

Commentary

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Persistent IFN- γ signaling in acquired resistance to PD-(L)1 blockade in NSCLC

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Abstract

Immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) have transformed the therapeutic landscape of non-small cell lung cancer (NSCLC), producing durable responses in a subset of patients. Yet for most, clinical benefit is undermined by the development of acquired resistance (AR), a phenomenon that continues to limit the long-term success of immunotherapy. Recent analyses have drawn attention to persistent interferon- γ (IFN- γ) signaling as a paradoxical hallmark of AR: a cytokine typically associated with effective antitumor immunity that, when chronically engaged, sustains immune dysfunction. In this commentary, we synthesize existing literature to expand upon this model. We review molecular and cellular mechanisms by which chronic IFN- γ drives resistance through the signal transducer and activator of transcription 1 (STAT1)/interferon regulatory factor 1 (IRF1) axis, epigenetic stabilization of exhaustion, antigen-presentation loss, and metabolic suppression. We extend the discussion to innate immunity, bystander T-cell responses, and stromal regulation, emphasizing spatial heterogeneity as a critical mediator of IFN- γ biology. Finally, we explore translational strategies - including rational checkpoint combinations, radiotherapy-immunotherapy sequencing, epigenetic modulation, and innate immune engagement - that may reprogram IFN- γ -permissive resistance states. We argue that IFN- γ persistence should not be viewed as an isolated mechanism but as a central hub in a broader resistance network, and we propose a phenotype-guided framework for therapeutic intervention in AR NSCLC.

Keywords: Non-small cell lung cancer, PD-1, PD-L1, interferon- γ , acquired resistance, STAT1, IRF1, tumor microenvironment



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INTRODUCTION

The introduction of immune checkpoint inhibitors (ICIs) targeting the programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) axis has transformed the treatment paradigm for advanced non-small cell lung cancer (NSCLC), offering unprecedented improvements in survival and quality of life for a subset of patients^[1-3]. However, the enthusiasm surrounding these therapies has been tempered by the reality that most individuals either fail to respond initially or relapse after a period of clinical benefit. This phenomenon of acquired resistance (AR) represents one of the most pressing challenges in oncology today. Classical models of resistance centered on antigen loss, impaired IFN- γ signaling, or immune-excluded phenotypes. Yet many AR lesions remain highly inflamed, enriched for CXCL9/CXCL10 expression, T-cell infiltration, and immune activation signatures^[1,4,5]. This paradox - “inflamed yet ineffective” - suggests a more complex biology.

Memon *et al.*^[1] crystallized this paradox in their analysis of patient-derived data, describing two major resistance phenotypes: “interferon- γ (IFN- γ)-increased” and “IFN- γ -stable.” Both states are defined by the persistence of IFN- γ signaling within the tumor microenvironment (TME), yet both are associated with therapeutic failure. This observation underscores the ambivalent nature of IFN- γ - essential for initiating antitumor immunity, yet, when chronically sustained, a driver of immune dysfunction, antigenic invisibility, and stromal suppression^[5-7].

This commentary builds upon the work of Memon *et al.*^[1] by broadening the mechanistic lens through which persistent IFN- γ signaling is interpreted. We expand upon the role of the STAT1/IRF1 axis in sustaining inhibitory receptor expression and promoting dysfunctional T-cell states, incorporate emerging evidence on the epigenetic stabilization of exhaustion programs^[5-7], and integrate current insights into antigen-presentation loss, NK-cell suppression, bystander T-cell activity, and stromal-mediated immune mislocalization^[8-12]. We also highlight the limitations of bulk transcriptomic analyses, which obscure spatial and functional heterogeneity within resistant lesions, underscoring the need for spatial profiling and single-cell-resolved approaches^[10-13]. Finally, we review mechanistic and clinical data supporting strategies such as radiotherapy-ICI sequencing, checkpoint diversification, and innate immune engagement^[5,11-14], proposing an integrative framework in which persistent IFN- γ signaling acts as a central hub within the broader resistance network.

IFN- γ -driven resistance phenotypes: increased vs. stable

One of the major insights from Memon *et al.*^[1] is that persistent IFN- γ signaling does not manifest uniformly across resistant NSCLC lesions, as instead, two broad resistance phenotypes can be identified. In the “IFN- γ -increased” phenotype, tumors show progressive amplification of IFN- γ -responsive genes such as CXCL9, CXCL10, GBP family members, and IDO1, indicating ongoing antigenic stimulation and T-cell infiltration despite continued therapeutic failure^[13,14]. In contrast, “IFN- γ -stable” tumors maintain persistently elevated, but non-escalating, IFN- γ transcriptional programs, which is consistent with a chronic cytokine environment that has driven the immune system into a stable, dysfunctional equilibrium^[5-7,14].

These patterns reflect distinct underlying biology. In IFN- γ -increased tumors, continued antigenic engagement promotes escalating inhibitory pathways, including PD-L1 upregulation on tumor and stromal cells, diversification of T-cell inhibitory receptors such as LAG3, TIM-3, and TIGIT, and metabolic suppression through IDO1-dependent tryptophan depletion^[11-15]. In IFN- γ -stable tumors, chronic exposure induces durable epigenetic remodeling, locking T cells into exhaustion states characterized by stable chromatin accessibility at loci such as TOX, NR4A, and PDCD1, limiting reinvigoration even with PD-1 blockade^[5-7,15]. In practice, these states may coexist within different regions of the same tumor, reflecting

significant spatial heterogeneity^[10-13].

From a therapeutic perspective, distinguishing these phenotypes is critical. Tumors with increased IFN- γ activity may respond to strategies targeting feedback inhibition - such as dual checkpoint blockade or metabolic modulation - while stable phenotypes may require interventions that restore antigen presentation or engage innate immunity such as NK-directed therapies^[11-14].

Mechanistic routes from chronic IFN- γ to immune dysfunction

Persistent IFN- γ signaling establishes a multilayered resistance program that undermines PD-(L)1 blockade through transcriptional, epigenetic, antigenic, and metabolic mechanisms. At the center of this process lies the STAT1/IRF1 axis, which normally promotes antigen presentation and chemokine-driven recruitment of effector T cells. Under acute exposure, this axis enhances major histocompatibility complex (MHC) class I (MHC I) expression, mobilizes cytotoxic lymphocytes, and strengthens antitumor immunity^[4-6,11]. However, chronic IFN- γ stimulation alters the equilibrium, producing constitutive STAT1 phosphorylation and sustained IRF1 activity. This prolonged activation drives persistent upregulation of inhibitory receptors such as PD-1, LAG3, and TIM-3, along with negative regulators including suppressor of cytokine signaling 1 (SOCS1), suppressor of cytokine signaling 3 (SOCS3), and protein tyrosine phosphatase non-receptor 2 (PTPN2)^[5-7,11,14]. Rather than fueling effective cytotoxicity, this feedback loop entrenches a dysfunctional phenotype in which T cells remain present but progressively exhausted. The paradox is striking: IFN- γ , once a linchpin of immune activation, becomes a central driver of immune paralysis.

A second layer of resistance emerges at the epigenetic level. Chronic IFN- γ reshapes the chromatin architecture of infiltrating T cells, establishing open regions at exhaustion-defining loci (TOX, NR4A family, PDCD1 enhancers) while closing those associated with effector programs^[5-7,15]. These modifications stabilize a state of functional exhaustion, rendering T cells resistant to reinvigoration even after checkpoint blockade. While interventions such as bromodomain and extraterminal domain (BET) and histone deacetylase (HDAC) inhibitors have demonstrated transient capacity to reverse these patterns in preclinical models, their long-term durability remains uncertain. The evidence suggests that epigenetic “hard-wiring” of exhaustion is a major barrier to restoring durable effector activity in the IFN- γ -persistent state.

The third mechanism involves loss of antigen presentation. Mutations in beta-2-microglobulin (B2M), human leukocyte antigen (HLA) class I disruption, or inactivating mutations in Janus kinase 1 (JAK1)/Janus kinase 2 (JAK2) prevent tumor cells from presenting antigens effectively, even in the face of abundant IFN- γ signaling^[3,4,8]. This disconnect produces a misleading phenotype in which tumors appear inflamed by transcriptomic analysis but remain invisible to CD8⁺ T cells^[3,4,8]. Importantly, such lesions are not “cold” tumors in the traditional sense; rather, they are “inflamed but ineffective,” misleading both clinicians and researchers who rely solely on transcriptional markers.

Finally, IFN- γ persistence drives metabolic counterprogramming that suppresses immune function. Sustained cytokine exposure induces enzymes such as IDO1, depleting tryptophan and producing immunosuppressive kynurenine metabolites^[14,15]. Meanwhile, hypoxia, glucose deprivation, and adenosine accumulation within the TME converge to deprive lymphocytes of metabolic resources necessary for cytotoxic activity. The combination of nutrient scarcity and metabolite-mediated suppression locks effector T cells into an energy-deficient, nonfunctional state^[10,14]. Additionally, IFN- γ -driven upregulation of PD-L1 on dendritic cells has been shown to further suppress T-cell priming and contribute to dysfunctional immune activation, adding another layer to IFN- γ -mediated resistance mechanisms^[16]. Together, these mechanisms illustrate that chronic IFN- γ signaling is not a single failure point but a multidimensional

network of resistance that reinforces itself across transcriptional, epigenetic, antigenic, and metabolic domains.

Beyond T cells: innate immunity, bystander T cells, and stromal circuits

Although CD8⁺ T cells dominate the narrative of checkpoint resistance, IFN- γ persistence also intersects with other immune and stromal actors. NK cells, critical for recognizing MHC I-deficient tumors, can be inhibited by IFN- γ -induced upregulation of HLA-E, which engages the inhibitory receptor NKG2A (NK group 2 member A)^[10-12,14]. In contexts of B2M loss, this axis becomes particularly relevant, as tumors exploit non-classical MHC ligands to suppress innate immunity. Therapeutically, NKG2A blockade or interleukin (IL)-15-based activation strategies may restore NK function^[10,13].

Another overlooked contributor is the population of bystander T cells - clonotypes that are not tumor-specific but retain cytokine-secreting capacity. These cells, often viral-specific or low-affinity, can maintain IFN- γ production without executing cytotoxic programs, artificially inflating inflammatory signatures while diluting true antitumor responses^[6,12].

Finally, stromal and myeloid elements impose structural and chemical barriers that blunt effector function. M2-like macrophages and myeloid-derived suppressor cells secrete transforming growth factor- β (TGF- β), IL-10, and arginase, while cancer-associated fibroblasts remodel the extracellular matrix and generate CXC motif chemokine 12 (CXCL12) gradients that mislocalize T cells^[10,11,13,14]. Together, these circuits decouple cytokine presence from cytotoxic efficacy, sustaining an inflamed but ineffective microenvironment.

Spatial heterogeneity and sampling limitations

An important caveat is that most evidence of IFN- γ persistence comes from bulk RNA sequencing, which averages across spatially diverse niches. Within a single lesion, peripheral regions may display IFN- γ -increased states, while central hypoxic cores manifest IFN- γ -stable exhaustion^[10-13]. Misclassification is therefore a real risk when pre- and post-resistance biopsies are non-colocalized or temporally misaligned with therapeutic transitions^[1,11,14]. Advances in multiplex immunohistochemistry (IHC), spatial transcriptomics, and paired circulating tumor DNA (ctDNA) profiling are beginning to resolve these complexities, enabling more precise dissection of whether IFN- γ reflects productive immunity, exhausted stasis, or bystander activation^[10-13].

TRANSLATIONAL IMPLICATIONS AND THERAPEUTIC STRATEGIES

Recognizing IFN- γ persistence as a hub rather than an isolated mechanism reframes therapeutic decision-making. One strategy is checkpoint diversification, adding LAG3, TIGIT, or TIM-3 blockade to PD-1 inhibition in IFN- γ -increased tumors with receptor diversification^[11,12]. Another is radiotherapy, which can re-prime antigen presentation, release neoantigens, and activate cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes (cGAS-STING) pathways, thereby converting IFN- γ -stable exhaustion into a reprimable state^[5,13]. Epigenetic therapies, including BET and HDAC inhibitors, hold promise for loosening exhaustion-associated chromatin and restoring effector potential when combined with PD-(L)1 blockade^[5-7,15]. These pathways collectively shape dysfunctional CD8⁺ T-cell compartments within resistant tumors^[17].

For tumors with B2M/HLA or Janus kinase (JAK) pathway defects, NK-based approaches - such as NKG2A blockade or IL-15 agonists - may bypass the requirement for classical antigen presentation^[10,13,17]. Finally, metabolic rewiring through IDO1, adenosine, or glycolytic modulation could complement these strategies, although careful biomarker selection is essential given prior mixed trial outcomes^[14,15]. To concisely

integrate the mechanistic and therapeutic dimensions of persistent IFN- γ signaling, Table 1 summarizes the principal resistance pathways, representative biomarkers, and emerging therapeutic strategies implicated in AR to PD-(L)1 blockade in NSCLC.

Evidence base and limitations

Despite expanding insights, much of the evidence supporting IFN- γ persistence as a resistance mechanism derives from bulk transcriptomic inference, which is limited in its ability to resolve functional interactions or spatial heterogeneity within tumors^[10-12]. Functional measurements - such as phospho-STAT1, IRF1 activity, chromatin accessibility, or spatial mapping - are underrepresented in most datasets^[1,4-8].

Additionally, critical contributors such as NK cells, bystander T cells, fibroblasts, and myeloid populations remain incompletely characterized in most resistance analyses^[10-14]. Heterogeneity in biopsy timing, site selection, and sequencing platforms further complicates comparisons across cohorts^[11,12,17].

Single-cell sequencing has demonstrated substantial intratumoral variation in IFN- γ response states^[18], while systemic immune activity is also essential for maintaining antitumor responses to checkpoint blockade^[19]. Moreover, future studies should integrate spatial transcriptomics, single-cell profiling, paired ctDNA analysis, and prospective phenotypic stratification to validate IFN- γ -based resistance states and test phenotype-guided treatment strategies^[10-13,18,19].

CONCLUSION

Persistent IFN- γ signaling has emerged as one of the most defining and paradoxical features of acquired resistance to PD-(L)1 therapy in NSCLC. While acute IFN- γ activity promotes antigen presentation, immune recruitment, and effector activation, chronic IFN- γ signaling becomes maladaptive - driving T-cell exhaustion, diminishing antigen visibility through MHC-I loss, and shaping stromal and innate suppressive programs that collectively blunt therapeutic efficacy^[1,4-7,10-14,17,19].

Moreover, distinguishing IFN- γ -increased from IFN- γ -stable states provides a conceptual framework for tailoring therapeutic approaches, including dual checkpoint blockade, radiotherapy sequencing, epigenetic reprogramming, and NK-cell-directed strategies. Furthermore, aligning interventions with the dominant resistance axis offers a pathway toward more durable and personalized treatment responses.

To fully utilize these insights, the field must move beyond correlational transcriptomics and adopt functionally and spatially resolved analyses that map IFN- γ dynamics in real time. Similar efforts such as these will advance predictive precision, guide adaptive clinical trial design, and ultimately enable the rational manipulation of IFN- γ signaling to restore therapeutic sensitivity^[14,17].

Viewed in this light, IFN- γ persistence is no longer merely a biomarker of resistance but a central biological lever - one that, if properly contextualized and therapeutically engaged, may define the blueprint for overcoming PD-(L)1 failure in NSCLC.

Table 1. Mechanistic axes of persistent IFN- γ signaling and corresponding therapeutic implications in acquired resistance to PD-(L)1 blockade

Mechanistic axis	Representative molecules/pathways	Functional consequence in TME	Potential therapeutic interventions
STAT1/IRF1 transcriptional feedback	STAT1, IRF1, PD-L1, LAG3, TIM-3	Sustained checkpoint expression; effector T-cell exhaustion and diminished cytotoxicity	Dual PD-1 + LAG3 or TIGIT blockade; selective JAK-STAT modulation
Epigenetic stabilization of exhaustion	TOX, NR4A, BATF, EZH2, DNMT3A	Fixed chromatin accessibility at exhaustion loci; loss of effector memory potential	HDAC or BET inhibitors; epigenetic reprogramming combined with ICI
Antigen-presentation disruption	HLA-A, B2M, JAK1/2, TAP1	Reduced MHC-I expression; loss of tumor visibility despite inflammation	STING agonists; NK-cell-based or HLA-independent therapies
Metabolic counterprogramming	IDO1, ARG1, HIF1A, ADORA2A	Nutrient depletion (tryptophan, arginine); adenosine accumulation; immune anergy	IDO1/arginase inhibitors; adenosine receptor antagonists; metabolic adjuvants
Innate and stromal feedback loops	HLA-E/NKG2A, IL-6, TGF- β , CXCL12	NK-cell suppression; fibroblast-derived exclusion zones; immune mislocalization	NKG2A blockade; IL-15 agonists; stromal-targeted therapies; TGF- β inhibition
Phenotypic divergence	IFN- γ -increased vs. IFN- γ -stable signatures	Distinct transcriptional, metabolic, and spatial patterns of resistance	Phenotype-guided therapeutic sequencing; radiotherapy-ICI integration

IFN- γ : Interferon-gamma; PD-(L)1: programmed cell death protein 1/ligand 1; TME: tumor microenvironment; STAT1: signal transducer and activator of transcription 1; IRF1: interferon regulatory factor 1; LAG3: lymphocyte activation gene-3; TIM-3: T cell immunoglobulin and mucin-domain containing-3; TIGIT: T cell immunoreceptor with immunoglobulin and ITIM domain; JAK-STAT: Janus kinase - signal transducer and activator of transcription; TOX: thymocyte selection-associated high-mobility group box; NR4A: nuclear receptor subfamily 4A; BATF: basic leucine zipper ATF-like transcription factor; EZH2: enhancer of zeste homolog 2; DNMT3A: DNA methyltransferase 3A; HDAC: histone deacetylase; BET: bromodomain and extraterminal domain; ICI: immune checkpoint inhibitor; HLA: human leukocyte antigen; B2M: beta-2-microglobulin; JAK: Janus kinase; TAP1: transporter associated with antigen processing 1; MHC-I: major histocompatibility complex class I; STING: stimulator of interferon genes; NK: natural killer; IDO1: indoleamine-2,3-dioxygenase 1; ARG1: arginase 1; HIF1A: hypoxia-inducible factor 1-alpha; ADORA2A: adenosine A2A receptor; NKG2A: NK group 2 member A; IL-6: interleukin-6; TGF- β : transforming growth factor- β ; CXCL12: CXC motif chemokine 12.

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Authors' contributions

Made substantial contributions to the conception and design of the study, as well as to data analysis and interpretation: Karri V, Dalia SM

Performed data acquisition and provided administrative, technical, and material support: Karri V, Dalia SM

Drafted the article, revised it critically for important intellectual content, and approved the final version for publication: Karri V, Dalia SM

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All authors declared that there are no conflicts of interest.

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REFERENCES

1. Memon D, Schoenfeld AJ, Ye D, et al. Clinical and molecular features of acquired resistance to immunotherapy in non-small cell lung cancer. *Cancer Cell.* 2024;42:209-224.e9. DOI PubMed PMC
2. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell.* 2017;168:707-23. DOI PubMed PMC
3. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. *Br J Cancer.* 2018;118:9-16. DOI PubMed PMC
4. Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med.* 2016;375:819-29. DOI PubMed PMC
5. Gao J, Shi LZ, Zhao H, et al. Loss of IFN- γ pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. *Cell.* 2016;167:397-404.e9. DOI PubMed PMC
6. Benci JL, Xu B, Qiu Y, et al. Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. *Cell.* 2016;167:1540-1554.e12. DOI PubMed PMC
7. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science.* 2018;362:eaar3593. DOI PubMed PMC
8. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell.* 2016;165:35-44. DOI PubMed PMC
9. Gettinger SN, Wurtz A, Goldberg SB, et al. Clinical features and management of acquired resistance to PD-1 axis inhibitors in 26 patients with advanced non-small cell lung cancer. *J Thorac Oncol.* 2018;13:831-9. DOI PubMed PMC
10. Thorsson V, Gibbs DL, Brown SD, et al; Cancer Genome Atlas Research Network. The immune landscape of cancer. *Immunity.* 2018;48:812-830.e14. DOI PubMed PMC
11. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science.* 2018;359:1350-5. DOI PubMed PMC
12. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. *Immunity.* 2018;48:434-52. DOI PubMed PMC
13. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β -catenin signalling prevents anti-tumour immunity. *Nature.* 2015;523:231-5. DOI PubMed
14. Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - a target for novel cancer therapy. *Cancer Treat Rev.* 2018;63:40-7. DOI PubMed PMC
15. Peng D, Kryczek I, Nagarsheth N, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature.* 2015;527:249-53. DOI PubMed PMC
16. Oh SA, Wu DC, Cheung J, et al. PD-L1 expression by dendritic cells is a key regulator of T-cell immunity in cancer. *Nat Cancer.* 2020;1:681-91. DOI PubMed
17. Li H, van der Leun AM, Yofe I, et al. Dysfunctional CD8 T cells form a proliferative, dynamically regulated compartment within human melanoma. *Cell.* 2019;176:775-789.e18. DOI PubMed PMC
18. Guo X, Zhang Y, Zheng L, et al. Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing. *Nat Med.* 2018;24:978-85. DOI PubMed
19. Spitzer MH, Carmi Y, Reticker-Flynn NE, et al. Systemic immunity is required for effective cancer immunotherapy. *Cell.* 2017;168:487-502.e15. DOI PubMed PMC