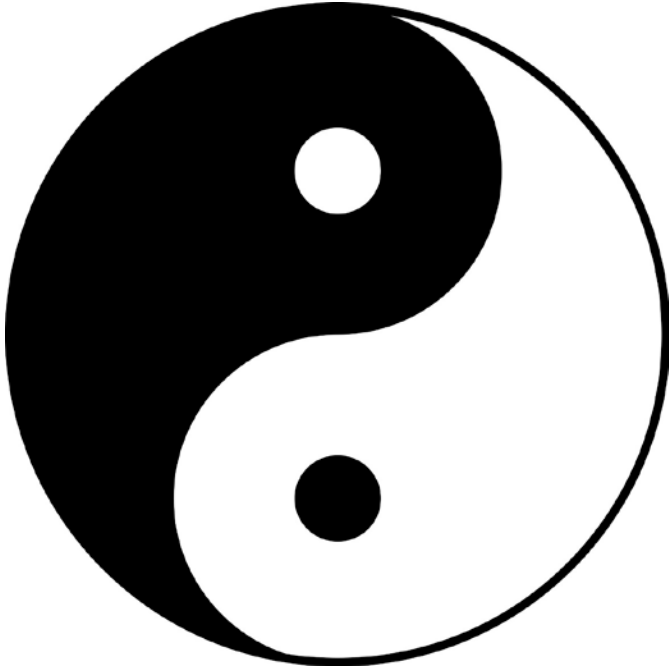


# Localized Regulated Expression of IL-12 as a Gene Therapy Concomitant with Blockade of PD-1 for Treatment of Glioma

John A. Barrett, Hongliang Cai, John Miao, Margaret Murray, Paul Gonzales, Suma Krishnan, Francois Lebel, Laurence J.N. Cooper

# Immuno-oncology

**Immune Activation**



**Immune Suppression**

## **CTLA4: Brakes**

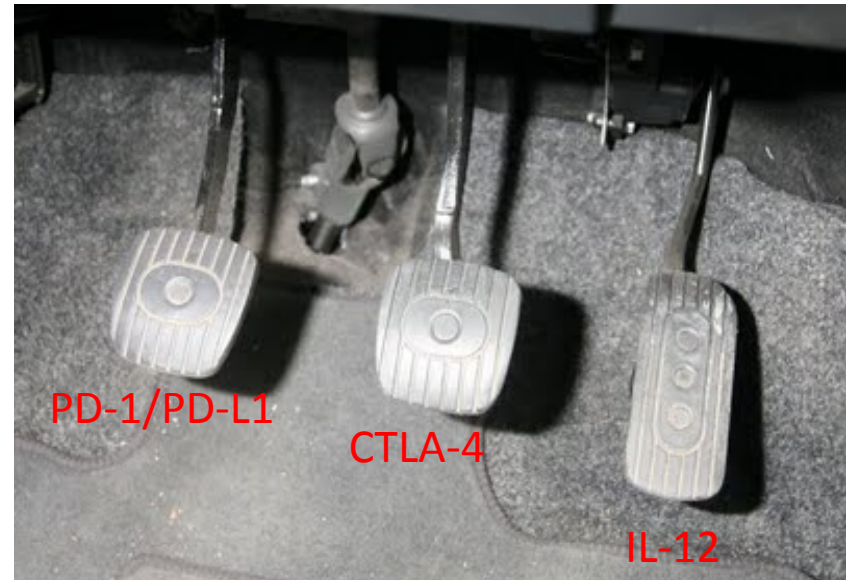
- Proportionally dampens cytotoxic T cell activation

## **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

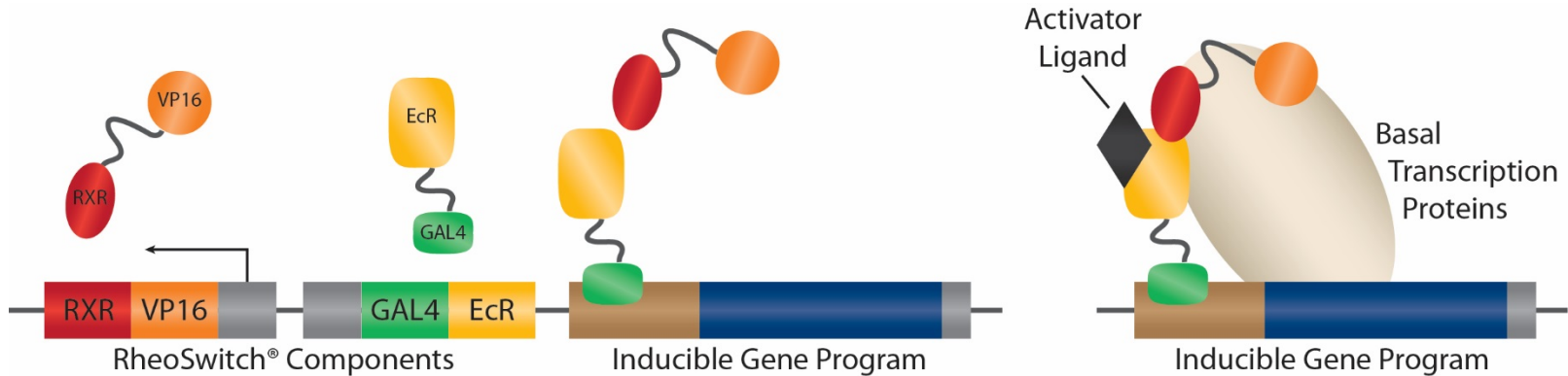


# Rationale for tumor IL-12 production in combination with anti-PD-1 therapy

- **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 engagement 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.

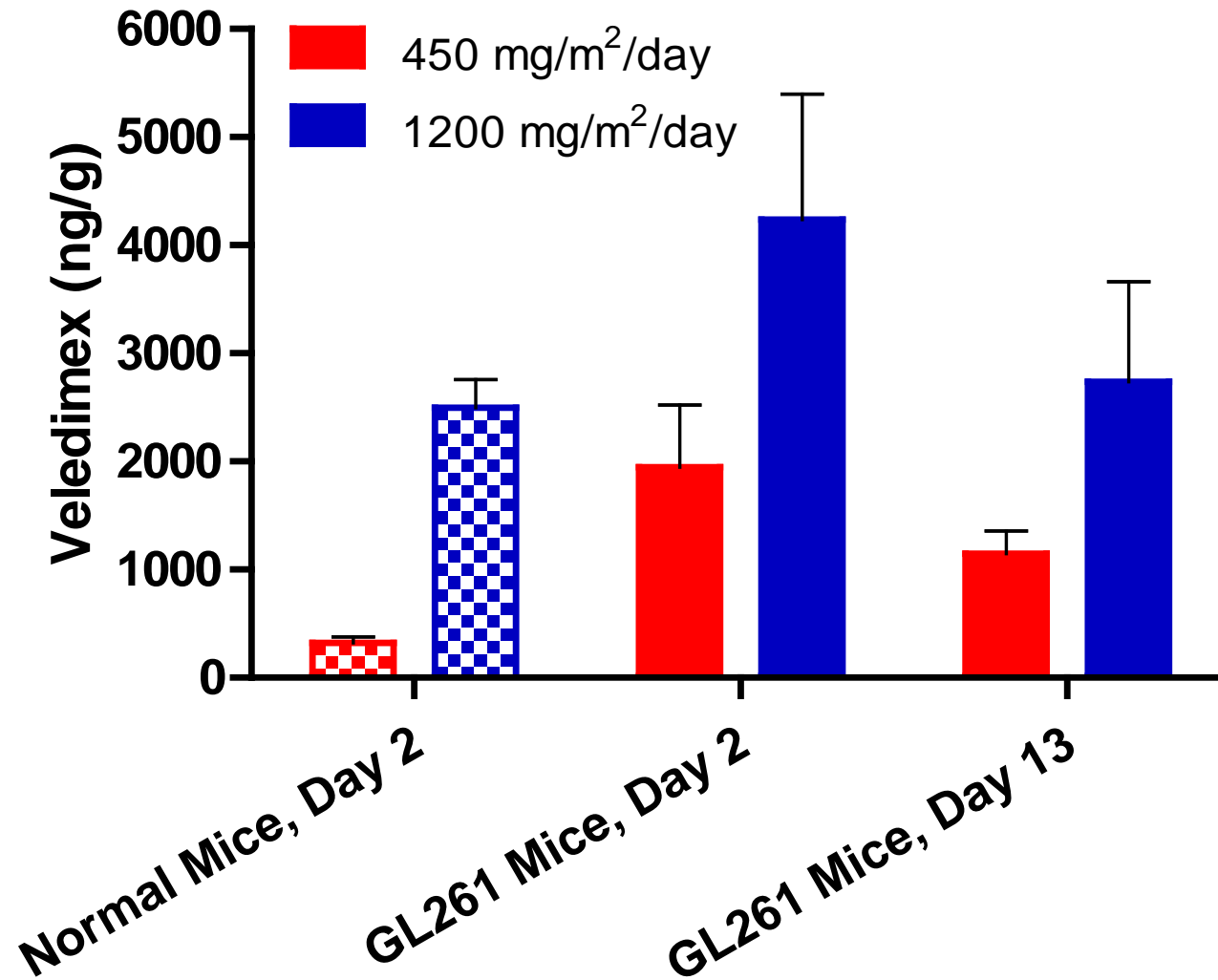
# Inducible Gene Regulation: RheoSwitch Therapeutic System<sup>®</sup>

RheoSwitch Therapeutic System<sup>®</sup> (RTS<sup>®</sup>) is a 3-component transcriptional regulator



- 1. The Switch Components:** The RTS<sup>®</sup> 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.

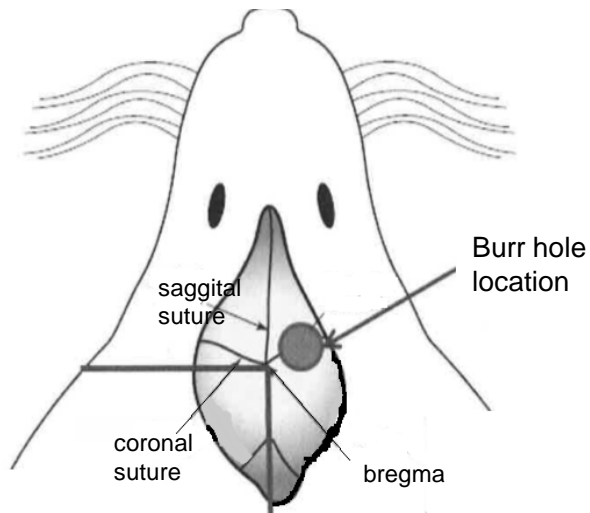
# Veledimex Crosses the Blood Brain Barrier in GL261 Orthotopic Glioma and Normal Mice



Veledimex levels at 24 hr posttreatment

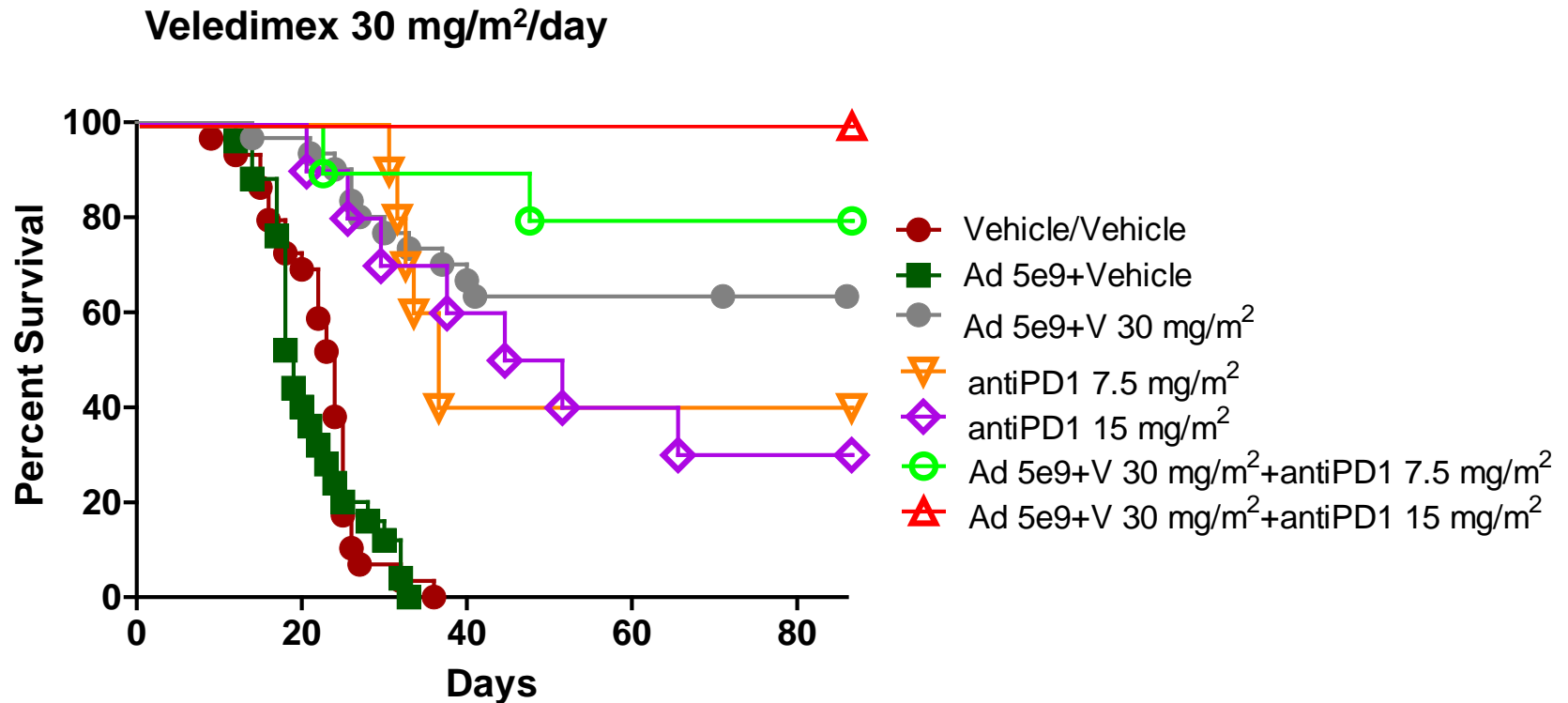
# Ad-RTS-mIL12 + Veledimex in Combination with anti PD-1 in GL-261 Orthotopic Model

## GL-261 Orthotopic Glioma Model



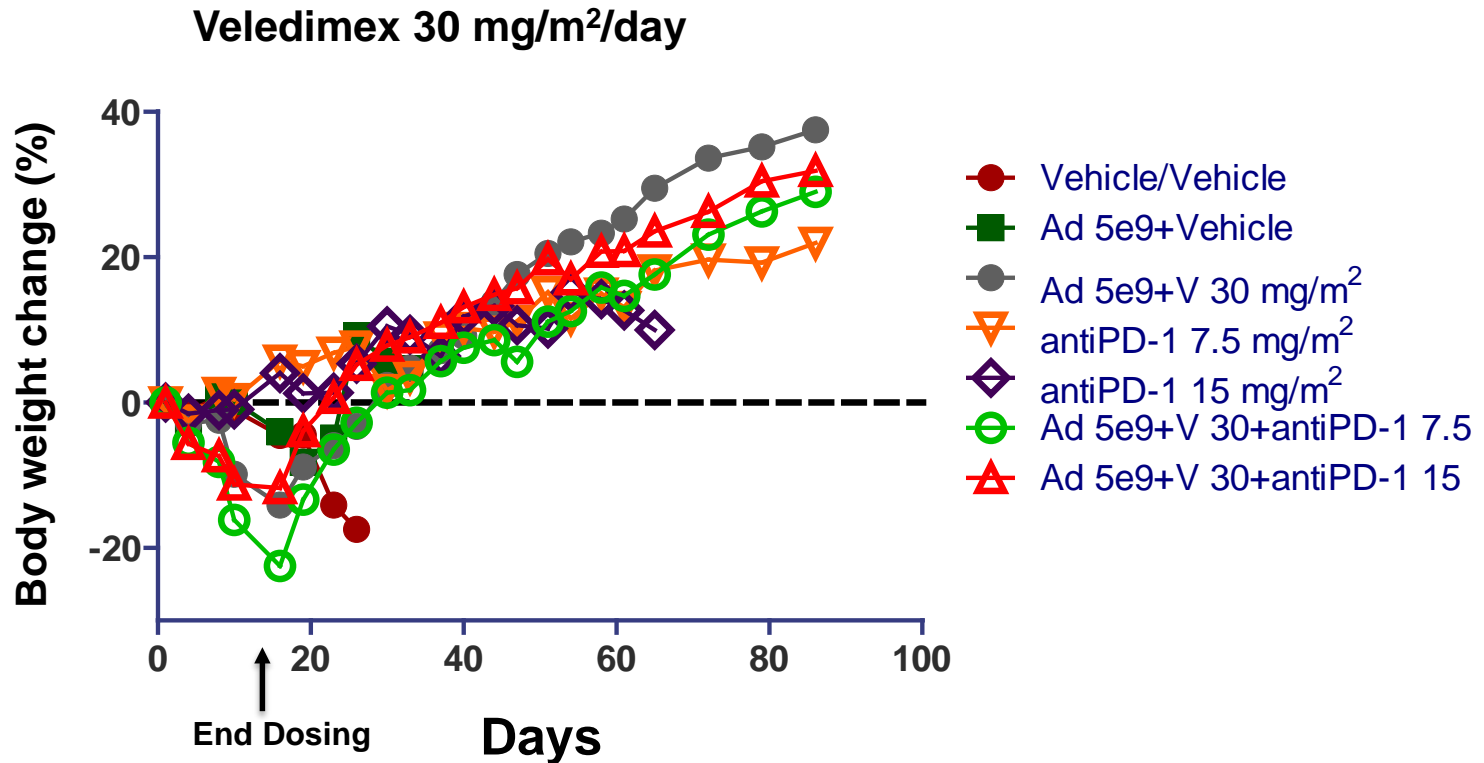
| iCPI     | Antibody | Route | Schedule | Dose (mg/m2) |    |
|----------|----------|-------|----------|--------------|----|
| antiPD-1 | RMP 1-14 | i.p.  | Q4Dx5    | 7.5          | 15 |

# 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/m<sup>2</sup>/day + anti PD1 15mg/m<sup>2</sup> results in 100% survival

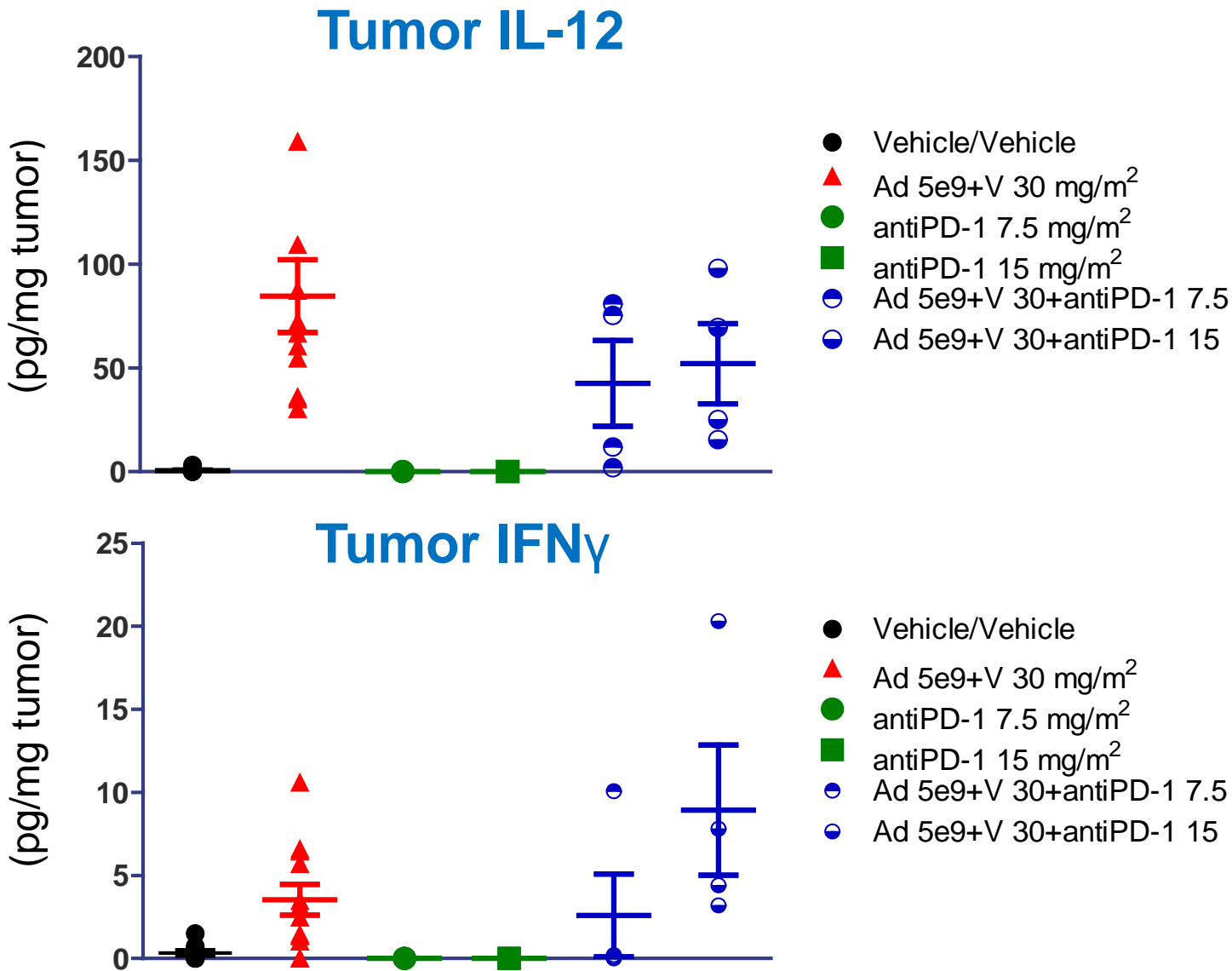
# Ad-RTS-mIL-12 + Veledimex + Anti-PD-1: Body Weight Change



- Ad-RTS-mIL-12 + veledimex + anti-PD-1 therapy augmented the reduction in body weight over monotherapy.
- All groups recovered when veledimex was discontinued.

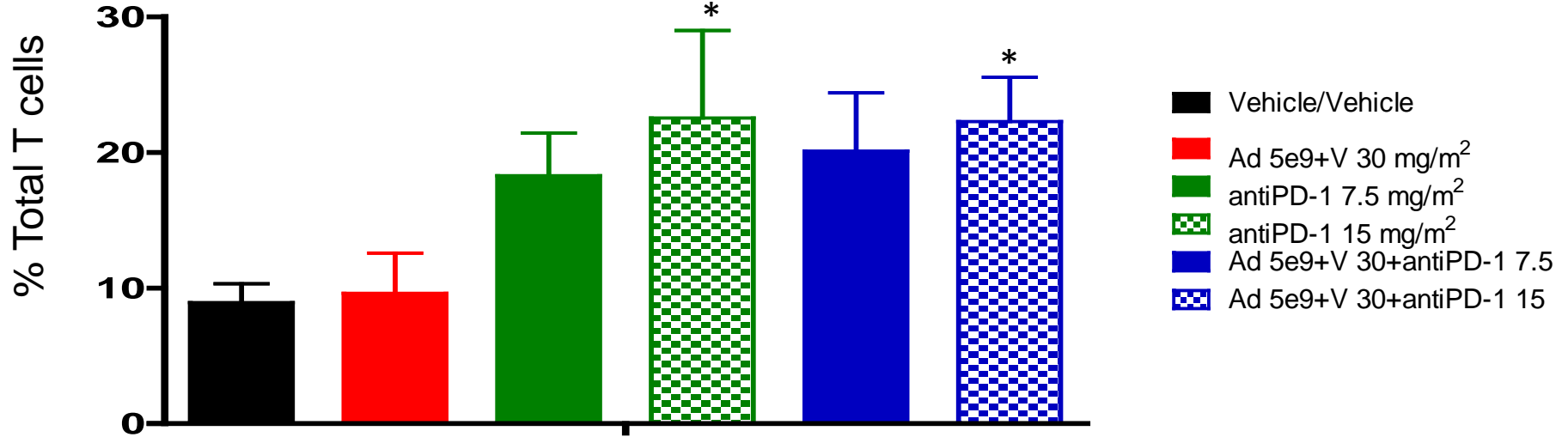


# Ad-RTS-mIL-12 + Veledimex in Combination with Anti-PD-1 (RPM 1-14) on Tumor Cytokines

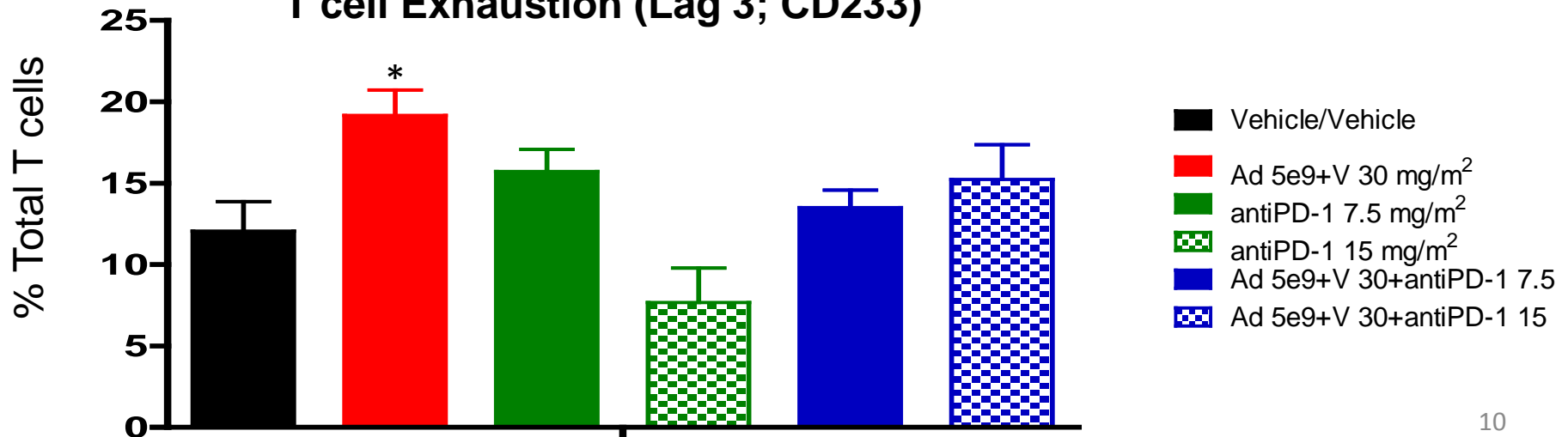


# Ad-RTS-mIL-12 + Veledimex + Anti-PD-1 (RPM 1-14) Tumor FACS

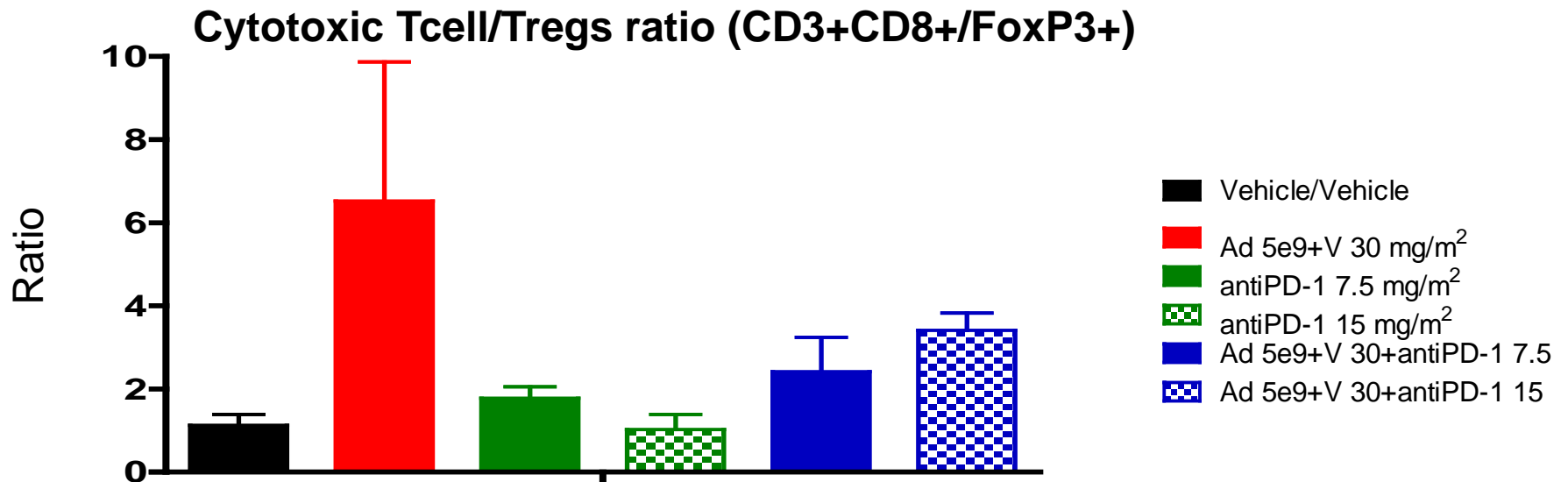
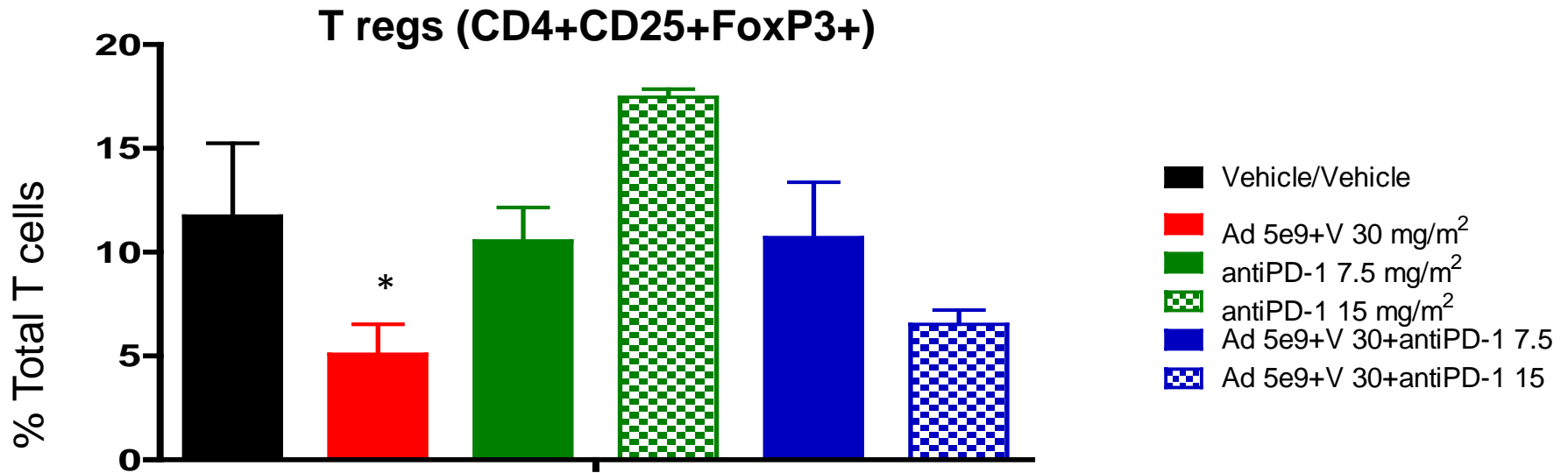
## Cytotoxic T cells (CD3+CD8+)



## T cell Exhaustion (Lag 3; CD233)



# Ad-RTS-mIL-12 + Veledimex + Anti-PD-1 (RPM 1-14) Tumor FACS



# 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**