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The Gut–NET–ICB Axis: Microbial Metabolite-Mediated Regulation of NETosis and Implications for Cancer Immunotherapy

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Immune checkpoint blockade (ICB) has transformed oncology, but its efficacy is limited by primary and acquired resistance. The gut microbiome is a established determinant of ICB response, while neutrophil extracellular traps (NETs) have recently emerged as key mediators of an immunosuppressive tumor microenvironment (TME) and ICB resistance. The mechanistic link between these two phenomena remains elusive. We hypothesize that gut microbial metabolites serve as critical systemic messengers that modulate NET formation (NETosis), thereby influencing ICB outcomes.

Methods & Findings: Through a synthesis of recent literature, we delineate a novel "gut-NET-ICB" axis. We provide evidence that specific microbiota-derived metabolites directly regulate NETosis. Short-chain fatty acids (SCFAs) like butyrate and polyamines such as spermidine suppress NET formation by inhibiting histone deacetylases (HDACs), reactive oxygen species (ROS) production, and peptidyl arginine deiminase 4 (PAD4) activity. Conversely, certain secondary bile acids can promote NETosis. We propose a model wherein a favorable gut microbiome generates a metabolite profile that systemically suppresses pathological NETosis, thereby remodeling the TME to enhance CD8+ T-cell infiltration and function, creating a state permissive to ICB. Conversely, dysbiosis fosters a pro-NETotic environment that drives resistance.

Conclusion: The modulation of NETosis by gut microbial metabolites represents a crucial mechanism underlying the microbiome's impact on cancer immunotherapy. This mechanistic insight positions the gut microbiome and NETosis as complementary therapeutic targets. Strategies to promote a NETosis-suppressive metabolite profile through dietary interventions, pre/probiotics, or postbiotic supplements or to directly inhibit NETosis (e.g., with PAD4 inhibitors) hold significant promise for overcoming ICB resistance and improving patient outcomes.

Keywords: Gut microbiome; microbial metabolites; neutrophil extracellular traps (NETs); NETosis; immune checkpoint blockade; cancer immunotherapy; tumor microenvironment.

1. INTRODUCTION

The advent of immune checkpoint blockade (ICB), which targets regulatory pathways such as PD-1/PD-L1 and CTLA-4 to reinvigorate anti-tumor immunity, represents a paradigm shift in oncology (Ribas and Wolchok, 2018). Despite producing remarkable and durable responses in a subset of patients, ICB therapy is hampered by the fact that a significant majority of individuals experience primary or acquired resistance (Mariniello et al., 2025). This stark variability in treatment outcomes has spurred intensive research into the factors that govern ICB efficacy, leading to the seminal discovery that the gut microbiome is a critical determinant of therapeutic success.

Compelling evidence from both preclinical models and clinical studies has established a causal relationship between the composition of the gut microbiota and response to ICB. Fecal microbiota transplantation (FMT) from ICB responders can convert non-responders into responders, demonstrating the microbiome's potent role in modulating the anti-tumor immune response (Routy et al., 2018, Davar et al., 2021). Concurrently, the role of innate immune players in shaping the tumor microenvironment (TME) has gained prominence. Neutrophils, and specifically their ability to release neutrophil extracellular traps (NETs), have been implicated in cancer progression and therapy resistance. NETs are web-like structures composed of decondensed chromatin decorated with histones and cytotoxic granule proteins that are extruded by neutrophils in a process called NETosis (Liu et al., 2025). While physiologically a host defense mechanism, aberrant NET formation in the TME promotes metastasis, angiogenesis, and, crucially, immunosuppression by physically impeding T-cell infiltration and directly suppressing T-cell cytotoxic function (Ayodele, 2025, Teijeira et al., 2020). Recent work has directly correlated elevated levels of NETs with resistance to ICB in both murine models and cancer patients (Yang et al., 2020).

A crucial, yet under-explored, link connecting these two fields is the functional output of the gut microbiome: its dynamic metabolome. Gut bacteria metabolize dietary and host-derived substrates into a vast array of small molecules, including short-chain fatty acids (SCFAs), secondary bile acids, polyamines, and tryptophan metabolites that can enter systemic

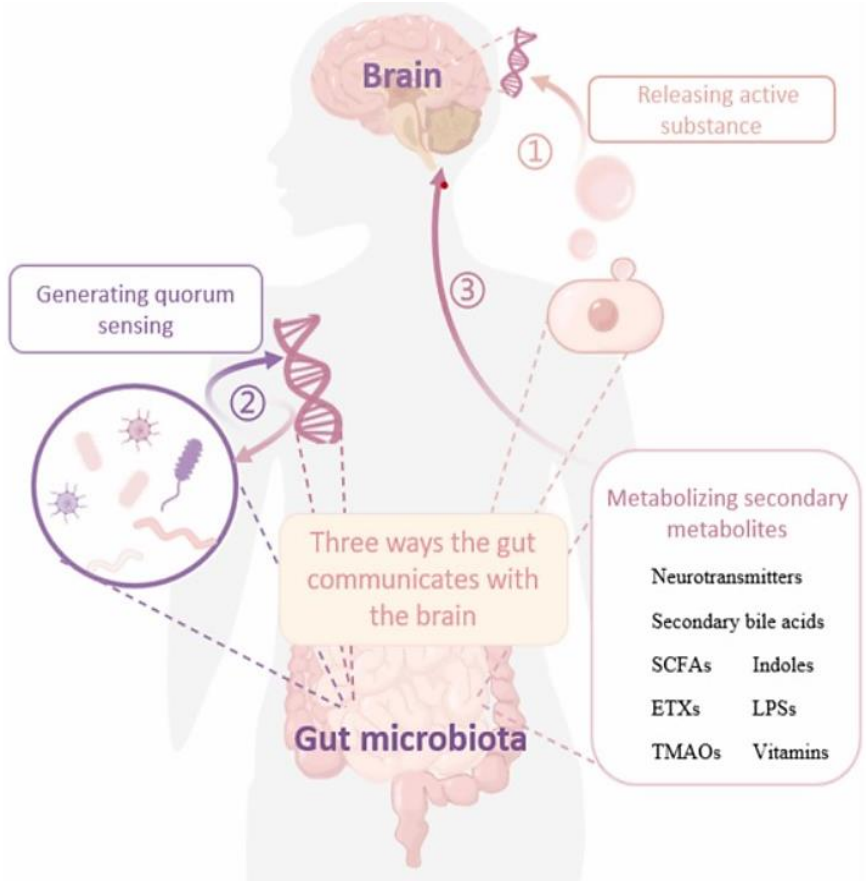

Fig. 1. The Human GUT (Zhang et al., 2025)

circulation and directly influence the function of immune cells, both within and outside the gut (Kaltenmeier et al., 2021, Parada Venegas et al., 2019, Ajibola and Ayodele, 2025, Jia et al., '). We hypothesize that these microbial metabolites serve as systemic rheostats for neutrophil activity and NETosis.

This review aims to synthesize emerging evidence and provide a comprehensive mechanistic framework for the "gut-NET-ICB" axis. We will explore how specific gut microbial metabolites directly and indirectly modulate the molecular pathways of NETosis. Furthermore, we will propose an integrated model wherein a favorable microbiome, through its metabolite profile, suppresses pathological NET formation, thereby remodeling the TME to be more permissive to T-cell-mediated killing and enhancing ICB efficacy. Elucidating this axis not only deepens our fundamental understanding of immunotherapy resistance but also unveils novel therapeutic strategies to modulate the immune system for improved cancer treatment.

2. GUT MICROBIAL METABOLITES: KEY MEDIATORS OF HOST IMMUNITY

The gut microbiome exerts a profound influence on host physiology not merely through its presence but through its immense metabolic activity. It functions as a virtual endocrine organ, converting dietary components and host-derived substances into a diverse array of small molecules that serve as key communicators with the host's immune system (Ayodele, 2025). These metabolites can mediate their effects locally within the gastrointestinal tract or, upon absorption into the portal circulation, systemically throughout the body, thereby influencing immune responses in distant sites, including the tumor microenvironment (TME) (Jia et al., 2018).

Table 1. Key classes of microbiota-derived metabolites and their immunomodulatory mechanisms

Metabolite Class	Key Examples	Bacterial Origin / Precursor	Primary Immunologic Mechanisms	Impact on Immunity
Short-Chain Fatty Acids (SCFAs)	Acetate, Propionate, Butyrate	Fermentation of dietary fiber by genera like Faecalibacterium, Roseburia, Lachnospiraceae (Mager et al., 2020)	Roseburia, Lachnospiraceae (Mager et al., 2020). - HDAC inhibition (Mager et al., 2020) - Signaling via GPCRs (GPR41, GPR43, GPR109a) (Ayodele et al., 2025) - Energy source for colonocytes & immune cells.	Generally Anti-inflammatory: Promote Treg differentiation, maintain gut barrier integrity, modulate neutrophil chemotaxis and function.
Secondary Bile Acids	Deoxycholic Acid (DCA), Lithocholic Acid (LCA), Ursodeoxycholic Acid (UDCA)	Bacterial dehydroxylation of primary bile acids (cholic acid, chenodeoxycholic acid) by species like Clostridium cluster XIVa (Parada Venegas et al., 2019).	- Signaling via nuclear receptor FXR & membrane receptor TGR5 (Ma et al., 2025).	Context-Dependent: Can be pro-inflammatory (DCA) or anti-inflammatory (UDCA). Regulate metabolic homeostasis and macrophage differentiation.
Polyamines	Spermidine, Spermine, Putrescine	Decarboxylation of amino acids (e.g., arginine, ornithine) by bacteria like Bifidobacterium and Lactobacillus (Zhang et al., 2025).	- Regulation of autophagy, translation (via hypusination) (Qi et al., 2025) - Anti-oxidant effects - Modulation of ion channels.	Immunomodulatory: Support T-cell differentiation and function, suppress pro-inflammatory cytokine production, can inhibit NETosis.
Tryptophan Catabolites	Indole, Indole-3-aldehyde, Indolepropionic Acid (IPA)	Tryptophan metabolism by bacteria (e.g., Lactobacillus spp.) expressing tryptophanase (Tofalo et al., 2019).	- Activation of the Aryl Hydrocarbon Receptor (AhR) (Puleston et al., 2019).	Barrier Fortification & Immune Regulation: AhR activation strengthens epithelial barriers, modulates Th17/Treg balance, and suppresses innate immune activation.
Other Bioactive Metabolites	Inosine, D-lactate, Trimethylamine N-oxide (TMAO)	Purine metabolism (Inosine), fermentation (D-lactate), choline/carnitine metabolism (TMAO).	- Adenosine A2A receptor signaling (Inosine) (Kullberg et al., 2025) - Precursor to pro-atherogenic TMAO.	Variable: Inosine enhances Th1 differentiation and ICB response; TMAO is generally pro-inflammatory and linked to cardiovascular disease.

2.1 Short-Chain Fatty Acids (SCFAs): Microbial Fermentation Products with Systemic Reach

SCFAs are the most extensively studied microbial metabolites. Butyrate is a primary energy source for colonocytes, crucially maintaining gut barrier function and preventing systemic inflammation. Beyond this local role, SCFAs function as potent epigenetic regulators via HDAC inhibition, leading to increased histone acetylation and altered gene expression in immune cells (Ajibola and Ayodele, 2025, Mager et al., 2020). This mechanism promotes the differentiation of regulatory T cells (Tregs), which are critical for maintaining immune tolerance. SCFA signaling through GPCRs like GPR43 on neutrophils can also dampen their inflammatory potential and chemotaxis, representing a direct pathway for systemic immunomodulation relevant to NETosis (Ayodele et al., 2025).

2.2 Secondary Bile Acids (BAs): From Digestion to Immunomodulation

Primary bile acids, synthesized in the liver, are transformed into secondary bile acids by gut bacteria. This process significantly alters their signaling properties. Secondary BAs act as signaling molecules through the nuclear receptor FXR and the G-protein-coupled receptor TGR5, regulating not only metabolic homeostasis but also immune cell function (Ma et al., 2025). For instance, specific secondary BAs can promote the differentiation of anti-inflammatory macrophages, while others, in excess, can be cytotoxic and induce inflammatory stress responses.

2.3 Polyamines: Crucial for Cellular and Immune Homeostasis

Polyamines are essential for cell proliferation and are produced by both mammalian cells and gut bacteria. Bacterial-derived polyamines can influence host immunity by modulating autophagy, a process critical for immune cell function and antigen presentation (Qi et al., 2025). Spermidine, in particular, has been shown to have anti-inflammatory and life-span-extending effects, partly through its ability to induce autophagy and suppress oxidative stress.

2.4 Tryptophan Metabolites: AhR Ligands and Barrier Regulators

Gut bacteria that metabolize the essential amino acid tryptophan into ligands for the AhR play a vital role in immune education and barrier integrity. AhR activation in intestinal epithelial cells and intraepithelial lymphocytes strengthens the mucosal barrier and helps maintain a balanced immune response, preventing aberrant inflammation (Puleston et al., 2019). The systemic immunologic impact of these metabolites is an area of active investigation.

The gut microbial metabolome constitutes a critical interface between the external environment and the host immune system. The balance and composition of these metabolites directly influence the functional state of innate and adaptive immune cells, creating a systemic milieu that can either favor or suppress anti-tumor immunity. The subsequent sections will delve into how these specific metabolites interact with the process of NETosis to shape this immunologic landscape (Puleston et al., 2019).

3. MECHANISTIC INTERPLAY: MICROBIAL METABOLITES AS REGULATORS OF NETOSIS

The central hypothesis of the gut-NET-ICB axis posits that gut microbial metabolites systemically influence the propensity of neutrophils to undergo NETosis, thereby shaping the immune landscape of the tumor microenvironment (TME). This interaction is not merely correlative; a growing body of evidence demonstrates direct and indirect mechanistic pathways through which these bacterial byproducts modulate the molecular machinery of NET formation. The balance between NETosis-suppressive and NETosis-promoting metabolites can critically determine the outcome of anti-tumor immunity (Ma et al., 2025).

3.1 Suppressive Metabolites: SCFAs and Polyamines as NETosis Brakes

Short-Chain Fatty Acids (SCFAs): Butyrate has been demonstrated to robustly inhibit NETosis induced by various stimuli, including PMA and LPS, in human neutrophils in vitro (Zhang et al., 2025). The primary mechanism is epigenetic: as potent HDAC inhibitors, SCFAs lead to hyperacetylation of histones. This increased negative charge makes the chromatin less accessible to PAD4, thereby preventing the histone citrullination that is essential for chromatin decondensation. Furthermore, SCFA signaling through GPR43 on the neutrophil surface directly suppresses phorbol ester-induced ROS production, dismantling a critical trigger for the NETotic cascade (Cai et al., 2025).

Table 2. Mechanisms of Gut Microbial Metabolites in Regulating NETosis

Metabolite Class	Net Effect on NETosis	Direct Molecular Mechanisms	Indirect Immunological Mechanisms
Short-Chain Fatty Acids (SCFAs)	Potently Suppressive	- HDAC Inhibition: Hyperacetylation of histones and other proteins interferes with PAD4 activity and chromatin decondensation (Zhang et al., 2025). - GPCR (GPR43) Signaling: Downregulates NADPH oxidase assembly and ROS production, a key trigger for suicidal NETosis (Cai et al., 2025). - Inhibition of NF-κB Pathway: Reduces transcription of pro-inflammatory cytokines that prime neutrophils.	- Promotes differentiation of anti-inflammatory Tregs, reducing the cytokine drive for NETosis. - Enhances gut barrier integrity, reducing systemic translocation of LPS, a potent NETosis inducer.
Polyamines	Potently Suppressive	- Direct Inhibition of PAD4: Competitively binds to the enzyme's active site, preventing histone citrullination (Perrone and D'Angelo, 2025). - Suppression of ROS: Inhibits the assembly and function of the NADPH oxidase complex. - Induction of Autophagy: Autophagic flux can clear damaged mitochondria (mitophagy), preventing mtROS-induced NETosis (Saha and Goswami, 2025).	- Modulates macrophage polarization towards an M2-like, anti-inflammatory phenotype. - Supports T-cell function, creating a cytokine milieu less conducive to persistent neutrophil activation.
Tryptophan Metabolites (AhR Ligands)	Suppressive	- AhR Activation: AhR signaling in neutrophils directly upregulates anti-apoptotic genes and downregulates pro-inflammatory pathways, raising the activation threshold for NETosis (Zhao et al., 2025).	- AhR activation in dendritic cells and T cells promotes immune tolerance and a balanced cytokine response. - Strengthening of the gut barrier reduces systemic inflammatory tone.
Secondary Bile Acids	Context-Dependent (Often Promotive in Dysbiosis	- Mitochondrial Stress: High concentrations of DCA act as detergents, disrupting mitochondrial membranes and inducing mitochondrial ROS (mtROS), which drives NETosis (Zhao et al., 2025). - NLRP3 Inflammasome Activation: Can prime and activate the inflammasome in immune cells, leading to IL-1β release, a potent NETosis trigger.	- Certain BAs can have anti-inflammatory effects. An imbalanced ratio of pro- to anti-inflammatory BAs creates a systemic environment that favors neutrophil activation.

Polyamines: Spermidine emerges as a powerful, direct inhibitor of NETosis. It exerts its effect by targeting two key nodes in the process. First, it directly inhibits PAD4 enzymatic activity, preventing the initiation of chromatin decondensation (Perrone and D'Angelo, 2025). Second, it suppresses the generation of ROS by the NADPH oxidase complex. Additionally, its well-established role as an inducer of autophagy (Saha and Goswami, 2025, Lu et al., 2024) helps maintain cellular homeostasis in neutrophils by clearing damaged organelles that could otherwise serve as intrinsic inducers of NETosis.

3.2 Inductive Metabolites: The Detrimental Role of Dysbiosis

Secondary Bile Acids: In a state of dysbiosis, an overabundance of certain secondary bile acids like deoxycholic acid (DCA) can have the opposite effect. At high concentrations, DCA can disrupt mitochondrial membranes in neutrophils, leading to the leakage of mitochondrial DNA and the generation of mitochondrial ROS (mtROS). This mtROS can act as a potent trigger for NETosis, independent of or synergistic with the NADPH oxidase pathway (Saha and Goswami, 2025). This highlights that the impact of microbial metabolites is not universally beneficial and is entirely dependent on the compositional and metabolic balance of the gut community.

3.3 Indirect Modulation via the Tumor Microenvironment

Beyond direct actions on neutrophils, microbial metabolites sculpt the broader TME, creating feedback loops that influence NETosis. For instance, SCFAs and inosine promote the activation and tumor infiltration of CD8+ T cells and Th1 cells (Zhao et al., 2025). These activated T cells produce IFN-γ, which can have complex effects on neutrophils but can also contribute to an anti-tumor immune cycle that suppresses the pro-tumorigenic functions of neutrophils, including excessive NET formation. Conversely, a metabolite profile that favors Treg expansion and M2 macrophage polarization can create an immunosuppressive environment that indirectly permits sustained NETosis.

Gut microbial metabolites function as master regulators of NETosis through a multi-pronged strategy involving direct epigenetic and enzymatic interference, receptor-mediated signaling, and systemic modulation of inflammatory tone. A favorable microbiome generates a metabolite profile that acts as a "brake" on pathological NETosis, while a dysbiotic microbiome often releases the "accelerator," contributing to an ICB-resistant TME (Saha and Goswami, 2025, Korbecki et al., 2025).

4. THE GUT-NET-ICB AXIS: AN INTEGRATED MODEL

We propose a comprehensive model wherein the gut microbiome dictates clinical response to Immune Checkpoint Blockade (ICB) through the systemic regulation of Neutrophil Extracellular Trap (NET) formation, with microbial metabolites serving as the primary signaling intermediaries. This "Gut-NET-ICB Axis" provides a mechanistic framework to explain how the intestinal microbiota can influence anti-tumor immunity in distant tissues. The model pivots on the balance between two contrasting states: a NETosis-Suppressive, ICB-Permissive state and a NETosis-Promotive, ICB-Resistant state.

The following table outlines the contrasting characteristics of these two states within our integrated model.

4.1 The ICB-Permissive Cycle: A Favorable Microbiome Suppresses Nets

In this virtuous cycle, a diverse and balanced gut microbiome, characterized by taxa such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and Bifidobacterium spp., generates a metabolite profile rich in SCFAs, polyamines, and AhR ligands.

Metabolite Production: These microbes ferment dietary fiber to produce high levels of butyrate and other SCFAs. They also generate immunomodulatory polyamines like spermidine and tryptophan-derived AhR ligands.

Systemic Signaling: These metabolites are absorbed into the portal circulation and exert systemic effects. They reach the bone marrow and the TME, where they encounter neutrophils.

NETosis Suppression: As detailed in Section 4, these metabolites directly "raise the threshold" for NETosis. Butyrate (via HDACi) and spermidine (via PAD4 inhibition) disrupt the core biochemical pathways required for chromatin decondensation and NET release.

TME Remodeling: The consequent low level of NETs in the TME dismantles a major immunosuppressive barrier. CD8+ T cells can effectively infiltrate the tumor parenchyma, encounter their antigen, and execute cytotoxic functions without being suppressed by NET-derived components like histones and proteases.

Effective ICB Response: In this permissive environment, the administration of ICB can effectively reinvigorate these functional, tumor-infiltrating T cells, leading to robust tumor control and clinical response (Zhao et al., 2016, Mager et al., 2020, Xi et al., 2022).

4.2 The ICB-Resistant Cycle: Dysbiosis Promotes Net-Driven Immuno-suppression

Conversely, a state of dysbiosis, potentially driven by antibiotics, a Western diet, or disease, initiates a vicious cycle that culminates in ICB resistance.

Dysbiosis and Metabolite Imbalance: The gut community is depleted of beneficial metabolite producers. This leads to low systemic levels of SCFAs and spermidine. Concurrently, there may be an expansion of bacteria that produce pro-inflammatory metabolites or, through a weakened gut barrier, allow the translocation of microbial products like Lipopolysaccharide (LPS) (Ajibola and Ayodele, 2025).

Loss of NETosis Suppression: The absence of the "NETosis brake" (SCFAs, spermidine) leaves neutrophils hypersensitive to activation signals. Furthermore, circulating LPS and certain secondary bile acids provide direct triggers for NETosis.

NET-Driven Immunosuppressive TME: Neutrophils in the TME readily undergo NETosis, leading to high NET density. These NETs physically trap T cells and prevent their infiltration (Deng et al., 2025). More critically, NET components have been shown to directly promote T cell exhaustion by upregulating PD-1 expression and suppressing their proliferative and cytotoxic capacity (Cai et al., 2025).

ICB Failure: In this scenario, the TME is characterized by excluded and profoundly exhausted T cells. Even when PD-1 is blocked by ICB, the T cells are too dysfunctional or physically sequestered to mount an effective anti-tumor response, resulting in primary therapeutic resistance.

Table 3. The Two States of the Gut-NET-ICB Axis

Feature	NETosis-Suppressive, ICB-Permissive State	NETosis-Promotive, ICB-Resistant State
Gut Microbiome Profile	"Favorable" / Eubiotic. Enriched with SCFA-producers polyamine-producers, and AhR-ligand generators.	"Unfavorable" / Dysbiotic. Enriched with pathobionts, reduced microbial diversity, and bacteria producing pro-inflammatory metabolites.
Systemic Metabolite Profile	High circulating levels of Butyrate, Propionate, Spermidine, and Indoles. Balanced secondary bile acid ratio.	Low levels of beneficial metabolites. High levels of pro-inflammatory secondary bile acids.
Impact on Neutrophils & NETosis	Suppressed Pathological NETosis. Metabolites directly inhibit PAD4, reduce ROS, and block HDACs. Neutrophils are less prone to excessive NET release.	Promoted Pathological NETosis. Lack of suppression and presence of inflammatory inducers lead to rampant NET formation.
Tumor Microenvironment (TME)	- Low NET Density - Enhanced T-cell Infiltration: Physical barrier is removed. - Functional T-cells: Reduced T-cell exhaustion and impairment. - Favorable CD8+/Treg ratio.	- High NET Density - Impaired T-cell Infiltration: NETs form a physical barrier. - T-cell Exhaustion: NET components directly suppress T-cell function. - Immunosuppressive milieu.
Final ICB Outcome	Responsive. Checkpoint inhibitors effectively reinvigorate pre-existing, functional, and infiltrated T cells, leading to tumor cell killing.	Refractory. T cells are excluded, dysfunctional, and exhausted; ICB fails to initiate an effective anti-tumor response.

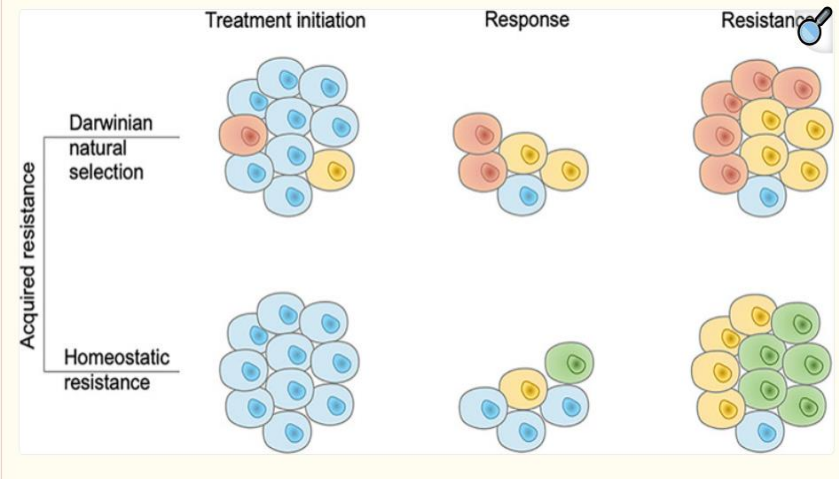

Fig. 2. Acquired resistance to immune checkpoint blockades (Bi et al., 2025)

4.3 Therapeutic Implications of the Axis

This model directly suggests novel therapeutic strategies aimed at interrupting the resistant cycle and promoting the permissive one. These interventions can target different nodes of the axis:

Targeting the Gut: Using prebiotics, probiotics, or FMT to establish a NETosis-suppressive microbiome.

Targeting the Metabolite: Using postbiotic supplements to directly deliver the inhibitory signals.

Targeting NETosis: Using pharmacological PAD4 inhibitors (e.g., GSK484) or DNase I to directly prevent NET formation or degrade existing NETs, thereby breaking the immunosuppressive barrier and potentially synergizing with ICB (Bi et al., 2025, Zhang et al., 2025).

The Gut-NET-ICB Axis provides a powerful, mechanistic model that integrates the fields of microbiome research, neutrophil biology, and cancer immunotherapy (Zhou et al., 2021). It explains how a systemic factor of the gut microbiome can locally determine treatment efficacy by controlling a specific innate immune process, NETosis, that directly shapes the adaptive anti-tumor response (Cai et al., 2025, Pirini et al., 2025).

5. CONCLUSION

The intricate interplay between the gut microbiome, host immunity, and cancer therapy continues to redefine our understanding of oncobiology. This review has synthesized compelling evidence to establish a novel and critical pathway: the Gut-NET-ICB Axis. We have delineated a mechanistic framework wherein gut microbial metabolites serve as pivotal systemic regulators of neutrophil extracellular trap (NET) formation, which in turn exerts a profound influence on the efficacy of immune checkpoint blockade (ICB).

The model posits two divergent states. A favorable gut microbiome, characterized by producers of short-chain fatty acids (SCFAs), polyamines, and AhR ligands, generates a metabolite profile that systemically suppresses pathological NETosis. This creates a tumor microenvironment (TME) that is permissive to T-cell infiltration and function, thereby enabling a robust response to ICB. Conversely, a state of dysbiosis fails to provide these suppressive signals and may even promote NETosis, leading to a NET-rich, immunosuppressive TME that fosters T-cell exclusion and exhaustion, ultimately resulting in ICB resistance.

This paradigm shift from viewing the microbiome as a simple correlate to understanding it as an active modulator of specific innate immune effector mechanisms like NETosis has significant implications. It moves the field beyond taxonomic associations and towards a functional, metabolite-driven understanding of therapy response. The axis provides a unifying explanation for how a distal community of microbes can precisely tune the immune landscape of a distant tumor.

Looking forward, this knowledge opens up a promising frontier for clinical translation. The Gut-

NET-ICB axis is not merely a explanatory model; it is a roadmap for therapeutic innovation. Strategies targeting the gut microbiome, its metabolic output (postbiotics), or the downstream NETotic process itself (PAD4 inhibitors, DNase) represent tangible, combinatorial approaches to overcome immunotherapy resistance. Future research must focus on validating these mechanistic links in human cohorts, identifying predictive metabolite and NET-based biomarkers, and launching clinical trials that strategically manipulate this axis. By harnessing the power of the gut microbiome to disarm a key mechanism of immunosuppression, we can ultimately expand the reach and success of cancer immunotherapy for a greater number of patients.

5.1 Future Perspectives

The elucidated Gut-NET-ICB axis provides a powerful, mechanistic framework that connects the gut microbiome's metabolic output to a specific innate immune process, NETosis, which critically determines the efficacy of adaptive immunotherapy. This model not only deepens our fundamental understanding of ICB resistance but also unveils a suite of novel, actionable strategies to improve clinical outcomes. Based on the evidence synthesized in this review, we put forth the following recommendations for future research and clinical translation:

1. Prioritize Functional Metabolomics in Clinical Cohorts: Future clinical studies correlating the microbiome with ICB response must move beyond 16S rRNA sequencing and integrate deep metagenomics with metabolomic profiling of patient serum and feces. The primary goal should be to define a "NETosis-Suppressive Metabolite Signature" that can serve as a superior predictive biomarker compared to taxonomic data alone.

2. Establish Causality in Gnotobiotic Models: To move from correlation to causation, well-defined gnotobiotic mouse models should be employed. Colonizing germ-free mice with consortia of bacteria engineered to produce specific metabolites (e.g., SCFA-producers versus non-producers) and subjecting them to ICB and NETosis assays will provide direct proof of the metabolite-NET link.

3. Explore Combinatorial Clinical Trials: The most immediate translational path involves designing clinical trials that combine ICB with interventions targeting the Gut-NET axis. We recommend prioritizing:

ICB + Pre/Postbiotics: Testing high-fiber diets or SCFA/spermidine supplements in conjunction with anti-PD-1 therapy.

ICB + NETosis Inhibition: Investigating the safety and efficacy of PAD4 inhibitors or DNase I in overcoming resistance in selected cancer types.

4. Develop Standardized NET Biomarkers: The field requires a concerted effort to standardize the measurement of NETs in human samples. Validated assays for circulating NET remnants are needed to correlate systemic NET load with metabolite levels and ICB response in patient cohorts.

5. Personalize Microbiome-Modulating Therapies: Recognizing that a "one-size-fits-all" microbiome intervention may be ineffective, we recommend developing diagnostic platforms to match patients with dysbiotic, NETosis-promoting profiles to tailored interventional strategies, such as FMT from donors with a validated beneficial metabolome.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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