Controlled Local Expression of IL-12 as Gene Therapy Concomitant with Systemic Chemotherapy Improves Survival in Glioma (IMMU-33)

Lomustine: is a lipid soluble alkylating nitrosourea compound which crosses the BBB and used in the treatment of glioma. Lomustine causes interstrand and intrastrand cross-linking of DNA resulting in cell death.

Ad-RTS-IL-12 + Veledimex: controlled local expression of IL-12 via a RheoSwitch Therapeutic System® (RTS®) is a 3-component transcriptional regulator
• Five Days prior to therapy 1 x 10^5 GL-261 glioma cells volume 3 µl were administered into the brain of C57BL/6 mice.
• On Day 1 a single dose of Ad-RTS-mIL-12 at 5x10^9vp 5 µl followed by the activator ligand, veledimex p.o. QDx14 CCNU i.p. QDx5.
• The time to disease progression and death was studied.
Expression of Tumor IL-12 & IFNγ with Ad-RTS-mIL-12 + Veledimex Alone or in Combination with CCNU

Serum levels of IL-12 and IFNγ ~ 30 times lower than tumor
• * P<0.05 vehicle vs treatment
• NS= No significant differences between Ad-RTS-mIL-12 + veledimex vs. combination
Ad-RTS-mIL-12 + veledimex increases tumor cytotoxic T cells while decreasing Tregs via FACS

CD3+CD8+ (tumor)

Day 7

% total leukocytes (CD45+)

CD4+CD25+FoxP3+ (tumor)

Day 7

% CD3+CD4+ T cells

* P< 0.05 one-way analysis of variance; Dunnets test
Survival: Ad-RTS-mIL-12 + Veledimex or CCNU Alone & In Combination

### Diagrams:
- **Ad-RTS-mIL-12+Veledimex 3 mg/m²**
  - Vehicle/Vehicle
  - Ad 5e9+V 3 mg/m²
  - CCNU 9 mg/m²
  - CCNU 18 mg/m²
- **Ad-RTS-mIL-12+Veledimex 10 mg/m²**
  - Vehicle/Vehicle
  - Ad 5e9+V 10 mg/m²
  - CCNU 9 mg/m²
  - CCNU 18 mg²

### Table:
<table>
<thead>
<tr>
<th>Treatment (mg/m²)</th>
<th>Median Survival (Days)</th>
<th>Increase in Life Span (ILS) (%)</th>
<th>Percent Survival at Day 85</th>
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<tbody>
<tr>
<td>Vehicle/Vehicle</td>
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<td>Ad 5e9+V 3 mg/m²</td>
<td>37</td>
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**a** ILS=%T/C -100%; T/C = quotient of median survival of treated vs. control

**b** Animals survived to the end of the study (Day 85), TTE >85 days & ILS >286%
Survival rates and life span increases for different treatments:

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<sup>a</sup> ILS = %T/C - 100%; T/C = quotient of median survival of treated vs. control

<sup>b</sup> Animals survived to the end of the study (Day 85), TTE >85 days & ILS >286%
Summary

• **Cytokines:**
  • Ad-RTS-mIL-12 + veledimex increased tumor cytokines in a dose-related manner
  • CCNU (lomustine) alone does not affect tumor cytokine levels at the doses studied
  • Ad-RTS-mIL-12 + veledimex + CCNU does not enhance tumor cytokines when compared to Ad-RTS-mIL-12 + veledimex alone

• **Tumor FACS:**
  • Ad-RTS-mIL-12 + veledimex increased tumor cytotoxic T cells with concomitant decrease in tumor Tregs
  • CCNU alone had no effect on tumor cytotoxic T cells or tumor Tregs
  • Ad-RTS-mIL-12 + veledimex + CCNU does not further increase cytotoxic T cells when compared to Ad-RTS-mIL-12 + veledimex alone

• **Survival:**
  • Ad-RTS-mIL-12 + veledimex demonstrated dose-related increase in survival vs. vehicle
  • CCNU 18 mg/m² demonstrated minimal increase in survival vs. vehicle
  • Ad-RTS-mIL-12 + veledimex + CCNU resulted in an increase in survival over Ad-RTS-mIL-12 + veledimex monotherapy. 100% survival with Ad + V 10 mg/m² + CCNU 18 mg/m²

• **Controlled local immunostimulation with IL-12 combined with CCNU, warrants further nonclinical investigation**