Controlled Expression of IL-12 Improves Survival in Glioma by Activating the Immune Response in Mice and Humans (IMMU-34)

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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: for example, 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.
Protocols: Mouse GBM Model & GBM Patients

Mouse GBM Model

• 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

• The time to disease progression and death was studied.

GBM Clinical Trial

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<thead>
<tr>
<th>Mouse Dose (mg/m^2)</th>
<th>HED (mg)</th>
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<tr>
<td>3</td>
<td>6</td>
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<td>10</td>
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Ad-RTS-IL-12 2x10^{11}vp
20mg
30mg
40mg
Tumor Peak Cytokines in GL-261 Orthotopic Mouse Model

Each histogram is the mean ± SEM N=6; * P<0.05
Peak Serum Cytokine in GL-261 Mice & in GBM Patients

**GL261 Mice**

Peak Serum IL-12

- Cytokines in mouse tumor were ~32x higher than serum for IL-12 & 43x for IFNγ at veledimex 10mg/m²

Peak Serum IFNγ

**GBM Patients**

Peak Serum IL-12

- *P* < 0.05

Peak Serum IFNγ

* P< 0.05
Ad-RTS-IL-12 + V Demonstrates Prolonged Tumor T Cell Infiltration in GL-261 Mouse & GBM Patients

GL-261 Mice
14 Days Posttreatment (N=4-6)

GBM Patients
Biopsy Days 135-175 (N=3)
Summary of Survival in GL-261 Mice & GBM Patients

GL-261 Mice

Survival at end of study

- Vehicle/Vehicle
- Ad 5e9+V 1 mg/m²
- Ad 5e9+V 3 mg/m²
- Ad 5e9+V 10 mg/m²
- Ad 5e9+V 30 mg/m²
- antiPD-1 15 mg/m² q4dx5
- Bevacizumab 30mg/m² biwkx3
- Temozolomide 300mg/m² qdx5
- Lomustine 18 mg/m² qdx5

10mg/m² (HED 20mg) 67% at Day 85 with majority of the mice tumor free.

GBM Patients

Veledimex 20 mg Cohort

20 mg cohort, based on Kaplan-Meyer plot, estimated Median OS (mOS) is 12.5 months and mean follow-up of 11.1 months with 6 of 15 subjects alive 18 Oct 2017
Median Overall Survival (mOS) in GL-261 Mice & GBM Patients

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### mOS in GL-261 Mice (days)

- **Ad-RTS-mIL-12 + V 10mg/m²**
  - Vehicle/vehicle, n=42
  - Lomustine, n=12
  - Bevacizumab, n=24
  - Temozolomide, n=21
  - antiPD-1, n=34

- **>85 days**

### mOS in GBM Patients (months)

- **Ad-RTS-hIL-12 + V 20 mg**
  - Polymer placebo
  - Lomustine
  - Bevacizumab
  - Physician’s choice

- **12.5 Months**

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**Notes:**
- * Ad-RTS-mIL-12 5e9 vp + veledimex 10 mg/m² qdx14; Lomustine 18 mg/m² qdx5; bevacizumab 30 mg/m² biwkx3; temozolamide 300 mg/m² qdx5; antiPD-1 15 mg/m² q4dx5 (End of Study D 85)
- * Median OS (mOS) is at 12.5 months with a mean follow up of 11.1 months (range: 1.8, 28.2)
- * 6 of 15 subjects alive in 20 mg veledimex cohort (18 Oct 2017)
Conclusions

• Ad-RTS-IL-12+veledimex treatment demonstrated that veledimex crosses BBB in mice and patients

• RTS® gene switch controls the expression of IL-12 in both mice and humans

• In mice and patients we have observed an increase in overall survival when compared to current therapies