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CAR-T CELL THERAPY: A REVOLUTIONARY CANCER TREATMENT

Svetlana Guthrie[1], Janek Belekov[2], Sabirakhan Mamatova[3], Lazokatkhan Dzhumaeva[4]

[1]Student of Medical Science, International Medical Faculty, Osh State University Kyrgyzstan.

[2]MD, Ph.D. Professor, Doctor Of Medical Sciences, Director of the Cancer Center Kyrgyzstan.

[3]MD, Ph.D.Candidate of Medical Sciences, International Medical Faculty, Osh State University, Department of clinical Disciplines, Kyrgyzstan.

[4]MD, Ph.D. International Medical Faculty, Osh State University, Kyrgyzstan.

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ABSTRACT :

Cancer is one of the world's leading causes of mortality. A number of conventional cytotoxic approaches for neoplastic diseases have been developed over the years. However, due to their limited effectiveness due to the heterogeneity of cancer cells, there is a constant search for therapeutic approaches with improved outcomes, such as immunotherapy, which utilizes and enhances the patient's immune system's normal capacity.Recently,Adoptive T cell immunotherapy has sparked a lot of interest in cancer treatment. Chimeric antigen T cell (CAR T) treatment has emerged in recent years as an advantageous therapy for treating cancer. CAR-T cell therapy is thought to be an effective treatment for relapsed or refractory tumors. Though the initially approved CAR-T anti-CD19 therapy produced impressive results, retrogressions, including high relapse rates and resistance, led to the need for detection of engineered cells more therapeutically efficient. Significant efficacy and persistence improvements in the design and manufacture of CAR-T cells have been achieved.

KEYWORD :- CAR-T cell therapy,function, side effects, CRS, Neurotoxicity, cellular structure, efficacy, safety, failure.

INTRODUCTION:

Cancer therapy has progressed over the past few decades globally, and cell therapy has come into view as a new modality to treat cancer patients. The main form of treatment for any cancer was surgery, chemotherapy, radiation, and bone marrow transplantation. However, these procedures were the pillars of any cancer treatment but were ineffectual for advanced and relapsed stages of cancer. Considering the complex functioning of cancer tumors, the present-day advancement and expansion involve cell-based immunotherapy that aims at tumors at both the cellular and genetic levels. Out of them, chimeric antigen receptor T-cell therapy (CAR-T therapy)is the most active and advanced. It is hailed as the newest approach for the treatment of cancer. The concept of CAR- T uses genetically engineered T cells with CARs. CAR-T cell therapy gears up the immune system to attack cancerous

cells. Unlike traditional treatments, CAR-T therapy is administered only once from the patient's T cells.

CAR-T CELL THERAPY:

Chimeric antigen receptor T cells are genetically modified T cells used to treat Malignancies. This is an experimental form of gene therapy that can reconfigure T lymphocytes to fight and eradicate cancer cells.

LEUKAPHERESIS:

Leukapheresis or apheresis is the first step in this therapy.
T lymphocytes, or T cells, are a type of leukocyte that plays an important role in the immune system. The procedure begins with collecting leukocytes from the patient via leukapheresis or apheresis. During the collection process, blood is drawn, and the T cells are separated from the other components of the blood. The leukapheresis separation process can take up to 4 hours to ensure that there enough T cells, and it must be coordinated with the patient's ongoing care. To obtain enough T cells, 3-6 times the total blood volume of the patient should be processed. The patient's remaining blood is then reintroduced into his or her system.

ACTIVATION OF T CELLS:

Collected T cells are transported to a specialized cell therapy manufacturing factory, where they are genetically "reprogrammed" to become CAR T cells, and receptors are added to the T cells to help identify and fight against the target cells contained on the surface. T Cells with specific antigens, including normal cells and malignant cells.T cell activation is necessary for in vitro amplification of T cells and CAR cDNA transduction through retroviral vectors, Lead to permanent genome modification and sustained CAR expression. T cell activation requires major-specific signals and costimulatory signals through T cell receptors, such as
CD28, 4-1BB, or OX40. The different methods of T cell activation are cell-based T cell activation, bead-based T cell activation, Expamer technology.

T CELL EXPANSION:

A variety of culture systems can expand the cells to produce a therapeutic number of CAR T cells for treatment. GE bioreactors, G-Rex bioreactors, and Clinimacs Prodigy are examples of different expansion platforms.

QUALITY ASSESSMENT:

Further to amplification, the cells are washed and concentrated, and samples are collected for quality assurance. The quality management system must ensure continuous control, traceability, as well as documentary evidence of all processes, including product approval or rejection. After ensuring the final product's safety, purity, sterility, and efficacy, it should be frozen and shipped to the infusion site.

PREPARING THE PATIENT FOR TREATMENT:

The patient receives preconditioning chemotherapy known as “lymphodepletion” in the days preceding the infusion therapy. This helps make room in the patient’s immune system for reprogrammed T cells and target cancer cells. Lymphodepletion also causes the release of endogenous intracellular inflammatory cytokines, which promote CAR T cell activity after cell

infusion. The preconditioning modes differ considerably based on the protocol and the individual patient.

INFUSION OF CAR T CELL THERAPY:

At the treatment center, patients receive their personalized CAR T cells in a single dose. The procedure is generally completed in an hour or less. CAR T cells may then multiply and distribute throughout the body, attacking target cells.

PATIENT MONITORING :

Patients undergoing CAR T cell therapy are carefully scrutinized by their medical team for potentially severe, life-threatening,or fatal side effects. The duration of stay in the hospital will vary depending on the patient's response to treatment. Patients must stay at the treatment center for four weeks and may return home when their doctor says it is safe to do.

THE FUNCTION OF CAR-T CELL THERAPY:

Patients with DLBCL who have: Failed first-line chemotherapy, Failed second or higher lines of chemotherapy,Prior therapies must have included an anti-CD20 antibody and an anthracycline if the patient relapsed within 12 months of getting an autologous stem cell transplant.ALL patients who have a refractory, second, or subsequent relapse.

CAR T CELL THERAPY SIDE EFFECTS:

CAR T cell therapy has a slew of negative side effects.Patients should be aware of two potentially serious side effects:

1. **Cytokine Release Syndrome (CRS):** CRS is a systemic inflammatory response that can occur within a few days to several weeks after a patient's CAR T cells are reintroduced into their body. Fever is one of the symptoms, but it is not the only one (pyrexia) Fatigue, nausea, chills, and other symptoms Headache, swelling, and low blood pressure (hypertension).Rapid heartbeat (tachycardia),Muscle/joint pain (myalgia/arthralgia),Weakness (asthenia),Lowoxygen level (hypoxia),Difficulty breathing (dyspnea),Stomach pain Perplexity

2. **Neurotoxicity:** Neurotoxicity can alter the function or structure of the nervous system. It can occur anywhere from a few days to several weeks after a patient's CAR T cells are reintroduced into their body. The following symptoms may occur but are not confined to confusion, difficulty or inability to speak, difficulty staying awake, and fatigue. Agitation, difficulty walking, Shaking, Loss of coordination Seizures ara type of seizure that occurs when there is a headache Memory lapses.

THE CELLULAR STRUCTURE OF CAR-T CELLS:

The autologous T cells of CAR-T cell therapy are modified to express a CD19 chimeric antigen receptor (CAR), which is useful for the removal of B cells (including malignancies). **CARs made up of five parts are used in the current CAR-T cell therapies:**

- Extracellular receptor that binds to the antigen of interest
- Extracellular hinge component that allows for greater flexibility to improve binding affinity, The transmembrane domain of the molecule that anchors it to the T cell, the Intracellular costimulatory domain that promotes CAR-T cell survival, Intracellular signaling domain that starts the biochemical cascade that leads to immune activation.

FACTORS INFLUENCING CAR -T CELL EFFICACY:

Many known and unknown factors are likely to contribute to the observed variability in clinical responses across trials and between individual patients. Despite the fact that disparity in clinical protocols makes direct comparisons impossible, clinical data collectively point to T cell expansion and persistence after adoptive transfer as key critical factors for achieving effective cancer clearance. The in vivo fate of T cells is influenced by a variety of factors, including the design of the car, the composition of the infused T cells, the tumor type and microenvironment, and the recipient preconditioning regimen a Savoldo and colleagues elegantly demonstrated the importance of incorporating costimulatory domains into second-generation CARs by treating six lymphoma patients with a mix of first and second-generation CAR T cells, demonstrating the improved persistence of T cells in which the last CAR configuration is expressed. The majority of second-generationCARs studied in clinical trials contain either CD28 or 4-1BB signaling domains, and preclinical and emerging clinical data suggest that CD28-containing constructs expand more quickly and then decline, whereas 4-1BB CARs have a longer persistence. Third-generation CARs incorporating CD28-4-1BB or CD28-OX40 in combination have demonstrated sustained T cell activation, but their efficacy must still be determined in clinical trials. A clinical trial using a CD20-redirected third-generation CD28-4-1BB-CD3 signaling CAR failed to produce dramatic results in a receptor configuration that provides CD28 costimulation via the CAR endodomain and 4-1BB ligand coexpressed with the CAR cell surface; Zhao and colleagues recently demonstrated superior performance in the combination. The in vivo proliferative capacity is also affected by the T cell composition in the infused product. Central memory T cells, which have longer telomeres and higher proliferation than more differentiated effector T cell populations, provide long-termpersistence and function. The majority of clinical trials today use unselected, ex vivo expanded T cells derived from patient's peripheral blood mononuclear cells.The usage of paramagnetic beads covalently conjugated with agonistic CD3 and CD28 antibodies, such as CTS Dynabeads CD3/CD28, in conjunction with the CTS DynaMag magnet adapted for culture bags, has been successfully implemented in the clinic because it allows for the simultaneous isolation and activation of T cells from PBMC. Ex vivo expansion for a short period of time, typically around ten days, finally results in a T cell drug with both CD4 and CD8 phenotypes that exhibits early memory phenotypes and the ability to expand in patients' blood and generate long-term memory. Methods for isolating defined T cell subsets under good manufacturing practices (GMP) conditions have recently been developed in order to control the phenotype of transferred T cells better.They demonstrated superior efficiency in a murine lymphoma tumor model using a CAR T cell formulation consisting of CD4 T cells obtained from the naive CD4 T cell pool and CD8 T cells obtained from central memory CD8 T cells at a 1:1 ratio, in comparison to the unselected batch T cells and CD8 or CD4 cells only. Memory stem T cells, ICOS costimulated Th17-polarized T cells, and virus-specific memory T cells have all piqued the interest of researchers as viable T cell populations with high replicative potential. The use of lymphodepletion chemotherapy in patients preceding T cell infusion is one factor that has been shown to influence T cell engraftment and proliferation. This preconditioning allows infused cells to grow, limits competition for homeostatic gamma chain cytokines IL-7 and IL-15, depletes regulatory T cells, and turns on the innate immune system. Finally, relapse with CD19-negative tumor cells following CAR T cell therapy is still a problem. Singletarget therapy may select for and result in the escape of variants, whereas targeting multiple antigens on tumors increases the likelihood of therapeutic efficiency. Combining CARs with different specificities or using bispecific tandem CARs, which join two antigen recognition moieties, may help 03to prevent relapses due to variant escape, but more research is needed.While solid tumors have been largely resistant to T cell therapy, promising preclinical and clinical data to support further research. Solid tumors are difficult to treat because their microenvironment is hostile, causing T cell anergy. Some of the technological advances required to improve CAR T cell function include strategies to increase T cell traffic, T cell resistance

to an immunosuppressive environment, and the recruitment of other immune effectors and survival in solid tumors. T cells must survive and withstand an environment characterized by oxidative stress and hypoxia, the existence of suppressive immune cells and aspects, and T cell-intrinsic negative regulatory mechanisms such as inhibitory receptor upregulation.CAR design has been modified to produce "Trucks" (T cells redirected to universal cytokine-mediated killing) and "Armored CARs" these express cytokines and chemokine receptors and have recently been modified to coexpress catalase to protect T cells from oxidative stress-mediated repression and heparanase to improve T cell penetration through tumor stroma and infiltration. Combining ATC with checkpoint inhibitor blockade using antagonistic antibodies against the negative regulators CTLA-4 and PD1/PD1-L has also been proposed, and it has been demonstrated that specific blockade of the PD-1 immunosuppressive pathway significantly improved the function of HER2 redirected CAR-expressing T cells, resulting in improved tumor eradication in immunocompetent HER2 trajectories. Recently, studies have shown that T cells with genes modified with fusion receptors containing the extracellular domain of PD-1 connected to the cytoplasmic domain of CD28 have a promising potential for reversing the inhibitory effects of PD-1 binding.

FACTORS THAT AFFECT SAFETY OF CAR T CELL THERAPY:

Given the extreme potency of CAR-modified T cells, the use of this therapy carries a high risk of toxicity. Toxicities range from potentially fatal cytokine release syndromes (CRS) and macrophage activation syndromes (MAS) to off-target toxicity, neurotoxicity, and tumor lysis syndrome (TLS). CRS and neurotoxicity appear to be common in B-cell malignancies, but they are usually treatable and reversible. CRS is connected with elevated circulating levels of several cytokines, including interleukin-6 (IL-6) and interferon-, and appears to be associated with high antitumor activity and tumor burden. CRS is usually accompanied by MAS, which may be exacerbated by elevated IL-6 levels. Tocilizumab, a monoclonal antibody that restricts the action of IL-6 and reduces inflammation, can be used to treat both CRS and MAS. The mechanisms underlying neurologic symptoms such as aphasia, tremor, and seizures are still unknown; however, it has been reported that MAS can cause neurological toxicity. Off-target toxicity of CAR-modified T cells was first reported in a phase-I clinical trial of patients with renal cell carcinoma treated with T cells expressing a CAR recognizing carbonic anhydrase IX (CAIX). Several patients in this study experienced significant liver toxicity as a result of CAIX expression on normal bile duct epithelium, necessitating treatment discontinuation. The first fatal adverse event caused by off-tumor recognition by a CAR occurred in a colorectal cancer patient who was treated with a high number of T cells expressing a third-generation CAR targeting ERBB2/HER2. Shortly after the T cell transfer, the patient experienced respiratory distress and cardiac arrests and died five days later from multisystem organ failure.The CAR T cells were thought to have recognized ERBB2 expressed at low levels in the lung epithelium, resulting in pulmonary toxicity and a cascading cytokine storm with a fatal outcome. Predicted off-target toxicity with depletion of normal B-cells has been reported in nearly all patients treated with CD19 CAR T cells, and B-cell aplasia can last months to years depending on the CAR configuration. Patients receive monthly immunoglobulin replacement to mitigate this toxicity; however, long-term follow-up is required to assess the long-term effects of B-cell aplasia.Because few CARs are truly tumor-specific and recognize both normal and malignant cells, strategies to improve specificity are necessary. Affinity-tuned CARs with low-affinity scFv recognition have been shown to improve tumor specificity for overexpressed targets when compared to normal tissues expressing the same target at physiological levels. Various dual targeting strategies have also been developed to improve specificity and safety. One strategy involves T cells that have been modified with two different CARs, with CAR number one providing the CD3 signal and initiating killing and CAR number two transmitting the costimulation signal. CAR T cell activation and function are fully realized when the T cell is activated by both CAR antigens. Furthermore, in preclinical mouse models, inhibitory

CARs (iCARs) that harness natural T cell inhibition exerted by PD-1 and CTLA-4 have been shown to protect normal tissue from off-target effects. The CAR T cell's inhibitory function is the result of checkpoint inhibition initiated as a reaction to an antigen found on typical tissue but not on the tumor.Other approaches rely on switchable CARs (sCARs) and multichain CARs (mcCARs), which are only activated in thepresence of intermediate switch molecules.While the sCAR design is based on the co-infusion of antibody-based switch molecules that connect the target cell and the sCAR-expressing T cell, mcCARs are only fully activated in the presence of a small-molecule drug, such as rapamycin. In an immunocompetent mouse model of CD19 targeting, the switch approach was used to achieve reversible control of sCAR T cell activity. The sCARs principle also allows for the simultaneous targeting of multiple tumor antigens bysimply infusing switch molecules with two or more specificities, such as CD19 and CD22. The severity of chronic toxicities can be reduced by incorporating suicide genes into the CAR gene transfer vector or allowing surface coexpression of binding epitopes for depleting antibodies already in clinical use, such as EGFR and CD20. Other methods rely on self-limiting, transiently expressed CARs, or the administration of blocking antibodies and steroids. Finally, integrating vectors used to facilitate CAR gene transfer into T cells may pose a clinical risk due to the theoretical possibility of insertional mutagenesis, as demonstrated in stem cell gene therapy studies in primary immunodeficiencies. Despite the fact that numerous studies with over 500 patient-year follow-ups have demonstrated the safety of retroviral gene transfer into mature T cells, it is still too early to draw any conclusions.

FAILURE OF CAR-T THERAPY:

Most cancer treatments have failed or relapsed, and CAR T cell therapy is no exception, as individual immunity and comorbid conditions differ across cohorts. Understanding these events are the next step in improving the efficacy of this therapy.Long-term survival studies in CAR T cell therapy have revealed cases of disease relapse within one year of treatment. In a rare instance, one patient who initially did not respond to therapy achieved complete remission after the clonal evolution of one of the CAR T cell clones with a hypomorphic mutation in one of its tumor suppressor genes. On the contrary, a relapsed case of B cell acute lymphoblastic Leukemia with aberrant myeloperoxidase expression after CAR T cell therapy was reported. These findings highlight the importance of conducting mechanistic studies on CAR T cell therapy with more cases in order to understand the altered gene expression exhibiting two opposing phenomena- one remission and the other relapse after therapy.To obtain a complete picture of the events that occur during failure and relapses, the strategies used by cancer cells to avoid CAR T cells require special attention. In general, tumor cells escape through lineage switching, loss of tumor antigens, such as CD 19, or epitope hiding from recognition, immunomodulation of host immune cells to avoid detection, T cell exhaustion, and epigenomic landscape modulation. Lineage markers, including myeloid conversion, are seen in patients following CD19 CAR therapy in murine adult acute lymphoblastic leukemia models afterthe long-term effects of CD19 CAR-T cells. In addition, a CD19-negative myeloid phenotype is responsible for the immune escape of mixed lineage leukemia (MLL) from CD19 CAR-T-cell therapy.

SUMMARY:

Chimeric antigen receptor T cell (CAR T) therapy has emerged as a potential cancer treatment in recent years. CAR-T cell therapy is considered to be an appropriate cure for relapsed or refractory tumors. This is an experimental method of gene therapy that reprogrammes T lymphocytes to attack and destroy cancer cells. CAR-T is a concept in which CARs on genetically modified T cells are used to attack cancerous cells. It has been dubbed the most cutting-edge path to cancer care.

At the start of the operation, leukocytes are obtained from the patient through leukapheresis or apheresis. It may take up to 4 hours to ensure that there are adequate T cells.

The patient takes preconditioning chemotherapy known as "lymphodepletion" in the days leading up to the infusion treatment. CAR T cells can then disperse across the body, attacking specific cells. If their doctor agrees, patients must remain at the treatment center for four weeks before going home. Patients undergoing CAR T cell therapy are carefully watched by their care staff for severe, life-threatening, or lethal adverse effects. The duration of the medical stay would be dictated by how much the patient responds to the medication. The treatment is normally completed in an hour or less.

The Aim of CAR-T Cell Therapy Prior treatments would have involved an anti-CD20 antibody and an anthracycline if the patient relapsed within 12 months of undergoing an autologous stem cell transplant. Patients who have undergone a refractory, second, or subsequent relapse are qualified.

CAR T Cell Therapy's Side Effects Patients should be aware of two highly harmful side effects: Cytokine Release Syndrome (CRS) and Neurotoxicity. Patients should be aware of two highly harmful side effects: Cytokine Release Syndrome (CRS) and Neurotoxicity. The architecture of the vehicle, the composition of the infused T cells, as well as the tumor type and microenvironment, all have an effect.

The majority of second-generation CARs now in clinical trials have either CD28 or 4-1BB signaling domains. Core memory T cells, which have longer telomeres and higher proliferation than more distinct effector T cell species, have long-term survival and activity. The use of lymphodepletion chemotherapy in patients prior to T cell infusion has been shown to cause T cell engraftment and proliferation. Although single-target therapy may select for and cause variants to escape, targeting numerous antigens on tumors increases the likelihood of therapeutic efficacy. Relapse with CD19-negative tumor cells after CAR T cell therapy is still a problem.

As a result of car design improvements, "Trucks" (T cells redirected to universal cytokine-mediated killing) and "Armored CARs" have been produced. Because of the high efficacy of CAR-modified T cells, using this procedure carries a high risk of toxicity. Cytokine release syndromes (CRS), off-target toxicity, neurotoxicity, and tumor lysis syndrome are also potentially fatal toxins (TLS) The mechanisms behind neurologic conditions such as aphasia, tremor, and epilepsy are also unknown.

The first death caused by CAR off-tumor identification occurred in a colorectal cancer patient contaminated with a significant number of T cells expressing a third-generation CAR targeting ERBB2/HER2. The woman died five days later from multisystem organ failure. Few CARs are fully tumor-specific, distinguishing both healthy and malignant cells, necessitating the development of techniques to improve precision. Affinity-tuned CARs with low-affinity scFv recognition have been shown to improve tumor sensitivity for overexpressed targets.

Long-term CAR T cell therapy survival studies have revealed cases of disease relapse following one year of treatment. In one instance, during the clonal evolution of one of the CAR T cells with a hypomorphic mutation in one of its tumor suppressor genes, a patient who had previously not responded to therapy underwent complete remission. On the other side, a relapsed case of B cell acute lymphoblastic leukemia with aberrant myeloperoxidase expression after CAR Tcell therapy was recorded. Understanding these incidents is the next step toward enhancing the treatment's efficacy.

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