INTRATUMORAL REGULATED EXPRESSION OF IL-12 AS A GENE THERAPY APPROACH TO IMMUNOTHERAPY

John Nemunitis¹, John A. Barrett², Francois Lebel², Thomas D. Reed³, E. Antonio Chiocca⁴, Antonio M. Omuro⁵, Larry Norton⁵, Jonathan Lewis²

¹ Mary Crowley Cancer Research Centers, Dallas, TX, United States, 75201
² ZIOPHARM Oncology Inc., Boston, MA, United States, 02129
³ Intrexon Corporation, Germantown, MD, United States, 20876
⁴ Brigham and Woman’s Hospital, Harvard Medical School, Boston, MA, United States, 02115
⁵ Memorial Sloan-Kettering Cancer Center, New York, NY, United States, 10065
Background & Rationale IL-12 in Glioma

• Tumors 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.
Background & Rationale IL-12 in Glioma

- Roy & Kranz (University of Illinois) IL-12. *Journal of Immunology*, 2000, 165: 7293–7299. **Model:** SV11 Transgenic mouse administered recombinant mIL-12 i.c. **Findings:** Increased survival infiltration of activated CD8 and CD4 T cells.

- Vom Berg & Becher (University of Zurich) J Exp Med, 2013, 210: 2803-2811. **Model:** GL261 transduced to constitutively express IL-12 i.c. **Findings:** Increased survival combination of IL-12 + CTLA4 elicits decrease Tregs while increasing Teff.

- Dimeco & Olivi (Johns Hopkins School of Medicine & Istituto Nazionale Tumori, Milan, Italy) *J Neurosurg* 2000, 92:419–427. **Model:** Rat 9L gliosarcoma cells expressing IL-12. **Findings:** Local delivery of IL-12 in rat brain prolongs survival in animals challenged i.c. with a malignant glioma cells.

- Sonabend & Lesniak (University of Chicago). *Anti-Cancer Drugs* 2008, 19:133–142. **Model:** GL-261 orthotopic glioma model. **Findings:** Synergy in survival with locally administered pmIL-12/PPC + biodegradable carmustine

- Markert & Whitley (University of Alabama) *Journal of Virology* 2012, 86: 5304–5313 **Model:** 4C8 glioma cells orthotopic B6D2F1 mouse. **Findings:** Hsv mutant M002 expressing IL-12 demonstrated prolonged survival vs. control

- Liu & Yu (Cedars Sinai Medical Center) *Cancer Gene Therapy* (2002) 9, 9–15 **Model:** GL-26 orthotopic mouse. Ad5-mIL-12 **Findings:** Survival was significantly prolonged in Ad-mIL-12–treated animals with increased CD4+ and CD8+ T- cell infiltration
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.
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

**RTS gDNA**

- **IL-12 RNA**

- **Tumor Veledimex Level**
  - Vehicle/Vehicle
  - Vehicle/Veledimex 150 mg/m²
  - Ad (1e10) + Veledimex 15 mg/m²
  - Ad (1e10) + Veledimex 30 mg/m²
  - Ad (1e10) + Veledimex 75 mg/m²
  - Ad (1e10) + Veledimex 150 mg/m²

- **Tumor IL-12 Protein Level**
Ad-RTS-mIL-12 + Veledimex Increases Tumor CD8⁺ & CD4⁺ While Decreasing CD4⁺ Fox P3⁺ TILs in the 4T1 Syngeneic Mouse

**Vehicle**

**Ad-RTS-mIL-12**

1 x 10¹⁰ vp

+ Veledimex 150 mg/m²
Dose-Dependent Anti-Tumor Activity of Ad-RTS-mIL-12 + Veledimex (AL) in Murine 4T1 Model

Tumor volume reached 100-200 mm³
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
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

Treatment For 14 days
Day 74 (end of study)

Vehicle
BID x 14

Ad-RTS-mIL-12 1x10^{10}vp
+ AL 450 mg/m^2/day
BID x 14
Ad-RTS-mIL-12 + Veledimex (in Chow) Results in Increased Survival in the GL261 Orthotopic Glioma Mouse Model

Veledimex administered *ad lib* in chow from Day 4 to EOS at ~ 675 mg/m²/day
Ad-RTS-mIL12 administered on Day 5
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/m²) 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-mIL-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.