Bahrami, 2019. Journal of Genes and Cells, 5(1): p, 01-06
doi: 10.15562/gnc.67

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Mini-Review Article

Comparison of CAR-T Cells Engineering using CD19 and CRISPR/Cas9

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Submitted 01 January 2019 *Accepted 01 March 2019*

ABSTRACT

Cancer is the second largest cause of mortality in the world accounting more than 13% deaths. Currently, cancer is being treated by several hazardous approaches such as chemotherapy or radiotherapy and much attention has been seen in last decades on the development of novel targeted therapeutic approaches. Clinical data have suggested the engineering immunity as a one of the most promising approach in cancer treatment where engineering T cells has shown >99% efficiency to cure cancer. T-cells are being engineered for CD19 based chimeric antigen receptors by using lentiviral vectors and also for T-cell receptors using ribonucleoprotein based CRISPR/Cas9 gene editing tool. Activation of T-cells via engineering as immunotherapeutic cancer approach could create a new horizon of targeted therapeutic research in coming years and recent huge investment by global leading pharmaceutical companies in T-cell engineering have proven that a live cell-based drug in near future for cancer cure is not a myth anymore.

Key words: CAR-T Cells Engineering, CRISPR/Cas9, CD19, Chimeric Antigen Receptors, Lentiviral Vectors

Introduction

Cancer is defined as the abnormality of cells in which cells failed to follow the cell cycle checkpoint pathways and start dividing in an uncontrolled manner. Millions of cells are dying daily, and these cells are being replaced by newly divided cells to regulate body functions normally. Somewhere these cells skip the controlled and regulated division mechanism and start developing massive structures containing cancer cells called tumors by some unwanted mutations (1). As these

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mutations are making cells abnormal or cancerous, T cells of body are aimed to kill the cancerous or mutated cells whenever they developed in any part of the body. When the number of abnormal or cancer cells increased rapidly, these cells as a part of their survival mechanism, start to modulate T cell function and these modulated T cells failed to kill the cancerous cells allowing them to grow rapidly leading to metastasis (2). A number of therapeutic approaches such as radiotherapy, chemotherapy etc. are being applied in clinical practices to remove tumors or kill tumor cells (3). Understanding the signalling network of T cells activation and their compromised function in cancer cells identification have been proposed as a promising approach to develop a cell based targeted cancer therapeutic approach.

T-cell Activation and Signalling Pathways

Human immune defence system is a T-cells activation dependent mechanism which is accompanied by two signalling pathways (3);

1. **Antigen-dependent Signalling:** In this signalling pathway, T-cells activated when the cell surface receptors of T-cells (T-cell receptor or TCR) identify the antigenic peptide/major histocompatibility complex (MHC) and start apoptosis mechanism.

2. **Antigen-independent Signalling:** In this pathway, co-stimulation of signalling molecules on antigen-presenting cells (APCs) and T-cell receptors happen which activates the T-cell to enhance immunity and recognise infected cells and kill them.

CD19-CAR-T Cell Engineering

Adoptive transfer of T cells is the current most promising approach used to treat leukaemia as it is the genetically activation of allogenic T cells of patients which are engineered to identify and target cancer cells in the body (4). In this regard, T cells are engineered for CD19 based chimeric antigen receptors (CAR), CD19-CARs results in the activation of immunomodulated T cells and their introduction in patient's body have proved an ideal targeted cancer therapeutic agent. CARs are composed of single chain variable fragment (scFv) derived from a monoclonal antibody which is designed for a specific tumor cell-surface molecule for T-cell signalling and to activate T cell functions. Studies have shown that CD19-CARs and endogenous T-cell surface receptors (TCR) on effector T cells (T_E) demonstrate equal activation functions of T cells based on their functional analysis of cell signalling (5). This approach till now has been proven a promising approach to target different type of cancers as shown in table 1.

Table 1: Published reports of clinical trials using genetically redirected T-cells for cancer therapy

Sr. No.	Type of Cancer in Clinical Trials (CT)	No of patients	Year of CT report	Ref.
1.	AML	4	2013	(6)
2.	Colorectal and breast	27	2002, 2010, 2011	(7-10)
3.	Leukemia and lymphoma	106	2008, 2010, 2011, 2013	(11-17)
4.	Melanoma &sarcoma	142	2006, 2009, 2010, 2011, 2013	(18-24)
5.	Multiple myeloma	11	2012	(25)
6.	Neuroblastoma	25	2007, 2011	(26, 27)
7.	Ovarian	12	2006	(28)
8.	RCC	11	2011	(29, 30)
9.	Prostate	5	2013	(31)

CRISPR/Cas9 CAR-T Cell Engineering

The CRISPR/Cas9 is the most advanced form of a genome editing tool which has been used to edit many cell lines and several efforts are underway to edit human cells and primary tissues directly. This genome editing tool has a promising potential in cancer immunotherapies where cancer killing cells whose functions have been modulated or compromised by the advanced developed cancer and these cells are unable to identify and target cancer. This tool may help to edit the modulated T cells at genomic level and their functions can be restored and these cells can be transplanted again to the same patients for enhanced recovery (32). The concept of engineering patients own immune system to target cancer is no more a sci-fi hypothesis, but a near to be implemented as CRISPR has been used successfully to edit human cell lines and now the system is all set to edit T cells are their genomic level. Cas9 ribonucleoproteins (RNPs), an advanced version of CRISPR engineering, can act as a precise programmable tool to substitute mutated nucleotide sequences in the genome of a mature immune cell (i.e. primary T cell) but their efficiencies have to be determined in this regard (33). The comparative differences of CAR-T cell engineering using CD19 and CRISPR/Cas9 is showing in figure 1.

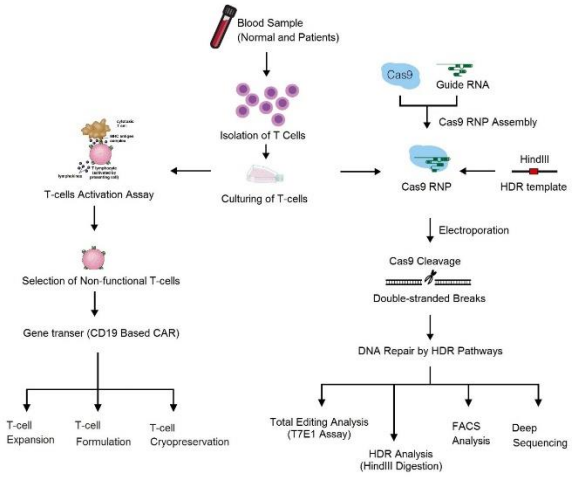

Figure 1: Comparative differences of CAR-T cell engineering (Isolation, engineering and analysis) using CD19 and CRISPR/Cas9 approaches.

Perspective of CAR-T Cell Engineering:

Immunotherapeutic approaches have always been considered the gold standard and huge research has been done in this discipline to target cancer. It has been shown that T cells isolated from the cancer patients can be trained and introduction of CAR on the T-cell surface enhances T cells function and their introduction in the patient's circulatory system results in the identification of cancerous cells and killing them (34). This approach has been known as the adoptive cell transfer or adoptive cell therapy (T cells) and has shown 99% promising results in >100 clinical trials done so far. Adoptive cell transfer which is also known as adoptive cell therapy, is the reactivation and transfer of modulated T cells of cancer patients (35). The manufacturing of cellular products under current good manufacturing practices (cGMPs) for commercial applications is under process by a number of pharmaceutical firms working for the large-scale production of clinical-grade cells (i.e. TILs, virus-specific and genetically modified CAR or TCR transduced T cells) to bring these cellular products in clinics (36). Yescarta™ was the first approved FDA approved drug as CAR-T cell therapy to treat the patients with B-cell lymphoma. Another CAR-T cell drug, Kymriah™ has been approved by FDA recently to treat the acute lymphoblasticleukemia (ALL) (37, 38). Further clinical trials are going on to determine its efficacy in cancer treatment and results obtained yet, have shown the perspective of adoptive cell therapy as the future approved cancer therapy (39).

Conflict of Interest:

I, Monireh Bahrami, declare no conflict of interest with any individual or organization regarding this manuscript.

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