Company Profile

Altor BioScience Corporation

Altor BioScience Corporation is a privately held, development-stage company engaged in the discovery and development of targeted immunotherapeutic agents for the treatment of cancer, viral infection and inflammatory diseases. Altor currently has three targeted immunotherapeutic products in clinical development. Two of these products are monoclonal antibodies, but the third is a novel molecule that utilizes targeting based on Altor's single-chain T-cell receptor (scTCR) STAR™ technology. In addition to therapeutic applications, this scTCR STAR technology has enabled Altor to develop novel reagents for research use to identify disease-associated antigens derived from intracellular proteins as biomarkers.

Altor, based in Miramar, Florida, USA, was formed in 2002 as a spin-off from Sunol Molecular Corporation. Altor is utilizing its novel Soluble T-Cell Antigen Receptor (STAR™) platform technology to capture the precise, disease-targeting properties of T-cell receptors (TCRs) in creating next-generation therapeutic molecules and novel reagents for research use.

Monoclonal antibodies are well known as targeted immunotherapeutics and have enjoyed enormous success in the clinic and marketplace. Annual sales for the antibody therapeutics approved for clinical use were estimated to be US\$32 billion in 2008. However, many valuable targets are inaccessible to antibodies because these targets are not displayed on diseased cell surfaces. These antibody-inaccessible targets are processed into small peptides by the diseased cells and displayed on the cell surface in the context of MHC molecules (or HLA molecules in humans). Through this antigen-presenting pathway, each cell in the body is able to display a cell-specific set of peptide antigen–MHC complexes. Antigen-specific TCR molecules on T cells then recognize such antigen–MHC complexes. Every T cell produces TCRs with unique and specific binding properties, providing the immune system with the potential to survey and react to many different disease antigens, including antigens derived from intracellular protein targets. The ability of TCRs to bind a wide range of peptide antigens presented in the context of MHCs on tumor or virus-infected cells highlights their

potential as targeting agents for cancer and viral therapeutics. Altor has captured this potential with the development of its STAR or single-chain TCR (scTCR) molecules, which Altor is using to guide therapeutic drugs directly to the disease sites where they will be most effective. These scTCR molecules also have utility as diagnostic reagents for detecting disease-associated biomarkers.

Since a targeted immunotherapeutic only benefits patients with the corresponding targets on their diseased tissues, companion reagents are needed to identify or screen eligible patients for treatment or clinical trials. For instance, a patient's tumor sample is tested for the Her2/neu marker prior to treatment with Herceptin®, an antibody specific for Her2-positive breast cancer [1]. Similarly, diseased tissues from patients require verification that the target peptide is presented in the context of the appropriate HLA before treatment using a TCR-based immunotherapeutic. Altor's STAR multimer reagents and methods are designed to meet this need.

Altor's approach

With its unique proprietary STAR technology, Altor has developed the means for producing biologically active, soluble TCR molecules in a single-chain format. The resulting scTCR or STAR molecules retain the TCR's ability to specifically recognize novel antigen targets on virus-infected or cancerous cells, and can be readily produced on a commercial scale. Importantly, the MHC-restricted, peptide-specific

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binding activity of these scTCRs is retained even when fused or coupled with a detectable label, an effector molecule or a drug. This further simplifies the process of producing biologically functional molecules in a very cost-effective manner.

STAR multimer technology for biomarker detection

The cellular expression of specific peptides in a complex with MHC class I molecules plays a major role in stimulating and maintaining immune responses to cancer and infectious diseases. In fact, the presence of disease-related peptide–MHC complexes on the surface of tumor- or virus-infected cells represents a unique target for vaccine and other immunotherapeutic approaches. As indicated, most MHC-presented peptides are derived from the degradation of

intracellular proteins by the proteasome complex (FIGURE 1). The resulting peptide fragments are transported into the lumen of the endoplasmic reticulum where they are bound to the antigen-binding groove of the MHC molecule. The peptide–MHC complex is then transported to the cell surface for presentation to the adaptive immune system.

STAR multimer reagents allow detection of peptide–MHC complexes based on the ability of a given TCR to recognize a specific peptide presented in the context of a particular MHC molecule (FIGURE 1). STAR multimers are comprised of a soluble scTCR containing a biotin moiety. The biotinylated TCR molecules are multimerized following addition to streptavidin carrying detectable labels. Although the binding affinities between peptide–MHC

Figure 1. Specific detection of disease-related peptide–MHC complexes by single-chain T-cell receptor multimers.

SA-PE: Streptavidin-phycoerythrin; scTCR: Single-chain T-cell receptor.

complexes and TCRs are generally low, the increased avidity of STAR multimers results in an apparent higher peptide–MHC affinity than that observed for the monomeric TCR. Thus, STAR multimers are able to specifically and stably bind endogenous peptide–MHC complexes under a variety of experimental conditions [2]. For the clinical studies of Altor's lead TCR-based product, STAR multimers have been used to identify patients with tumors displaying the p53 peptide/HLA-A*0201 complex (FIGURE 2), demonstrating the utility of this method in a clinically relevant setting.

STAR multimers represent a breakthrough technology, allowing for the first time the direct detection of endogenous peptide antigen presentation on diseased cells and tissues. STAR multimers with appropriate fluorophores or contrast reagents have demonstrated versatile utilities in quantifying, validating and monitoring tumor and viral antigen presentation, imaging, and in the design and optimization of vaccine-based approaches using flow cytometric analysis, fluorescent microscopic or immunohistochemistry techniques on cells, formalin fixed or frozen tissues [2]. In many cases, staining with STAR multimers detected peptide–MHC complexes in tumor samples that would not be identified using antigen-specific antibodies. Thus, Altor's STAR technology will provide a powerful tool to elucidate those tumor or viral antigens that may be broadly applicable targets for immunotherapy.

STAR multimers also have uses in characterizing antigen-presenting cells (FIGURE 3), such as dendritic cells (DCs). Vaccination with DCs presenting tumor antigens has been shown to induce tumor-specific immunity and provide clinical benefit in some cancer patients [3]. However, optimization and standardization of these approaches generally relies on indirect and lengthy methods, such as CTL-based cytotoxicity assays, to assess antigen presentation. Altor has found that STAR multimer staining methods are as sensitive as other current methods for detecting antigen presentation on cells (FIGURE 3) and may provide a more general and direct approach to optimizing and validating the production of antigen-presenting cell therapies. This is illustrated by the success of using STAR reagents to quantitate peptide–MHC complexes on

the surface of DCs in an *in vivo* imaging approach to reveal the relationship of T-cell activation and antigen dose levels presented by DCs [4,5]. Finally, these reagents can be employed in a variety of methods to characterize TCR–MHC–peptide interactions and identify novel peptide antigens.

Altor has launched commercial sales of its first-in-class research reagents based on the STAR multimer technology. Altor's product line includes STAR multimers specific to two p53-antigen epitopes, melanoma antigen recognized by T cells (MART)-1 and gp100 tumor antigens and a cytomegalovirus antigen, as shown in TABLE 1. This portfolio will soon be expanded to include reagents targeting HIV, hepatitis C virus (HCV) and other tumor antigens, such as melanoma-associated antigen (MAGE)-A3, and with a focus on reagents for tumor antigens that are actively being investigated for vaccine- and cell-based therapies. Additionally, the HIV/HCV-specific reagents are being developed to monitor changes of epitope presentation by the infected cells occurring during acute and chronic infection and in certain patient populations to aid vaccine design and development.

STAR fusions in targeted therapeutic approaches

In addition to the STAR multimers, Altor's scientists have created several formats of STAR hybrid agents, including fusions to molecules such as cytokines (STAR-Ck), immunoglobulin constant regions (STAR-IgG1), and single-chain antibodies (STAR-scAb), and conjugates with anticancer or antiviral agents or to nanoparticles encapsulating these agents (STAR-Rx) (FIGURE 4). In each of the fusions, the scTCR component of the STAR hybrid molecule functions to guide and deliver the therapeutic agent or effector function to the tumor or infected cells, while the cytokines or other immuno-molecules stimulate and augment an antitumor or antiviral immune response. For STAR-Rx, the payloads can include anticancer or antiviral drugs, such as cytokines, cytotoxic drugs, radioisotopes or imaging agents, which could either elicit immune responses, cause direct toxicity or function as contrast agents once they reach the intended target tissues. Current development is focused on improving the

Figure 2. Study demonstrating peptide antigen presentation in human tumor tissue samples using p53-specific STAR™ multimer (264scTCR/multimer) by immunohistochemistry. (A) The normal tissue shows no staining with the horseradish peroxidase-conjugated p53-specific 264scTCR/multimer. **(B)** Formalin-fixed cancerous breast and colorectal tissues stained positively with horseradish peroxidase-conjugated 264scTCR/multimer, labeled with anti-p53 and anti-HLA-A*0201 antibodies. A control scTCR/multimer specific for a cytomegalovirus antigen shows no staining in either the breast cancer or colorectal cancer tissue. Anti-HLA-A2.1: Anti-HLA-A*0201 antibody; Anti-p53: Anti-p53 antibody; CMV: Cytomegalovirus; scTCR: Single-chain T-cell receptor. Reproduced with permission from [2].

US FDA-approved agents such as IL-2 (Proleukin®), GM-CSF (Leukine®), doxorubicin or promising drug candidates, such as IL-15, for treatment of malignant tumors, and IFN- α (Pegasys®), and small molecule viral inhibitors for chronic viral infections in humans. scTCRs are employed as a drug delivery system to enhance efficacy, lower the therapeutic dosage of these currently

approved drugs, provide more convenient dosing regimens and decrease their toxic side effects.

Lead STAR fusion drugs for cancer in clinical development

Altor's lead anticancer STAR fusion targets cancer cells through the tumor-associated antigen, p53 [6]. The p53 antigen is

Figure 3. Study comparing level of STAR™ multimer staining with amount of peptide used in loading antigen-presenting cell preparation. PE: Phycoerythrin.

Table 1. STAR™ multimer reagents available from Altor.

Protein	AA	Peptide sequence	Labels	HLA
p53	264–272	LLGRNSFEV	HRP and PE	HLA-A*0201
p53	149–157	STTPPPGTRV	HRP and PE	HLA-A*0201
MART-1	26–35	ELAGIGILTV	PE	HLA-A*0201
gp100	209–217	IMDQVPFSV	PE	HLA-A*0201
CMV pp65	495–503	NLVPMVATV	HRP and PE	HLA-A*0201
HIV Vpr	59–67	AL9: AIIRILQQL	PE	HLA-A*0201

AA: Amino acid; CMV: Cytomegalovirus; HLA: Human leukocyte antigen; HRP: Horseradish peroxidase; MART: Melanoma antigen recognized by T cells; PE: Phycoerythrin.

overexpressed in approximately 50% of all human cancers and in many cases correlates with poor prognosis, making it an ideal target candidate for cancer-specific therapies [7–10]. However, p53 cannot be used as a target for antibody-based therapies because it is not displayed on the tumor cell surface. Altor has developed a high-affinity scTCR that recognizes a peptide antigen derived from p53 and is displayed in the context of HLA-A*0201. ALT-801 is a fusion of that p53-specific scTCR and the approved anticancer drug, IL-2 [6], and is designed to deliver IL-2 activity directly

to the tumor site [11]. In numerous animal studies, ALT-801 exhibits targeted, potent anticancer activity against p53⁺ xenograft tumors [6,11,12].

In a recently completed Phase I/IIa clinical trial, ALT-801 was well tolerated at a level that exhibits evidence of clinical benefit, including tumor shrinkage, to patients with metastatic malignancies. A Phase II clinical trial for ALT-801 in combination with chemotherapy in patients with metastatic melanoma is currently underway and additional Phase II clinical trials in other cancer indications, including prostate,

Figure 4. Altor's single-chain T-cell receptor-based therapeutic product candidates.

ADCC: Antibody-dependent cellular cytotoxicity; HLA: Human leukocyte antigen; NK: Natural killer.

head and neck and renal cell carcinoma, will follow. A US\$3 million National Institutes of Health (NIH)/National Cancer Institute (NCI) Small Business Innovation Research (SBIR) Bridge Grant awarded to Altor in September 2009 supports these trials.

Additional anticancer product candidates based on the STAR fusion technology in preclinical development include ALT-802, a fusion of the p53-specific scTCR to the IgG1 heavy chain constant domains [13], and ALT-804, a fusion of the p53-specific scTCR to a super agonist mutant of IL-15 [14]. Altor has also developed a large portfolio of STAR immunotherapeutics against HIV, HCV and

cytomegalovirus. These programs are supported by various grants from NIH/NCI, NIH/National Institute of Allergy and Infectious Diseases (NIAID) and the Gates Foundation.

Future perspective

STAR reagents promise to provide a valuable new tool for the discovery of biomarkers, vaccines and therapeutics for cancer and as antiviral treatments. Altor has been collaborating with many leading researchers in its focal areas to vigorously pursue the creation of STAR reagents. A sizable portfolio of STAR reagents is anticipated to be commercially available for the research community.

Executive summary

- Altor has utilized its single-chain T-cell receptor (scTCR)-based technology to successfully develop reagents capable of detecting novel tumor and viral antigens. Specifically:
 - Soluble scTCR/multimers were generated, which specifically detect endogenous HLA-A*0201/p53 (amino acid 264–272) peptide complexes on human tumors cells, xenograft tumors, human colorectal and breast cancer specimens, and tumors obtained from patient biopsies.
 - Very sensitive staining methods were developed to detect the HLA-A*0201-displayed cognate peptide at peptide concentration as low as 12 nM.
 - First-in-class research reagents based on antigen-specific TCR/multimers have been commercialized by Altor. Custom reagents have also been developed.
 - An expanded panel of scTCR-based staining reagents recognizing other tumor antigens and HIV and hepatitis C virus antigens are currently being developed.
 - Strategies, such as affinity improvement of TCR and enhanced reagent labeling, to improve TCR-based staining reagents have proven to be productive.
 - Soluble scTCR/multimers have the potential to be a novel class of diagnostic reagents useful in monitoring cancer and viral infection, and guiding vaccine and immunotherapeutic approaches.

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