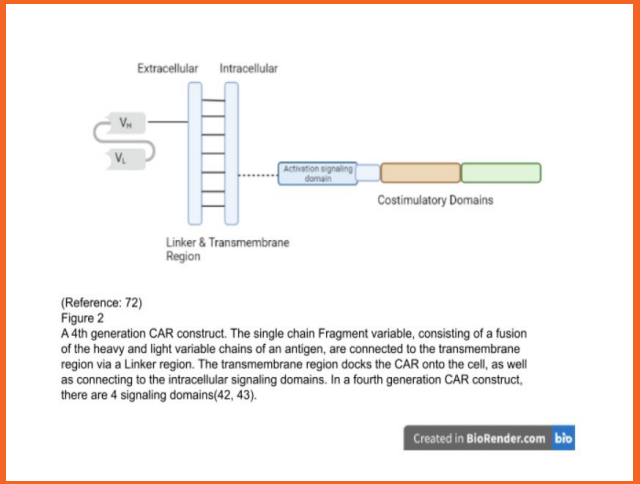

(Reference: 72)
Figure 2
A 4th generation CAR construct. The single chain Fragment variable, consisting of a fusion of the heavy and light variable chains of an antigen, are connected to the transmembrane region via a Linker region. The transmembrane region docks the CAR onto the cell, as well as connecting to the intracellular signaling domains. In a fourth generation CAR construct, there are 4 signaling domains(42, 43).

Treating Diffuse Large B Cell Lymphoma Using HLA Class I Molecule Deficient Anti CD19 CAR-NK Cells

Suraj Das

Statement of Problem

- There are many shortcomings of current modern-day treatments for cancers such as Diffuse Large B-cell Lymphoma
  - For example
    - Human Leukocyte Antigen Mismatch Restriction
    - Accessibility of autologous NK cells to use for CAR(Chimeric Antigen Receptor) Nk cell therapy
    - Targeting specificity of Chemotherapies
- How can I use CRISPR Cas9 gene editing to create a CAR Nk cell therapy that bypasses such restrictions

Hypothesis

- By creating HLA Class I-deficient anti CD19 CAR-NK cells through CRISPR/Cas9 gene-editing technology and using allogeneic NK cells derived from Induced Pluripotent Stem Cells(IPSCs) as a source, it would be possible to develop an “off-the-shelf” CAR-based immunotherapy to target DLBCL in patients that maximizes cost-efficiency, bypasses HLA mismatch restriction, lowers the risk of Graft versus Host Disease and Cytokine Release Syndrome, all the while targeting malignant B-cells.

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CRISPR Cas9 Gene Editing

- Clustered Regularly Interspaced Palindromic Repeats
- Cas9 is recognized as a CRISPR associated protein
- Type II System, one of the simpler systems of CRISPR
- Originally discovered in bacteria as a mechanism of immunity
    - Bacterium would integrate viral DNA into these spaced sequences, and would later use these regularly spaced sequences to determine the targeting specificity of the Cas9 protein to sever the DNA at the location of the viral integration
- This mechanism has been replicated in human settings to use a method of inhibiting gene expression, replacing a gene, as well as various other functionalities
    -

Chimeric Antigen Receptor Cells

- Chimeric Antigen Receptors(CAR) are attached onto different immune cells in order to determine targeting specificity, and to allow for researchers to be able to target the elimination of cells that these immune cells might normally ignore
- CAR-based immunotherapies are a novel treatment in modern medicine, with CAR-T Cell therapy rising in popularity
    - There are some shortcomings however
        - Autologous T-cells must be used which is very expensive
        - Graft versus. Host Disease and Cytokine Release Syndrome have high possibilities of appearing in patients
- CAR Nk cells can fill in the gaps here through some modifications mentioned in the Cell Design slide
-

Cell Design

- CAR Nk cell
- Anti-CD19 receptor
  - CD19 is recognized to be related to the prevention of Nk cells from attacking malignant B-cells
- Suicide iCasp9 gene
  - In the case of adverse side effects, this gene has been integrated into the CAR Nk cells in order to induce cell lysis of all allogeneic CAR-Nk cells
- B2M gene knockout
  - This prevents surface expression of HLA Class I molecules, allowing for the bypassing of the HLA mismatch restriction

Experimental Design

- 2 rounds of CRISPR Cas9 based gene editing
- Round 1 will target the gene for the PD-1 inhibiting receptor
    - This is important as malignant B-Cells have been noted to have a high overexpression of PD-1
- Round 2 will target the B2M gene
    - This gene is responsible for the surface expression of HLA Class I molecules, so eliminating this gene allows us to bypass the HLA mismatch restriction
- Round 1 will insert an HLA Class E molecule to be coexpressed by the Nk cell, which allows for further insurance against HLA mismatch
- Round 2 will insert the CAR into our Nk cell, which will determine the targeting specificity of the Nk cell

Conclusion

Until now, the prognosis of patients with DLBCL resistant to conventional therapies has been incredibly poor with little to now hope in becoming better. However, with the innovations of CRISPR Cas9 gene editing technology, as well as Chimeric Antigen Receptors, alternative treatments for DLBCL, as well as other lymphomas can only get better. With the introduction of CAR-T cell therapies into commercial medical-care one might believe that an optimal alternative treatment has been found, but this is not the case. The CAR-T cells currently used still have many flaws, such as requirement of autologous T-cells for sourcing to prevent HLA mismatch, and adverse side-effects of Cytokine Release Syndrome, and Graft versus. Host Disease. With my proposed cell design of allogeneic IPSC-derived HLA Class I molecule deficient anti CD19 CAR-NK cells, I predict that these constraints that pose problems for the applications of CAR-T cells in the medical field will be bypassed by my cell treatment. By inhibiting HLA Class I molecule surface expression, as well as coexpressing a single-chain HLA Class E molecule, the cell will be able to bypass the NK cell inhibiting receptors that interact with HLA Class I molecules, and instead recognize the expression of the single-chain HLA Class E molecule, which will inhibit the NK cell's ability to induce cell-dependent cytotoxicity. Furthermore, with the addition of an anti CD19 CAR that also contains a transmembrane domain and costimulatory domain of NKG2A and 4-1BB respectively as well as the breaking of the PD-1 receptor, the cell's cytotoxic functionality and targeting of CD19 on malignant B-cells will marginally increase. In the case of mutation of abnormal behavior, the inducible suicide gene iCasp9 can be activated, causing near-immediate lysis of all the cells contains CARs. Finally, by using an induced pluripotent stem cell line as a source of NK cells, we are afforded much more freedom and flexibility with regards to the availability of my treatment commercially. To summarize, this experimental cell I have proposed here has many prospective applications in the medical field. It will also possibly serve as a jumping point to advance the current research there is available on CAR-NK cells. At the current rate technology and science is advancing, I predict that there will be immense innovation in these two fields, and that the prognosis of lymphoma patients will increase exponentially.