Localized Regulated Expression of IL-12 as a Gene Therapy Concomitant with Blockade of PD-1 for Treatment of Glioma

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Immuno-oncology

**Immune Activation**

- CTLA4: Brakes
  - Proportionally dampens cytotoxic T cell activation

- PD-1/PD-L1: Clutch
  - Regulates cytotoxic T cell activity to decrease tissue damage

- Local IL-12: Gas/GPS
  - Activates cytotoxic T cell
  - Decreases T regs, T cell homing

**Immune Suppression**
Rationale for tumor IL-12 production in combination with anti-PD-1 therapy

• Interleukin-12 (IL-12)
  – Master regulator of cell-mediated immunity to pathogens and malignant cells
  – Produced by innate immune cells in response to pathogens

• PD-1
  – Negative regulator of lymphocyte activation
  – PD-1 is expressed on activated CD4 & CD8 T cells after MHC-TCR engraftment modulates & T cell activation.
  – Inhibition of PD-1 reverses the immunosuppression

• Tumor immunostimulation via IL-12 coupled with the reduction in innate tumor immunosuppression by anti-PD-1 should result in enhanced efficacy over monotherapy.
**Inducible Gene Regulation: RheoSwitch Therapeutic System®**

RheoSwitch Therapeutic System® (RTS®) is a 3-component transcriptional regulator

1. **The Switch Components**: The RTS® gene program includes 2 receptor protein fusions: VP16-RXR (co-activation partner, CAP) and Gal4-EcR (ligand-inducible transcription factor, LTF). In the absence of ligand, the LTF binds to the inducible promoter and does not form a stable interaction with the CAP.

2. **The Inducible Promoter**: A customizable promoter to which basal transcription proteins are recruited and the target gene is transcribed.

3. **The Activator Ligand (veledimex)**: An ecdysone analog, diacylhydrazine-based small molecule functions as an activator. In the presence of the ligand, a conformational change in the LTF leads to a stable, high affinity interaction with the CAP.
Veledimex Crosses the Blood Brain Barrier in GL261 Orthotopic Glioma and Normal Mice

Veledimex levels at 24 hr posttreatment
Ad-RTS-mIL12 + Veledimex in Combination with anti PD-1 in GL-261 Orthotopic Model

GL-261 Orthotopic Glioma Model

GL261 1e5 cells Start Therapy End Study Day 90

<table>
<thead>
<tr>
<th>iCPI</th>
<th>Antibody</th>
<th>Route</th>
<th>Schedule</th>
<th>Dose (mg/m2)</th>
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<td>antiPD-1</td>
<td>RMP 1-14</td>
<td>i.p.</td>
<td>Q4Dx5</td>
<td>7.5</td>
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Ad-RTS-mIL-12 + Veledimex + Anti-PD-1:
Overall Survival

- Ad-RTS-mIL-12 + veledimex dose-related increase in survival
- Ad-RTS-mIL-12 + veledimex + anti PD1 increased survival over monotherapy
- Ad-RTS-mIL-12 + veledimex 30mg/m²/day + anti PD1 15mg/m² results in 100% survival
Ad-RTS-mIL-12 + Veledimex + Anti-PD-1: Body Weight Change

- Ad-RTS-mIL-12 + veledimex + anti-PD-1 therapy augmented the reduction in body weight over monotherapy.
- All groups recovered when veledimex was discontinued.
Ad-RTS-mIL-12 + Veledimex in Combination with Anti-PD-1 (RPM 1-14) on Tumor Cytokines

**Tumor IL-12**

**Tumor IFNγ**

- Vehicle/Vehicle
- Ad 5e9+V 30 mg/m²
- antiPD-1 7.5 mg/m²
- antiPD-1 15 mg/m²
- Ad 5e9+V 30+antiPD-1 7.5
- Ad 5e9+V 30+antiPD-1 15
Ad-RTS-mIL-12 + Veledimex + Anti-PD-1 (RPM 1-14) Tumor FACS

Cytotoxic T cells (CD3+CD8+)

T cell Exhaustion (Lag 3; CD233)
Ad-RTS-mIL-12 + Veledimex + Anti-PD-1 (RPM 1-14) Tumor FACS

T regs (CD4+CD25+FoxP3+)

% Total T cells

Cytotoxic Tcell/Tregs ratio (CD3+CD8+/FoxP3+)

Ratio
Conclusions

• Controlled tumor IL-12 production was able to stimulate the immune system in the presence of innate tumor immunosuppression

• The addition of anti-PD-1 therapy resulted in a reduction in tumor innate immunosuppression

• The combination of both therapies resulted in decreased immunosuppression coupled with local immunostimulation proved to be beneficial in the treatment of glioma with a profound increase in survival over controls & monotherapy

• **Clinical study design to assess combination therapy in recurrent GBM in progress**