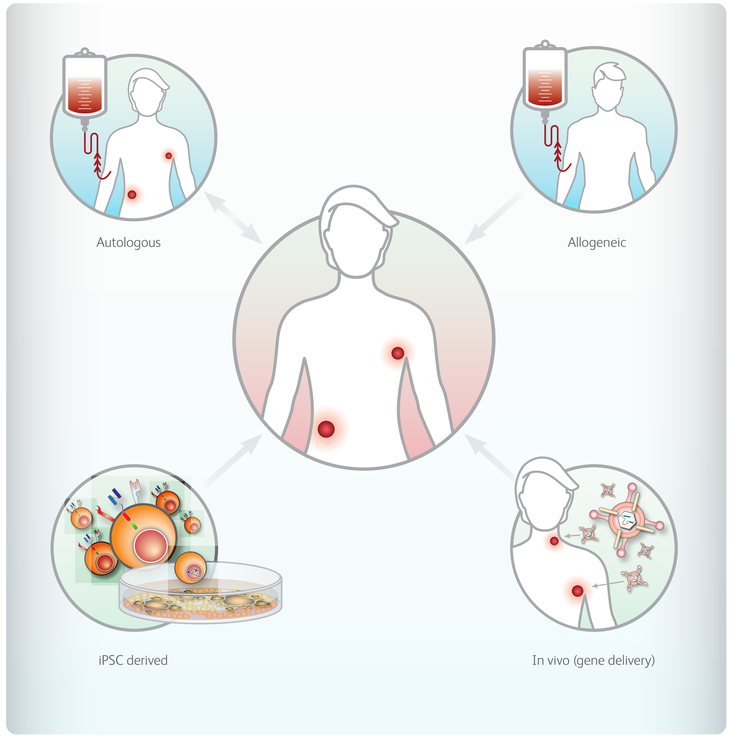
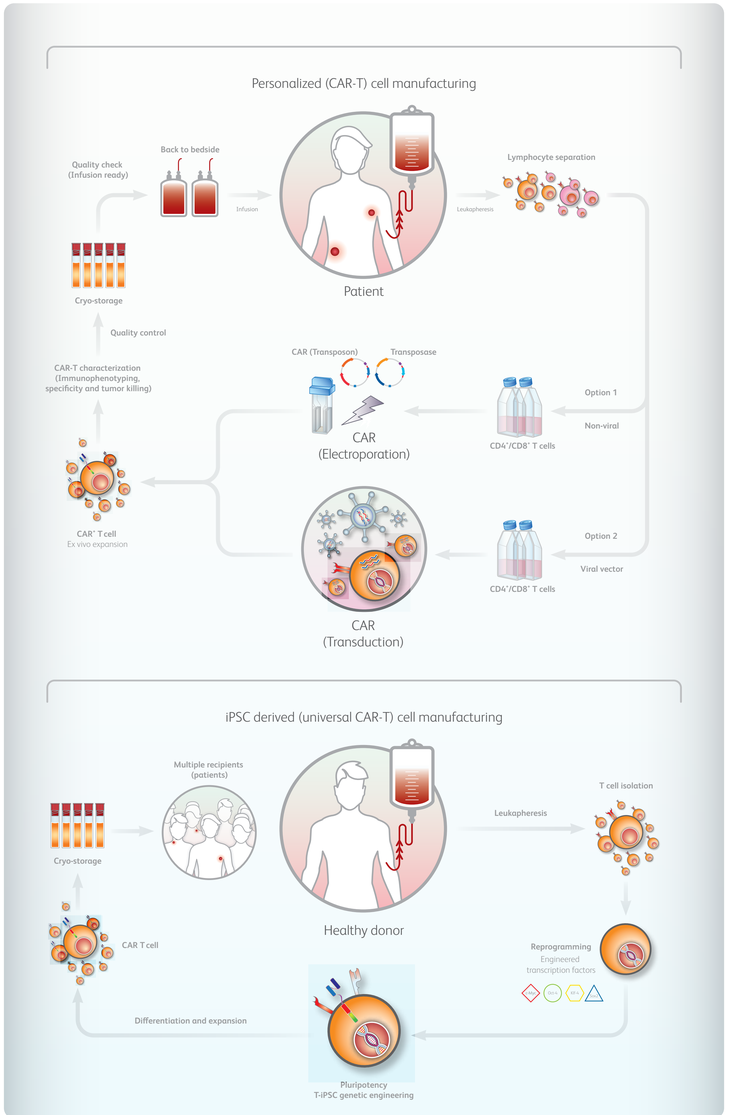
# Cancer immunotherapy

## Engineered immune cells for anti-tumor immunity

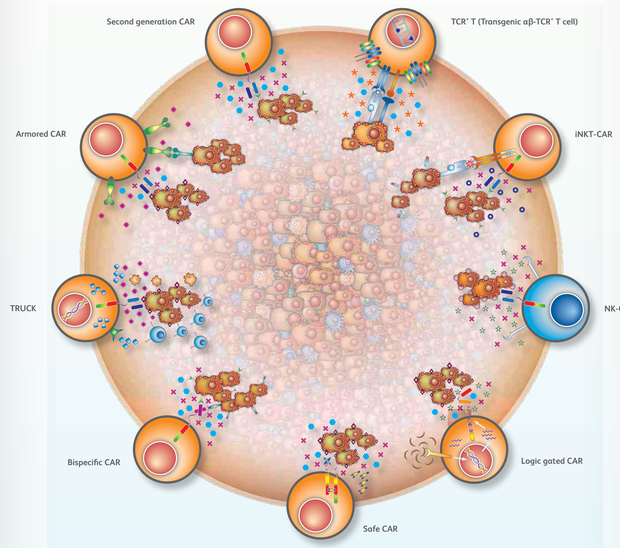
### Immune cell source




### Gene modified cell manufacturing




### Immune cell engineering




Engineered CAR/TCR counters tumor complexity

**Immunotherapy is a rapidly growing branch of cancer medicine that treats cellular malignancies by empowering the body's immune system. Cell-based immunotherapy involves adoptive transfer of tumor targeting cytotoxic immune cells (with or without gene modification) for tumor neutralization. Tumor antigen targeting gene engineered T cells, natural killer (NK) cells and other unconventional immune cells such as invariant natural killer T cells (iNKT) or γδ T cells have been successfully used for treatment of various tumor malignancies. This poster provides an overview of cell-based cancer immunotherapy illustrating immune cell engineering and gene modified cell manufacturing methods currently in practice.**

#### Cell therapy

An immunotherapy approach based on the use of live immune cells (with or without genetic modification) for treatment of cancer and other cellular disorders. Major steps involved in cell therapy are cell isolation, genetic modification, ex vivo culture, immunophenotyping and functional characterization of tumor targeting effector cells. One notable example of cell-based immunotherapy is CART-19 (CD19 specific chimeric antigen receptor T cell) for treatment of B cell tumors such as B-ALL, CLL and DLBCL.

#### CAR-T

Chimeric antigen receptor expressing T cells (CAR-T) refer to T cells modified with the synthetic CAR gene. Structurally, a CAR consists of an scFv (single chain fragment variable comprising of VH and VL fragments derived from a monoclonal antibody) joined in frame with the T cell transmembrane domain and intracellular signaling domains. Expression of a CAR on the T cell surface helps in antigen recognition independent of MHC. CD19CD28z CARs or CD19 4-1BBz CARs are notable examples of CAR-T products used in the clinic for treatment of specific B cell tumors.

#### Autologous

Autologous cell refers to a patient-derived immune cell applied as a therapeutic agent in the same patient. In autologous T cell therapy, cells are isolated from a patient's own leukapheresis product followed by CAR gene modification, ex vivo expansion and characterization before infusion in patient. Current FDA approved CAR-T products use T cells derived from autologous sources.

#### Allogeneic

Allogeneic cell refers to a non-self donor-derived immune cell used as a therapeutic agent in a patient. Allogeneic T cells are derived from an HLA-matched sibling and gene modified to express tumor antigen targeting CAR. Allogeneic T cells derived from unrelated sources are gene modified to disrupt expression of intact αβ-TCR and HLA on the cell surface to avoid causing graft-versus-host disease (GVHD) and/or rejection in the host. Besides T cells, other immune cell types such as NK cells and γδ T cells derived from allogeneic sources are also used for anti-tumor application.

#### iPSC derived

Induced pluripotent stem cell (iPSC) technology shows that by using specific transcription factors (Oct4, Sox2, Klf4, c-Myc) both mouse and human somatic cells can be reprogrammed to pluripotency. Based on iPSC technology, researchers have made CAR-T and CAR-NK from iPSC derived T cells or NK cells. To enable T iPSC-CAR for therapeutic applications, multiple gene engineering strategies are needed. Among those, engineering to disrupt HLA-Class I, genomic deletion of TCR-α or β and stable expression of CAR in T-iPSC clones are important. A regulatory compliant manufacturing process and methods to scale up the T-iPSC derived CAR-T cells are needed in ensuring generation of a universal CAR-T product that is ready to be used as an off-the-shelf drug.

#### In vivo gene delivery

An alternate approach to use of ex vivo expanded CAR-T or CAR-NK is in vivo delivery of synthetic gene(s) along with oncolytic viruses (OV) that replicate within the tumor and elicit systemic anti-tumor responses. Selective expression of tumor antigens via OV can enhance trafficking of T cells to the tumor site. Engineered OVs are effective in delivering BiTE (bispecific T cell engager), a specific engineered antibody that enhances the action of tumor infiltrating lymphocytes (TILs) and elicits stronger anti-tumor immunity. OVs can be engineered to deliver immunostimulatory cytokines on tumor tissues and are being tested in combination with existing cancer immunotherapy modalities like CAR-T to treat refractory solid tumors.

#### Personalized CAR-T cell manufacturing

Personalized CAR-T therapy uses a patient's own peripheral blood mononuclear cells (PBMCs) to make CAR-T cells. Patient PBMC derived T cells are modified to express the CAR transgene either by viral transduction or non-viral DNA plasmid (transposon) electroporation. Genetically modified T cells are then assessed for stable CAR expression and evaluated for specificity, immunophenotyping and CAR-specific effector function. Next, CAR-T cells are expanded to large numbers in bioreactors or in tissue culture flasks under GMP condition and checked for quality. Cryopreserved CAR-T vials are transferred to designated treatment sites for infusion in patients.

#### iPSC derived universal CAR-T manufacturing

iPSC-based off-the-shelf cell banks aim to provide a steady source of therapeutic grade tumor neutralizing cells to patients on demand. This requires creation of master universal cell banks by reprogramming antigen specific T cell using iPSC technology. CARs can be expressed on iPSC-T cells through genetic engineering. Multiple genome engineering strategies are required to make iPSC CAR-T cells immunologically relevant. Establishment of regulatory compliant clinical grade cell manufacturing processes and safety of iPSC CAR-T cells are major research areas in this field.

#### Second generation CAR

Second-generation CARs represent a molecular design that possesses two different T cell signaling endo-domains embedded downstream of the CAR molecular construct. First generation CARs contain only one CD3ζ signaling domain. Inclusion of additional T cell co-stimulatory domain in second generation CARs improve persistence of CAR-T cells in vivo and facilitates long-term anti-tumor immunity to the host. Choice of a second co-stimulatory domain in CAR-T cells include CD28, 4-1BB or OX-40 among others. FDA-approved CD19-CAR T products belong to the second generation CAR that targets CD19 on B cell tumors and secretes IL-2 and IFN-γ upon antigen recognition.

#### Armored CAR

Tumors display various immune evasion mechanisms like MHC downregulation or expression of inhibitory receptors, secretion of suppressive cytokines which ultimately dampen the activity of cytotoxic T cells. Armored CAR-T cells are designed to counteract such barriers so that they function effectively within the tumor microenvironment (TME). A classic example of tumor immune escape is upregulation of PD-L1 on the tumor cell surface that interacts with the inhibitory PD-1 displayed on activated T cells. To remove the inhibitory effect of the PD-1/PD-L1 interaction, researchers transduced a PD-1 double negative receptor along with second generation CAR expressed on the same T cell in parallel. The engineered negative receptor can successfully saturate ligand PD-L1 thereby blocking its interaction with inhibitory PD-1 on the T cell, a strategy shown to boost anti-tumor efficacy of CAR-Ts. Armored second-generation CAR unleashes effector function through secretion of cytokines IL-2, IFN-γ and TNF after tumor antigen recognition via CAR.

#### TRUCK

T cell redirected universal cytokine killing (TRUCK) is a specific type of armored CAR-T cell. TRUCKs can secrete specific cytokines (IL-12 or IL-18) upon engagement with TAA that helps proliferation, survival and TRUCK effector function. This strategy is effective for solid tumors as the TRUCK-secreted cytokines enhance increased infiltration of bystander innate immune cells to influence stronger anti-tumor immunity.

#### Bispecific CAR

Bispecific CARs are designed to negotiate the antigen-escape mechanism evolved by tumors. Bispecific CARs consist of two different antigen targeting moieties (scFv1 and scFv2) embedded on the same CAR molecular construct. Cytokine secretion abilities of bispecific CARs are like that of second-generation CARs. Preclinical studies have shown improved efficacy of bispecific CARs targeting CD19 and CD22 in B-ALL and lymphoma. Clinical trials involving various bispecific CARs are underway and may provide further insights into the efficacy of this approach.

#### Safe CAR

CAR-T cells have strong tumoricidal properties along with the ability to proliferate upon tumor antigen recognition. Theoretically CAR-T cells may persist in the host for a long time after infusion. However, over activation and proliferation of CAR-T cells in vivo can pose a threat to the patient. Scientists have introduced features in CAR constructs to ensure spatial and temporal control of infused CAR-T products. One safe CAR design entails the ability to recognize TAA but execute its effector function only after sensing an exogenously administered drug. This design regulates CAR function in a controlled manner by functioning as a safety switch.

#### Logic gated CAR

Most tumor antigens are shared among normal cells, which poses a challenge to develop effective CAR-Ts that do not cause harmful side effects. For logic gated CAR, activation occurs only in presence of a second antigen, thereby limiting the action of CAR-Ts on tumor cells only. Researchers have explored the use of synthetic Notch receptors on which antigen A binding releases a transcription factor that provides stimulus for expression of CAR targeting antigen B expressed on tumor. Thus, logic CARs exercise full functionality via cytokine (GM-CSF, IL-2, IFN-γ) secretion and tumor neutralization but only after conditional recognition of antigen.

#### NK-CAR

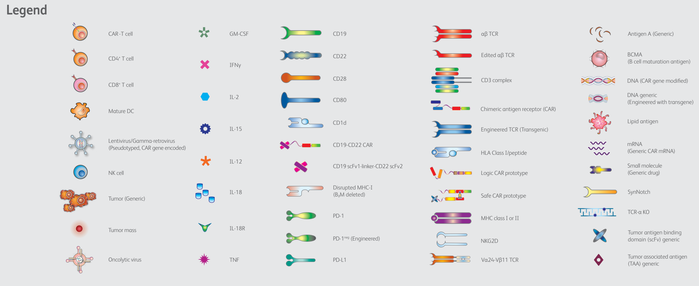
CARs can be expressed on NK cells and infused in patients similar to CAR-Ts. NK-CAR constructs contain tumor targeting scFv, NK cell specific transmembrane domain NKG2D and intracellular domain 2B4 for optimal signaling. NK-CARs targeting the B cell maturation antigen (BCMA) on multiple myeloma is one example currently under evaluation.

#### iNKT-CAR

iNKT cells are CD3+ T cells that express invariant αβ-receptors on cell surface. iNKT cells could be engineered to express CAR with specificity toward a tumor antigen. In addition to recognition of lipid antigens presented by non-polymorphic CD1d molecules, iNKT-CAR can be directed against tumors expressing a specified antigen target. Anti-tumor immunity of iNKT-CAR is driven by both natural cytotoxicity against CD1d+ tumor and CAR specific antigen expression. iNKT-CARs show efficacy against B cell tumors and certain brain tumors like glioblastoma.

#### TCR+-T

TCR+ T cells are pMHC (peptide-MHC complex) specific CD3+ T cells expressing transgenic αβ-TCR that are identified through a rigorous screening process and cloned into T cells. TCR+-T acts by precise recognition and affinity of the TCR to the neoantigen. TCR+-T cells have defined specificity to antigens such as NY-ESO-1, MART-1 and WT-1. Mechanistically, TCR+-T has an advantage over CAR-T as it can recognize peptide antigens presented in the MHC groove, while CAR-T recognizes cell surface antigens only. Use of TCR+-T is an effective immunotherapeutic agent for treatment of solid tumors.




## Abbreviations

**αβ-TCR**, Alpha beta T cell receptor; **B-ALL**, B cell acute lymphoblastic leukemia; **CAR**, Chimeric antigen receptor; **CAR-T**, Chimeric antigen receptor expressing T cell; **CD19 4-1BBz CAR**, CD19 4-1BB zeta chimeric antigen receptor; **CD19 CD28z CAR**, CD19 CD28 zeta chimeric antigen receptor; **CD3z**, CD3 zeta; **CLL**, Chronic lymphocytic leukemia; **DLBCL**, Diffuse large B cell lymphoma; **γδ T cell**, Gamma delta T cell; **GM-CSF**, Granulocyte-macrophage colony-stimulating factor; **GMP**, Good manufacturing practice; **HLA**, Human leukocyte antigen; **IFN-γ**, Interferon gamma; **IL**, Interleukin (e.g., IL-2 Interleukin 2); **iNKT**, Invariant natural killer T cell; **iPSC**, Induced pluripotent stem cell; **MHC**, Major histocompatibility complex; **NKG2D**, Natural killer group 2D; **PD-1**, Programmed cell death protein 1; **PD-L1**, Programmed cell death ligand 1; **scFv**, Single chain fragment variable; **TCR+ T**, Transgenic αβ-TCR expressing T cell; **TNF**, Tumor necrosis factor; **VH**, Variable heavy chain; **VL**, Variable light chain