DEMONSTRATION OF SYSTEMIC ANTITUMOR IMMUNITY VIA INTRATUMORAL REGULATED EXPRESSION OF IL-12 IN ADVANCED BREAST CANCER AND MELANOMA PATIENTS

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Abstract

Immunotherapy is an attractive approach to cancer treatment. Localized controlled production of IL-12 in a tumor generates both local and systemic antitumor immune responses. We have developed an adenoviral vector, Ad-RTS-IL-12 (AD), administered intratumorally under control of the RheoSwitch Therapeutic System® (RTS®) expression platform. Gene expression and subsequent IL-12 protein production is tightly controlled by oral administration of the small molecule activator ligand veledimex (AL).

We have shown on mechanism biologic activity and safety with this system in syngeneic mouse studies of melanoma and breast cancer (BC). Those studies demonstrated an AL dose-related increase in tumor IL-12 mRNA and IL-12 protein expression. In addition, a return to baseline IL-12 mRNA and IL-12 protein expression was observed on cessation of AL. These changes correlate with a local and systemic immune and antitumor response.

Subsequently, two open label, phase 2 trials evaluating the safety of inducible IL-12 expression in heavily pretreated subjects with recurrent/metastatic breast or melanoma cancer with surface accessible lesions were performed. The results of these studies showed biologic activity with an acceptable therapeutic index. Treatment with Ad-RTS-hIL-12 + veledimex resulted in an increase in IL-12 and downstream IFN-gamma production and was followed by a rapid increase in IL-10 and IP-10 (indicating IL-12 biologic activity). Tumor lesion results from these two maximum tolerated dose studies are encouraging. In the breast cancer study, 12 subjects were administered Ad-RTS-IL-12 + veledimex. A total of 16 non-injected lesions in 7 breast cancer subjects were evaluated. Of the 16 non-injected lesions, 4 increased in diameter >20%, 6 increased in diameter 0-20% with observed decreases in lesion diameters of 1 lesion 12%, 2 lesions 30-49% and 3 lesions 50-100%. Injected lesions were not evaluated in this study. In the melanoma study, 26 subjects were administered Ad-RTS-IL-12 + veledimex. A total of 70 non-injected lesions in 19 melanoma subjects were evaluated. Of the 70 lesions, 23 increased in diameter >20%, 30 increased in diameter 0-20% with observed decreases in lesion diameters of 7 lesions 10% to 19%, 4 lesions 20-29%, 4 lesions 30-49%, and 3 lesions 50-100%. In the same 19 melanoma subjects, a total of 33 injected lesions were evaluated. Of the 33 lesions, 11 increased in diameter >20%, 9 increased in diameter 0-20% with observed decreases in lesion diameters of 3 lesions 10% to 19%, 1 lesion 25%, 4 lesions 30-49% and 5 lesions 50-100%. Most common ≥ Grade 3 treatment emergent adverse events in BC and melanoma included neutropenia and hyponatremia (16% each), hypotension, cytokine release syndrome, AST increase (11% each), dehydration, fatigue, pyrexia (8% each). All TEAEs and SAEs ≥ Grade 3 reversed rapidly upon discontinuation of veledimex

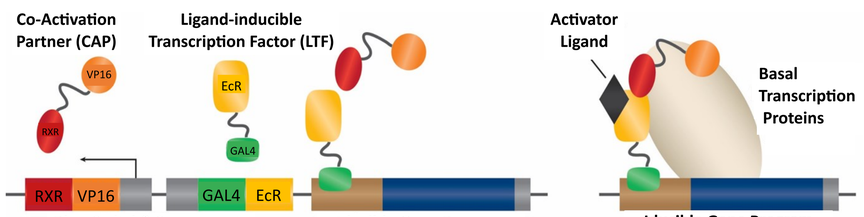
In conclusion, regulated and controllable IL-12 expression using Ad-RTS-hIL-12 + veledimex in all advanced breast cancer and melanoma patients is promising with an acceptable and rapidly reversible adverse experience profile upon discontinuation of veledimex. The administration of Ad-RTS-hIL-12 + veledimex has shown biologic activity and warrants further clinical investigation.

IL-12 and Cancer Immunotherapy

- Interleukin-12 (IL-12)
  - Pro-inflammatory cytokine
  - Master regulator of cell-mediated immunity to pathogens and neoplastic transformation
  - Naturally produced by innate immune cells in response to pathogens
  - Leads to production by T- and natural killer (NK) cells of
    - Interferon-gamma (IFNγ)
    - Tumor necrosis factor alpha (TNFα)
  - Studies confirmed significant systemic toxicities
    - Prompted investigation of alternative delivery routes, e.g., sub-cutaneous, intratumoral, etc.
- Ad-RTS-IL-12 + veledimex explores regulated local treatment strategy
  - Goal is extending the IL-12 therapeutic window
  - Reducing systemic toxicity
  - Ad-RTS-IL-12 injected into tumor
  - IL-12 transcription initiated only in presence of activator ligand Veledimex
  - IL-12 expression level can be modulated by dose and frequency of veledimex administration

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 (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.

Study Design (Melanoma and Breast)

Melanoma: Phase 1/2, Unresectable Stage III/IV Melanoma

- Phase 1: 13 subjects enrolled in a 21 day cycle, intratumoral injection of Ad-RTS-hIL-12 and oral escalating veledimex dose (5-160 mg QD) for a total of 7 doses per cycle
- Phase 2: 13 subjects enrolled using a 28 day cycle, for up to 6 cycles; intratumoral injection of Ad-RTS-hIL-12 and oral veledimex dose (80 - 160 mg QD or QOD) for a total of 7 doses per cycle

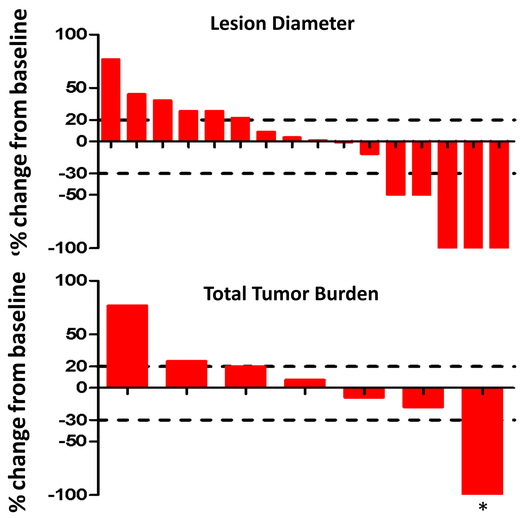
Breast Cancer: Phase 2, Recurrent/Metastatic Breast Cancer

- 12 subjects enrolled on 21 day cycle, for up to 6 cycles; intratumoral injection of Ad-RTS-hIL-12 occurred on Day 1 and oral veledimex (80-140 mg) daily on Days 1-7

Patient Demographics (Melanoma and Breast)

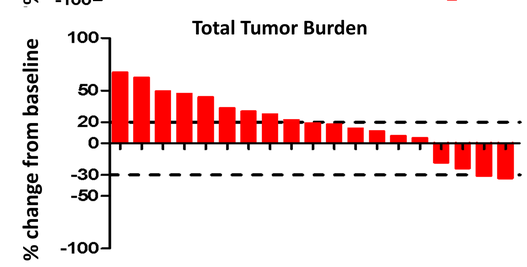
Patient Demographics and Baseline Characteristics	ATI001-101 Melanoma Study N= 26	ATI001-201 Breast Cancer Study N= 12
Age in years - Median (Min, Max)	64 (22-94)	63 (38-88)
Gender Male : Female	18 : 8	1 : 11
Time Since Initial Diagnosis (months) Median (Min, Max)	24 (1.3-133)	111 (8-264)
Stage at Study Entry IIIB IV	 0 26	 1 11
Classification HER2 Breast Cancer HR status ER or PR Breast Cancer Triple-Negative Breast Cancer (TNBC)	NA	25% HER 2+; 67% HER 2-; 8% ND 58% HR+; 42% HR- 25% TNBC
ECOG Performance Status ≤1	26	12
Patients with Visceral Metastatic Sites	92% (24/26)	92% (11/12)
Prior Therapy Mean	6	8

Best Overall Response from Noninjected Lesions and Total Tumor Burden in Evaluable Breast Cancer Subjects



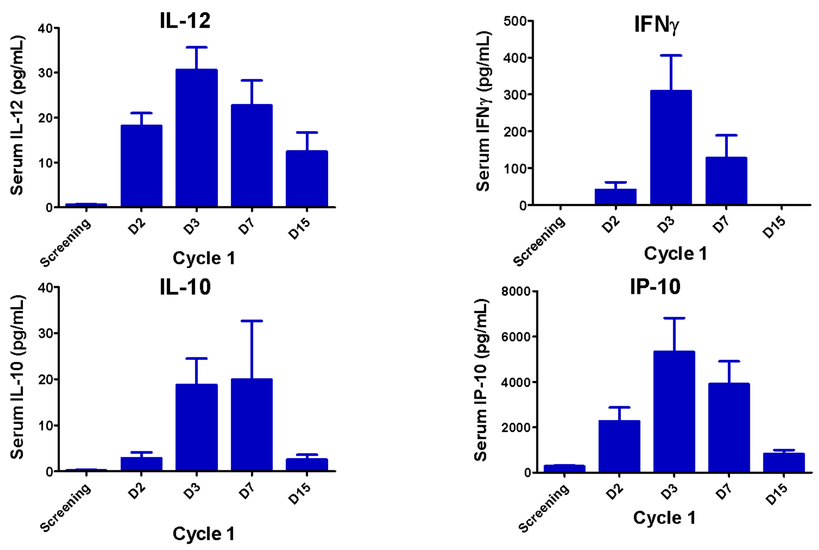
Top Panel: waterfall plot of best overall response on a per lesion basis. On average there were 2 lesions per patient (range: 1-4). Lesions were measured per RECIST 1.1 guidance approximately 10 weeks after initiation of therapy. **BottomPanel:** best overall response in total tumor burden on a per patient basis. In this study, total of 16 non-injected lesions in 7 breast cancer subjects were evaluated. * New lesion C6D7.

Best Overall Response from Noninjected Lesions and Total Tumor Burden in Evaluable Melanoma Subjects

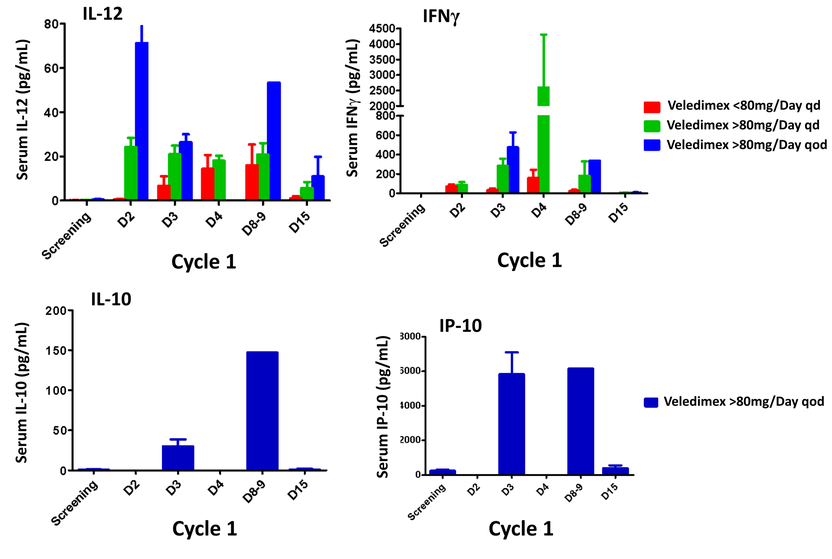


Top Panel: waterfall plot of best overall response on a per lesion basis. On average there were 4 lesions per patient (range: 1-11). Lesions were measured per RECIST 1.1 guidance approximately 10 weeks after initiation of therapy. **Bottom Panel:** best overall response in total tumor burden on a per patient basis. In this study, total of 76 non-injected lesions in 19 melanoma subjects were evaluated.

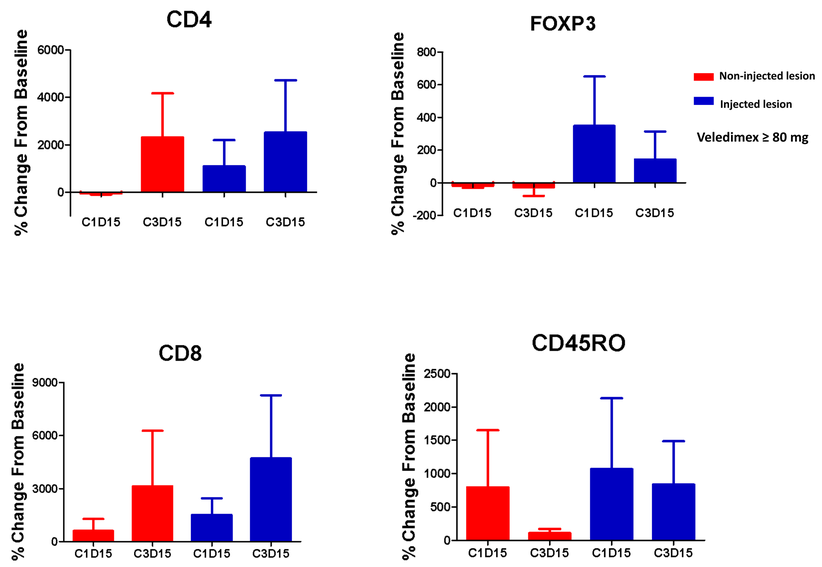
Functional IL-12 Expression in Breast Ca Patients Veledimex > 80mg



Cytokine Expression in Melanoma Patients



Increased TILs with Decreased Tregs in Melanoma Patients



Change in pharmacodymanic (PD) markers after the intratumoral administration (IT) of Ad-RTS-hIL-12 + orally administered veledimex in metastatic advanced stage melanoma (N=26) and breast cancer (N=12) subjects. Depicted in the upper panel is the time course of serum IL-12 protein production after a single IT administration of Ad-RTS-hIL-12 $1x10^{12}$ vp + veledimex administered orally QDx7 at doses equal to or greater than 80mg. The IL-12 that was produced was biologically active as demonstrated by the increased production of IFNγ and inhibitory cytokines IL-10 and IP-10. Depicted in the lower panel is the tumor flow cytometry results from both injected and noninjected lesions in the melanoma study. The results show increased CD4+ and CD8+ cytotoxic T cells upon initiation of therapy. In addition therapy elicited a reduction in FoxP3+ Tregs. Taken together these changes in PD markers demonstrate systemic immune activation with increased tumor infiltrating T cells and clinical effect in advanced, heavily pretreated melanoma patients.

Most Frequent Treatment Related AEs Grade ≥ 3 in Melanoma and Breast Cancer Studies (N=38)*

Adverse Event	Grade ≥ 3
Neutropenia/Febrile Neutropenia	6 (16%)
LFTs Increased	6 (16%)
Hyponatremia	4 (11%)
Cytokine Release Syndrome	4 (11%)
Pyrexia	4 (11%)
Hypotension	4 (11%)
Lymphopenia	4 (11%)
Anemia	4 (11%)
Dehydration	4 (11%)
Fatigue	3 (8%)
Delirium/Confusion	3 (8%)
Leukopenia	2 (5%)
Mucosal Inflammation/ Mucosal Oedema	2 (5%)

* Modified from J.J Nemuaitis, *et al.* AACR December 3, 2014 Abstract #B11

Conclusions

- **Expression of functional IL-12 in human subjects by direct intratumoral injection of Ad-RTS-hIL-12 + oral veledimex generates downstream IFNγ production and rapid elevation of IL-10 and IP-10**
- **Results show systemic immune activation and clinical effect in advanced, heavily pretreated melanoma and breast cancer patients; with increased tumor infiltrating T cells in melanoma patients**
- **The adverse event profile, including cytokine release syndrome, of Ad-RTS-hIL-12 + veledimex is predictable, controllable, and fully reversible upon stopping veledimex**
- **Findings are being translated in a study of subjects with metastatic breast cancer to explore further TILs in patients showing a response to standard therapy. In addition, a Phase 1 study in malignant high grade glioma is underway**