A Phase I Open-Label Study of Ad-RTS-hIL-12, an Adenoviral Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand in Subjects with Unresectable Stage III/IV Melanoma

G.P. Linette[1], O. Hamid[2], E.D. Whitman[3], J.J. Nemunaitis[4], J. Chesney[5], S.S. Agarwala[6], A. Starodub[7], J.A. Barrett[8], A. Marsh[9], L. Martell[8], A. Cho[9], T.D. Reed[9], H. Youssoufian[6], A. Vergara Silva[8]

[1]Washington University in St. Louis, St. Louis, MO; [2]The Angeles Clinic and Research Institute, Los Angeles ,CA; [3]Atlantic Melanoma Center, Morristown, NJ; [4]Mary Crowley Cancer Research Center, Dallas, TX; [5]University of Louisville, Louisville, KY; [6]St Luke's Hospital and Health Network, Newark, Easton, PA; [7]Indiana University Health Goshen Center for Cancer Care, Goshen, IN; [8]ZIOPHARM Oncology, Boston, MA; [9]Intrexon Corporation, Blacksburg, VA

Abstract

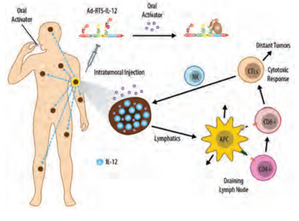
Background: IL-12 is a pleiotropic cytokine with known antitumor activity; however, clinical use has been limited by toxicity when delivered systemically. Ad-RTS-hIL-12 is an adenoviral vector engineered for controlled expression of IL-12 with the RheoSwitch Therapeutic System® (RTS®) technology and an oral activator, INXN-1001.

Methods: In a Phase I, 3+3 dose escalation study, subjects with unresectable stage III/IV melanoma were administered 1x10^{12} viral particles (Ad-RTS-hIL-12; INXN-2001) intratumorally on the first day of up to six 21-day cycles, and INXN-1001 (5, 20, 100, and 160 mg) orally on Days 1 to 7 of each cycle.

Results: Dose escalation is complete with 14 subjects treated. Median prior therapeutic agents was 3 (range 1-4). Common related adverse events included chills (11, 78.6%), pyrexia (11, 78.6%), fatigue (10, 71.4%), and nausea (10, 71.4%). With a biologically effective dose of 160 mg, MTD for INXN-1001 was not reached. One death unrelated to study drug was secondary to septicemia. Clinical activity was observed in 5 of 7 subjects treated at doses of INXN-1001 ≥100 mg, but not at <100 mg, and included prominent inflammatory responses in injected and non-injected lesions, decreases in size of injected and non-injected lesions, and reduction in tumor-associated pain. One subject at the 160 mg dose had stable disease for 20 weeks. Clinical activity in dose cohorts ≥100 mg coincided with a 4-fold median increase from baseline in peak serum levels of IL-12 and IFN-γ compared with lower dose cohorts. Flow cytometric analyses of PBMCs revealed 7-fold (≥100 mg dose cohorts) median increases from baseline in peak levels of absolute numbers of CD3$^+$ and CD8$^+$ T-cells. ELISPOT and T-cell proliferation assays for antigen-specific responses are ongoing.

Conclusions: Intratumoral delivery of IL-12 via an adenoviral vector with RTS®-enabled finely-controlled expression of IL-12 levels by an oral ligand. Ad-RTS-hIL-12 plus INXN-1001 (160 mg) was well tolerated and induced biological and clinical activity in subjects with advanced melanoma. Phase II studies are ongoing at the biologically effective dose.

Background



- An adenoviral vector engineered to express IL-12 (Ad-RTS-hIL-12) utilizing the RheoSwitch Therapeutic System® (RTS®) technology is injected intratumorally
- Expression of IL-12 is controlled through the administration of an oral activator ligand (INXN-1001)

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

- Localized production of IL-12 leading to enhanced antigen presenting cell activity and T-cell activation toward tumor-associated antigens, locally and systemically
- T-cell activation toward tumor-associated antigens
- Influx of cytotoxic CD8$^+$ T-cells coupled with an influx of CD45RO$^+$ memory T-cells

Inducible Gene Regulation: RheoSwitch Therapeutic System

RheoSwitch Therapeutic System® (RTS®) Technology is a 3-Component Transcriptional Regulator

1. **The Switch Components:** The RTS® gene program includes 2 receptor protein fusions: VP16-RXR and Gal4-EcR. These exist as 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 from which target gene transcription is activated.
3. **The Activator Ligand:** 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.

Study Design

- Phase I, 3+3 dose escalation
- Subjects with unresectable stage III/IV melanoma, n=14 (Phase I) + n=3 (Phase II, ongoing)
- Intratumoral injection of 1x10^{12} Ad-RTS-hIL-12 viral particles (INXN-2001)
- INXN-1001 (5, 20, 100, or 160 mg) on Days 1-7 of each cycle
- Up to six 21-day cycles

Primary Objective

- Evaluate the safety and tolerability of intratumoral injections of 1x10^{12} viral particles Ad-RTS-hIL-12 (INXN-2001) in combination with escalating doses of INXN-1001

Secondary Objective

- Inform the selection of an INXN-1001 dose for further study in combination with INXN-2001

Inclusion Criteria

- Males or females of all races ≥18 years of age
- Unresectable Stage III or Stage IV melanoma arising from any site other than ocular melanoma
- A minimum of 2 accessible lesions (shortest diameter ≥1 cm) or palpable tumor-involved lymph nodes (shortest diameter ≥1.5 cm)
- ECOG performance status of 0 or 1
- Adequate bone marrow, liver, and renal function

Demographics (n=17)

Gender (n)	
Female	6
Male	11
Age (years)	
Median (range)	64 (22 to 94)
Race (n)	
Caucasian	16
Pacific Islander	1

Overall Response Rate

Derived Best Response Rate by mRECIST

Response	Evaluable Patients (n = 13)
CR	0
PR	0
SD	6 (46%)
PD	2 (15%)
uPD	5 (39%)

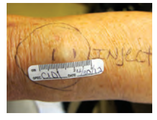
uPD = unconfirmed progressive disease

Modified RECIST (mRECIST) = Immune-related response criteria[1] modified by replacing WHO Criteria with RECIST v1.1. Thirteen subjects were evaluable for response assessment per mRECIST.

[1] Wolchok JD, Hoos A, O'Day S et al. Clin Cancer Res. 2009;15:7412-7420

Prominent Inflammatory Response Correlates with High Levels of IL-12 and IFN-γ

C1D1



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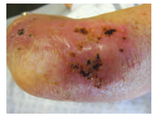
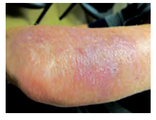


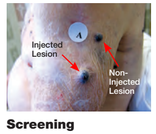
C2D1



- Subject 107, 60 y/o female, 100 mg INXN-1001
- Initial increase in lesion size due to inflammatory response seen at Cycle 1 Day 15
- Lesion was undetectable at Cycle 2 Day 1
- Subject ultimately progressed and was taken off study

Flattening and Depigmentation of Injected and Non-Injected Tumors After a Single Dose of INXN-1001 (160 mg)

Screening



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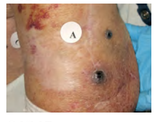
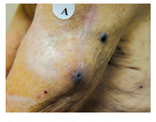
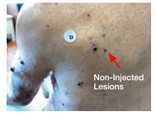


8 Weeks Post Tx



Screening



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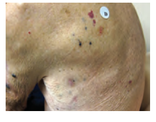
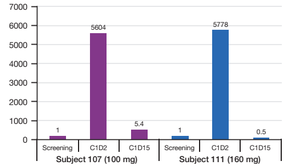


- Subject 115, 90 y/o male, 160 mg INXN-1001
- Stage IV Melanoma, T4a N3 M1c, treatment naïve
- Received only one dose of the activator ligand at 160 mg (C1D1)
- Very high levels of IL-12 and IFN-γ

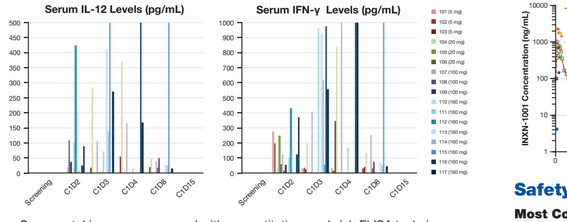
Regulated Expression of IL-12 in the Tumor Following Ad-RTS-hIL-12 + INXN-1001 Administration



Subjects received intratumoral injection of 1x10^{12} viral particles (INXN-2001) on Day 1 of each cycle and INXN-1001 on Days 1-7 of each cycle. Genomic DNA and total RNA were extracted and analyzed as described.[2]

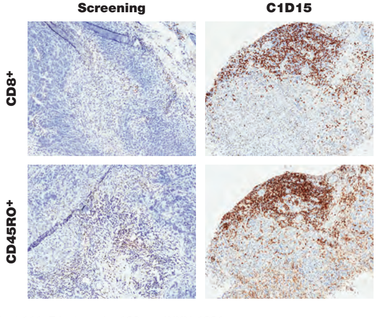
[2]Livak KJ and TD Schmittgen. 2001. Methods 25(4):402-8.

Serum Cytokine Levels (Cycle 1)



- Serum cytokines were measured with a quantitative sandwich ELISA technique per vendor instructions (R and D Systems, Minneapolis MN). Optical density was read in a microplate reader at 450 nm.
- Values at Screening and C1D15 were below the limit of quantitation. ULOQ was 500 pg/mL for IL-12, 1000 pg/mL for IFN-γ.

Cytotoxic T-Cells & Memory T-Cells Increase in Tumor Following Treatment with Ad-RTS-hIL-12



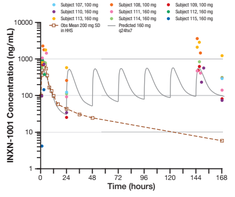
- Subject 111, 74 y/o male, 160 mg INXN-1001
- Images were obtained using an Aperio ScanScope XT whole-slide imager and digitized at 20x

Increases in Immune Cell Populations in Subjects in Higher Dose Cohorts of INXN-1001 (100 and 160 mg)

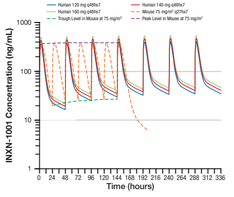
	Absolute CD3$^+$ (T-Cells)	Absolute CD8$^+$ (Cytotoxic T-Cells)
% Increase in Peak Median Cell Number (5 and 20 mg INXN-1001)	13%	52%
% Increase in Peak Median Cell Number (100 and 160 mg INXN-1001)	85%	219%

- Blood cells were analyzed with a FACSCanto II flow cytometer
- Percent increase was calculated by comparing peak values of immune cell populations at either Cycle 2 Day 15 or Post Treatment Safety Assessment visit (28 days following last dose of INXN-1001) to screening values

Observed and Predicted INXN-1001 Human Plasma PK Profiles Oral INXN-1001 Daily for 7 Days



Predicted INXN-1001 PK in Human Every Other Day for 14 Days vs. Mouse at Efficacious Dose of 75 mg/m^2



Safety

Most Common (≥20% of Subjects) TEAEs

Chills, Fatigue, and Pyrexia (73% each); Nausea (67%); Anorexia and Anemia (40% each); Vomiting (33%); and Diarrhea, Peripheral edema, Hyponatremia, Arthralgia, Pain in extremity, Dizziness, Headache, and Pruritus (20% each)

Most Common (≥20% of Subjects) Related TEAEs

Chills and Pyrexia (73% each), Nausea (67%), Fatigue (60%), Vomiting (33%), Anorexia (27%), Arthralgia and Diarrhea (20% each)

≥ Grade 3 Related TEAEs

Hyponatremia, Fatigue, Pyrexia, Aspartate aminotransferase increased, White blood cell count decreased, and Dehydration were assessed as related to study drug

Related SAEs (5 Subjects)

Pyrexia, Dehydration, Hypotension, Febrile neutropenia, Altered mental status, Failure to thrive, Pancytopenia and Hyponatremia related to study drug

Fatal SAE

Septicemia, 1 subject, unrelated

Conclusions

- INXN-1001 + Ad-RTS-hIL-12 induces production of IL-12 mRNA in the tumor microenvironment (switch on). Upon removal of INXN-1001, IL-12 mRNA returns to baseline (switch off).
- Following treatment with INXN-1001 + Ad-RTS-hIL-12, increases in TILs (CD8$^+$, CD45RO$^+$) were observed in the tumor microenvironment
- Clinical activity was observed in injected and non-injected lesions primarily at the higher doses of INXN-1001 (100 and 160 mg)
  - Inflammation, shrinkage, flattening, and depigmentation of lesions correlated with the highest serum levels of IFN-γ
- Ad-RTS-hL-12 + INXN-1001 was generally well-tolerated and its safety profile is consistent with other immunotherapies
- Ongoing in Phase II
  - Optimization of schedule guided by pharmacokinetics and tolerability using every other day dosing