

# The tumor microenvironment and its immunosuppressive characteristics-IFN- $\gamma$ immunotherapy approaches

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**Abstract.** Advancements in cancer research and technology are driving a significant paradigm shift from traditional, one-size-fits-all treatments toward a more personalized and precise medical approach. This modern strategy, often termed precision medicine, offers tailored therapeutic care by addressing the specific genetic, molecular, and epidemiologic factors unique to each patient's disease. Among the most promising emerging strategies is immunotherapy, which represents a fundamental departure from conventional therapies like chemotherapy. Immunotherapy leverages detailed knowledge of the tumor's genetic makeup and its surrounding microenvironment to target key mutations. The primary goal is to enhance the body's own immune system, empowering it to effectively detect and eradicate cancer cells. However, tumors are notoriously adept at developing resistance; they dynamically reshape their local microenvironment into an immunosuppressive fortress to evade immune detection. This paper specifically analyzes the complex mechanisms of this immunosuppressive tumor microenvironment and investigates how scientists are employing various immunomodulators to overcome these defensive strategies, thereby restoring the immune system's ability to fight the cancer.

## 1. Introduction

The human body has an innate and adaptable defense system, with the capability of recognizing and memorizing pathogens. After repeated exposure, our learned defense leads a faster response and elimination of the foreign cells. A hallmark of tumor cells is when the cell no longer obeys the checkpoints. These abnormal cells can present with resistance to apoptosis, uncontrolled growth, and dysregulation in signal transduction [1, 2]. Deviations from the normal cellular processes hinders the immune system's ability in detecting and eradicating cancer cells.

Traditional cancer treatment's objective is to eradicate any tumorous cells in the body and prevent its relapse. Treatment should therefore eliminate as many cells as possible, right? Well, chemotherapy has the potency to kill cancer cells; however, the high doses can kill healthy cells and leave adverse side effects. Prolonged exposure may also result in drug resistance. With advancements in cancer research, personalized treatments are employed in the treatment of various diseases [3]. For cancer specifically,

personalized treatments take into account the genetic makeup of a person's tumor and the variabilities in its mutations and environment [4].

When using combination therapies or immunotherapy approaches, we need to consider the efficacy and safety of the treatment. Increasing medical treatments taken at the same time, especially one as aggressive as immunotherapy, has adverse side effects and a high risk of drug toxicity. Alternatively, using single-agent treatments may lack the potency to eliminate cancer cells — the patient then risks disease progression. In this paper, we will evaluate methods of tumor escape and its implications for immunotherapy treatment.

## 2. Mechanisms of Tumor Evasion

Tumor cells are invasive by nature, driven by its need to survive. In order to meet its high proliferation rate, the tumor compensates with a high metabolic rate. The tumor microenvironment (TME) consists of a variety of cells, blood vessels, and the extracellular matrix [1, 5, 6]; these interactions create a unique tumor physiology [7, 8]. Its diverse environment enables

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crosstalks, interconnections among signaling pathways. Malignant cancers will exploit these interactions and reprogram its molecular characteristics and the microenvironment.

## 2.1 Glucose Metabolism

The Warburg effect demonstrates how tumor cells metabolize differently from normal cells. Even in the presence of oxygen, tumor cells favor aerobic glycolysis compared to mitochondrial oxidative phosphorylation (OXPHOS) [5]. Although glycolysis produces less glucose molecules, glucose reuptake occurs at a much faster rate, as well as the production of lactate [5, 9]. This metabolic shift also provides intermediates for lipid and amino acid synthesis, which are critical for sustaining tumor proliferation. The tumor's reprogrammed growth is a form of immunosuppression. Despite the presence of oxygen, normal cells are not able to proliferate and function normally because tumor cells consume nutrients in their microenvironment.

In the beginning stages of tumor growth, before angiogenesis occurs, the tumor cells need to adapt to a hypoxic environment. Metabolic stress and low oxygen conditions can activate hypoxia-inducible factor-1 (HIF-1), which initiates the transcription of genes involved in vascularization [5, 10, 11]. HIF-1 contributes to the reprogramming of a cell's metabolic behavior. By preventing pyruvate's conversion to acetyl-CoA, HIF-1 redirects pyruvate away from the citric acid cycle and into lactate production [5, 9, 12]. Excess lactate accumulation turns the microenvironment acidic — directly impairing cytotoxic T-cell function and creating an immunosuppressive environment that favors tumor progression [5, 13].

## 2.2 Angiogenesis

As the cancerous growth increases in abnormal structure, the environment becomes hypoxic. The mass is too thick for nutrients and oxygen to diffuse through [14]. To overcome this, vascularization or angiogenesis occurs to create blood vessels through the tumor. The observance of angiogenesis often indicates malignant tumor progression [15]. With increased surface area in the epithelial capillaries, there is a higher risk of cancer cells crossing into blood vessels and metastasizing.

Vasculogenesis occurs first, where angioblasts are used to form a primitive vascular plexus. Pro-angiogenic activity in the TME is then mediated by vascular endothelial growth factor (VEGF), which promotes endothelial cell proliferation and migration

into the pre-existing vascular network [10]. Expression of VEGF is regulated by HIF-1, a transcription factor that becomes stabilized under low oxygen conditions and initiates the transcription of angiogenic genes. HIF-1 also upregulates plasminogen activator inhibitor (PAI) 1, which helps break down the extracellular matrix protein [10]. VEGF activity can be observed at the sprouting tips of blood vessels, where endothelial cells extend and branch toward hypoxic regions. These cells continue to proliferate and migrate until a complex, but highly disorganized and leaky vascular network is formed. The tumor has regulations in place to prevent the vessels from becoming too unstable and lead to the vessels collapsing. The progression of cancer is characterized by repeated cycles of hypoxia and subsequent increases of oxygen levels. This growth is perpetuated by angiogenesis and the suppressive microenvironment. The newly formed blood vessels supply nutrients to the microenvironment, which are rapidly consumed by the tumor, necessitating vascular growth. Furthermore, a leaky vascular system can promote tumor escape and metastasis.

## 3. IFN- $\gamma$ and Its Principle of Action

Interferon-gamma (IFN- $\gamma$ ) is an important cytokine in our immune system, produced by activated T lymphocytes in adaptive immunity and natural killer (NK) cells from innate immunity. IFN- $\gamma$  binds to a heterodimeric receptor, composed of two subunits (IFNGR1 and IFNGR2), which activates Janus kinase, JAK1 and JAK2, respectively. This activation leads to the phosphorylation of the signal transducer and activator of transcription (STAT1), which dimerizes and translocates into the nucleus to induce the transcription of IFN- $\gamma$  induced genes that regulate antigen presentation, immune cell amplification, and apoptosis. Among these genes, interferon regulatory factor-1 (IRF-1) upregulates the major histocompatibility complex (MHC) class 1 and 2, which enhances tumor cell recognition. IFN- $\gamma$  not only activates the body's immune response and immune cell differentiation, but also prevents T-cell exhaustion. Now, with IFN- $\gamma$ , the immune system could counteract the tumor's escape mechanisms. Within the TME, pro-inflammatory pathways reinforce the positive feedback loop of IFN- $\gamma$  signaling, primarily through the canonical JAK-STAT pathway, though non-canonical pathways, such as P13K/Akt or MAPK signaling, contribute. The presence of non-canonical pathways may be from crosstalks in our diverse immune responses and tissue-specific conditions.

In the TME, high concentrations of nitric oxide (NO) generated by IFN- $\gamma$ -activated macrophages or

tumor cells expressing elevated inducible nitric oxide synthase (iNOS) exert anti-tumorigenic effects by inducing DNA damage, mitochondrial dysfunction, and apoptosis. Additionally, IFN- $\gamma$  can inhibit angiogenesis by suppressing VEGF expression. Conversely, low levels of NO may have pro-tumorigenic effects, as the stabilization of HIF-1 $\alpha$  promotes angiogenesis.

### 3.1 IFN- $\gamma$ 's Role in Glucose Metabolism

Chattopadhyay et al studied IFN- $\gamma$  concentrations on various in vitro cell lines in order to understand its implications as an immunotherapy. The canonical IFN- $\gamma$  signaling cascade activates the JAK-STAT pathway. Once phosphorylated, STAT1 $\alpha$  induces a primary response with a secondary response driven by IRF-1. Resulting from cross-talks of infiltrating immune cells, IFN- $\gamma$  induces nitric oxide synthase (NOS) 2 expression which biosynthesizes NO, a reactive oxygen species (ROS). Upon IFN- $\gamma$  activation, H6 (hepatoma) tumor cells displayed reduced mitochondrial activity and oxygen consumption, accompanied by an increased glycolytic flux and extracellular acidification. In this experiment, the ROS acts as an anti-tumorigenic effector, causing DNA damage and tumor cell apoptosis.

The changes in metabolism reflect the Warburg effect, in which cells primarily rely on glycolysis, in spite of the presence of oxygen. The data indicates that IFN- $\gamma$ 's signal amplification impaired tumor growth through oxidative stress. This reveals a discrepancy in IFN- $\gamma$ 's function: while promoting pro-inflammatory metabolism, it simultaneously inhibits tumor proliferation.

### 3.2 IFN- $\gamma$ 's Role in Angiogenesis

At the hypoxic core of solid tumors, iNOS activity is generally limited. Under these conditions, low concentrations of NO and ROS stabilizes HIF-1 $\alpha$ , which upregulates glycolytic enzymes, such as HK2. NO and ROS contribute to HIF-1 $\alpha$  accumulation [5, 9]. The shift in metabolism causes the extracellular environment to become acidic, allowing tumors to facilitate matrix degradation, creating possible sites for angiogenesis. ROS-driven stabilization of HIF-1 $\alpha$  promotes glucose reuptake, lactate production, and angiogenesis, all of which are pro-tumorigenic characteristics.

In contrast, IFN- $\gamma$  can also suppress angiogenesis by downregulating VEGF transcription and promoting vessel regression through immune activation and NO signaling. IFN- $\gamma$  can elevate intracellular ROS and reactive nitrogen species to induce apoptosis and

ferroptosis in tumor and stromal cells, while it concurrently stimulates antioxidant defenses to prevent excessive oxidative injury to healthy cells. It also reduces mitochondrial respiration and reinforces glycolytic flux that drives metabolic exhaustion and growth inhibition.

### 3.3 Duality of IFN- $\gamma$

Although IFN- $\gamma$  has been recognized for its anti-tumorigenic properties, there are still experiments that indicate its role in tumor progression. Sun et al. examined the impact of IFN- $\gamma$  on tumor-associated macrophages (TAMs) within the TME. Using a gallbladder cancer model, they demonstrated that IFN- $\gamma$  stimulation enhanced the recruitment of monocytes and macrophages to the tumor site. While monocytes and macrophages are not inherently pro-tumorigenic, within the TME, they can differentiate into angiogenesis promoting TAMs. Interestingly, Sun et al. reported that despite the increased recruitment of monocytes and macrophages, IFN- $\gamma$  treatment significantly reduced their differentiation into TAMs. This suggests that IFN- $\gamma$  may exert an antitumor effect by preventing the polarization of macrophages toward a proangiogenic, tumor-supportive phenotype.

The same immune mechanisms that enable IFN- $\gamma$  to suppress tumors can also facilitate tumor progression, under certain TME conditions. Wawrzyniak and Hartman reviewed multiple studies demonstrating that low-level or chronic exposure to IFN- $\gamma$  during early tumor development can promote immune evasion. Specifically, IFN- $\gamma$  upregulates inhibitory checkpoint inhibitors (ICI) such as programmed death-ligand (PD-L) 1, PD-L2, and cytotoxic T-lymphocyte associated (CTLA) protein 4, which leads to a dampened immune response. The TME can grow desensitized and resistant to IFN- $\gamma$  expression after chronic exposure and signal amplification. It is paradoxical that IFN- $\gamma$  is capable of activating immune responses that suppress tumor growth and can simultaneously induce immune-regulatory pathways that allow cancer cells to escape immune surveillance.

Both Sun et al. and Wawrzyniak and Hartman recommend further evaluation on the behavior of IFN- $\gamma$  and its implications. This uncertainty prompted researchers to develop a more nuanced understanding of IFN- $\gamma$ , whether the medication's effectiveness is dependent on dose, genetic makeup, or the TME.

## 4. IFN- $\gamma$ Immunotherapy

Immunotherapy's goal is to increase the magnitude of the cell's immune response, whether by restoring T-

Cell activity or inhibiting the environment's suppressive characteristics. Strzelec et al. reviewed promising immunotherapies, with a focus on immunomodulators. Progression and growth of cancer cells are driven by dysregulated and amplified positive feedback loops. The multifaceted immune system and its many pathways offer numerous possible targets and treatments — modulating the immune environment to stimulate or inhibit the body's immune system.

In an experiment comparing nivolumab (PD-1 inhibitor) and ipilimumab (CTLA-4 inhibitor) as combination or monotherapy approaches, Wolchock et al. followed up on results after 6 years. For participants in the experimental group (nivolumab and ipilimumab), the frequent reason for discontinuation was study drug toxicity. Those in the monotherapy groups experienced increased rates of drug progression — with a greater number of patients in the ipilimumab alone group. Other immunotherapy research has indicated the presence and increased expression of IFN- $\gamma$ . Although these experiments' focus was also on PD-L1 and CTLA-4 inhibitors, the observed upregulation of IFN- $\gamma$  suggests its potential effectiveness against the elimination of cancer cells. The following clinical trials investigate how IFN- $\gamma$  can be used in clinical settings.

At this time period, the majority of immunotherapy trials in metastatic prostate cancer were uncontrolled compared to our current standard. Gleave et al. 1998 studied the effect of IFN- $\gamma$  in metastatic prostate cancer in a controlled, randomly assigned clinical trial. There were no significant differences in clinical efficacy observed between the experimental and placebo groups; the incidence and severity of adverse events were comparable, with most toxicities being mild to moderate (grade I–II). Despite the lack of results on IFN- $\gamma$ 's effectiveness on treating tumors, this experiment demonstrates the need to continue researching IFN- $\gamma$ 's interaction with tumors.

Reijers et al. proposed using stage III melanoma patient's interferon- $\gamma$  (IFN- $\gamma$ ) gene signature to stratify them into different cohorts. Patients whose tumors exhibited high IFN- $\gamma$  expression were randomized either to nivolumab monotherapy (anti-PD-1) or nivolumab + domatinostat (a class I histone deacetylase inhibitor), whereas low IFN- $\gamma$  tumors were assigned to more aggressive regimens, including nivolumab + domatinostat or triplet therapy (nivolumab + ipilimumab + domatinostat). The goal for this experiment is to see whether IFN- $\gamma$  expression could predict which patients required intensified immunotherapy versus those for whom monotherapy would suffice, with additional assessments including pathologic response and safety. Patients with high IFN- $\gamma$  scores achieved high pathological response rates with nivolumab monotherapy. This indicates that

the patient may not require a harsher, more toxic combination treatment. Interestingly, in low IFN- $\gamma$  expression cohorts there were patients who experienced an increase in IFN- $\gamma$  production. They reported pathologic responses of 50% in arm C (nivolumab + domatinostat) and 80% in arm D (ipilimumab + nivolumab + domatinostat). Those patients with sustained (IFN- $\gamma$  low  $\rightarrow$  low) had a 0% pathological response rate. These results provide compelling clinical evidence for the importance of personalized treatment strategies.

Zibelman et al. conducted a phase I, dose-escalation trial combining interferon-gamma (IFN- $\gamma$ ) with nivolumab in 26 patients with metastatic solid tumors, primarily to evaluate safety and determine a recommended phase II dose (RP2D) of IFN- $\gamma$ . The combination was generally well tolerated, with a notably lower incidence of serious immune-related adverse events (irAEs) compared to the ~10–15% typically observed with nivolumab monotherapy. Zibelman et al. suggests that IFN- $\gamma$ -induced chemokines may help restrain autoimmunity, indicated from this improved safety profile. However, they caution that higher or prolonged IFN- $\gamma$  exposure could lead to T-cell exhaustion, potentially dampening immune responses. Overall, the study demonstrates that this combination therapy shows preliminary signs of clinical efficacy. Limitations of the trial include its small sample size and heterogeneous patient population, which primarily consisted of renal cell, gastroesophageal, breast, and ovarian cancers. Future studies could benefit from focusing on a single tumor type or enrolling a larger cohort to better evaluate efficacy outcomes.

Most colorectal cancers (CRC) are proficient in mismatch repair (pMMR) and poorly responsive to ICIs because they lack immune infiltration and antigenicity. Tang et al. hypothesized that local intratumoral delivery of IFN- $\gamma$  could drive immune infiltration and sensitize tumors to anti-PD1 therapy. The combination of intratumoral IFN- $\gamma$  + anti-PD1 produced significantly greater tumor suppression than either agent alone, indicating a synergistic effect *in vivo* and *in vitro*. The control (anti-PD1 alone) arm showed limited efficacy, consistent with the known resistance of pMMR CRC to checkpoint monotherapy. Compared to that, the experimental arm (IFN- $\gamma$  + anti-PD1) achieved enhanced tumor control and increased T cell infiltration and cytotoxicity — effectively overcoming some intrinsic resistance mechanisms.

Hosseinzadeh et al. investigated whether combining IFN- $\gamma$  with a STING agonist and PD-1 checkpoint blockade could strengthen antitumor immunity in gastric cancer (GC). The rationale stems from the observation that GC has limited T-cell infiltration and moderate responses to PD-1 inhibitors.

The study evaluated cross-talks of the STING pathway and IFN- $\gamma$  signaling in patient samples and GC cell lines, followed by in-vitro and in-vivo murine models testing single agents, dual combinations, and the full triple-therapy regimens. The experimenters noted in co-cultured groups treated with IFN- $\gamma$ , there were increased rates of cell cycle arrest and death—demonstrating IFN- $\gamma$ 's treatment efficacy in combined therapies.

## 5. Conclusion

The number of clinical trials experimenting with IFN- $\gamma$  remains limited. Most studies consist of early-phase designs or small patient cohorts, several of which were conducted years ago or withdrawn due to insufficient efficacy data or toxicity concerns. Despite these constraints, IFN- $\gamma$  continues to represent a critical component of our immune system and a promising immunotherapeutic medication. Current immunomodulatory trials show encouraging outcomes, yet patient selection and individualized treatment planning remain pertinent. Not every medication and treatment will benefit the patient identically. The genetic makeup of a patient's cancer is determinative in their treatment's effectiveness. Personalized medicine allows physicians to tailor therapies according to the patient's tumor characteristics and comorbidities.

Looking forward, further investigation is needed to understand IFN- $\gamma$ 's dosing and its interactions with different tumor variations. Future studies could explore its role in non-metastatic cancers, where early immunomodulation of the TME could potentially be clinically significant. Because IFN- $\gamma$  has been shown to suppress VEGF expression, it may limit angiogenesis, and consequently, metastatic potential. By targeting these early pro-tumor pathways, IFN- $\gamma$  based strategies could not only reduce disease progression, but enhance responsiveness to existing immunotherapy treatments. The cytokine's capability of both suppressing and promoting tumor growth still remains a controversy, but also an opportunity for future research. As our understanding depends, IFN- $\gamma$ 's immunomodulatory potential may significantly refine cancer treatment and improve patient outcomes.

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