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An Integrated Approach for T Cell Receptor Mimicking Antibody-Based Cancer Immunotherapy: From Biomarker-Driven Antibody Discovery to Personalized Treatment Strategies

Dua-E-Fatima [a]

[a] *Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology (NUST), Islamabad, Pakistan*

A B S T R A C T

This proposed framework outlines a personalized immunotherapy strategy using T cell receptor mimicking antibodies (TCRmAbs). It utilizes a pre-existing therapeutic strategy; however, it relies on enhanced target discovery using a patient specific approach, such that the tumor cells, and subsequently produced TCRmAbs, are specific to the individual in need of the therapy. It begins with the collection of patient-derived cancer biopsies and uses administration of certain agents to enhance Major Histocompatibility Complex Class I (MHC I) expression, both during the discovery phase of MHC-I conjugated peptides, and later during the treatment phase. This proposed therapy demands rigorous preclinical validation, including that on animal models and cell lines, before clinical consideration. This framework emphasizes a cautious and systematic approach aligned with established scientific and ethical standards.

Keywords: T cell receptor mimicking antibodies, Personalized immunotherapy

1. Introduction:

Cancer immunotherapy is a rapidly evolving discipline with various targeted therapeutic strategies under development. One of the most distinguished features of cancer immunotherapy is the recognition and subsequent targeting of biomarkers and surface antigens unique to cancerous cells. Each cancer cell expresses certain antigens which are abnormal with respect to either their structure, or their relative expression (Baba AI et al., 2007). These antigens can be targeted either for delivery of a drug, or for immune system mediated destruction of the cells expressing them.

The majority of cancer antigens are intracellular ones. (He et al., 2019) These include a wide array of various signalling proteins which have accumulated pro-cancerous mutations and are contributing to tumorigenicity. Due to their intracellular nature, these proteins or peptides are processed within the cell and displayed on the cell surface conjugated to an MHC-1 molecule. MHC conjugated antigens require T cell receptor (TCR) mediated recognition in order to be viewed as cancerous, and therefore destroyed. However, the highly immunosuppressive tumour microenvironment renders T-cells less efficient at being able to provoke an anti-tumour immune response. Thus, this article proposes the use of combinatorial biologics to target personalized antigens specific to an individual's cancer.

2. A Novel Design at the Intersection of Humoral and Cell Mediated Immunity:

Within the immune system, Cytotoxic T cells (CTLs or $CD8^+$ cells), and B cells, each provide immunity against slightly different categories of antigens. These cells each require a different combination of circumstances in order to initiate protection against a particular type of antigen. Cytotoxic T cells are capable of recognizing peptides conjugated to MHC class one molecules and use this binding affinity between their receptors and the peptide-HLA complex as a signal to kill the cell to which they have bound (Neefjes et al., 2011). (Unless this process is inhibited due to the presence of inhibitory cytokines, or other suppressive mechanisms, which as stated above, is often the case with immunosuppressive tumor microenvironments.) Whereas B cells require other activation signals as a result of which they secrete antibodies. Which then direct destruction or functional blockage of target cells in a number of ways.

Within cancerous cells, many transformations occur at the intracellular level. These transformations are not necessarily good drug targets due to their sequestered nature. Additionally, targeting these changes within cancer cells requires a high degree of precision, or the presence of cancer cell specific surface proteins in order to mediate endocytosis/delivery of a therapeutic molecule within cells. However, another means of targeting intracellular aberrations can be accomplished by combining the antigen detection method of CTLs, and the cytotoxic mechanisms of secreted antibodies. By making antibody like structures that mimic T cell receptors in terms of their affinity towards antigenic peptide sequences conjugated onto MHC class one molecules, it is possible to target these otherwise evasive targets (Trenevska et al., 2017).

3. The need for Targeting Intracellular Proteins:

T cell therapies have been developed, which can prime either whole T cells (as is the case of TILs and CAR-T) or specifically receptors of T cells (as is the case of TCRmAbs), to target tumor cells (Zhang et al., 2020). While CAR-T cells generally target extracellular proteins, a large number of cancer antigens include intracellular peptides conjugated to MHC-1. Such antigens represent a therapeutic target which CAR-T cells cannot utilize as a binding site. MCH-1 conjugated; tumor specific, intracellular peptides are thus targeted by TCR mimicking antibodies (He et al., 2019).

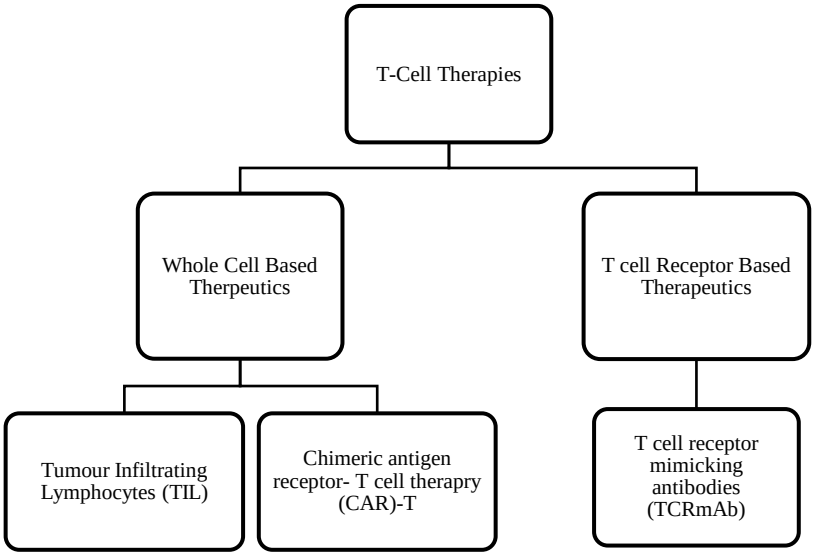

Figure 1: T-Cell based Therapies

4. Overcoming MHC-1 Downregulation:

As with the majority of immunotherapies, administration of agents such as antibodies, or primed immune cells is insufficient to cause target destruction, or even appropriate recognition of cancer cells. Tumor cells have deployed many mechanisms to evade immune responses. These include the induction of highly immune suppressive microenvironments, which by virtue of cells such as T-regulatory cells, myeloid derived suppressor cells, and tumor associated macrophages, provide signals, cytokines, metabolites and other signaling factors which render cytotoxic immune responses incapable of efficiently targeting cancer cells (Cortellino et al., 2023). Apart from these, tumor cells have developed individual mechanisms by which they escape recognition by the immune system as well. Among the most common of such strategies is the downregulation of MHC-1 molecules, which prevents the display of peptide fragments from mutated proteins being displayed and consequently being recognized by cytotoxic T lymphocytes. (Leone, 2013) Overcoming this barrier is imperative for the success of TCRmAbs, which cannot function unless an MHC-conjugated peptide is available as their target. Thus, in this paper, a combinatorial, targeted therapy of TCRmAbs alongside MHC-I upregulation agents have been proposed as part of the process to develop patient specific TCRmAbs.

5. A Conceptual Framework for the Proposed Therapeutic Strategy:

A conceptual framework is proposed, involving patient biopsy, MHC-I upregulation, isolation of MHC-I peptides, phage display library construction, exposure to MHC-I peptides, phage selection, validation on normal cells, engineering TCR mimicking antibodies, and personalized patient administration.

5.1 Biopsy from Patient with Cancer:

Performing a minimally invasive or surgical procedure to collect a representative-tissue biopsy from the patient's tumor and ensuring proper handling and preservation of the biopsy to maintain the integrity of the cellular components. As opposed to conventional TCRmAb design, this approach does not begin with pre-identified peptide sequences, against which antibodies are designed.

5.2 Administration of MHC I Upregulators and Extraction of MHC I Peptides

Carefully selecting MHC-I up-regulating agents based on the specific characteristics of the patient's cancer. This is to be followed by treatment of the biopsy-obtained cells with selected agents. The next step is extraction and purification of MHC-I conjugated peptides from the cancer cells for downstream analysis.

5.3 Phage Display Library Construction and Exposure of Phage Display Library to MHC I Peptides:

Developing a diverse phage display library by incorporating a repertoire of antibody fragments and ensuring that the library represents a wide range of potential binding specificities. This is to be followed by exposing the phage library to the isolated MHC I conjugated peptides from cancer cells and allowing sufficient interaction time for antibody fragments to bind specifically to the MHC I peptides.

5.4 Phage Selection:

Implementing a stringent selection process to isolate phages displaying antibody fragments with high affinity and specificity for the MHC-I peptides followed by multiple rounds of selection to enrich for the desired binding characteristics.

5.5 Validation on Normal Cells Using Cell Binding Assays:

To do so, procurement of non-neoplastic cells from the corresponding tissue (e.g., non-malignant liver cells for hepatocellular carcinoma) should be followed by performing detailed binding assays to assess whether the selected antibody fragments also bind to such non-cancerous cells. If MHC-I up-regulators are used prior to isolating MHC-I conjugated peptides from cancer cells, the same procedure should be repeated prior to testing for unwanted binding during this step.

5.6 Engineering, Cloning and Expression of TCR Mimicking Antibodies:

If necessary, modifying the antibody sequences in order to enhance TCR mimicking properties while minimizing the binding to normal cells. This is to be proceeded by cloning the optimized antibody fragments into expression vectors and expressing the TCR mimicking antibodies in an appropriate expression system.

5.7 Patient Administration via an Individualized Treatment Plan:

Developing a personalized treatment plan based on the patient's characteristics, including dosing and administration schedule while ensuring compliance with ethical and regulatory standards. TCRmAbs are to be administered to the patient ensuring simultaneous injection with MHC-I up-regulators and an appropriate adjuvant at the tumor site. The co-administration strategy should be optimized to enhance immune response.

Note:

The proposed therapeutic strategy delineated above represents a conceptual framework that requires meticulous validation and extensive preclinical investigation before translation into clinical applications. It is imperative to underscore that the journey from concept to clinical viability necessitates rigorous validation in animal models and comprehensive testing across relevant cell lines. This process mandates years of systematic research to elucidate the potential efficacy, safety, and mechanistic nuances of the devised therapeutic approach. It is of paramount importance to acknowledge the inherent risks associated with therapeutic development, and as such, a rigorous risk-mitigation strategy involving iterative refinement based on preclinical outcomes must precede any consideration of its application in human subjects. This conceptualization, while promising, is part of an evolving scientific inquiry and underscores the imperative for a measured and stepwise progression in accordance with established scientific and ethical standards.

6. RL21A-A TCR Mimicking Antibody against Macrophage Inhibitory Factor:

Expression of Target Protein:

Located on the 22nd chromosome, the macrophage migration inhibitory factor gene codes for a protein, MIF, also known as Glycosylation inhibiting factor (GIF) which functions as a cytokine and is expressed by various immune cells including T cells, B cells, macrophages and other cells of both the innate and adaptive immune system. (Calandra & Roger, 2003)

MIF in Cancerous Cells:

MIF is also expressed by other cell types throughout the body including epithelial cells. Notably, a higher-than-normal level of MIF expression is observed in several cancerous cells including breast cancer cells. This expression is in accordance with its inductive effects on proliferative signaling in cells. MIF has been shown to induce cell division via the ERK1/2-MAPK pathway. (Calandra & Roger, 2003)

Targeting MIF through A TCR Mimicking Antibody:

In light of its pro-cancerous role, Hawkins et al devised an experimental design in order to target breast cancer cells by using MIF as a target. Since MIF is an intracellular protein, peptides derived from its digestion are present on cell surfaces in an MHC conjugated form, making this a good candidate for TCR mimicking antibodies. However, since MIF is a protein expressed in normal body tissues, in order for it to have true therapeutic relevance, a specific MHC conjugated peptide fragment would need to be isolated, which showed expression in a cancer cell exclusive manner. Hawkins et al found such a peptide, which also showed anti-tumor activity (Hawkins et al., 2011).

7. Concluding Remarks: Benefits of a Personalized Approach

While MIF is not a tumor specific protein, as seen in the experiment conducted by Hawkins et al, a certain peptide fragment of this protein was exclusively being expressed in an MHC conjugated manner in certain breast cancer cell lines. This implies that proteins apart from tumor specific antigens also have the potential to become targets for biological therapies. This observation from their study, paired alongside the longstanding notion of tumor heterogeneity, points towards the need to develop TCRmAbs as a part of personalized medicine. Thus, it is hoped that the proposed approach allows for finding useful and previously unknown targets for a promising immunotherapeutic approach against cancer.

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