# Novel chimeric antigen receptor T cells based on T-cell receptor-like antibodies

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**Abstract**

The need for novel therapeutics against human cancers such as leukemias and solid tumors is well recognized. Human T cells are poised to make a fundamental change in the therapeutic approach. T-cell interaction with a tumor cell is a critical event and primarily driven by T-cell receptor (TCR) recognition of peptide in the pocket HLA. However, among TCR-based T-cell therapies, either TCR mismatching or the low density of major histocompatibility complex causes tumor cells to escape from the immune response. TCR molecules have low binding affinities, preventing their recognitions. Undoubtedly, antibody therapeutics is an effective treatment for cancer. As the new generation of monoclonal antibodies, TCR-like antibodies can mimic TCR recognition but are not susceptible for mechanisms of tumor evasion from the immune response. As chimeric antigen receptor (CAR) structure expressed on the surface of T cells, TCR-like antibodies can confer antigen specificity to T cells. The new TCR-like CAR may be important to drive new technologies of adoptive cell therapy, in particular, T-cell therapy, and open possibilities to target endogenous tumor-specific antigens.

**Keywords:** Antibody, CAR, T cell, TCR-like antibody

## 1. INTRODUCTION

Peptide/major histocompatibility complex (pMHC) I molecules are very important in the presentation of aberrant proteins in tumor cells. T-cell receptor (TCR) on the surface of T lymphocytes can recognize peptide fragments derived from endogenous proteins in complex with MHC[1] (Fig. 1). The expression of pMHC constitutively occurs on all nucleated cells. Disease-specific pMHC as T-cell epitopes are desirable targets for immunotherapies. A number of well-defined tumor-associated antigens were discovered in different tumor types and recognized by T cells. These T-cell epitopes are classified into tumor-specific shared antigens, differentiation antigens, antigens resulting from mutations, overexpressing antigens, and viral antigens. Among these groups, highly specific antigens have been identified, such as (i) WT1, gp100 and MART-1, melanocyte differentiation antigens; (ii) β-catenin, a colon-specific antigen arising from mutated antigens; (iii) HER2/neu, an antigen derived from gene overexpression or amplification; and (iv) NY-ESO-1, a cancer testis antigen expressed only by tumor cells and spermatogenic cells from the testis. Despite the presence of cytotoxic T cells (CTLs) specific for these pMHC epitopes in cancer patients, immune response against tumors is insufficient to cause tumor regression. Lots of researchers are exploring strategies for enhancing T-cell responses to these T-cell epitopes, including vaccination,[2] adoptive T-cell transfer,[3] and TCR engineering.[4]

In cancer patients, however, native TCRs usually fail to mediate T-cell recognition and activation process.[5] Tumor cells effectively escape from T-cell response by regulatory mechanisms, such as downregulated expression of pMHC, in tumor environment. It has been widely believed that the interaction of TCR and pMHC is lower avidity. Though some TCRs can target pMHC epitopes, their therapeutic potential is limited due to their inherent low binding affinities. The low density of MHC molecules on tumor cell surface causes constraints on low-affinity TCR recognition. Protein engineering of TCRs is often difficult due to loss of peptide specificity, resulting in dramatic increased nonspecific reactivity. In most cases, native or engineered TCRs are obviously insufficient to detect and effectively eliminate malignant cells.

## 2. TCR-LIKE ANTIBODIES

Therapeutic antibodies have been highly successful for cancer treatment since the past decade. As a novel class of antibodies, TCR-like antibodies can imitate the fine specificity of T-cell recognition toward antigen-derived pMHC molecules on the cell surface (Fig. 1).[6] Functional improvement of TCR-like antibodies is easier than native TCR molecules by rational antibody engineering approach.[7,8] The structural data can further benefit to design high-affinity antibodies, keeping the specificity unchanged.[9] To obviate the obstacles of native TCR,
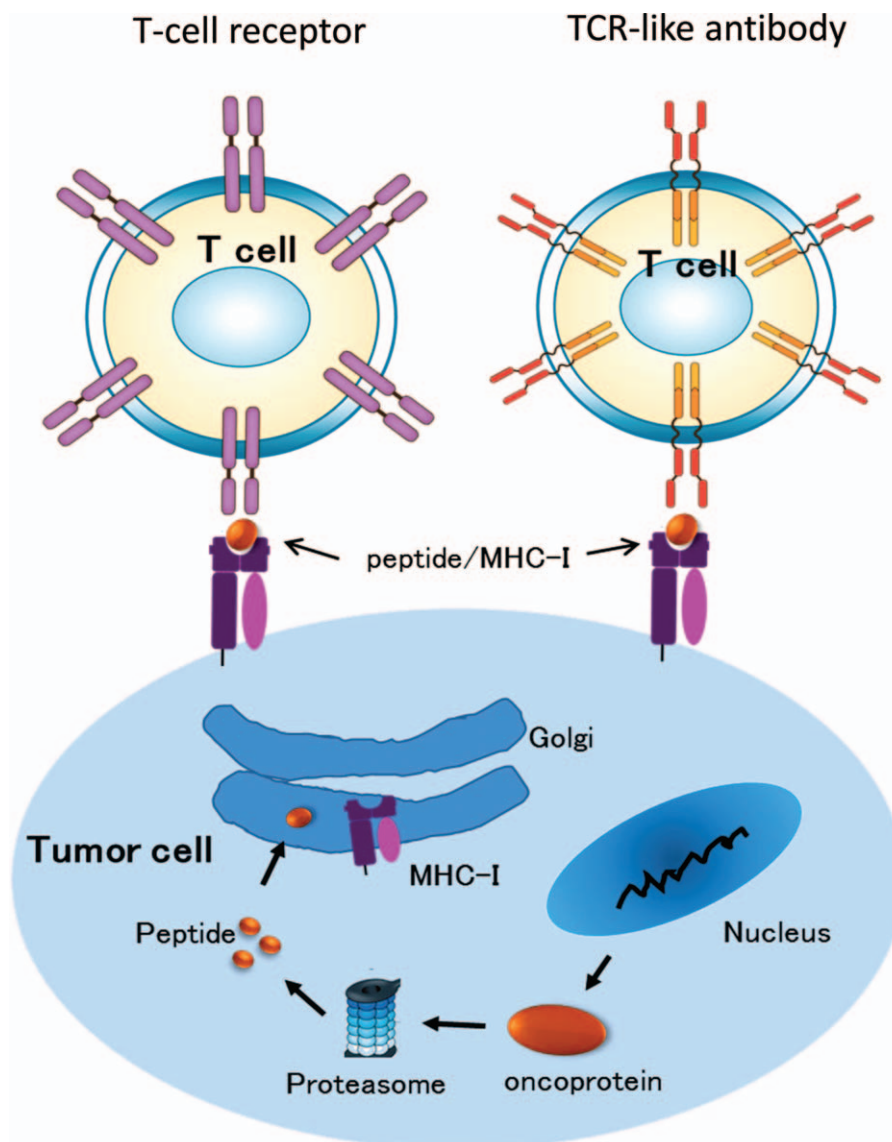
**Figure 1.** Recognition of TCR molecule and TCR-like antibody to a peptide/MHC complex on a cancer cell. Endogenous proteins are processed by the proteasome and presented on the cell surface as small peptides in the pocket of MHC class I molecules (HLA in humans). Peptides are recognized by T-cell receptors (TCRs). TCR-mimic antibodies mimic the fine specificity of T-cell recognition.

lots of TCR-like antibodies targeting pMHC epitopes derived from tumor-specific antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2[10–15] have been identified. By phage-display selection, TCR-like antibodies could be feasibly selected entirely in vitro, dramatically increasing the efficiency of selection compared with isolation of TCR. Tumor-specific antigens include the leukemia antigen WT1 or proteinase 3, testicular cancer antigen NY-ESO-1, and the melanoma antigens gp100, telomerase, MART1. They actually enhance cytotoxic T-cell response or induction of immune effectors. These TCR-like antibodies mimic the recognition of TCRs to particular pMHC complexes on tumor cells. In general, TCR-like antibodies typically have far better antigen binding affinities than native TCR molecules. TCR-like antibodies could be ideal therapeutics with high affinity and controlled specificity. They will be valuable tools in

developing new immunotherapy agents and studying antigen presentation.

## 3. TCR-LIKE CAR-T CELLS

Recently, monoclonal antibodies have become successful is the use of chimeric antigen receptor (CAR)-modified T cells.[16,17] A CAR construct usually uses an antibody-derived single-chain variable fragment (scFv), a transmembrane region (TM), and signaling regions of activating T cells. CAR-T therapy has recently emerged as a potentially curative therapy in clinical trials of leukemias and some type of solid tumor. For example, anti-CD19 CAR-T cells show remarkable clinical results in B-lineage malignancies.[18] T cells are genetically engineered to express CAR by viral transduction. These engineered T cells are ex vivo expanded and then infused into the patient. After the infusion, T

cells in the patient's body recognize and kill cancer cells that harbor the antigen on their surfaces. Although a CAR in a non-HLA complex-restricted manner can generally target tumor-specific antigens on cell surface, sources of such surface antigens are limited. TCR-like antibodies just provide a possible insight into intracellular tumor-associated antigens. Like traditional monoclonal antibodies, TCR-like antibodies could also be fused with intracellular signal transduction moieties to generate TCR-like CARs. When T cells carry TCR-like-CAR, they can be efficiently delivered to desired T-cell epitopes on tumors.[19] Once a TCR-like antibody binds to a pMHC on cell surface of tumor, the intracellular domain of TCR-like CAR transduces activating and proliferating signals to trigger T cells to lyse target tumor cells. Therefore, this TCR-like CAR could execute TCR's function while circumventing the drawbacks of transgenic TCR. This unique TCR-like chimeric receptor is in fact analogous to the use of affinity-enhanced TCRs. The design of TCR-like antibody-based CARs will improve the T-cell recognition while remaining the specificity of antigen.[20] Recently, several TCR-like CARs have been developed to target tumor-associated epitopes in MHC complexes, including WT1,[8,21] NY-ESO-1,[22] PR1,[23] alpha-fetoprotein,[24] and gp100.[25] These novel CARs demonstrate that TCR-like antibodies are able to redirect T cells to kill cancer cells expressing low pMHC densities.

One example is Wilms tumor 1 (WT1) that is found in hematologic malignancies and some types of solid tumors, including acute lymphocytic leukemia (ALL), and some solid tumor (e.g., breast cancer).[26] It is listed as a high-priority target for cancer drugs by the National Cancer Institute pilot project.[27] Significantly, a 9-mer WT1-derived peptide (residue positions 126–134, RMFPNAPYL) (WT1126) in the context of HLA-A2 molecules induced specific cytotoxic T-cell responses in acute lymphoblastic leukemia (ALL) patients.[28] Two CARs encoding anti-WT1 TCR-like antibodies have been designed to express on the surface of T cells.[8,21] CAR-modified T cells could mediate specific and potent cytotoxicity toward different types of WT1-positive tumor cells, including ALL. These T-cell epitopes overexpressed by tumor cells to date have become targetable by CAR T cells.

## 4. CONCLUSION AND PERSPECTIVE

These TCR-like antibodies offer the combined features of antibody therapies and T cells, joining the functions of TCR recognition of an endogenous antigen. Using these reagents, the antigenic repertoire of CARs is expanded to target intracellular antigens. However, there is the difference of antigen affinity for TCR (micromolar level) and TCR-like antibody (nanomolar level).[8] This high avidity of multiple TCR-like molecules on T cells potentially affects the binding specificity, leading to cross-reactivity with HLA-A2 complexes presenting irrelevant peptides.[29] Therefore, it is important to address whether it exists at maximal receptor affinity thresholds for achieving T-cell function while keeping the fine specificity. Although some questions are concerned, TCR-like antibodies represent a next generation of antibodies that offers a new option for personalized therapy and diagnosis of HLA-matched patients.

## ACKNOWLEDGMENTS

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