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 delivery of IL-12 in a tumor generates both local and systemic antitumor immune responses. We have developed an adenoviral vector (Ad) engineered to express Activator Ligand (aledimex) under the control of the RheoSwitch® Transcription System (RTS®) platform. Gene expression and subsequent IL-12 protein expression is tightly controlled by a small molecule activator ligand (aledimex). We have shown on mechanical biologic activity and safety with this system in syngeneic mouse models of melanoma and breast cancer. These studies demonstrated an AL dose-related increase in tumor IL-12 mRNA and IL-12 protein expression, in addition to a return to baseline IL-12 mRNA and IL-12 protein expression was observed in sites of AL. These changes correlate with a local and systemic immune and antitumor response.

1. 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 toxicities
    - Prompted investigation of alternative delivery routes, e.g., subcutaneous, 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

- Co-Activation Partner (CAP)
  - Ligand-induced Transcription Factor (LITF)
  - Activator Ligand (AL)

- LITF binds to CAP in the absence of AL, and forms a stable complex in the presence of AL.

- The Activator Ligand (AL) is a small molecule that can be administered systemically or by local injection.

Study Design (Melanoma and Breast)

Melanoma: Phase 1/2, Unselectable 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 8 cycles; intratumoral injection of Ad-RTS-hIL-12 and oral veledimex dose (80-160 mg QD) 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)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ATTHO-101 Melanoma Study (N=26)</th>
<th>ATTHO-101 Breast Cancer Study (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>Median (Min, Max)</td>
<td>66 (22-94)</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>10</td>
</tr>
<tr>
<td>Time Since Diagnosis (months)</td>
<td>Median (Min, Max)</td>
<td>24 (1-133)</td>
</tr>
<tr>
<td>Prior Therapy</td>
<td>Mean</td>
<td>6</td>
</tr>
</tbody>
</table>

Most Frequent Treatment-Related AEs Grade ≥3

- Delirium/Confusion
- Hypokalemia
- Hypoalbuminemia
- Hypomagnesemia
- Hypocalcemia
- Hypoglycemia
- Hypocholesterolemia
- Anemia
- Increased LFTs
- Neutropenia
- Febrile neutropenia
- Hypertension
- Nausea
- Diarrhea
- Thrombocytopenia
- Dehydration
- Mucosal inflammation/mucosal oedema
- Delirium/Confusion
- Dehydration
- Anemia
- Increased LFTs
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- Delirium/Confusion
- Dehydration
- Anemia

Increased TILs with Decreased Tregs in Melanoma Patients

Change in pharmacodynamic (PD) markers after the intratumoral administration (IT) of Ad-RTS-hIL-12 or 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 1x1017vp + veledimex administered orally QDx7 at doses equal to or greater than 80mg. The IL-12 that was produced was bioactively active as demonstrated by the increased production of IFNγ and inhibitory cytokines IL-9, 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.

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

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

Graphs showing the increase in IL-12 expression and IFNγ production in breast cancer patients receiving oral veledimex doses greater than 80mg.

Change in serum IL-12 protein levels in breast cancer patients receiving oral veledimex doses greater than 80mg.