Ad-RTS-hIL-12 + Veledimex Regulation of IL-12 Expression in Advanced Breast Cancer and Melanoma

ZIOPHARM Oncology, Inc.

ZIOPHARM Oncology, Inc.
1 First Avenue, Parris Building #34, Navy Yard Plaza, Boston, MA 02129
Main 617-259-1970 Fax 617-241-2855 www.ziopharm.com

INTREXON

Intrexon Corporation
20358 Seneca Meadows Parkway, Germantown, MD 20876
Phone 301-556-9900 Fax 301-556-9901 www.dna.com

John J. Nemunaitis[1], Gerald P. Linette[2], Haythem Ali[3], Troy Guthrie[4], Francois Lebel[5], John A. Barrett[5], Thomas Reed[6], Suma Krishnan[6], Jonathan Lewis[5], Larry Norton[7]

[1] Mary Crowley Cancer Research Center, Dallas, TX , [2] Washington University, Saint Louis, MO, [3] Henry Ford Hospital, Detroit, MI, [4] Baptist Cancer Institute, Jacksonville, FL , [5] ZIOPHARM Oncology Inc., Boston, MA, [6] Intrexon Corporation, Germantown, MD, [7] Memorial Sloan-Kettering Cancer Center, New York, NY

Abstract

Background: IL-12 triggers an acute inflammatory environment that reverses stromal cell dysfunction within tumors, enabling them to efficiently cross-present naturally occurring tumor antigens. This recognition of cross-presented tumor antigens by $CD8^+$ T cells maybe the critical initial step that allows for $CD8^+$ T cell immune response against tumor antigens. Indeed, controlled IL-12 expression may be one of the best ways to augment adoptive cellular cancer immunotherapy. We have previously demonstrated, in human subjects administered Ad-RTS-hIL-12 intratumorally under the control of RheoSwitch Therapeutic System (RTS®), acute inflammation and increase in $CD8^+$ T cells. Animal studies have demonstrated that tight control of localized IL-12 production results in a dose-related increase in tumor IL-12 and downstream IFNγ with levels in the tumor being approximately 10 times greater than the serum. These cytokine increases eliicited an influx of cytotoxic T cells along with a reduction in MDCs and Tregs. Here we report the clinical experience of Ad-RTS-hIL-12 + veledimex in dose cohorts of advanced breast cancer and melanoma.

Methods: The studies evaluated heavily pre-treated breast cancer and melanoma subjects. Multiple treatment cycles up to 3 or 6 cycles were planned; each cycle consisted of intratumoral injection of Ad-RTS-hIL-12 (10^{10} vp) on Day 1 and administration of oral veledimex QD for 7 days or QOD for 14 days of each cycle. The primary objectives were safety and tolerability and the assessment of preliminary anti-tumor activity according to RECIST 1.1. Serum IL-12 and (downstream) IFNγ were used to monitor the expression activity of the RTS® switch and functionality of the IL-12 protein.

Results: 38 subjects were treated with veledimex from 5 mg to 160 mg administered as QD or QOD schedules; subject characteristics include 11 female and 1 male in breast cancer; 8 female and 18 male in melanoma; median age in breast cancer and in melanoma was 64 (range 22-94). All subjects had heavy tumor burden and disease progression at the time of enrollment. Treatment with Ad-RTS-hIL-12 + veledimex resulted in an increase in IL-12 and (downstream) IFNγ peak at Day 3 of 31 +/- 4 pg/ml and 313 +/- 91 pg/ml, respectively which was followed by rapid increase in serum IL-10 and IP-10. One triple negative breast cancer subject crossed the primary endpoint of 16 week PFS. In melanoma, of 15 evaluable subjects there is 1 PR and 1 SD. Most common ≥ Grade 3 TEAEs in breast cancer 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.

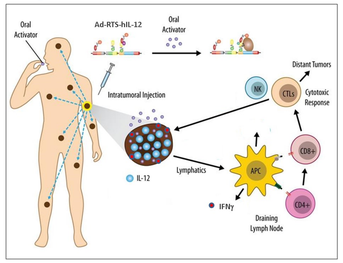
Conclusions: The administration of Ad-RTS-hIL-12 + veledimex has shown biologic activity demonstrated by the expression of functional IL-12 as manifested in secretion of IFNγ, acute inflammation, and increase in intratumoral $CD8^+$ T cells. All AEs were consistent with IL-12 immune activation, and rapidly reversed upon discontinuation of veledimex. Controlled and regulated IL-12 expression using Ad-RTS-hIL-12 + veledimex is a promising immunotherapy for breast cancer and melanoma. This approach warrants further clinical investigation, in particular in combination with other therapies where an increase in tumor-specific $CD8^+$ T cells has been shown to be predictive of positive outcomes.

Introduction

IL-12 reverses tumor immune escape mechanisms, unmasks the immunogenicity of tumor cells and alters the intrinsic capabilities of resident APCs in the tumor microenvironment. Rosenberg and co-workers recently showed that IL-12 can reverse immune escape mechanisms induced by MDSCs and DCs and significantly improve the function of activated $CD8^+$ T cells resulting in the collapse of the vascularized stroma of solid tumors.[1] We have previously demonstrated that intratumoral administration of Ad-RTS-IL-12 results in targeted tumor cytotoxicity and the induction of systemic T cell memory.[2]

IL-12 delivered by systemic infusion in patients with a variety of advanced tumors has shown positive clinical responses, but yielded unacceptable treatment-related toxicities. The sub-optimal therapeutic index has been attributed in part to the limited penetration of the solid tumor matrix. We and others (Rosenberg and co-workers, Brentjens and co-workers) are exploring cell-based delivery of IL-12.[3,4] We have reported increased control and safety with delivery of IL-12 directly to the tumor site when under the control of the RheoSwitch Therapeutic System® (RTS®) gene switch.[5,6]

Background



- An adenoviral vector engineered to express IL-12 (Ad-RTS-hIL-12) utilizing the RheoSwitch Therapeutic System® (RTS®) gene switch is injected intratumorally
- Expression of IL-12 is controlled through the administration of an oral activator ligand veledimex

- Localized production of IL-12
- T cell activation toward tumor-associated antigens
- Influx of cytotoxic $CD8^+$ T cells coupled with a reduction in $CD4^+$ regulatory T cells

Regulated intratumoral expression of IL-12 promotes activation of tumor-infiltrating lymphocytes to drive a cytotoxic immune response against distant tumors

Study Designs

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

- Phase 1: 13 subjects enrolled where using a 21 day cycle, intratumoral injection of Ad-RTS-hIL-12 occurred on Day 1 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 occurred on Day 1 and oral veledimex dose (160 mg QD - 4 subjects and 80-160 QOD - 9 subjects) for a total of 7 doses per cycle

Breast Cancer: Phase 2, Recurrent/Metastatic Breast Cancer

- Monotherapy Arm – 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

Primary Objective

- To evaluate the safety and tolerability of intratumoral injections of Ad-RTS-hIL-12 + veledimex
- Assess the efficacy of repeated cycles of intratumoral injections of Ad-RTS-hIL-12 + veledimex as monotherapy as measured by the 16-week progression–free (PFS) survival rate (Breast)

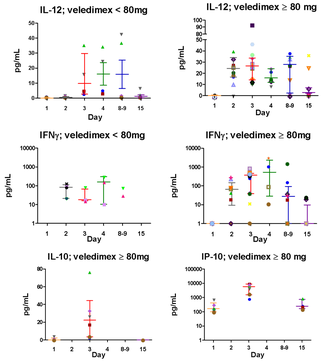
Key Inclusion Criteria

- Males or females of all races ≥ 18 years of age
- ECOG 0, 1 (and 2 for Breast)
- Adequate bone marrow, liver, and renal function
- Melanoma:
  - –Unresectable Stage III or Stage IV melanoma arising from any site other than ocular melanoma
  - – A minimum of 2 accessible non-visceral lesions (shortest diameter ≥ 1 cm) or palpable tumor-involved lymph nodes (shortest diameter ≥ 1.5 cm)
- Breast:
  - – Confirmed adenocarcinoma of the breast, either locally recurrent or metastatic disease with minimum of 2 lesions
  - – Subject has failed or progressed on at least 1 prior systemic CT regimen

Patient Demographics

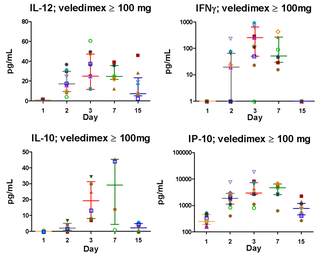
Patient Demographics and Baseline Characteristics	ATI001-101 Melanoma Study N= 26	ATI001-201 Breast Cancer Study N= 12
Age in years - Median (Min, Max)	64 (22, 94)	63 (38, 88)
Gender Male : Female	18 : 8	1 : 11
Time Since Initial Diagnosis (months) Median (Min, Max)	24 (1.3, 89.9)	109 (8, 261)
Stage at Study Entry		
IIIB	0	1
IIIC	1	0
IV	25	11
Classification		
HER2 Breast Cancer	NA	25% HER 2+; 67% HER 2-; 8% ND
HR status ER or PR Breast Cancer	NA	58% HR+; 42% HR-
Triple-Negative Breast Cancer (TNBC)	NA	25% TNBC
ECOG Performance Status :	N=26	N=12
0	16	5
1	10	7
Patient Baseline LDH above ULN (%)	57%	NA
Patients with Visceral Metastatic Sites	73% (19/26)	50% (6/12)
Prior Therapy Mean (Standard Error)	10 (1)	14 (2)

Serum Cytokine Expression in Melanoma Patients Cycle 1



- Effects of Ad-RTS-hIL-12 + veledimex on serum cytokines in melanoma patients. Intratumoral injection of Ad-RTS-hIL-12 occurred on Day 1 and oral escalating veledimex dose (5-160 mg QD or QOD) for a total of 7 doses. Serum cytokines were measured with a quantitative sandwich ELISA technique (R&D Systems, Minneapolis MN). Optical density was read in a microplate reader at 450 nm. Each symbol depicts the individual subject data. Lines depict the median ± interquartile range; For veledimex <80mg N=6; for veledimex ≥80mg N=19.

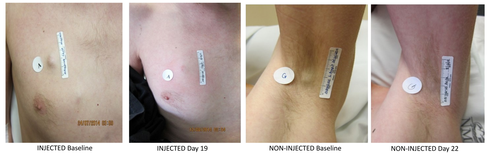
Serum Cytokine Expression in Breast Cancer Patients Cycle 1



- Effects of Ad-RTS-hIL-12 + veledimex on serum cytokines in melanoma patients. Intratumoral injection of Ad-RTS-hIL-12 occurred on Day 1 and oral escalating veledimex dose (100-140 mg QD) for a total of 7 doses. Serum cytokines were measured with a quantitative sandwich ELISA technique (R&D Systems, Minneapolis MN). Optical density was read in a microplate reader at 450 nm. Each symbol depicts the individual subject data. Lines depict the median ± interquartile range; For veledimex ≥100mg N=12.

Evidence of Local and Systemic Biologic Activity in Melanoma

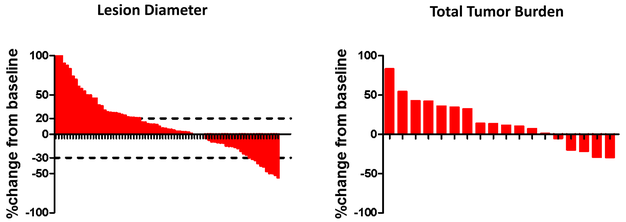
27 year old male diagnosed with MM in 2011. History of treatment with ipilimumab, IL-2 and vemurafenib, abrafenib and trametinib, and whole brain radiation; secondary adrenal insufficiency from ipilimumab on steroid maintenance. Third dose of veledimex was withheld (C1D5) due to leukopenia and cytokine release syndrome.



INJECTED Baseline | INJECTED Day 19 | NON-INJECTED Baseline | NON-INJECTED Day 22

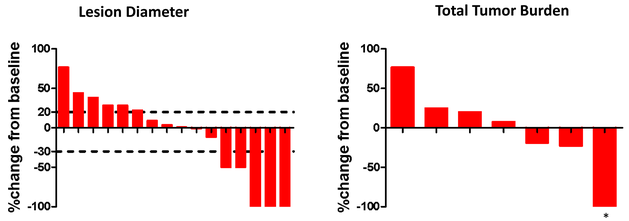
Cytokine	IL-12	IFN-γ	IL-10	IP-10
Normal range	up to 3.58 pg/mL	up to 15.6 pg/mL	< 3 pg/mL	38-361 pg/mL
Screening	0.50	< 8.0	< 0.78	226
Cycle1 Day 3	95.05	1367.5	48.43	9897
Cycle1 Day 8	53.40	335.9	146.98	6158
Post Treatment	0.77	< 8.0	3.58	491

Best Overall Response by Lesion and Total Tumor Burden in Evaluable Melanoma Subjects



- On the left, waterfall plot of best overall response on a per lesion basis expressed as a percent change from the baseline image. On average there were 4 lesions per patient (range: 1-11). Lesions were measured by unidimensional longest diameter method including target and nontarget lesions ~ 10 weeks after initiation of therapy for 18 subjects with metastatic melanoma. On the right, change from baseline in total tumor burden on a per patient basis. Lesions were assessed as described above.

Best Overall Response by Lesion and Total Tumor Burden in Evaluable Breast Cancer Subjects

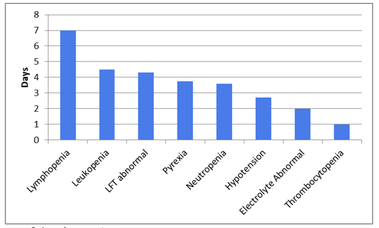


- On the left, waterfall plot of best overall response on a per lesion basis expressed as a percent change from the baseline image. On average there were 2 lesions per patient (range: 1-4). Lesions were measured by unidimensional longest diameter method including target and nontarget lesions ~ 10 weeks after initiation of therapy for 7 subjects with metastatic breast cancer. On the right, change from baseline in total tumor burden on a per patient basis. Lesions were assessed as described above. * Three measured target lesions regressed completely; this patient ultimately progressed.

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

Adverse Event*	Grade ≥ 3
Neutropenia/febrile neutropenia	8 (21%)
Electrolyte abnormalities	8 (21%)
LFTs increased	6 (16%)
Leukopenia	5 (13%)
Cytokine release syndrome	4 (11%)
Pyrexia	4 (11%)
Hypotension	4 (11%)
Lymphopenia	4 (11%)
Anemia	4 (11%)
Fatigue	3 (8%)
Dehydration	3 (8%)
Mucosal inflammation/mucosal oedema	2 (5%)
Delirium/confusion	2 (5%)

Melanoma and Breast Cancer Studies: Mean Time to Resolution of Grade ≥ 3 TEAEs*



Serious adverse events are:

- Predictable, reversible and typical of IL-12 immune activation
- Reverse within days after stopping veledimex dosing

*Recovery is potentially shorter as assessments were captured at the next scheduled visit

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
- This results in systemic immune activation and clinical effect in advanced, heavily pretreated melanoma and breast cancer patients
- The adverse event profile, including cytokine release syndrome, of Ad-RTS-hIL-12 + veledimex is predictable, controllable, and fully reversible on stopping veledimex
- These findings will next be translated in a study of subjects with early metastatic breast cancer, in those responding to standard therapy
- Additional studies using this control system for IL-12 cell-based therapies are being planned

References

1. S.P. Kerkar, R.S. Goldszmid, P. Muranski, D. Chinnasamy, Z. Yu, R.N. Reger, A.J. Leonardi, R.A. Morgan, E. Wang, F.M. Marincola, G.Trinchieri, S.A. Rosenberg, and N.P. Restifo. *IL-12 triggers a programmatic change in dysfunctional myeloid-derived cells within mouse tumors.* J Clin Invest. 2011;121(12):4746–4757.
2. J.A. Barrett, J. Sun, M. Griepsiadis, C. Furlan-Freguia, T. Chan, W. Fogler, L. Humeau, R. Khosravi Far, R.A. Morgan, and H. Youssoufian. *Intratumoral-Regulated Expression of IL-12 as a Potential Immunotherapeutic Strategy for the Treatment of Breast Cancer.* American Association for Cancer Research (AACR), April 9,2013.
3. S.P. Kerkar, A.J. Leonardi, N. Panburana, L. Zhang, Z. Yu, J.G. Crompton, J.H. Pan, D.C. Palmer, R.A. Morgan, S.A. Rosenberg and N.P. Restifo. *Collapse of the Tumor Stroma is Triggered by IL-12 Induction of Fas.* Molecular Therapy. 2012;21(7):1364–1373.
4. H.J. Pegram, J.C. Lee, E.G. Hayman, G.H. Imperato, T.F. Tedder, M. Sadelain and R.J. Brentjens. *Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning.* Blood. 2012;119(18):4133-41.
5. G.P. Linette, D. Hamid, F.D. Whitman, J.J. Nemunaitis, J. Cheney, S.S. Agarwala, A. Starodub, J.A. Barrett, A. Marsh, J. Martell, A. Cho, T.D. Reed, H. Youssoufian, and A. Vergara Silva. *A Phase I Open-Label Study of Ad-RTS-hIL-12, an Adenoviral Vector Controlled by Ligand-Inducible Gene Switch, in Combination with an Oral Activator Ligand in Subjects with Unresectable Stage IIIB/IV Melanoma.* Annual Meeting of the American Society of Clinical Oncology (ASCO), May 31-June 4, 2013.
6. J.J. Nemunaitis, G.P. Linette, D. Hamid, S.S. Agarwala, A. Starodub, L. Sun, F. Lebel, J. A. Barrett, and J. Lewis. *Regulated intratumoral expression of IL-12 as a basis for combination therapy in melanoma.* Melanoma Bridge Conference, Cancun, December 4-6, 2013.
7. J.J. Nemunaitis, H. McArthur, C. Hudis, T. Reed, S. Broder, F. Lebel, J.A. Barrett, J. Lewis, and L. Norton. *Regulated intratumoral expression of IL-12 in combination with cytotoxic agents as a strategy for the treatment of metastatic breast cancer.* San Antonio Breast Cancer Symposium. December 10-14, 2013.