The Libyan International Medical University

Faculty of Basic Medical Science

Chimeric Antigen-Receptor T Cells Immunotherapy

(CAR T Cells Immunotherapy)

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Abstract:

Cancer has been identified as the most common cause of death worldwide. Throughout the past years, numerous traditional treatments and cytotoxic immunotherapies for neoplastic diseases have been developed. Considering the behavior of the tumors and the involvement of several genetic and cellular factors and basis in the growth of the tumor and its metastasis, there must be a promising immunotherapy to target the tumor at genetic and cellular levels. One of these immunotherapies is the adoptive T cell transfer (ACT), this report will cover one type of this method, which is CAR T cells, including components, materials used to initiate the treatment, mechanism of action, and its side effects.

Introduction :

Researches have shown clinically effective approaches to cancer immunotherapy, for example monoclonal antibodies directed against CTLA-4 and PD-1, which are inhibitory to costimulatory response of the T cells, and antibodies against these proteins block their inhibitory response[(1)]. And by doing complicated ex vivo culture and cellular engineering approaches to adoptive T cell transfer (ACT), which is another form of immunotherapy[(2)].

ACT is a new branch of transfusion medicine that involves the infusion of lymphocytes to express antitumor, antiviral, or anti-inflammatory effects. Three forms of ACT are being developed as cancer therapy; including tumor-infiltrating lymphocytes (TILs), T cell receptor (TCR) T cells, and CAR T cells[(2)].

In CAR t cells immunotherapy, T cells are redirected to certain antigenic targets by cellular engineered chimeric antigen-receptors (CARs), which are synthetic receptors that target antigens in their native conformation without the involvement of MHC, unlike TCRs[(3)]. CAR T cells have been identified as powerful therapies and have shown positive results in remission of hematologic malignancies, such as B cell malignancies and acute lymphoblastic leukemia (ALL)[(1)(3)].

Materials and methods:

To produce CAR T cells, several carefully performed steps were required. First, leukapheresis was used to remove and separate WBCs from the patient's body and the return of the remainder blood to the circulation again. After a sufficient number of leukocytes was obtained, the leukapheresis product was enriched for T cells. Then the cells were washed out of the leukapheresis buffer. Enrichment of lymphocytes can be achieved subsequently by counterflow centrifugal elutriation, that separates the cells by size and density and maintains their viability. T cells were separated at the level of CD8/CD4 composition using specific antibodies markers [(4)].

The autologous antigen-presenting cells (APCs) were purified from the patient to be used for T cells activation. During the activation process, the T cells are incubated with the viral vector encoding the CAR, after few days the vector was washed out of

the culture by dilution. The viral vector uses viral mechanism to attach and enter to the patient cells, then genetic material was introduced to the patient cells in the form of RNA encoding the CAR. The RNA was reverse-transcribed into DNA and permanently integrated into the genome of patient's cells. Then the CAR was transcribed and translated by the patient's cells and finally expressed on the cell surface [(4)].

Results:

CAR T cells immunotherapy has demonstrated clinical efficacy in hematologic malignancies, such as B cell malignancies and ALL.

Discussion:

CAR contains antigen-binding domains, a single-chain variable fragments (scFv) that are derived from the variable domains of antibodies with the signaling domains of the TCRs and additional costimulatory domains from receptors [(2)]. CARs have much higher and broader range of affinity than the other immunotherapies that will engage the target without necessarily causing cross-reactivity problems [(3)].

The results from initial procedures using first-generation of CARs in patients with different types of cancers were a bit disappointing, Therefore, second-generation was invented targeting CD19 and encoding costimulatory domains was said to be the lead type of engineered T cell therapies [(3)]. Many features make CD19 a good or ideal target for CARs. It is highly expressed in B cell malignancies, it is needed for B cells development, and it can't be found in other than B cell lineage, that's why patients treated with CD19 CARs usually develop B cells aplasia with preservation of plasma cells and the humoral immunity, but this can be managed by replacement therapy by intravenous globulin [(3)].

Early results from CAR T cell trials watching other targets have shown that the CD19 off-tumors cross-reactions doesn't happen to a single target, but may occur in other lineage-dependent targets. Multiple myeloma, which expresses lower levels of

CD19, has shown a positive response to CD19 CAR T cell therapy as one of the examples of the off-tumor cross-reactions of this therapy. Another example is in trials of CARs targeting B cell maturation antigen (BCMA) in advanced myeloma, the non-malignant plasma cells that also express BCMA are affected along with malignant myeloma cells. The tolerability of off-tumor reaction depends on the types of non-cancerous cells that are targeted, this tolerability is the main aim of the recent studies and the development of new CARs generations [(3)].

Some adverse effects are expected to occur while using CARs, the most common of which is the onset of immune activation known as cytokine release syndrome (CRS). CRS was seen after the development of second generation of CAR design that have additional costimulatory signaling which translated to improved T cell activation, cytokine production, and therefore improved antitumor response. The hallmark of CRS is immune activation and elevated inflammatory cytokines. Another adverse effects are neurological toxicities, the causative pathophysiology of these problems is unknown. On-target\of-tumor problem is another adverse effect of CARs, and it is caused by shared expression of the target antigens on both pathogenic and non-pathogenic tissues. Other adverse effects include anaphylactic responses and graft versus host diseases [(5)].

Conclusion:

In conclusion, immunotherapies are the therapies used to prevent or to cure a specific disease using the individual's own immunity and the autologous cells by modifying them, as in CAR T cells immunotherapy where the autologous T cells are directed against some antigens located on tumors cells surfaces by synthetic receptors (CARs) to destroy these tumors by attacking their malignant cells. Although these modified or genetically engineered cells a range of mild to severe side effects, they have shown positive results in treating some types of cancer and reducing their remission.

References:

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