Available online on 15.06.2025 at ijmspr.com

International Journal of Medical Sciences and Pharma Research

Open Access to Medical Science and Pharma Research

Open Access **Review Article**

N1 and N2 Neutrophils in Breast Cancer: Mechanisms, Clinical Relevance, and Therapeutic Potential

Emmanuel Ifeanyi Obeagu [1]*, Isaac Isiko [2]

[1] Department of Biomedical and Laboratory Science, Africa University, Zimbabwe

[2] Department of Community Medicine, Axel Pries Institute of Public Health and Biomedical Sciences, Nims University, Jaipur, Rajasthan State, India

Article Info:

Article History:

Received 02 Jan 2025
Reviewed 26 Jan 2025
Accepted 18 Feb 2025
Published 15 June 2025

Cite this article as:

Obeagu EI, Isiko I, N1 and N2 Neutrophils in Breast Cancer: Mechanisms, Clinical Relevance, and Therapeutic Potential, International Journal of Medical Sciences & Pharma Research, 2025; 11(2):1-11 *DOI: http://dx.doi.org/10.22270/ijmspr.v11i2.141*

*Address for Correspondence:

Emmanuel Ifeanyi Obeagu, Department of Biomedical and Laboratory Science, Africa University, Zimbabwe

Abstract

Neutrophils are key components of the immune system and play a significant role in the tumour microenvironment (TME) of breast cancer. These cells can undergo polarization into two distinct phenotypes: N1 and N2 neutrophils. N1 neutrophils are typically associated with antitumor immunity, characterized by the production of pro-inflammatory cytokines and reactive oxygen species (ROS), which help inhibit tumour growth and metastasis. On the other hand, N2 neutrophils contribute to tumour progression by secreting immunosuppressive cytokines, promoting angiogenesis, and enhancing metastatic spread. The balance between these two phenotypes can have significant implications for cancer progression and treatment outcomes in breast cancer patients. The polarization of neutrophils is regulated by a complex network of cytokines, growth factors, and signalling pathways in the TME. Factors such as IL-12, IFN-γ, and GM-CSF promote N1 polarization, while IL-10, TGF-β, and VEGF are key drivers of N2 polarization. These pathways influence neutrophil recruitment, activation, and survival within the TME. Strategies targeting neutrophil polarization could offer new opportunities for breast cancer treatment, particularly for patients with aggressive or metastatic disease.

Keywords: N1 Neutrophils, N2 Neutrophils, Breast Cancer, Tumor Microenvironment, Immunotherapy

Introduction

Neutrophils, as the most abundant type of white blood cell in the human body, are crucial players in the immune response and have garnered significant attention in cancer research.[1]These cells are traditionally associated with combating infections through phagocytosis, the release of antimicrobial agents, and the formation of neutrophil extracellular traps (NETs).[2] However, their role in cancer, particularly in breast cancer, extends far beyond these classical functions. Neutrophils are found in large numbers in the tumour microenvironment (TME), where they exhibit a dual role, either promoting tumour progression or aiding in immune-mediated tumour suppression. This review focuses on the two distinct polarization states of neutrophils in breast cancer—N1 and N2—and their impact on tumourbehaviour, progression, and therapy response.[3,4] Neutrophil polarization refers to the ability of neutrophils to adopt different functional phenotypes in response to various signals from the TME.[5] N1 neutrophils are considered the "classically activated" phenotype and are characterized by their ability to produce pro-inflammatory cytokines, reactive oxygen species (ROS), and cytotoxic molecules that exert antitumor effects.[6,7] These neutrophils promote the recruitment of other immune cells to the tumor site and participate in the elimination of tumor cells, thus contributing to the suppression of tumor growth. In contrast, N2 neutrophils are associated with the "alternatively activated" phenotype and exhibit immunosuppressive functions.[8,9] These neutrophils are involved in creating a pro-tumorigenic environment by secreting anti-inflammatory cytokines, promoting angiogenesis, and supporting metastasis.[10]

The transition between N1 and N2 polarization is governed by complex signaling pathways and factors present in the TME. Tumor cells and stromal cells produce cytokines, growth factors, and extracellular matrix components that influence neutrophil recruitment, activation, and polarization. For example, cytokines such as IL-12, IFN-γ, and GM-CSF promote N1 polarization, while factors like IL-10, TGF-β, and VEGF are known to drive N2 polarization. Additionally, hypoxia, which is common in rapidly growing tumours, can also influence neutrophil behavior and polarization.[11] Understanding the molecular mechanisms that regulate neutrophil polarization is critical for developing strategies to manipulate these cells in a way that favourstumor suppression rather

than tumor promotion.[12,13] The impact of neutrophil polarization on breast cancer progression has profound implications for patient prognosis and therapeutic outcomes. A higher proportion of N1 neutrophils in the TME has been linked to improved clinical outcomes, as these cells help eliminate cancer cells and reduce metastasis.[14,1] On the other hand, an abundance of N2 neutrophils correlates with tumor progression, poor prognosis, and resistance to therapy. N2 neutrophils contribute to an immunosuppressive microenvironment that hinders the activity of cytotoxic T cells, natural killer cells, and other immune effector cells. Furthermore, N2 neutrophils facilitate the creation of pre-metastatic niches and promote angiogenesis, which is essential for tumor growth and the spread of cancer cells to distant organs.[15,3]

Recent studies have highlighted the potential of targeting neutrophils to improve breast cancer treatment.[16] Therapeutic strategies that enhance the antitumor activity of N1 neutrophils or reprogram N2 neutrophils into an N1-like state may provide a novel avenue for cancer therapy. One potential strategy is the use of cytokines or small molecules that can skew neutrophil polarization toward the N1 phenotype, thereby enhancing the immune response against the tumor.[17,18] Conversely, inhibiting the signals that promote N2 polarization or neutralizing the immunosuppressive factors produced by N2 neutrophils could prevent tumor progression and improve the effectiveness of current therapies, including chemotherapy, immunotherapy, and targeted therapies.[19] Moreover, the dynamic nature of neutrophil polarization in the TME poses challenges in the development of targeted therapies.[20,21] The plasticity of neutrophils, the heterogeneous nature of tumors, and the complex interactions between neutrophils and other immune cells contribute to the difficulty in identifying therapeutic windows for modulating neutrophil function. Additionally, neutrophils in different stages of cancer or in response to specific therapies may adopt different polarization states, further complicating the clinical application of neutrophil-targeting strategies.[22,13]

Aim

The aim of this review is to comprehensively explore the role of N1 and N2 neutrophils in the tumor microenvironment (TME) of breast cancer, with a particular focus on their mechanisms of polarization, clinical relevance, and therapeutic potential.

Rationale

Neutrophils are critical components of the immune system and play an essential role in the tumor microenvironment (TME), where they can either promote or inhibit tumor progression depending on their polarization state.[16] In the context of breast cancer, neutrophils exhibit a complex duality: N1 neutrophils typically exert antitumor activity by activating immune responses and directly attacking tumor cells, while N2 neutrophils are often associated with pro-tumor functions, including immune suppression, promoting angiogenesis, and facilitating metastasis.[23]This dual role makes neutrophils a key target for therapeutic interventions aimed at reprogramming their function to improve cancer treatment outcomes.[24] Given that neutrophils can significantly influence the effectiveness of therapies such as chemotherapy, targeted treatments, and immunotherapy, understanding how their polarization contributes to cancer progression is vital for identifying new therapeutic strategies.[25] The potential to manipulate neutrophil polarization—either by enhancing N1 activity or reprogramming N2 neutrophils to a more antitumor phenotype—presents an innovative approach to cancer immunotherapy. Additionally, the development of biomarkers to assess neutrophil polarization could enable personalized therapeutic strategies and improve treatment efficacy.[26] This review is thus crucial in synthesizing current knowledge about the molecular mechanisms driving neutrophil polarization in breast cancer, the clinical implications of their behavior in the TME, and the therapeutic potential of modulating these immune cells.[27,13] By bridging the gap between basic immunology and clinical oncology, this review aims to provide insights that can guide the development of novel immunotherapies to improve breast cancer treatment outcomes.[28,24]

Review Methodology

This review was conducted through a systematic approach, synthesizing relevant literature on the role of neutrophils, specifically N1 and N2 phenotypes, in breast cancer. The methodology involved an extensive search and selection of peer-reviewed articles from multiple scientific databases such as PubMed, Scopus, and Google Scholar. [29,17]We focused on studies published in the last decade to ensure the inclusion of the most recent and relevant findings on neutrophil polarization, mechanisms, and their implications for breast cancer therapy.[30] The selection process involved the use of specific keywords such as "N1 neutrophils," "N2 neutrophils," "tumor microenvironment," "breast cancer," "neutrophil polarization," and "cancer immunotherapy."[31] Only articles that provided insights into the molecular pathways of neutrophil polarization, their role in breast cancer progression, and their potential therapeutic implications were included. We excluded studies that were not focused on breast cancer or did not address the role of neutrophils in the tumor microenvironment.[32] Additionally, research articles discussing neutrophil-targeting therapies, both experimental and clinical, were carefully evaluated for their relevance to the therapeutic potential of neutrophil modulation in cancer treatment.[33]

Neutrophil Polarization in Breast Cancer

Neutrophils, as a key component of the innate immune system, exhibit remarkable plasticity in response to the signals within the tumor microenvironment (TME).[34] This plasticity allows them to adopt different functional phenotypes, known as polarization states, which play a pivotal role in cancer progression. In the context of breast cancer, neutrophils can polarize into two distinct subtypes—N1 and N2 neutrophils—each with unique

characteristics and effects on tumor development.[35] Understanding the polarization of neutrophils and the mechanisms driving this process is critical to unraveling their contributions to breast cancer progression and therapy response.[36] N1 neutrophils, often referred to as the "classically activated" phenotype, exhibit pro-inflammatory and antitumor properties.[37,38] These cells are characterized by the production of reactive oxygen species (ROS), pro-inflammatory cytokines, and cytotoxic molecules, such as tumor necrosis factor (TNF)-α and interleukin (IL)-12, which can directly kill cancer cells.[38] N1 neutrophils can also recruit and activate other immune cells, such as T lymphocytes and natural killer (NK) cells, thereby enhancing the overall immune response against the tumor. This polarization state is generally considered to have antitumor effects, contributing to tumor regression and preventing metastasis. Furthermore, N1 neutrophils help shape an immune environment that is hostile to cancer cell survival, providing a protective mechanism against tumor progression.[39] In contrast, N2 neutrophils represent the "alternatively activated" phenotype, which promotes tumor progression and metastasis. N2 neutrophils are immunosuppressive, secreting anti-inflammatory cytokines like IL-10 and transforming growth factor-beta (TGF-β), which dampen the immune response.[40] They also facilitate tumor growth by promoting angiogenesis, tissue remodeling, and immune evasion. These cells contribute to the establishment of a pro-tumorigenic microenvironment that supports cancer cell survival, proliferation, and dissemination. N2 neutrophils are particularly implicated in the creation of pre-metastatic niches, which enhance the ability of cancer cells to migrate and colonize distant organs. The presence of a high number of N2 neutrophils in the TME is often associated with poor prognosis, chemotherapy resistance, and worse clinical outcomes.[41]

The polarization of neutrophils into either the N1 or N2 phenotype is influenced by a combination of intrinsic and extrinsic factors within the TME. Cytokines and growth factors released by tumor cells, stromal cells, and other immune cells are critical regulators of neutrophil polarization.[42] For example, cytokines such as IL-12, interferon-gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) promote N1 polarization, while factors such as IL-10, TGF-β, and vascular endothelial growth factor (VEGF) favor N2 polarization.[43] Additionally, environmental factors like hypoxia, metabolic changes, and extracellular matrix components contribute to the polarization process. This complex interplay of signals determines whether neutrophils adopt an antitumor or pro-tumor phenotype, influencing tumor growth and metastasis.[44] The polarization of neutrophils in the breast cancer TME has significant implications for tumor progression. As N1 neutrophils exhibit antitumor activity, their presence is generally associated with a better prognosis and improved response to therapies. On the other hand, an increased abundance of N2 neutrophils is linked to tumor progression, immune suppression, and resistance to treatment.[45] The balance between N1 and N2 neutrophils may therefore serve as a predictor of breast cancer aggressiveness, metastasis, and response to treatment. Furthermore, the dynamic nature of neutrophil polarization suggests that the TME is constantly influencing and shaping the immune landscape, which may change throughout disease progression or in response to therapeutic interventions.[46]

Role of N1 Neutrophils in Antitumor Immunity

N1 neutrophils, also referred to as "classically activated" neutrophils, are integral components of the innate immune system with potent antitumor properties. These neutrophils play a crucial role in initiating and sustaining antitumor immune responses through a variety of mechanisms, ultimately contributing to tumor suppression. Their role in antitumor immunity has been highlighted in various studies, emphasizing their ability to modulate both the immune microenvironment and directly target cancer cells.[47,48]

Mechanisms of N1 Neutrophil Antitumor Activity

The antitumor effects of N1 neutrophils primarily arise from their ability to generate reactive oxygen species (ROS), release pro-inflammatory cytokines, and induce direct cytotoxicity against tumor cells. One of the defining features of N1 neutrophils is their potent ROS production, which is toxic to cancer cells.[48] This oxidative burst can induce apoptosis or necrosis of malignant cells, thereby reducing tumor growth. Additionally, N1 neutrophils produce cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-12, which not only enhance their own cytotoxicity but also activate other immune cells, including T lymphocytes and natural killer (NK) cells, in the TME. This coordinated immune response amplifies the antitumor immune surveillance, making N1 neutrophils essential players in tumor control.[49] Moreover, N1 neutrophils can promote the recruitment of other immune cells to the TME through the secretion of chemokines like CXCL8 and CCL2, which act as signals to attract T cells, dendritic cells, and NK cells. The interaction between these immune cells and N1 neutrophils further strengthens the overall immune response against the tumor.[50] By fostering a T cell-mediated immune response, N1 neutrophils contribute to the establishment of a protective immune network that limits tumor escape mechanisms and hinders cancer progression. Their ability to collaborate with other immune effectors highlights the importance of N1 neutrophils in orchestrating a multifaceted immune response against breast cancer.[51]

N1 Neutrophils in Tumor Regression and Prevention of Metastasis

In addition to direct tumor cell killing, N1 neutrophils contribute to tumor regression by inhibiting angiogenesis and preventing the formation of pre-metastatic niches. N1 neutrophils express high levels of angiostatic factors such as thrombospondin-1, which inhibit the growth of blood vessels that are essential for

tumor survival and expansion. By preventing angiogenesis, N1 neutrophils limit the tumor's ability to sustain its metabolic demands, thereby restricting its growth. Furthermore, N1 neutrophils can modulate the extracellular matrix (ECM) environment through the production of matrix metalloproteinases (MMPs), which help to degrade the ECM and limit cancer cell invasion into surrounding tissues.[52,13] This action is vital in preventing cancer cell dissemination and metastasis, particularly in early-stage tumors. Another significant aspect of N1 neutrophil function is their role in fostering immune surveillance during the early stages of tumorigenesis. They can contribute to the detection and elimination of nascent cancer cells before they accumulate mutations or proliferate into clinically detectable tumors. This proactive surveillance mechanism further supports the notion that N1 neutrophils are crucial for early tumordefense and maintaining immune homeostasis within the TME. The ability of N1 neutrophils to limit both local tumor growth and metastasis underscores their importance as a potent antitumor immune cell subset.[53]

Clinical Relevance of N1 Neutrophils in Breast Cancer

The clinical relevance of N1 neutrophils in breast cancer is becoming increasingly recognized. Studies have shown that the presence of N1 neutrophils in the TME correlates with a favorable prognosis and better therapeutic responses, particularly in immunotherapy and chemotherapy. The ability to mobilize or enhance the function of N1 neutrophils could offer a promising strategy for improving outcomes in patients with breast cancer, especially in those with high metastatic potential or resistant tumors.[54] Moreover, N1 neutrophils have been implicated in the response to targeted therapies, such as those involving immune checkpoint inhibitors, as they can modulate the immune response to enhance the efficacy of these therapies. However, the therapeutic manipulation of N1 neutrophils in clinical settings remains complex. A better understanding of the factors that drive neutrophil polarization and their interactions with other immune and stromal cells in the TME is essential to harness their full therapeutic potential. Strategies aimed at enhancing N1 neutrophil function, such as cytokine-based therapies, or inhibiting pathways that drive N2 polarization (which induces an immunosuppressive TME), may offer novel avenues for boosting antitumor immunity. [55]While these approaches hold promise, further preclinical and clinical studies are required to refine strategies that effectively exploit N1 neutrophils as therapeutic agents in breast cancer management.[56]

Role of N2 Neutrophils in Tumor Progression

N2 neutrophils, also referred to as "alternatively activated" neutrophils, are a subset of neutrophils that, in contrast to their N1 counterparts, tend to promote tumor progression rather than tumor suppression. Their polarization is driven by signals within the tumor microenvironment (TME), including cytokines like IL-4, IL-10, and TGF-β, which lead to a shift towards an immunosuppressive phenotype. These N2 neutrophils are involved in various processes that facilitate tumor growth, metastasis, and immune evasion, making them key players in the progression of cancer.[57]

Mechanisms of N2 Neutrophil-Mediated Tumor Progression

N2 neutrophils promote tumor progression primarily through their ability to suppress effective antitumor immunity and enhance tumor cell survival. One of the primary ways N2 neutrophils contribute to cancer progression is by secreting immunosuppressive cytokines such as IL-10 and TGF-β, which inhibit the activation of cytotoxic T cells and NK cells. These cytokines create an immunosuppressive microenvironment that allows tumor cells to escape immune surveillance and proliferate unchecked. N2 neutrophils can also suppress dendritic cell function, impairing antigen presentation and further hindering the ability of the immune system to mount a robust antitumor response.[58] Moreover, N2 neutrophils support tumor growth by promoting angiogenesis—the formation of new blood vessels to supply the growing tumor. They produce factors such as vascular endothelial growth factor (VEGF), which stimulates endothelial cell proliferation and blood vessel formation. This angiogenesis not only provides the tumor with the oxygen and nutrients it requires but also facilitates the dissemination of cancer cells into the bloodstream, supporting metastasis. Additionally, N2 neutrophils are involved in remodeling the extracellular matrix (ECM) by secreting matrix metalloproteinases (MMPs). This degradation of the ECM allows tumor cells to invade surrounding tissues, making it easier for them to migrate to distant organs and establish secondary tumors.[59]

Impact of N2 Neutrophils on Tumor Immunity

N2 neutrophils play a significant role in shaping the immune landscape of the TME in a way that supports tumor progression. They are often found in large numbers in tumors with high metastatic potential, correlating with poor prognosis in several cancers, including breast cancer. By secreting pro-tumor factors, N2 neutrophils contribute to immune evasion mechanisms such as the induction of regulatory T cells (Tregs) and the inhibition of effector T cell activity. This immunosuppressive role is crucial for tumors to avoid immune detection and destruction, thus facilitating tumor progression.[60] N2 neutrophils also contribute to the creation of a permissive environment for tumor cell invasion and metastasis. The secretion of MMPs and the induction of ECM remodeling enhance tumor cell motility and invasion, enabling the cancer cells to breach the basement membrane and spread to distant tissues. Moreover, N2 neutrophils may help tumor cells to evade apoptosis, thus contributing to the survival of malignant cells in the face of immune and therapeutic interventions. Through these mechanisms, N2 neutrophils facilitate the spread of breast cancer to distant organs, making them key contributors to metastasis.[61]

Therapeutic Targeting of N2 Neutrophils in Cancer

Given their role in supporting tumor progression and metastasis, N2 neutrophils represent a potential therapeutic target for combating cancer. Strategies aimed at modulating the polarization of neutrophils from the immunosuppressive N2 phenotype to the tumor-suppressive N1 phenotype have gained attention in recent years. Various approaches, including the use of cytokine inhibitors, immune checkpoint blockade, and small molecules, are being explored to disrupt the pro-tumor activities of N2 neutrophils. For example, targeting TGF-β signaling pathways or IL-10 could reduce the immunosuppressive effects of N2 neutrophils and enhance the effectiveness of existing therapies, including immune checkpoint inhibitors and chemotherapy. In addition to direct modulation of N2 neutrophil polarization, strategies aimed at reducing the recruitment and infiltration of N2 neutrophils into the TME are also under investigation.[62] By blocking the chemokines and receptors that attract N2 neutrophils, such as CXCR2 and CCL2, it may be possible to limit their contribution to tumor progression and metastasis. Combining these strategies with other immunotherapeutic approaches could result in a more robust and effective treatment for breast cancer patients, particularly those with advanced or metastatic disease.[63]

Mechanisms Driving N1 and N2 Neutrophil Polarization

Neutrophils exhibit remarkable plasticity, which allows them to polarize into distinct subtypes in response to signals within their environment. The polarization of neutrophils into N1 or N2 phenotypes is a complex, multifactorial process influenced by cytokines, signaling molecules, and interactions with other immune and tumor cells. These two polarized states, N1 and N2, have distinct functional roles in the immune response, particularly in cancer, where they can either promote tumor suppression (N1) or tumor progression (N2).[64]

1. Polarizing Signals for N1 Neutrophils

N1 neutrophils, often described as pro-inflammatory or antitumorigenic, are typically induced by cytokines and signals that promote a classical immune activation. Key polarizing factors for N1 neutrophils include interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), **and** lipopolysaccharides (LPS). These factors trigger the activation of signaling pathways, such as the JAK-STAT pathway, which lead to the upregulation of pro-inflammatory cytokines like IL-12, TNF-α, and reactive oxygen species (ROS). These N1 neutrophils are characterized by their ability to produce pro-inflammatory cytokines and chemokines that promote immune activation, recruitment of other immune cells, and direct cytotoxicity against tumor cells.[65] Upon activation, N1 neutrophils exhibit increased phagocytic activity and can directly kill tumor cells via the release of reactive oxygen species (ROS), degranulation, and the production of proteolytic enzymes. Additionally, N1 neutrophils promote the recruitment of effector T cells and natural killer (NK) cells to the tumor microenvironment (TME), contributing to enhanced antitumor immunity. They also activate antigen-presenting cells (APCs), facilitating an adaptive immune response against tumor cells. N1 neutrophils' ability to induce the release of pro-inflammatory cytokines and chemokines further enhances the immune response, creating an environment that is hostile to cancer cell survival.[65,66]

2. Polarizing Signals for N2 Neutrophils

In contrast, N2 neutrophils, which are often associated with immune suppression and tumor progression, are driven by cytokines that favor an anti-inflammatory and tissue-repair environment. The key signals that drive N2 polarization include IL-4, IL-10, and TGF-β. These cytokines, particularly IL-4 and IL-10, are commonly produced by tumor cells, regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. These cytokines activate pathways such as the STAT6 pathway (induced by IL-4) and SMAD signaling (mediated by TGF-β), which induce a shift towards the N2 phenotype.[67] N2 neutrophils typically exhibit immunosuppressive functions that aid in tumor progression. Rather than promoting an inflammatory immune response, they secrete cytokines like IL-10 and TGF-β, which inhibit the activity of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. These cytokines help create a microenvironment that suppresses effective antitumor immunity and supports tumor survival. In addition, N2 neutrophils promote angiogenesis through the secretion of vascular endothelial growth factor (VEGF), and they express matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM), facilitating tumor invasion and metastasis.[68]

3. Crosstalk Between Tumor Cells and Neutrophils

The polarization of neutrophils into either the N1 or N2 phenotype is not a one-way process. Tumor cells can actively influence neutrophil polarization through direct cell-cell interactions and the secretion of soluble factors. In the TME, tumor cells often produce chemokines such as CXCL1 and CXCL8, which recruit neutrophils to the tumor site. These signals can drive neutrophil polarization towards the N2 phenotype by interacting with **CXCR2**, a receptor expressed by neutrophils. Additionally, tumor-derived exosomes and microvesicles can transfer immunosuppressive molecules to neutrophils, further promoting their N2 polarization and supporting tumor growth.[69] The immune cells present in the TME, such as regulatory T cells (Tregs) and MDSCs, also play a significant role in inducing N2 polarization. Tregs, in particular, secrete IL-10 and TGF-β, which can influence neutrophil differentiation and function, pushing them toward an N2 phenotype. This immunosuppressive environment hampers the recruitment and function of antitumor immune cells, such as cytotoxic T cells and NK cells, thereby facilitating the immune evasion of tumor cells.[70]

4. Molecular Pathways Governing Neutrophil Polarization

The polarization of neutrophils into either N1 or N2 subsets is tightly regulated by intracellular signaling pathways. The NF-κB pathway, which is activated in response to inflammatory cytokines such as TNF-α and IL-1β, is crucial for the activation of N1 neutrophils and the production of pro-inflammatory cytokines. On the other hand, the PI3K/Akt pathway, activated by IL-4 and IL-10, plays a pivotal role in promoting N2 polarization and the secretion of immunosuppressive cytokines. The JAK/STAT signaling pathways are also central to the regulation of neutrophil polarization, with STAT1 and STAT3 driving N1 and N2 polarization, respectively.[71] These signaling networks ensure that neutrophils respond appropriately to the changing conditions in the tumor microenvironment. However, when these pathways are dysregulated, they can contribute to tumor progression by fostering an environment that supports immune evasion, tissue remodeling, and metastasis. Therefore, understanding the molecular mechanisms that regulate neutrophil polarization could provide valuable insights into developing novel therapeutic strategies that aim to manipulate neutrophil function in cancer.[72]

Clinical Relevance of N1 and N2 Neutrophils in Breast Cancer

Neutrophils play a pivotal role in the tumor microenvironment (TME) of breast cancer, with their polarization into N1 and N2 subsets having significant implications for tumor progression, immune response, and treatment outcomes. The clinical relevance of these two neutrophil subsets lies in their contrasting functions—N1 neutrophils generally promote antitumor immunity, while N2 neutrophils are associated with immune suppression and tumor progression. Understanding the mechanisms that drive N1 and N2 polarization and their impact on clinical outcomes has become crucial for developing more targeted and effective therapies for breast cancer patients.[73]

1. N1 Neutrophils and Their Antitumor Effects

N1 neutrophils exhibit pro-inflammatory and antitumorigenic properties. They are typically associated with the suppression of tumor growth and the promotion of a robust immune response. These neutrophils are activated by cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), leading to the production of reactive oxygen species (ROS), pro-inflammatory cytokines, and the recruitment of other immune cells such as cytotoxic T cells and natural killer (NK) cells. Clinically, the presence of N1 neutrophils within the TME correlates with a favorable prognosis in breast cancer, as these cells contribute to the effective recognition and elimination of cancer cells. N1 neutrophils also stimulate the activity of antigen-presenting cells (APCs), which further enhances the adaptive immune response and supports tumor eradication. Therefore, the ability to increase N1 neutrophil function in the TME may be an important therapeutic strategy for improving treatment outcomes in breast cancer.[74]

2. N2 Neutrophils and Their Role in Tumor Progression

Conversely, N2 neutrophils are predominantly associated with immune suppression and tumor progression. These cells are activated by cytokines such as interleukin-4 (IL-4), IL-10, and transforming growth factor-beta (TGF-β), which promote anti-inflammatory responses and tissue remodeling. N2 neutrophils secrete immunosuppressive cytokines, such as IL-10 and TGF-β, that inhibit the cytotoxic activity of T cells and NK cells, ultimately allowing the tumor to evade immune surveillance. Furthermore, N2 neutrophils promote metastasis by secreting matrix metalloproteinases (MMPs), which degrade the extracellular matrix, facilitating tumor cell migration and invasion.[75] Clinically, high levels of N2 neutrophils in the TME are often associated with poor prognosis, higher tumor stage, and reduced survival in breast cancer patients. The presence of N2 neutrophils contributes to a more immunosuppressive environment, making it difficult for the body's immune system to mount an effective defense against the tumor.[76]

3. Implications for Treatment Response and Prognosis

The balance between N1 and N2 neutrophils in the TME has profound implications for the treatment response and prognosis of breast cancer patients. A higher proportion of N1 neutrophils is generally associated with a better response to immunotherapies, such as checkpoint inhibitors or cancer vaccines, due to their ability to promote a more robust antitumor immune response. In contrast, a shift toward N2 polarization can limit the efficacy of such therapies, as N2 neutrophils actively suppress immune function and promote tumor progression. Furthermore, therapies that target the mechanisms driving N2 polarization, such as inhibiting cytokine signaling (e.g., IL-4, IL-10, or TGF-β) or neutralizing immunosuppressive factors, may enhance the effectiveness of existing cancer treatments and improve patient outcomes.[77] The clinical relevance of N1 and N2 neutrophils extends beyond their role in immune modulation. The levels and polarization state of neutrophils can serve as potential biomarkers for patient prognosis and treatment monitoring. For instance, an increase in N1 neutrophils could be indicative of a favorable response to immune-based therapies, while a predominance of N2 neutrophils may suggest the need for strategies that counteract immune suppression and promote a more inflammatory tumor environment. Thus, assessing neutrophil polarization status in breast cancer patients may offer valuable insights for personalized treatment strategies and better management of the disease.[78]

Therapeutic Potential of Targeting Neutrophil Polarization in Breast Cancer

Neutrophil polarization into distinct subsets, primarily N1 and N2 neutrophils, has emerged as a critical

determinant in shaping the immune landscape of breast cancer. While N1 neutrophils are associated with tumor suppression and antitumor immunity, N2 neutrophils play a role in immune suppression, tumor progression, and metastasis. Given their pivotal functions in the tumor microenvironment (TME), targeting neutrophil polarization presents a promising strategy for enhancing cancer therapy. By modulating the balance between N1 and N2 neutrophils, therapeutic interventions could improve the efficacy of existing treatments, reduce metastasis, and overcome immune resistance in breast cancer.[79]

1. Enhancing Antitumor Activity of N1 Neutrophils

One of the most attractive therapeutic approaches for breast cancer involves enhancing the activity and accumulation of N1 neutrophils in the TME. These cells are capable of promoting antitumor immunity through the secretion of pro-inflammatory cytokines, recruitment of cytotoxic immune cells (e.g., T cells and NK cells), and direct cytotoxic activity against tumor cells. Strategies to enhance N1 neutrophil function might include the use of cytokines such as interferon-gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), or toll-like receptor (TLR) agonists.[80] These approaches could activate N1 neutrophils to secrete pro-inflammatory cytokines and increase their cytotoxic potential, thus promoting a more favorabletumor immune environment. Additionally, direct modulation of neutrophil recruitment to the tumor site through chemokine receptor agonists, such as CXCR2 ligands, could further bolster N1 neutrophil numbers and function, amplifying their antitumor effects. Another promising strategy involves leveraging immune checkpoint inhibitors to unleash the full potential of N1 neutrophils. Immune checkpoint blockade, which has shown success in various cancers, could be combined with therapies that stimulate N1 polarization to create a powerful synergistic effect. By inhibiting checkpoint molecules like PD-1 or CTLA-4, these treatments would not only increase T cell activity but could also enhance the effectiveness of N1 neutrophils in combating the tumor. This dual approach could improve overall therapeutic outcomes and provide a more effective strategy to overcome the immunosuppressive tumor microenvironment in breast cancer.[81]

2. Reprogramming N2 Neutrophils to N1 Phenotype

In contrast to promoting N1 polarization, another approach is to reprogram N2 neutrophils to adopt an antitumor N1 phenotype. N2 neutrophils are typically activated by immunosuppressive cytokines such as IL-4, IL-10, and TGF-β, which contribute to a tumor-promoting environment. These cells not only suppress T cell function but also promote angiogenesis and metastasis by releasing matrix metalloproteinases (MMPs). Reversing this polarization by targeting the signaling pathways that drive N2 differentiation could significantly reduce tumor progression and metastasis.[82] Several molecular targets hold promise in reprogramming N2 neutrophils. For example, inhibiting IL-4 or IL-13 signaling could prevent the induction of N2 polarization, while the use of TGF-β inhibitors could block the immunosuppressive effects of N2 neutrophils. Additionally, small molecules or antibodies that target specific transcription factors, such as STAT6, could modulate the differentiation of neutrophils from an N2 to an N1 phenotype. By reprogramming N2 neutrophils, it may be possible to enhance their pro-inflammatory, antitumor activity and restore immune surveillance, ultimately leading to improved tumor control and reduced metastasis.[83]

3. Targeting Neutrophil-Mediated Immune Suppression

While enhancing N1 neutrophil activity and reprogramming N2 neutrophils hold significant promise, it is also essential to directly target the immune-suppressive mechanisms mediated by N2 neutrophils. These cells contribute to tumor progression through the secretion of immunosuppressive cytokines (e.g., IL-10, TGF-β) and the inhibition of cytotoxic T lymphocytes and NK cells. One potential therapeutic strategy involves neutralizing these cytokines or blocking their signaling pathways to prevent N2 neutrophils from creating a tolerogenic environment that promotes immune evasion. For example, TGF-β inhibitors, such as fresolimumab, have been shown to suppress tumor-promoting activities and enhance antitumor immunity in preclinical models.[84] Additionally, targeting the chemokine receptors and adhesion molecules that drive N2 neutrophil infiltration into the tumor could limit their immunosuppressive functions. Chemokine receptor antagonists, such as those targeting CCR2, could reduce the recruitment of N2 neutrophils to the tumor site, thus decreasing their contribution to immune suppression. In combination with other therapies, such as checkpoint inhibitors or anti-cancer antibodies, targeting N2 neutrophil trafficking could synergize to reduce the immunosuppressive influence of the TME and enhance therapeutic outcomes.[85]

4. Combination Therapies Involving Neutrophil Modulation

The therapeutic potential of targeting neutrophil polarization extends beyond single-agent therapies. Combination strategies that incorporate neutrophil modulation with other cancer treatments, such as chemotherapy, radiation therapy, and immune checkpoint inhibitors, could have synergistic effects. For instance, chemotherapy drugs like cyclophosphamide have been shown to modulate the TME by depleting immunosuppressive cells and enhancing the recruitment of immune effector cells, including N1 neutrophils. When used in combination with N1-enhancing therapies, chemotherapy could improve the tumor's susceptibility to immune-mediated clearance.[86] Moreover, the combination of neutrophil-targeted therapies with immune checkpoint inhibitors could help overcome resistance mechanisms that limit the efficacy of checkpoint blockade alone. By reprogramming N2 neutrophils and enhancing N1 neutrophil function simultaneously, this combination approach could significantly improve the tumor response, reduce

metastasis, and promote long-term immune memory, leading to durable clinical outcomes.[87]

Conclusion

Neutrophils, with their ability to polarize into distinct N1 and N2 phenotypes, play a pivotal role in shaping the tumor microenvironment (TME) and influencing the progression of breast cancer. While N1 neutrophils contribute to antitumor immunity by promoting inflammation, enhancing immune cell activity, and directly attacking tumor cells, N2 neutrophils have a more complex role in supporting tumor progression, metastasis, and immune suppression. Understanding the molecular mechanisms driving the polarization of neutrophils and their functional effects in the TME is crucial for developing novel therapeutic strategies.

Targeting neutrophil polarization, by either enhancing N1 neutrophil activity or reprogramming N2 neutrophils into an N1-like state, holds significant therapeutic potential. These approaches can help reshape the TME into a more immunogenic environment, enhance the efficacy of immunotherapies, and reduce tumor metastasis. However, challenges remain in overcoming the complexity of neutrophil behavior within the TME, where factors such as cytokine milieu and tumour-specific characteristics can influence neutrophil polarization and function. Additionally, the need for precise strategies to modulate neutrophil activity without eliciting unwanted side effects presents an ongoing challenge in the clinical translation of these approaches.

Conflict of Interest: Author declares no potential conflict of interest with respect to the contents, authorship, and/or publication of this article.

Source of Support: Nil

Funding: The authors declared that this study has received no financial support.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting in this paper are available in the cited references.

Ethics approval: Not applicable.

References

1. Gong YT, Zhang LJ, Liu YC, Tang M, Lin JY, Chen XY, Chen YX, Yan Y, Zhang WD, Jin JM, Luan X. Neutrophils as potential therapeutic targets for breast cancer. Pharmacological Research. 2023:106996. https://doi.org/10.1016/j.phrs.2023.106996 PMid:37972723

2. Sounbuli K, Mironova N, Alekseeva L. Diverse neutrophil functions in cancer and promising neutrophil-based cancer therapies. International Journal of Molecular Sciences. 2022;23(24):15827. https://doi.org/10.3390/ijms232415827 PMid:36555469 PMCid:PMC9779721

3. Antuamwine BB, Bosnjakovic R, Hofmann-Vega F, Wang X, Theodosiou T, Iliopoulos I, Brandau S. N1 versus N2 and PMN-MDSC: a critical appraisal of current concepts on tumor-associated neutrophils and new directions for human oncology. Immunological Reviews. 2023;314(1):250-279. https://doi.org/10.1111/imr.13176 PMid:36504274

4. Masucci MT, Minopoli M, Carriero MV. Tumor associated neutrophils. Their role in tumorigenesis, metastasis, prognosis and therapy. Frontiers in oncology. 2019;9:1146. https://doi.org/10.3389/fonc.2019.01146 PMid:31799175 PMCid:PMC6874146

5. Zhang X, Zhang W, Yuan X, Fu M, Qian H, Xu W. Neutrophils in cancer development and progression: roles, mechanisms, and implications. International journal of oncology. 2016;49(3):857-867. https://doi.org/10.3892/ijo.2016.3616 PMid:27573431

6. Yang S, Jia J, Wang F, Wang Y, Fang Y, Yang Y, Zhou Q, Yuan W, Bian Z. Targeting neutrophils: Mechanism and advances in cancer therapy. Clinical and Translational Medicine. 2024;14(3):e1599. https://doi.org/10.1002/ctm2.1599 PMid:38450975 PMCid:PMC10918741

7. Mouchemore KA, Anderson RL, Hamilton JA. Neutrophils, G-CSF and their contribution to breast cancer metastasis. The FEBS journal. 2018 ;285(4):665-679. https://doi.org/10.1111/febs.14206 PMid:28834401

8. Ocana A, Nieto-Jiménez C, Pandiella A, Templeton AJ. Neutrophils in cancer: prognostic role and therapeutic strategies. Molecular cancer. 2017;16:1-7. https://doi.org/10.1186/s12943-017-0707-7 PMid:28810877 PMCid:PMC5558711

9. Subhan MA, Torchilin VP. Neutrophils as an emerging therapeutic target and tool for cancer therapy. Life Sciences. 2021;285:119952. https://doi.org/10.1016/j.lfs.2021.119952 PMid:34520766

10. Zheng C, Xu X, Wu M, Xue L, Zhu J, Xia H, Ding S, Fu S, Wang X, Wang Y, He G. Neutrophils in triple-negative breast cancer: an underestimated player with increasingly recognized importance. Breast Cancer Research. 2023;25(1):88. https://doi.org/10.1186/s13058-023-01676-7 PMid:37496019 PMCid:PMC10373263

11. Zhang J, Gu J, Wang X, Ji C, Yu D, Wang M, Pan J, Santos HA, Zhang H, Zhang X. Engineering and Targeting Neutrophils for Cancer Therapy. Advanced Materials. 2024;36(19):2310318. https://doi.org/10.1002/adma.202310318 PMid:38320755

12. Grecian R, Whyte MK, Walmsley SR. The role of neutrophils in cancer. British Medical Bulletin. 2018;128(1):5-14. https://doi.org/10.1093/bmb/ldy029 PMid:30137312 PMCid:PMC6289220

13. Timaxian C, Vogel CF, Orcel C, Vetter D, Durochat C, Chinal C, NGuyen P, Aknin ML, Mercier-Nomé F, Davy M, Raymond-Letron I. Pivotal role for Cxcr2 in regulating tumor-associated neutrophil in breast cancer. Cancers. 2021;13(11):2584. https://doi.org/10.3390/cancers13112584 PMid:34070438 PMCid:PMC8197482

14. Hajizadeh F, Maleki LA, Alexander M, Mikhailova MV, Masjedi A, Ahmadpour M, Hashemi V, Jadidi-Niaragh F. Tumor-associated neutrophils as new players in the immunosuppressive process of the tumor microenvironment in breast cancer. Life sciences. 2021;264:118699. https://doi.org/10.1016/j.lfs.2020.118699 PMid:33137368

15. Liu S, Wu W, Du Y, Yin H, Chen Q, Yu W, Wang W, Yu J, Liu L, Lou W, Pu N. The evolution and heterogeneity of neutrophils in cancers: origins, subsets, functions, orchestrations and clinical applications. Molecular Cancer. 2023 ;22(1):148. https://doi.org/10.1186/s12943-023-01843-6 PMid:37679744 PMCid:PMC10483725

16. Gomes T, Várady CB, Lourenço AL, Mizurini DM, Rondon AM, Leal AC, Gonçalves BS, Bou-Habib DC, Medei E, Monteiro RQ. IL-1β blockade attenuates thrombosis in a neutrophil extracellular trap-dependent breast cancer model. Frontiers in immunology. 2019;10:2088. https://doi.org/10.3389/fimmu.2019.02088 PMid:31552036 PMCid:PMC6737452

17. Jia J, Wang Y, Li M, Wang F, Peng Y, Hu J, Li Z, Bian Z, Yang S. Neutrophils in the premetastatic niche: key functions and therapeutic directions. Molecular Cancer. 2024;23(1):200. https://doi.org/10.1186/s12943-024-02107-7 PMid:39277750 PMCid:PMC11401288

18. Ohms M, Möller S, Laskay T. An attempt to polarize human neutrophils toward N1 and N2 phenotypes in vitro. Frontiers in immunology. 2020;11:532. https://doi.org/10.3389/fimmu.2020.00532 PMid:32411122 PMCid:PMC7198726

19. Tyagi A, Sharma S, Wu K, Wu SY, Xing F, Liu Y, Zhao D, Deshpande RP, D'Agostino Jr RB, Watabe K. Nicotine promotes breast cancer metastasis by stimulating N2 neutrophils and generating pre-metastatic niche in lung. Nature communications. 2021;12(1):474. https://doi.org/10.1038/s41467-020-20733-9 PMid:33473115 PMCid:PMC7817836

20. Dutta A, Bhagat S, Paul S, Katz JP, Sengupta D, Bhargava D. Neutrophils in Cancer and potential therapeutic strategies using neutrophil-derived exosomes. Vaccines. 2023;11(6):1028. https://doi.org/10.3390/vaccines11061028 PMid:37376417 PMCid:PMC10301170

21. Rapoport BL, Steel HC, Theron AJ, Smit T, Anderson R. Role of the neutrophil in the pathogenesis of advanced cancer and impaired responsiveness to therapy. Molecules. 2020 ;25(7):1618. https://doi.org/10.3390/molecules25071618 PMid:32244751 PMCid:PMC7180559

22. Yan M, Zheng M, Niu R, Yang X, Tian S, Fan L, Li Y, Zhang S. Roles of tumor-associated neutrophils in tumor metastasis and its clinical applications. Frontiers in Cell and Developmental Biology. 2022;10:938289. https://doi.org/10.3389/fcell.2022.938289 PMid:36060811 PMCid:PMC9428510

23. Wu G, Pan B, Shi H, Yi Y, Zheng X, Ma H, Zhao M, Zhang Z, Cheng L, Huang Y, Guo W. Neutrophils' dual role in cancer: from tumor progression to immunotherapeutic potential. International Immunopharmacology. 2024;140:112788. https://doi.org/10.1016/j.intimp.2024.112788 PMid:39083923

24. Chen Q, Yin H, Liu S, Shoucair S, Ding N, Ji Y, Zhang J, Wang D, Kuang T, Xu X, Yu J. Prognostic value of tumor-associated N1/N2 neutrophil plasticity in patients following radical resection of pancreas ductal adenocarcinoma. Journal for immunotherapy of cancer. 2022;10(12). https://doi.org/10.1136/jitc-2022-005798 PMid:36600557 PMCid:PMC9730407

25. Li Y, Li M, Su K, Zong S, Zhang H, Xiong L. Pre-metastatic niche: from revealing the molecular and cellular mechanisms to the clinical applications in breast cancer metastasis. Theranostics. 2023;13(7):2301. https://doi.org/10.7150/thno.82700 PMid:37153744 PMCid:PMC10157731

26. Al Qutami F, AlHalabi W, Vijayakumar A, Rawat SS, Mossa AH, Jayakumar MN, Samreen B, Hachim MY. Characterizing the Inflammatory Profile of Neutrophil-Rich Triple-Negative Breast Cancer. Cancers. 2024;16(4):747. https://doi.org/10.3390/cancers16040747 PMid:38398138 PMCid:PMC10886617

27. Mahmud Z, Rahman A, Mishu ID, Kabir Y. Mechanistic insights into the interplays between neutrophils and other immune cells in cancer development and progression. Cancer and Metastasis Reviews. 2022;41(2):405-432. https://doi.org/10.1007/s10555-022-10024-8 PMid:35314951

28. Lecot P, Sarabi M, Pereira Abrantes M, Mussard J, Koenderman L, Caux C, Bendriss-Vermare N, Michallet MC. Neutrophil heterogeneity in cancer: from biology to therapies. Frontiers in immunology. 2019;10:2155. https://doi.org/10.3389/fimmu.2019.02155 PMid:31616408 PMCid:PMC6764113

29. Zhao J, Xie X. Prediction of prognosis and immunotherapy response in breast cancer based on neutrophil extracellular traps-related classification. Frontiers in Molecular Biosciences. 2023;10:1165776. https://doi.org/10.3389/fmolb.2023.1165776 PMid:37304069 PMCid:PMC10250592

30. Wang X, Qiu L, Li Z, Wang XY, Yi H. Understanding the multifaceted role of neutrophils in cancer and autoimmune diseases. Frontiers in immunology. 2018;9:2456. https://doi.org/10.3389/fimmu.2018.02456 PMid:30473691 PMCid:PMC6237929

31. Shaul ME, Fridlender ZG. Cancer-related circulating and tumor-associated neutrophils-subtypes, sources and function. The FEBS journal. 2018;285(23):4316-4342. https://doi.org/10.1111/febs.14524 PMid:29851227

32. Awasthi D, Sarode A. Neutrophils at the crossroads: unraveling the multifaceted role in the tumor microenvironment. International Journal of Molecular Sciences. 2024;25(5):2929. https://doi.org/10.3390/ijms25052929 PMid:38474175 PMCid:PMC10932322

33. Raftopoulou S, Valadez-Cosmes P, Mihalic ZN, Schicho R, Kargl J. Tumor-mediated neutrophil polarization and therapeutic implications. International Journal of Molecular Sciences. 2022;23(6):3218. https://doi.org/10.3390/ijms23063218 PMid:35328639 PMCid:PMC8951452

34. Cristinziano L, Modestino L, Antonelli A, Marone G, Simon HU, Varricchi G, Galdiero MR. Neutrophil extracellular traps in cancer. InSeminars in cancer biology 2022;79:91-104. Academic Press. https://doi.org/10.1016/j.semcancer.2021.07.011 PMid:34280576

35. Gianni C, Palleschi M, Schepisi G, Casadei C, Bleve S, Merloni F, Sirico M, Sarti S, Cecconetto L, Di Menna G, Schettini F. Circulating inflammatory cells in patients with metastatic breast cancer: Implications for treatment. Frontiers in oncology. 2022;12:882896. https://doi.org/10.3389/fonc.2022.882896 PMid:36003772 PMCid:PMC9393759

36. Saraiva DP, Correia BF, Salvador R, de Sousa N, Jacinto A, Braga S, Cabral MG. Circulating low density neutrophils of breast cancer patients are associated with their worse prognosis due to the impairment of T cell responses. Oncotarget. 2021;12(24):2388. https://doi.org/10.18632/oncotarget.28135 PMid:34853660 PMCid:PMC8629401

37. Han Q, Wang M, Dong X, Wei F, Luo Y, Sun X. Non-coding RNAs in hepatocellular carcinoma: Insights into regulatory mechanisms, clinical significance, and therapeutic potential. Frontiers in Immunology. 2022;13:985815. https://doi.org/10.3389/fimmu.2022.985815 PMid:36300115 PMCid:PMC9590653

38. Guo F, Li H, Wang J, Wang J, Zhang J, Kong F, Zhang Z, Zong J. MicroRNAs in Hepatocellular Carcinoma: Insights into Regulatory Mechanisms, Clinical Significance, and Therapeutic Potential. Cancer Management and Research. 2024:1491-1507. https://doi.org/10.2147/CMAR.S477698 PMid:39450194 PMCid:PMC11499618

39. Rui XU, Zehao WA, Jiong WU. Advances in the role of tumor-associated neutrophils in the development of breast cancer. China Oncology. 2024;34(9):881-889.

40. Pylaeva E, Lang S, Jablonska J. The essential role of type I interferons in differentiation and activation of tumor-associated neutrophils. Frontiers in immunology. 2016;7:629. https://doi.org/10.3389/fimmu.2016.00629 PMid:28066438 PMCid:PMC5174087

41. Giese MA, Hind LE, Huttenlocher A. Neutrophil plasticity in the tumor microenvironment. Blood, The Journal of the American Society of Hematology. 2019;133(20):2159-2167. https://doi.org/10.1182/blood-2018-11-844548 PMid:30898857 PMCid:PMC6524564

42. Lau D, Lechermann LM, Gallagher FA. Clinical translation of neutrophil imaging and its role in cancer. Molecular Imaging and Biology. 2022;24(2):221-234. https://doi.org/10.1007/s11307-021-01649-2 PMid:34637051 PMCid:PMC8983506

43. Munkácsy G, Santarpia L, Győrffy B. Therapeutic potential of tumor metabolic reprogramming in triple-negative breast cancer. International Journal of Molecular Sciences. 2023;24(8):6945. https://doi.org/10.3390/ijms24086945 PMid:37108109 PMCid:PMC10138520

44. Chen Q, Zhang L, Li X, Zhuo W. Neutrophil extracellular traps in tumor metastasis: pathological functions and clinical applications. Cancers. 2021;13(11):2832. https://doi.org/10.3390/cancers13112832 PMid:34204148 PMCid:PMC8200981

45. Treffers LW, Hiemstra IH, Kuijpers TW, Van den Berg TK, Matlung HL. Neutrophils in cancer. Immunological reviews.

2016;273(1):312-328. https://doi.org/10.1111/imr.12444 PMid:27558343

46. SenGupta S, Hein LE, Xu Y, Zhang J, Konwerski JR, Li Y, Johnson C, Cai D, Smith JL, Parent CA. Triple-negative breast cancer cells recruit neutrophils by secreting TGF-β and CXCR2 ligands. Frontiers in immunology. 2021;12:659996. https://doi.org/10.3389/fimmu.2021.659996 PMid:33912188 PMCid:PMC8071875

47. Buzaglo GB, Telles GD, Araújo RB, Junior GD, Ruberti OM, Ferreira ML, Derchain SF, Vechin FC, Conceição MS. The Therapeutic Potential of Physical Exercise in Cancer: The Role of Chemokines. International Journal of Molecular Sciences. 2024;25(24):13740. https://doi.org/10.3390/ijms252413740 PMid:39769501 PMCid:PMC11678861

48. Bareke H, Akbuga J. Complement system's role in cancer and its therapeutic potential in ovarian cancer. Scandinavian Journal of Immunology. 2018;88(1):e12672. https://doi.org/10.1111/sji.12672 PMid:29734524

49. Andzinski L, Kasnitz N, Stahnke S, Wu CF, Gereke M, von Köckritz-Blickwede M, Schilling B, Brandau S, Weiss S, Jablonska J. Type I IFN s induce anti-tumor polarization of tumor associated neutrophils in mice and human. International journal of cancer. 2016;138(8):1982-1993. https://doi.org/10.1002/ijc.29945 PMid:26619320

50. Ji H, Hu C, Yang X, Liu Y, Ji G, Ge S, Wang X, Wang M. Lymph node metastasis in cancer progression: molecular mechanisms, clinical significance and therapeutic interventions. Signal Transduction and Targeted Therapy. 2023;8(1):367. https://doi.org/10.1038/s41392-023-01576-574 PMid:37752146 PMCid:PMC10522642

51. Soto-Perez-de-Celis E, Chavarri-Guerra Y, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor-associated neutrophils in breast cancer subtypes. Asian Pacific journal of cancer prevention: APJCP. 2017;18(10):2689.

52. Chen Q, Yin H, Liu S, Shoucair S, Ding N, Ji Y, Zhang J, Wang D, Kuang T, Xu X, Yu J. Prognostic value of tumor-associated N1/N2 neutrophil plasticity in patients following radical resection of pancreas ductal adenocarcinoma. Journal for immunotherapy of cancer. 2022;10(12). https://doi.org/10.1136/jitc-2022-005798 PMid:36600557 PMCid:PMC9730407

53. Zeindler J, Angehrn F, Droeser R, Däster S, Piscuoglio S, Ng CK, Kilic E, Mechera R, Meili S, Isaak A, Weber WP. Infiltration by myeloperoxidase-positive neutrophils is an independent prognostic factor in breast cancer. Breast cancer research and treatment. 2019;177:581-589. https://doi.org/10.1007/s10549-019-05336-3 PMid:31267330

54. Tyagi A, Sharma S, Wu K, Wu SY, Xing F, Liu Y, Zhao D, Deshpande RP, D'Agostino Jr RB, Watabe K. Nicotine promotes breast cancer metastasis by stimulating N2 neutrophils and generating pre-metastatic niche in lung. Nature communications. 2021;12(1):474. https://doi.org/10.1038/s41467-020-20733-9 PMid:33473115 PMCid:PMC7817836

55. Yin H, Gao S, Chen Q, Liu S, Shoucair S, Ji Y, Lou W, Yu J, Wu W, Pu N. Tumor-associated N1 and N2 neutrophils predict prognosis in patients with resected pancreatic ductal adenocarcinoma: A preliminary study. MedComm. 2022;3(4). https://doi.org/10.1002/mco2.183 PMid:36349143 PMCid:PMC9632487

56. Zhao J, Xie X. Prediction of prognosis and immunotherapy response in breast cancer based on neutrophil extracellular traps-related classification. Frontiers in Molecular Biosciences. 202;10:1165776. https://doi.org/10.3389/fmolb.2023.1165776 PMid:37304069 PMCid:PMC10250592

57. Gong YT, Zhang LJ, Liu YC, Tang M, Lin JY, Chen XY, Chen YX, Yan Y, Zhang WD, Jin JM, Luan X. Neutrophils as potential therapeutic targets for breast cancer. Pharmacological Research. 2023:106996. https://doi.org/10.1016/j.phrs.2023.106996 PMid:37972723

58. Grassadonia A, Graziano V, Iezzi L, Vici P, Barba M, Pizzuti L, Cicero G, Krasniqi E, Mazzotta M, Marinelli D, Amodio A. Prognostic relevance of neutrophil to lymphocyte ratio (NLR) in luminal breast cancer: a retrospective analysis in the neoadjuvant setting. Cells. 2021;10(7):1685. https://doi.org/10.3390/cells10071685 PMid:34359855 PMCid:PMC8303552

59. Soto-Perez-de-Celis E, Chavarri-Guerra Y, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor-associated neutrophils in breast cancer subtypes. Asian Pacific journal of cancer prevention: APJCP.;18(10):2689.

60. Saraiva DP, Correia BF, Salvador R, de Sousa N, Jacinto A, Braga S, Cabral MG. Circulating low density neutrophils of breast cancer patients are associated with their worse prognosis due to the impairment of T cell responses. Oncotarget. 2021;12(24):2388. https://doi.org/10.18632/oncotarget.28135 PMid:34853660 PMCid:PMC8629401

61. Zhang W, Shen Y, Huang H, Pan S, Jiang J, Chen W, Zhang T, Zhang C, Ni C. A rosetta stone for breast cancer: prognostic value and dynamic regulation of neutrophil in tumor microenvironment. Frontiers in immunology. 2020;11:1779. https://doi.org/10.3389/fimmu.2020.01779 PMid:32849640 PMCid:PMC7426521

62. Chung AW, Anand K, Anselme AC, Chan AA, Gupta N, Venta LA, Schwartz MR, Qian W, Xu Y, Zhang L, Kuhn J. A phase 1/2 clinical trial of the nitric oxide synthase inhibitor L-NMMA and taxane for treating chemoresistant triple-negative breast cancer. Science translational medicine. 2021;13(624):eabj5070. https://doi.org/10.1126/scitranslmed.abj5070 PMid:34910551

63. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. Nature Reviews Cancer. 2016;16(7):431-446. https://doi.org/10.1038/nrc.2016.52 PMid:27282249

64. Mouchemore KA, Anderson RL, Hamilton JA. Neutrophils, G-CSF and their contribution to breast cancer metastasis. The FEBS journal. 2018;285(4):665-679. https://doi.org/10.1111/febs.14206 PMid:28834401

65. Hajizadeh F, Maleki LA, Alexander M, Mikhailova MV, Masjedi A, Ahmadpour M, Hashemi V, Jadidi-Niaragh F. Tumor-associated neutrophils as new players in immunosuppressive process of the tumor microenvironment in breast cancer. Life sciences. 2021;264:118699. https://doi.org/10.1016/j.lfs.2020.118699 PMid:33137368

66. Al Qutami F, Al Halabi W, Hachim MY. Identification of breast cancer LCK proto-oncogene as a master regulator of TNBC neutrophil enrichment and polarization. International journal of molecular sciences. 2023;24(17):13269. https://doi.org/10.3390/ijms241713269 PMid:37686072 PMCid:PMC10487917

67. Zheng C, Xu X, Wu M, Xue L, Zhu J, Xia H, Ding S, Fu S, Wang X, Wang Y, He G. Neutrophils in triple-negative breast cancer: an underestimated player with increasingly recognized importance. Breast Cancer Research. 2023;25(1):88. https://doi.org/10.1186/s13058-023-01676-7 PMid:37496019 PMCid:PMC10373263

68. Shaul ME, Fridlender ZG. Tumour-associated neutrophils in patients with cancer. Nature reviews Clinical oncology. 2019;16(10):601-620. https://doi.org/10.1038/s41571-019-0222-4 PMid:31160735

69. Schernberg A, Blanchard P, Chargari C, Deutsch E. Neutrophils, a candidate biomarker and target for radiation therapy?. Acta oncologica. 2017;56(11):1522-1530. https://doi.org/10.1080/0284186X.2017.1348623 PMid:28835188

70. Li Z, Zhao R, Cui Y, Zhou Y, Wu X. The dynamic change of neutrophil to lymphocyte ratio can predict clinical outcome in stage I-III colon cancer. Scientific reports. 2018;8(1):9453. https://doi.org/10.1038/s41598-018-27896-y PMid:29930287 PMCid:PMC6013456

71. Wang P, Xu MH, Xu WX, Dong ZY, Shen YH, Qin WZ. CXCL9 Overexpression Predicts Better HCC Response to Anti-PD-1 Therapy and Promotes N1 Polarization of Neutrophils. Journal of Hepatocellular Carcinoma. 2024:787-800. https://doi.org/10.2147/JHC.S450468 PMid:38737384 PMCid:PMC11088828

72. Masucci MT, Minopoli M, Carriero MV. Tumor associated neutrophils. Their role in tumorigenesis, metastasis, prognosis and therapy. Frontiers in oncology. 2019;9:1146. https://doi.org/10.3389/fonc.2019.01146 PMid:31799175 PMCid:PMC6874146

73. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, Sautès-Fridman C, Ma Y, Tartour E, Zitvogel L, Kroemer G. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology. 2012;1(8):1323-1343. https://doi.org/10.4161/onci.22009 PMid:23243596 PMCid:PMC3518505

74. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nature Reviews Cancer. 2020;20(11):662-680. https://doi.org/10.1038/s41568-020-0285-7 PMid:32753728

75. Xu W, Weng J, Xu M, Zhou Q, Liu S, Hu Z, Ren N, Zhou C, Shen Y. Chemokine CCL21 determines immunotherapy response in hepatocellular carcinoma by affecting neutrophil polarization. Cancer Immunology, Immunotherapy. 2024;73(3):56. https://doi.org/10.1007/s00262-024-03650-4 PMid:38367070 PMCid:PMC10874310

76. Huang YC, Chang CY, Wu YY, Wu KL, Tsai YM, Lee HC, Tsai EM, Hsu YL. Single-cell transcriptomic profiles of lung pre-metastatic niche reveal neutrophil and lymphatic endothelial cell roles in breast cancer. Cancers. 2022;15(1):176. https://doi.org/10.3390/cancers15010176 PMid:36612175 PMCid:PMC9818165

77. Wu Y, Liu H, Sun Z, Liu J, Li K, Fan R, Dai F, Tang H, Hou Q, Li J, Tang X. The adhesion-GPCR ADGRF5 fuels breast cancer progression by suppressing the MMP8-mediated antitumorigenic effects. Cell Death & Disease. 2024;15(6):455. https://doi.org/10.1038/s41419-024-06855-8 PMid:38937435 PMCid:PMC11211477

78. Gianni C, Palleschi M, Schepisi G, Casadei C, Bleve S, Merloni F, Sirico M, Sarti S, Cecconetto L, Di Menna G, Schettini F. Circulating inflammatory cells in patients with metastatic breast cancer: Implications for treatment. Frontiers in oncology. 2022;12:882896. https://doi.org/10.3389/fonc.2022.882896 PMid:36003772 PMCid:PMC9393759

79. Jadoon, S.K., Soomro, R., Ahsan, M.N., Khan, R.M.I., Iqbal, S., Yasmin, F., Najeeb, H., Saleem, N., Cho, N., Shaikh, T.G. and Hasan, S.F.S., Association of neutrophil-to-lymphocyte ratio with clinical, pathological, radiological, laboratory features and disease outcomes of invasive breast cancer patients: A retrospective observational cohort study. Medicine, 2023;102(20):e33811. https://doi.org/10.1097/MD.0000000000033811 PMid:37335707 PMCid:PMC10194494

80. Tabariès S, Ouellet V, Hsu BE, Annis MG, Rose AA, Meunier L, Carmona E, Tam CE, Mes-Masson AM, Siegel PM. Granulocytic immune infiltrates are essential for the efficient formation of breast cancer liver metastases. Breast cancer research. 2015;17:1-8. https://doi.org/10.1186/s13058-015-0558-3 PMid:25882816 PMCid:PMC4413545

81. Hu P, Liu Q, Deng G, Zhang J, Liang N, Xie J, Zhang J. Radiosensitivity nomogram based on circulating neutrophils in thoracic cancer. Future Oncology. 2019;15(7):727-737. https://doi.org/10.2217/fon-2018-0398 PMid:30693787

82. Wang J, Ocadiz-Ruiz R, Hall MS, Bushnell GG, Orbach SM, Decker JT, Raghani RM, Zhang Y, Morris AH, Jeruss JS, Shea LD. A synthetic metastatic niche reveals antitumor neutrophils drive breast cancer metastatic dormancy in the lungs. Nature communications. 2023;14(1):4790. https://doi.org/10.1038/s41467-023-40478-5 PMid:37553342 PMCid:PMC10409732

83. Schmidt E, Distel L, Erber R, Büttner-Herold M, Rosahl MC, Ott OJ, Strnad V, Hack CC, Hartmann A, Hecht M, Fietkau R. Tumor-Associated Neutrophils Are a Negative Prognostic Factor in Early Luminal Breast Cancers Lacking Immunosuppressive Macrophage Recruitment. Cancers. 2024;16(18):3160. https://doi.org/10.3390/cancers16183160 PMid:39335132 PMCid:PMC11430230

84. Grassadonia A, Graziano V, Iezzi L, Vici P, Barba M, Pizzuti L, Cicero G, Krasniqi E, Mazzotta M, Marinelli D. Prognostic relevance of neutrophil to lymphocyte ratio (NLR) in luminal breast cancer: a retrospective analysis in the neoadjuvant setting. Cells. 2021;10(7):1685. https://doi.org/10.3390/cells10071685 PMid:34359855 PMCid:PMC8303552

85. Liu S, Wu W, Du Y, Yin H, Chen Q, Yu W, Wang W, Yu J, Liu L, Lou W, Pu N. The evolution and heterogeneity of neutrophils in cancers: origins, subsets, functions, orchestrations and clinical applications. Molecular Cancer. 2023;22(1):148. https://doi.org/10.1186/s12943-023-01843-6 PMid:37679744 PMCid:PMC10483725

86. Azab BN, Bhatt VR, Vonfrolio S, Bachir R, Rubinshteyn V, Alkaied H, Habeshy A, Patel J, Picon AI, Bloom SW. Value of the pretreatment albumin to globulin ratio in predicting long-term mortality in breast cancer patients. The American Journal of Surgery. 2013;206(5):764-770. https://doi.org/10.1016/j.amjsurg.2013.03.007 PMid:23866764

87. Zhang J, Yu D, Ji C, Wang M, Fu M, Qian Y, Zhang X, Ji R, Li C, Gu J, Zhang X. Exosomal miR-4745-5p/3911 from N2-polarized tumor-associated neutrophils promotes gastric cancer metastasis by regulating SLIT2. Molecular Cancer. 2024;23(1):198. https://doi.org/10.1186/s12943-024-02116-6 PMid:39272149 PMCid:PMC11396805