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NAVIGATING THE COMPLEXITY: CHALLENGES AND ADVANCEMENTS IN CAR T-CELL THERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT
Chimeric antigen receptor (CAR) T cell therapy is a groundbreaking treatment that involves modifying a patient's T cells to target tumors, with notable success in hematologic malignancies such as non-Hodgkin lymphomas and acute lymphoblastic leukemia (ALL). This approach engineers T cells with CARs, enabling them to specifically identify and destroy cancer cells. While CAR T cell therapy has been highly effective against blood cancers, its application to solid tumors faces significant challenges. The therapy shows promise but is also associated with serious limitations, including cytokine release syndrome (CRS), neurotoxicity, and high costs, which restrict its accessibility. Ongoing research focuses on enhancing efficacy, minimizing side effects, and broadening the therapy's applicability to various cancers through combination treatments, innovative CAR designs, and improved manufacturing processes. The goal is to make CAR T cell therapy more effective and accessible to a wider range of patients.

KEYWORDS: Car T cell, Lymphoma, Leukemia, Acute lymphoblastic leukemia(ALL), **C**ar-Tcell biomarkers, Car T cell infusion.

INTRODUCTION

Cell Therapy by CAR T cells

"Chimeric antigen receptor" (CAR) T cell therapy is a novel manufacturing process for therapeutic T cells that involves modifying patient-derived T cells in vitro to develop manufactured receptors specific to a tumor. Remission rates for hematologic malignancies have decreased by 80% in the past several years because of this medication. Particularly beneficial outcomes have been shown in the management of non-Hodgkin lymphomas, like large lymphoma B cell and acute lymphoblastic leukemia (ALL).[1] While there is value in employing CAR T cells to treat solid tumors because of these promoting advantages, it has been difficult to consistently demonstrate clinical effectiveness in this context yet.[2]

A chimeric antigen receptor (CAR) can be expressed on T cells, enabling them to be genetically altered to specifically target malignancies. T-cell receptors that have high affinity towards genetically altered T cells & are only relevant to a diverse patient population when they present antigens that match that of human leukocytes. In addition, they may react with a variety of typical antigens as they identify small peptide epitopes.[3]

T-cells with CAR have specific epitopes which is immunotherapy targeted for cancer cells which was the lentivirus-mediated conversion of self-produced t cells and it is one of the first human gene therapy techniques that has wide therapeutic effectiveness. Less precise and less successful methods of boosting antitumor immunity have been employed in previous gene therapy experiments.[4]

Acute lymphoblastic leukemia

“ALL (Acute lymphoblastic leukemia) is an especially prevalent illness among kids and one of the leading causes of morbidity and mortality, even though it has a cure rate of over 90Over the last ten years, there has been notable progress in our comprehension of the genetic foundation of leukemogenesis and the reaction to treatment in patients with ALL. “ALL can be divided into several subgroups, each of which is characterized by a unique set of somatic structural DNA rearrangements and sequence abnormalities that generally affect

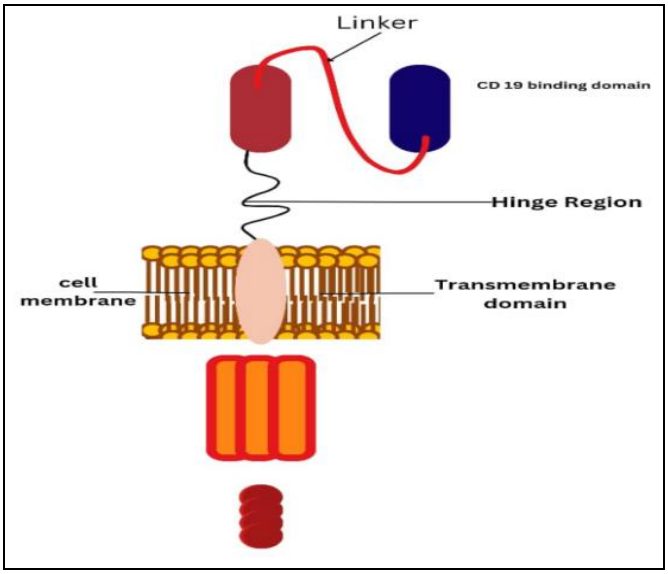
lymphoid development, cytokine receptors, tumor suppression, kinase and Ras signaling, and chromatin modification. Recent studies have improved our understanding of the genetic foundations of clonal formation and relapse as well as the function of inherited genetic polymorphisms in leukemogenesis".[5]

T-CELL THERAPY'S IMPACT ON ACUTE LYMPHOBLASTIC LEUKEMIA

STRUCTURE OF CAR T CELL

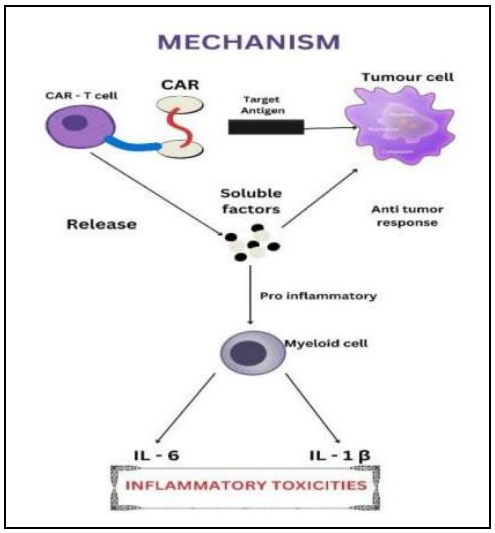
In general terms, CARs consist of four domains or regions:

- the hinge region,
- the intracellular domain of T cell signaling,
- the transmembrane as well as antigen recognition.[6]



Mechanism OF The CAR T Cell Therapy[7]

When cells of car T recognize a specific antigen, they produce soluble components in response. Myeloid cells can be triggered or anti-tumor responses can be supported by substances that are soluble. Pro-inflammatory cytokines like IL-6 and IL-1b are released by activated myeloid cells as a result, which has developed inflammatory toxicities in CAR-T cell recipients' patients.



Limitations

Antigen release

CAR-T cell therapies are targeting multiple antigens, such as CD19 and CD22 for ALL and DLBCL and CD19 and BCMA for multiple myeloma. The benefits in terms of efficiency are evident from the initial findings of clinical trials. CARs have demonstrated superior efficacy in solid tumors as compared to single antigen therapies in HER2/MUC1 (breast cancer) and HER2/IL13Ra2 (glioblastoma) anti-tumor responses.

Effects of on-target off-tumor

The major focus has been on the post-translational changes unique to tumors. Four main targets have been investigated with CAR-T cell therapy: TAG72, B7-H3, MUC1, and MUC16.

Mobility in CAR-T cells and tumor invasion

As compared to the systemic distribution of CAR-T cells in clinical therapy, direct injection locally—such as intrapleural for mesothelioma and intraventricular for glioblastoma/brain tumors—showed higher therapeutic success. The effectiveness of antitumor trafficking is improved through the expression of CXCR1/CXCR2 in CAR-T cells or Integrin αvβ6-CAR-T cells that have been modified to express CXCR2. These CAR-T cells have chemokine receptors on them, and they bind to and respond to chemokines generated by tumors.

Optimizing CAR-T cells' ability to traverse physical barriers (tumor stroma): Research has demonstrated that CAR-T cells producing heparanase or fibroblast stimulating protein have enhanced antitumor effectiveness and infiltration.

Immunosuppressive microenvironment

Checkpoint reduction paired with CAR-T cells is called combination immunotherapy. Hematological malignancies outcomes improved and B-ALL patients who received concurrent treatment with CD19 CAR-T cells & PD-1 inhibition showed an improvement in the durability of CAR-T cells. For solid tumors, a plethora of research is now evaluating combination therapy.

Synthesizing CAR-T cells that generate the immunostimulatory signals known as cytokines, or CARs tolerant to immunosuppressive drugs. Research has demonstrated that the creation of CARs to produce immunostimulatory signals is associated with increased survival, proliferation, and anticancer activity. IL-12 secretion, IL-15 synthesis, and immunosuppressive cytokine rerouting (e.g., IL-4) have all contributed to this. By avoiding TGF-β-mediated inhibitory signals, it has been demonstrated that it is feasible to produce CARs that are immune-suppressive factor-resistant, even in the unfavorable tumor microenvironment.

Consequences of using CAR-T cells

"The reactivity of the CAR antigen-binding domain has been decreased to a micromolar level by altering the structure of the protein in order to minimize toxicity. The discharge of cytokines can be regulated through changes to the transmembrane and CAR hinge. Numerous factors, including the target antigen-antigen binding domain pair, antigen density, cancer type, tumor load, and potential toxicity, are taken into account when customizing the costimulatory domain. By using human or modified antibody fragments rather than murine-derived CARs, CAR immunogenicity can be decreased.

When lenzilumab is used to inhibit the cytokine GM-CSF, which encourages macrophages and monocytes, T cell modification, neurological injury caused by CAR CRS, and neurotoxicity decrease, whereas CAR-T cell activity is improved. An aspect of neuroinflammation can be decreased in mouse models of leukemia/lymphoma by IL-1 receptor antagonists".[8]

DIFFICULTIES WITH EVERYONE'S CAR T-CELL THERAPY

Access Issue Treatment availability is limited because patients cannot afford the high manufacturing costs.

Eligibility Barrier Enrollment is restricted by certain requirements for admission or exclusion.

Severe Side Effects Pyroptosis and neurotoxicity are only a couple of the severe side effects that might result from CAR T-cell treatment.

Primary Resistance Primary resistance is the inability to reach remission following infusion.

Types of Relapse Relapses that are Antigen-positive when target antigen is present, and Relapses that are Antigen-negative when antigen expression is minimal or nonexistent.[9]

Excluded Before Infusion Patients may still be disqualified from clinical trials prior to infusion if they get an infection, their illness worsens, or their production fails. Patients typically have lymphodepletion following apheresis, which leaves them susceptible to infection. As a rescue strategy, enrolled patients are often in poor health since ALL advances quickly. They therefore run the risk of developing infections or experiencing illness worsening while the product is being manufactured. However, there is a possibility of a manufacturing error.[10,11] Because of the development of largely positive experiences and the suggestion of production standards, the danger of production failure brought on by advancements in manufacturing technology and transportation is steadily declining.[12,13]

RECENT ADVANCES AND STRATEGIES

To get a better and longer-lasting result, combination treatments that can lower drug resistance and increase medication efficacy should be used. studies are being carried out to evaluate the effectiveness of different drug combinations in frontline and relapse prevention environments. The combined effects of these two drugs on apoptosis were demonstrated by the findings of a phase 2 monocentric trial, which was reported by Short et al. at ASH 2021. Bonatumomab stimulates an anticancer response against B cells that express CD19, whereas ponatinib inhibits BCR-ABL kinases.[14]

Novel car design and targets

Ninety-nine studies for CAR-T treatments in ALL were enrolled or ongoing as of April 2020 on clinicaltrials.gov. These studies cover targets other than CD19, such as CD22 and CD38, in addition to CAR-T therapies for ALL. While T cell-directed CAR-T products could be constrained by the toxicity of T cell depletion, methods such as transplanting CD7-edited CARs or using other similar approaches with a "kill-switch" to eliminate the CAR-T cells during remission are being investigated.[15]

Venetoclax is a BH3-mimetic that inhibits BCL2. Preclinical research has demonstrated BCL-2 dependency in ALL cell lines, which prompted research into this agent's potential for treating R/R ALL. Early results indicate that it could have some positive effects when used with lower-intensity regimens like hyper-CVD, but more research is required. But in general, it's still important to target the apoptotic machinery in ALL, which includes BCL-2, CDK-9, MCL-1, and other proteins.[16]

Access initiatives and affordability programs for all

Over the past 50 years, pediatric acute lymphoblastic leukemia (ALL) has seen significant improvements due to improved supportive care, efficient delivery of traditional chemotherapeutic medicines, and new diagnostic technology. The goal is to increase survival rates to 100% and reduce side effects, as high-income nations now have above 90% 5-year survival rates.[17]

Biomarkers for predicting response in acute lymphoblastic leukemia

The hematologic malignancy known as precursor B cells acute lymphoblastic leukemia (B-ALL) is characterized by aberrant B-cell precursor formation in the bone marrow. With a nearly 90% long-term survival rate for newly diagnosed patients, B-ALL has become the most curable malignancy in children due to advancements in biology, prognostic and predictive biomarkers, and tailored risk-adjusted treatment. But among young individuals and adolescents, returned B-ALL remains the most common cause of mortality associated with cancer. White blood cell count, early treatment response, chromosomal abnormalities, and age are the factors that go into risk classification. Recent molecular diagnostic technology has led to the discovery of genetic biomarkers that can improve risk prediction, treatment, and clinical outcomes.[18]

DIFFICULTIES FOLLOWING FROM THERAPY of CAR-T CELL

T-cell therapy combined with CAR (chimeric antigen receptor) targeting is a novel way to treat cancer. T-cell therapy is a cutting-edge method of treating cancer. However, there have also been other adverse events (AEs) linked to CAR T-cell therapies. These include cytokine release syndrome (CRS) and neurologic issues (previously known as immune effector cell-associated neurotoxicity syndrome [ICANS] or, more recently, CAR T-cell-related encephalopathy syndrome [CRES]). With the right treatment approaches, these side effects are manageable and even reversible; nevertheless, if neglected, they may worsen.[19]

CYTOKINE RELEASE SYNDROME

After CAR-T cell injection, CRS is the most often reported adverse event. The four primary signs and symptoms of CRS are end-organ toxicity, fever, hypotension, and hypoxia. Chronic rejection syndrome (CRS) is a potentially lethal immunological response that has positive feedback between immune system cells and cytokines.

Following CAR-T therapy, the cytokines associated with CRS are produced directly by CAR-T cells upon activation through engagement with the target antigen, as well as by macrophages/monocytes and dendritic cells.[20]

As CAR T cells become activated and undergo proliferation, they release cytokines, which in turn activate and stimulate other immune cells like macrophages and endothelial cells.[21] Among the cytokines that have been identified to be raised include TNF-α, interferon-gamma, granulocyte-macrophage–colony-stimulating factor (GM-CSF), interleukin-2, interleukin-6 (IL-6), and interleukin-8.[22]

NEUROLOGICAL TOXICITIES

ICANS, or immune effector cell-associated neurologic syndrome, is the name given to the abrupt development of neurological toxicities that occur eight weeks after the last two weeks of CAR infusions. The second most common adverse event, also known as ICANS, can occur prior to or subsequent to CRS.[23]

ICANS, or immune effector cell-associated neurotoxicity syndrome, is characterized by neurological symptoms such as disorientation., encephalopathy, deafness, lethargy, agitation, tremors, seizures, and, in rare cases, cerebral edema. Furthermore, even though they are rather prevalent, headaches might not always indicate neurotoxicity.[24]

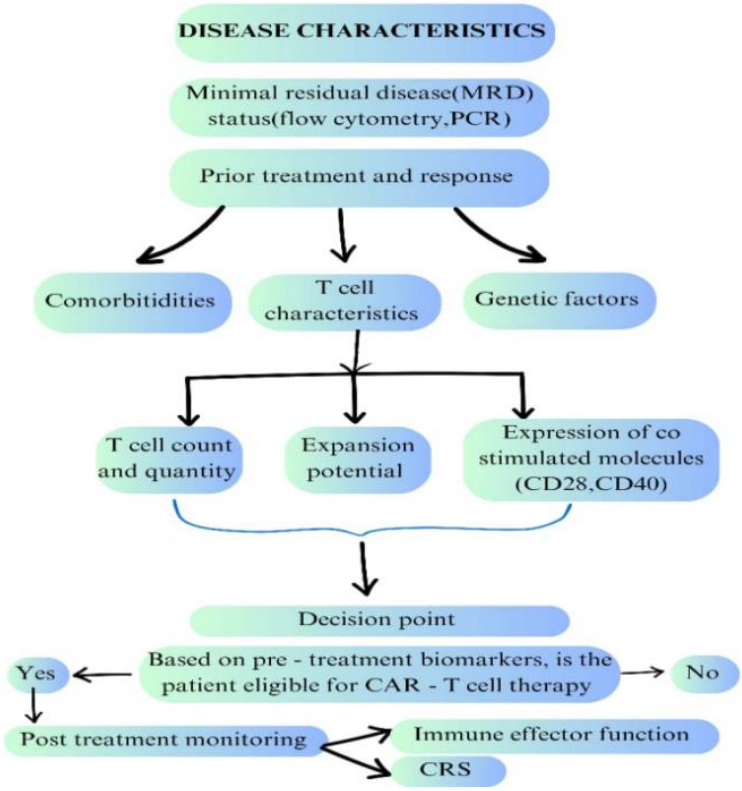
Although the exact mechanisms of neurotoxicity are still not fully known, evidence from studies on animals and patient cases points to a potential disturbance of the blood-brain barrier. This impairment is linked to higher levels of cytokines in the blood and brain fluid and endothelial activation. Neurotoxicity is now treated with corticosteroids, interleukin-6-targeted treatments, along with supportive care. However, there isn't much conclusive evidence of their effectiveness.[25]

ROLE OF CAR-T CELL BIOMARKERS

Maintaining a significant therapeutic result and a long-lasting remission requires an understanding of CAR-T cell activity. According to research, immunological barriers such as PD-1, TIM-3, and LAG-3, as well as the immune microenvironment, can all have a significant impact on the ability of CAR-T cells to effectively destroy tumors, proliferate, and endure over time.[26]

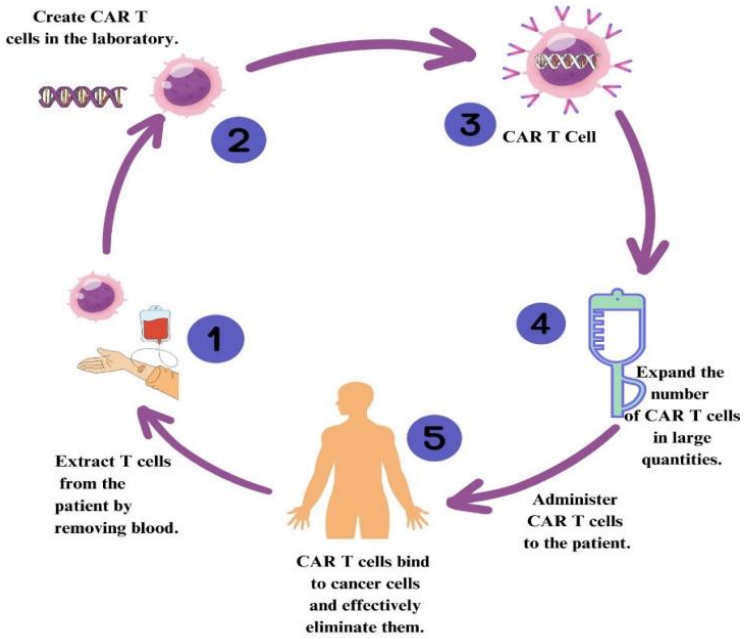
When compared to other memory T cell subsets, TSCM (T memory stem cells) exhibit a higher degree of stem cell-like characteristics. As such, CAR-T cells that originate from less differentiated T cell subsets that are stem-like and naïve may exhibit higher rates of proliferation and longer survival. Marianna S. et al. used a mouse model of B-ALL to investigate the efficacy of CD8+ TSCM cells transformed with CD19 CAR. Their

findings showed that these CAR-T cell products produced better survival rates and evoked a longer antitumor response when compared to CD8plus T cells originating from different T cell subsets.[27]



CAR T CELL MANUFACTURING

An identical procedure was used in the production of the CAR-T cells. This method comprises sourcing and evaluating T-cells and their selection, large-scale development, genetic alteration using a CAR cDNA as well as final composition to produce CAR-T cells. To ensure the product's integrity the production process and quality control release testing are tightly related.[28]



PRE-TREATMENT LYMPHODEPLETION

The effectiveness of lymphodepletion in enhancing the antitumor responses of transferred CD8+ T cells may be attributed to several factors, including homeostatic expansion and T cell activation. Additionally, experiments suggest that lymphodepletion improves antitumor efficacy by reducing competition for antigen-presenting cell surfaces. Regulatory T cells, crucial for maintaining tolerance to self and tumor antigens, are also believed to be pivotal in this context, their depletion being considered a significant mechanism contributing to lymphodepletion's effectiveness.[29]

CYCLOPHOSPHAMIDE WITH OR WITHOUT FLUDARABINE

Cyclophosphamide and fludarabine, at dosages and times tailored to individual clinical trials and CAR-T cell products, are the mainstays of the usual lymphodepletion chemotherapy therapy. Higher degrees of lymphodepletion have also been associated with a degradation of cytokine release syndrome (CRS), in addition to the duration of low blood cell counts and the degree of chemotherapy.[30]

A treatment plan that started with cyclophosphamide and fludarabine and was followed by CD19 CAR-T cell therapy on the fifth day showed greater efficacy in terms of tumor reduction in size and survival rates.[31]

It is common to observe variations in the dosages of Fludarabine and Cyclophosphamide (ranging from 25/250 mg/m2 to 30/750 mg/m2), the duration of their administration (3 to 5 days for Fludarabine and 1 to 3 days for Cyclophosphamide), and the interval between their administration and CAR-T cell transfusion (from 1 day up to 14 days).[32]

Tisagenlecleucel (KYMRIAH®, Novartis Pharmaceuticals Corporation) is a newly FDA-approved immunotherapy for patients under 25 years old with refractory B cell ALL or those experiencing their second or subsequent relapse, providing a novel treatment choice for this demographic.[33,34]

CAR T CELL INFUSION

TISAGENLECLEUCEL

An immunotherapy called tirandeleasecleucel genetically modifies T cells to target CD19. Autologous T cell lines are altered via a lentiviral vector to enable the expression of a CAR (chimeric antigen receptor) that targets CD19. Human CD8 hinge and transmembrane segments, intracellular signaling domains from 4-1BB (CD137), and CD3 zeta are all combined into a single protein to form this CAR. and a murine mAb-derived single-chain fragment (scFv) specific for CD19. By sticking to CD19-presenting cells and initiating a signaling cascade, the CAR promotes T-cell proliferation, activation, and elimination of target cells.[35]

MANAGEMENT OF CRS

IL-6 has become increasingly recognized as a crucial cytokine that exacerbates the development of cytokine release syndrome (CRS) in therapies involving T cells. Recent research in both experimental models and clinical trials has revealed an increasing awareness of its significance in the pathophysiology of CAR T cell treatments.[37]

TOCILIZUMAB

Tocilizumab has been approved by the FDA to treat severe or potentially fatal CRS (cytokine release syndrome) in adults and children two years of age and older as a result of CAR T-cell therapy. By competing with IL-6 to bind to its receptor, IL-6R, tocilizumab, a monoclonal antibody, inhibits IL-6 signalling in effector cells. When IL-6 receptors are attached to either the membrane-bound (traditional signalling) or the soluble (sIL-6R, trans-signaling) IL-6 receptor, the JAK/STAT system is stimulated.[38]

CORTICOSTERIODS

Clinical observations have shown that corticosteroids effectively treat CRS, and a quick tapering of steroids over several days typically prevents CRS from recurring.

Standard beginning doses for corticosteroids often start with methylprednisolone at two milligrams/kilogram/day and can be tapered gradually over a few days, however, each patient should have their own customized dosage and selection. In patients with severe neurological symptoms, dexamethasone at 0.5 mg/kg (maximum 10 mg/dosage) may be taken into consideration because of its improved blood-brain barrier crossing capacity. However, As of this now, there is insufficient data to prove that dexamethasone and methylprednisolone function differently in this circumstance.[39]

MANAGEMENT OF NEUROLOGIC TOXICITIES (NEUROTOXICITY OR ICANS)

Dexamethasone and methylprednisolone are the two corticosteroids most frequently employed in this situation, with dexamethasone potentially favored due to its superior ability to penetrate the central nervous system.

The ideal standard dosage of corticosteroids remains undefined; however, past literature has suggested administering intravenous dexamethasone at 10 mg every 6 hours alongside supportive care for managing neurological toxicity.[40]

ANTI SEIZURE MEDICATION

Seizures are treated with antiepileptic medications such as levetiracetam, phenobarbital. Benzodiazepines may be employed for immediate seizure control when needed. Neurological toxicity is generally addressed with supportive care and diagnostic assessments including electroencephalogram to detect electrical seizures, along with brain imaging to check for cerebral edema. Many

centers initiate prophylactic levetiracetam on the infusion day for CAR T products with a high incidence of ICANS, while others start it after ICANS symptoms begin.[41]

Levetiracetam, at a dosage of 750 mg twice day, was administered to most patients as a prophylactic against seizures before to the development of neurotoxicity or at the beginning of early neurological symptoms.[42]

POST TREATMENT FOLLOW UP

Following infusion, evaluations have to include standard bone marrow aspirate and trephine biopsy analyses, enhanced, based on institutional capacities, by either multiparameter flow cytometry (MFC) or molecular measurable residual disease (MRD) testing.[43] For those who are overweight (Actual weight > 20% of optimum weight or BMI > 30 kg/m²), the dose should be modified in accordance with the ideal adjusted weight, which may be calculated using the method below

Ideal adjusted weight = Ideal weight + 0.4 x (real weight - ideal weight).[44]

Patients with fibrinogen levels, fibrinogen replacement therapy (FFP), or severe CRS should have their fibrinogen levels constantly checked for elevated fibrinogen levels, PT or INR, and aPTT, as required by their physician. Checking fibrinogen levels often, ideally daily, was advised while administering fibrinogen replacement for severe bleeding or hypofibrinogenemia. After starting the fibrinogen replacement infusion, the first check should be completed within 30 minutes.[45]

CAR T CELL THERAPY'S FUTURE

Nowadays, T cells that have undergone permanent modification to produce CD19 CARs are the main focus of clinical experience with chimeric antigen receptor therapy for leukemia. However, targeting a variety of antigens is theoretically feasible. Research is looking for novel strategies to get beyond resistance and broaden the application of cellular immunotherapy to treat other kinds of cancer. By altering the process of introducing genetic information into cells as well as the domain that recognizes antigens, therapeutic efficacy may be increased and adverse effects can be decreased.[46]

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