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CAR T Cell: A Novel Treatment Regime for Cancer

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Abstract

Cancer is always being a matter of concern and when it comes to its treatment Chimeric antigen receptor (CAR) T cell therapy is an approaching and is found to be a promising cancer treatment. In this review, we focus on the production and design of CAR T cell and proposed mechanism of action to kill the tumors as well as strategies to way out safe and effective CAR T cells, including vector designs to increase both the efficacy and safety. All these approaches promote the evolution and development of CAR T cell therapy and move toward our final goal: curing cancer with extent to its safety, high efficacy, and low cost.

Keywords: Cancer; Chimeric antigen receptor; T cell therapy

Abbreviations

ERT: Efficient Reverse Transcription; Gag: Group Specific Antigen; Pol: Polymerase; Env: Envelope; VSV-G: Vesicular Stomatitis Virus; ELISA: Enzyme Linked Immune Sorbent Assay; QPCR: Quantitative Polymerase Chain Reaction; SIV: Simian Immunodeficiency Virus; FIV: Feline Deficiency Virus; EIAV: Equine Infectious Anemia Virus; LTR: Long Terminal Repeat; Gvhd: Graft Versus Host Disease; GVL: Graft-Versus-Leukemia; HSCT: Hematopoietic Stem Cell Transplantation; ACT: Adoptive Cell Therapy; MPM: Malignant Pleural Mesothelioma; DLBCL: Diffuse Large B –Cell Lymphoma; PMBCL: Primary Mediastinal B-Cell Lymphoma; FL: Follicular Lymphoma; Aka: Also Known As

Introduction

Immunotherapy has found to be suitable and promising novel cancer treatment and is about to enter a new era of rapid growth. Different from traditional therapies for cancer, such as surgery, radiotherapy and chemotherapy, immunotherapy is based on the knowledge of the basic mechanisms of the immune system and anti-tumor immune response and aims to harness the immune system to eliminate tumors effectively.

Over the decades, successful translation of immunotherapeutic agents to clinic with remarkable response rates in solid tumors, including lung cancer, has reinvigorated the interest in further exploiting the discoveries in tumor immunology. Immunotherapy uses and activates components of the immune system, such as antibodies, dendritic cells, and T lymphocytes, to treat cancer. Dr. William Coley in 1893 made a first documented attempt of immune stimulation as a cancer treatment. Dr. Coley treated patients diagnosed with sarcoma and carcinoma using cultures of *Streptococcus pyogenes* [1]. This concept of immunotherapy has now been advanced and is all set to make the treatment of cancer effective and rapid. One of the major setbacks in this therapy came up with engineering of T cells with chimeric antigen receptors. Chimeric antigen receptors (CARs, aka chimeric immunoreceptors, chimeric artificial T cell receptors, T cell receptors or CAR-T) are engineered receptors which graft an arbitrary specificity onto an immune effector cell (T cell). Typically, these receptors are used to graft the specificity of a monoclonal antibody linked to co stimulatory and activation domains of the T cell receptor (TCR) complex [3], using the transfer of their coding sequence facilitated by retroviral vectors. The receptors are called chimeric because they are composed of parts from different sources. US Food and Drug Administration have recently approved CAR therapy for cancer using a technique called adoptive cell transfer [2] for use against acute lymphoblastic leukemia (ALL). In this therapy T cells are removed from a patient's blood and are modified in order to express receptors that are specific to the patient's particular cancer. T cells, which can then recognize and kill the cancer cells, are reintroduced into the patient. Modifications of T-cells sourced from donors other than the patient are also under investigation. Zelig Eshhar was the first to discover that CARs can redirect T cell activation to surface antigens in 1989 [3,4].

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Two different approaches exist to redirect T cell specificity. First is the introduction of chimeric antigen receptors (CARs) to recognize TAAs *via* specific single chain variable fragments [9-13], and second is gene modification over highly reactive T cell receptors (TCRs), in which α and β chains of variable domains are sub-clones from tumor-associated antigen (TAA) specific T cell receptors with high-affinity [7-9,13].

CAR T cell therapy that is a GCT (gene based cell therapy) product subtype has been tested for its promising efficacy in cancer treatment in many clinical trials globally. CARs generally contain two domains, signaling and an antigen recognition domain, inserted by gene transfer, through which the T cells can specifically recognize and attack unwanted target cells. These CAR T cells are developed for the treatment of malignancies, with B-cell ALL one of the main indications [13]. Instead of the demonstrated efficacy, it has not resulted in MAA (marketing authorization approval) [14]. Various hurdles are experienced in the clinical development with GCTs in general and with CAR T cells specifically [15-16]. Nevertheless, multiple CAR T-cell products are expected to receive U.S. Food and Drug Administration and European Medicines Agency approval in coming years [17]. CD19 targeting CAR-T cells are likely to be the first engineered T cells to receive marketing authorization [18]. This review is intended to provide an overview of why these CAR T cells became the most effective and most significant approach opted to cure cancer.

Car T Cell Design

Chimeric Antigen receptor or CARs are recombinant molecules that consist of antigen-binding variable regions of a monoclonal antibody [19]. The activation and Co stimulatory domains of the T cell receptor complex are linked to these CARs. They are composed of an extracellular single-chain variable fragment (scFv) derived from an antibody, joined to a transmembrane domain and a hinge peptide, which is further linked to the intracellular T cell signaling domains of the T cell receptor. Cytotoxic and memory functions of T cells are combined with the specificity of an antibody are combined by CAR t cells [20].

Fc receptor gamma or CD3 Zeta cytoplasmic signaling domains are the most common signailing domains used. In current clinical trials CARs also include cytoplasmic signaling domains of T cell co-stimulatory receptors. In order to target a tumor-associated antigen in a human leukocyte antigen (HLA) independent manner T cell specificity are redirected by CARs [21,22]. Extracellular domain provides CAR specificity for a tumor antigen. Designed intracellular domain helps to replicate the normal series of events by which T cells are activated.CAR T cell therapies have the potential to kill targeted cancer cells that have evolved to escape immune surveillance through mechanisms such as down-regulation of HLA expression or proteasomal antigen processing, or in tumors that express negative co-stimulatory molecules that inhibit T cell activity (eg, PD-L1) only because these therapies are able to recognize tumor antigens independent of HLA and provide their own co-stimulatory signaling. CAR T cell therapies may also be used in combination therapy to complement immunotherapies that performs the modification of the tumor microenvironment.

Targeting element

CARs include an extracellular single chain variable fragments or targeting element that recognizes a specific tumor antigen. Targeting domain is derived from a scFv from the variable heavy and variable light chains of a monoclonal antibody. When are expressed on the Surface of a T cell, the cell is allowed to recognize and bind to the antigen on the cancer cell by the targeted domain independent of triggering the process of T cell activation, peptide HLA interaction. B cell-specific tumor-associated antigens as expressed by B cell malignancies may serve as potential targets for CAR-mediated adoptive immunotherapy. CD22 and CD19 are B cell antigens found on the surface most B cell malignancies and differentiated B cells. Hematopoietic stem cells express no antigen or other essential cell types.

Spacer and trans-membrane domain

The extracellular targeting domain and the trans membrane domain are connected by the spacer, which may affect T cell proliferation, tumor recognition and T cell cytokine production. The trans membrane domain may play an important role in CAR function by influencing CAR expression on the surface of the T cell [23].

Costimulatory domain

The construct for CAR includes one or more co-stimulatory domains that provide a second signal to stimulate full T cell activation and the intracellular signaling domain (CD3 zeta) of the native T cell receptor complex. Evidence suggests that co-stimulatory domains facilitate T cell replication and may also increase CAR T cell cytokine production. Either 4-1BB or CD28 are included in all CAR constructs currently used in clinical trials as co-stimulatory domains. The clinical significance of the co-stimulatory domain used in a CAR T construct is not known.

Costimulatory domain-4-1BB

The tumor necrosis factor (TNF) super family includes 4-1BB as a member, and is an inducible glycoprotein receptor *in vivo* that is primarily expressed on antigen-activated CD4 and CD8 T cells [24,25].

4-1BBL, a ligand usually expressed on antigen-presenting cells (APCs) binds to 4-1BB. Overall,

CAR constructs with the 4-1BB co-stimulatory domain have been associated with effector function and gradual, sustained expansion, enriched central memory cells (T_{CM}), and increased persistence in the T cell subset composition in preclinical studies. 4-1BB co-stimulatory signaling domain in CAR T cell therapy clinically significance is not known.

Costimulatory domain-CD28

Immunoglobulin (Ig) super family includes CD28 as the member. Constitutively expressed on resting and activated CD8 and CD4 T cells and plays a critical role in activation of T cell by stimulating the PI3K-AKT signal transduction pathway. Without CD28 signaling, activation of the T cell receptor by an antigen results in T cell exhaustion or death.

CD28 binds to the B7 (CD80 or CD86) ligands, which are found on the surface of APCs. On antigen-activated T cells, the receptor-ligands interaction amplifies expression of pro-inflammatory cytokines such as IL-2, which drives the proliferation of both CD4 and CD8 T cells.

CD28 CAR T cells have been associated with decreased persistence, rapid effector function, decreased persistence, and enriched effector memory cells (TEM) in the T cell subset composition in preclinical

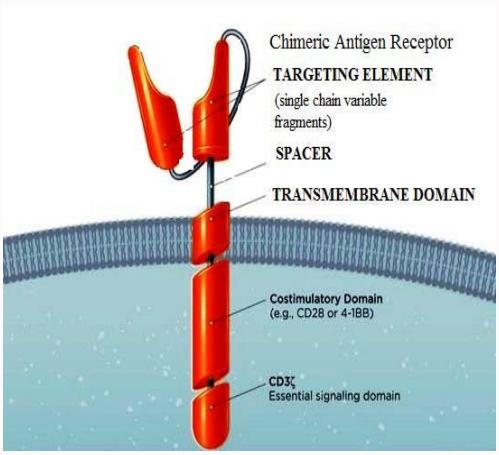

Figure 1: Car T Cell Design.

studies. The CD28 co-stimulatory signaling domain in CAR T cell therapy significance clinically is not known.

CAR T Cell Therapy: For Whom

Kymriah is only for children and young adults up to age 20 who have B-cell ALL that has not responded to standard treatments or that has relapsed at least twice. Most children with B-cell ALL respond to standard treatment and do not relap.

Development of CAR Tcell therapies

The steps involved in the CAR T cell therapies include:

- Apheresis
- Processing
- T cell composition
- Stimulation and Transduction
- *ex vivo* expansion
- Conditioning
- Infusion
- *In vivo* expansion

Apheresis

CAR T cell therapy development begins with collection of the patient's blood and separate of its components through Apheresis. Clinicians then coordinate collection based on the patient's treatment regimen in order to ensure the presence of sufficient numbers of T cells. The peripheral blood mononuclear cell (PBMC) fraction containing the lymphocytes is then delivered to a centralized laboratory. The cells may be shipped frozen or refrigerator prior to transport.

Processing

The reached a pheresis product may or may not be processed depending on subsequent procedures and laboratory. Contaminants of RBCs and platelets may be removed. Cell fractionation based on size may be incorporated to isolate lymphocytes and deplete monocytes. One possible reason for the selection of T cells is to remove monocytes that can inhibit T cell expansion and activation.

T cell composition

CD8 and CD4 T cells are functionally distinct subsets differing in their ability to persist and proliferate in the body, and their relative composition may affect the T cell antitumor response. Adult patients with B cell malignancies that are pre treated heavily have highly variable numbers and proportions of CD8 and CD4 subsets, with most patients having a higher percentage of CD8 cells and a lower percentage of CD4 cells than normal controls. The subtypes may be separated during purification as part of manufacturing process for investigational CAR T cell therapies, to adjust their relative composition either before or after expansion. The clinical importance of T cell composition in CAR T cell therapy is under investigation [26].

Stimulation and transduction

Activation is required for transduction of the CAR gene construct *via* retroviral vectors into the genome of the patient's T cells. Several different activation approaches may be used, including beads-based T cell activation and cell-based T cell activation (using native or artificial APCs). Beads-based activation uses anti-CD3 antibody stimulation in combination with anti-CD28 co-stimulation. DNA encoding the CAR construct can be transduced *ex vivo* into T cells using several methods, including gamma-retroviral or lent viral recombinant vectors or a transposon system. The clinical importance of the transduction method used has not been determined. Virtually all CAR constructs currently in clinical trials have used retroviral vectors. Both gamma vectors and lent viral vectors deliver high rates of CAR gene expression. Gamma vectors offer flexibility because they are available in several different packaging cell lines. Lent viral vectors can transduce non-dividing cells and may be less likely to cause insertional transformation.

Ex vivo expansion

T cells with expressed CAR are expanded, or grown; outside the body to the appropriate patient- specific therapeutic dose. Studies in adult B-ALL, pediatric B-ALL, CLL, and B-NHL have not identified a clear correlation between CAR T cell persistence or CAR T cell dose and efficacy. Several studies have reported a correlation between CAR T cell dose and the severity of its potential risk *i.e.*; cytokine-release syndrome, majorly in patients with higher levels of disease burden at the time of infusion.

Conditioning

Use of conditioning with lympho depleting chemotherapy to reduce tumor load and deplete lymphocytes prior to CAR T cell therapy may improve the enlargement potential (persistence and expansion) of CAR T cells. Lympho depleting chemotherapy may eradicate regulatory T cells, eliminate other immune cells that compete for homeostatic cytokines, prevent transgene rejection and enhance APC activation.

Infusion

The CAR T cells are administered by infusion as a single dose or multiple doses over 1-3 days. Patients may be admitted to the hospital for monitoring for treatment-related toxicities. Serious toxicities, including cytokine release syndrome, neurotoxicity, and B cell aplasia, are known to occur with CAR T cell therapies. These toxicities may require immediate medical attention and sometimes result in death.

In vivo expansion

Once inside the body, the CAR-modified T cells expand exponentially and are available to spread throughout the circulation. There they are thought to attack targeted cells and

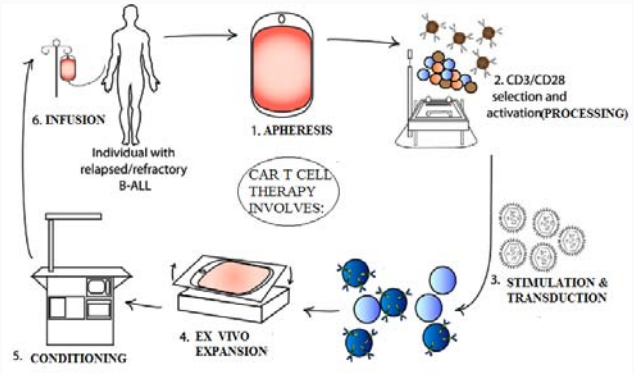

Figure 2: Development of CAR T cell therapy.

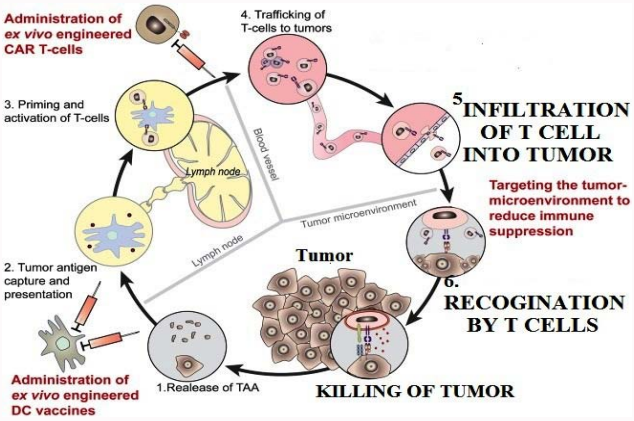

Figure 3: Mechanism of action of CAR T cell therapy.

differentiate into memory cells. These memory cells may live in the body and potentially establish immune memory. Expansion and persistence in the body are linked to several factors, including co-stimulatory signaling domain (i.e., 4-1BB vs. CD28), cell lineage/state of differentiation, cell product composition, cell dose, tumor burden, and the conditioning regimen used to provide lympho depletion. A pictorial representation of development of CAR T cell therapy is shown in Figure 2.

Proposed Mechanism of Action

Engineered CAR T cells mechanism of action is not fully understood. Evidence indicates that CAR T cell therapies stimulate HLA-independent, targeted T cell responses against tumor cells.

The mechanism follows as:

- The CAR T cell recognizes and binds to targeted antigen on tumor cells.
- A conformational change is usually induced by binding that transmits the binding signal through the cell membrane and into the cell.
- Second-generation receptors inside the cell, deliver both a co-stimulatory signal through the CD28 or 4-1BB domains in the cytoplasmic tail as well as a primary activation signal domain through the CD3 zeta domain.
- Cytokines are then released by the activated CAR T cells and transcription factors that may induce cytotoxic activities against tumor cells and promote cell survival and function:

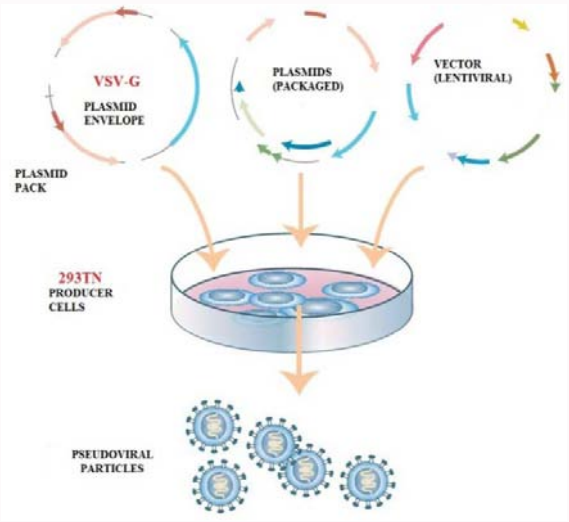

Figure 4: Lentiviral production in a culture medium.

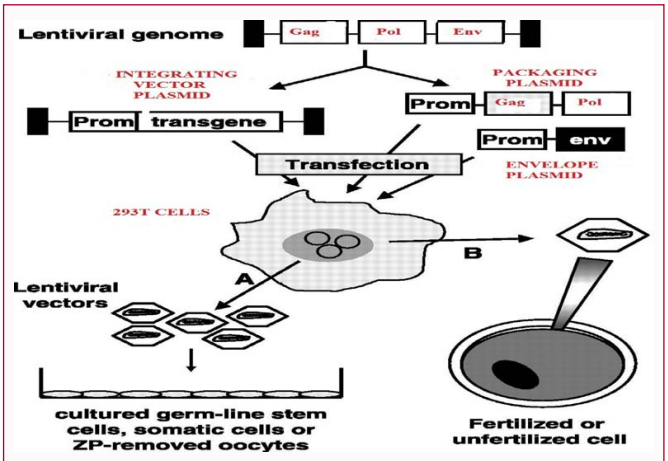

Figure 5: A General mechanism for production.

- Innate immune response activation by interferon (IFN)-gamma,
- T cell survival as promoted by interleukin-2 (IL-2).
- Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) and Fas Ligand (FasL) may induce tumor cell apoptosis.

A pictorial representation is shown in Figure 3.

Optimal Vector Use: Consistent Cell Processing

The viral vector is considered to be as a chief raw material for the transduction of CAR into T cells of the CAR T cell manufacturing process. And the modified T cell is the final investigational product, also known as the medicinal product in the European Union [27]. The CAR T cell product must be generated individually for each patient, but the vector by which CAR has been encoded can be made in large quantities and stored at -80 C for 4 years. Frozen viral vectors are stable for about 9 years at this temperature [27-29]. The vector to be used must be sterile because the final product (CAR T cell) so produced can't be sterilized by filtration or by any other sterility means. Use of a third generation minimal lentiviral vector, enhances safety [30,31]. The minimum of 2 weeks are required for the batch

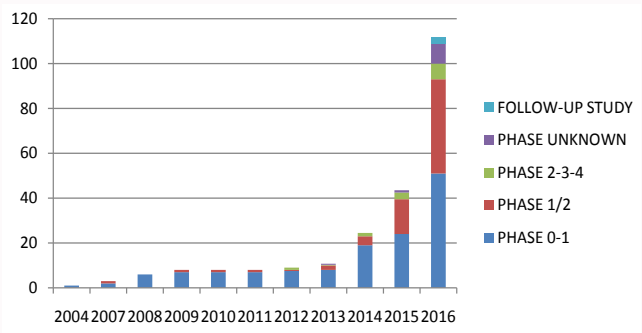

Figure 6: Phases of CAR T cell therapy trials.

manufacture of viral vector for cellular therapies. The major time is spent to grow adequate HEK293T cells in order to produce large quantities of replication-defective viral vector [32]. The whole process start with a cryopreserved aliquot from a suitable working cell bank and are allowed expanding to an appropriate number in a culture for several days for final production (Figure 4).

Plasmids are then transfected with the produced cells; the minimal lentiviral vector is produced as a result typically these plasmids are:

1) A packaging construct Gag/Pol packaging used to encode the structural (viral) protein (Gag) And enzyme (Pol);

2) A construct encoding appropriate envelope glycoprotein from a heterologous source, which Results pseudotyping of vector particle such as VSV-G;

3) A viral accessory expressing construct Rev; along with,

4) A plasmid vector which encodes the CAR construct and also other sequences essential for RNA, integration and ERT packaging [33].

Lentivirus vector usually comprise of three or four plasmid expression systems (Table 1).

In order to prevent reacquisition of replication competence the system of vector should employ several key safety features that prevent them collectively. CAR-expressing lentiviral vector begins to release from the production cells within 48 hr of transfection and is collected from the culture medium [34]. Multiple batches of vector containing medium are then harvested (typically two). It is then filtered in order to make it free from debris and production cells. Finally purification is performed through downstream processing to make the viral vector enrich and to formulate the vector into an appropriate storage buffer. After complete production the vector is made cryopreserved until later use (Figure 5, Table 2).

Lentivirus Limitations

The major risks associated with the use of lentiviral vectors are as follows:

- Sometimes, lentiviral vectors may induce oncogenesis through insertional mutagenesis.
- These vectors have the potential of generating replication competent lentivirus.

Due to the above mentioned concerns and to avoid the risk of lethal human infection, scientists prefer to use non human lentiviral vectors, such as FIV, SIV and EIAV [35-47].

Table 1: Plasmid expression.

Transfer vector plasmid	Comprises the LTR, psi – sequence and therapeutic gene sequence.
Packaging vector plasmids	Comprises the elements required for virus packaging, such as genes coding for structural proteins, other genes (except the Env gene). This plasmid lacks the packaging signal owing to which the virus is rendered incapable of reproduction after it has infected host cell.
Envelope gene plasmid	Encodes the viral envelope proteins.

Table 2: Lentivirus production.

Titer	10^6-10^7 TU/ml	10^8-10^9 TU/ml
Quantity	5 × 200 µl	2 × 100 µl
Turnaround time	2 – 3 weeks	2 – 3 weeks
Analysis options	Infectious Titre Physical Titre (QPCR, P24 ELISA)	Infectious Titre Physical Titre (QPCR, P24 ELISA)
Purification	Clarified supernatant	Purified viral particles

Managing Toxicity

CAR T cell therapy has been found beneficial in hematologic malignancies. But the intense activation of immune system has resulted in many unwanted side effects despite optimism that direct targeting of tumor cells would avoid systemic toxicity. Successful clinical application of CAR T cells comes with the management of these toxicities (Table 3). Several methods have been proposed in order to control the toxicity, these methods includes:

Clinical Development

CD19-targeted CAR T cell therapy for hematologic malignancies as a first clinical trial results were reported in 2010. Since then, several additional studies have been published. The studies have been small, with limited follow-up, but CAR T cell therapy potential has generated excitement due to the duration and rates of complete remission observed in patients with relapsed or refractory disease who had already been received multiple lines of treatment [48]. A number of CAR T cell investigational agents have received breakthrough therapy designation from the FDA. Breakthrough therapy designation is for a drug that treats a serious or life-threatening condition and preliminary clinical evidence indicates that the drug may demonstrate substantial improvement on at least one clinically significant endpoint over available therapies [49,50].

Serious toxicities, requiring immediate medical attention and sometimes resulting in death, are known to occur with CAR T cell therapy. Reported treatment-related toxicities have been similar across CD19-targeted CAR T trials for hematologic malignancies. They have included cytokine.

Release syndrome (CRS), neurotoxicities, and B cell aplasia, although the incidence and severity of the toxicities has differed across trials [48-51].

Although the early results have been promising, the clinical safety and efficacy profiles of these investigational agents will only be established when the results of ongoing trials are available (Figure 6).

Target Antigens for CARS

ACT using CAR T cells using ACT is a growing strategy that is being investigated for the treatment of solid tumors including lung cancer and MPM. Although finding a suitable target antigen has

Table 3: Toxicity Management.

METHODS	COMMENTS
Suicide Genes	Suicide gene integration to allow for selective depletion of CAR T cells may prove to be an essential component in the evolution of this technology. Herpes simplex thymidine kinase was the first suicide gene evaluated in human trials [42-44]. The connective link between toxicity and clinical efficacy when applies CAR-redirected T cells for the treatment for cancer resembles the case for HSCT, in which the GVL effect and GVHD are strongly linked phenomenon's. Since suicide gene therapy for HSCT has demonstrated the potential to operate a meaningful dissociation between the GVL effect and GVHD, it is reasonable to implement suicide gene in CAR-redirected T cells which may help to mitigate their risks, and In order to allow selective depletion of CAR T cells preserves their therapeutic effect. This therapy is most appropriate particularly when targeting oncoantigens and because of its leaky expression present it is therefore present, albeit at lower levels, on healthy tissues. suicide genes will work as an universal surrogate for safety, suicide genes may proved to be of great help in managing intermediate and late toxicities not only during the different phases of clinical development, but especially, and hopefully, in the case CAR-redirected T cells and would finally translate into mainstream medicine.
Pharmacological Immunosuppression	Suppressing immune system with systemic corticosteroid also found useful in improving the symptoms of CRS, also dexamethasone as a first logical first choice agent because of its superior central nervous system penetration. The prolonged use of systemic corticosteroids has been shown to diminish the persistence and potentially, the efficacy of CAR T cells.[45]
Targeted Activation	The intensity or toxicity control of T-cell activation is functionally possible through the inclusion of an "on-switch" in CAR design. Response of T cell can be controlled through combinatorial antigen targeting with separation of T-cell activation signals [46,47].

Table 4: Current CAR T cell clinical trial.

TARGET ANTIGEN	MALIGNANCIES	NCT#	PHASE	STATUS	LOCATION
MUC 1	Hepatocellular, pancreatic, breast(triple negative)	NCT02587689	½	Recruiting	China
Mesothelin	Breast, lung, metastases, malignant pleural disease, mesothelioma	NCT02414269	1	Recruiting	MSKCC
Mesothelin	Mesothelin Cervical, lung, mesothelioma, ovarian, pancreatic	NCT01583686	½	NCI	
Mesothelin	Mesothelin Mesothelioma, metastatic pancreatic, ovarian	NCT02159716	1	Ongoing, Not recruiting	UPENN
Mesothelin	Mesothelin Breast (triple negative), endometrial, mesothelioma, ovarian, pancreatic	NCT02580747	1	Recruiting	China
Mesothelin	Mesothelioma	NCT01355965	1	Ongoing, Not recruiting	UPENN
ROR 1	Leukemia's, breast	NCT02706392	1	Enrolling (by invitation only)	NCI
CEA	Gastric, lung, colorectal and pancreatic	NCT02349724	1	Recruiting	China
FAP	Mesothelioma	NCT01722149	1	Recruiting	Zurich
VEGFR 2	Metastatic cancer, renal, melanoma	NCT01218867	½	Completed	NCI
EGFR	EGFR positive malignancies (ovarian, renal etc.)	NCT01869166	½	Recruiting	China
GD2	Solid tumors	NCT02992210	½	Recruiting	China

been one of the greatest challenges in the development of CAR T cell therapy for these cancers (Table 4).

Race with the Market: CAR T Cells

In the evolving field of immuno-oncolytic therapeutic development, the clinically advanced CAR T cells are currently those engineered against CD19, a receptor for cell surface constitutively expressed in hematologic malignancies. Several pharmaceutical companies raced to be the first to secure the regulatory approval, ultimately Novartis' CTL019 (tisagenlecleucel; Kymriah) was the first to- market advantage. Tisagenlecleucel was approved for the treatment which includes young-adult (up to 25 years) and pediatric patients with relapsed and refractory B cell precursor ALL. To about 68 young-adult and pediatric patients with CD19-positive B-cell precursor ALL were infused with a single dose of CAR T cells. 63 patients treated with tisagenlecleucel were evaluable for response (after follow up of 3 month); 83% achieved complete remission (CR) or incomplete CR within 3 months of infusion with minimal residual disease (MRD)–negative status achieved in the bone marrow. With Novartis there was Kite Pharma, whose CD19-targeted CAR T-cell therapy, received approval as treatment for adults with relapsed or refractory non-Hodgkin lymphoma based on results of the ZUMA-1 trial in October.

Till January 2017, 101 patients were enrolled in the ZUMA-1 trial and were treated with axi-cel. After 6 months the study completed its primary endpoint of combined objective response rate (ORR) for patients with PMBCL, DLBCL or transformed FL. The majority of the population were patients with DLBCL (n = 77) with the CR and OR Rrates, 49% and 82%, respectively.

Both tisagenlecleucel and axicabtagene ciloleucel are also being evaluated in clinical trials for additional indications. Juno Therapeutics' CD19-targeted CAR T-cell therapy, JCAR015, was originally slated to secure regulatory approval first. But, the development of JCAR015 was discontinued after clinical holds placed on the phase II ROCKET trial, in which an adverse safety profile revealed an increased incidence of deaths due to cerebral edema.

Future Goals

CAR T cell therapies is coming with lot of promises for future, researchers are looking for ways to make these immunotherapy better at recognizing and destroying leukemia cells. That is also trying to figure out how to get gene therapy to work in solid tumors, which has been challenging. To combat solid tumors, the CAR-T treatment being designed must not only be specific and effective but also be administered within the window in which the patient is preconditioned. New studies have however found that when used in combination with adenovirus, CAR-T therapy with anti-PD1/PDL1 and CLTA4 strategy can be more effective. Ad5Δ24, an oncolytic virus that contains the chemokine RANTES along with IL15 cytokine has shown via the intratumoral release of these two substituent's, to not only attract CAR-T cells to the tumor, but also promote their local survival in the immunosuppressive environment. The non-HLA

CAR-T target of stromal solid tumor (*i.e.* ErbB) has been in study. By combining Ad5Δ24 with CAR-T treatment, while the study will need to progress further before clinical trials, it does shed light on the future of CAR-T cell therapy's role in treating solid tumors.

Conclusion

Using engineered T cells with chimeric antigen receptor is a powerful and effective strategy for cancer therapy. Toxicities leading to risks as caused by CAR T cells are diverse and not fully understood. Intensive care and monitoring is required for their management. As demonstrated by the clinical that the prudent choice of target antigens is the fundamental step and crucial to the success of CAR T therapy. Managing and working effectively upon the toxicity is one of the major avenues for the improvement of this field of CAR T cell therapy.

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