

Overview

Hot and cold tumor are crucial in assessing the efficacy of existing cancer immunotherapies. A hot tumor is rich in tumor immune microenvironment (TME), PD-L1 overexpression, genomic instability. Example of hot tumors include melanoma and lung cancer. In contrast, non-T- cell inflamed cancers, such as Triple Negative Breast Cancer (TNBC), prostate or pancreas cancers fall into the category of “cold tumors”. Simultaneously, tumor-associated macrophages (TAMs), T/B regulatory cells (T/Bregs), myeloid-derived suppressor cells (MDSCs) have been identified as contributor to the formation of a specific TME in “cold tumors”. Factors affecting tumor virulence include immune checkpoints (PD-1, PD-L1, CTL4, TIM-3, and LAG-3), tumor infiltrating lymphocytes (TIL), tertiary lymphoid tissue (TLS), microsatellite status (MSI), tumor mutational burden (TMB) and gene mutations (for ex. TP53, BRCA1).

Immune checkpoint inhibitors (ICIs) have shown success in improving the survival of cancer patient. ICI-mediated antitumor responses depend on the infiltration of T cells that identify and eliminate cancer cells. Therefore, ICIs are less efficacious in “cold tumors” which are characterized by the lack of T-cell infiltration.

Specific Aims

The aims of this project are to analyze the current landscape of strategies designed in “warming up” cold tumors to immune checkpoint inhibitors (ICIs); to introduce an innovative approach utilizing nanoparticles, viral vectors, or polymeric particles as potential solutions.

Research Strategy

a) Significance

According to the National Cancer Institute, in 2020, cancer-related healthcare expenses in the U.S. reached \$208.9 billion. Since their peak in 1991, there has been a 33% decline in the rates of most common cancers, including lung, colorectal, breast and prostates, The trend has been attributed to a combination of factors, such as reduced smoking rates, advanced in therapies like ICIs, and the development of improved diagnostic and prognostic biomarkers. Nonetheless, cancer incidence rates have increased in breast, uterine, melanoma and prostate cancers [1].

In last 10 years, the FDA has approved an increasing number of Immune Checkpoint Inhibitors (ICIs) following successful clinical trials. These treatments have significantly enhanced long-term survival rates for metastatic patients and prolong progression-free survival for those in early stages of the disease. Cancer cell can escape detection and destruction by activating different molecules, such as PD1 or CTLA-4 on the surface of the T cells, inhibiting their activity. ICIs work by blocking the interaction between checkpoint molecules and their ligands found on the surface of the cancer cells, allowing T cells to remain activated. However, cold tumors are characterized by a deficiency in T cells, and in the absence of T cells, there are no checkpoint inhibitors to activate.

[illegible]

Colony-Stimulating Factor-1 Receptor (CSF1R) and its binding molecule CSF1 are prevalently observed in many cancers, including breast, prostate, pancreas, renal and ovary cancers. The inhibition of the CSF-1/CSF1R axis has demonstrated significant impact on the recruitment, and transformation of M2-like TAMs, showcasing potential therapeutic effects that could be contingent upon specific TME and cancer subtype. In various preclinical models, such as mouse models of glioblastoma (GBM) and malignant meningiomas, blocking CSF1 has shown promise in “reeducation” of M2-like TAMs towards an antitumoral M1-like phenotype, leading to tumor reduction; additionally, encouraging preliminary antitumor activity were observed in GBM, and NSCLC. In recent years, a variety of small-molecule CSF1R inhibitors have been proposed and entered clinical trials. Nevertheless, despite the initial encouraging breakthrough in the management of TGCT, a non-malignant tumor, the translation of such therapies into effective monotherapies for malignant solid tumors has often been disappointing.

Yves Greatti

severe adverse events. Similarly, combining CSF-1/CSF1R inhibitors with conventional treatments like chemotherapy, radiotherapy or targeted therapies have yielded mixed results.

b) Innovation

The Poly (ADP-ribose) polymerase (PARP) family has many crucial functions in cellular processes, including the regulation of transcription, apoptosis and promotes the repair of DNA. PARP inhibitors (PARPi) are effective against homologous recombination repair of cancer cells. By blocking PARP, a PARPi-derived drug could trigger DNA damage accumulation, leading to synthetic lethality in cancer cells with defects in DNA repair mechanisms. Additionally, PARPi can upregulate PD-L1 expression and PD-L1 upregulation can contribute to an inflammatory feedback loop that enhances T cell infiltration [21].

Research indicates that PARPi can facilitate the recruitment and activation of CD4+ and CD8+ T cells through neoantigen generation and the release of cytokines and chemokines like INF- γ , CCL5, and CXCL10 [22, 23].

In cancer therapy, inhibiting CSF-1R has demonstrated to augment the efficacy of PARP inhibitors (PARPi) [23]. This inhibition disrupts the recruitment and activity of tumor-associated macrophages (TAMs), which are often immunosuppressive and promote tumor progression. By targeting CSF-1R, the presence of these TAMs in the tumor microenvironment can be diminished.

Research suggests that the pairing CSF-1R inhibition with PARP inhibitors can lead to synergistic effects, effectively restraining tumor growth and improving treatment outcomes. This combination therapy not only bolsters the anti-tumor immune response but also increases tumor sensitivity to PARP inhibition.

We intend to enhance the therapeutic potential of exosomes derived from iPSC-MSC by utilizing them as carriers for PARPi cargo. These exosomes will be further modified by conjugating them with a CSF-1R inhibitor to target tumor-associated macrophages (TAMs) and cancer cells. To increase specificity and minimize off-target effects, we propose surface modifications of the exosomes derived from MSCs. This modification will involve conjugating the exosome surface with CSF1R, as well as markers specific to TAMs, such as CD68 or CD163, and markers specific to various cancer cell types. For instance, epithelial-derived tumors may be targeted using EpCam, breast cancer using HER2, or ovarian cancer using CA125.

c) Research Outline

Aim 1: Development and Characterization of Exosome-based Therapeutic for PARPi and CSF-1R Inhibitors.

Sub-aims:

- 1.1. Generate exosomes from iPSC-MSCs and load them with PARPi cargo.
- 1.2. Conjugate the surface of the exosomes with CSF-1R inhibitors and markers specific to TAMs (CD68, CD163) and cancer cells (EpCam, HER2, CA125).

Justification: Efficient loading of PARPi into exosomes is critical for maximizing the therapeutic potential of the drug while minimizing the required dose and associated side effects. This step is fundamental to ensuring that the exosomes can serve as effective drug carriers to the tumor microenvironment.

Benchmarks for Success:

- Successful encapsulation of PARPi into exosomes with over 80% efficiency; demonstration of targeted binding and uptake by TAMs and cancer cells in vitro.
- Verification of targeted conjugation via immunofluorescence staining and Western blotting for TAM and cancer cell markers.

Aim 2: In Vitro Evaluation of Therapeutic Efficacy and Specificity

Sub-aims:

2.1. Assess the cytotoxic effects of the engineered exosomes on a panel of cancer cell lines in vitro.

2.2. Evaluate the recruitment and activation of immune cells by treated cancer cells, focusing on CD4+ and CD8+ T cells and the impact on TAMs.

Justification:

This aim tests the hypothesis that the engineered exosomes can selectively target cancer cells and TAMs, inducing an anti-tumor immune response while sparing healthy cells.

Benchmarks for Success:

- Demonstrated specificity and cytotoxicity via cell viability assays.
- Increased infiltration and activation of T cells, observed through flow cytometry and cytokine profiling.

Aim 3: In Vivo Efficacy and Safety Evaluation

Sub-aims:

3.1. Conduct preclinical trials using relevant animal models to assess the therapeutic efficacy of the exosome-based delivery system.

3.2. Evaluate the safety profile and potential off-target effects of the treatment in animal models.

Justification: This aim addresses the translational potential of the research.

Benchmarks for Success:

- Significant tumor growth inhibition and improved survival rates in treated animals.
- Minimal adverse effects and evidence of targeted delivery to tumor sites.
- No significant toxic effects observed in normal tissue; a detailed mechanistic understanding of how the therapy modulates the tumor microenvironment, supported by changes in immune cell populations and cytokine profiles.

If time allows, we also investigate:

Aim 4: Evaluation of Combination Therapies with Immune Checkpoint Inhibitors to Enhance Antigen-Specific T-cell Activation.

Sub-aims:

4.1. Formulate combination therapy protocols that integrate the use of PD-L1/PD-1 inhibitors with anti-CTLA4 alongside the exosome-based delivery therapeutic agent carrying PARPi and CSF-1R inhibitors.

4.2. Investigate the effects of combination therapies on the priming and activation of antigen-specific CD4+ and CD8+ T-cells in vitro and in vivo.

Justification: The rationale behind this aim is to leverage the complementary mechanisms of action between immune checkpoint inhibitors and the targeted delivery of PARPi and CSF-1R inhibitors via exosomes.

Benchmarks for Success:

- Enhanced activation and proliferation of antigen-specific T-cells, as measured by flow cytometry and ELISPOT assays.
- Synergistic reduction in tumor growth and increased survival in relevant animal models treated with the combination therapy compared to monotherapies.
- Documentation of the immune cell infiltration within the tumor microenvironment through immunohistochemistry.

Facts And Figures Cancer Related References

[1] American Cancer Society – Cancer Facts & Figures 2024

[2] Asher Mullard “22 FDA approvals” nature reviews drug discovery

Significance References

1. Turning cold tumors into hot tumors by improving T cell infiltration

Yuan-Tong Liu, Zhi-Jun Sun doi: 10.7150/thno.58390

2. Turning cold tumors hot: form molecular mechanisms to clinical applications

Jiahui Zang et al. Trend in Immunology

3. Strategies for Heating Up Cold Tumors to Boost Immunotherapies

Danie Zabranksy et al. Annual Review of Cancer Biology

a) with chemotherapies to improve patient outcomes.

4. Hot and cold tumors: Immunological features and the therapeutic strategies

L Wang, H Geng, Y Liu, L Liu, Y Chen, F Wu, Z Liu

<https://doi.org/10.1002/mco2.343>

5. Combining in site vaccination and immunogenic apoptosis to treat cancer.

Arman Lamai, Mehdi Shalgolzari – Future Medecine – Immunotherapy – Vol.15, Issue 5

6. Dendritic cells and natural killer cells: The road to a successful oncolytic virotherapy

Matin Ghasemi et al. Frontiers in Immunology

7. Pouring Petrol on the flames: Using oncolytic virotherapies to enhance tumor immunogenicity.

Alicia Teijeira Crespo et al. Wiley Library, Immunology

8. Unlocking the potential of antibody-drug conjugates for cancer therapy

Joshua Z. Drago et al. doi:10.1038/s41571-021-00470-8.

9. Tumor Targeting of a Sting Antagonist with an Antibody-Drug Conjugate Elicits Potent Anti-Tumor Immune Responses.

10. Antibody-Drug Conjugates: A Review of Approved Drugs and Their Clinical Level of Evidence

Review by Pooja Gogi et al. Cancers

11. The Evolution of Antibody-Drug Conjugates: A Positive Inflexion Point

Anthony W. Tolcher - ASCO Educational Book

Research Previous Approaches

12. Igniting Hope for Tumor Immunotherapy: Promoting the “Hot and Cold” Tumor Transition

Chen Wei et al., DOI: 10.1177/11795549221120708

Sage Journal: Clinical Medicine Insights: Oncology Volume 16.

13. Advancing cellular immunotherapy with macrophages

Alok K. Mishra et al. DOI: <https://doi.org/10.1016/j.lfs.2023.121857>

14. Targeting tumor-associated macrophages in hepatocellular carcinoma: biology, strategy, and immunotherapy

Hongyu Zheng et al. Cell Death Discovery (2023(9: 65) Nature

Doi: <https://doi.org/10.1038/s41420-023-01356-7>

15. CSF1R inhibitors are emerging immunotherapeutic drugs for cancer treatment

Jiachen Wen et al., DOI: 10.1016/j.ejmech.2022.114884

Elsevier - European Journal of Medicinal Chemistry

16. Targeting tumor-associated macrophages for successful immunotherapy of ovarian carcinoma

Iva Truxova et al., DOI: 10.1136/jitc-2022-005968

Journal for Immunotherapy 2023

17. Sophisticated genetically engineered macrophages, CAR-Macs, in hitting the bull's eye for solid cancer immunotherapy.

Nese Unver, DOI: <https://doi.org/10.1007/s10238-023-01106-0>

Clinical and Experimental Medicine 2023

18. Engineering extracellular vesicles derived from macrophages for tumor therapy.

Ying Yan et al., DOI: 10.1039/D2MA00961G

Royal Society of Chemistry

19. Recent advances in macrophage-derived exosomes as delivery vehicles

Shumin Wang et al. DOI: <https://doi.org/10.26599/NTM.2022.9130013>

20. Therapeutic Targeting of DNA Damage Repair in the Era of Precision Oncology and Immune Checkpoint Inhibitors

Curis Clark et al., DOI: <https://doi.org/10.36401/JIPO-22-15>

21. Dual antitumor immunomodulatory effects of PARP inhibitor on the tumor microenvironment: A counterbalance between anti-tumor and pro-tumor

Xiao-Fang Yi et al., DOI: 10.1016/j.biopha.2023.114770

ELSEVIER - Biomedicine & Pharmacotherapy 163

22. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy.

Havel JJ, Chowell D, Chan TA (2019), Nat Rev Cancer 19(3):133–

150. <https://doi.org/10.1038/s41568-019-0116-x>

23. Beyond DNA repair, the immunological role of PARP-1 and its siblings.

Rosado MM, Bennici E, Novelli F, Pioli C (2013) Immunology 139(4):428–

437. <https://doi.org/10.1111/imm.12099>