IL-12 and Cancer Immunotherapy

- Interleukin-12 (IL-12)
  - Pro-inflammatory cytokine
  - Master regulator of cell-mediated immunity to pathogens and neoplastic transformation
  - Produced by innate immune cells in response to pathogens
  - Leads to production of T- and natural killer (NK) cells
  - Interferon gamma (INFγ)
  - Tumor necrosis factor alpha (TNFα)
  - Studies confirmed significant systemic toxicities

- AD-RTS-L12 + veledimex explores regulated local treatment strategy
  - Goal is extending the IL-12 therapeutic window
  - Reducing systemic toxicity
  - AD-RTS-L12 injected into tumor
  - IL-12 transcription upregulated only in presence of activator ligand veledimex
  - IL-12 expression level can be modulated by dose and frequency of veledimex administration.

Veledimex is Orally Absorbed & Crosses the Blood Brain Barrier

- Inducible Gene Regulation: RheoSwitch Therapeutic System®
  - RheoSwitch Therapeutic System® (RTS) is a 3-component transcriptional regulator
  - Co-Activation Partner (CAP)
  - Ligand-Inducible Transcription Factor (LITF)
  - RTF binds to the LITF and forms a stable interaction with the CAP

Veledimex was administered orally either as a single dose at 225 and 450 mg/m²/day to C57BL/6 mice or as BID doses at 450 and 1200 mg/m²/day with 4h between doses. Terminal plasma, CSF, and brain samples were collected from each animal at 1, 2, 4, 5 (BID only), 6, 8 (BID only), 10 (BID only), 24, and 48h post dose. Samples were processed and analysed for veledimex using LC-MS/MS.

Summary of Veledimex Plasma, Brain & CSF Pharmacokinetics

<table>
<thead>
<tr>
<th>Phase</th>
<th>Time (h)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>AUC(0-24h) (ng*h/mL)</th>
<th>CL/F (L/kg)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2h</td>
<td>200</td>
<td>6h</td>
<td>1000</td>
<td>12h</td>
<td>3000</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Brain</td>
<td>2h</td>
<td>200</td>
<td>6h</td>
<td>1000</td>
<td>12h</td>
<td>3000</td>
<td>50</td>
<td>20</td>
</tr>
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<td>CSF</td>
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<td>12h</td>
<td>3000</td>
<td>50</td>
<td>20</td>
</tr>
</tbody>
</table>

BALT/c mice were s.c. inoculated with 5x10⁸ CT26 cells in the right flank. On Day 12, when tumors reached 50-100mm³, animals were randomized to one of the treatment groups. AD-RTS-L12 was administered intratumoral at 1x10⁶ vp with veledimex p.o. administered continuously beginning Day 1 for the duration of the study. Tumor volumes were monitored using LxW²/2 and normalized to the individuals starting volume. Arrows depict administration of AD-RTS-L12.

Ad-RTS-mL12 + Veledimex Induces Sustained Reduction in Tumor Growth in C57B16 Syngeneic Mouse Melanoma Model

- Treatment with AD-RTS-L12 + veledimex 1x10¹⁰ vp p.o. improved survival over bevacizumab 30 biwkx3 and SB415286/Medroxyprogesterone 10 and 50 mg/kg/day.

Ad-RTS-mL12 + Veledimex Pretreatment Induces Systemic Tumor Response in GL261 Orthotopic Glialoma Mouse Model

- Treatment with AD-RTS-L12 + veledimex 1x10¹⁰ vp p.o. improved survival over bevacizumab 30 biwkx3 and SB415286/Medroxyprogesterone 10 and 50 mg/kg/day.

Conclusions

- Veledimex exhibited dose-induced toxicity in plasma and brain tissue exposure.
- The increase in tumor veledimex levels in combination with AD-RTS-mL12 resulted in an increase in expression of IL-12 mRNA leading to an increase in tumor IL-12p70 expression with minimal increase in serum IL-12.
- Dose-response was established, the optimal dose of orally administered veledimex was 450 mg/m²/day Qdx14 were rechallenged with 1x10⁸ 5x10⁴ GL-261 cells at the site of original implantation vs. age-matched controls administered 1x10⁸ GL-261 cells.

- Ad-RTS-mL12 + veledimex demonstrated systemic memory upon rechallenge in multiple syngeneic mouse models.
- These findings support the utility of localized regulatable IL-12 production as an approach for the treatment of malignant glioma.