Novel usage of CAR T cell therapy in treatment of hematological malignancies and solid tumors: Shift in paradigm of biomarkers

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ABSTRACT:
Hematological malignancies and solid tumors are the common form of cancers increasing by annual rate of 7.9% every year. In past few decades, with new advent of chimeric antigen receptor (CAR-T) cell therapy, T cells are engineered in such a way that it becomes more specific, selective as well as efficient targets to treat different types of cancers. Biological markers are naturally occurring molecules, genes or characteristics by which particular disease can be identified. However, biological markers (CD19) produced in past are found to have no capability to kill solid tumors and associated with some toxicities in treating hematological malignancies. Hence, recently, group of biomarkers associated with surface of malignant cells have been emerged which serve as targets for directing the cytotoxic T cells towards CD5, IL3Rα, CD33, CD70, CD38, BCMA. These are some of surface targets having unprecedented clinical outcomes in the treatment of hematologic malignancies and solid tumor. Furthermore, these biomarkers are known to have capacity to kill tumors with no toxicities. This review demarked the newly emerged biomarkers which are useful for treatment of hematological malignancies and solid tumors.

1. INTRODUCTION:
Lymphocyte T cells (T cells) perform a significant function in the immune response as it track and destroy malignant cells [1]. Many therapeutic approaches have been established for the development, and/or improving T cells against tumours over the past few years [2]. This adoptive immunotherapy focused on T cells, which creates novel approaches to malignancies, in particular hematological cancers. This new therapy comprises of 3 models: tumor infiltrating lymphocytes, T-cell receptors & CART-T cell therapy [3]. The first two approaches do not allow a major change in T cells compared to CAR-T therapy and thus they are not as efficacious as CAR-T cell therapy. The development process, low success levels, and vaccine dependency often hinder their production. CAR-T cell therapy has been studied for more than 25 years as an effective clinical treatment. The TCR segment is substituted with CAR. Two domains: an extracellular as well as an intracellular is present in CAR. In this extracellular domain is usually an antibody fragment of a single-chain. It acts against a cell antigen specifically. On the other hand intracellular domain consists of fused signals from a normal TCR complex molecule. Different intracellular portions reflect various generations of CAR-T cells [4, 5]. The configuration extends from the CD3z signaling domain in the first-generation CARs to those in CD3z-fused signalling of costimulatory molecules such as CD28, CD134 or CD137[1, 6].
Cancer is the leading cause of death in the world population [1]. Death due to cancer estimated to be 9.6 million in 2019. The most important kind of cancer is hematological malignancies and solid tumours. It is distributed most widely around the world [7]. The discovery of driver biomarkers has facilitated the clinical development of targeted therapies, especially CAR T cell therapy. CAR T cell therapy is novel and innovative immunotherapy in which T cells are modified in such a way that T cells become more selective, specific and effective in targeting the cancer cells [8]. However, due to poor antigen recognition, poor trafficking it was difficult to treat hematological malignancies and solid tumors. But in recent researches, scientists were able to treat such type of cancers by inventing new biomarkers. Such type of newly emerged biomarkers have passed the clinical trials for clinical use [9]. Hence novel biomarkers have triggered the paradigm shift in treatment of cancer. This review will give an emphasis on new clinical biomarkers and to improve the therapeutic efficacy of CAR-T in malignancies [10].

2. Production & Mechanism of CAR-T cell:

CAR-T cell development process is very complicated. First, phlebotomy or leukapheresis are performed to extract T cells from peripheral blood, followed by apheresis with no introduction of the granulocyte colony activating Factor[1]. The explanation for the absence of the granulocyte colony stimulating factor is that it might impair the proliferation and response of T-cells. The different T cells are then moved to the CAR (retroviral or lentiviral) or nonviral vector with artificial insertion of a portion of the genome DNA[11]. The further and crucial move is to assess the efficacy of this new adoptive immunotherapy by ex vivo expansion & purification. The appropriate dosage in human bodies is 1 to 5×10^8 cells, but this is not equal to the number of CAR-T cells. Eventually, cell quality tests and sterility that take 2-4 weeks to complete are required[12]. A testing, including lymphodepleting, should be performed 2 days ahead of time for a more proliferation of the T cells prior to transduced T cells being administered. This form of immunotherapy is widely used in the treatment of hematological diseases such as acute lymphoblastic leukemia, lymphoma & multiple myeloma [7, 13]. The most common goal of such therapy is attachment with CD19, and to get overall efficacy for ALL should be positive. There is some progress with other targets including CD 20, CD23, CD30 and 138[14]. Strong tumours, including melanoma, sarcoma and breast cancer are yet another arena for the treatment of CAR-T. Unlike hematological tumors, most solid tumor therapy has not succeeded in targeting and regulating the tumor environment because of inadequate and untypical molecular targets for CAR-T cells. It is a promising resource for potential adoptive cancer immunotherapy, despite several protection and efficacy concerns [15].

CAR-T cell therapy utilizes CAR-engineered T-cells for the treatment of cancer. CAR-T immunotherapy involves the alteration of T cells to recognise cancer cells in order to attack and kill them more efficiently[16]. In order to combat the tumor, scientists extract T cells from humans, genetically modify them and then infuse the resultant CAR-T cells into patients. CAR-T cells can either be extracted from the patient's own blood T cells or extracted from other stable donor T cells[17, 18]. When removed from a human, the T cells are genetically modified to different CARs that bind to a specific antigen located on the tumor surface. Once CAR-T cells have been injected into a patient, they become a "living weapon" against cancer cells. CAR-T cell
attaches to intended antigen present on a cell surface and gets activated then leads to proliferate & become cytotoxic. CAR-T cell kills cells via numerous pathways, including extensively induced cell proliferation, enhances the degree to which CAR-T cells become cytotoxic[19, 20].

![Application of CAR T cell therapy](image)

**Fig. Application of CAR T cell therapy**

### 3. Biomarkers for solid tumors:

Biomarkers can be a good source for the management of cancer. It can be used in use in the risk identification, screening, diagnostic differentials, drug reaction prediction, and tracking of disease progress. A new class of targetable biomarkers has recently emerged with the advent of Chimeric Antigen Receptor (CAR) T cell therapy[21]. This biomarkers are linked to malignant cell surfaces & serve as targets for cytotoxic T cells. CD19 is kind of B cell marker that was strongly expressed in malignant B cells. It was considered as first biomarker considered for cell therapy. Since the popularity of CD19, an explosion of new biomarkers targeting human malignancies has occurred over the past decade. Such surface targets have allowed direct, targeted treatment to reduce the degradation of healthy tissues and to conserve the immune system of patient during treatment[22].

#### 3.1. Fr-alpha:

Folate receptor-alpha (FRα) sometimes recognized as FOLR1 is a glycosylphosphatidyl inositol linked protein encoded with the gene FOLR1. This receptor is a membrane- protein that have strong affinity to bind and bring physiological folate concentrations into cells[23]. FRα is overexpressed in ovarian cells and many other epithelial diseases, like breast cancer, renal cancer & lung cancer, in contrast to normal cells. FR is an attractive choice in cancer therapy for many experimental approaches[24]. One new strategy is to direct T cells to FR using bifunctional antibodies on the surface of ovarian carcinomas. This approach culminated in selective tumor cell growth inhibition. In xenogeneic model and in patients having ovarian cancer these chimeric antibodies that bind with both FR and CD3 or CD28 show promising effects[25]. A single chain,
Fv anti-FR antibody was also successful in inhibiting in vivo tumor growth. Alternatively, single chain FR-rich tumor follicle acid conjugates may mobilize the T-cell receptor antibody. FRα can thus be used as a biomarker in CART cell therapy[26].

3.2. Her2:
HER2 (human epidermal growth factor 2) belongs to ERBB family is a membrane-bound tyrosine kinase. It doesn't have a specific ligand also it does not heterodimerizes that is ligand-binding with the other members of the ERBB family to facilitate the various intracellular signals in cell growth[27]. In different types of cancer, HER2 protein overexpression, gene amplification and mutation have been reported. Therefore assessment of her2 level is indeed important as a supportive diagnosis for anti her 2 targeted treatment[28]. There are two separate HER2 targeting methods widely used in the clinic: Antibodies guided towards the receptor's extracellular domain & Tyrosin Kinase Inhibitors (TKIs) small molecule working in the domain of intracellular kinase. Several HER2 positive malignant targeting agents (trastuzumab and pertuzumab) have been authorized[29].

The HER2 gene produces HER2 proteins. HER2 proteins are also present in breast cell receptors. Typically, HER2 receptors help in maintaining the development, division and regeneration of a healthy breast cell[30]. However, in approximately 20% of breast cancers, the HER2 gene does not function properly and generates too many versions of itself. All of these HER2 extra genes instruct breast cells to create so many HER2 receptors (HER2)[31]. This allows breast cells to expand in an uncontrolled way. HER2-positive cancers in the breast continue to develop rapidly and propagate more often than HER2-negative cancers in the breast. Therefore her2 is considered as a potential biomarker in CAR-T cell therapy[32].

3.3. GD2:
GD2 is a sialic acid-containing, clinically and pathologically essential glycosphingolipid. In the endoplasmic reticulum and Golgi apparatus, GD2 is synthesized & then passed to the outer layer of the plasma membrane[33]. On the cell surface GD2 plays a key function in signal transduction & cell-cell adhesion processes leads to cell proliferation, immune escape and invasion & neoangiogenesis. It is distributed primarily on the surface of the cell and is predominantly present in the central nervous system and in small quantities in the peripheral nerves & skin melanocytes[34]. One of the pathological effects of GD2 is the existence in large numbers in several forms of tumors like breast cancer cells[35].

GD2 is found predominantly in neuroblastomas as well as certain melanomas, and to a varying degree in a number of other cancers, such as bone, small lung cell cancer & brain tumors. The involvement of GD2 in binding tumor cells with extracellular matrix proteins is assumed to play a significant function, thereby causing tumorigenesis[36]. GD2 can be used as a valuable biomarker for multiple cancers owing to its occurrence in numerous tumor cells. In a recent report, GD2 has been significantly increased in a variety of
patients with breast cancer. This result prompted researchers to determine its significance for breast cancer as a biomarker[37].

3.4. MUC-1:

The MUC1 gene encodes a cell-surface glycoprotein with a diverse cytoplasmic domain that is part of a signal transduction. In epithelial cancers, like epithelial ovarian cancer, this gene is frequently overexpressed and over-expression is correlated with reduced viability[38]. The MUC1 gene is also act as an essential adhesion or metastasis modulator. Several additional MUC1 isoforms produced by RNA (mRNA) messengers are characterized as A, B, C, D, X, Y, Z, SEC & REP[39]. Cancer cells produce up to a 100-fold increase in MUC1 protein compared to regular cells. Cancer cells typically have irregular MUC1 protein glycosylation. It has been shown that the development of MUC1-related antibodies and cellular immune responses have a beneficial effect on cancer patients[40]. Hence MUC1 related immunity that can be increased by the use of vaccinations, MUC1-specific antibodies & transfer of T cells is an effective technique to fight against cancer[41].

The overexpression of abnormally glycosylated MUC1 acts as an interesting target for the treatment of chemotherapy. It is available in the majority of solid tumors and some hematological malignancies. MUC1's investigated to function in cancer growth, invasion, metastases, angiogenesis and chemical resistance [42]. MUC1 is shed and may impede binding / recognition of the tumor antibody so it can create some kind of trouble as it may also inhibit the role of T cells and thus encourage anti-inflammatory TME. Consequently, CAR T-cell therapy targeted using MUC1 give rise to variety of challenges such as steric weakness and heterogeneity correlated with glycosylation [43]. MUC1-CAR T cells have shown substantial delays in tumor development in case of xenograft models on mice after a CAR optimization. This process was associated with tripartite endodomas and strong affinity screening of successful ScFv fragments [1, 44]. In accordance with in vitro cell regulation, cells of MUC1-CAR T causes increase in proliferation, IFN-gamma secretion, or antitumor efficacy. Based on their performance, many clinical trials targeting MUC1 were conducted for many cancer forms, including the preclinical MUC1-CAR T cell. Initial adverse side effects and patient cytokine profiles were not identified in early phase I clinical trials, suggesting a favourable outcome[45].

3.5. CD133:

Human CD133 is five single-chain transmembrane glycoprotein belonging to the prominin family with two wide, extracellular and two narrow intracellular loops. One of the most important biomarkers used for isolation of cancer stem cells (CSCs) is pentaspan membrane glycoprotein CD133 (promin-1)[1, 46]. Collected evidence has demonstrated that CSCs Tumours, Metastases, and Chemoresistance can be triggered by CD133. Since the discovery of CSCs in 1998 & till the date these cells have shown a particular interest as they are capable of self-renewal and tumor development[47]. Recent studies shown that there are several solid tumors of CSCs during the last two decades. CD133 is one of the prominent biomarkers of stem cells. In multiple tissues such as the liver, breast, colon, & pancreas, CD133 was found in various CSCs originating tumors[48]. Although the functions of this protein is not fully understood, but its function in tumor propagation have been reported in various studies. Moreover, it may induce cell proliferation via Wnt and Notch signals. CD133 also prevents
apoptosis and upregulates FLIP, which contributes to chemo-resistance. Based on these different functions of CD133, it seems to be a suitable biomarker for CAR-T cell therapy[49].

3.6. EPH A2:

EPHA2 receptor belongs to receptor tyrosine kinase family that is classified on the basis of sequence homology & ligand binding affinity into two subfamilies collectively called as ephrins (EPHA & EPHB) [50]. In cancer cell development, migration & invasiveness, ephrin-dependent signalling is played a leading role in multiple pathways, particularly RAS and AKT, that leads to adhesion & transition. Specific ephrins and its receptors like EPHA2 are triggered in a variety of malignancies, potentially resulting in higher malignancy and poor prognosis[51]. It has been found that EPHA2 and ephrin A1 are overexpressed in colorectal cancer in phase I & II compared to Phase III-IV, which indicates its possible role in initial stages of the development of the disease. EPHA2 was also associated with weak stage II and III prognosis. EPHA2 is commonly over-expressed in multiple human malignancies, including the CRC, in which an alternative adverse prognostic marker has previously been proposed for Early Stage disease[52]. The role of EPHA2 in cancer resistance to different targeted therapies in melanoma, nonsmall cell lung cancer and breast cancer was confirmed by recent findings[53].

4. ADVANTAGES OF CAR-T CELL THERAPY:

4.1. Short treatment time:

One of the main benefits of CAR T-cell treatment is the short time required to treat it – given with a single injection that may take a maximum of 2 weeks of hospital care, and then treated. Because aggressive chemotherapy is not used, most patients are treated much more rapidly than after the transplantation of stem cells. Hence CAR T-cell therapy may also replace certain forms of transplants in the future. But till now this therapy is only approved for the treatment of those patient who are not cured by transplantation method.

4.2. Specificity:

A CAR is an artificial framework that stimulates the activation of T cell receptors when expressed in T cells and directs specifically to a given antigen. In the case of cancer treatment, an extracellular ligand binding network specific to a tumor cell surface antigen is connected to an intracellular signal module, which stimulates T cells after the binding to a antigen. This shows that CAR-T cell therapy is very specific it specifically target a particular antigen that is present on tumor cell surface[54].

4.3. Easily recognize potential antigens:

CAR-T cells without the expression of MHC can identify cell surface molecules that are present on tumor cells. Versatility of intracellular CAR signalling domains helps the cell to directly or indirectly overcome the downregulation of co-stimulatory molecules triggered by cancer cells. It is worth noting that therapy using CAR-T cells useful for identification of antigens, carbohydrates, lipids and antigens [55].
4.4. Safety:

As CAR has high specificity for T cells that is the reason for the improved efficacy of CAR T-cell therapy compared to chemotherapy. The conventional therapies including different types of chemotherapy are not specific these target both cancerous and healthy cells leads to casualties like hair loss. But with the advent of CAR-T cell therapy patients are no longer worry about the adverse side effects of traditional cancer therapies, including hair loss. Moreover CAR T-cell therapy is very much convenient over chemotherapy. Patients must undergo routine treatments with chemotherapy. In comparison, CAR T cell therapy patients need only one time treatment because CAR T cells autonomously fight cancer till eradication [1, 56, 57].

5. CHALLENGES OF CAR-T CELL THERAPY:

It has been shown that the stimulation of antitumor activity of engineered T cells leads to broad and powerful cytokine-driven effects during treatment of hematological malignancy using CAR-T cells, such as macrophage activation, CRS & hemophagocytic lymphohistiocytosis. CRS provides a clinical reaction to elevated cytokine and involves certain symptoms like hypotension, fatigue, Hypoxia & neurological changes. In response to the increased incidence of significant CRS identified in patients who display higher burden of underlying disease conditions, dose selection activity were investigated utilising risk-adapted CAR-T higher doses administered. Systemic corticosteroids help to minimize hyper-proliferative activity of CAR-T cells as first aid therapy. It can be useful for patients with life-threatening CRS. However, the drawback has also been found: rapid removal of engineered T cells can reduce CD19 CAR-T's anti-tumor efficacy and cause relapse or recurrence. Neurotoxicity is severe potential toxicity resulting from CAR-T cell therapy and was found in many CD19 CAR-T cell-treated patients. The clinical evidence of neurotoxicity is endothelial dysfunction. Apart from this vascular instability, blood-brain disturbance and disseminated intravascular coagulation are other kind of evidence of neurotoxicity. These side effects limit the use of CAR-T cells usage therefore more research is required to overcome these challenges.[1, 58, 59].

6. Conclusion:

The compelling success of biomarkers identification in hematologic malignancies and solid tumors is propelling the development of CARs that show potential actions in destroying such type of cancers. The ability to genetically manipulate infused CAR-T cells followed by targeting the novel biomarkers has provided limitless opportunities to cure HM and ST along with strong hope for future success.

References


[55] !!! INVALID CITATION !!! {Abken, 2013 #137; Hillerdal, 2015 #139; Zhao, 2018 #1828}.


