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

John A. Barrett Ph.D.
Ziopharm Oncology, Boston MA 02129
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.
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

**RTS gDNA**

**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

**IL-12 RNA**

**Tumor IL-12 Protein Level**

- Vehicle
- AL 150 mg/m²
- AL 15 mg/m² + Ad 1e10vp
- AL 30 mg/m² + Ad 1e10vp
- AL 75 mg/m² + Ad 1e10vp
- AL 150 mg/m² + Ad 1e10vp

**Time (days)**

Relative Expression

Note: The graphs illustrate the change in RT S gDNA, Tumor Veledimex Level, IL-12 RNA, and Tumor IL-12 Protein Level over different time points and with varying doses of Veledimex and AL.
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 10^{10} 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 mm$^3$
Rechallenge Study

Ad-RTS-mIL12 Developed Protective Immunity Against Colon Cancer

(Day 15) Ad-RTS-IL12

RIGHT Flank

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

(Day 43)

2

(Day 47) Rechallenged with CT26Luc

LEFT Flank

Control animals all developed tumor nodule
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

**IL-12**

**IFNγ**

(pg/mL vs. Veledimex (mg))
Effects of Ad-RTS-hIL-12 + Veledimex (AL) in Melanoma Patient

- 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

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

Tumor & Plasma INXN-1001 $C_{\text{trough}}$ Day 4

- INXN-1001, ng/mL

- Tumor INXN-1001
- Plasma INXN-1001

RTS gDNA

- RTS copy number/µg DNA
- Day 4

Tumor IL-12 mRNA

- Relative Expression
- Day 4

Tumor IL-12 protein

- pg/mg
- Day 4

Ad1e10+AL 450mg/m²

$1.0 \times 10^0$

$1.0 \times 10^2$

$1.0 \times 10^4$

$1.0 \times 10^6$

$1.0 \times 10^8$
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-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.