

### *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).

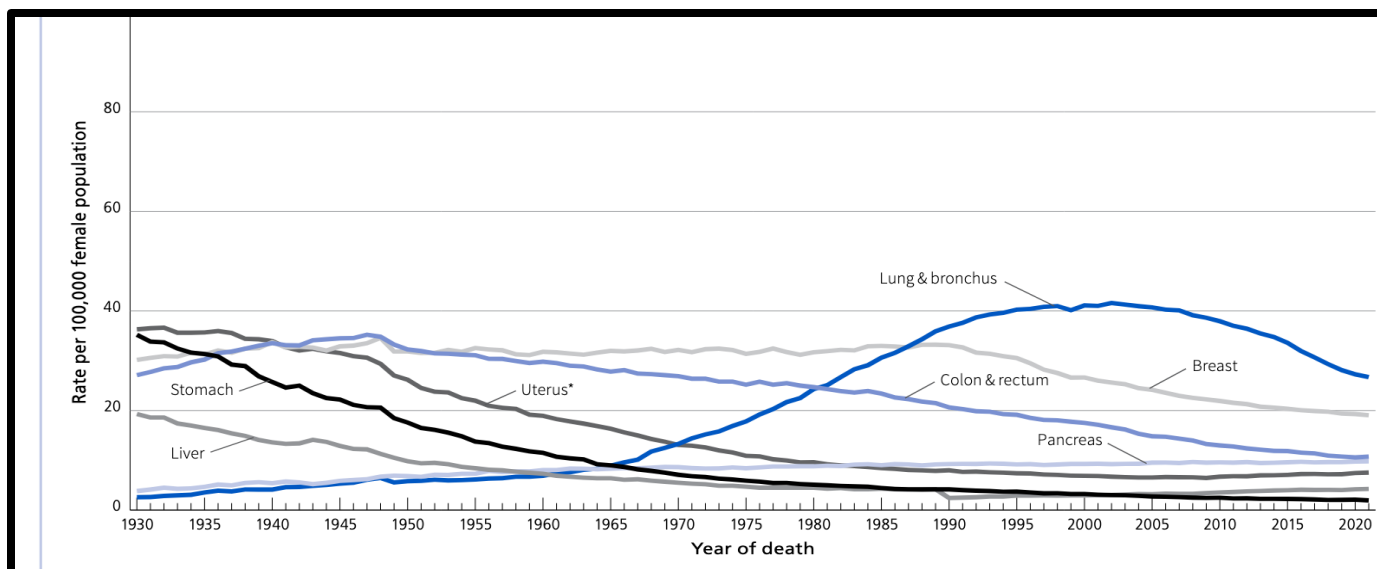
Immune checkpoint inhibitors (ICIs) have shown success in improving the survival of cancer patient. ICI-mediated antitumor responses depend on the infiltration of T cells that identify and eliminate cancer cells. Therefore, ICIs are less efficacious in “cold tumors” which are characterized by the lack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

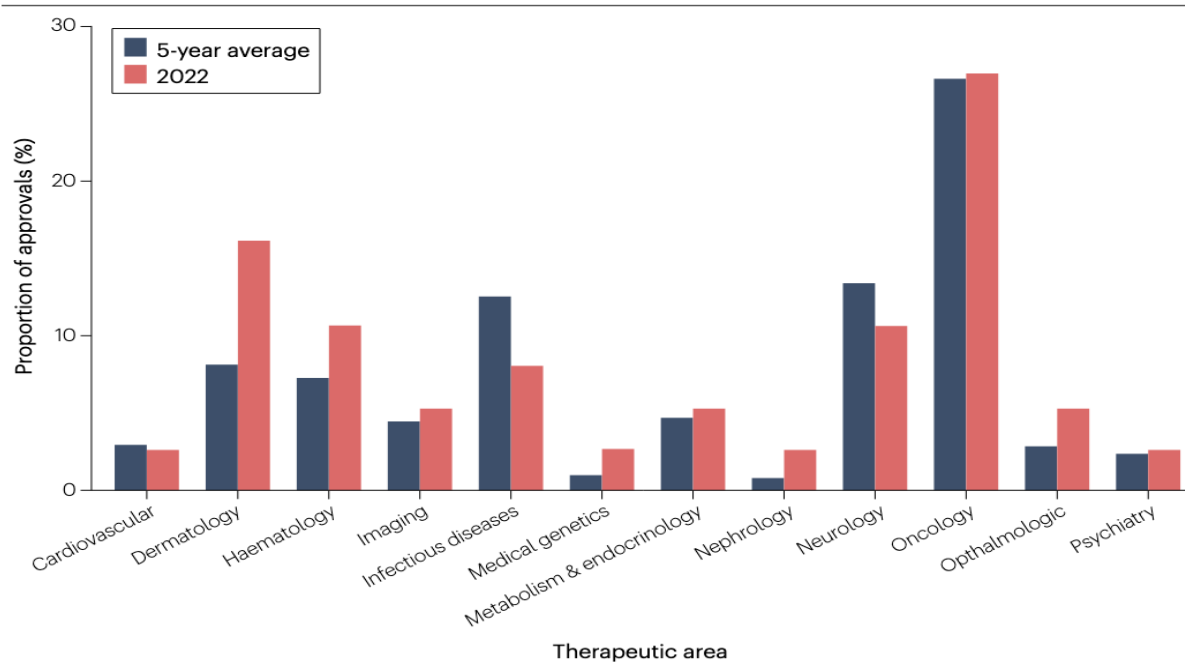
According to the National Cancer Institute, in 2020, cancer-related healthcare expenses in the U.S. reached \$208.9 billion. Since their peak in 1991, there has been a 33% decline in the rates of most common cancers, including lung, colorectal, breast and prostates, The trend has been attributed to a 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 recognize effectively 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 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.
- 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 exclude T cells from the tumor by promoting angiogenesis through factors like VEGF and MMP9.
- 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.

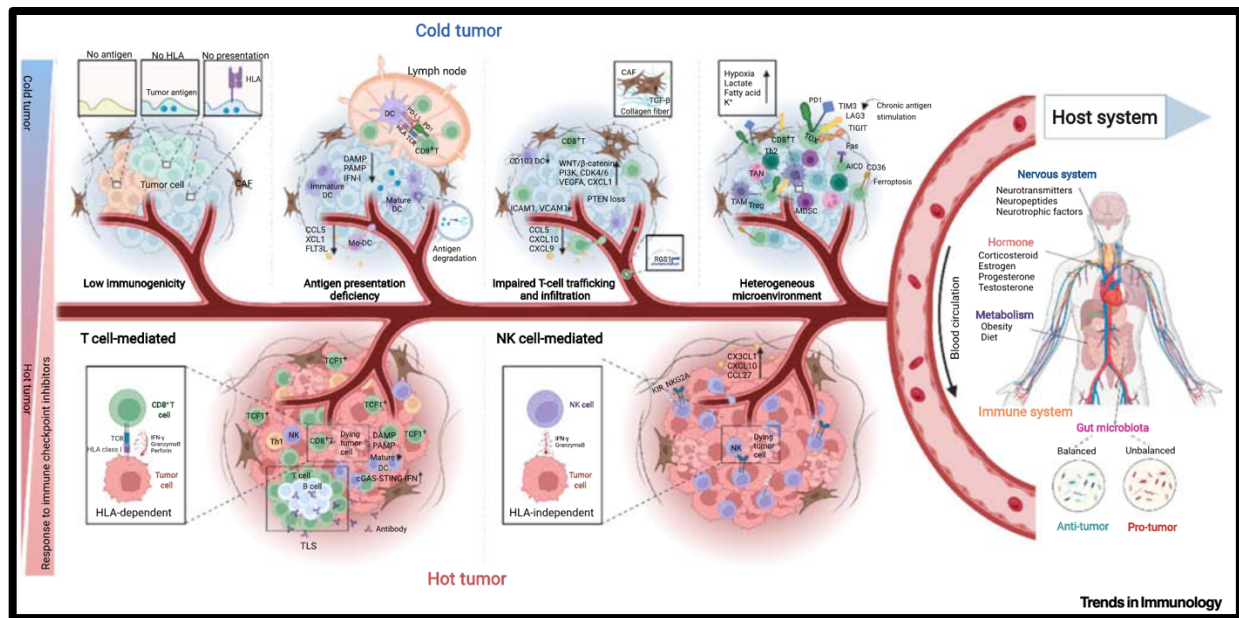


Figure 3: Mechanisms characterizing hot tumor vs. cold tumor.

Converting cold tumor to hot tumors will involve understanding less the immune response to cancer but more about the biology of cancer cells themselves and how to modulate or stimulate their activity, including:

- **Mechanism of Immune activation:** understanding the specific mechanism by which cold tumors are converted into hot tumors will provide valuable insights into immune activations and tumor recognition, such as elucidating the roles of various immune cells, cytokines, chemokines, and other signaling molecules involved in initiating and sustaining an anti-tumor immune response.
- **Immune Memory and long-term protection against cancer recurrence:** research in this area could help understanding the durability of anti-tumor immune responses, the formation of memory T cells, and the establishment of immune monitoring mechanisms to prevent tumor relapse.
- **Optimization of existing Immunotherapy strategies:** Elucidating how the immune system can be triggered to turn cold tumors to hot tumors will complement the ongoing research in immunotherapy which have shown significant response rates for hot tumors (i.e., PD-L1-positive). This includes studying mechanisms of immune evasion, and resistance to immunotherapies and the role of the TME in promoting treatment resistance. Combination therapies, such as PD-L1 or PD-1 inhibitors with anti-CTLA4 which enhance priming and activation of antigen-specific T-cells, are examples of strategies being explored in this context.
- **Precision Medicine:** Understanding how to turn a cold tumor into hot will enhance our ability to predict patient responses to immunotherapy based on tumor profiles, facilitating more precise personalized treatment approaches. This may involve designing better biomarkers or integrating multi-omics data to better match patients with the most effective treatments.

- [1] American Cancer Society – Cancer Facts & Figures 2024
- [2] Asher Mullard “2-22 FDA approvals” nature reviews drug discovery