

REVIEW ARTICLE

CAR T Cells: Precision Cancer ImmunotherapyAnna Meiliana^{1,2,*}, Nurrani Mustika Dewi², Andi Wijaya^{1,2}¹Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Jl. Eijkman No.38, Bandung, Indonesia²Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung, Indonesia

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Abstract

BACKGROUND: Current cancer drugs and treatments are aiming at eradicating tumor cells, but often are more toxic than effective, killing also the normal cells and not selectively the tumor cells. There is good personalized cancer therapy that involves administration to the cancer-bearing host of immune cells with direct anticancer activity, which called adoptive cell therapy (ACT). A review of the unique biology of T cell therapy and of recent clinical experience compels a reassessment of target antigens that traditionally have been viewed from the perspective of weaker immunotherapeutic modalities.

CONTENT: Chimeric antigen receptors (CAR) are recombinant receptors which provide both antigen-binding and T cell-activating functions. Many kind of CARs has been reported for the past few years, targeting an array of cell surface tumor antigens. Their biologic functions have extremely changed following the introduction of tripartite

receptors comprising a costimulatory domain, termed second-generation CARs. The combination of CARs with costimulatory ligands, chimeric costimulatory receptors, or cytokines can be done to further enhance T cell potency, specificity and safety. CARs reflects a new class of drugs with exciting potential for cancer immunotherapy.

SUMMARY: CAR-T cells have been arising as a new modality for cancer immunotherapy because of their potent efficacy against terminal cancers. They are known to exert higher efficacy than monoclonal antibodies and antibody-drug conjugates, and act via mechanisms distinct from T cell receptor-engineered T cells. These cells are constructed by transducing genes encoding fusion proteins of cancer antigen-recognizing single-chain Fv linked to intracellular signaling domains of T cell receptors.

KEYWORDS: chimeric antigen receptor, CAR T cells, adoptive cell therapy, ACT, T cell receptor, TCR, cancer, immunotherapy

*Indones Biomed J. 2018; 10(3): 203-216***Introduction**

Current cancer drugs and treatments such as chemotherapy aiming at eradicating tumor cells, but often are more toxic than effective, killing also the normal cells and not selectively the tumor cells. Actually our body have own immune system that can target tumor cells and kill them, but more often the immune responses were suppressed within a tumor and its surrounding.(1) New approaches involving immunotherapy to solve this immunosuppression problem

via the programmed cell death protein 1 (PD-1)-receptor pathway, but a risk of immunological side effects have to be considered.(2,3) Up to now, there are three types of adoptive cell transfer (ACT) using effector T cells that are in path favoring to regulatory approval, including antigen-specific T cell therapy, using endogenous T cells sourced from peripheral blood, redirect T cells to tissue by transferring chimeric antigen receptors (CARs) or T cell receptors (TCRs), and tumor-infiltrating lymphocytes (TILs), utilize lymphocytes expanded from biopsy sample. TIL is slow developed but keep progressing during decades.(4)

The most interest of ACT drew to CAR- and TCR-engineered T cells (TCR-T) currently, going uphill from basic theory to clinical immunotherapy. Combining the principles of synthetic biology, immunology, and genetic engineering, enhanced ACT of T cells engineered to express artificial receptors that target certain selected cells. This become very promising for treating cancer, chronic infection, or even autoimmunity. Clinical trials using cluster of differentiation (CD)19-specific CAR T cells to treat patients with advanced B cell leukemias and lymphomas induced durable remissions in adults and children, dawning attention of pharmaceutical industry.(4)

Kymriah (tisagenlecleucel, formerly known as CTL019) was just approved by the USA Food and Drug Administration (FDA) recently. A CAR-T therapy, Kymriah scratched a history by showing good result on children and young adult patients with refractory or relapsing B cell precursor acute lymphoblastic leukemia (ALL). It became the first gene therapy to the United States also marks a new frontier in medical innovation with the ability to reprogram a patient's own cells to attack a deadly cancer.(5)

CAR-T cells are alive T cells taken from the blood, then genetically engineered by introducing DNA to them, so they could express synthetic, target-specific CARs on the cells surface. For tisagenlecleucel, T cells taken autologous from the patients themselves, then modified to target CD19 in a manufacturing center, and sent back to the hospital and reinfused to the patients, performing the magic trick, which is recognizing the patient's target antigen present on tumor cell, proliferate, and kill the tumor cells upon antigen contact. (6) Many questions to be explored further, are the CAR-T performance on solid tumor cells, the more efficient CAR-T isolation procedures and the possibilities to manufacturing CAR-T either from autologous or preparing the off-the-shelf products from allogeneic donors, then finally the safety and cost concerns related to the processes.(6)

Adoptive Immunotherapy for Cancer

Before become apparent, many microscopic tumors have been eliminated by our immune surveillance. Some investigations proposed that tumors experience immunoediting.(7-9) Thus, some tumor cells escape the recognition by eliminating antigenic targets that they express, and even co-opt or deliver the host adaptive immunity to become insufficient, and lastly the tumor mass grow furiously, in the end killing their host.(10-

12) Several hundred billion of T cells in our lymphoid tissues protect us all over our lives, they circulate through our bloodstream, detect and destroy any diseased cells. Diseased cells expressed major histocompatibility complex (MHC) molecules, which will become the antigen for TCR engagement, and mediating the T cells recognition and action.(13)

Then how majority of tumor cells could escape the immunity? These ingenious cells subvert the normal immune process, downregulate the antigen presentation by reducing antigen processing or MHC expression so the T cells misguided and do not recognized them as the diseased cells.(14) On the other hands, they may co-opt growth factors and immunosuppressive compounds from macrophages or granulocytes to downregulate immune activity and the tumor cells could grow.(15) ACT refer to utilizing large number of activated tumor-specific T cells from *ex vivo* expansion injected and induced complete and more stable regression for certain advanced cancers. The reinfused cells will traffic to the tumor and mediate its destruction. Genetically engineered, ACT from autologous T cells can be directed to express particular TCRs or CARs to fight diverse targets. (16-22)

Preparative lymphodepletion is a temporary ablation of the immune system by destruction of lymphocytes and T cells using chemotherapy alone or in combination with irradiation, prior to immunotherapy to enhanced persistence of the transferred T cells due to no circulating leukocytes, very few regulatory cells and higher than normal amounts of cytokines that promote T cell survival. Combining lymphodepleting preparative regimen with ACT and the administration of the T cell growth factor interleukin (IL)-2 give advantage in prolonged tumor eradication either in metastatic melanoma or other tumor histologies (including leukaemias and synovial cell sarcomas).(16-25) Unfortunately, it is also possible for unwanted and unanticipated autoimmune adverse events resulting from T cell recognition of antigens expressed by normal tissues (26-31).

Other forms of immunotherapy for cancer usually rely on sufficient numbers of active antitumor T cells developed *in vivo*. Beyond this, ACT has many advantages because antitumor lymphocytes (up to 10¹¹) has been selected for high-avidity recognition of the tumor and expanded *in vitro* so any inhibitory factors existed *in vivo* were abolished, while the favorable microenvironment was set to support better antitumor immunity. ACT can proliferated *in vivo* while maintaining their antitumor effector functions, thus it

is a living” treatment.(32) One critical point in determining the successful of ACT in human is the identification of cells, which is selectively target cancer antigens and not the essential normal tissues.(32-37)

T cells were reprogrammed through genetic engineering to recognize and destroy cancer cells and the malignancies (Figure 1).(13) It is important to determine the target antigen for ACT specifically expressed by tumor and not healthy cells, to reduce chance of cross-reactivity against epitopes in unintended targets, which is further make a risk for autoimmune.(38,39)

Basic Principles of CAR

Not only applied to enforce tumor antigen recognition, genetic reprogramming also improves T cell survival, boost T cell expansion, generate memory lymphocytes and offset T cell death, anergy and immune suppression. Other than that, genetic modified T cells could be used to track T cell migration *in vivo*, introduce safety or recall mechanism into

T cells, to harness T cell responses. The main objective, which is the recognition of tumor antigen, is achieved by expressing antigen receptors, including either physiological, MHC-restricted TCRs or non-MHC-restricted CARs.(40)

CARs, the living drug, are recombinant receptors for antigen, that redirect specificity and function of T lymphocytes or other immune cells using a single molecule. The application in cancer immunotherapy mainly aimed to generate immediate and long-term effects of tumor-targeted T cells rapidly, to bypass the barriers and incremental kinetics of active immunization.(41,42) Stable gene is required in T cell transduction to empower sustained CAR expression in clonally expanding and persisting T cells. Any cell surface molecule in principle can be targeted through a CAR, furthermore T cell reactivity scope could be limited by considering the tolerance to self-antigens and the antigen recognition gaps in the physiologic T cell repertoire.(43)

CARs composed of several fusion molecules, including an specific extracellular single chain variable fragment (scFv) of a monoclonal antibody (mAb) for a surface molecule on the tumor cell, a spacer domain to provides T

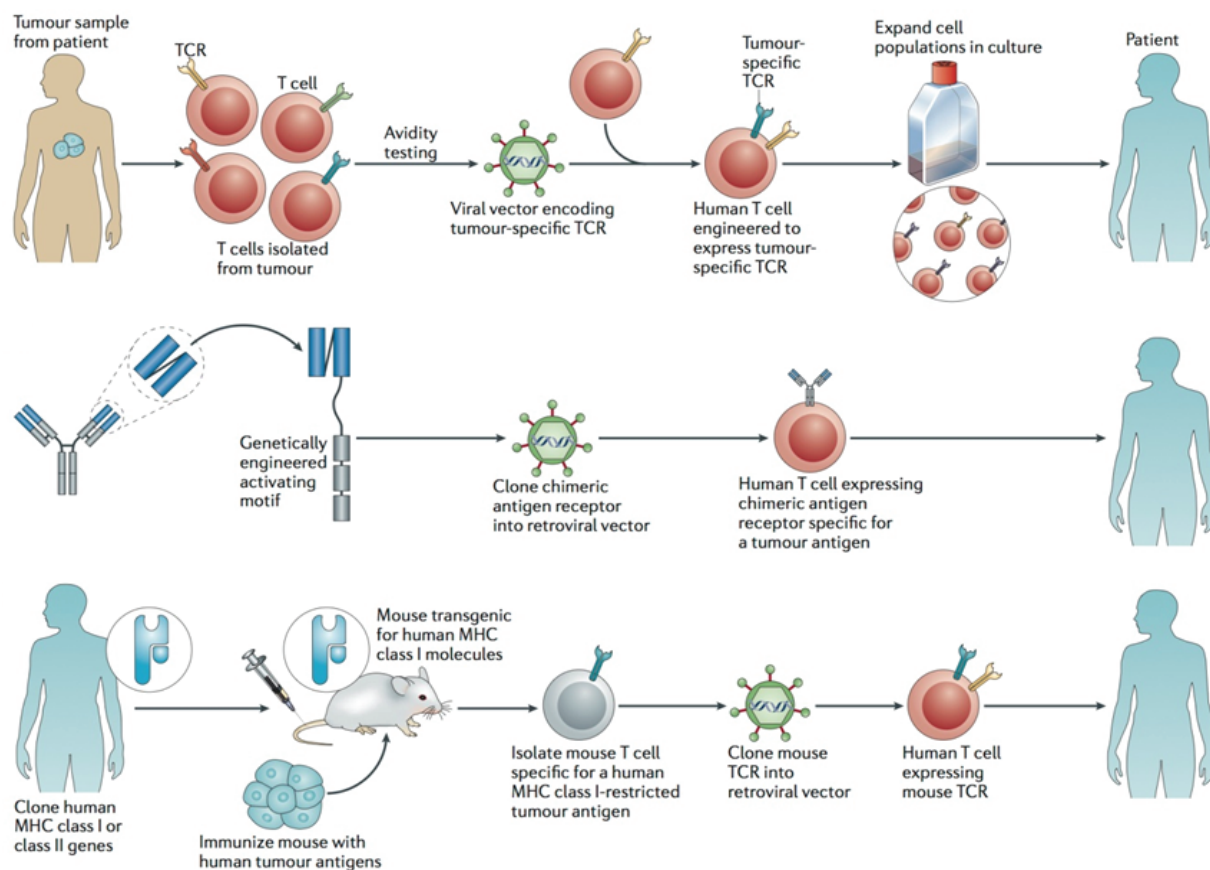


Figure 1. Three ways to genetically engineer T cells to confer specificity for tumour-associated antigens.(12) (Adapted with permission from Springer International Publishing AG).

cell flexibility and optimization, a transmembrane domain, and signaling modules to trigger T cell effector functions (Figure 2). Several other newer ligands are developing for clinical applications, but recently scFvs as ligand binding for tumor associated molecules have advantages of the high specificity and prevalence of mAbs.(44)

While TCRs have been refined for their safety and efficiency all over the time, most CARs were empiric based, constructed synthetically and assembly of an optimal receptor. Ligand binding of a CAR is different from TCR in receptor affinity, antigen density and spatial properties. An optimal CAR relied on functional assays of transduced T cells *in vitro* or in human tumor xenograft models.(45) CARs have two domains (Figure 3), the first one is an extracellular antigen-recognition domain most-commonly consists of an scFv, usually has a hinge to anchor to the cell, and/or transmembrane domain which is binds to the tumour-associated antigen (TAA), and the second one is an intracellular signaling domain for T cells activation and determine the CAR-T classification as first-, second- and third-generation.(46,47)

First-generation CARs noticed by utilizing the CD3z signaling chain, to activate signal 1, but clinical trials of this generation result in low of anti-tumor efficacy, apparently due to activation-induced cell death (AICD) of the transplanted T cells, or because of the shortage of long-term T cell expansion.(48-51) On the contrary, in the case of human immunodeficiency virus (HIV) treatment, CD4-specific CAR T cells can have a half-life of more than 16 years (52).

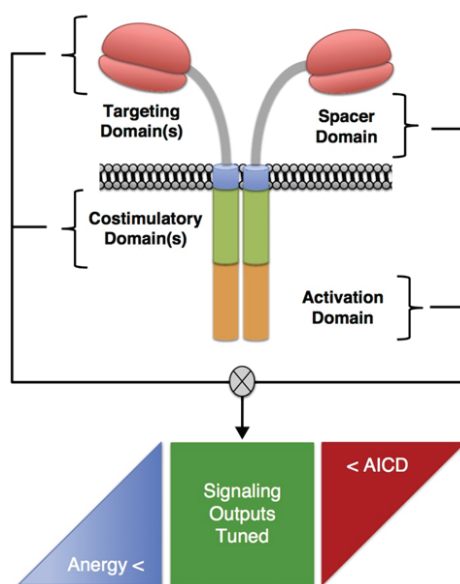


Figure 2. The structural elements of CAR.(45) (Adapted with permission from Elsevier Ltd).

The second-generation CARs improving the first one with additional co-stimulatory signaling domain, named signal 2 as the second signal then, the same receptors delivers two signals include both a CD3z and a CD28 signaling to optimally activated the T cell. The second generation specific for CD19, showed better persistence and proliferation compared to the first one, when infused simultaneously into patients with non-Hodgkin lymphoma (NHL) at the Baylor College of Medicine, Texas, USA.(51) In last 5 years, the second generation CD19-targeted CAR T cells with either CD28 or 4-1BB (CD137) co-stimulatory signaling domains demonstrated clinical efficacy in treating B-ALL, but the optimal second signal moiety remain to be determined.(53-55)

Third generation CARs contains two co-stimulatory domains besides a CD3z domain, including CD28, 4-1BB, or OX40 (CD134). The preclinical studies showed preferable antitumour efficacy compared to the other ones (NCT01853631).(56-58) Thus, these give insight about designing future CARs with one or two co-stimulatory signaling domains to treat most tumor types.(13)

CAR T Cells for Adoptive Cell Therapy

The history of ACT was opened by hematopoietic stem cell transplantation.(59) Adoptive T cell transfer involves the isolation and reinfusion of T lymphocytes into patients to treat disease, conceptually similar to T cell immunization, associated with vaccine-based strategies, *i.e.*, required *de novo* activate and expansion of a tumor antigen-specific T cell response in patients who was usually immune compromised and deeply tolerant to cancer antigens or to antigens that are expressed during chronic infection.(60)

Basic discoveries in immunology fueled the development of another class of off-the-shelf targeting reagents that combined the antigen recognition domain of antibodies with the signaling domains of T cells. Such receptors, known as CARs, entered clinical trials in more than 15 years since first being described (61,62), paralleled by continued improvements in design and efficacy.(63)

CAR-Ts act via different mechanisms from TCR-T to recognize complexes of tumor antigens. Unlike TCR-T, which were processed in antigen-presenting cell (APC) cells and presented on APC cell surfaces with MHC class molecules, CAR-Ts do not require processing and presentation of tumor antigen-recognizing moieties with MHC molecules.(64) Thus, CAR-T have wider eligible patients group.(47)

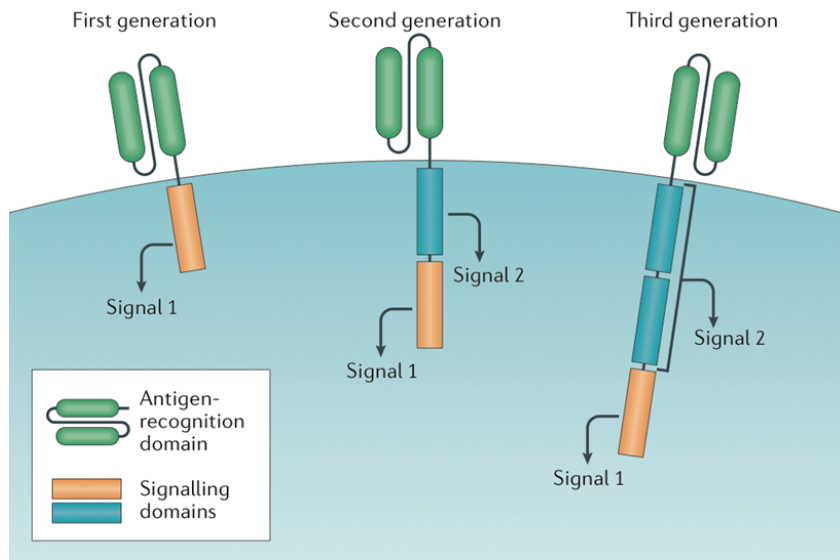


Figure 3. CAR-T-cell design. All chimeric antigen receptor (CAR) designs contain an antigen-recognition domain and a signaling domain that provides 'signal 1' to activate T cells.(46) (Adapted with permission from Springer International Publishing AG).

Contrary to the responses elicited by therapeutic tumor vaccines which need several months, adoptive T cell transfer therapy can be observed within days to weeks. Pro-inflammatory immune state will be developed as a predicted consequence of target antigen-driven activation of infused T cells or because of secondary immune activation triggered by the primary T cell activation event.(60) Of course, several consideration about the infused product has to be raised, including the most effective strategies for cell expanding, defining the subpopulation for central and/or effector memory subsets (65), virus-specific T cells (66), the impact of tumor-driven immunosuppressive mechanisms, or potentially products derived from engineered T cell stem cell precursors (67) important to resolve. Data showed small numbers of engineered T cells is enough to deliver potent and persistent antitumor activity (37,68), implied that the critical point accentuate quality over quantity. Tumor burden in theoretical also affects the complex decision where higher tumor burden will yield in most effective T cell activation and the therapies may paradoxically be less effective or require higher doses at earlier stages of disease.

Another controversial issue to address is to maintain a long-term persisting memory T cells in patients in the case of tumor dormancy, given that human tumors can remain dormant more than 16 years.(59) A clinical study performed by Kalos, *et al.*, with CAR engineered cells that target CD19 demonstrated a favorable molecular remission with persisting engineered T cells for at least 2 years after treatment, but B cells aplasia also developed resulting from targeting of normal CD19-positive B cells, encourage the next development to specific ablate engineered cells and enable normal B cell reconstitution.(60,69)

With recent advanced technology transfer, adoptive T cell therapy give a strategic opportunity for combination with other antitumor therapies such as therapeutic vaccination, checkpoint inhibition, agonistic antibodies, small molecule inhibitors of tumors, and targeting of tumor stroma and neo-vasculature, moreover the possibility of automated cell culture system development.

Cell Sources and Clinical Manufacturing of CAR T Cells

ACT via genetic engineering utilize the naturally occurring endogenous tumor-infiltrating lymphocytes or T cells to express either TCRs (70) or CAR (43). The promising clinical outcomes in phase ½ clinical studies attract the interest of many pharmaceutical and biotechnological industries (71-74) to manufacture the clinical grade CAR-T cells under current good manufacturing procedure (cGMP). Currently, CAR-T cell-manufacturing platforms are labor intensive, and the most extensive experience in CAR-T manufacturing still lies in the academic centers, while the industries company just step in.(75-77) Automated, powerful, and cost-effective cell production platforms compliant with cGMP coupled with robust analytics, ensure reproducible cell quality was now on hunted to commercialize these potent personalized, therapeutic modalities in an efficient, effective manner.(78,79)

The fusion proteins genetically engineered in CARs including: (1) an antigen domain, derived from a monoclonal antibody and (2) intracellular T cell signaling and costimulatory domains.(44,62,80-82) The development of

CAR T cell therapy has now expanded beyond phase 1 trials and moved into phase 2 multi-site trials (NCT02435849 and NCT02228096).(83)

CAR-Ts are manufactured using three consecutive steps (84-86): (1) generate genetic constructs of CAR to encode tumor antigen-specific Fv linked to signaling sequences of T cell receptors; (2) Transduction of T cells with CAR using viral (commonly used retroviral or lentiviral), non-viral (using plasmid DNA or RNA) via electroporation (87-91) or physical methods; and (3) CAR-T cells cultivation. CAR-T cells production demands several carefully performed steps, accompany with quality control testing throughout the entire protocol.(86) The first step involves removing blood from the patient's body with leukapheresis, separate the leukocytes, and return the remainder of the blood to the circulation (92) until a sufficient number of leukocytes have been harvested, and continue with T cell enrichment to the lymphocytes while washing the buffer and anticoagulants out (Figure 4).(93) The enrichment process can be performed through counterflow centrifugal elutriation, which separates cells by size and density and maintains cell viability.(94) In some cases, additional step such as separation of T cell subsets at the level of CD4/CD8 composition using specific antibody bead conjugates or markers may be performed.(95) A potent CAR-T cell product is difficult to be collected from purified autologous antigen-presenting cells (APCs) (86) and need some extra steps to attain a standard activated T cells, for example Life Technologies developed beads coated with anti-CD3/anti-CD28 monoclonal antibodies.(83)

T cells were incubated with the viral vector encoding the CAR, to induce the activation. Lentiviral vectors is commonly used including CTL019, rather than gamma-retroviral vectors because if the safety integration. The vectors then be washed out in a way of medium exchange. The viral vectors attach to the patient cells using its machinery, and introduces genetic material encodes CAR in the form of RNA.(96) The RNA is reverse-transcribed into DNA, integrates into the patient cells' genome permanently. Thus, every time the cells divide in the bioreactor, CAR expression is maintained, until the adequate cells number is reached. After transcribed and translated by the patients cell, the CAR is expressed on the cell surface.(97,98) Another methods of gene transfer used is the Sleeping Beauty transposon system or mRNA transfection.(99,100)

With high interest and investments on this, transferring the CAR T production protocols from academic institution to industrial manufacture, a highly controlled for each process had to be implemented across the collection,

manufacturing, and treatment. A clear understanding of quality attributes both for the process and the products should be established.(83)

CAR-T cell appertained on the fast track of FDA approval for B-cell malignancies, but many active clinical trials and investigations were keep routing to build better CAR-T cells for treating hematologic malignancies and solid tumors.(101) Figure 5 shows some major steps in CAR-T-cell manufacturing process.

Clinical Application of CAR T Cells

CARs are synthetic receptors proteins that have been engineered to give T cells the new persistent ability to target a specific protein.(41,102) In contrast to generic T cell receptors, CARs doesn't independent on MHC to bind with cell surface molecules, means that CARs can target patient's proteins, carbohydrates, or glycolipids and function regardless of patient human leukocyte antigen (HLA) haplotype. After binding to antigen, T cell become active mediated by the cytoplasmic domain of the CD3z chain.(103-107) Costimulatory domain was needed to provide the expansion of T cells to retain their functionality upon repeated exposure to antigen (108-112), and bring up the second generation CARs as the more persistence T cells (40), prospecting for treating solid tumors (113-116). Autologous modified T cells targeting CD19 showed a promising results in patients with refractory B-cell hematologic malignancies (117), also in children and adults with relapsed B-ALL, chronic lymphocytic leukemia (CLL), and B-NHL. However, each institution designed their own methods for T cell activation and transduction, as well as the cell doses.(118) Standard treatment for B cell malignancy consist of chemotherapy, radiotherapy, haematopoietic stem cell transplantation auto and allogeneic, and donor lymphocyte infusion. Refractory disease or still relapsing after all treatments do something, change to live to enroll on CAR T cell.

A powerful CAR is not enough powerful enough in clinical reality, but a suitable target which is specific expressed in the tumor cells but not in normal cells is needed. CD19 is promising, due to its signaling pattern and its cell-surface expression in most leukemia and lymphomas.(119-122) Unfortunately, CD19 targeting also induces a B cell aplasia (123-126), although it is clinically manageable for a limited time therapy. Some studies suggested that this B cell elimination has the intent to prevent the appearance of suspected anti-CAR antibodies.(127-129) Another issues is

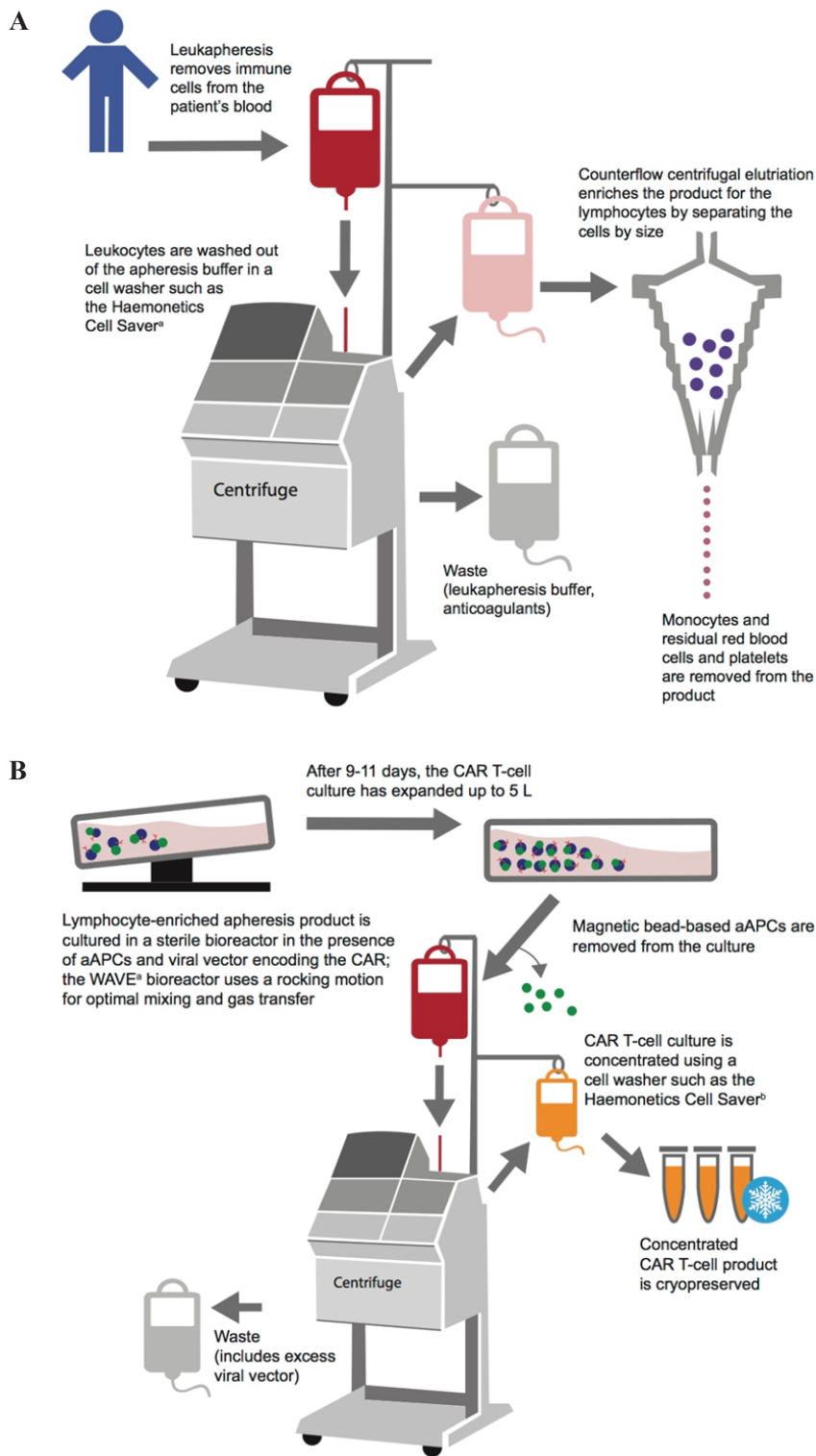


Figure 4. CAR-T manufacturing steps.
 A: Leukapheresis and T cell isolation.
 B: Cell culture and transduction.(83)
 (Adapted with permission from Elsevier).

how about the long-term clinical outcome. Several strategies to improve the performances of CAR-T19 therapy has been implemented, those are increasing efficacy against indolent B cell leukemia and lymphomas, avoiding or preventing antigen-loss relapses, toxicity reduction and management, and CART therapy on routine clinical practice.

However, until recently, no single ideal antigen has yet been identified for solid tumors. The investigating CAR targets including gene products arising from genetic mutations or altered splicing (EGFRvIII), altered glycosylation patterns (MUC1), cancer-testis antigen-derived peptides (MAGE), overexpressed differentiation

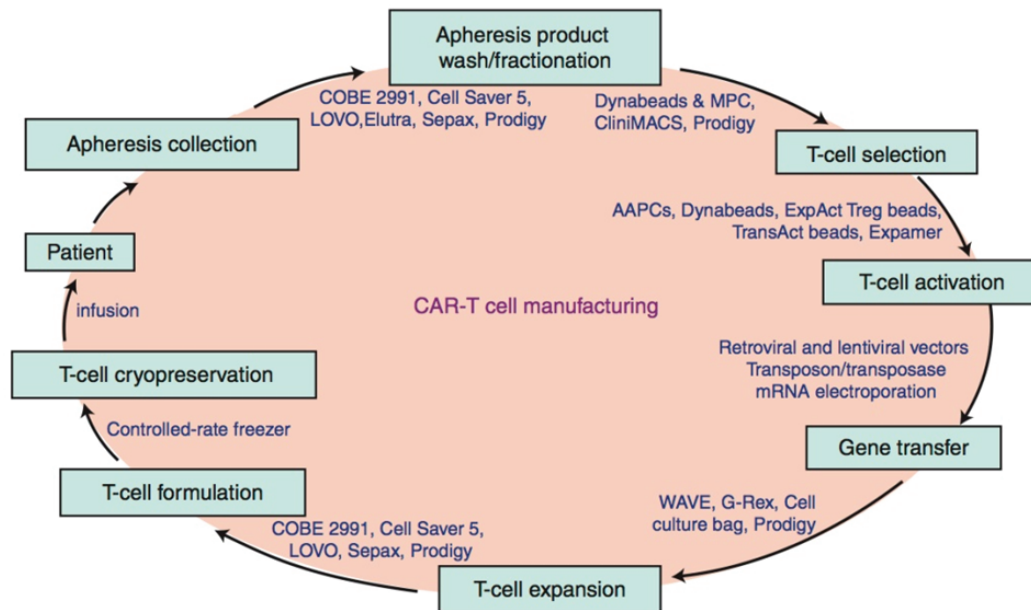


Figure 5. Major steps in chimeric antigen receptor-T-cell manufacturing process and examples of available technologies and devices.(75) (Adpted with permission from Springer International Publishing AG).

antigens (CEA, PSMA, GD2, MUC16, HER2/ERBB2, and mesothelin (MSLN)), or tumor-associated stroma (FAP and VEGFR).(116) If we could relate the target antigen with tumor invasion or metastasis formation, we could use CAR therapy for directing the more aggressive cancer cells and be less vulnerable to tumor relapse. MSLN is a glycoprotein initially synthesized as a 69 kDa cell-surface protein, cleaved by the furin protease at the amino terminus results in a 40-kDa C-terminal fragment attached to the plasma membrane by a glycoposphatidyl inositol (GPI) domain, and a soluble 32-kDa N-terminal fragment, named megakaryotic-potentiating factor (MPF), is released.(130) There has been some therapy clinical trials done which are related to antigens targeted in solid tumor CAR T cell (Figure 6).

MSLN knockout in mice does not exhibit any differences in development, reproduction, and blood cell count, suggest that this protein do nothing essential in normal tissues.(131) Nevertheless, in tumor cells, aberrant MSLN expression actively role in both tumor malignant transformation by committing to local invasion and metastasis, also contribute to tumor aggressiveness by promoting cancer cell proliferation, and supplying cell resistance to apoptosis induced by cytotoxic agents.(132-135) Its high expression in consort with the low expression in normal tissues, increase a consideration of making MSLN for a targeted immunotherapy. However, currently, CD19 still become the holy grail of CAR therapy.(136)

Cytokine Release Syndrome (CRS)

In contempt of its early success, together with the limitation of available targeted antigens, CAR T cell therapy had to be considered for its toxicity, including bystander effects leading to systemic inflammatory reactions such as cytokine release syndrome (CRS) besides the neurologic toxicities, hypersensitivities or autoimmunity or graft-versus-host disease (GVHD) caused by T cell products, on- and off-target effects of CARs, possibilities of mutagenesis mediated by transgene delivery, autonomous CAR signaling, and generation of a replication-competent virus.(137,138)

EGFRvIII in glioblastoma, not like the ideal criteria of CAR T ideal antigen, also present at some level in normal tissues. CAR T cell activation is mainly drove by direct engagement of the scFv with its cognate antigen. Thus, the co-expression of this antigen on any non-tumor cell could eliminate both tumor and non-tumor targets, although the affinity of the CAR, the antigen expression level on the healthy tissue, the CAR potency and the relative functional importance of the healthy tissue target also determine the degree of on-target toxicity.(139)

CAR have benefit because its specificity is edicted by antibody-like binding, not by the MHC expression. Unlike TCR-modified T cell therapies, so far CAR T cell therapy has not demonstrated any inappropriate scFv recognition of a non-target antigen.(139) Mostly the modified T cell

infusion addressed tolerable adverse effects (140), only two cases of serious adverse events reported in 2010 (141,142), both were caused related to a systemic cytokine release that has been termed CRS. According to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAEs) Version 4.0, CRS defined as a disorder characterized by nausea, headache, tachycardia, hypotension, rash, and shortness of breath caused by the release of cytokines from the cells.(143) This could happen because of an excessive release of cytokines and chemokines from systemic immune response mediated by T cells, B cells, NK cells and monocytes/macrophages. CRS common occurs in many clinical setting, such as GVHD after transplantation, severe bacterial and viral infections, hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS) and mAb therapy.(144-148) Acute inflammatory response after cytokine released will spread into systemic and induce endothelial and organ damage, result in microvascular leakage, heart failure and even death.(149-151) That's why CRS should be well managed during CAR-T therapy.(152)

The classic and basic design of a CAR includes a scFv targeting TAA, an extracellular spacer/hinge region, a trans-membrane domain and an intracellular signaling domain. Once the CAR-T cells encountered tumor cells, the scFv is engaged by the TAA sending the activation signal to the immunoreceptor tyrosine-based activating motif of the CD3z chain, continues to provide signal 1 which activate T cell, cytokine secretion and kill the target cell.(106,153) The second generation CARs were enhanced with the co-stimulatory incorporating such as CD27, CD28, 4-1BB and ICOS.(61,154-156) As the CAR-T cells activated, a variety of inflammatory cytokines, including interferon (IFN)- γ , tumor necrosis factor (TNF)- α , IL-1 β , IL-2, IL-6, are released. IFN- γ activates the macrophages which release more cytokines including TNF- α , IL-1 β , IL-6, IL-8 and IL-10, which further enhancing the positive feedback loop for activation, proliferation and more cytokine secretion of T cells except IL-10 because IL-10 act as the immune suppressor and play a limited role in this arena. This formation therefore, induce the cytokine storm.(152)

Similar to sepsis, the cytokine storm could promote systemic inflammatory response and induce fever, headache, dizziness, nausea, rigors, chills, rash, hypotension, tachycardia and dyspnea. This syndrome also associated with arrhythmia and cardiac arrest, hepatic, and renal failure. Acute vascular leak could happen and leads to fluid retention, causing pulmonary and general edema like ARDS. Usually hydration will be given due to hypotension

symptoms, but this will even worsen the situation.(152) CRP biomarkers can be applied as a marker for CRS risk. CRS have a positive correlation with tumor burden, in other words, lower tumor burden will give a lower risk of CRS. (53,46)

Side effects of CAR T cell therapy can be classified into 3 side effects, which are CRS, nervous side effect and B aplasia. CSR toxicity consist of grade 1, grade 2, grade 3 and grade 4. Despite of all risk of adverse reaction and toxicities, T cell therapy grows rapidly and have tremendous potencies as the living drug for treating and offers the possibility of dramatically extending the lives of patients with cancer.(139)

Conclusion

Immunotherapy has undergone a long road before come to success. A major step of hope come as CAR-based technology. These cells can be modified as a synthetic identical receptor, as long as we can get the specific lymphocytes (autologous or allogeneic) regardless of the HLA context, makes this therapy become very universal. The ultimate goal for this therapy is for treating cancer, either alone or be combined with current cancer therapies. CAR T-cell therapy respond, 70-94% complete remission rate for B cell ALL, CLL overall response with 70% respond lasting more than 9 months, for diffuse large B-cell lymphoma (DLBCL) at six month 30% patients in complete response with 70 % relapse free rate. Numerous clinical trials were still ongoing to gather us more information for getting the best strategy to achieve this goal.

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