Poster #883

Rheoswitch®-Mediated Regulation of IL-12 Protein Delivered Using an Adenoviral Vector Results in Anti-Tumor Effects Across a Spectrum of Tumor Types

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Abstract

One in four deaths in the United States is due to cancer. Despite the advent of advanced surgery, radiation, biological and chemotherapeutic agents, there remains a great medical need for improved therapy. Intrexon is employing a novel, regulated expression system using direct, intratumoral (IT) adenovirus-mediated delivery of interleukin-12 (IL-12) to enhance cancer treatment.

IL12 is a novel cytokine with potent antitumor activity. However, the utility of recombinant IL12 protein therapy in clinical settings is limited due to systemic toxicity. To improve the therapeutic benefit and avoid toxicity, Intrexon developed the RTS™ (RheoSwitch Therapeutic System™) inducible gene regulation system that utilizes an adenoviral vector (Ad-RTS-IL12) to mediate expression of IL12 that is controlled by an inducible promoter, conditionally activated by an orally bioavailable small molecule activator ligand (L), RG115932.

Currently, our focus is to the development of direct, IT AdRTS-IL12 delivery for cancer treatment. Adenoviral vectors encoding the human or mouse IL-12 coding regions under the control of the RTS promoter were generated and characterized. *In vitro* transduction of the human fibrosarcoma cell line, HT1080, with human IL-12 (Ad-RTS-hIL12) at an MOI of 400 pfu, resulted in a >6000-fold induction of human IL-12 expression above background levels in the presence of the activator ligand, while no induction of IL-12 was detected when the activator molecule was omitted. Since human IL-12 (hIL-12) does not function on murine cells, whereas mouse IL-12 (mIL12) acts on both human and mouse cells, Ad-RTS-mIL-12, encoding mouse IL-12 was generated and the therapeutic benefit of regulated IL-12 gene expression was evaluated in relevant mouse tumor models. First, the dose response for the activator ligand was investigated. Significant tumor inhibition (91-98%) in the B16F0 melanoma model was observed when Ad-RTS-mIL12 (1 x 10^{10} viral particles (vp) plus activator ligand was delivered at doses ranging from 100-1000mg/kg of rodent feed, without observable reduction in animal body weight, demonstrating that the activator ligand has a broad therapeutic window. Next, the effect of varying the Ad-RTS-mIL12 virus vector dose was explored using a constant activator ligand dose of 1000 mg/kg of rodent feed. Ad-RTS-mIL12 doses ranged from 1x10^{7}- 5x1010 vp. All Ad-RTS-mIL12 doses above 1x10^{7} vp displayed a therapeutic effect on tumor growth inhibition in the B16F0 melanoma mode. At a low 1x10^{8} vp dose, a 73% tumor growth inhibition was observed, while higher doses of 1x10^{9} to 5x10^{10} vp displayed a 95-99% tumor reduction (p<0.005), demonstrating that Ad-RTS-mIL12 displays a broad therapeutic window . These data suggest that RTS controlled- IL12 expression delivered through direct IT adenoviral administration is effective and tolerable. Finally, IT Ad-RTS-mIL12 with activator ligand (1000mg/kg in rodent feed) treatment resulted in significant tumor growth inhibition in several relevant murine tumor models including melanoma, colon, lung, leukemia, breast and pancreatic cancer models.

These data highlight the potential therapeutic benefit of direct adenoviral based immune-therapy with Ad-RTS-mIL12 plus activator ligand and warrant further evaluation in the clinic.

A Phase I clinical trial is currently in progress in patients with advanced melanoma cancer who were treated with dentritic cells (DC) transduced with the adenoviral vector encoding inducible IL-12. A poster presentation reporting insights into the mechanism of action of DC as well as direct adenoviral vector delivery of IL-12 will be presented by Dr. Herberman et al., Poster #899, Friday 5/20/11, Late Abstract Session II.

RheoSwitch Therapeutic System™ (RTS™) Inducible Gene Regulation System

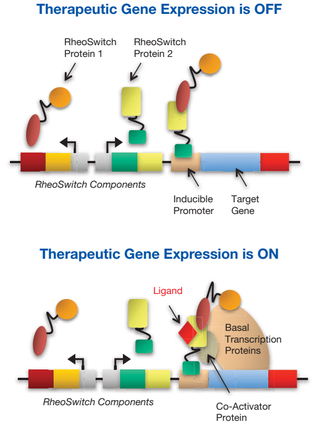
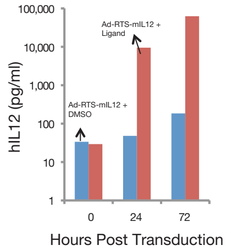


Figure 1: Schematic representation of recombinant adenovirus incorporating the RheoSwitch Therapeutic System (RTS) inducible generRegulation system. In cancer cells transduced with Ad-RTS-mIL12, the Gal4-EcR and VP16-RXR fusion proteins are expressed under the control of constitutive promoters. These proteins form heterodimers in the presence of the activator ligand, resulting in the conditional activation of mIL-12 transcription from the responsive/ inducible RTS promoter.

IL-12 Induction *In Vitro*

Figure 2: *In vitro* conditional expression of hIL-12 in cancer cells using a recombinant adenovirus incorporating the RheoSwitch Therapeutic System (RTS) inducible gene regulation system. The cancer cells, fibrosarcoma, HT1080, were infected with AdRTS-hIL12 at 400 MOI with or without activator ligand (75nM). The secreted hIL12 was measured using ELISA. The AdRTS-hIL12 vector with ligand produced hIL12 abundantly relative to vector with no ligand.



Ad-RTS-IL-12 has a Broad Activator Dose Window in Melanoma Cancer (B16F0) Model

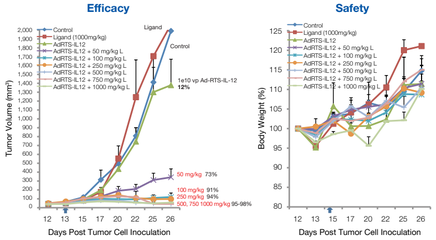


Figure 3: The potency of Ad-RTS-mIL12 with various doses of activator ligand (L) in melanoma (B16F0) tumor. The B16F0 tumors were grown in C57BL6 mice (n=5). The tumor bearing mice were separated into control (no treatment), the activator ligand (L) alone, Ad-RTS-mIL12 and Ad-RTS-mIL12 with activator ligand (50, 100, 250, 500, 750 and 1000mg/kg). A single injection of Ad-RTS-mIL12 (arrow) was given intratumorally (IT) at a dose level of 1x10^{10} vp. Tumor sizes are shown as mean ±SE. The treatment with Ad-RTS-IL12 plus activator ligand significantly inhibited the tumor growth (91-98%) compared to controls. This data suggests Ad-RTS-mIL12 is effective with a wide range doses of activator ligand (100-1000 mg/kg of rodent chow).

Ad-RTS-mIL12 has a Broad Therapeutic Window in Melanoma Cancer (B16F0) Model

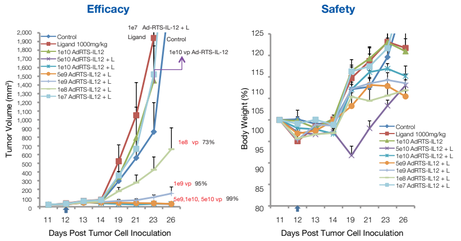


Figure 4: The dose response of Ad-RTS-mIL-12 in melanoma (B16F0) tumor. The B16F0 tumors were grown in C57BL6 mice. The tumor bearing mice were randomized into groups (n=5) control (no treatment), the activator ligand (L) alone, Ad-RTS-mIL-12 (1x10^{10} vp) and Ad-RTS-mIL-12 with activator ligand (1x10^{7}, 1x10^{8}, 1x10^{9}, 5x10^{9}, 1x10^{10} and 5x10^{10} vp). A single injection of Ad-RTS-mIL-12 (arrow) was given IT. Tumor sizes and body weight (%) are shown as mean ±SE. The treatment with Ad-RTS-mIL-12 plus activator ligand led to substantial tumor growth inhibition (95-99%) at dose ≥ 1x10^{9} vp (p<0.005). No major body weight loss was observed. This data suggests Ad-RTS-mIL-12 plus activator ligand is effective and tolerable and has broad therapeutic window in melanoma cancer model.

Anti-Tumor Activity of AdRTS-mIL12 with Activator Ligand in Various Tumor Models

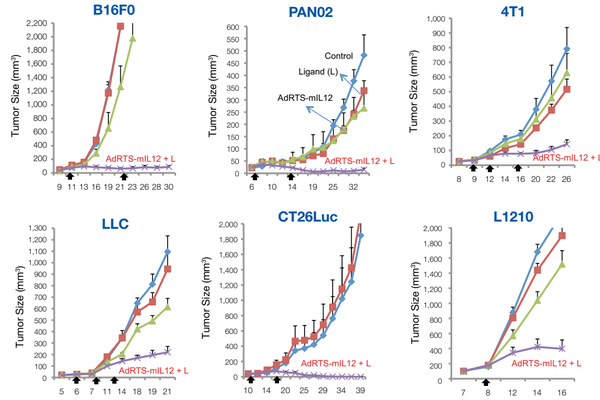


Figure 5: Antitumor activity of AdRTS-mIL12 with activator ligand in various cancer models. Subcutaneous melanoma (B16F0), colon (CT26Luc), lung (LLC), breast (4T1), leukemia (L1210) and pancreatic (PAN02) cancer models were developed in the respective immune competent mice (C57BL/6 or BALb/c). The tumors were treated with AdRTS-mIL12, ligand alone, combination of AdRTS-mIL12 and ligand or no treatment (control). AdRTS-mIL12 was administered via IT injections (arrows) at a dose of 1x10^{10} vp. The activator ligand was delivered through rodent feed (1000 mg RG119532/kg of feed) 24hr prior to vector injection until end of study. The tumor size and body weights were measured as an indicator of efficacy and tolerability. Each time point represents tumor size mean ± SE. Of note, the tumors grew rapidly in animals with no treatment, ligand or AdRTS-mIL12 alone . Importantly, animals treated with AdRTS-mIL12 plus ligand showed statistically (p<0.05 – p<0.005) significant anti-tumor activity relative to control groups. The treatment was well tolerated (data not shown).

Summary

- Activator ligand can induce the RTS™ inducible gene regulation system in AdRTS-mIL12 to express IL-12 protein both *in vitro* and *in vivo*
- Evidence of a broad therapeutic window for both activator ligand and AdRTS-mIL12 in B16F0 melanoma cancer model
- Combination of AdRTS-IL12 and activator ligand showed strong anti-tumor activity in various cancer models in mice including: melanoma, colon, lung, breast, pancreas and leukemia
- AdRTS-IL12 and activator ligand therapy was well tolerated