

### *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).

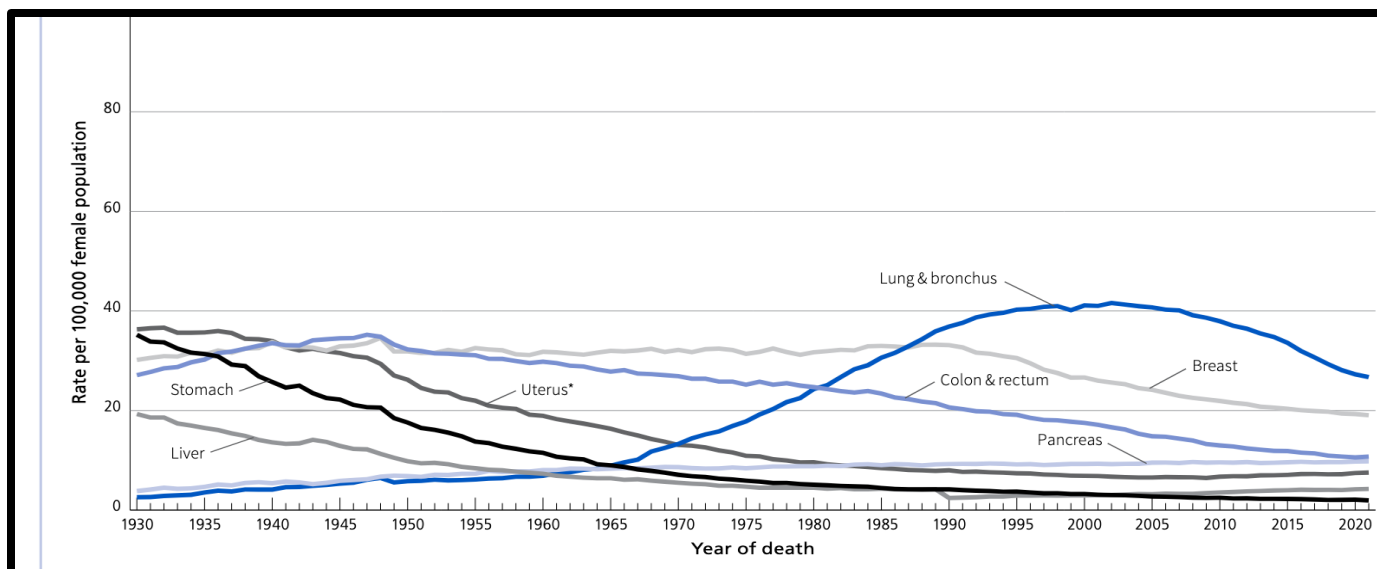
Immune checkpoint inhibitors (ICIs) have shown success in improving the survival of cancer patient. ICI-mediated antitumor responses depend on the infiltration of T cells that identify and eliminate cancer cells. Therefore, ICIs are less efficacious in “cold tumors” which are characterized by the lack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

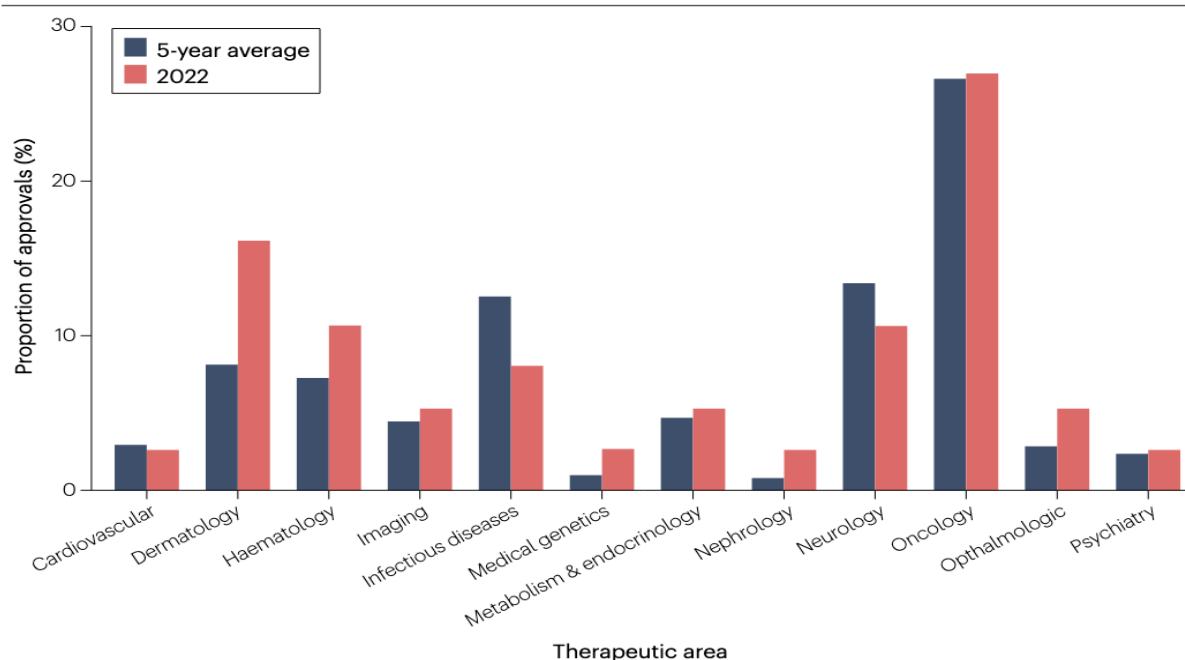
According to the National Cancer Institute, in 2020, cancer-related healthcare expenses in the U.S. reached \$208.9 billion. Since their peak in 1991, there has been a 33% decline in the rates of most common cancers, including lung, colorectal, breast and prostates, The trend has been attributed to a 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).



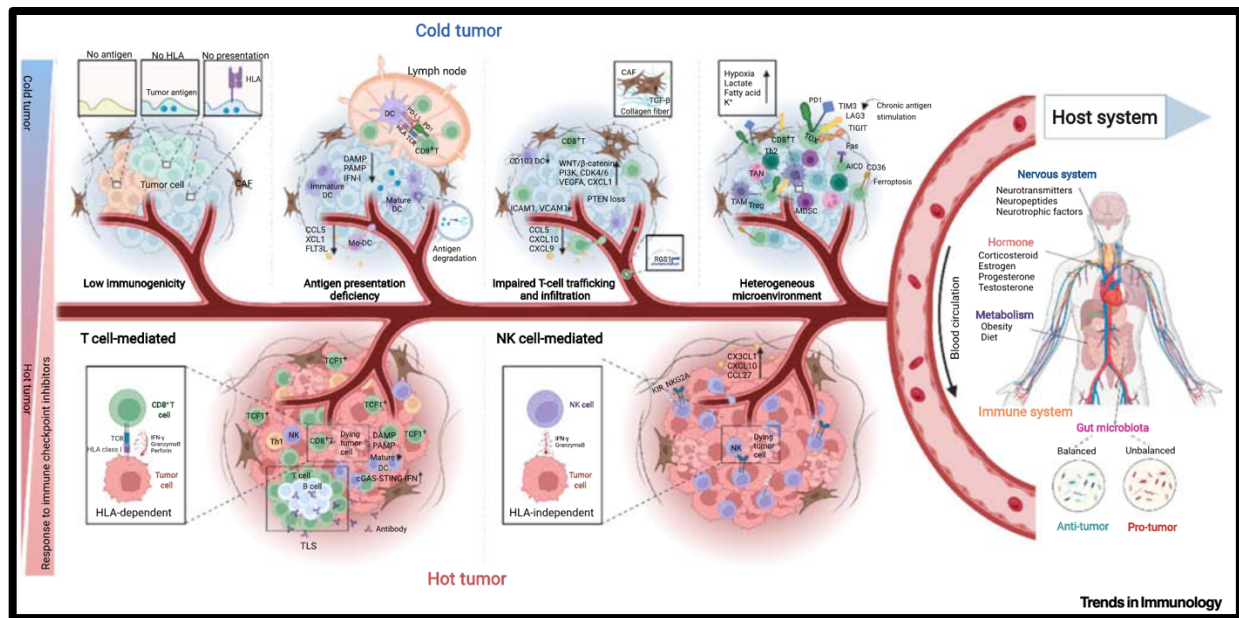
**Fig. 2 | CDER approvals by therapeutic area.** Indications that span multiple disease areas are classified under only one. Source: *Nature Reviews Drug Discovery*, FDA.

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.



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

We will look further at the strategies to target TAMS, which constitutes a significant source of tumor immunosuppression. And we will propose a novel approach targeting TAMS to transform cold tumor into hot tumor.

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. Employing a combination of these inhibitors alongside TAMS inhibitors, within the drug tolerance limits, could offer synergistic benefits.

### *Research Previous Approaches*

#### **1. Igniting Hope for Tumor Immunotherapy: Promoting the “Hot and Cold” Tumor Transition**

Chen Wei et al., DOI: [10.1177/11795549221120708](https://doi.org/10.1177/11795549221120708)

Sage Journal: Clinical Medicine Insights: Oncology Volume 16.

Good references on current approaches to driving T-cell proliferation in the TME (Table 1).

#### **2. Advancing cellular immunotherapy with macrophages**

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

- Review of chimeric antigen receptor-expressing macrophages (CAR-M) therapy. - - Discussion of the challenges and opportunities of this therapy.

### **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, ad 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](https://doi.org/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](https://doi.org/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), bisphosphonates, Trabectedin, CAR T therapy, Anti-TREM2 mAbs (3) TAM reprogramming/repolarization via TLR agnostic, PI3K inhibitors, and agonistic anti-CD40 mAbs, (4) restoring the antitumor functions of TAMS with SIRPalphai 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](https://doi.org/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.

#### **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.

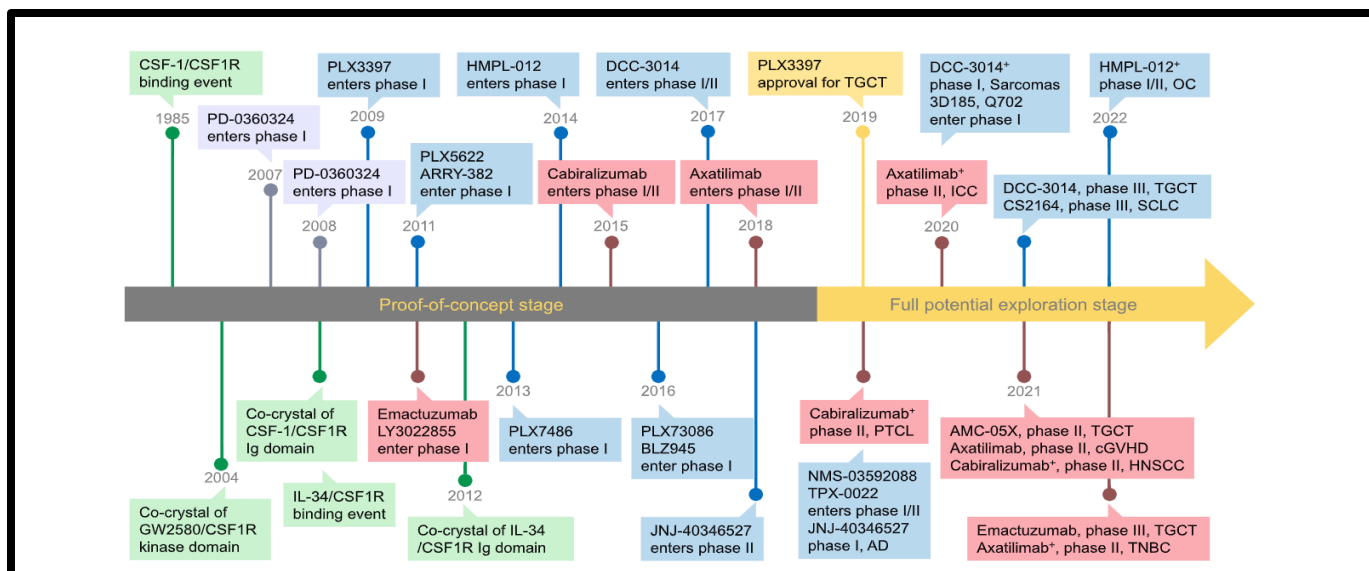
In various preclinical models, such as mouse models of glioblastoma (GBM) and malignant meningiomas, blocking CSF1 has shown promise in `reeducation` of M2-like TAMs towards an antitumoral M1-like phenotype, leading to tumor reduction; additionally, encouraging preliminary antitumor activity were observed 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.

CSF1 inhibition has rarely led to tumor regression. As combinatorial therapy, the outcomes were more encouraging: combining CSF1R inhibitor (PLX3397) with checkpoint inhibitors like PD-1 or CTLA-4 antibodies reduced tumor progression by more than 90%. However, most of the clinical trials were stopped due to observed severe adverse events. Similarly, combining CSF-1/CSF1R inhibitors with conventional treatments like chemotherapy, radiotherapy or targeted therapies have yielded mixed results. While preclinical studies have shown prolonged survival rates, clinical responses in certain cancers, such as metastatic breast cancer, have remained modest with only a 16% ORR observed in a phase II study.

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. Employing a combination of these inhibitors alongside TAMs inhibitors, within the drug tolerance limits, could offer synergistic benefits.
- **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.





**Figure 4: CSF1R inhibitor timeline**

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. PD-L1 on cancer cells binds to PD-1 receptors on T cells, leading to T cell exhaustion. This can trigger the release of additional inflammatory cytokines and chemokines by activated T cells, further promoting T cell recruitment into the tumor microenvironment.

Inhibiting CSF-1R (Colony-Stimulating Factor 1 Receptor) has been shown to enhance the efficacy of PARP inhibitors (PARPi) in certain contexts, particularly in the context of cancer treatment. CSF-1R inhibition can lead to decreased recruitment and function of tumor-associated macrophages (TAMs), which are often immunosuppressive and promote tumor growth and progression. By targeting CSF-1R, it is possible to reduce the presence of these immunosuppressive TAMs within the tumor microenvironment. Studies have demonstrated that combining CSF-1R inhibition with PARP inhibitors can lead to synergistic effects in inhibiting tumor growth and improving treatment outcomes. This combination therapy has been shown to enhance the anti-tumor immune response and increase the sensitivity of tumors to PARP inhibition.

Using an antibody-drug conjugate (ADC) 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.

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

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

**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

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**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**

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

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Anthony W. Tolcher - ASCO Educational Book