

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.

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].

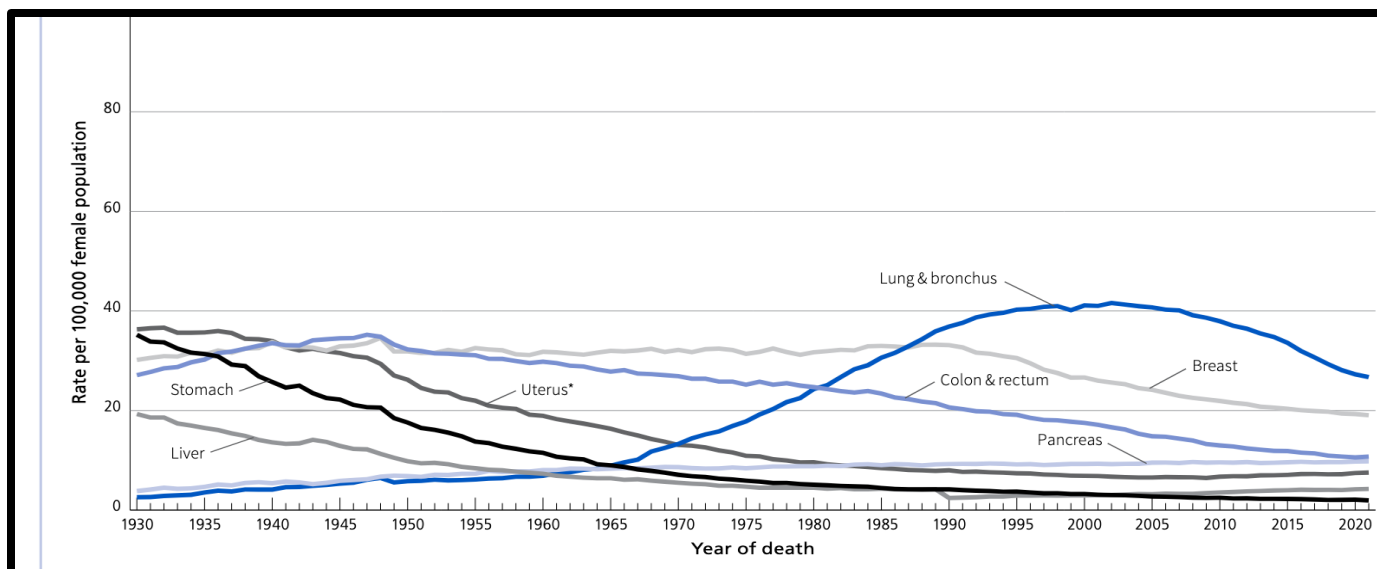


Figure 1: Trends in Age-adjusted Cancer Death Rates by Sites, Females, US, 1930-2021

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; yet some patients fail to respond to these strategies; in addition, these drugs are ineffective for certain tissue or tumor types. For 2024, in the United States there will be around 2 million new cancer diagnoses, with an estimated of 9,620 cases affecting children and 5,290 adolescents. This year is also expected to bring about approximately 611,720 cancer-related deaths, including 1,040 children and 550 adolescents.

In 2022, the FDA approved 37 new drugs, with ICIs leading the charge. Among these approvals, there were new checkpoint inhibitors like LAG-3, as well novel antibodies, and targeted gene therapies. Notably, some of these therapies were aimed at addressing specific mutations, such as KRAS G12C mutation in NSCLC [2]. However, while these therapies hold great promise, patients can still experience autoimmune side effects causing life-threatening complications affecting various organs (please refer to side effects reported by American Cancer Society).

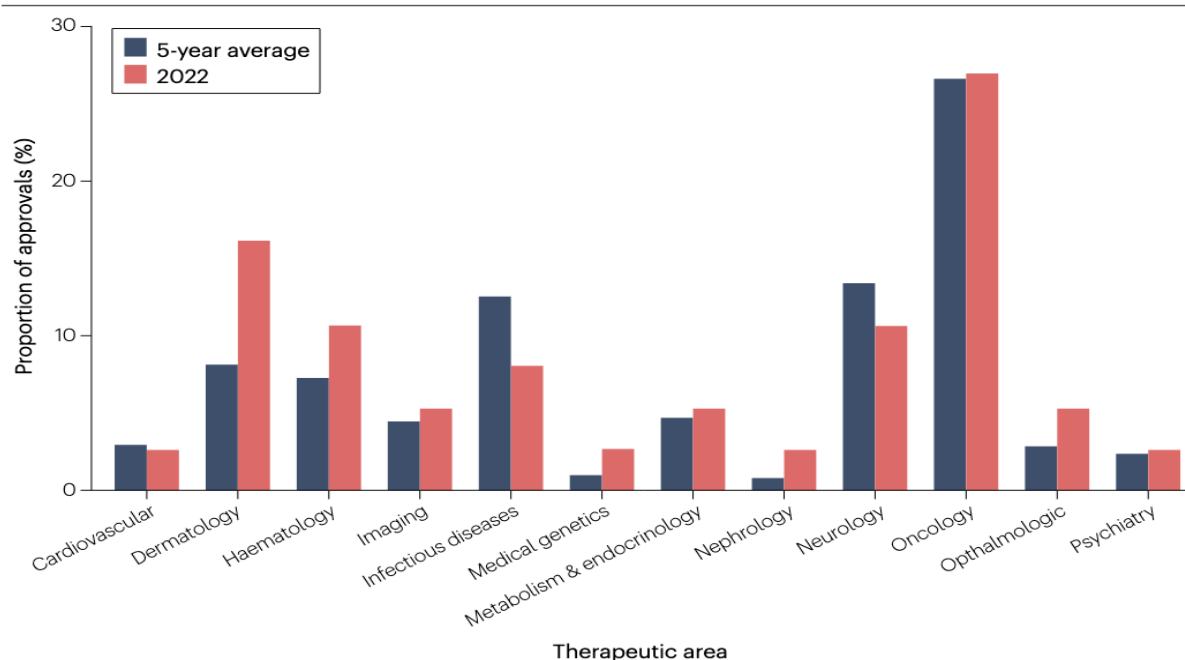


Fig. 2 | CDER approvals by therapeutic area. Indications that span multiple disease areas are classified under only one. Source: *Nature Reviews Drug Discovery*, FDA.

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.

Several key factors contribute to this shortage of T cells, including:

- **Insufficient tumor antigens:** cold tumors exhibit a scarcity of tumor-specific antigens, hindering the immune system's ability to effectively recognize tumor antigens.
- **Downregulation of MHC-I molecules:** reduction in class I MHC molecules limits the presentation of tumor antigens, diminishing the immune system's ability to target the tumor cells. In addition, tumor cells can inhibit DC phagocytosis and escape the immune system.
- **Low Tumor Mutational Burden (TMB):** cold tumors typically have fewer mutations and a lower load of neoantigens, making them less recognizable to the immune system.

- **A variety of oncogenic pathways:** such as WNT/Beta-catenin, KRAS or MYC; could be activated to modulate the immune response. This activation may lead to decreased recruitment of DCs, deficient phagocytosis of tumor cells, increased neutrophil levels, or reduced infiltration of T cells. Factors such as low levels of pro-inflammatory cytokines (CXCL9, CXCL10, CCL4, CCL5, CXCL16) can contribute to immunosuppression. By contrast, stromal cells (CAFs) can redirect Cytotoxic T Lymphocytes (CTLs) toward the cancer stroma preventing them from entering the tumor. Elevated expression of CXCL8 has been linked to a decrease in T cell presence and an increase in neutrophils within the tumor microenvironment (TME).
- **Inadequate vasculature:** resulting from ineffective aggregation of vascular endothelial adhesion cells, can impair T-cell trafficking to the tumor. Vascular endothelial growth factor (VEGF) plays a crucial role in angiogenesis, promoting the formation of new blood vessels, which affects the migration of T cells toward the tumor. Furthermore, factors such as hypoxia driven by the transcription factors HIF-1, along with acidosis, and necrosis, contribute to the recruitment of immunosuppressive cells within the TME. These conditions also induce angiogenesis through molecules like CCL28 and VEGF, further influencing T-cell trafficking and impacting the presence of inflammatory molecules like CD39 and CD73. CD39 and CD73 by converting extracellular ATP, into adenosine, have potent immunosuppressive effects.
- **Immunosuppressive cells and factors:** within the cancer stroma, cancer-associated fibroblasts (CAFs) play a pivotal role as they produce an extracellular matrix that acts as a physical barrier. CAFs also release CXCL12, which decreases T-cell response, and produce TGF-beta, which limits the proliferation of CD4+ T cells. TGF-beta achieves this by inhibiting production of IL-2 and promoting the conversion of naïve CD4 + into Treg. In addition, TAMs can reduce T cells infiltration within the TME by promoting angiogenesis through factors like colony-stimulating factor 1 (CSF-1), VEGF and MMP9. Tumor cells can release CSF1, which interacts on monocytes or macrophages, inducing recruitment and differentiation of TAMs. We will look further at the strategies to target TAMs, which constitutes a significant source of tumor immunosuppression, and targeting TAMs represents a promising strategy to transform cold tumor into hot tumor which we will research.
- **Elevated glycolysis activity:** and the subsequent accumulation of lactate (Warburg effect) are associated with a negative correlation to the infiltration of CD8+ cells, often indicated by high expression of LDH-A. Similarly, heightened cholesterol levels have been observed to downregulate MHC-I levels resulting in a decreased infiltration of CTLs.

3. 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>

- This is study on targeting TAM for liver cancer (HCC), reviewing small molecule drug, immune checkpoint inhibitors strategies, antibodies, tumor vaccines, and nanocarriers for the drug delivery system.
- This review mentions the promising potential of adoptive cell therapy (CAR-M) for clinical application. However, it emphasizes that there are still major challenges to overcome, particularly in the realms of biosafety and understanding the intricate synergistic effects involving macrophages. This paper emphasizes that it is critical to ascertain whether depletion of TAMs can occur without adversely impacting monocytes/macrophages in normal tissue. Additionally, there is a pressing need to understand whether anti-tumor phenotypes can be regulated over the long term, and to dig deeper into the complexities of macrophage subtypes beyond the simplistic “M1/M2” macrophage dichotomy.

4. 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

- Detailed review of a variety of CSF1R inhibitors completed and still active in clinical trials, with their limitations.

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

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

Journal for Immunotherapy 2023

- This article presents a detailed comparison of the characteristic of M-1like vs. M2-like macrophages (refer to Table 1).
- It then describes the TAMs antitumor response in ovarian TME, focusing on their involvement in angiogenesis, invasion and metastasis.
- The article highlights the critical impact of TAMs in recent immunotherapeutic advancements; emphasizing the need for further investigation into the detailed mechanisms by which TAMs affect the outcomes of these therapies.
- It categorizes TAM-targeting strategies into several approaches: (1) Preventing TAM recruitment through agents like CSF1(R)i, CCL2-CCR2i, CXCR4i, Ang2i (2) Depleting TAMs/ reducing their survival using CSF1(R)i, bisphosphonates, Trabectedin, CAR T therapy, Anti-TREM2 mAbs (3) TAM reprogramming/repolarization via TLR agonistic, PI3K inhibitors, and agonistic anti-CD40 mAbs, (4) Restoring the antitumor functions of TAMs with SIRP α i, and SIGLEC10 inhibitors, and (5) Limiting the tumor-promoting activity of TAMs through anti-PD-L1 mAbs, IDOi and VEGFi

6. 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

- This review describes how CAR-Macrophages (CAR-Macs) , a novel approaches for manipulating M2-like or M2-like macrophages, the components of CAR-Macs platforms, and their approaches.

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

Ying Yan et al., DOI: 10.1039/D2MA00961G

Royal Society of Chemistry

- This article explores engineered macrophages-derived extracellular vesicles (EVs) designed to mimic natural EVs but with enhanced yield, and efficient targeting capabilities. These advancements can make them ideal for targeted drug delivery, and immunotherapy.

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

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

- This article describes recent advances using extracellular vesicles derived from macrophages as drug delivery.
- Gunassekaran et al. developed a targeted cancer immunotherapy based on the biological properties of exosomes derived from M1 macrophages.

9. 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>

- Elaboration on DNA-damage response (DDR) mechanisms the impact of DNA single-strand break (SSB) damage in cancer.
- Review of loss of functions in hallmark genes such as BRCA1/2, ATM, ATR, CHEK1/2, BRD4, PALB2, RAD51, BARD1, FANC, PTEN, or TP53.
- Overview of current PARP inhibitors (including Olaparib, rucaparib, niraparib, talazoparib) along with ongoing clinical trials.
- Discussion of immunotherapies and strategies targeting DDR pathways.
- Examination of innate and/or acquired resistance mechanisms to single-agent PARPi therapies.

10. 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

- The article provides an overview of the dual immunomodulatory effects of PARP inhibitors (PARPi) within the tumor microenvironment (TME).
- PARPi enhances the TME by fostering the activation, mobilization, and efficacy of immune cells while reducing tumor angiogenesis.
- Conversely, PARPi exert a negative regulatory influence on the TME by disrupting DNA repair mechanisms in immune cells and inducing upregulation of PD-L1 expression in tumor cells.
- There is a need for further investigation into the potential synergistic effects of combining PARPi with immune checkpoint inhibitors (ICIs) and other agents known to modulate the TME.

Outline Method to Tackle Problem

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.

The Poly (ADP-ribose) polymerase (PARP) family has many crucial functions in cellular processes, including the regulation of transcription, apoptosis and promote the repair of DNA. PARP inhibitor (PARPi) have been shown to be efficient against homologous recombination repair of cancer cells. Additionally, PARPi can upregulate PD-L1 expression and PD-L1 upregulation can contribute to an inflammatory feedback loop that enhances T cell infiltration. This can trigger the release of additional inflammatory cytokines and chemokines by activated T cells, further promoting T cell recruitment into the tumor microenvironment.

Using an antibody-drug conjugate (ADC) or a macrophage-derived exosome to combine CSF-1R inhibition and PARP inhibition (PARPi) could be a promising strategy for targeting associated macrophages (TAMs) and cancer cells within the TME.

This project presents significant challenges: first, designing an effective delivery mechanism and second, devising an experiment encompassing aspects such mouse model selection, cell line or tissue selection, CSF-1R knock out in animal model, monitoring tumor growth, TAMs infiltration, and antigen expression analysis. Additionally, the success of this innovative approach is not guaranteed, and integrating with PD-1 inhibitor therapies poses additional complexities.

Questions for professors:

1. How can we effectively structure an experiment to ensure its success?
2. What strategies can be employed to develop an efficient novel drug therapy that enhances survival rates when used in conjunction with checkpoint immunotherapy inhibitors?

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 Zabransky 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

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

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

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

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