>6 days Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 µM Resminostat</td>
<td>52 %</td>
<td>30 %</td>
</tr>
<tr>
<td>1.0 µM Resminostat</td>
<td>68 %</td>
<td>33 %</td>
</tr>
<tr>
<td>3.0 µM Resminostat</td>
<td>98 %</td>
<td>39 %</td>
</tr>
</tbody>
</table>
Sustained reduction of secreted IL-31 protein level following compound withdrawal

% Reduction of IL-31 protein level

<table>
<thead>
<tr>
<th>Compound</th>
<th>5 days x 5 h treatment</th>
<th>5 days withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 µM Resminostat</td>
<td>11%</td>
<td>20%</td>
</tr>
<tr>
<td>1.0 µM Resminostat</td>
<td>28%</td>
<td>28%</td>
</tr>
<tr>
<td>3.0 µM Resminostat</td>
<td>57%</td>
<td>26%</td>
</tr>
<tr>
<td>6.0 µM Resminostat</td>
<td>80%</td>
<td>35%</td>
</tr>
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</table>

Sustained reduction of secreted IL-31 protein after resminostat removal
What is the mechanism?

- IL-31 mRNA
  - Preliminary data

- Hyperacetylation

- Maintained effects not observed on acetylation and mRNA level
- Mechanism? IL-31 protein level, secretion
Summary and Outlook

• Resminostat reduces the production of the itch-mediator IL-31

• The decrease of IL-31 is maintained after compound removal
  o Sustained and durable effect on IL-31 reduction in cell lines

➔ Resminostat has the potential to improve itching in CTCL

• Our findings support a treatment break in the clinical schedule

IL-31 level from serum will be determined as part of the biomarker program in the ongoing RESMAIN trial in CTCL (NCT02953301)
Mode of action of resminostat in CTCL cells

Resminostat’s mode of action in CTCL cells

Debulking effects

Modulation of cell phenotype

Cell death / Apoptosis

TUMOR CELL

Hyperacetylation
of histone and non-histone proteins

Cell Proliferation

Immune Modulation
NK cell response

Gene Regulation
Th1/Th2 Skin homing receptors

IL-31 cytokine reduction

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Thank you!
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