

IL-15 Technology

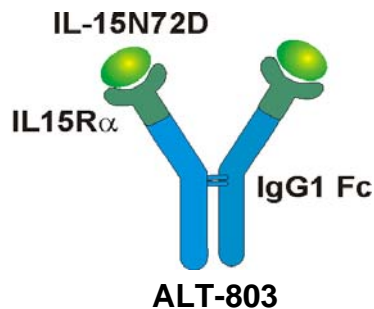
Interleukin-15 (IL-15) is a critical factor for the development, proliferation and activation of effector natural killer (NK) and CD8⁺ memory T cells. IL-2 and IL-15 are related cytokines capable of stimulating immune cells via interactions with shared signaling receptor components, IL-2R β and IL-2R γ_c , and unique α chain subunits (IL-2R α and IL-15R α) that are required for high affinity binding to the IL-2R $\beta\gamma$ complex. Based on its ability to provide durable responses in a subset of patients, IL-2 has been approved by the FDA for use in treatment of metastatic renal cell carcinoma and malignant melanoma. However, the broad effectiveness of IL-2 as an anti-cancer therapeutic has been questioned based on its pivotal role in the maintenance of CD4⁺CD25⁺ T-regulatory cells and in activation-induced cell death (AICD) – a process that leads to the elimination of stimulated T cells and induction of T-cell tolerance thereby limiting memory T cell responses. In contrast, IL-15 inhibits IL-2-induced AICD and supports long lasting CD8⁺ T cell memory and effector responses against diseased cells, suggesting that IL-15 may be superior to IL-2 for the treatment of cancers. Indeed, a recent NCI review listed IL-15 as the most promising product candidate among twelve immunotherapy drugs that could potentially cure cancer. IL-15 has a novel mechanism of action in which IL-15 and IL-15R α are coordinately expressed by antigen-presenting cells (monocytes and dendritic cells), and IL-15 bound to IL-15R α is presented *in trans* to neighboring NK or CD8⁺ T cells expressing only the IL-2R $\beta\gamma$ receptor. As a co-stimulatory event occurring at the immunological synapse, IL-15 trans-presentation appears to be a dominant mechanism for IL-15 action *in vivo* and appears to play a major role in tumor immunosurveillance.

The extracellular domain of IL-15R α has been shown to bear most of the structural elements responsible for high affinity (K_d 100 pM) cytokine binding, can be produced as a recombinant soluble protein and used to form stable heterodimeric complexes in solution. These complexes are capable of modulating (*i.e.* either stimulating or blocking) immune responses via the IL-2R $\beta\gamma$ complex. In fact, it has been shown in some studies that the biological activity of IL-15 could be increased 50-fold by administering preformed complexes of IL-15 and soluble IL-15R α through a mechanism that is likely due in part to the longer half-life of the complex compared to IL-15 alone. Recent studies in mouse tumor models have demonstrated that the efficacy of IL-15 can be dramatically increased by preassociating it with soluble IL-15R α either in a single chain format or as an IL-15R α -Fc fusion. These responses were found to be mediated by either NK cell or T cells, including tumor-resident effector cells, depending on the tumor model.

ALT-803: a novel IL-15 super agonist complex: Based on these findings, we have been interested in evaluating immunotherapeutics comprising IL-15. However, there are several limitations in developing IL-15-based approaches that include the difficulty in producing large amounts of this cytokine in standard mammalian cell expression systems and the requirement for high doses to achieve functional responses. To contend with these shortcomings, we have identified a novel IL-15 mutant with increased ability to bind IL-2R $\beta\gamma$ and enhanced biological activity. This super agonist mutant of IL-15 was described in a publication (J Immunol 2009 183:3598) and is the subject of several patent applications. As shown in numerous studies conducted at Altor, combining this IL-15 super agonist with a soluble IL-15 α receptor fusion protein (IL-15R α -Fc) results in a protein complex with highly potent IL-15 activity *in vitro* and *in vivo*. This IL-15 super agonist complex (IL-15N72D/IL-15R α -Fc) is referred to as ALT-803. Pharmacokinetic

analysis indicated that the complex had a half-life in mice of 11.5 hours following i.v. administration. In various aggressive solid and hematological tumor models in immunocompetent mice, ALT-803 exhibits impressive anti-tumor activity as a monotherapy using a weekly i.v. dose regimen. The ALT-803 anti-tumor response is also durable. Mice implanted with various tumors and found to be tumor-free following ALT-803 treatment are highly resistant to re-challenge with the same tumor cells indicating that ALT-803 induces effective immunological memory responses against these re-introduced tumor cells. The mechanisms of action of ALT-803 against tumors are currently under intensive investigation at Altor. ALT-803 is also well tolerated in mice using a weekly dosing regimen.

Clinical development schedule of ALT-803: Since the efficacy and safety results of ALT-803 in animal models are very encouraging, Altor has given ALT-803 a high priority for clinical development. A high productivity recombinant CHO cell line has been developed to produce ALT-803 in suspension in large-scale fermentations in defined serum-free and protein-free media. A robust proprietary, industrial-scale protein purification procedure has also been developed by Altor to generate highly purified ALT-803 to support clinical development. The cell banking of the production cell line is underway and the cGMP manufacture of clinical material is scheduled in the 4th quarter of 2011 at a CMO already selected by Altor. The cGMP material will be available for clinical trials in the first quarter of 2012. The pre-clinical pharmacology and toxicology studies will be completed in 2011 and an IND filing to the US FDA for ALT-803 development is scheduled in early first quarter of 2012. Patient enrollment for clinical trials against cancer is projected in the 2nd quarter of 2012.



Targeted IL-15 mutein/IL-15R α as a Multi-specific Scaffold:



We are also using the IL-15 superagonist and soluble IL-15R α domains as fusion partners to single chain T-cell receptors (scTCRs), single-chain antibodies and other binding proteins to extend our targeted cytokine fusion protein approach. For example, when the TCR/IL-15 and TCR/IL-15R α fusion proteins are generated and combined, the IL-15 and IL-15R α domains interact with high affinity to form a dimeric complex containing two TCR binding domains. We have found that the p53-specific TCR fusion complex binds to target cells displaying the p53 peptide/HLA-A2 complex better than the monomeric TCR/IL-15 fusion. Additionally higher order multimeric (*i.e.*, tetramers) forms of the TCR/IL-15+TCR/IL-15R α complex also showed enhanced staining of p53 peptide/HLA-A2-positive cells. The TCR/IL-15+TCR/IL-15R α complex is capable of supporting growth of various cytokine-dependent cells, demonstrating functional activity of the IL-15 domain. The IL-15/TCR+IL-15R α domains have also been used to create functional TCR heterodimeric chains and to combine scTCR and single-chain CD8 molecules together to form a more effective peptide-MHC binding reagent. This work has been described in a recent publication (Protein Eng Des Sel 2011 24:373) and was supported by a Phase I SBIR grant.

The TCR/IL-15+TCR/IL-15R α fusion protein complex shows anti-tumor activity in the adoptive NK cell transfer studies in mice bearing lung tumors and as monotherapy in subcutaneous xenograft tumor models. We are evaluating the anti-tumor activity of the TCR/IL-15 fusions against aggressive mouse tumors in immunocompetent mice to confirm their value for clinical development.

IL-15 mutein/IL-15R α Antagonistic Complexes for Autoimmune/Inflammatory Diseases

Altor scientists have also identified novel IL-15 **antagonists** that could be useful as immunosuppressive agents for autoimmune disease.

In summary, we have successfully created a technology platform based on IL-15. This platform promises to deliver novel targeted or non-targeted immunotherapeutic and multi-functional molecules for treatment of cancer, infectious and inflammatory diseases and autoimmune diseases. The IL-15 super agonist may also serve a role as a potent adjuvant in vaccine applications. We believe that we have secured an intellectual property position on this exciting and promising cytokine for development of second-generation human therapeutics.

Altor is seeking a partner that will license, complete clinical development and commercialize the product. The following non-confidential information can be provided and sent as e-mail attachments:

1. Publications:
 - Zhu, X., W. D. Marcus, W. Xu, H. I. Lee, K. Han, J. O. Egan, J. L. Yovandich, P. R. Rhode, and H. C. Wong. 2009. Novel human interleukin-15 agonists. *J Immunol* 183:3598.
 - Wong, R.L., B. Liu, X. Zhu, L. You, L. Kong, K-P. Han, H. Lee, P-A. Chavaillaz, M. Jin, Y. Wang, P. Rhode and H. Wong. 2011. Interleukin-15:interleukin-15 receptor a scaffold for creation of multivalent targeted immune molecules. *Protein Eng Des Sel* 24:373.
2. Altor White Papers relating to:
 - p53 as a target
 - STAR-Ck Technology
 - STAR-Ck Mechanism of Action
 - STAR Diagnostics
3. Keystone Symposium Oral Presentation in 2009 on IL-15 superagonist mutant and TCR/IL-15 fusion.

For more information, contact Altor BioScience Corporation at (954) 443-8600, ext. 832 or e-mail deantaylor@altorbioscience.com. More detailed documentation can be provided upon execution of a confidentiality agreement.