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CAR T CELL THERAPY – NOVEL IMMUNOTHERAPY FOR REFRACTORY MALIGNANCIES

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INTRODUCTION

The number of cases of cancer seems to be increasing be it due to better investigating approaches, more awareness or the sheer longevity of cancer patients. The management of early-stage cancer seems to be encouraging but it is the advanced stage cancer whose treatment with the prevailing modalities of therapy be it surgery, cytotoxic chemotherapy or radiotherapy seems to be frustrating. Sooner or later these cancers seem to relapse and many times with more vengeance and it is at this particular time when we don't have much to offer except palliative therapy. The failure of cytotoxic chemotherapy in advanced stage, lack of sustained remission and the toxicity profile of cytotoxic chemotherapy prompted researchers to use the body's immune mechanism to fight against cancer, hoping that the relapses would be more sustained and toxicities fewer. Hence, the concepts of Immunotherapy were born. Chimeric Antigen Receptor T cell (CAR-T Cell therapy) therapy is the one of the latest immunotherapies with a very promising future in the setting of Refractory or Relapse Malignancies.[1]

PATHOPHYSIOLOGY

Cancer cells like any other foreign substances are attacked and destroyed by the body's immune mechanism either the innate immunity or the acquired immunity. So, for cancer cells to survive it has to immunomodulate themselves by a series of process which can be easily summed up as 3Es, Elimination, Equilibrium, and Escape.[2] The first stage of elimination when the cancer cells are identified and destroyed by the innate immunity cells or the adaptive immunity mechanism. Till this stage cancer is not visible and generally under control of the body's immunity. However, at times few of the cancer cells survive the elimination stage.

Cancer cells are rapidly dividing and there is a lack of effective DNA damage control mechanism and thus they can survive the elimination stage and enter the Equilibrium stage. The equilibrium stage is the longest and can go up to almost 20 years till the time cancer becomes big enough to be clinically detected. The exact transition from the Equilibrium stage to the Escape Stage can never be forecasted. Whenever the critical number of genetic and epigenetic changes have occurred, these cancer cells are unleashed into the body with no control of the body's immunity on them and thus Escape from the clutches of immunity.

Scientists for ages are trying to figure out the mechanism by which these cancer cells can evade the immune mechanism. Some of the proposed mechanism is that these cancer cells can change their recognition pattern by altering the expression of major HLA Complexes on them and hence body's immune mechanism fails to recognize them and hence cannot destroy them. The second mechanism proposed was that these cancer cells produce certain cytokines that deactivate the entire T cell mechanism which could destroy them.[3] So the role of immunotherapy is to either make theses cancer cells "visible" again, so that immune cells are able to detect and kill them and the second mechanism was to alter the cytokine milieu in the tumor so as to make the body's immune mechanism to become more active by preventing the action of various inherent mechanisms to keep the rapid multiplication of the immune cells i.e. checkpoint inhibitors.

Adoptive T cell therapy

"Adoptive transfer "was originally coined by Billingham to describe allograft rejection and adoptive immunotherapy is the term used to infusion of selected immunocompetent cells to control infections or malignancies.[4]

The idea for the use of the body's own T cell and its ex vivo manipulation, Selection based on target specificity), expansion and reinfusion were being studied for many years. It was in 1980 that Rosenberg showed that these selected T cells were able to kill tumor cells from metastatic sites.[5] There were further studies on Lymphokine activated T cells (LAK cells) which in the presence of Interleukin 2 (IL 2) were able to kill tumor cells in Melanoma. Thus, came the understanding that these rare Activated lymphocytes can kill tumor cells (Tumor-infiltrating Lymphocytes i.e TILs). However,

these activated lymphocytes were effective in laboratory settings but their role in clinical settings was not very encouraging.

By 1990 there was another addition in the armamentarium as cytokine-induced killer cells (CIK) which were lymphocytes which were co-cultured with anti -CD3 antibody.[6] Almost at the same time, researchers realized the importance of genetically modified T cells to target which was more specific and did not require de novo T cell activation in patients.[7] Another an important aspect of these newer form of therapy was to make these newer or genetically modified T cells which are MHC independent and hence the tumor cells cannot escape. And then came the era of Chimeric Antigen Receptor T Cell therapy (CAR T Cell Therapy).

Chimeric Antigen Receptor T Cell therapy (CAR T Cell Therapy)

CAR T cells are autologous T cells that are harvested through leukapheresis, genetically modified (viral or non-viral transfection method) ex vivo then multiplied the number of times in culture media. These genetically modified T cells then pass through a lot of quality control. The patient meanwhile is given lymphodepleting chemotherapy followed by CAR T cell infusion. It was in 1989 that Eshhar's group in Israel made the first CAR T cell.[8]

It is important to understand the structure of CAR as the functionality of the CAR depends mostly on the genetically engineered domains of the T cell receptor. The extracellular domain consists of two sub-parts the antigen binding moiety and the spacer. The antigen-binding moieties can be of[9]

1. Single chain friable Fragment (scFv) is a monoclonal antibody fragment derived from either mouse monoclonal antibodies, humanized Abs or fully human Abs.
2. Human Fab fragment which is selected from phage display libraries
3. Nature ligands

The advantage these CAR cell has been that they can recognize unprocessed antigens hence there is no requirement of antigen presentation through MHC. This property of CAR cells helps them to bypass the restriction of both Classes I and Class II restriction and hence CAR T cells of both CD8+ as well as CD4+ are recruited against Tumor cells. These CAR cells use then use two mechanisms for destroying the tumor cells by either perforins/ granzymes exocytosis or by death receptor signaling (Fas L / TNF -R).[10]

The spacer is a connection between the antigen-binding domain and the TM (transmembrane Domain). It is a hinge-like structure that connects to intracellular signaling moiety like CD 3 complex. The developments in the structure of intracellular domain divide the various generation of CAR T cells.[11]

1. First-generation: Consist of T cell activating domain (zeta chain of CD3) and extracellular immunoglobulin derived heavy and light chains. These first-generation CAR T cells do not have any sustained T cell responses as they have limited signaling capacities.
2. Second generation: Dual signal for activation (on produced by antigen recognition and second by co-stimulatory molecule such as CD 28/B7. The activation of CD 28/B7 promoted the synthesis of IL 2 which complemented the activation of Cells and avoided apoptosis. These second-generation CAR T cells are called the "**living drugs**" and are the foundation of current CAR T cell therapies.
3. Third generation -Multiple co-stimulatory signals combined, thereby increasing cytokine production / T cell proliferation.

There have been further developments in the form of CAR T cells redirected for universal cytokine killing (TRUCK). These cells produce IL 12 which activates the innate immune response and IFN gamma which contribute to antigen-independent destruction of tumor cells.[12]

Researchers have now developed biphasic CAR T cells (**Tandem CAR – TAN CAR**) whose transgenic receptors can recognize two sets of antigenic receptors. In cases where one type of receptor is down-regulated / mutated giving the tumor cells the advantage of escaping immune cells, TAN CAR is still functional and retains the ability to destroy the tumor cells.[13]

In the latest series of developments, to enhance the specificity of the CAR T cells dual antigen coding (Tumor barcoding) Is being developed. There is also an antigen-specific inhibitory CAR T cells (**iCAR**) which regulate the release of cytokine secretion in case of interaction with off-target tissue antigen.[14] Further to this there also studies of using checkpoint inhibitors along with CART cells.

How is CAR T cell manufactured?

There are various methods for engineering CAR T cells. They require gene transfer techniques like viral/non-viral transduction, transposons, and mRNA transfection methods.

1. Viral transduction

They are the most preferred procedures to equip T cells with CAR. "Retroviridae" family is most commonly used. The advantage of using viruses from this family is that most of the viral genome can be replaced by the transgene of interest and on transduction viral genome is permanently integrated to the host genome.[15]

2. Transposons

They have two mobile genetic elements consisting of one plasmid carrying CAR (transposon) and other carrying transposase. Transposase flank the CAR sequence

leading to excision and subsequent integration in the target cell genome. They are more effective and less toxic. These transposons were earlier referred to as sleeping beauty and piggyback system.[16]

3. CRISPR/Cas 9

This is a mechanism of gene editing where Cas 9 functions as endonuclease and then a donor template in the form of a plasmid is integrated at the desired transgene using homology-directed repair.[17]

4. Non-Viral Transfer Methods

It uses the non-viral transfer of plasmid DNA or IVT-mRNA. It represents a cytoplasmic expression system and does not need to enter the nucleus to mediate its function. The delivery of mRNA can be mediated by endocytosis using several nanoparticles, like polymeric nanoparticles. Gold nanoparticles or lipofectamine.[18]

Therapeutic Role

Presently the role of CAR T cells has been studied in hematolymphoid malignancies like relapse/refractory B cell malignancies like B Cell non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukemia (ALL) and Chronic lymphocytic leukemia (CLL). The response of anti-CD 19 CAR T cells in these above-mentioned malignancies was between 70-94% in different trials.[19] The various targets that have been targeted are CD 19 for FL/CLL/ALL and in Multiple myelomas.[20] The newer targets being studied are CD138 and B Cell maturing antigen (BCMA) for Multiple myelomas and CD 33 and CD 123 for AML.[21]

CD19 CAR T cells

CD19 CAR T cells received first FDA approval for investigational studies in 2007. The initial protocols used retroviral or lentiviral vectors encoding for CARs. The costimulatory domains targeted were either CD28 or 4-1BB costimulatory domains.[22]

"Tisagelecleucel T" Kymriah produced by Novartis is the first CAR T cell to be used commercially. It got its FDA approval on 30 Aug 2017 with an estimated price of $475,000. It is a CD19 directed genetically modified T cell immunotherapy used in

1. Relapse /refractory B cell ALL for patients up to the age of 25 in second or later relapse
2. DLBCL (NOS/ High Grade/ DLBCL arising from follicular Lymphoma)

Kymriah is not used for Primary CNS lymphoma (PCNSL), however, there are newer forms of CARs that are tried in PCNSL. It is a single dose for infusion.

The dosage ranges from 1X106 -25X106 CAR positive viable T cells per kg body weight for pediatric and young adult ALL. In cases of DLBCL, the dosing is about 3.6X108 Car positive viable T cells.

Lymphodepletion with Fludarabine and cyclophosphamide is done before the administration of CAR T cells. In cases of Refractory DLBCL which cannot tolerate cyclophosphamide due to hemorrhagic cystitis or previous failure to cyclophosphamide, Bendamustine (90mg/m2) for two days prior administration of CART cell therapy is given.

Depending on the protocol followed CART cells are infused between 2-11 days post lymphodepletion. The patient should recover from the effects of lymphodepletion before the infusion of CAR T cells.

The main adverse reactions of KYMRIAH are as follows

1. Cytokine Release Syndrome (Table 2)
2. Neurological Toxicities
3. Infections and Febrile Neutropenia
4. Prolonged Cytopenias
5. Hypogammaglobulinemia

The second drug which received its FDA approval was called "axicabtagene ciloleucel" (Yescarta) from Kite Pharma. This drug was used in the setting of Relapse/ Refractory aggressive B cell NHL (post two lines of therapy and ineligible for the transplant), Mantle cell lymphoma and indolent lymphoma like follicular lymphoma. This drug was priced at $373,000. Like Kymriah they also had side effects of CRS / Neurotoxicity and risk of second malignancies.[23]

Novel CAR T cells

CD 19 CAR therapy is the most successful and most studied. However various newer targets have been identified and are being studied. In multiple myeloma various new targets like kappa light chain, CD138, Lewis Y antigen, CS1 (cell surface glycoprotein), BCMA, SLAM F7. Several trials targeting these novel antigens are underway.[24]

Similarly, for AML the newer targets being studied are CD 33, CD70, Tim 3, Lewis Y antigen, CD123 folate receptor B.

CAR T cell therapy in solid tumors

CAR T cells are facing a lot of challenges in the management of solid tumors. Though we have studies going on in the field of glioblastoma multiforme / pancreatic cancer and recently Her2 directed CARs in Breast cancer, the results of which are promising but not to the extent of CD19 CAR.

The main reasons which have been hypothesized for the ineffectiveness of CAR T cells in solid tumors are the tumor heterogeneity, lack of specific antigens and the inability of the CAR T cells to reach the tumor milieu which is hypoxic, acidic and contain cytokines which make the T-cells ineffective.[25] Most of the T cells die due to cross-reaction with "on-target off-tumor antigens"[26]

To overcome these barriers lot of different techniques are being used like, iCARs, logic gated CARs and using CAR T cells along with other immunomodulatory drugs like checkpoint inhibitors and cytokines.[27]

The various targets which are being studied for using CAR T cells in solid tumors are

1. Epidermal growth factor receptor (EGFR)
2. interleukin-13Ra2 (IL13Ra2) for glioblastoma
3. Human epidermal growth factor receptor 2 (HER2)
4. Carcinoembryonic antigen (CEA)
5. Prostate-specific membrane antigen (PSMA)
6. Disialoganglioside 2 (GD2) for neuroblastoma

A lot of studies are being carried out in GBM and even in ovarian tumors based on these targets, though the final results of these trials are pending, they seem to be promising.

Safety Concerns of CAR T cell Therapy

Like all other therapeutic modalities for cancer treatment CART cells also have a fair share of toxicities. [Table 1] Cytotoxic chemotherapies have off-target toxicity causing long term damage but CAR T cells have an on-target effect and their toxicities are generally reversible. B cell aplasia is one of the important on target, off-tumor adverse effect of CARs.

Cytokine release syndrome (CRS) is an important side effect associated with CD19 CARs and B cell maturation antigen (BCMA) CARs. CRS is further divided into various garde depending upon the sign and symptoms. Depending on the extent of tumor burden, the extent of CRS can vary from a non-infectious flue like syndrome to life-threatening capillary leak syndrome manifesting with hypoxia and hypotension.[28] (table 2)The main pathology behind CRS is high levels of cytokines including IL6 and IFN gamma. Anti IL6 receptor antagonist tocilizumab is FDA approved treatment for CRS.

Most of the researches involved with CD19 and BCMA CAR T cells have shown some extent of neurotoxicity. The central nervous system is generally involved. However, to date, the exact pathophysiology of neurotoxicity is not known.

The most important aspect of management of CRS or neurotoxicity was to recognize these symptoms early and treating them effectively. The role of steroids and tocilizumab form the cornerstone of the management of CRS and neurotoxicity. Indication and usage of these medications have been highlighted in Table3.

Prolonged cytopenias leading to infections and febrile neutropenias are another important side effect of this form of therapy. Many a time requiring prolonged admission to hospital and supportive care.

Future Challenges

CAR T cell therapy is the first form of commercially viable gene transfer therapy. Though its role in hematolymphoid malignancies has gained acceptance in solid malignancies a lot of research needs to be done. We need to search for the targets in solid tumors which are effective and have minimum off-tumor activity. Presently we have almost 250 trials on CAR T cell therapy, mostly being conducted in the USA, China, Japan and few centers in Europe.

CAR T cell therapy a has brought a new hope in the lives of patients having refractory lymphoma / leukemias/ and a few solid tumors. However, a lot need to be done to increase their specificity and to decrease their toxicity and side effects.