REVIEW ARTICLE

CAR T Cells: Precision Cancer Immunotherapy

Anna Meiliana1,2,*, Nurrani Mustika Dewi2, Andi Wijaya1,2

1Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Jl. Eijkman No.38, Bandung, Indonesia
2Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung, Indonesia

*Corresponding author. E-mail: anna.meiliana@prodia.co.id

Received date: Nov 7, 2018; Revised date: Dec 17, 2018; Accepted date: Dec 19, 2018

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

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 immunoeediting.(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
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 CD3ζ 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).

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 CD3ζ 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 CD3ζ 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)
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 NCT02280966). 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. 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 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). The enrichment process can be performed through counterflow centrifugal elutriation, which separates cells by size and density and maintains cell viability. 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. A potent CAR-T cell product is difficult to be collected from purified autologous antigen-presenting cells (APCs) 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.

T cells were incubated with the viral vector encoding the CAR, to induce the activation. Lentiviral vectors are commonly used including CTL019, rather than gammaretroviral vectors because of 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. 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. Another methods of gene transfer used is the Sleeping Beauty transposon system or mRNA transfection.

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.

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. 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. 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. Costimulatory domain was needed to provide the expansion of T cells to retain their functionality upon repeated exposure to antigen, and bring up the second generation CARs as the more persistence T cells, prospecting for treating solid tumors. Autologous modified T cells targeting CD19 showed a promising results in patients with refractory B-cell hematologic malignancies, 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. 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. Unfortunately, CD19 targeting also induces a B cell aplasia, 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. Another issues is
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
antigens (CEA, PSMA, GD2, MUC16, HER2/ERBB2, and mesothelin (MSLN)), or tumor-associated stroma (FAP and VEGFR). 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 glycoprophatidyl inositol (GPI) domain, and a soluble 32-kDa N-terminal fragment, named megakaryotic-potentiating factor (MPF), is released. 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. 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. 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.

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.

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.

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. 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-1β, IL-2, IL-6, are released. IFN-γ activates the macrophages which release more cytokines including TNF-α, IL-1β, 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, rigor, 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.

**References**

1. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. Ann Oncol. 2016; 27: 1482-92.
2. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol. 2015; 26: 2375-91.
3. Melief CJM. Cancer: precision T-cell therapy targets tumours. Nature. 2017; 547: 165-7.
4. Barnett DM, Grupp SA, June CH. Chimeric antigen receptor- and TCR-modified T cells enter main street and wall street. J Immunol. 2015; 195: 755-61.
5. U.S. Food and Drug Administration [Internet]. FDA approval brings first gene therapy to the United States [updated 2017 Aug 30; cited 2018 Apr 27]. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm.
6. Yang KH. A new model T on the horizon? Cell. 2017; 171: 1-3. doi: 10.1016/j.cell.2017.09.011.

7. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity’s roles in cancer suppression and promotion. Science. 2011; 331: 1565-70.

8. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012; 482: 400-4.

9. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. Nature. 2012; 482: 405-9.

10. Staveley-O’Carroll K, Sotomayor E, Montgomery J, Borrello I, Hwang L, Fein S, et al. Induction of antigen-specific T cell anergy: an early event in the course of tumor progression. Proc Natl Acad Sci USA. 1998; 95: 1178-83.

11. Meilliana A, Dewi NM, Wijaya, A. Cancer Immunotherapy: A Review. Indones Biomed J. 2016; 8: 1-20.

12. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol. 2012; 12: 269-81.

13. Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. Nat Rev Cancer. 2013; 13: 525-41.

14. Restifo NP, Marincola FM, Kawkamayi T, Taubenberger J, Yannelli JR, Rosenberg SA. Loss of functional β 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. J Natl Cancer Inst. 1996; 88: 100-8.

15. Motz GT, Coukos G. The parallel lives of angiogenesis and immunoediting: cancer and other tales. Nature Rev Immunol. 2012; 12: 11: 702-11.

16. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010; 116: 4099-102.

17. Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood. 2011; 118: 4817-28.

18. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011; 17: 4550-7.

19. Porter DL, Levine BL, Kalos M, June CH. T cell immunotherapy for human cancer: harnessing the T cell response. Nat Biotechnol. 2013; 31: 999-1008.

20. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011; 29: 917-24.

21. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, et al. B-Cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood. 2011; 119: 2709-20.

22. Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA, et al. Long-term outcome of EBV-speciﬁc T cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010; 115: 925-35.

23. Paulos CM, Wrzesinski C, Kaiser, A, Hinrichs CS, Chiappa M, Cassard L, et al. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. J Clin Invest. 2007; 117: 2197-204.

24. Antony PA, Piccirillo CA, Akpinarli A, Finkelstein SE, Speiss PJ, Surman DR, et al. CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. J Immunol. 2005; 174: 2591-601.

25. Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Speiss PJ, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med. 2005; 202: 907-12.

26. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009; 114: 535-46.

27. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan D-AN, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther. 2011; 19: 620-6.

28. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother. 2013; 36: 133-51.

29. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurenzot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010; 18: 843-51.

30. Linette GP, Stadtmueller EA, Maas MV, Rapoport AP, Levine BL, Emery L, et al. Cardiovascular toxicity and titin cross-reactivity of affinity enhanced T cells in myeloma and melanoma. Blood. 2013; 122: 863-71.

31. Cameron BJ, Gerry AB, Dukes J, Harper JV, Kannan V, Bianchi FC, et al. Identification of a titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3-directed T cells. Sci Transl Med. 2013; 5: 197ra103. doi: 10.1126/scitranslmed.3006034.

32. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015; 348: 62-82.

33. Rosenberg SA, Packard BS, Abersold PM, Solomon D, Topalian SL, Toy ST, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med. 1988; 319: 1676-80.

34. Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med. 2008; 358: 2698-703.

35. Butler MO, Friedlander P, Milstein MI, Mooney MM, Metzler G, Murray AP, et al. Establishment of antitumor memory in humans using in vitro-educated CD8+ T cells. Sci Transl Med. 2011; 3: 80ra34. doi: 10.1126/scitranslmed.3002207.

36. Chapuis AG, Thompson JA, Margolin KA, Rodmyre R, Lai IP, Dowdy K, et al. Transferred melanoma-specific CD8+ T cells persist, mediate tumor regression, and acquire central memory phenotype. Proc Natl Acad Sci USA. 2012; 109: 4592-7.

37. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013; 368: 1509-18.

38. Hinrich CS, Restifo NP. Reassessing target antigens for adoptive T-cell therapy. Nat Biotechnol. 2013; 31: 999-1008.

39. Yee C. Adoptive T-cell therapy for cancer: boutique therapy or treatment modality? Clin Cancer Res. 2013; 19: 4550-2.

40. Sadalain M, Brentjens R, Riviere I. The promise and potential pitfalls of chimeric antigen receptors. Curr Opin Immunol. 2009; 21: 215-23.

41. Sadalain M, Riviere I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer. 2003; 3: 35-45.
42. Ho WY, Blattman JN, Dossett ML, Yee C, Greenberg PD. Adoptive immunotherapy: engineering T cell responses as biologic weapons for tumor mass destruction. Cancer Cell. 2003; 3: 431-7.

43. Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Dis. 2013; 3: 388-98.

44. Kahlon KS, Brown C, Cooper LJ, Raubitschek A, Forman SJ, Jensen MC. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. Cancer Res. 2004; 64: 9160-6.

45. Jensen MC, Riddell SR. Designing chimeric antigen receptors to effectively and safely target tumors. Curr Opin Immunol. 2015; 33: 9-15.

46. Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR T-cells forward. Nat Rev Oncol. 2016; 13: 370-83.

47. Kim MG, Kim D, Suh SK, Park Z, Choi MJ, Oh YK. Current status and regulatory perspective of chimeric antigen receptor-modified T cell therapeutics. Arch Pharm Res. 2016; 39: 437-52.

48. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, et al. Antitumor rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. Biol Blood Marrow Transplant. 2010; 16: 1245-56.

49. Lamers CHJ, Sleijfer S, Vulto AG, Kruit WHJ, Kliffen M, Debets R, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carcinoic anhydride IX: first clinical experience. J Clin Oncol. 2006; 24: e20-2.

50. Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008; 112: 2261-71.

51. Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest. 2011; 121: 1822-6.

52. Scholler I, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci Transl Med. 2012; 4: 132ra153. doi: 10.1126/scitranslmed.3003761.

53. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. 2013; 5: 177ra138. doi: 10.1126/scitranslmed.3005930.

54. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014; 371: 1507-17.

55. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukemia in children and young adults: a phase I dose-escalation trial. Lancet. 2014; 385: 517-28.

56. Carpenito C, Milone MC, Hassan R, Simoni JC, Lakhal M, Suhoski MM, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc Natl Acad Sci USA. 2009; 106: 3360-5.

57. Zhong XS, Matsushita M, Plotkin J, Riviere I, Sadelain M. Chimeric antigen receptors combining 4-1BB and CD28 signaling domains augment PI3Kinase/AKT/Bcl-XL activation and CD8+ T cell-mediated tumor eradication. Mol Ther. 2010; 18: 413-20.

58. US National Library of Science [Internet]. Activated T-Cells Expressing 2nd or 3rd Generation CD19-Specific CAR, Advanced B-Cell NHL, ALL, and CLL (SAGAN) (SAGAN) [updated 2018 Jan 23; cited 2018 Apr 27]. Available from: https://clinicaltrials.gov/ct2/show/NCT01853631.

59. Melenhorst JJ, Levine VL. Innovation and opportunity for chimeric antigen receptor targeted T cells. Cytotherapy. 2013; 15: 1046-53.

60. Kalos M, June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunology. 2013; 39: 49-60.

61. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci USA. 1989; 86: 10024-8.

62. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006; 12: 6106-15.

63. Jensen MC, Riddell SR. Design and implementation of adoptive therapy with chimeric antigen-receptor-modified T cells. Immunol Rev. 2014; 257: 127-44.

64. Kershaw MH, Westwood JA, Slaney CY, Darcy PK. Clinical application of genetically modified T cells in cancer therapy. Clin Transl Immunol. 2014; 3: e16. doi: 10.1038/cti.2014.7.

65. Turtle CJ, Riddell SR. Genetically retargeting CD8+ lymphocyte subsets for cancer immunotherapy. Curr Opin Immunol. 2011; 23: 299-305.

66. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008; 14: 1264-70.

67. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell-like properties. Nat Med. 2011; 17: 1290-7.

68. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med. 2011; 3: 95ra73. doi: 10.1126/scitranslmed.3002842.

69. MacKe RM, Reid R, Janor B. Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. N Engl J Med. 2003; 348: 567-8.

70. Yee C. The use of endogenous T cells for adoptive transfer. Immunol Rev. 2013; 257: 250-63.

71. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014; 6: 224ra225. doi: 10.1126/scitranslmed.3008226.

72. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RPT, Carpenter RO, Stetler-Stevenson M, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015; 33: 540-9.

73. Fleming A. Deal watch: Pfizer and GSK join race for T cell cancer therapies. Nat Rev Drug Discov. 2014; 13: 568-9.

74. June, CH, Riddell, SR and Schumacher, TN (2015). Adoptive cellular immunotherapy: a race to the finish line. Sci Transl Med. 2015; 7:280ps287. doi: 10.1126/scitranslmed.aaa3643.

75. Wang X, Riviere I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. Mol Ther. 2016; 3: 16015. doi: 10.1038/mt.2016.15.

76. Walker A, Johnson R. Commercialization of cellular immunotherapies. Mol Ther. 2017; 25: 1067-8.

77. Kuwana Y, Asakura Y, Utsunomiya N, Nakaniishi M, Arata Y, Itoh S, et al. Expression of chimeric receptor composed of immunoglobulin-
80. Finney HM, Lawson AD, Bebbington CR, Weir AN. Chimeric receptors providing both priming and costimulatory signaling in T cells from a single gene product. J Immunol. 1998; 161: 2791-7.

81. Maude SL, Pulsipher MA, Boyer MW, Grupp SA, Davies SM, Phillips CL, et al. Efficacy and safety of CTL019 in the first US phase II multicenter trial in pediatric relapsed/refractory acute lymphoblastic leukemia: results of an interim analysis. Blood. 2016; 128: 2801.

82. Grupp SA, Laetsch TW, Buechner J, Bittencourt H, Maude SL, Vermeris MR, et al. Analysis of a global registration trial of the efficacy and safety of CTL019 in pediatric and young adults with relapsed/refractory acute lymphoblastic leukemia. Blood. 2016; 128: 221.

83. Levine BL, Miskin I, Wonnacott C, Keir C. Global manufacturing of CAR T cell therapy. Mol Ther Methods Clin Dev. 2017; 4: 92-101.

84. Lee DW, Barrett DM, Mackall C, Orentas R, Grupp SA. The future is now: chimeric antigen receptors as new targeted therapies for childhood cancer. Clin Cancer Res. 2012; 18: 2780-90.

85. Wang X, Rivire I. Manufacture of tumor- and virus-specific T lymphocytes for adoptive cell therapies. Cancer Gene Ther. 2015; 22: 85-94.

86. Levine BL. Performance-enhancing drugs: design and production of redirected chimeric antigen receptor (CAR) T cells. Cancer Gene Ther. 2015; 22: 79-84.

87. Huang G, Yu L, Cooper LJ, Hollomon M, Huls H, Kleinerman ES. Genetically modified T cells targeting interleukin-11 receptor a-chain kill human osteosarcoma cells and induce the regression of established osteosarcoma lung metastases. Cancer Res. 2012; 72: 271-81.

88. Kumaresan PR, Manuri PR, Albert ND, Maiti S, Singh H, Mi T, et al. Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection. Proc Natl Acad Sci USA. 2014; 111: 10660-5.

89. Wang C, Hu W, Shen L, Dou R, Zhao S, Shan D, et al. Adoptive antitumor immunotherapy in vitro and in vivo using genetically activated erbB2-specific T cells. J Immunother. 2014; 37: 351-9.

90. Wang Y, Zhang W, Han Q, Liu Y, Dai H, Guo Y, et al. Effective response and delayed toxicities of refractory advanced diffuse large B-cell lymphoma treated by CD20-directed chimeric antigen receptor-modified T cells. Clin Immunol. 2014; 155: 160-70.

91. Zhao YE, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, et al. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. Cancer Res. 2010; 70: 9053-61.

92. Smith JW. Apheresis techniques and cellular immunomodulation. Ther Apher. 1997; 1: 203-6.

93. Lee G, Arepally GM. Anticogulation techniques in apheresis: from heparin to citrate and beyond. J Clin Apher. 2012; 27: 117-25.

94. Powell DJ, Brennan AL, Zheng Z, Huynh H, Cotte J, Levine BL. Efficient clinical-scale enrichment of lymphocytes for use in adoptive immunotherapy using a modified counterflow centrifugal elutriation program. Cytotherapy. 2009; 11: 923-35.

95. Riddell SR, Sommermeyer D, Berger C, Liu LS, Balakrishnan A, Salter A, et al. Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. Cancer J. 2014; 20: 141-4.

96. Coffin J, Hughes S, Varmus H. Retroviruses. New York: Cold Spring Harbor Laboratory Press; 1997.

97. McGarry RJ, Hoyah G, Winemiller A, Andre K, Stein D, Blick G, et al. Patient monitoring and follow-up in lentiviral clinical trials. J Gene Med. 2013; 15: 78-82.

98. Montini E, Cesana D, Schmidt M, Sanvito F, Bartholomae CC, Ranzani M, et al. The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. J Clin Invest. 2009; 119: 964-75.

99. Huls MH, Figliola MJ, Dawson MJ, Olivesares S, Kebricaie P, Shpall EJ, et al. Clinical application of Sleeping Beauty and artificial antigen presenting cells to genetically modify T cells from peripheral and umbilical cord blood. J Vis Exp. 2013; 72: e50070. doi: 10.3791/50070.

100. Singh H, Figliola MJ, Dawson MJ, Olivesares S, Zhang L, Yang G, et al. Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PLoS ONE. 2013; 8: e64138. doi: 10.1371/journal.pone.0064138.

101. Maus MV, June CH. Making better chimeric antigen receptors for adoptive t-cell therapy. Clin Cancer Res. 2016; 22: 1875-84.

102. Eshhar Z, Bach N, Fitzter-Attas CJ, Grosse G, Lustgarten J, Waks T, et al. The T-body approach: potential for cancer immunotherapy. Springer Semin Immunopathol. 1996; 18: 199-209.

103. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell. 1991; 64: 891-901.

104. Yang C, Seed B. Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. Cell. 1991; 64: 1037-46.

105. Letourneur F, Klausner RD. T-cell and basophil activation through the cytoplasmic tail of T-cell-receptor zeta family proteins. Proc Natl Acad Sci USA. 1991; 88: 8905-9.

106. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci USA. 1993; 90: 720-4.

107. Brocker T, Peter A, Trautwecker A, Kajalainen K. New simplified molecular design for functional T cell receptor. Eur J Immunol. 1993; 23: 1435-9.

108. Brocker T, Kajalainen K. Signals through T cell receptor-zeta chain alone are insufficient to prime resting T lymphocytes. J Exp Med. 1995; 181: 1653-9.

109. Gong MC, Latouche JB, Krause A, Heston WDW, Bander NH, Sadelain M. Cancer patient T cells genetically targeted to prostate-specific membrane antigen and umbilical cord blood. J Vis Exp. 2013; 72: e50070. doi: 10.3791/50070.

110. Krause A, Guo HF, Latouche JB, Tan C, Cheung NKV, Sadelain M. Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. J Exp Med. 1998; 188: 619-26.

111. Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human chimeric antigen receptor expression and release cytokines in response to prostate-specific membrane antigen. Neoplasia. 1999; 1: 123-7.

112. Brocker T. Chimeric Fv-zeta or Fv-epsilon receptors are not sufficient to induce activation or cytokine production in peripheral T cells. Blood. 2000; 96: 1999-2001.

113. Krause A, Guo HF, Latouche JB, Tan C, Cheung NKV, Sadelain M. Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. J Exp Med. 1998; 188: 619-26.
116. Morello A, Sadelain M, Adusumilli PS. Mesothelin-targeted CARs: driving T cells to solid tumors. Cancer Discov. 2016; 6: 133-46.

117. Sadelain M, Brentjens RJ, Rivie`re I, Park J. CD19 CAR therapy for acute lymphoblastic leukemia. Am Soc Clin Oncol Educ Book. 2015; 35: e360-3.

118. Park JH, Geyer MB, Brentjens RJ. CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date. Blood. 2016; 127: 3312-20.

119. Brentjens RJ, Latouche JB, Santos E, Marti F, Gong MC, Lyddane C, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med. 2003; 9: 279-86.

120. Carter RH, Fearon DT. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. Science. 1992; 256: 105-7.

121. Engel P, Zhou L1, Ord DC, Sato S, Koller B, Tedder TF. Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. Immunity. 1995; 3: 39-50.

122. Rickert RC, Rajewsky K, Roes J. Impairment of T-cell-dependent B-cell responses and B-1 cell development in CD19-deficient mice. Nature. 1995; 376: 352-5.

123. Kuchenderfer JN, Yu Z, Frasher D, Restifo NP, Rosenberg SA. Adoptive transfer of syngeneic T cells transduced with a chimeric antigen receptor that recognizes murine CD19 can eradicate lymphoma and normal B cells. Blood. 2010; 116: 3875-86.

124. Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood. 2012; 119: 4133-41.

125. Davila ML, Kloss CC, Gunset G, Sadelain M. CD19 CAR-targeted T cells induce long-term remission and B cell aplasia in an immunocompetent mouse model of B cell acute lymphoblastic leukemia. PLoS ONE. 2013; 8: e61338. doi: 10.1371/journal.pone.0061338.

126. Paszkiewicz PJ, Fräisle SP, Srivastava S, Sommermeyer D, Hudecek M, Dreixel I, et al. Targeted antibody-mediated depletion of murine CD19 CAR T cells permanently reverses B cell aplasia. J Clin Invest. 2016; 126: 4262-72.

127. Lamers CHJ, Willemsen R, van Elzakker P, van Steenbergen-Langeveld S, Broertjes M, Oostervij-Wakkia J, et al. Immunee responses to transgene and retrovector in patients treated with ex vivo-engineered T cells. Blood. 2011; 117: 72-82.

128. Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, et al. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res. 2013; 1: 26-31.

129. Ruella M, June CH. Chimeric antigen receptor T cells for B cell neoplasms: choose the right CAR for you. Curr Hematol Malig Rep. 2016; 11: 368-84.

130. Pastan I, Hassan R. Discovery of mesothelin and exploiting it as a target for immunotherapy. Cancer Res. 2014; 74: 2907-12.

131. Bera TK, Pastan I. Mesothelin is not required for normal mouse development or reproduction. Mol Cell Biol. 2000; 20: 2902-6.

132. Servais EL, Colovos C, Rodriguez L, Bograd AJ, Nitadori J, Sima C, et al. Mesothelin overexpression promotes mesothelioma cell invasion and MMP-9 secretion in an orthotopic mouse model and in epithelioid pleural mesothelioma patients. Clin Cancer Res. 2012; 18: 2478-89.

133. Kachala SS, Bograd AJ, Villena-Vargas J, Suzuki K, Servais EL, Kadota K, et al. Mesothelin overexpression is a marker of tumor aggressiveness and is associated with reduced recurrence-free and overall survival in early-stage lung adenocarcinoma. Clin Cancer Res. 2014; 20: 1020-8.

134. Rizk NP, Servais EL, Tang LH, Sima CS, Gerdes H, Fleisher M, et al. Tissue and serum mesothelin are potential markers of neoplastic progression in Barrett’s associated esophageal adenocarcinoma. Cancer Epidemiol Biomarkers Prev. 2012; 21: 482-6.

135. Tozbikian G, Brogi E, Kadota K, Catalano J, Akram M, Patil S, et al. Mesothelin expression in triple negative breast carcinomas correlates significantly with basal-like phenotype, distant metastases and decreased survival. PLoS One. 2014; 9: e114900. doi: 10.1371/journal.pone.0114900.

136. Riviere I, Sadelain M. Chimeric antigen receptors: a cell and gene therapy perspective. Mol Ther. 2017; 25: 1117-24.

137. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. Mol Ther Oncolytics. 2016; 3: 1601. doi: 10.1038/mtol.2016.11.

138. Brudno JN, Kuchenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016; 127: 3321-30.

139. Bedoya F, Frugault MJ, Maus MV. The flipside of the power of engineered t cells: observed and potential toxicities of genetically modified t cells as therapy. Mol Ther. 2015; 23: 314-20.

140. Xu XJ, Zhao HZ, Tang YM. Efficacy and safety of adoptive immunotherapy using anti-CD19 chimeric antigen receptor transduced T-cells: a systematic review of phase I clinical trials. Leuk Lymphoma. 2012; 54: 255-60.

141. Brentjens R, Yeh R, Bernal Y, Riviere I, Sadelain M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. Mol Ther. 2010; 18: 666-8.

142. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010; 18: 843-51.

143. National Institutes of Health [Internet]. Common Terminology Criteria for Adverse Events (CTCAE) [updated 2018 Mar 1; cited 2018 Apr 28]. Available from: http://ctep.cancer.gov/protocolDevelopment/ electronic_applications/etc.htm.

144. Ferrara JL, Abhyankar S, Gilliland DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. Transplant Proc. 1993; 25: 1216-7.

145. Xu XJ, Tang YM, Liao C, Song H, Yang SL, Xu WQ, et al. Inflammatory cytokine measurement quickly discriminates gram-negative from gram-positive bacteremia in pediatric hematologic/oncology patients with septic shock. Intens Care Med. 2013; 39: 319-26.

146. De Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJD, Chau TNB, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med. 2006; 12: 1203-7.

147. Xu XJ, Tang YM, Song H, Yang SL, Xu WQ, Zhao N, et al. Diagnostic accuracy of a specific cytokine pattern in hemophagocytic lymphohistiocytosis in children. J Pediatr. 2012; 160: 984-990.e1.

148. Bugelski PJ, Achuthanandam R, Capocasale RJ, Treacy G, Bouman-Thio E. Monoclonal antibody-induced cytokine-release syndrome. Exp Rev Clin Immunol. 2009; 5: 499-521.

149. Wang H, Ma S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. Am J Emerg Med. 2008; 26: 711-5.

150. Lee WL, Slutsky AS. Sepsis and endothelial permeability. N Engl J Med. 2010; 363: 689-91.

151. Rudiger A, Singer M. Mechanisms of sepsis-induced cardiac dysfunction. Crit Care Med. 2007; 35: 1599-608.
152. Xu XJ, Tang YM. Cytokine release syndrome in cancer immunotherapy with chimeric antigen receptor engineered T cells. Cancer Lett. 2014; 343: 172-8.

153. Heuser C, Hombach A, Lösch C, Manista K, Abken H. T-cell activation by recombinant immunoreceptors: impact of the intracellular signalling domain on the stability of receptor expression and antigen-specific activation of grafted T cells. Gene Ther. 2013; 10: 1408-19.

154. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther. 2009; 17: 1453-64.

155. Song DG, Ye Q, Poussin M, Harms GM, Figini M, Powell DJ. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood. 2012; 119: 696-706.

156. Shen CJ, Yang YX, Han EQ, Cao N, Wang YF, Wang Y, et al. Chimeric antigen receptor containing ICOS signaling domain mediates specific and efficient antitumor effect of T cells against EGFRvIII expressing glioma. J Hematol Oncol. 2013; 6: 33. doi: 10.1186/1756-8722-6-33.