Module 9 Assignment

585.751 Immunoenginnering

1. (40 points) Design a cancer vaccine. In consideration of your design, specify design constraints, such as:

   a. Type of antigen (i.e. peptide, DNA, mRNA, etc.) and adjuvant
   b. Biophysical properties (examples: size, shape, stiffness, etc.)
   c. Material properties
   d. Safety

   For the material, we utilize Mesoporous Silica Rods (MSRs) with high aspect ratio to allow large sporous space. These rods feature macropore of size large enough for cellular infiltration, and a high volume that facilitates controlled drug release. Mesoporous silica is biocompatible and biodegradable. Upon injection, these MSRs spontaneously self-assemble into a 3D-scaffold structure. This structure serves as a site where DCs can be recruited, allowing the uptake of antigen and adjuvant. This process matures the DCs, to enabling them to present MHC-peptides, and express lymph node homing receptors, preparing for deployment to the lymph node. The pore sizes within the rods are typically in the range of 2-50 nanometers, ideal for loading with various therapeutic agents. The mechanical stiffness of the MSRs can be adjusted through the synthesis process.
   The payload includes:
   - **GM-CSF**: This is utilized for recruitment of DCs to the scaffold.
   - **CpG ODN**: It activates these DCs, enhancing their ability to process and present antigens.
   - **OVA**: is a well characterized model antigen, it serves as a safe, initial step to assess the vaccine's mechanisms of actions and efficacy.
   Following the initial evaluation using OVA, we plan to transition from OVA to:
   - **Irradiated tumor cells**: these cells will be used as a source of tumor antigens.
   The vaccine containing the MSRs with their payload, is injected near the site of the tumor or in a lymphatic area to maximize exposure to immune cells.

2. (50 points) CAR T cell therapy has been effective for treating some liquid tumors but has some shortcomings/concerns, such as:

   a. Ineffectiveness for solid tumors
   b. Toxicities, including cytokine release syndrome, neurological toxicity, on-target/off-tumor recognition (i.e. recognizing the correct antigen on noncancerous cells), and anaphylaxis
   c. Safety concerns associated with viral transduction
   d. High cost

   Choose one of the shortcomings listed or one identified in your own research and explain why the issue exists and describe how CAR T cell therapy could potentially be modified or enhanced to address that issue.

CAR-T therapy has not been very successful against solid tumors in the clinics [1], [2], [3]. The unsuccessful outcomes are due to a variety of factors:

Tumor antigen heterogeneity: One significant obstacle for CAR-T therapy in detecting and eliminating cancer cells, particularly in solid tumors, is the variability of tumor antigens. This variability often leads to inadequate detection of cancer cells by CAR-T cells. Moreover, in the context of solid tumors, the antigens that are targeted are frequently not crucial for the tumor's growth and progression. Additionally, several studies have highlighted that the low-level expression of antigens targeted by CAR-T cells can further diminish the efficacy of this therapy. In some cases, targeting a specific antigen with CAR-T cells can even trigger the downregulation of that antigen on the tumor cells, effectively making the CAR-T therapy less effective or even incapable of targeting the tumor cells.

Poor tumor site CAR-T trafficking: Contrary to non-solid tumors, such as blood cancers, CAR-Ts encounter their target cells in the bloodstream or the lymphatic system, in solid tumor CAR-T therapy, CAR-Ts have limited accessibility to the tumor site rendering CAR-T effect ineffective and insufficient.

Reduce CAR-T Cell Migration and Penetration: The ability of CAR-T cells to penetrate and migrate into solid tumor sites is crucial for their therapeutic effectiveness. This process is heavily reliant on specific types of chemokines that facilitate the movement and infiltration of these immune cells into tumor tissues. However, in many solid tumors, the expression of these chemokines is downregulated or entirely absent. Additionally, scientific evidence has shown that the WNT/beta-catenin signaling pathway, active in various tumor types, can block T-cell infiltration, including CAR-T cells, into the tumor site.

Enhancing the efficacy of CAR-T therapy in targeting solid tumors can be achieved through several strategies:

Inducing Target Antigen Expression: one approach is to induce the expression of specific CAR target antigens within the tumor itself.

Regional delivery of CAR-T Cells: Researchers have proposed the direct delivery of engineered CAR-Ts into the cerebrospinal fluid (CSF), intracerebroventricular or intertumoral administration.

Improving CAR-T Infiltration: Addressing the issue of poor infiltration rates of CAR-T cells into solid tumors could involve integrating specific chemokine receptors into the CAR construct. For example, genetically modifying CAR-T cells to express IL-8 receptors or the chemokine receptor CCR2b could enhance their migration towards and infiltration into tumor sites overexpressing these chemokines.

Leveraging Cytokines to Enhance the Tumoricidal activity: Utilizing cytokines that boost the tumoricidal activity of CAR-T cells can help counteract the immunosuppressive tumor microenvironment (TME), which contributes to CAR-T cell exhaustion.

Encouraging the infiltration and recruitment of a broad spectrum of endogenous T cells into the tumor site can help in preventing tumor antigen escape.

Boosting CAR-Ts Efficacy with Vaccines: Recent advancements include the development of a nanoparticle-based RNA vaccine designed to deliver CAR target antigen directly into the lymphoid compartment; another innovative approach involved a nanoemulsion-based vaccine intended to prime DCs to present specific antigens (e.g., OVA) to CAR-T cells engineered to express TCRs specific for these antigens.

3. (50 points) Describe checkpoint blockade therapy and specifically explain the mechanism of action for the FDA-approved checkpoint inhibitors, anti-PD-1/anti-PD-L1 and anti-CTLA4. Briefly describe an engineering design, either your own or from the literature, to enhance checkpoint blockade therapy (examples: local delivery, combination therapy that synergizes with checkpoint inhibition, etc.).

- In a healthy immune response, CTLA-4 functions as a brake on T cells to prevent overactivation, which could lead to autoimmunity. It competes with the stimulatory receptor CD28 for binding to B7 (CD80/CD86) on antigen-presenting cells (APCs). When CTLA-4 outcompetes CD28, it inhibits T cell activation, Tumors can create an immunosuppressive microenvironment that encourages the expression of CTLA-4. T cell activation requires two signals. Signal 1 is provided when the T cell receptor (TCR) recognizes an antigen presented by the MHC molecule on APCs. Signal 2 is the co-stimulatory signal provided by the interaction between CD28 on T cells and B7 on APCs.Anti-CTLA-4 therapy, such as ipilimumab, is designed so that it specifically binds to CTLA-4 on the surface of T cells. This binding prevents CTLA-4 from interacting with B7 molecules on APCs. By blocking CTLA-4, the therapy ensures that CD28 (instead of CTLA-4) binds to B7 molecules, Signal 2 is therefore not blocked, and T cell activation is restored.
- PD-1 is a receptor found on the surface of T cells and some other immune cells. Its ligands, PD-L1 and PD-L2, can be expressed on the surface of various cells, including some tumor cells. The interaction between PD-1 on T cells and its ligands serves as a checkpoint mechanism to prevent autoimmunity and to regulate the intensity of immune responses. Many types of cancer upregulate PD-L1, "instructing" T cells to turn off and allowing the tumor to escape immune detection and destruction. Immunotherapies, such as pembrolizumab (Keytruda) and nivolumab, can either bind to PD-1 on T cells, preventing its interaction with PD-L1 and PD-L2, or directly bind to PD-L1 on tumor cells, blocking their ability to send inhibitory signals to T cells.

Enhancement Solution

This research team [4] developed a two-part drug delivery system, a polymeric micelle for a simultaneous delivery of an anti-PD1 checkpoint inhibitor and the chemotherapeutic drug paclitaxel (PTX) for a synergistic effect in cancer treatment. This design involves encapsulating PTX within the core of a pH-sensitive micelle. This micelle is constructed from a copolymer of azide-terminated polyethylene glycol and polyaspartic acid (diisopropyl ethylenediamine co benzylamine) (Azide-PEG- PAsp(Dip/Bz)) via hydrophobic interactions and π-π stacking for encapsulation.

To attach anti-PD-1 antibodies to the micelle, a click chemistry reaction is employed, connecting the antibodies via a peptide linker sensitive to matrix metalloproteinase-2 (MMP-2) and incorporating a detachable long-chain PEG. This design takes advantage of PEG to increase circulation time of these "stealth" particles and to evade the immune recognition and engulfment by the MPS to reach the tumor.

Once these micelles accumulate at the tumor site, the acidic environment (approximately 6.5) and the high concentration of MMP-2, a protein often found in tumors, trigger the release of the aPD-1 antibodies, and cause the long-chain PEG to detach. This change not only releases the immune booster near the cancer cells but also alters the charge of the micelle from negative to positive. This charge switch helps the micelle to enter cancer cells more easily. Once inside,

the acidic conditions in the cell's carrier system (lysosome) allow a rapid PTX release, making treatment more targeted and effective.

[1] H. J. Jackson, S. Rafiq, and R. J. Brentjens, "Driving CAR T-cells forward," *Nat. Rev. Clin. Oncol.*, vol. 13, no. 6, pp. 370–383, 2016, doi: 10.1038/nrclinonc.2016.36

[2] P. S. Kozani, P. S. Kozani, M. A. Najafabadi, F. Yousefi, S. M. J. Mirarefin, and F. Rahbarizadeh, "Recent Advances in Solid Tumor CAR-T Cell Therapy: Driving Tumor Cells From Hero to Zero?," *Front. Immunol.*, vol. 13, p. 795164, 2022, doi: 10.3389/fimmu.2022.795164

[3] T. T. Spear, K. Nagato, and M. I. Nishimura, "Strategies to genetically engineer T cells for cancer immunotherapy," *Cancer Immunol., Immunother.*, vol. 65, no. 6, pp. 631–649, 2016, doi: 10.1007/s00262-016-1842-5

[4] Z. Su *et al.*, "Codelivery of Anti-PD-1 Antibody and Paclitaxel with Matrix Metalloproteinase and pH Dual-Sensitive Micelles for Enhanced Tumor Chemoimmunotherapy," *Small*, vol. 16, no. 7, p. e1906832, 2020, doi: 10.1002/smll.201906832