Chimeric antigen receptor

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Artificial T cell receptors (also known as **chimeric T cell receptors, chimeric immunoreceptors, chimeric antigen receptors (CARs)**) are engineered receptors, which graft an arbitrary specificity onto an immune effector cell.

Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by retroviral vectors. The receptors are **called chimeric because they are composed of parts from different sources.**

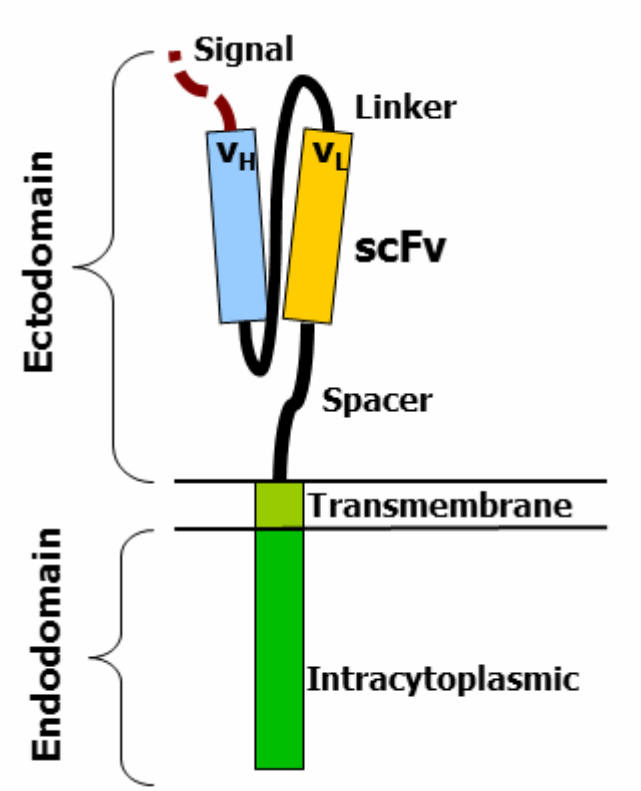
Artificial T cell receptors are under investigation as a therapy for cancer, using a technique called adoptive cell transfer.[1] T cells are removed from a patient and modified so that they **express receptors specific to the particular form of cancer.** The T cells, which can then recognize and kill the cancer cells, are reintroduced into the patient. Modification of T-cells sourced from donors other than the patient are also under investigation.

Structure

The **most common form** of these molecules are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies, fused to CD3-zeta transmembrane and endodomain. Such molecules result in the transmission of a zeta signal in response to recognition by the scFv of its target.

An example of such a construct is **14g2a-Zeta, which is a fusion of a scFv derived from hybridoma 14g2a (which recognizes disialoganglioside GD2)**. When T cells express this molecule (usually achieved by oncoretroviral vector transduction), they recognize and kill target cells that express GD2 (e.g. neuroblastoma cells). To target malignant B cells, investigators have redirected the specificity of T cells using a chimeric immunoreceptor specific for the B-lineage molecule, CD19.

The variable portions of an **immunoglobulin heavy and light chain are fused by a flexible linker to form a scFv.** This scFv is preceded by a signal peptide to direct the nascent protein to the endoplasmic reticulum and subsequent surface expression (this is cleaved). A flexible spacer allows the scFv to orient in different directions to enable antigen binding. The transmembrane domain is a typical hydrophobic alpha helix usually derived from the original molecule of the signalling endodomain which protrudes into the cell and transmits the desired signal.



Different components of an artificial TCR

The fact that these molecules actually work is at first glance surprising. At second glance one remembers that type I proteins are in fact two protein domains linked by a transmembrane alpha helix in between. The cell membrane lipid bilayer, through which the transmembrane domain passes, acts to isolate the inside portion (endodomain) from the external portion (ectodomain). It is not so surprising hence that attaching an ectodomain from one protein to an endodomain of another protein results in a molecule that combines the recognition of the former to the signal of the latter.

Ectodomain

Signal peptide

A signal peptide directs the nascent protein into the endoplasmic reticulum. This is **essential if the receptor is to be glycosylated** and anchored in the cell membrane. Any eukaryotic signal peptide sequence usually works fine. Generally, the signal peptide natively attached to the amino-terminal most component is used (e.g. in a scFv with orientation light chain - linker - heavy chain, the native signal of the light-chain is used).

Antigen recognition region

The antigen recognition domain is usually an scFv. There are however many alternatives. An antigen recognition domain from native T-cell receptor (TCR) alpha and beta single

chains have been described, as have simple ectodomains (e.g. CD4 ectodomain to recognize HIV infected cells) and more exotic recognition components such as a linked cytokine (which leads to recognition of cells bearing the cytokine receptor). **In fact almost anything that binds a given target with high affinity can be used as an antigen recognition region.**

Spacer

A spacer region links the antigen binding domain to the transmembrane domain. It should be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition. The simplest form is the hinge region from IgG1. Alternatives include the CH_2CH_3 region of immunoglobulin and portions of CD3. For most scFv based constructs, the IgG1 hinge suffices. However the best spacer often has to be determined empirically.

Transmembrane domain

The transmembrane domain is a hydrophobic alpha helix that spans the membrane. Generally, the transmembrane domain from the most membrane proximal component of the endodomain is used. Interestingly, using the CD3-zeta transmembrane domain may result in incorporation of the artificial TCR into the native TCR a factor that is dependent on the presence of the native CD3-zeta transmembrane charged aspartic acid residue[2] . Different transmembrane domains result in different receptor stability. The CD28 transmembrane domain results in a brightly expressed, stable receptor.

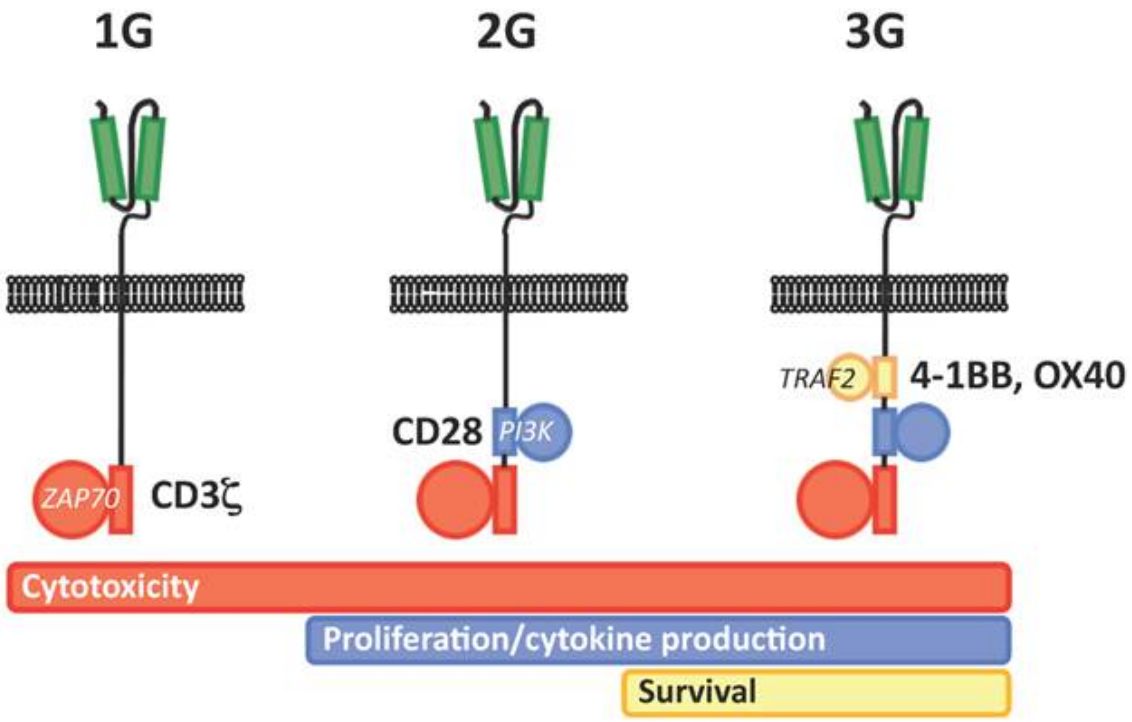
Endodomain

This is the "business-end" of the receptor. After antigen recognition, receptors cluster and a signal is transmitted to the cell. The most commonly used endodomain component is CD3-zeta which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signaling is needed. For example, chimeric CD28 and OX40 can be used with CD3-Zeta to transmit a proliferative / survival signal, or all three can be used together.

Evolution of CAR T-cell design

"First-generation" CARs typically had the intracellular domain from the CD3 ζ- chain, which is the primary transmitter of signals from endogenous TCRs. "Second-generation" CARs add intracellular signaling domains from various costimulatory protein receptors (e.g., CD28, 41BB, ICOS) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. Preclinical studies have indicated that the second generation of CAR designs improves the antitumor activity of T cells. More recent, "third-generation" CARs combine multiple signaling domains, such as CD3z-CD28-41BB or CD3z-CD28-OX40, to further augment potency. The evolution of CAR therapy is an **excellent example of the application of basic research to the clinic.** The PI3K binding site used was

identified in co-receptor CD28 {Rudd CE, Schneider H. (2003) Unifying concepts in CD28, ICOS and CTLA4 co-receptor signalling. Nat Rev Immunol. 3(7):544-56}, while the ITAM motifs were identified as a target of the CD4- and CD8-p56lck complexes {Rudd, C.E. (1999) Adaptors and molecular scaffolds in immune-cell Signaling. Cell 96, 5-8. PMID: 9989491}.



Depiction of first, second, and third generation chimeric antigen receptors with the scFv segments in green and the various TCR signalling components in red, blue and yellow.[3]

The **introduction of Strep-tag II sequence** (an eight-residue minimal peptide sequence (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) that exhibits intrinsic affinity toward streptavidin[4]) into specific sites in synthetic chimeric antigen receptors or natural T-cell receptors of diverse specificities provides engineered T cells with a marker for identification and rapid purification, a method for tailoring spacer length of chimeric receptors for optimal function, and a functional element for selective antibody-coated, microbead-driven, large-scale expansion.[5][6] Strep-tag can be used to stimulate the engineered cells, causing them to grow rapidly. Using a special antibody that binds the Strep-tag, the engineered cells can be expand by 200-fold. Unlike existing methods which stimulate all T cells, this technology stimulate just the cancer-specific ones. This new technology, is not yet tested in humans.

History

The concept of doctoring T-cells genetically was **first developed in the 1980s by Prof. Zelig Eshhar and colleagues at the Weizmann Institute of Science in Rehovot, Israel**. By 1989, Eshhar and his colleagues had created the first functional CAR T cells.

http://www.nejm.org/doi/full/10.1056/NEJMe1106965

Clinical studies

The use of chimeric antigen receptors in the clinic is based on reprogramming the T cell antigen receptor using a vector (for example viral) which is specific for malignant cells, enabling their destruction. CAR-modified T cells are a promising form of cancer immunotherapy. Pre-clinical and clinical trials have focused on determining the best possible structure and signalling.

The **first generation** of CAR-modified T cells have shown success in pre-clinical trials and have entered phase I clinical trials in ovarian cancer, neuroblastoma and various types of leukemia and lymphoma. To date, *these clinical trials have shown little evidence of anti-tumor activity, with insufficient activation, persistence and homing to cancer tissue.* Some anti-tumor responses have been reported in patients with B cell lymphoma (treated with alfaCD20-CD3zeta CARs-modified T cell) and some neuroblastoma patients (treated with ScFv-CD3zeta CARs-modified T cell) have reported partial response, stable disease and remission.

Second and third generation CAR-modified T cells are also capable of providing enhanced activation signals, proliferation, production of cytokines and effector function of CAR-modified T cells in pre-clinical trials. Both the second and the third generation CAR-modified T cells are entering clinical trials. The first clinical trial has shown some promising results. In a study with alfaCD19.4-1BB.CD3zeta T cells in **patients with chronic lymphocyte leukemia complete remission has been ongoing 10 months after treatment.** The CAR-modified T cells were found to expand 3-logs in these patients, and to have infiltrated and lysed cancer tissue. Interestingly, a fraction of these cell displayed a T cell memory phenotype for preventive tumor relapses. Although these CAR-modified T cells produced significant therapeutic effect, their activity led to life-threatening tumorlysis 3 weeks after the first infusion of CAR-modified T cells.

Recently, **adverse events were reported** that highlight the need for caution while using second and third generation of CAR-modified T cells. One **patient died 5 days after cyclophosphamide chemotherapy followed by infusion of CAR-modified T cells recognizing the antigen** ERBB2 (HER-2/neu).[7] The toxicity led to a clinically significant release of pro-inflammatory cytokines, pulmonary toxicity, multi-organ failure and eventual death of the patient. This "cytokine storm" was thought to be due to CAR T cell cytotoxicity against normal lung epithelial cells, known to express low levels of ERBB2. This and other adverse events highlight the need for caution when employing

CAR-modified T cells, as unlike antibodies against tumor-associated antigens, these cells are not cleared from the body quickly.

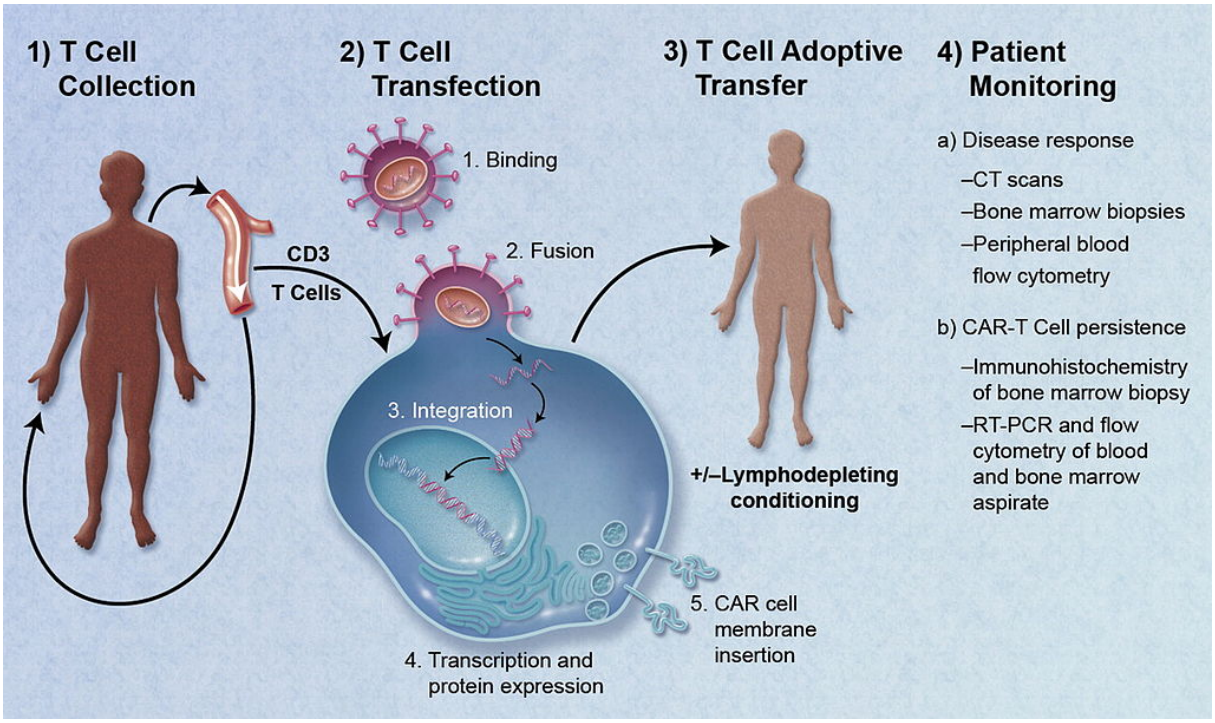
The great promise of cancer immunotherapy is to clear the tumor without the toxicity of conventional treatments. The treatment of cancer with modified T cells has several advantages: HLA-independent recognition of antigen, broad applicability for many patients and the rapid delivery of CAR-modified T cells. Successful application of these modified T cells will require the identification of the tumor-associated antigen, that are expressed only on tumor cells, thereby minimizing the risk of toxicity,[8][9]

Early examples

Adoptive transfer of CAR-modified cells as a cancer therapeutic

This section is **outdated**. Please update this article to reflect recent events or newly available information.

Last update: Kochenderfer JN, Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors Nature Reviews Clinical Oncology 10, 267-276 (May 2013) doi:10.1038/nrclinonc.2013.46 *(June 2013)*



Depiction of adoptive cell transfer therapy with CAR-engineered T cells

Adoptive transfer of T cells expressing chimeric antigen receptors is a promising anti-cancer therapeutic as CAR-modified T cells can be engineered to target virtually any tumor associated antigen. There is great potential for this approach to improve patient-specific cancer therapy in a profound way.

Following the collection of a patient's T cells, the cells are genetically engineered to express CARs specifically directed towards antigens on the patient's tumor cells, then infused back into the patient.[12]

Although adoptive transfer of CAR-modified T-cells is a unique and promising cancer therapeutic, there are significant safety concerns. Clinical trials of this therapy have revealed potential toxic effects of these CARs when healthy tissues express the same target antigens as the tumor cells, leading to outcomes similar to graft-versus-host disease (GVHD).

A potential solution to this problem is engineering a suicide gene into the modified T cells. In this way, administration of a prodrug designed to activate the suicide gene during GVHD triggers apoptosis in the suicide gene-activated CAR T cells.

This method has been used safely and effectively in hematopoietic stem cell transplantation (HSCT). Adoption of suicide gene therapy to the clinical application of CAR-modified T cell adoptive cell transfer has potential to alleviate GVHD while improving overall anti-tumor efficacy.[3]