

REVIEW ARTICLE

Neutrophil extracellular traps: A new frontier in breast cancer prognostics and therapy

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Abstract

Breast cancer has become the most frequently diagnosed malignancy worldwide, and its lethality is driven by cancer cells with high invasiveness, metastatic potential, and resistance to therapy. Neutrophil extracellular traps (NETs), web-like structures released by activated neutrophils and composed of histones, antimicrobial proteins, and DNA, play crucial roles in tumor metastasis and progression. In recent years, substantial efforts have been devoted to the development and validation of molecular biomarkers. These biomarkers can provide prognostic information and, more importantly, predict response to therapy. Multigene assays are increasingly being incorporated into the routine management of patients with early-stage breast cancer; however, their high cost remains a major barrier to widespread implementation. Therefore, there is an urgent need to develop and validate inexpensive, simple prognostic biomarkers. Among low-cost biomarkers, Ki-67 is one of the most widely used; however, inter-laboratory reproducibility of Ki-67 assessment is poor, and there is no universally accepted, clinically actionable cutoff. Emerging evidence increasingly indicates that NETs are closely associated with tumor dissemination, immune modulation, and the reactivation of dormant cancer cells. They are often regarded as significant correlates in the mechanisms of tumor progression. However, their validity as clinically actionable prognostic biomarkers or therapeutic targets in breast cancer remains to be further validated. This review systematically summarizes the molecular mechanisms proposed to link NETs with breast cancer progression and outlines their potential clinical implications within current evidentiary limits.

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1. Introduction

According to estimates from the Global Cancer Observatory (GLOBOCAN) 2022, breast cancer ranks among the most frequently diagnosed malignancies worldwide and is the fourth leading cause of cancer-related death, largely attributable to cancer cells with high invasiveness, strong metastatic potential, and resistance to therapy.¹ The advent of targeted therapies has substantially improved the overall survival of patients with breast cancer; however, effective treatment options for triple-negative breast cancer (TNBC) remain limited. Trastuzumab, an anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibody approved by the United States (US) Food and Drug Administration (FDA), is the most widely used targeted agent for HER2-positive (HER2+) breast cancer

and has achieved considerable success as standard therapy for HER2+ disease.^{2,3} Nevertheless, accumulating clinical evidence indicates marked heterogeneity in the response of HER2+ breast cancer to trastuzumab, with more than 50% of patients showing primary non-response or eventually developing resistance, leading to disease recurrence and progression.^{4,5} Conventional markers, such as Ki-67, have some value for prognostic stratification in early-stage estrogen receptor-positive/HER2-negative (ER+/HER2-) breast cancer, but substantial variability across laboratories and scoring methods limits their general applicability.^{6,7} Therefore, there is an urgent need to develop molecular biomarkers capable of predicting therapeutic efficacy and prognosis and to identify new therapeutic targets for breast cancer.

Neutrophils have traditionally been regarded as key cells in acute inflammation.^{8,9} They can rapidly eliminate pathogens through phagocytosis and can also exert antimicrobial effects by releasing granule enzymes, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs).^{10,11} NETs are complex web-like structures released by activated neutrophils and composed of histones, antimicrobial proteins, and deoxyribonucleic acid (DNA). Multiple studies have reported associations between elevated circulating NET markers and prognosis, metastasis, or therapeutic resistance in cancer, supporting their investigation as candidate diagnostic or prognostic correlates; however, specificity and inflammatory confounding require careful, disease-context validation.^{12,13} The persistently high global incidence and mortality of breast cancer are largely driven by the lack of effective therapies for TNBC and treatment resistance in HER2+ breast cancer.¹⁴ Consequently, the identification of new therapeutic approaches and prognostic biomarkers in breast cancer has become a central focus.¹⁵

Despite the rapidly expanding literature on NETs in cancer biology, the direct applicability of many NET-associated mechanisms to breast cancer remains incompletely defined. A substantial proportion of mechanistic insights into NET formation and function originates from studies in infectious diseases, inflammatory disorders, and non-breast cancer solid tumors. However, breast cancer represents a biologically heterogeneous disease characterized by marked molecular subtype diversity, distinct hormonal milieus, and organ-specific metastatic patterns. These features introduce disease-specific contexts in which NET-driven pathways may operate differently or exert non-uniform effects. Consequently, extrapolation of NET biology from other tumor entities to breast cancer should be approached with caution, and conclusions drawn without explicit consideration of disease-specific risk

overstating mechanistic certainty. Rather than providing an exhaustive catalog of all NET-associated molecules, this review adopted a selective, concept-driven approach. We focused on NET components and signaling axes for which at least one level of breast cancer-relevant evidence exists and that converge on key biological processes central to breast cancer progression, including tumor invasion, immune modulation, and metastatic dissemination. This strategy prioritizes mechanistic coherence and translational relevance over comprehensive coverage, recognizing that many NET-related pathways remain insufficiently validated in breast cancer-specific settings.

Guided by this framework, each subsequent section first outlines a proposed NET-associated mechanism and then explicitly weighs the supporting evidence according to source: human breast cancer samples or cohorts, murine breast cancer models, in vitro breast cancer assays, and extrapolation from other malignancies and inflammatory systems (Table 1). Throughout the review, causal language is reserved for breast cancer studies with direct functional perturbation, such as deoxyribonuclease (DNase)-mediated NET disruption, peptidylarginine deiminase 4 (PAD4) inhibition, and pathway blockade with phenotypic reversal, whereas correlative datasets are described as associations. We also integrated subtype context, model constraints, and assay heterogeneity into the interpretation of each mechanistic axis.

To improve analytical clarity, we applied the same evidence hierarchy across the manuscript. This framework does not rank biological importance alone; rather, it indicates how directly a claim has been tested in breast cancer-relevant systems. Claims supported mainly by extrapolated evidence were discussed as hypothesis-generating, whereas claims supported by interventional murine or in vitro breast cancer studies were presented as mechanistically stronger but still not equivalent to validated clinical causality.

2. Formation and functions of neutrophil extracellular traps

Neutrophil extracellular traps are composed of DNA, histones, and granule proteins, including neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, and other enzymatically active proteins.¹⁶ Current evidence indicates that NET formation occurs mainly through two pathways. The first is referred to as suicidal or lytic NETosis, which involves stimulation of protein kinase C (PKC) and rapidly accelerated fibrosarcoma (Raf)-mitogen-activated protein kinase kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling, leading to activation of NADPH oxidase (NOX) and the generation of ROS in neutrophils.^{17,18} ROS

Table 1. Evidence stratification of major NET-related claims in this review

Claim	H	M	I	E	Interpretation in this review
Elevated circulating or tissue NET burden is associated with adverse outcomes	Limited	Yes	Limited	Moderate	Plausible prognostic signal, but specificity and thresholds remain unsettled.
NE-containing NET-rich microenvironments reinforce invasion or EMT-like programs	Indirect	Yes	Yes	Extensive	Mechanistically plausible; human breast cancer causality not established.
NET-associated PD-L1 suppresses CD8+ T-cell function	Limited	Yes	Yes	Moderate	Strong preclinical rationale, sparse patient-level validation.
IL-8/CXCR1/CXCR2 signaling recruits neutrophils and amplifies NET–tumor crosstalk	Moderate	Yes	Yes	Extensive	Biologically consistent but pleiotropic; NET-specific contribution must be separated from broader IL-8 effects.
PAD4 inhibition or DNase-mediated NET targeting lowers metastatic burden	Minimal	Yes	Limited	Moderate	The best current interventional evidence is preclinical.
NET scaffolds capture circulating tumor cells through adhesion receptors	Minimal	Yes	Yes	Extensive	Strong experimental concept; human breast cancer vascular validation remains limited.
NET-derived analytes may support clinical stratification	Limited	Not primary	Assay-focused	Moderate	Candidate biomarkers, not standardized clinical tools.

Notes: H: Human breast cancer samples or cohorts; M: Murine breast cancer models; I: In vitro breast cancer assays; E: Extrapolated evidence from non-breast cancer or non-oncologic systems. Claims with strong M or I support, but limited H support, are interpreted as mechanistically plausible rather than clinically established.

Abbreviations: CXCR1/2: C–X–C chemokine receptor 1/2; DNase: Deoxyribonuclease; EMT: Epithelial–mesenchymal transition; IL-8: Interleukin 8; NE: Neutrophil elastase; NET: Neutrophil extracellular trap; PAD4: Peptidylarginine deiminase 4; PD-L1: Programmed death-ligand 1.

disrupt the integrity of granule and nuclear membranes, resulting in the release of NE and MPO, which are normally confined within azurophilic granules, thereby promoting histone cleavage and chromatin decondensation.¹⁹ This cascade ultimately leads to loss of neutrophil plasma membrane integrity, and the decondensed DNA is released into the extracellular space, together with granule contents, to form NETs.²⁰

The second pathway represents a non-lytic form of NETosis, which involves the mobilization of nuclear chromatin and the release of granule proteins through degranulation.²¹ This type of NETosis is independent of cell death, occurs more rapidly, typically within minutes, and can be triggered by activated platelets, microorganisms, and complement proteins.^{22–24} In contrast to the lytic pathway, non-lytic NETosis does not depend on NOX activity, and DNA-containing nuclear vesicles are extruded without disruption of the plasma membrane, thereby preserving neutrophil function²⁵ (Figure 1).

3. Components of neutrophil extracellular trap and their association with breast cancer

3.1. Neutrophil elastase

Neutrophil elastase is a major serine protease released by neutrophils and is frequently enriched within NETs.

It is an important mediator of chronic inflammation and tumor progression and plays a critical role in the initiation and development of breast cancer.^{26,27} NE is best viewed as a candidate effector within a broader neutrophil and NET program rather than as a fully validated standalone driver, because clinical studies usually measure intratumoral NE, neutrophil infiltration, or circulating NET markers in combination rather than isolating NE-specific activity.²⁸

Breast cancer-relevant experimental systems provide mechanistic support for NE-mediated malignant phenotypes. In cell lines and murine models, exogenous or neutrophil-derived NE can be internalized by tumor cells, reduce insulin receptor substrate 1 (IRS-1) abundance, and enhance phosphoinositide 3-kinase (PI3K)–Akt signaling, a pathway also implicated in therapy resistance.^{29–31} The enzymatic activity of NE is also linked to the regulation of apoptosis, mainly through disruption of cell-cycle regulatory proteins and consequent suppression of intracellular death pathways.^{32,33} In breast cancer cells, NE activity has been implicated in apoptosis regulation, in part by perturbing cell cycle regulatory proteins and death signaling pathways. Caruso *et al.*³⁴ reported that endogenous NE inhibitors can trigger retinoblastoma (Rb)-dependent growth arrest and induce caspase-dependent apoptosis in breast cancer cells, suggesting that inhibition of NE may shift tumor cells from a proliferative toward an apoptotic state.

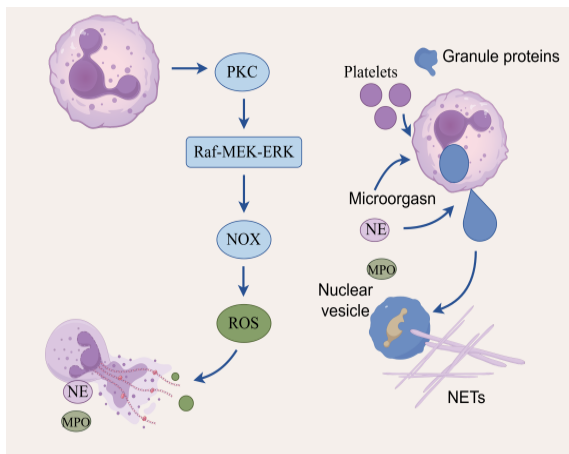


Figure 1. NETs are web-like extracellular structures composed of decondensed DNA, histones, and neutrophil granule enzymes (e.g., NE, MPO, and cathepsin G). NET formation proceeds via two major routes: suicidal/lytic NETosis, driven by PKC–Raf–MEK–ERK signaling and NOX-dependent ROS generation, which promotes NE/MPO release, histone processing, chromatin decondensation, and ultimately plasma membrane rupture; and rapid non-lytic NETosis, which is NOX-independent and preserves neutrophil viability by extruding DNA-containing nuclear vesicles and releasing granule proteins through degranulation. Image created by the authors with Figdraw.

Abbreviations: ERK: Extracellular signal-regulated kinase; MEK: Mitogen-activated protein kinase kinase; MPO: Myeloperoxidase; NE: Neutrophil elastase; NET: Neutrophil extracellular trap; NOX: NADPH oxidase; PKC: Protein kinase C; Raf: Rapidly accelerated fibrosarcoma; ROS: Reactive oxygen species.

Epithelial–mesenchymal transition (EMT) is the process by which tumor cells convert from an epithelial to a mesenchymal-like state, characterized by loss of adhesion and acquisition of enhanced migratory, invasive, and survival capacities.^{35,36} NE has been implicated in EMT-associated changes in breast cancer cells and may contribute to metastatic dissemination in preclinical models. It can activate inflammatory signaling in tumor and host cells, thereby upregulating EMT- and invasion-related genes and establishing a pro-metastatic microenvironment.³⁷ Specifically, tumor-associated neutrophils release NETs that provide a proinflammatory complex enriched in NE, MPO, and nucleic acids³⁸, which can directly induce I κ B kinase (IKK) activation in tumor cells and stromal cells through the toll-like receptor (TLR) 4/TLR9–myeloid differentiation primary response 88 (MyD88) axis and rapidly upregulate nuclear factor κ B (NF- κ B) target genes, such as those of interleukin (IL)-6, IL-8, and matrix metalloproteinases (MMPs).^{39–42} At the same time, the proteolytic activity of NE degrades basement membranes and components of the extracellular matrix (ECM), generating damage-associated molecular patterns (DAMPs), including high mobility group box 1 (HMGB1) and ECM fragments.^{43,44} These DAMPs activate

the receptor for advanced glycation end products (RAGE)/TLR signaling and further enhance NF- κ B activity, creating a local proinflammatory microenvironment that favors cell migration.⁴⁵ Another pathway involves the uptake of NE by tumor cells, followed by degradation of inhibitory molecules such as IRS-1 via the endosomal/lysosomal pathway, thereby relieving suppression of the PI3K–Akt pathway and indirectly amplifying NF- κ B-mediated survival and promigratory signaling via Akt-dependent IKK activation^{29,46–50} (Figure 2). NF- κ B activation, in turn, induces the secretion of neutrophil-attracting chemokines by tumor cells, recruiting additional neutrophils and promoting further NET formation, thus establishing a positive feedback loop between NE and NF- κ B (Table 2).

The functional relevance of NE in breast cancer is supported by a heterogeneous body of evidence spanning patient-derived observations, breast cancer-specific experimental models, and extrapolations from non-breast cancer systems.^{26,27} Clinical studies in breast cancer have reported associations between elevated neutrophil infiltration, increased circulating NET markers, or higher intratumoral NE expression and adverse outcomes, including increased metastatic burden and reduced survival. However, in most patient-based analyses, NE is assessed as part of broader neutrophil or NET signatures rather than as an isolated effector molecule. Consequently, it remains challenging to disentangle NE-specific effects from those mediated by other NET-associated components or by neutrophil-driven inflammation more broadly. Moreover, the extent to which NE activity directly reflects NET formation, rather than neutrophil degranulation independent of NETosis, is rarely addressed in clinical cohorts.

Breast cancer-specific preclinical models provide mechanistic support for a contributory role of NE in tumor progression. Studies using breast cancer cell lines and murine models have demonstrated that NE can be internalized by tumor cells and promote proliferative, invasive, and EMT-associated phenotypes through pathways such as PI3K–Akt and NF- κ B signaling.^{29,45} These findings support biological plausibility for NE as a contributor to malignant behavior in breast cancer contexts. Nonetheless, many experimental systems rely on exogenous NE exposure or neutrophil co-culture conditions that may not fully recapitulate the spatial, temporal, and quantitative dynamics of NET-associated NE delivery in vivo. In addition, the relative contribution of NE derived from NETs versus soluble or intracellular pools remains insufficiently resolved in breast cancer-specific settings.

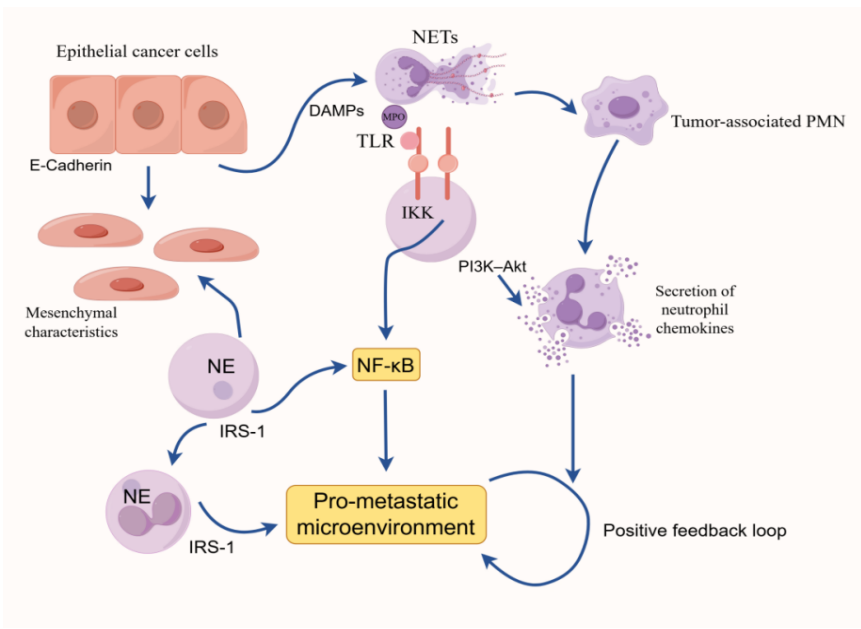


Figure 2. Proposed mechanisms by which NE-rich NETs from tumor-associated neutrophils may reinforce EMT-associated signaling and metastatic progression in breast cancer by triggering TLR4/TLR9–MyD88–IKK signaling and NF-κB-dependent expression of IL-6/IL-8/MMPs, while NE proteolysis remodels the ECM and releases DAMPs that amplify RAGE/TLR–NF-κB signaling. In parallel, tumor-cell uptake of NE drives IRS-1 degradation to activate PI3K–Akt and further enhance IKK–NF-κB, creating an IL-8–neutrophil–NET positive feedback loop. Evidence note: most pathway-level support derives from breast cancer preclinical models, with several signaling links extrapolated in part from other tumor systems. Image created by the authors with Figdraw.

Abbreviations: DAMP: Damage-associated molecular patterns; ECM: Extracellular matrix; EMT: Epithelial–mesenchymal transition; IKK: Induce IκB kinase; IL: Interleukin; IRS-1: Insulin receptor substrate 1; MMP: Matrix metalloproteinases; MPO: Myeloperoxidase; MyD88: Myeloid differentiation primary response 88; NE: Neutrophil elastase; NET: Neutrophil extracellular trap; NF-κB: Nuclear factor κB; PI3K: Phosphoinositide 3-kinase; PMN: Polymorphonuclear leukocyte; RAGE: Receptor for advanced glycation end products; TLR: Toll-like receptor.

Table 2. Proposed NET-associated NE mechanisms driving breast cancer progression

Pathological process	NET-associated mechanism	Key findings
Tumor cell proliferation/tumor growth	Neutrophil-derived NE enters tumor cells and degrades IRS-1	NE-mediated IRS-1 degradation relieves inhibition and activates the PI3K–Akt pathway, accelerating tumor cell proliferation ²⁹
Cell-cycle arrest and apoptosis	Pharmacologic inhibition of NET-released NE protease activity	NE inhibition triggers Rb-dependent growth arrest and induces caspase-dependent apoptosis, suggesting a shift from proliferative to apoptotic states ³⁴
Proinflammatory microenvironment formation → invasion/metastasis	NETs are enriched in NE, MPO, and nucleic acids; nucleic acids activate IKK via the TLR4/TLR9–MyD88 axis	NET exposure rapidly upregulates NF-κB target genes (e.g., those of IL-6, IL-8, and MMPs), fostering a pro-invasive, pro-metastatic niche ^{39–42}
EMT, migration, and tissue invasion → metastatic dissemination	Proteolytic NE activity generates ECM fragments and DAMPs (e.g., HMGB1), which amplify NF-κB signaling via RAGE/TLR pathways	Through an ECM degradation–DAMP–RAGE/TLR circuit, NE reinforces local inflammation and migratory cues, facilitating dissemination ^{43,44}
Survival, migration, and maintenance of metastatic phenotypes	Endocytosed NE promotes endosomal/lysosomal degradation of inhibitory molecules (e.g., IRS-1) → PI3K–Akt → Akt-dependent IKK → NF-κB	Intracellular NE relieves PI3K–Akt suppression and amplifies Akt–IKK–NF-κB pro-survival and pro-migratory programs, supporting a positive-feedback pro-metastatic state ^{29,46–50}

Note: Direct support is strongest in breast cancer preclinical models, whereas clinical evidence remains mainly correlative.

Abbreviations: DAMP: Damage-associated molecular patterns; ECM: Extracellular matrix; EMT: Epithelial–mesenchymal transition; HMGB1: High mobility group box 1; IKK: Induce IκB kinase; IL: Interleukin; IRS-1: Insulin receptor substrate 1; MMP: Matrix metalloproteinases; MPO: Myeloperoxidase; MyD88: Myeloid differentiation primary response 88; NE: Neutrophil elastase; NET: Neutrophil extracellular trap; NF-κB: Nuclear factor κB; PI3K: Phosphoinositide 3-kinase; RAGE: Receptor for advanced glycation end products; Rb: Retinoblastoma; TLR: Toll-like receptor.

Taken together, the NE axis is among the more biologically coherent NET-associated pathways in breast cancer, but its evidentiary profile remains mixed: mechanistically informative in vitro and murine data, limited correlative patient data, and substantial conceptual support borrowed from other tumor types. This evidence pattern justifies cautious translational interest but not the conclusion that NE uniformly drives breast cancer progression across clinical contexts.²⁹

Collectively, current data support a biologically relevant association between NE-rich, NET-enriched microenvironments and more aggressive breast cancer phenotypes. However, important gaps remain regarding the quantitative contribution of NE relative to other NET components, the dependence of NE-mediated effects on NETosis, and the extent to which mechanistic evidence derives from preclinical models, often using TNBC-like cell lines or murine systems rather than subtype-diverse clinical cohorts. Addressing these issues will require breast cancer-specific studies that integrate spatially resolved NET detection, functional perturbation of NE activity, and longitudinal clinical correlation across defined molecular subtypes.

3.2. Programmed death-ligand 1

Programmed death-ligand 1 (PD-L1) is the key immune checkpoint ligand in the programmed cell death protein 1 (PD-1) pathway and can be expressed by tumor cells and multiple immune cell subsets. Under tumor-associated inflammatory signaling, neutrophils upregulate PD-L1, and during NETosis, PD-L1 becomes colocalized or structurally associated with the extruded NET chromatin network. Functional studies indicate that NET-associated PD-L1 can engage PD-1 on CD8⁺ T cells, enhancing PD-1–dependent inhibitory signaling, thereby suppressing T-cell proliferation and effector functions and inducing exhaustion-like transcriptional and phenotypic changes.^{51,52} However, the ultrastructural basis for the sustained presence of PD-L1, a transmembrane protein, within NET structures remains incompletely defined and may involve membranous components or extracellular vesicles.

In the context of breast cancer, it has been reported that autophagosomes released by tumor cells can induce NET formation in neutrophils and generate PD-L1–bearing NETs through a HMGB1-mediated TLR4–MyD88–ERK/p38 signaling axis.⁵³ These “PD-L1-decorated NETs” can suppress T-cell effector responses both locally and in distant organs, promoting the establishment of an immunosuppressive pre-metastatic niche in the lung and facilitating the formation of metastatic lesions.^{54,55} Evidence supporting a functional link between NET-associated

PD-L1 and breast cancer progression is emerging but remains uneven across evidence levels. Patient-derived data in breast cancer demonstrate correlations between elevated NET burden, neutrophil infiltration, or immunosuppressive tumor microenvironments and reduced CD8⁺ T-cell activity or unfavorable clinical outcomes. However, direct detection of PD-L1 localized to NET structures within human breast cancer tissues has been reported in limited cohorts, and the majority of clinical studies infer immune suppression from broader NET or neutrophil signatures rather than PD-L1-specific NET markers. As a result, the contribution of NET-associated PD-L1 to immune evasion in patients remains largely correlative and insufficiently quantified.

Breast cancer-specific preclinical models provide mechanistic plausibility for NET-associated PD-L1 as a mediator of immune suppression.⁵¹ Experimental studies have shown that tumor-derived signals, including autophagosome-associated danger signals, can induce NET formation in neutrophils and promote the presentation or enrichment of PD-L1 within NET structures. In these models, this is associated with reduced CD8⁺ T-cell effector function and enhanced metastatic colonization, particularly in the lung. Collectively, these data support a preclinical causal framework in which NETs can act as spatial platforms that amplify checkpoint signaling within defined microenvironments. Nonetheless, most of these studies rely on murine models or in vitro systems, and the extent to which NET-associated PD-L1 operates independently of tumor-cell or myeloid-cell PD-L1 expression in breast cancer remains unclear.

Importantly, a substantial proportion of conceptual advances regarding NET-mediated immune checkpoint regulation derive from non-breast cancer contexts, including other solid tumors and inflammatory disease models. In these systems, NETs have been implicated in T-cell exhaustion and impaired cytotoxic immunity, but the molecular mechanisms governing PD-L1 retention, stability, and functional orientation within NET chromatin are not fully resolved. Moreover, breast cancer-specific variables, such as molecular subtype, baseline immune infiltration, and prior systemic therapy, may substantially influence whether NET-associated PD-L1 represents a dominant immunosuppressive axis or a secondary amplification mechanism.

Taken together, current evidence suggests that NET-associated PD-L1 constitutes a biologically plausible contributor to immune suppression in breast cancer, particularly within neutrophil-rich and metastatic microenvironments. However, its clinical relevance remains incompletely defined, in part because patient-

level validation is limited and standardized approaches for quantifying NET-associated PD-L1 in breast cancer specimens have not yet been established. Key unresolved questions include the quantitative contribution of NET-bound PD-L1 relative to other sources of checkpoint signaling, the reproducibility of NET-associated PD-L1 detection in patient samples, and the extent to which disrupting this axis enhances responses to immune checkpoint blockade. Addressing these gaps will require breast cancer-focused studies integrating spatially resolved immune profiling, functional perturbation of NET components, and longitudinal clinical validation (Table 3).

3.3. Chemokines

Chemokines embedded within NETs establish chemotactic gradients and couple endothelial adhesion with vascular responses, thereby reprogramming the recruitment and spatial positioning of immune cells in the tumor microenvironment and converting the sustained input of chronic inflammation into a driving force for tumor growth, invasion, and metastasis (Table 4).

C–X–C motif chemokine ligand 8 (CXCL8, also referred to as IL-8) is a prototypical neutrophil chemoattractant that can be produced by tumor cells and stromal cells and further amplified by immune cells within the tumor microenvironment.^{56,57} Through the C–X–C chemokine receptor 1/2 (CXCR1/CXCR2) axis, IL-8 can promote neutrophil recruitment, activation, and NETosis, forming a coupled inflammatory NET module.⁵⁶ The IL-8–CXCR1/2 axis is among the most frequently implicated chemokine

pathways linking neutrophil recruitment to NET formation and downstream breast cancer progression; however, the strength and specificity of evidence supporting a NET-dependent mechanism vary substantially across study types. At the experimental level, TNBC models indicate that IL-8 participates in paracrine interactions within the tumor-vascular unit and promotes invasion and angiogenesis.⁵⁶ In addition, IL-8 derived from tumor-associated adipocytes has been reported to promote EMT and metastasis and to alter T-cell infiltration, thereby linking IL-8 to invasive phenotypes and immune remodeling.^{58,59} In patient-derived breast cancer datasets, elevated circulating IL-8 levels, intratumoral IL-8 expression, and neutrophil-enriched inflammatory signatures have been associated with adverse clinical outcomes in selected cohorts. Tissue-based analyses further suggest that NET abundance may co-occur with IL-8-rich microenvironments and reduced CD8+ T-cell density, supporting a biologically plausible link between IL-8-driven neutrophil activity, NET formation, and local immune suppression. Nevertheless, in most clinical studies, IL-8 is measured systemically and is strongly influenced by tumor burden, treatment exposure, infection, metabolic inflammation, and other comorbid inflammatory conditions. These confounders limit causal inference and complicate the interpretation of IL-8 as a breast cancer-specific, NET-linked biomarker.

At the level of NET–tumor cell intrinsic crosstalk, tumor cell-derived factors, such as IL-8 and granulocyte colony-stimulating factor (G-CSF), can further enhance NET formation, establishing a positive feedback loop.^{60,61}

Table 3. NET-associated PD-L1 links neutrophil NETosis to immunosuppression and lung metastasis in breast cancer

Pathological process	NET-associated mechanism	Key findings
Tumor immune regulation: formation of an immunosuppressive microenvironment	During NETosis, PD-L1 is structurally associated with the extruded DNA–histone network	Neutrophils in tumor-associated inflammation can produce or carry PD-L1 and incorporate it into NET structures, providing a material basis for subsequent immunosuppressive functions ⁵¹
Immune evasion: reduced T-cell effector function/exhaustion-like changes	NET-associated PD-L1 engages PD-1 on T cells, enhancing PD-1-dependent inhibitory signaling	PD-L1 within NETs suppresses T-cell proliferation and effector activity and induces exhaustion-like phenotypes/transcriptional programs, indicating that NETs can function as an immunosuppressive platform rather than a passive scaffold ⁵²
Premetastatic conditioning: establishment of a lung premetastatic niche	TRAPs → HMGB1 → TLR4–MyD88–ERK/p38 signaling induces neutrophil NETosis and generates PD-L1-bearing NETs	Tumor-derived signals can drive neutrophils to form PD-L1-decorated NETs, defining an upstream pathway linking tumor cues to NET assembly ⁵³
Metastatic progression: immunosuppression enabling metastatic outgrowth	PD-L1-decorated NETs suppress T-cell activity locally and distally, promoting lung metastasis	PD-L1-decorated NETs not only suppress local anti-tumor immunity but also foster an immunosuppressive premetastatic ecosystem in distant organs, thereby facilitating lung metastasis ^{54,55}

Note: The strongest support comes from breast cancer-specific murine and in vitro studies, whereas direct validation in patient samples remains limited. Abbreviations: ERK: Extracellular signal-regulated kinase; HMGB1: High mobility group box 1; MyD88: Myeloid differentiation primary response 88; NE: Neutrophil elastase; NET: Neutrophil extracellular trap; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; TLR: Toll-like receptor; TRAP: Tumor cell-released autophagosome.

Stimulation with NETs strengthens the interaction between the NF- κ B essential modulator (NEMO) and IKK and activates NF- κ B.⁶² Blockade of the NEMO–IKK axis not only lowers IL-8 levels and reduces NET formation but also suppresses primary tumor growth and lung metastasis. Similarly, inhibition of PAD4-dependent NET formation attenuates NF- κ B activation and metastatic phenotypes.⁶² Breast cancer-specific experimental models have provided mechanistic support for IL-8 and CXCR1/2 as upstream drivers of neutrophil recruitment and NETosis, and as downstream amplifiers of NET-tumor crosstalk. In vitro and murine studies indicate that tumor- or stroma-derived IL-8 can recruit and activate neutrophils, promote NET formation, and, reciprocally, that NET exposure can enhance NF- κ B signaling and chemokine production in tumor cells, thereby reinforcing positive feedback loops. However, several key questions remain insufficiently resolved in breast cancer-relevant settings, including the quantitative contribution of IL-8 relative to other neutrophil chemoattractants, the dependency of observed phenotypes on NETosis versus neutrophil degranulation, and the degree to which IL-8 blockade suppresses tumor progression through NET-specific mechanisms rather than through broader effects on angiogenesis, stromal remodeling, or myeloid cell trafficking.

From the perspective of clinical prognosis, treatment cohorts of metastatic breast cancer suggest that lower circulating IL-8 levels during chemotherapy are associated with improved survival. Studies indicate that NETs show a heterogeneous distribution within breast cancer tissue, and that NET abundance correlates with local IL-8 expression and co-occurs with reduced CD8⁺ T-cell density, supporting the possibility that the IL-8–NET axis may weaken CD8-mediated immunity and associate with unfavorable outcomes.^{63–65} However, circulating IL-8 is readily influenced by confounding factors such as tumor burden, treatment exposure, and concomitant inflammatory conditions, and IL-8 itself is pleiotropic; its proangiogenic and proinvasive effects are not necessarily entirely dependent on NETs.⁶⁶ A major limitation of current evidence is the lack of longitudinally paired samples stratified by breast cancer subtype and organ-specific metastatic pattern. Multicenter cohort studies are needed to validate the spatial relationships among local IL-8, NETs, CD8⁺ T cells, and clinical outcomes, thereby distinguishing the degree of NET dependence. From a therapeutic standpoint in breast cancer, targeting the IL-8–CXCR1/2 axis and key steps in NET formation may provide new avenues for intervention.

A central limitation in interpreting this axis is the pronounced pleiotropy of IL-8 signaling. Beyond its

role as a neutrophil chemoattractant, IL-8 can directly influence tumor cells and stromal elements by promoting angiogenesis, endothelial activation, EMT-associated transcriptional programs, and therapy-associated inflammatory rewiring, effects that are not necessarily contingent on NET formation. Therefore, associations between IL-8 and poor prognosis cannot be assumed to reflect NET causality unless supported by concurrent NET quantification and functional perturbation. Moreover, the relevance of IL-8–CXCR1/2–NET coupling is likely context-dependent across breast cancer subtypes and metastatic niches, and may vary with systemic therapy regimens that modulate inflammatory tone.

Collectively, available evidence supports IL-8 and CXCR1/2 as biologically plausible upstream regulators and amplifiers of NET-related inflammation in breast cancer. However, the clinical interpretability of IL-8 as a NET-specific biomarker remains limited by confounding and pleiotropy. Future breast cancer-focused studies should prioritize longitudinal sampling with paired systemic and tissue measurements, rigorous adjustment for inflammatory comorbidities and treatment effects, and integrated designs combining spatial NET detection with targeted perturbation of the IL-8–CXCR1/2 axis to define the degree of NET dependence across molecular subtypes and organ-specific metastatic patterns.

3.4. Adhesion molecules

The DNA–histone backbone of NETs within the microvascular bed has the capacity to mediate the adhesion and retention of circulating tumor cells. In addition, matrix ligands present within NET structures, such as fibronectin, can interact with integrin receptors on the tumor cell surface, including the β 1 subunit and its heterodimers α 5 β 1 and α v β 3, thereby further strengthening tumor cell adhesion and capture.^{67,68} Experimental studies have indicated that the tumor cell membrane receptor coiled-coil domain-containing protein 25 (CCDC25) can recognize extracellular DNA and activate an adhesion and cytoskeletal remodeling pathway centered on integrin-linked kinase (ILK) and β -parvin, thereby enhancing the establishment of cell polarity and motility.⁶⁹ In animal models, NET-mediated adhesion has been shown to be closely associated with distant metastasis and adverse outcomes.^{67,70} However, substantial heterogeneity remains regarding the contribution of NET components (for example, the source and mode of presentation of fibronectin) and receptor dependence across different organ-specific microenvironments, and quantitative validation within the breast cancer microvascular niche is still required.

Table 4. Chemokine–NETs crosstalk and clinical implications in breast cancer

Pathological process	NET-associated mechanism	Key findings
Local progression: increased invasion and angiogenesis	CXCL8 (also known as IL-8) promotes endothelial migration and angiogenesis, creating a permissive inflammatory niche for neutrophil recruitment and NETosis	IL-8 promotes invasion and angiogenesis, positioning it at an “inflammation–vascular invasion” coupling node ⁵⁶
EMT and metastatic propensity; immune remodeling	IL-8 drives invasive programs and alters T-cell infiltration, indirectly favoring a NET-associated pro-metastatic ecosystem	IL-8 promotes EMT and metastasis and modulates T-cell infiltration, linking IL-8 to immune remodeling ^{56,57}
Inflammation amplification and dissemination priming; neutrophil recruitment/NETosis	Elevated IL-8 and/or G-CSF increases neutrophil recruitment and activation, enhancing NETosis and reinforcing a positive-feedback loop	Tumor-derived factors can reciprocally drive NET formation, supporting a self-amplifying “inflammation–NETs” circuit ^{60,61}
Primary tumor growth and lung metastasis	NETs activate the NEMO–IKK–NF- κ B axis; blockade with an NBD peptide lowers IL-8 and reduces NET formation	NEMO–IKK blockade reduces IL-8 and NETs and suppresses primary tumor growth and lung metastasis, indicating an actionable closed loop ⁶²
Clinical outcomes: survival/prognostic stratification	Circulating IL-8 serves as an integrated marker reflecting inflammatory drive and tumor burden, and is sensitive to treatment exposure	Lower circulating IL-8 during chemotherapy correlates with improved survival outcomes ⁶³
Immune microenvironment and prognosis	The IL-8–NET axis co-occurs with a CD8-restricted immune contexture	NETs display heterogeneous intratumoral distribution; NET abundance correlates with local IL-8 and coincides with reduced CD8+ T-cell density, consistent with impaired CD8 immunity and unfavorable outcomes ^{64,65}

Note: Chemokine-associated recruitment and amplification loops are supported by breast cancer preclinical studies and correlative clinical observations, but several effects are not NET-specific.

Abbreviations: CXCL8: C–X–C motif chemokine ligand 8; EMT: Epithelial–mesenchymal transition; G-CSF: Granulocyte colony-stimulating factor; IKK: Induce I κ B kinase; IL: Interleukin; NBD: NEMO-binding domain; NEMO: NF- κ B essential modulator; NET: Neutrophil extracellular trap; NF- κ B: Nuclear factor κ B.

Targeting the adhesion axis may represent a potential therapeutic strategy. Najmeh *et al.*⁶⁷ used DNase I to degrade and clear NETs and showed that disruption of NET structure attenuates tumor cell adhesion and reduces metastatic burden in preclinical models. Nevertheless, their work was conducted in inflammation- and perioperative-like models, and DNase I disrupts NETs globally rather than specifically blocking the adhesion axis, making it difficult to disentangle the direct contribution of adhesion from other NET-associated effects.^{67,70,71}

More precise quantification will require subtype-specific blockade of integrin-mediated adhesion. Monti *et al.*⁶⁸ demonstrated that NETs can present fibronectin and other adhesive ligands and significantly enhance tumor cell adhesion to NETs, whereas blockade of integrin $\alpha 5 \beta 1$ or $\alpha v \beta 3$ markedly reduces NET-mediated adhesion. Yang *et al.*⁶⁹ showed that knockout of *CCDC25* in tumor cells reveals NET-derived DNA as an upstream signal that activates the ILK– β -parvin adhesion and cytoskeletal remodeling pathway, thereby promoting migration and distant metastasis; *CCDC25* expression was also associated with poor prognosis. Dimitrov *et al.*⁷² reported that antibodies targeting integrin $\alpha 5 \beta 1$ or *CCDC25* reduce tumor cell adhesion to NETs and that prolonged

exposure to NETs can induce EMT-like changes in cell lines, including MCF7. However, the dosing parameters and optimal therapeutic time window for long-term NET exposure and intervention remain unclear and require further experimental confirmation (Table 5).

4. Discussion

Neutrophil extracellular traps have generally been regarded as byproducts of inflammatory responses; however, recent studies indicate that NETs connect immune activation with breast cancer progression through multiple mechanisms, including intrinsic tumor cell invasion, chemokine-driven recruitment, immune checkpoint-mediated suppression, and intravascular capture. This review, focusing on key NET components, delineates proposed mechanisms by which NETs may contribute to breast cancer progression and metastasis, and highlights candidate intervention points that warrant mechanistic and translational evaluation in breast cancer-relevant contexts.

Across the following paragraphs, recurrent pathways such as TLR-related signaling, NF- κ B activation, cytokine amplification, and neutrophil recruitment should be interpreted as overlapping components of a shared inflammatory amplification context rather than as fully separate linear mechanisms.

Table 5. NET-mediated adhesion axes and metastatic capture in breast cancer

Pathological process	NET-associated mechanism	Key findings
Early hematogenous dissemination: microvascular arrest/retention → extravasation priming	The DNA–histone scaffold provides a physical capture surface; NETs present matrix ligands (e.g., fibronectin) that bind tumor-cell integrins ($\alpha 5\beta 1$, and $\alpha v\beta 3$)	Beyond “entanglement,” integrin-dependent ligand presentation strengthens CTC arrest and capture, suggesting the feasibility of “NET disruption” or “anti-adhesion” strategies ^{67,68}
Perioperative/inflammatory window: enhanced CTC–NET binding → increased early metastatic burden	“NET disruption” intervention: DNase I degrades the NET DNA scaffold (non-specific anti-adhesion)	DNase I-mediated NET disruption attenuates CTC–NET adhesion and reduces metastatic burden, indicating that NETs promote early arrest and dissemination under inflammatory contexts ⁶⁷
Intravascular/tissue interface: capture/adhesion	“Anti-adhesion” intervention: NET-presented fibronectin and related ligands; tumor-cell integrins ($\alpha 5\beta 1/\alpha v\beta 3$) mediate adhesion	Blocking $\alpha 5\beta 1$ or $\alpha v\beta 3$ markedly reduces tumor-cell adhesion to NETs, supporting a dual-adhesion interface consisting of a DNA scaffold plus fibronectin ligands ⁶⁸
Pre-/post-extravasation: from arrest to motility/colonization (chemotaxis/motility → metastasis)	“Receptor/Signal blockade” intervention: CCDC25 recognizes extracellular DNA and activates ILK– β -parvin-centered focal adhesion/cytoskeletal remodeling	CCDC25 knockout weakens NET-DNA-induced migration and distant metastasis, supporting NET-DNA as an upstream motility cue and linking CCDC25 expression to poor prognosis ⁶⁹
Adhesive arrest; phenotype evolution after prolonged exposure (EMT/invasiveness)	Integrin/CCDC25-dependent adhesion; sustained adhesive signaling triggers phenotypic reprogramming (EMT-like changes)	Anti- $\alpha 5\beta 1$ or anti-CCDC25 antibodies reduce adhesion on NET-coated surfaces; prolonged NET exposure induces EMT-like changes in cell lines (including MCF7), although dose-dependence and optimal intervention windows remain unclear ⁷²

Note: Support is strongest in breast cancer preclinical models, with additional extrapolation from other malignancies; direct human evidence remains limited.

Abbreviations: CCDC25: Coiled-coil domain-containing protein 25; CTC: Circulating tumor cell; DNase: Deoxyribonuclease; EMT: Epithelial–mesenchymal transition; ILK: Integrin-linked kinase; NET: Neutrophil extracellular trap.

Neutrophil extracellular trap-associated constituents, such as NE, MPO, and nucleic acids, have been reported to activate NF- κ B via the TLR–MyD88–IKK axis and further amplify EMT- and invasion-promoting transcriptional programs through NE-mediated ECM degradation and DAMP release, and by relieving PI3K–Akt inhibition following NE internalization. However, the strength of support differs across pathways, with the clearest mechanistic evidence coming from breast cancer preclinical models and more limited confirmation in human breast cancer tissues. In addition, tumor cell-derived factors, such as IL-8 and G-CSF, feed back to promote neutrophil recruitment and NETosis, creating a positive feedback loop that sustains a pro-metastatic inflammatory microenvironment. Moreover, NETs can function as immunosuppressive platforms that carry or concentrate PD-L1, suppress CD8+ T-cell effector function, and contribute to pre-metastatic immune remodeling. Within the vascular bed, adhesion axes involving fibronectin–integrin interactions and DNA–CCDC25 recognition enhance the capture and seeding of circulating tumor cells. Building on this body of evidence, we further discuss the stratification potential of NET-related biomarkers and the therapeutic prospects of targeting PAD4, the NEMO–IKK axis, IL-8–CXCR1/2 signaling, NE activity, DNase-based NET degradation, and adhesion receptor axes within defined therapeutic windows.

Breast cancer ranks among the most frequently diagnosed malignancies worldwide and is the fourth leading cause of cancer-related death, largely owing to cancer cells with high invasiveness, strong metastatic potential, and resistance to therapy. NET-targeting strategies are under investigation in breast cancer, aiming to limit neutrophil recruitment and activation, inhibit NETosis, and enhance NET clearance; however, breast cancer-specific validation of efficacy, safety, and optimal therapeutic windows remains required. For example, interference with chemokine axes can reduce neutrophil recruitment to primary tumors and metastatic organs, indirectly limiting NET formation and dampening inflammatory amplification loops. Furthermore, modulation of G-CSF-dependent granulocyte mobilization and maturation pathways is being explored as an upstream approach to restrain NET accumulation, although careful evaluation of effects on host antimicrobial defense and optimization of the therapeutic window are required.

Histone citrullination by PAD4 is a key molecular step in NETosis, and pharmacologic or genetic inhibition of PAD4 reduces NET formation and metastatic risk in multiple tumor models, making it one of the most representative NETosis-targeting strategies. In parallel, interventions targeting ROS generation, NE activity, or the release of related granule proteases can attenuate NET

formation efficiency or the pathogenic effects of NETs at distinct stages. DNase-based approaches rapidly reduce NET-mediated physical trapping and inflammatory signal presentation by degrading the DNA backbone of NETs and may complement strategies that inhibit neutrophil recruitment and NET formation. However, DNase constitutes a broad NET-clearing tool rather than a pathway-specific intervention and may concurrently affect other DNA-related immune processes; thus, the optimal timing, dosing, and combination regimens remain to be defined. The PI3K–Akt axis is implicated both as a potential pathway of trastuzumab resistance and as a key node in NET-driven positive feedback. Inhibition of NE and the associated NF- κ B/PI3K–Akt signaling may ameliorate resistance, reduce invasiveness, and attenuate EMT.

Neutrophil extracellular traps are increasingly conceptualized not merely as correlates of inflammation but as potentially intervention-amenable hubs that may link innate immune activation to breast cancer metastasis, with functions spanning signal amplification, immune suppression, and intravascular capture. Further delineation of breast cancer-specific relationships between NET components and tumor biology may inform stratified biomarker frameworks, but will require standardized measurement, robust validation, and clinically feasible implementation pathways. Causal interrogation of breakable nodes, including neutrophil recruitment, NETosis, NE effector functions, NET-associated PD-L1, and adhesion axes, may ultimately inform improved strategies for metastasis prevention and for enhancing responses to immunotherapy in breast cancer.

Despite accumulating evidence implicating NETs in breast cancer progression, several important limitations and translational barriers constrain the interpretation and clinical applicability of current findings. A primary challenge lies in the heterogeneity of NET detection methodologies. Studies employ diverse approaches, including immunofluorescent colocalization of DNA with neutrophil enzymes, measurement of circulating MPO–DNA complexes, and surrogate inflammatory signatures, each capturing distinct aspects of NET biology. For example, cell-free DNA lacks NET specificity, assays for citrullinated histones (e.g., CitH3) vary across platforms, and clinically actionable thresholds remain undefined in most breast cancer cohorts. Variability in assay sensitivity, specificity, and tissue sampling introduces substantial inter-study heterogeneity and limits reproducibility, particularly in retrospective clinical analyses. The lack of standardized, validated NET quantification protocols hampers cross-cohort comparisons and weakens the robustness of prognostic associations.

Specificity represents a second major barrier to clinical translation. NET formation is not unique to malignancy and is readily induced by infection, tissue injury, metabolic inflammation, and therapy-associated stress responses. Consequently, elevated NET markers in patients with breast cancer may reflect systemic inflammatory states rather than tumor-specific biology. Disentangling cancer-driven NET formation from confounding inflammatory processes remains a critical unmet need, particularly when considering NETs as circulating biomarkers or therapeutic targets. Without rigorous adjustment for comorbidities and treatment-related inflammation, NET-associated signals risk being overinterpreted as tumor-intrinsic phenomena.

A further limitation concerns the distinction between correlation and causality. While numerous studies have demonstrated associations between NET abundance and aggressive disease features, direct causal evidence in human breast cancer remains limited. Preclinical models support mechanistic roles for NET components in invasion, immune suppression, and metastatic seeding; however, these systems often rely on supraphysiologic neutrophil activation, exogenous NET exposure, or acute inflammatory contexts that may not accurately reflect endogenous NET dynamics in patients. Moreover, the relative contribution of NETs compared with parallel inflammatory mechanisms—such as platelet activation, macrophage polarization, and endothelial dysfunction—has not been quantitatively established in breast cancer-specific settings.

From a therapeutic perspective, multiple translational challenges must be addressed before NET-targeted strategies can be considered clinically viable. Interventions aimed at inhibiting NET formation (e.g., PAD4 inhibition), dismantling existing NET structures (e.g., DNase-based approaches), or blocking downstream effector pathways may exert broad immunomodulatory effects that extend beyond the tumor microenvironment. In breast cancer, the most direct evidence currently supports DNase-based clearance and selected NETosis-inhibitory approaches in preclinical models, whereas several additional strategies remain supported mainly by non-breast cancer data or indirect inference. Key feasibility questions include the optimal therapeutic window (perioperative vs. metastatic settings), the delivery route and dosing for DNase-based agents, and the selection of endpoints that reflect NET-dependent biology. Such interventions carry the potential risk of impairing host defense, altering wound healing, or exacerbating susceptibility to infection, particularly in patients receiving cytotoxic or immunosuppressive therapies. In addition, many proposed targets lack specificity for tumor-associated NETs, raising concerns regarding on-target but off-tumor toxicity.

Patient stratification further represents a critical translational barrier. Breast cancer encompasses diverse molecular subtypes with distinct immune landscapes, inflammatory profiles, and metastatic behaviors. It is unlikely that NET-dependent mechanisms operate uniformly across all disease contexts, and this concern extends to differences between immunocompetent syngeneic models, xenografts, and experimental versus spontaneous metastasis settings. Identifying patient subsets in which NET biology meaningfully contributes to disease progression—and in which therapeutic modulation would confer net clinical benefit—remains an unresolved challenge. Addressing this issue will require integrated study designs that combine molecular subtype stratification, spatially resolved NET profiling, and longitudinal clinical outcome analysis.

Collectively, these limitations underscore that NETs in breast cancer currently represent biologically relevant correlates and mechanistic contributors rather than validated clinical biomarkers or therapeutic targets. Progress toward clinical translation will depend on standardized detection methodologies, rigorous causal interrogation in breast cancer-specific models, careful evaluation of safety and specificity, and the development of stratified frameworks to identify patients most likely to benefit from NET-directed interventions. Until such criteria are met, NET-based strategies should be viewed as an emerging conceptual framework that informs biological understanding and hypothesis generation, rather than as ready-to-implement clinical approaches.

5. Conclusion

Neutrophil extracellular traps are closely associated with inflammation, immune modulation, and metastatic progression in breast cancer. Current evidence suggests that NET-associated components, including NE, PD-L1, chemokine axes, and adhesion-related molecules, may participate in tumor invasion, immune escape, and the capture of circulating tumor cells. However, most mechanistic evidence remains preclinical or correlative, and NET-related biomarkers or therapeutic strategies have not yet been fully clinically validated in breast cancer. Future studies should further clarify breast cancer-specific causal mechanisms of NETs, incorporate molecular subtype-based stratification, and evaluate safety and feasibility to facilitate the clinical translation of NET-targeted strategies.

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