Localized Regulated Expression of IL-12 as a Gene Therapy Approach to Cancer Immunotherapy

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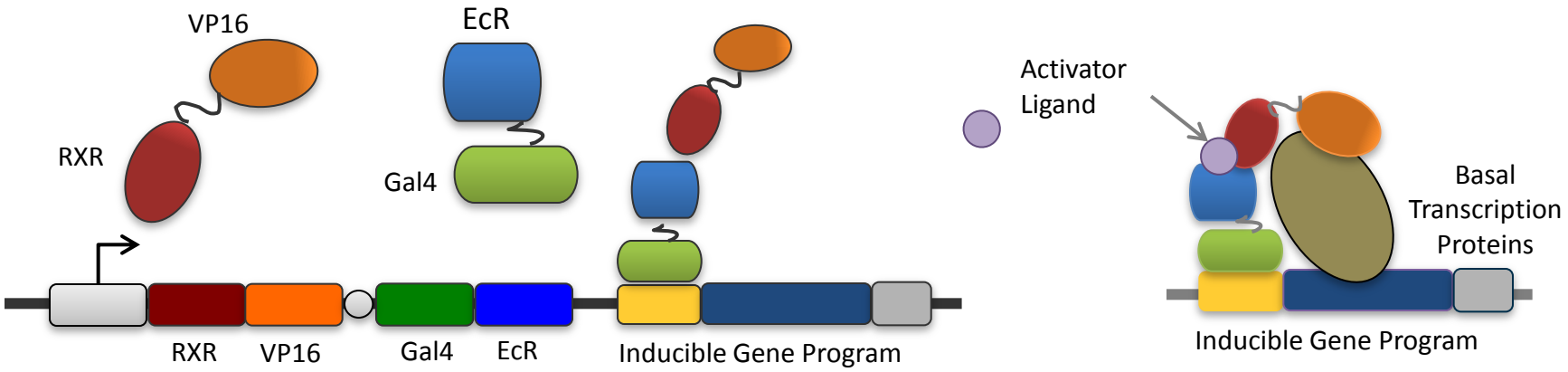
Background & Rationale IL-12 in Oncology

- Tumors grow & escape the immune system through the process of immunoediting. Thus, restoration of the immune system's ability to detect the tumor should result in improved treatment outcomes.
- Localized IL-12 administration has been shown to have antitumor activity that is mediated by direct tumor cell cytotoxicity, and enhancement of immuno-regulatory activities including activation of anti-tumor natural killer (NK) cells, $CD4^+$ T cells and $CD8^+$ T cells.

Inducible Gene Regulation: RheoSwitch Therapeutic System®

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

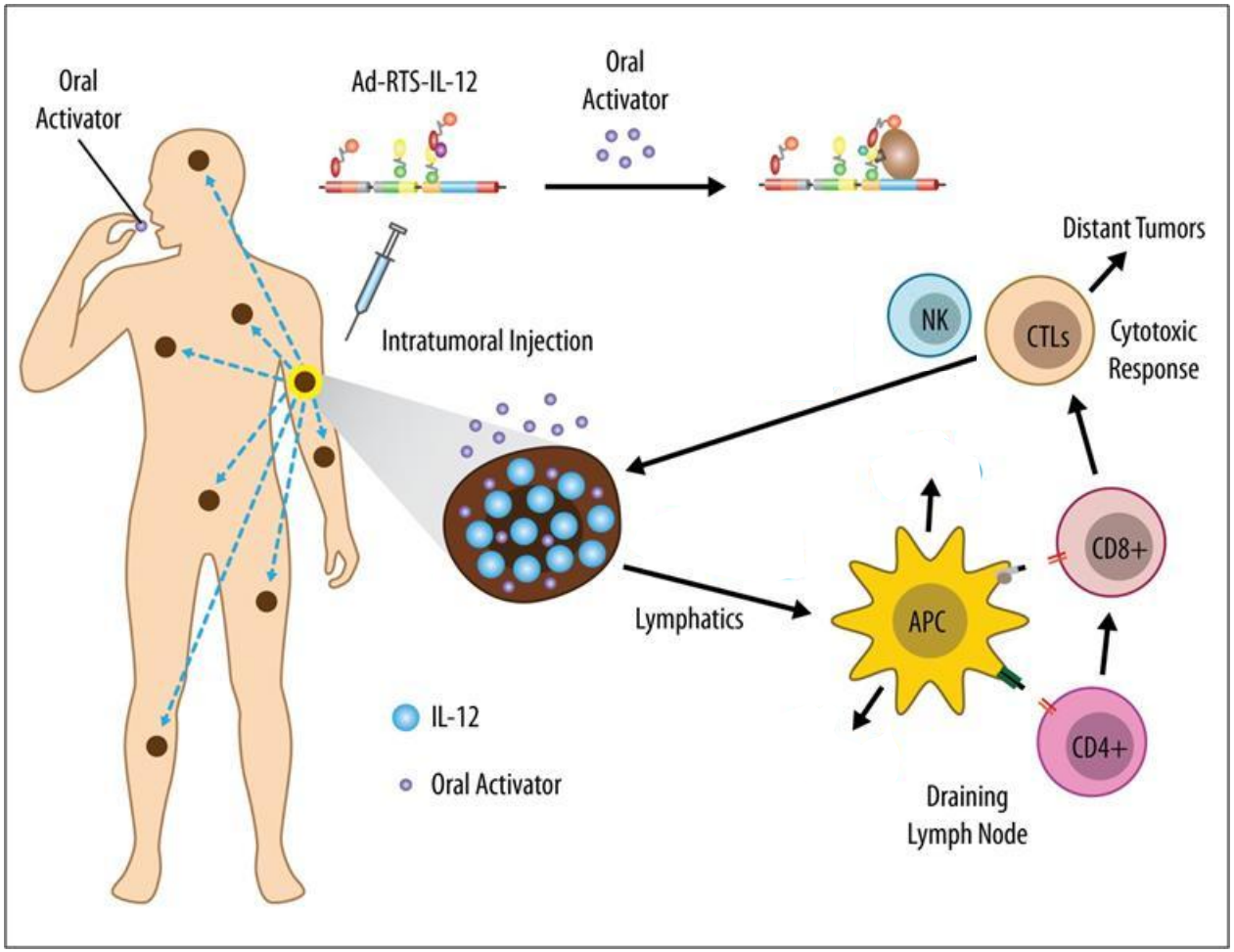


1. **The Switch Components**: The RTS® gene program includes 2 receptor protein fusions: VP16-RXR and Gal4-EcR. They form unstable and unproductive heterodimers in the absence of any ligand.

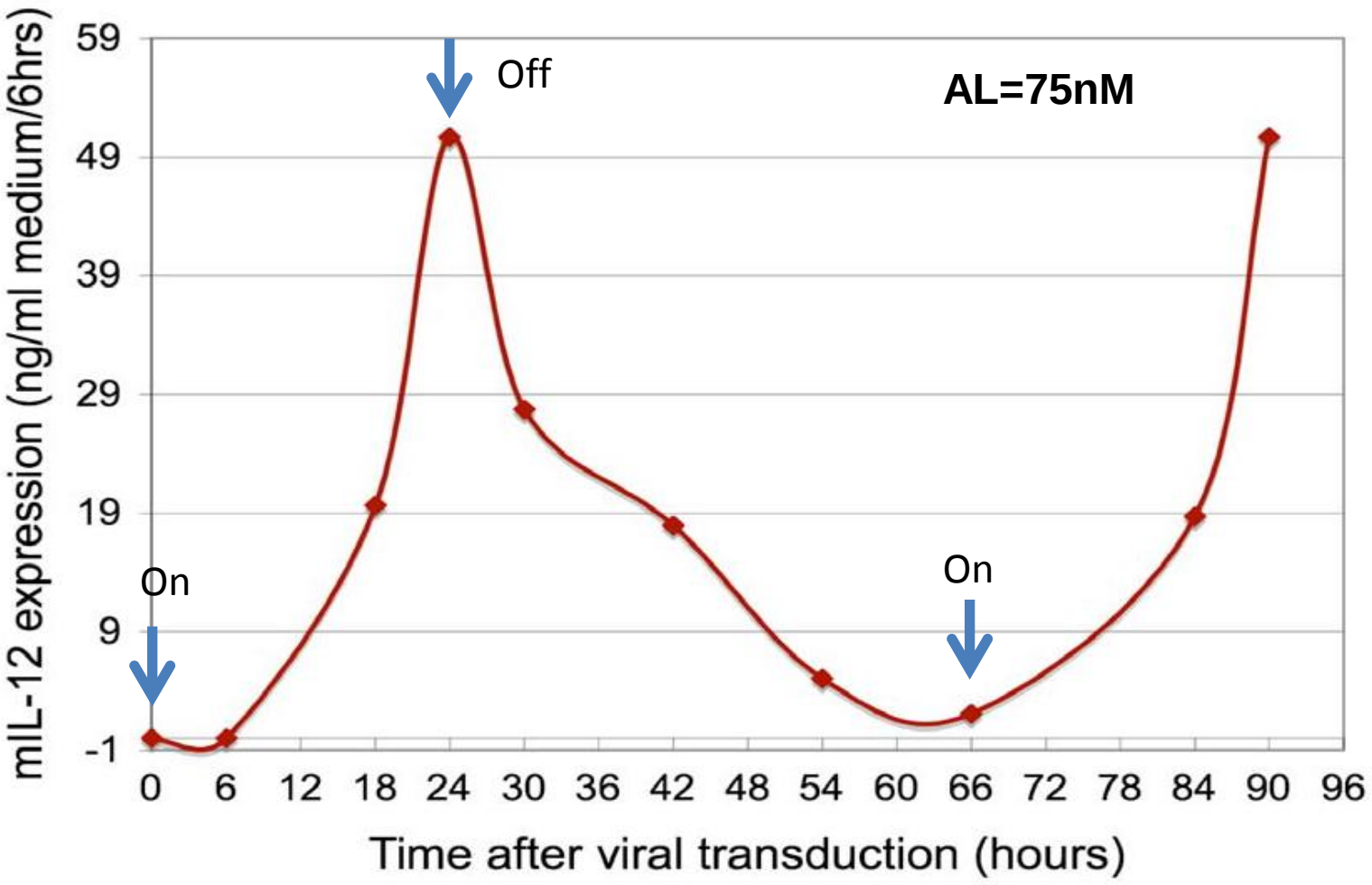
2. **The Inducible Promoter**: A customizable promoter to which basal transcription proteins are recruited and the target gene is transcribed.

3. **The Activator Ligand (veledimex):** An ecdysone analog, diacylhydrazine-based small molecule functions as an activator. In the presence of the ligand, the protein heterodimer changes to a stable conformation and binds to the inducible promoter.

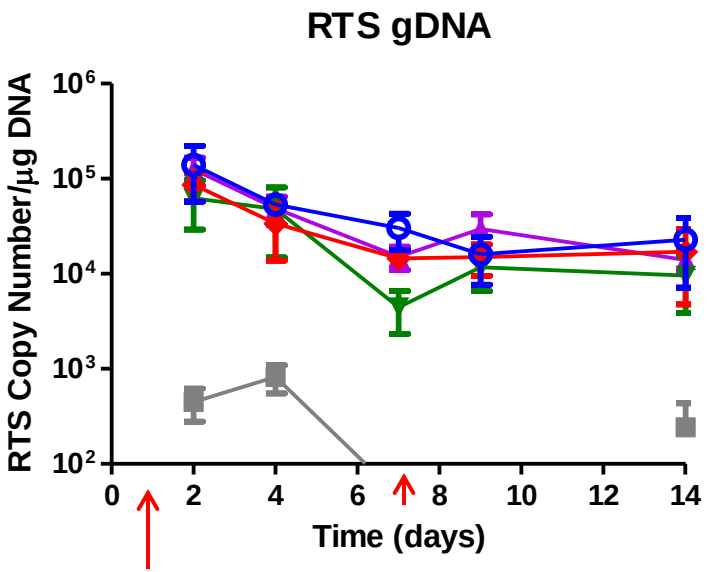
Regulated intratumoral expression of IL-12 promotes activation of TIL's to drive a cytotoxic immune response against distant tumors

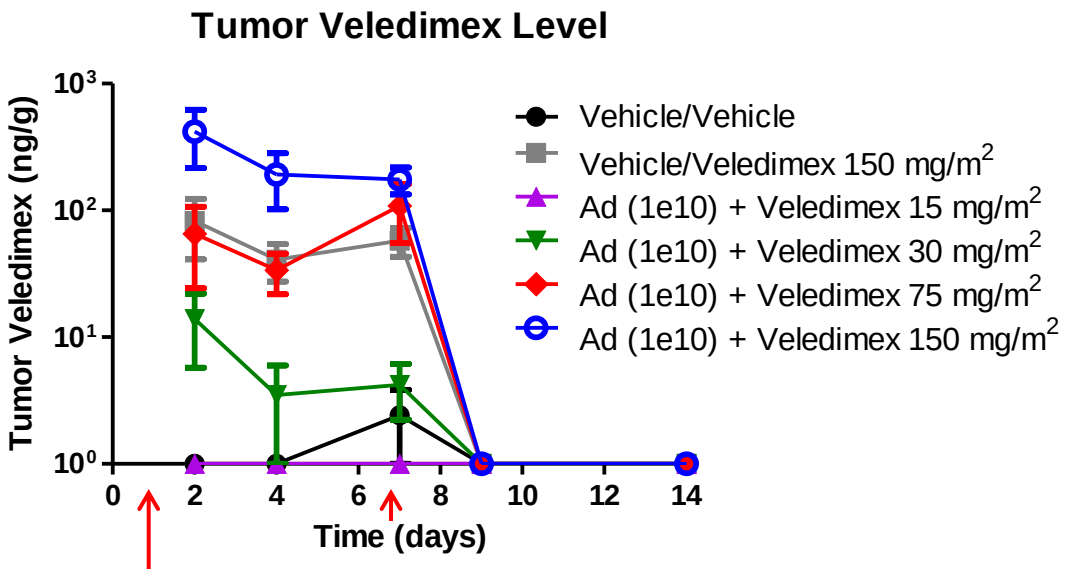


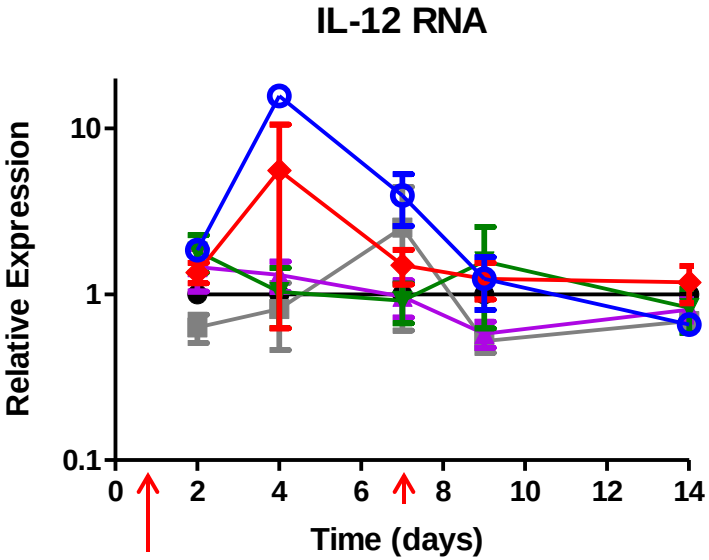
IL-12 Production is Modulated by Activator Ligand in HT 1080 Cells

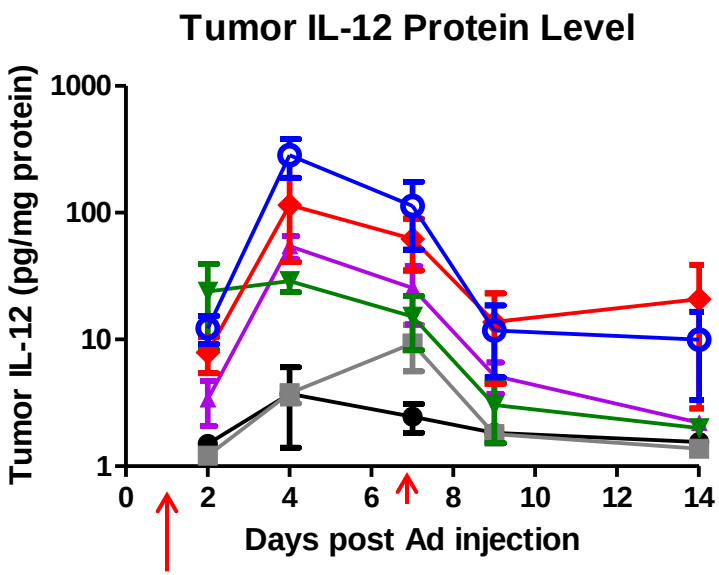


Dose-Dependent Increase in Expression of Tumor IL-12 mRNA & IL-12 Protein in Response to Veledimex

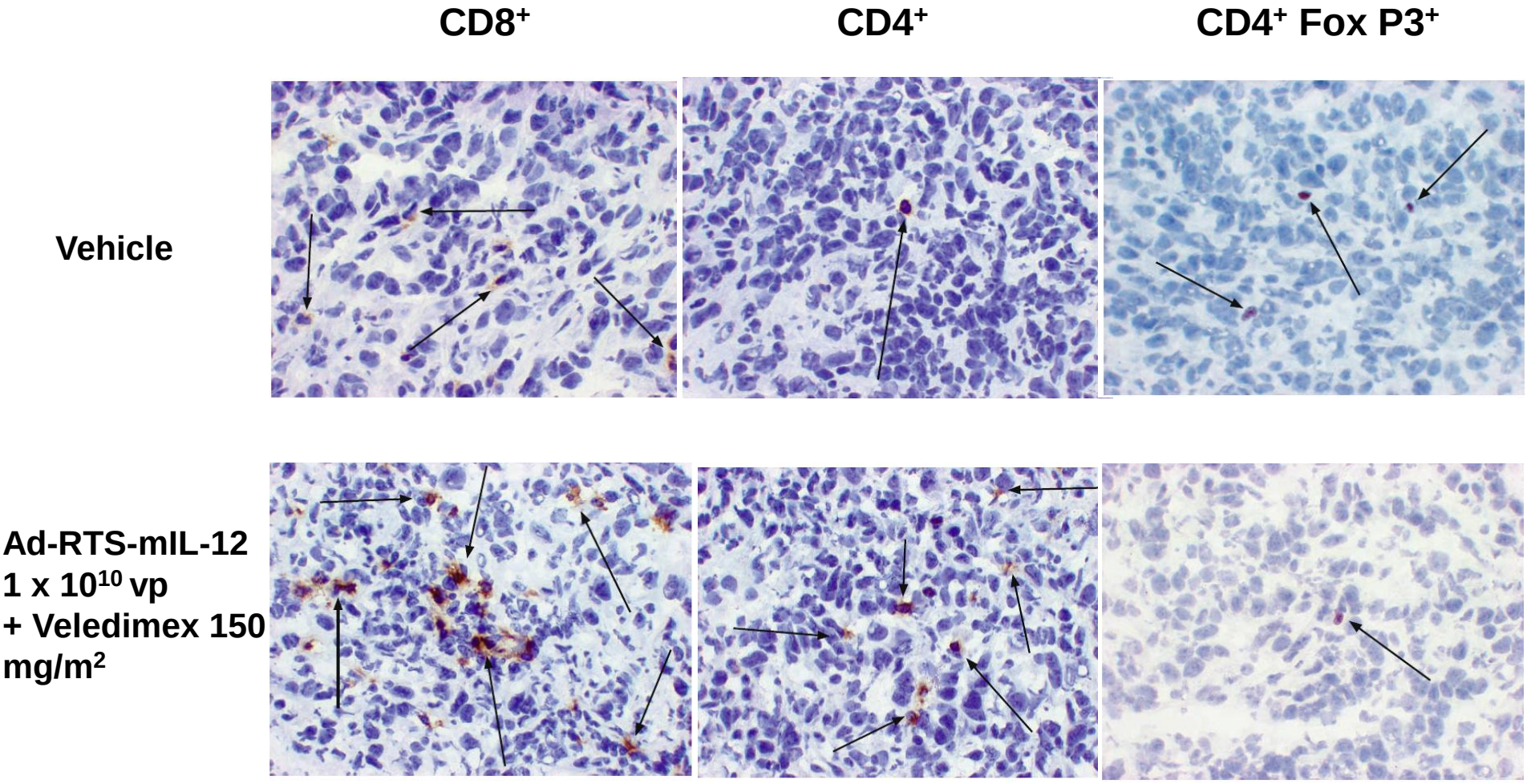




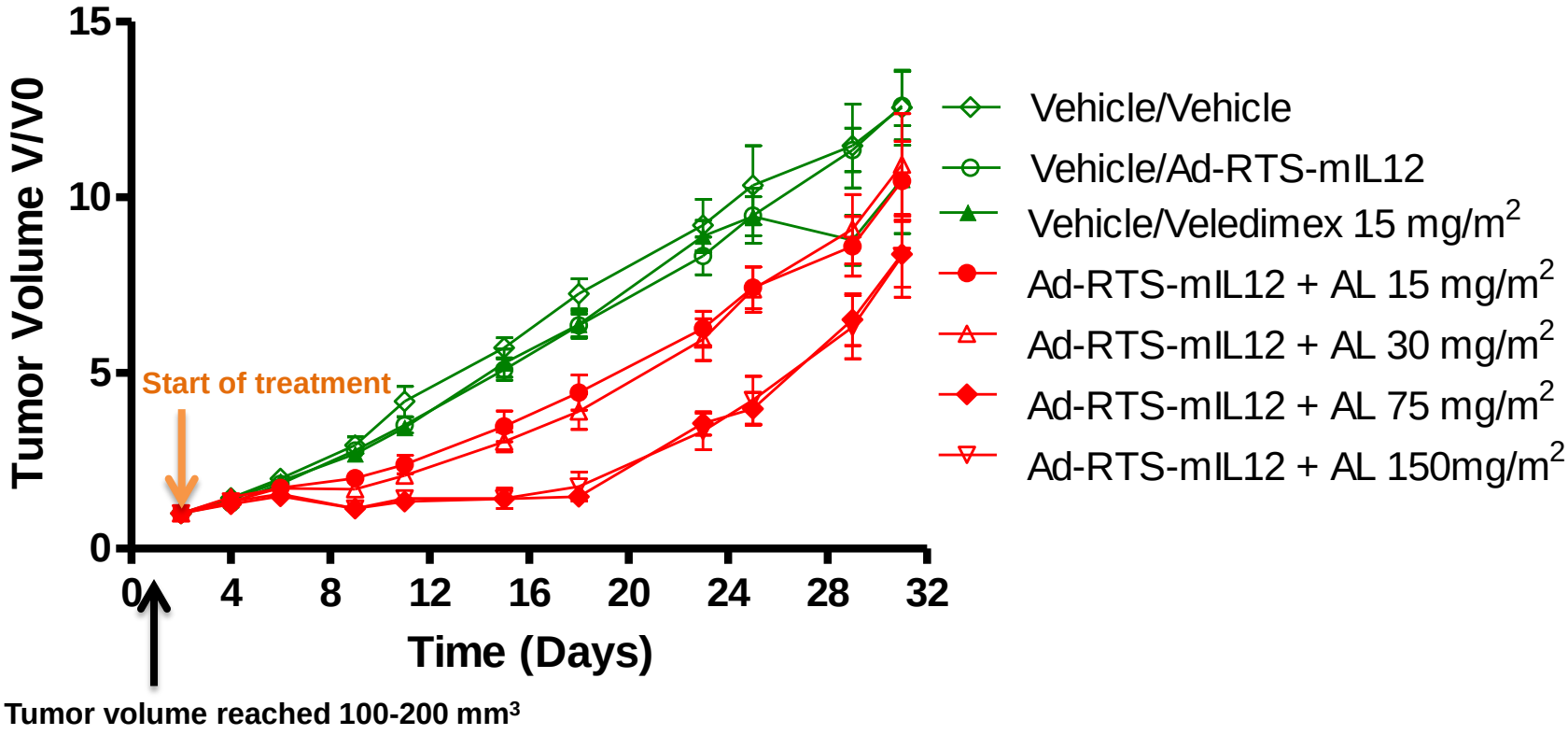




Ad-RTS-mIL-12 + Veledimex Increases Tumor CD8$^+$ & CD4$^+$ While Decreasing CD4$^+$ Fox P3$^+$ TILs in the 4T1 Syngeneic Mouse



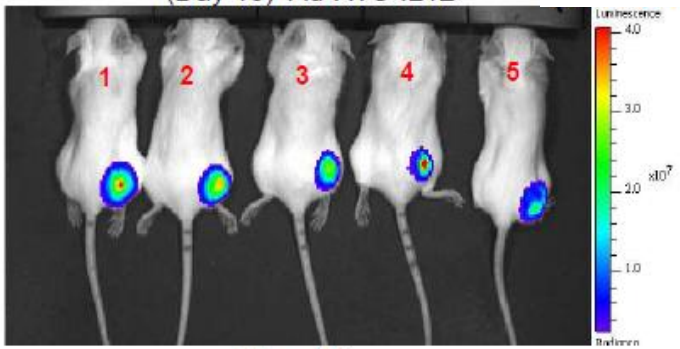
Dose-Dependent Anti-Tumor Activity of Ad-RTS-mIL-12 + Veledimex (AL) in Murine 4T1 Model



Rechallenge Study

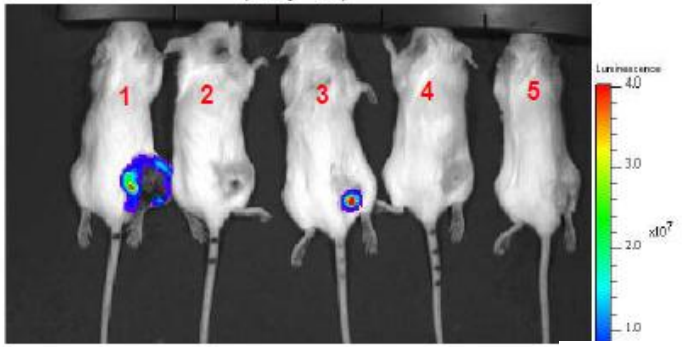
Ad-RTS-mIL12 Developed Protective Immunity Against Colon Cancer

(Day 15) Ad-RTS-IL12 **RIGHT Flank**



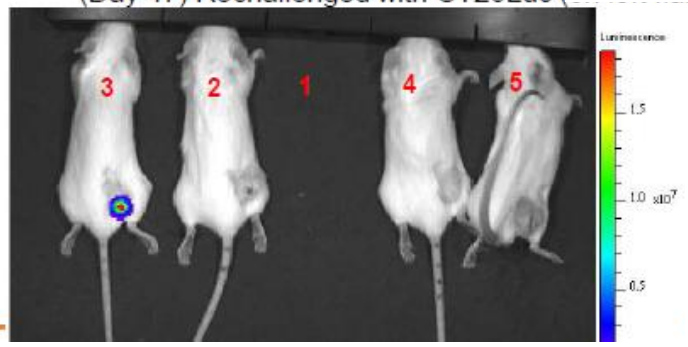
1

(Day 43)



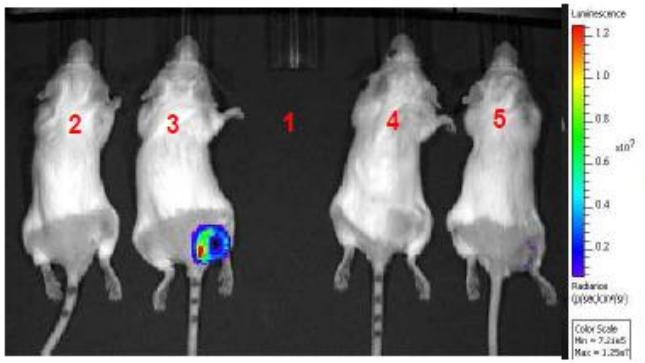
2

(Day 47) Rechallenged with CT26Luc (**LEFT Flank**



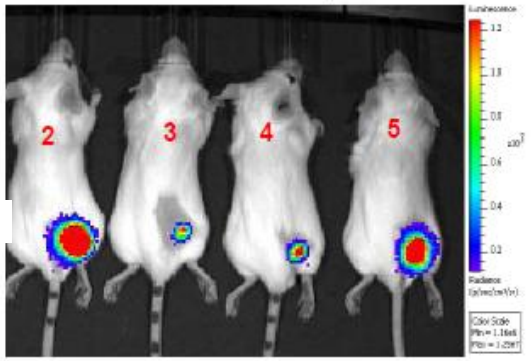
3

(Day 68) No tumor developed at 21 days post rechallenge



4

Control animals all developed tumor nodule



5

Clinical Observations to Date

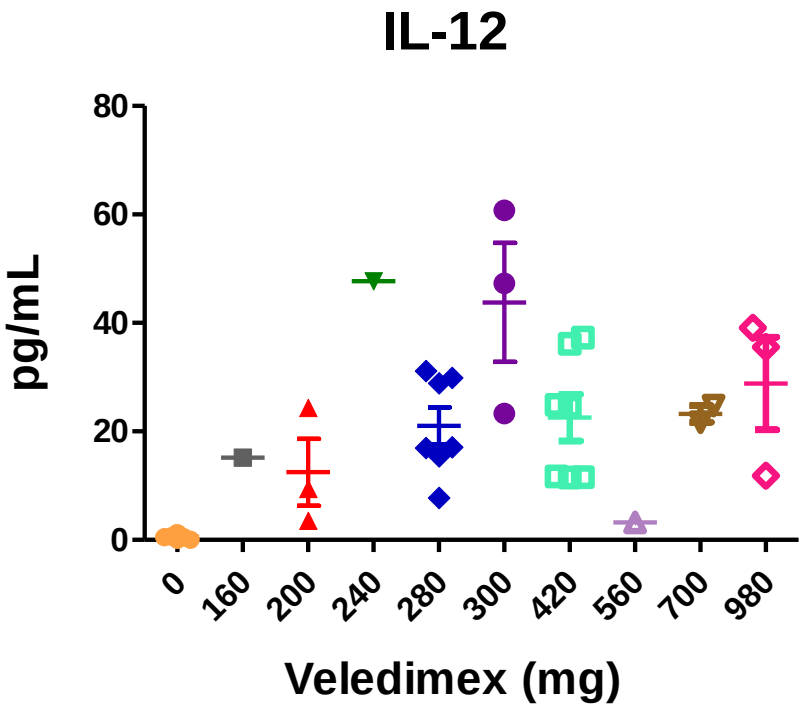
We can control gene expression to achieve a systemic immune response

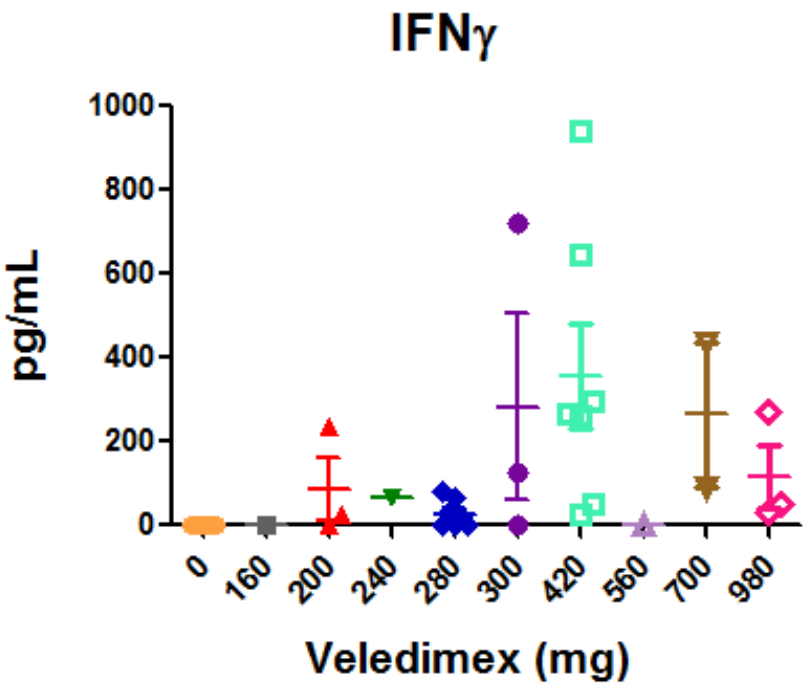
- High expression of IL-12 mRNA in tumors, tightly controlled by veledimex dose
- Tumor biopsies show increased tumor infiltrating lymphocytes in both injected and systemic non-injected lesions

We have seen systemic and fully reversible toxicity

- Serious adverse events are mechanism-based and consistent with immunotherapy (Fever, N&V, leukopenia, increased LFTs, hyponatremia, cytokine release response)
- Serious adverse events reversed within days after stopping veledimex dosing
- Subjects who have had IL-12 expression turned “off” have been redosed, and IL-12 turned “on” again

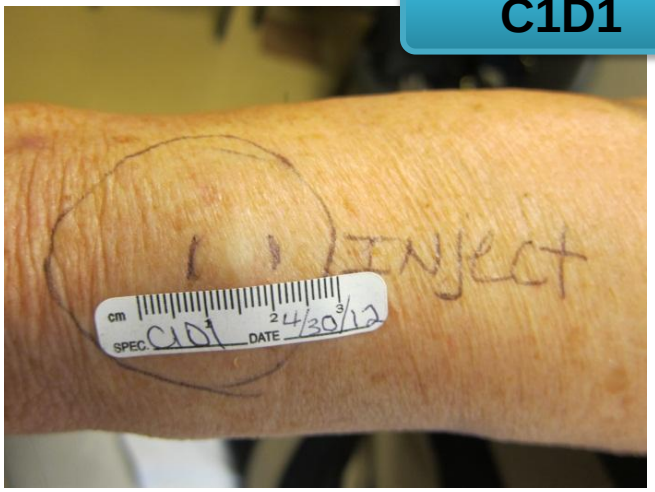
Increase in Patient Serum Cytokine Expression Cycle 1



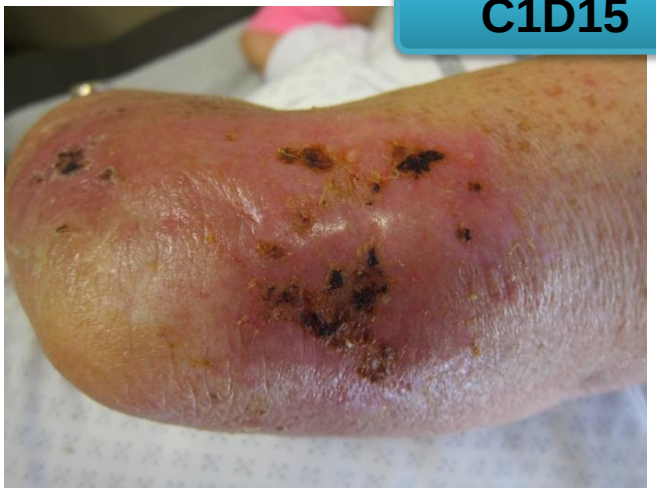


Effects of Ad-RTS-hIL-12 + Veledimex (AL) in Melanoma Patient

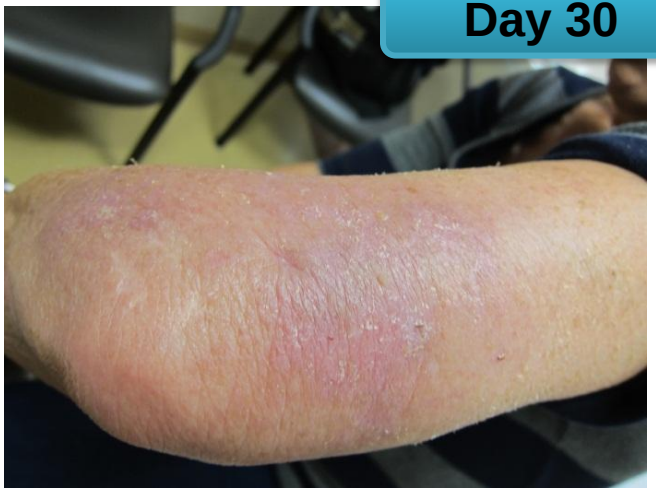
C1D1



C1D15

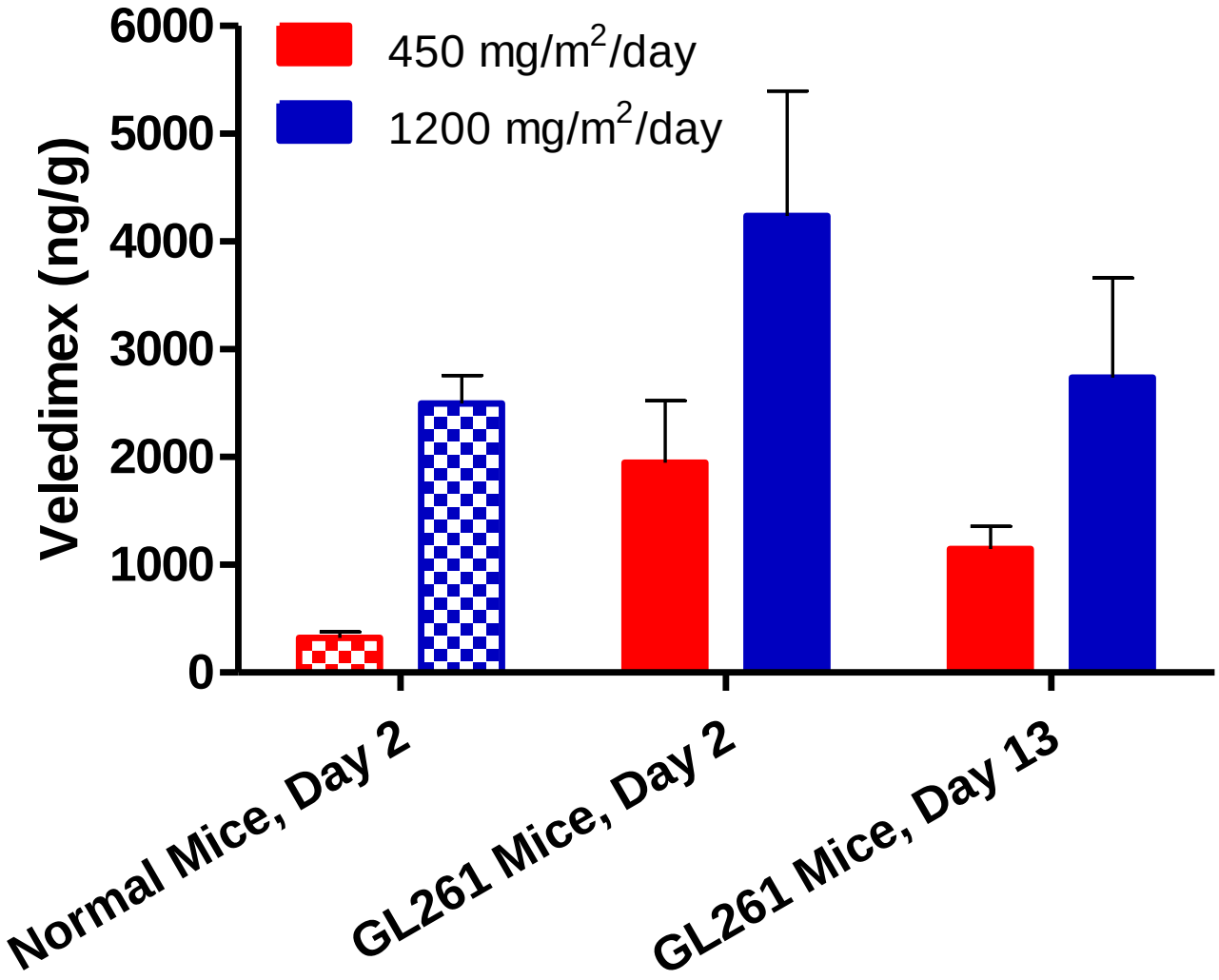


Day 30



- Initial increase in lesion size due to inflammatory response seen at Cycle 1 Day 15
- Lesion was undetectable at Day 30

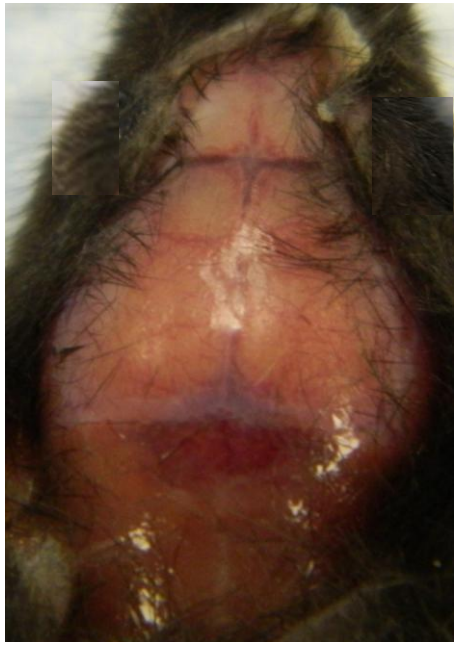
Higher Veledimex Levels Normal and in GL261 Orthotopic Glioma Mouse Brains



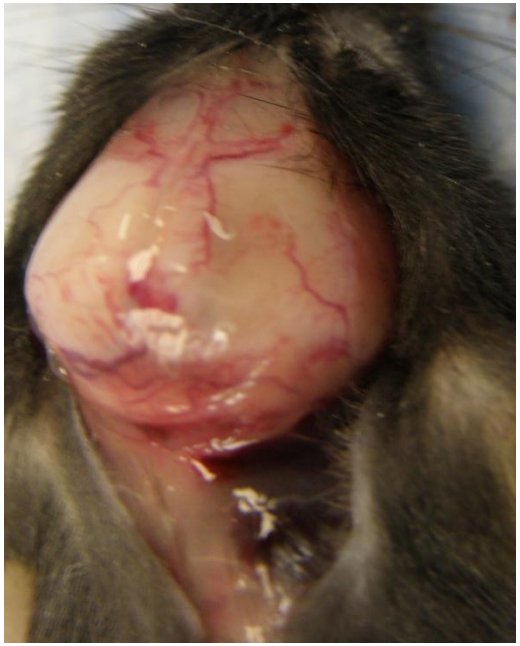
Veledimex levels at 24 hr posttreatment

Effects of Ad-RTS-mIL-12 + Veledimex (AL) in the Orthotopic GL261 Mouse

Normal Mouse

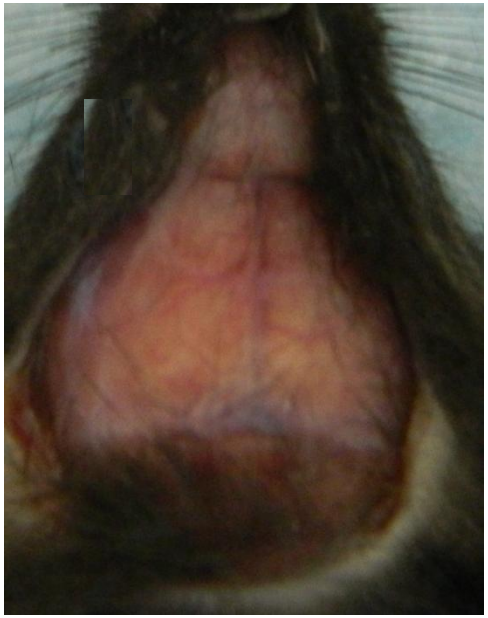


Control Day 20



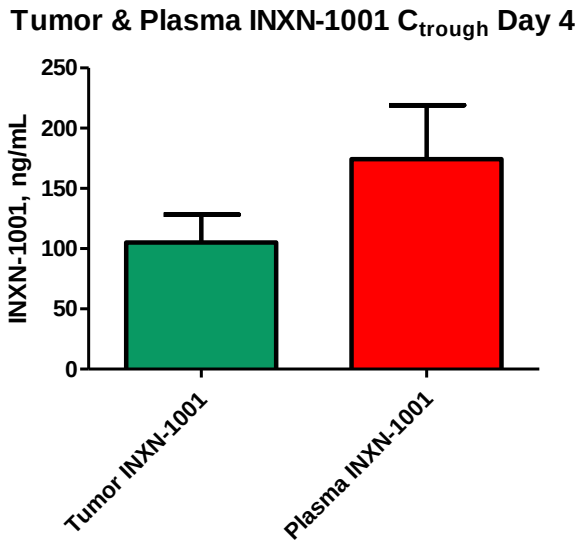
Vehicle
BID x 14

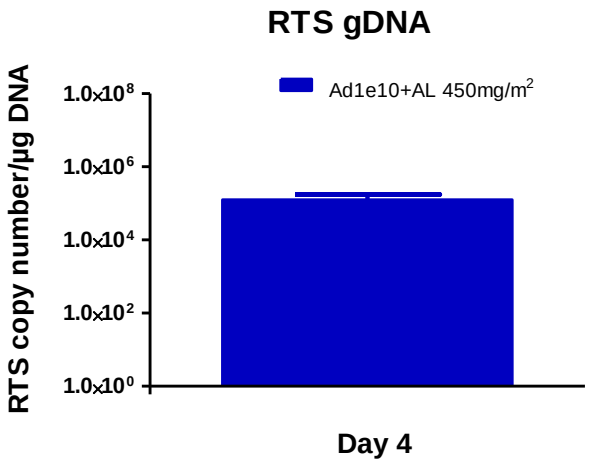
Treatment For 14 days
Day 74 (end of study)

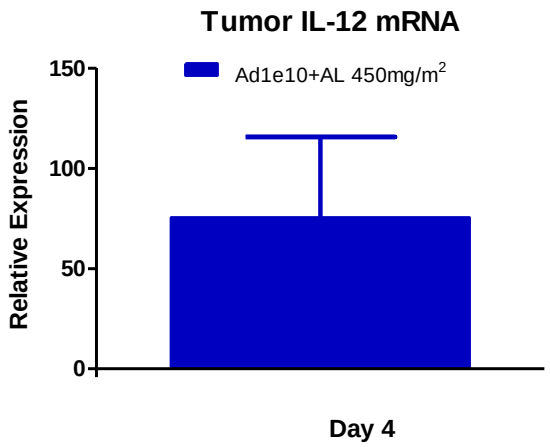


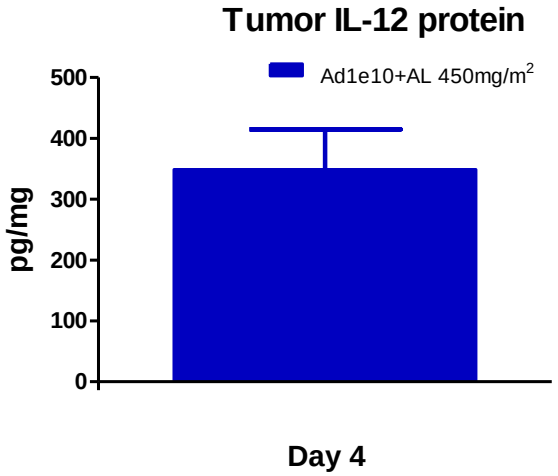
Ad-RTS-mIL-12 $1x10^{10}$vp
+ AL 450 mg/m^2/day
BID x14

Increased Expression of Tumor IL-12 mRNA & IL-12 Protein in Response to Veledimex

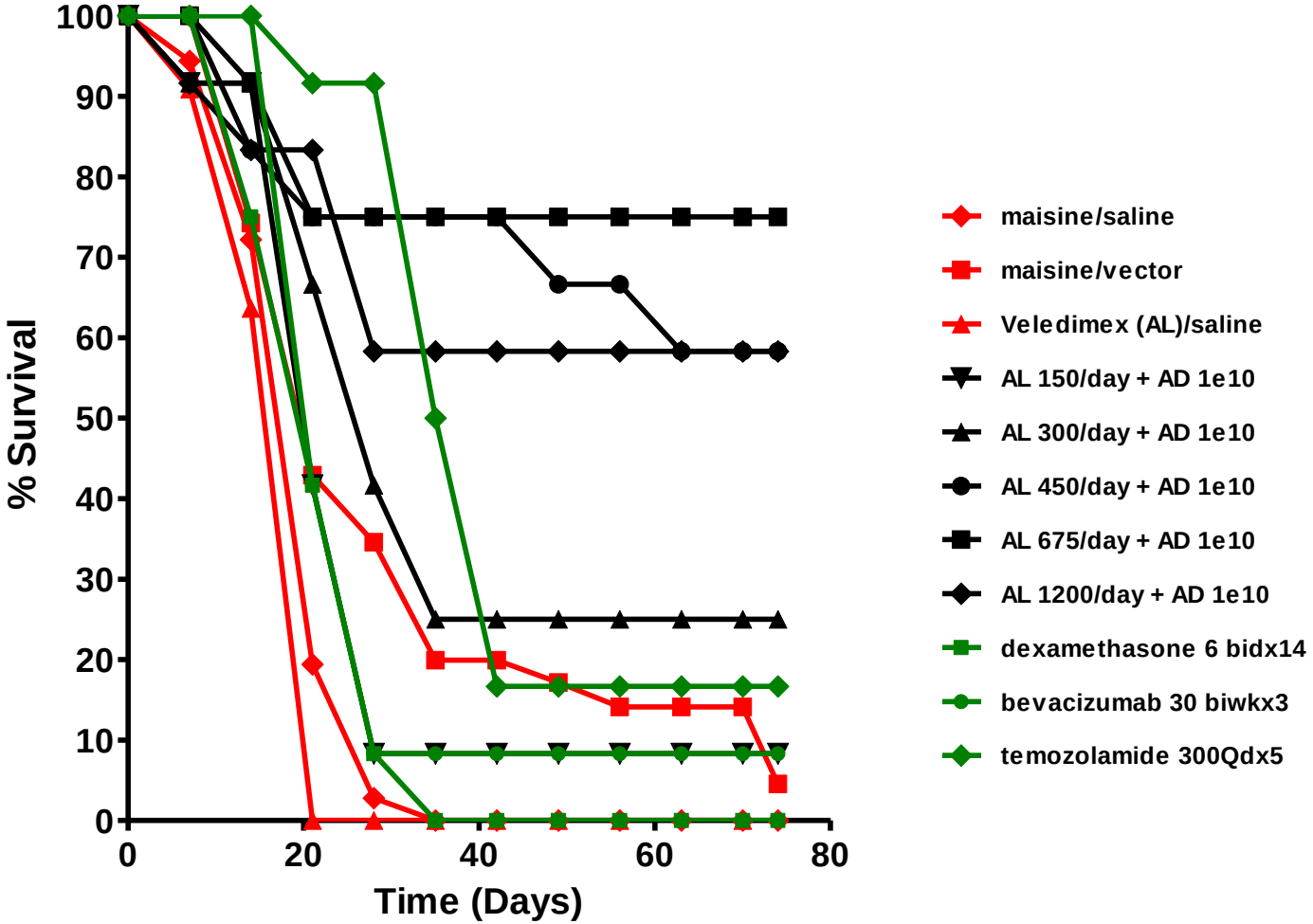






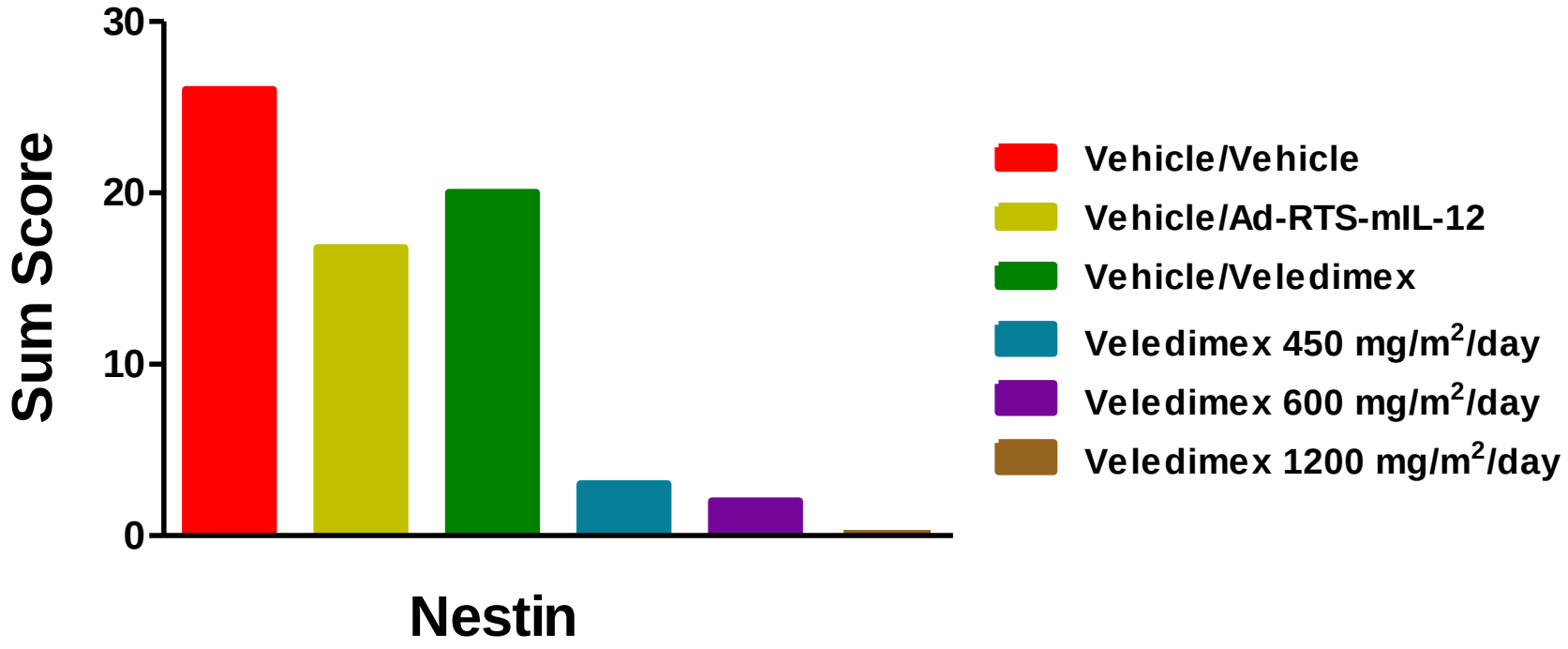


Ad-RTS-mIL-12 + Veledimex (AL) Results in Increased Survival When Compared to Control in the GL261 Orthotopic Glioma Mouse Model



Ad-RTS-mIL12 administered on Day 5 ; Veledimex (mg/m^2) administered BID for 14 days from Day 5;

Ad-RTS-mIL-12 + veledimex significantly reduces brain cancer stem cells in GL-261 Orthotopic Glioma Model



Nestin levels (marker for cancer stem cells)
inverse correlation with survival (Pearson r= 0.92)

Conclusions

- Ad-RTS-hIL-12 + veledimex PO exhibits controllable systemic immune activation in human subjects with melanoma and breast cancer.
- Veledimex exhibits dose-related increases in plasma and brain tissue exposure with no accumulation in brain.
- Ad-RTS-mIL-12 ($1x10^{10}$ vp) + veledimex PO improves survival over temozolomide, dexamethasone and bevacizumab.
- Ad-RTS-mIL-12 + veledimex significantly reduces brain cancer stem cells
- These findings support the utility of localized, regulatable IL-12 production as an approach for the treatment of malignant glioma in human subjects.