CellPress

Immunity

Perspective

From complexity to consensus: A roadmap for neutrophil classification

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SUMMARY

Neutrophils, previously considered a homogeneous immune cell population, exhibit substantial heterogeneity. Their diverse phenotypic and functional states are shaped by tissue microenvironments and disease-specific signals. However, the lack of robust fate-mapping methods and standardized classification criteria has led to overlapping and ambiguous descriptions of neutrophil heterogeneity. The growing number of neutrophil subpopulations reported in recent years highlights the need for a standardized framework to report how they might relate to each other. Here, we propose a framework that integrates maturation, tissue localization, and functional adaptations. This standardized system aims to harmonize research efforts, foster clearer cross-disciplinary communication, and accelerate both fundamental discoveries in neutrophil biology and the development of targeted therapies.

INTRODUCTION

Neutrophils are indispensable innate immune protectors against a wide variety of pathogens. As one of the first recruited cells at the inflammatory site, neutrophils, upon stimulation, rapidly secrete various pro-inflammatory mediators to neutralize threats. However, the discharge of noxious granule proteins, along with the release of potent reactive oxygen species (ROS) and neutrophil extracellular traps (NETs), can also cause collateral damage to surrounding tissues. Consequently, neutrophil activity is tightly regulated, a mechanism that preserves tissue homeostasis and prevents excessive inflammation. As millions of neutrophils circulate in the human blood and migrate into tissues, they are constantly replenished by bone marrow progenitors in a daily rhythm. Throughout their lifespan across tissues, neutrophils dynamically transit through a range of phenotypes

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https://doi.org/10.1016/j.immuni.2025.07.011

and functions, generating a constant flux of cell states within the neutrophil pool. The advent of advanced multi-omic technologies has enabled in-depth assessment of neutrophils, uncovering their molecular landscape through gene and protein expression studies at the single-cell level. They have also illuminated the mechanisms driving this phenotypic and functional heterogeneity.

Recent findings have demonstrated that neutrophils can adopt a myriad of functional states, shaped by tissue microenvironments and specific diseases. These states reflect not only their role in acute defense but also their contributions to chronic disease progression. Despite efforts to establish frameworks for neutrophil characterization, existing studies often describe different aspects of neutrophil heterogeneity without sufficient cross-mapping to other identified states, thereby resulting in a highly complex web of discordant descriptions. This ambiguity arises from the use of different technologies and techniques, along with the expansion into different pathological contexts, making it difficult to establish clear relationships between findings. Therefore, it is essential for research groups to adopt a standardized approach to report neutrophil subtypes, ensuring consistency, comparability, and effective communication across studies.

Immune cell subpopulations are conventionally identified through a combination of ontogenic, epigenetic, transcriptomic, phenotypic, and functional analyses. However, this approach presents unique challenges for neutrophils compared to other immune cell types. This difficulty arises from the neutrophil's intrinsic role as rapidly responding innate cells, capable of adapting to microenvironmental cues. Neutrophils express a wide variety of receptors, including pattern-recognition receptors (PRRs), phagocytic receptors, and cytokine and chemokine receptors, enabling them to swiftly detect and respond to external stimuli.[1] Upon stimulation, they transition into primed or activated states, partially in a tissue- or microenvironment-dependent manner, tailoring their responses to the local tissue and enabling them to influence other immune cells that amplify their impact beyond direct pathogen control. These dynamic changes complicate their classification, making it challenging to define distinct neutrophil subsets with precision.

Adding to this complexity is the inherent developmental heterogeneity of neutrophils originating from the bone marrow. Under homeostatic conditions, the release of mature neutrophils into the circulation, at least in mice, follows circadian fluctuations, maintaining a tight balance between neutrophil output and clearance.[2–4] By contrast, stress or inflammatory conditions

promote increased release of mature neutrophils, accompanied by the premature mobilization of immature neutrophils into the circulation (also known as "emergency granulopoiesis"). These factors, combined with tissue adaptations acquired throughout their lifespan, contribute to the diversity of the neutrophil pool at any given stage of the inflammatory cascade. Consequently, neutrophils with varying phenotypic and functional characteristics may coexist under both resting and stress conditions, making it difficult to precisely classify neutrophil heterogeneity observed across different studies. In this perspective article, we first highlight the key features of neutrophil biology that contribute to their complexity, including their developmental heterogeneity, dynamic responses to environmental cues, functional plasticity, and their capacity for prolonged survival under specific conditions. Subsequently, we propose a set of considerations to integrate these characteristics into a nomenclature system for neutrophil classification. Our goal is to establish a framework that serves as an inclusive and adaptable guideline for researchers studying neutrophil biology across different contexts and disease conditions.

MULTI-OMICS INTEGRATION REFINES NEUTROPHIL CLASSIFICATION BEYOND CLASSICAL DEFINITIONS

The diversity of neutrophil appearances has led to discrepancies among research groups utilizing different methodologies for their characterization. Historically, neutrophil heterogeneity was first described by hematologists: marrow smears and contrastive staining revealed the morphological attributes of the developing neutrophils, including their distinct nuclear segmentation and neutral-staining granules. Further characterization of granule composition improved our understanding of neutrophil maturation states based on the presence of primary, secondary, or tertiary granule types.[5] The segmentation of the neutrophil nucleus is a well-established hallmark of maturation and has been used as a clinical indicator of disease. For example, the presence of neutrophils with banded nuclei in the blood, commonly known as a "left shift" phenomenon, is a recognized indicator of infection.[6]

Characterizing the developmental heterogeneity of neutrophils has been a major point of contention, caused by the disconnect between the classical morphology-based descriptions and the identification of additional subpopulations through refined profiling by gene and protein expression. The traditional classification of neutrophil maturation states—myeloblast, promyelocyte, myelocyte, metamyelocyte, band cell, and segmented cell—has provided fundamental insights into the transcription factors and granule production essential for neutrophil development and function. Despite technological constraints, hematological characterization of neutrophils enriched our understanding of granulocyte cell states and revealed underpinning mechanisms in genetic disorders with aberrant neutrophil maturation or function.[7,8] Moreover, they have also led to the identification of the corresponding surface marker and bulk transcriptomic profiles of each morphological state.[9–11] These early approaches underscore how the classification of neutrophils can contribute to clinical applications, highlighting the importance of refining these definitions for better diagnostic and therapeutic strategies.

Advances in single-cell-based phenotyping strategies, such as single-cell RNA sequencing (scRNA-seq) and mass cytometry, have enabled in-depth characterization of the gene and surface protein expression landscape in neutrophil subsets across health and disease conditions. A typical workflow relies on selected marker genes and surface protein markers to delineate additional neutrophil subpopulations and to ascribe functions to them. However, this methodology has also introduced ambiguity, as differences in interpretation, marker selection, and validation strategies blur the distinction between subpopulations identified by different laboratories, leading to discrepancies and inconsistencies across the field (Figure 1). For example, the increased resolution afforded by single-cell phenotyping tools has led to the identification of precursor populations with phenotypic traits that overlap with existing classifications.[12–15] Such overlaps suggest that neutrophil heterogeneity may reflect a continuum rather than discrete subsets. Additionally, various approaches to studying bone marrow neutrophils have resulted in similar or overlapping subpopulations, complicating the historically well-established morphology-based nomenclature used to define neutrophil maturation (Figure 1). Although several attempts have been made to unify these emerging phenotypic definitions,[8,12,16–19] the adoption of these refined definitions has been challenged by the lack of full alignment between morphology-based and molecular classification systems. Moreover, the inherently low transcript counts in neutrophils compared with many other immune cells reduce the sensitivity and reliability of neutrophil detection and characterization by scRNA-seq, likely contributing to differences in neutrophil identification across studies.

NEUTROPHIL PLASTICITY ENABLES FUNCTIONAL ADAPTATION ACROSS INFLAMMATORY CONTEXTS

Neutrophils initially develop in a "naive" state within the bone marrow. Once in the bloodstream as mature cells, they undergo a priming phase while maintaining a degree of neutrality. This transitional "uncommitted state" allows neutrophils to remain highly adaptable, enabling them to respond effectively to environmental cues.[30,31] Circulating neutrophils are dynamically influenced by circadian rhythms, systemic factors, and interactions with the vasculature. For a detailed overview of these regulatory mechanisms, readers are encouraged to refer to a recent review.[32] As they circulate in the bloodstream, neutrophils continuously integrate signals from these cues, which shape their lifespan, drive adaptation, and prime them for heightened activity. Systemic signals—such as tumor-derived granulocyte colony-stimulating factor (G-CSF) or interleukin-6 (IL-6)—can initiate and promote neutrophil adaptations even before tissue infiltration, including within the bone marrow. This has been shown in mouse models[33] and circulating neutrophils of cancer patients, which elicit endoplasmic reticulum stress response changes, resulting in distinct metabolic and migratory activites.[34,35]

The dynamism of the circulating neutrophil population is illustrated in mice by the discovery that they can exhibit different behavioral states as determined by using 4D live imaging, which reveals morphodynamic patterns that predict the presence of at least three categories of neutrophils that could not be defined

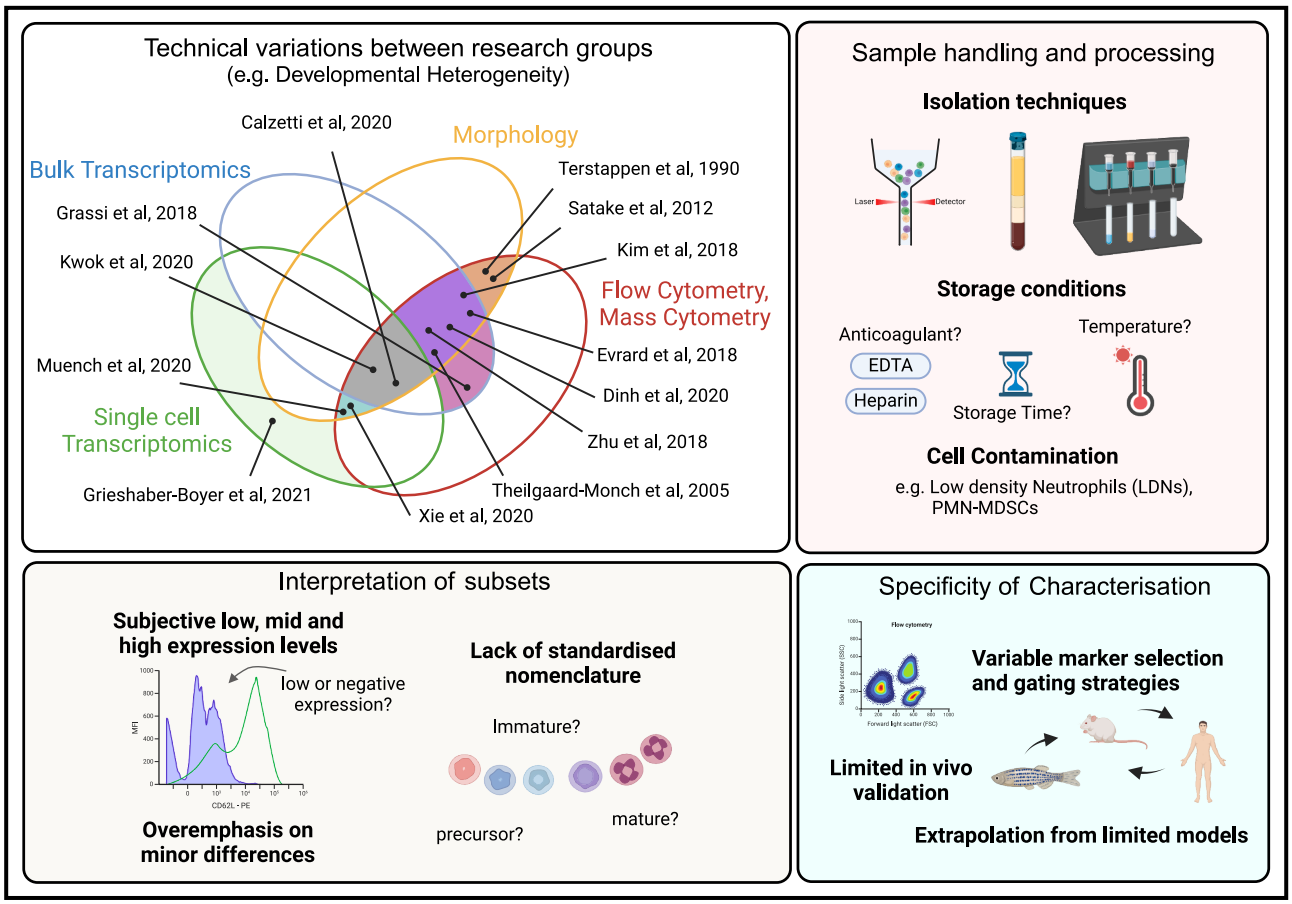

Figure 1. Sources of data variability contributing to overestimation of neutrophil heterogeneity

A common issue faced in cell biology is the variation of techniques and technologies used to characterize cell types. In studying neutrophil development, for example, research groups use a combination of techniques for the characterization of neutrophil intermediates. The use of single-cell transcriptomics[20–22] reveals finer details of neutrophil states compared with protein-based cytometric or morphology-based analyses.[10,23,24] Different pre-analytical choices, such as the method of isolation, the anticoagulant used, the handling time and temperature, and potential impurities, also contribute to differences in the raw data.[25,26] Interpreting these data can never be fully unbiased, contributing to highly variable definitions of neutrophil states that partially overlap between studies. Ly6G, for example, is expressed as a continuum in the mouse bone marrow and further influenced by inflammatory status. Demarcating a "positive" population depends on antibody staining concentrations, fluorophore selection, flow cytometer, and analytical strategy. As reactive cells, neutrophils can be challenging to handle.[27] Cell contamination, when isolating or sorting, can further lead to misrepresentation of neutrophil function and phenotypes.[26] Finally, both sex[28] and species can possess conserved and divergent features, and cross-species mapping is challenging.[20,29] Created with Biorender.com.

using traditional transcriptomic and phenotypic profiling.[36] Notably, these categories are functionally relevant as determined by their differential sensitivity to kinase inhibitors and causal association with inflammatory disease. We anticipate that the use of additional technologies will unveil layers of diversity, urging for a consistent framework that can embrace and incorporate these discoveries.

Upon tissue infiltration, neutrophils acquire diverse functional properties depending on the local cytokine and metabolic milieu, including pro-resolving, reparative, regulatory, or NETotic phenotypes.[31] This is true even under resting conditions, in which tissue-infiltrated mature neutrophils adopt diverse phenotypes and functional states depending on the local milieu.[37,38] In this resting state, neutrophil development in the bone marrow primarily favors maturation pathways that support immunoregulation and tissue maintenance. However, under stress conditions—such as infection, tissue injury, or systemic inflammation—this balance shifts, driving accelerated neutrophil production and the premature release of immature cells into circulation to meet the increased demand for immune defense. Once in the tissue, local inflammatory cues can further reprogram these recruited neutrophils.[20,29]

Independent of tissue influences, neutrophil identity is arguably defined by its maturation status. The prevailing paradigm suggests that, even during a challenge, neutrophils adhere to a developmental trajectory without skipping maturation stages. Collectively, the field has successfully isolated and validated distinct neutrophil subpopulations corresponding to different maturation stages, enabling the creation of a thorough catalog of surface markers and gene expression profiles.[11,13,14,21–24,39] These studies depict a linear developmental trajectory of increasing maturity and function, conserved across mice and humans.[20,29] In disease conditions, neutrophils at intermediate maturation stages have been detected in the circulation and within tissues.[20,29,40] Notably, their developmental maturity can influence their function, including antimicrobial efficacy, lifespan, and migratory responsiveness.[11,41] The availability of receptors

and ligands on each subpopulation, coupled with their inherent nuclear morphological characteristics,[42,43] determines their capacity to sense and adapt to the inflammatory stimuli within the local environment.

In addition to the influence of developmental maturation and tissue-specific cues, therapeutic interventions can also shape neutrophil functional properties. For example, certain cancer immunotherapies promote the emergence of neutrophil populations with anti-tumor functions.[44–46] Such findings highlight the plasticity of neutrophil responses and emphasize the importance of considering both endogenous developmental programs and exogenous therapeutic pressures when defining neutrophil subpopulations across health and disease.

From this collection of observations, an important question arises: how does the developmental trajectory of neutrophils align with their adaptation program? Notably, inflammatory signals introduce another layer of complexity to neutrophil heterogeneity. Recent evidence suggests that, in response to various cues, neutrophils can decouple their developmental trajectory from the subsequent acquisition of a terminal functional phenotype, thus exhibiting adaptive flexibility.[47] This decoupling enables them to respond to microenvironmental signals and achieve functional convergence at local effector sites, underscoring their capacity to act as both initiators and responders in immune cascades. We will explore this concept further in the next section.

REFRAMING NEUTROPHIL HETEROGENEITY THROUGH THE LENS OF FUNCTIONAL CONVERGENCE

Cellular diversity and adaptability are complementary rather than mutually exclusive features of the immune system. Immune cells utilize various mechanisms that allow them to effectively respond to a stimulus while maintaining heterogeneous states. Consider the macrophage: a diverse group of niche-imprinted specialists integral for tissue homeostasis.[48] In disease conditions, macrophages are known to acquire context-dependent states, mounting a concerted response despite their distinct developmental origins and tissue-specific functions.[49] Furthermore, these activated states are reinforced by recruited monocytes that differentiate into required states, enhancing or replacing existing pools.[50] In a similar vein, effector T cells achieve functional coherence through clonal expansion in response to cytokine cues, enabling an effective and targeted adaptive response against the specific foreign antigens. B cells similarly expand and refine immunoglobulin specificity and affinity within germinal centers, enabling them to produce antibodies with enhanced binding capabilities against specific antigens.

Following these other immune paradigms, neutrophils can exist as heterogeneous subpopulations while being able to mount cooperative responses against threats the body commonly faces. Since they are short-lived and do not divide after maturity, neutrophils rely on continuous replenishment from circulation to sustain their presence at localized sites. In a continual process, early-infiltrating subsets functionally adapt toward local cues or systemic signals, streamlining the overall response appropriate for the given environment and the nature of the threat. Notably, this local response appears to be independent of their maturation status, as recently shown in a model of pancreatic adenocarcinoma, in which both immature and mature neutrophils reprogram into a common terminal state attributed to tumor progression and growth.[30] Regardless of their initial developmental origin and subtype, a convergence of functional states underpins a perspective toward the apparent heterogeneity within tumors involving time kinetics and program fate. This sheds light on studies where tumor-infiltrating neutrophils are described to exist in four,[51] five,[52] six,[53,54] or even nine[55] transcriptional clusters. Dividing a heterogeneous snapshot of cell states into a fixed number of populations is inherently imprecise, as all such approaches rely on underlying assumptions and are not entirely unbiased. Consequently, no definitive number of cell states represents the "ground truth." Notably, RNA velocity or pseudotime analyses in these studies often implicate one cluster as the endpoint of neutrophil phenotypes in the tumor microenvironment.[30,55] It is likely that the majority of the transcriptional clusters identified in these studies represent transitionary states of neutrophils with varying maturity and stages of reprogramming. As such, among other possible mechanisms, the tumor microenvironment continuously drives neutrophils toward terminal functional states that are traceable by distinct surface markers.[30,55]

Achieving functional homogeneity in immune cells often requires strong signals that overpower competing cues to favor a common phenotype and function. At wound sites, the combination of acute pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1β, along with the presence of pathogens and associated microbial products, creates a potent stimulatory environment. The abundance of these signals within the localized tissue can considerably amplify the strength of these cues. Similarly, strong deprivation of certain signals can drive functional uniformity. Within tumors, factors such as hypoxia and nutrient deprivation can lead to cellular adaptations promoting homogeneity.[56,57] Cells are forced to adopt alternative metabolic pathways, resulting in changes in effector functions that require certain metabolites.[58] For example, neutrophils upregulate fatty acid transport protein 2 FATP2 (SLC27A2) to enhance fatty acid uptake, which drives increased fatty acid oxidation. This process, in turn, promotes immunosuppressive properties through the production of prostaglandin E_2.[59,60] This metabolic shift underscores how environmental pressures can unify neutrophils' roles across diverse contexts. Consequently, this synchronization of functions leads to further confusion, as similar functional states can be the result of transcriptomically heterogeneous subpopulations.

NAVIGATING THE CHALLENGES OF NAMING NEUTROPHIL POPULATIONS

The current nomenclature of neutrophil subpopulations remains inconsistent and ambiguous, highlighting the challenge of categorizing these highly dynamic and responsive cells. Specifically, the nomenclature reflects the fact that neutrophils exist along a spectrum of transcriptional and functional states influenced by developmental maturity, tissue microenvironment, molecular programming, and functional specialization. This contrasts with classification systems that apply rigid labels but fail to describe more dynamic states. Traditional approaches often define populations based on a single marker or isolated function, despite

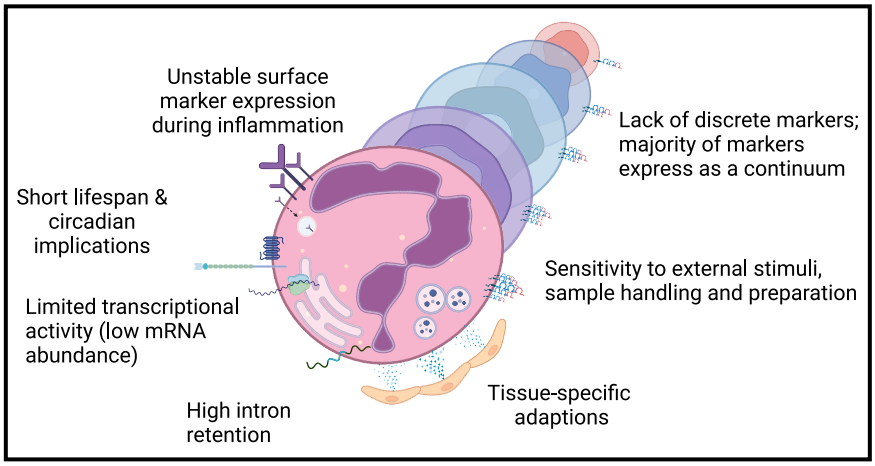

Figure 2. Challenges for a unified neutrophil classification

The inherent properties of neutrophils create many challenges, preventing robust characterization among research groups. Unlike other cell types, the many surface markers used for phenotyping can fluctuate in expression due to inflammatory cues or improper sample handling techniques. For instance, markers, such as Ly6G (mouse) or CD62L (mouse and human), do not typically follow a bimodal expression pattern during development. High rates of intron retention complicate the analysis and interpretation of transcriptomic data.[69] The expression of many markers as a continuous rather than clear bimodal distribution further complicates the assignment of neutrophils to finite clusters. Furthermore, the low transcriptional activity of neutrophils poses a technical challenge to attributing function and distinct states. Created with Biorender.com.

growing evidence that neutrophil markers and functions are dynamic and are heavily influenced by tissue context and disease state. Adding to the complexity, the same neutrophil population may be named differently across studies or characterized by diverse functions depending on the context under study.

For example, names like tumor-associated neutrophils (TANs) and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are frequently used interchangeably in mice studies. In humans, their relationship remains uncertain. Some evidence suggests that PMN-MDSCs instead may represent a subset of TANs, with distinct transcriptomic signatures, functional profiles, and surface markers like CD14 (in mice) and low-density lipoprotein receptor-1 (LOX-1) (in humans).[61–63] The term MDSC, which are also referred to as low-density neutrophils (LDNs), was originally coined in reference to the immunosuppressive abilities of myeloid cells in cancer.[64] However, this term has been applied to neutrophil populations across a wide range of other disease settings, and whether these populations are truly matched in terms of function in the absence of a tumor remains unclear.[65] An additional area of complexity derives from evidence that, in humans, LDNs displaying pro-inflammatory (not immunosuppressive) functions have also been discovered in patients with autoimmune diseases and named "low-density granulocytes" (LDGs).[66] Although substantial progress has been made in defining the specific signature of human mature PMN-MDSCs[67] and total LDGs,[15,64,68] a consensus on their molecular, phenotypic, and functional properties has yet to be established.

From these examples, we identify that the challenges in neutrophil subpopulation characterization can be broadly categorized as follows (Figure 2):

1. Methodological variability: differences in experimental techniques, analytical pipelines, and data interpretation can lead to differences in detected features, resulting in distinct classifications of neutrophils.
2. Heterogeneity and fluctuations in feature expression: many surface and intracellular markers used to define neutrophils have variable expression, partially already driven by stochasticity and varying further by activation status and differentiation stage. Species differences between mice and humans need to be considered as well.
3. Context-specific adaptation and overlapping states: neutrophils adapt dynamically to environmental cues, with specific cues driving particular functional states. Hence, these resulting states display both distinct and overlapping markers or functional profiles.

These overlapping descriptions and inconsistencies pose challenges for the clear delineation of boundaries in defining neutrophil subpopulations.

TOWARD A UNIFIED FRAMEWORK FOR NEUTROPHIL CLASSIFICATION AND FUNCTION

Effective communication of discoveries relies on a shared vocabulary. In the context of macrophages, researchers can define their developmental origins (embryonically or monocyte-derived), adapted functions, and specific niche localization. The widespread adoption of these terms has helped align the field and propelled macrophage research forward.[70] Similarly, T cells have benefited from a standardized nomenclature, relying on a hierarchical structure that takes into account key transcriptional programs, functional outputs, and marker expression.[71,72] Consensus statements have been proposed over a decade ago for both cell types, paving the way toward a shared framework with streamlined sets of requirements for researchers to qualify each subsequent discovery.[48,72,73]

With advances in multi-omics technologies, researchers now have access to large-scale quantitative datasets, allowing a broad characterization of cellular states and transitions. To fully harness this wealth of data, a recently proposed framework for cell classification advocates for a data-driven approach that integrates the diverse cell "observables" while accounting for the dynamic nature of cell states.[31,74] This proposed holistic strategy, which relies on the integration of multi-dimensional data, is invaluable for model development and hypothesis generation. However, it may not be the most suitable approach for establishing a standardized nomenclature for neutrophil subpopulations. A purely genomics data-driven classification, while powerful in capturing continuous cellular states, lacks biological anchoring in cellular identity. This limitation makes it difficult to distinguish stable subpopulations from transitional cellular

states. Additionally, neutrophils exhibit high plasticity and rapidly adapt to environmental cues with many post-translational effects, implying that transcriptional states are not sufficiently stable to define distinct subpopulations. Integrating surface marker expression profiles through cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) can help reconcile these transcriptomic states of neutrophils with existing phenotypic definitions.[22,75,76] However, existing datasets are still limited in scope, often lacking adequate coverage of surface markers and diverse disease contexts. As high-dimensional data continue to grow, recent advances in artificial intelligence and machine learning offer opportunities to accelerate the discovery of distinct neutrophil subpopulations through integrative and unbiased analysis across multi-omic platforms.

Given the challenges of developing a classification system that effectively conveys broad information about neutrophil subpopulations while remaining clear and accessible to all researchers, we draw inspiration from Herbert A. Simon's paper, "The Architecture of Complexity.[77]" This work explores how complex systems can be understood through hierarchical structures and modular organization, concepts that could provide valuable insights for establishing a structured framework for neutrophil characterization. Building on these principles, we propose a hierarchical system that integrates multiple biological dimensions, including maturity, transcriptional state, function, and tissue adaptation, with a clear ranking that minimizes redundancy and avoids overlapping descriptions. Further, our system follows the natural sequential flow of neutrophil lifespan—considering their developmental trajectory, the transcriptional regulatory networks shaping their functionality, and finally the influence of tissue adaptation on their functions. Here, we introduce a four-tiered framework designed to provide a thorough and adaptable approach for neutrophil classification.

Level 1—Developmental stage (maturation)

The first level captures the progression from progenitor stage to mature circulating neutrophils, as well as aged neutrophils, reflecting their dynamic developmental trajectory. As such, based on existing literature, we recommend the following categories in order: progenitor, precursor, immature, and mature. One can adopt the neutrotime transcriptional signature[20] to assess the maturation status of neutrophils. This approach utilizes a robust transcriptomic measure of maturation based on a curated signature set of genes.[20] Each neutrophil subpopulation queried is assigned both an "early" and "late" neutrotime score to define the extent of maturation, enabling "quantification" of neutrophil maturation. Additionally, surface markers such as CD101 in mice and CD10 in humans[78] serve as reliable markers of neutrophil maturity, complementing transcriptomic analyses. Furthermore, nuclear morphological phenotyping provides another layer of validation, offering a broad evaluation of neutrophil maturation in defining maturation status.[78] For human progenitors, the joint use of CD34 and CD66b can be beneficial.[19] Of note, aged neutrophils are classified within the mature category, as discriminatory gene sets to define neutrophil aging are still lacking. Moreover, although phenotypic markers CXCR4 and CD62L denote aged neutrophils, their differential expression toward inflammatory cues and tissue signals renders them unreliable as robust aged neutrophil markers.

Defining neutrophil maturation is important for accurately assessing neutrophil lifespan. For example, the lifespan of immature neutrophils will typically be longer than that of mature neutrophils, as the measurement begins at an earlier developmental stage. Without accounting for maturation status, this extended lifespan may be misinterpreted as increased longevity rather than an intrinsic difference in lifespan, as the endpoint remains unchanged. Therefore, consideration of maturation state is critical for the accurate interpretation of neutrophil longevity across studies.

Level 2—Functional module

This level defines neutrophil subpopulations based on transcription factors, signaling pathways, and metabolic shifts driving their functional states. A biologically meaningful approach is to utilize Gene Ontology (GO)[79] terms to categorize subpopulations rather than relying on broad and uninformative markers. In cases where transcriptomic data are unavailable, classification can be approximated using marker panels that describe neutrophil function rather than a single defining marker. By integrating functionally relevant marker combinations into the descriptors, this approach can help to bridge the gap caused by the absence of transcriptomic datasets. We propose a general classification spanning four overarching functional properties (Figure 3). These include neutral, inflammatory, immunosuppressive, and non-canonical functions. The first three represent well-established functional roles of neutrophils, while the latter refers to the emerging context-specific functions. By consolidating their diverse and specific roles, this approach aims to reduce the existing complexity in neutrophil subset nomenclature.

The non-canonical category is created to enable the annotation of subpopulations that display features more commonly associated with other immune cells rather than traditional neutrophil functions. When should the non-canonical tag be applied, and when should a more specific functional descriptor be used? To illustrate this, consider a recent transcriptomic analysis of skin neutrophils, which revealed an enrichment of matrix-related genes.[80] Without further functional validation, these cells would be classified as non-canonical neutrophils found in the skin. However, subsequent analysis demonstrates that *Col3a1*-expressing skin neutrophils play a crucial role in barrier function. With this functional evidence, a more precise descriptor, such as matrix-producing, can be applied. This approach accommodates the growing number of neutrophil adaptations, including studies that describe specialized functions that emerge under specific conditions, such as antigen presentation in tumors[55,81,82] and extracellular matrix transportation.[83] This system acknowledges that functional programs can be shared across multiple tissues, i.e., pro-inflammatory programs can occur both in circulation and at the infection site.

Level 3—A defining identifier

At this level, incorporating a distinctive identifier should enhance the precision of the descriptor denoted in level 2. This identifier may be a protein marker or a gene, such as a transcription factor, meeting at least one of the following criteria: (1) it is associated with a functionally distinct subset of neutrophils, (2) it plays a direct role in neutrophil activity or differentiation, and/or (3) it reflects adaptation to a specific tissue environment. While

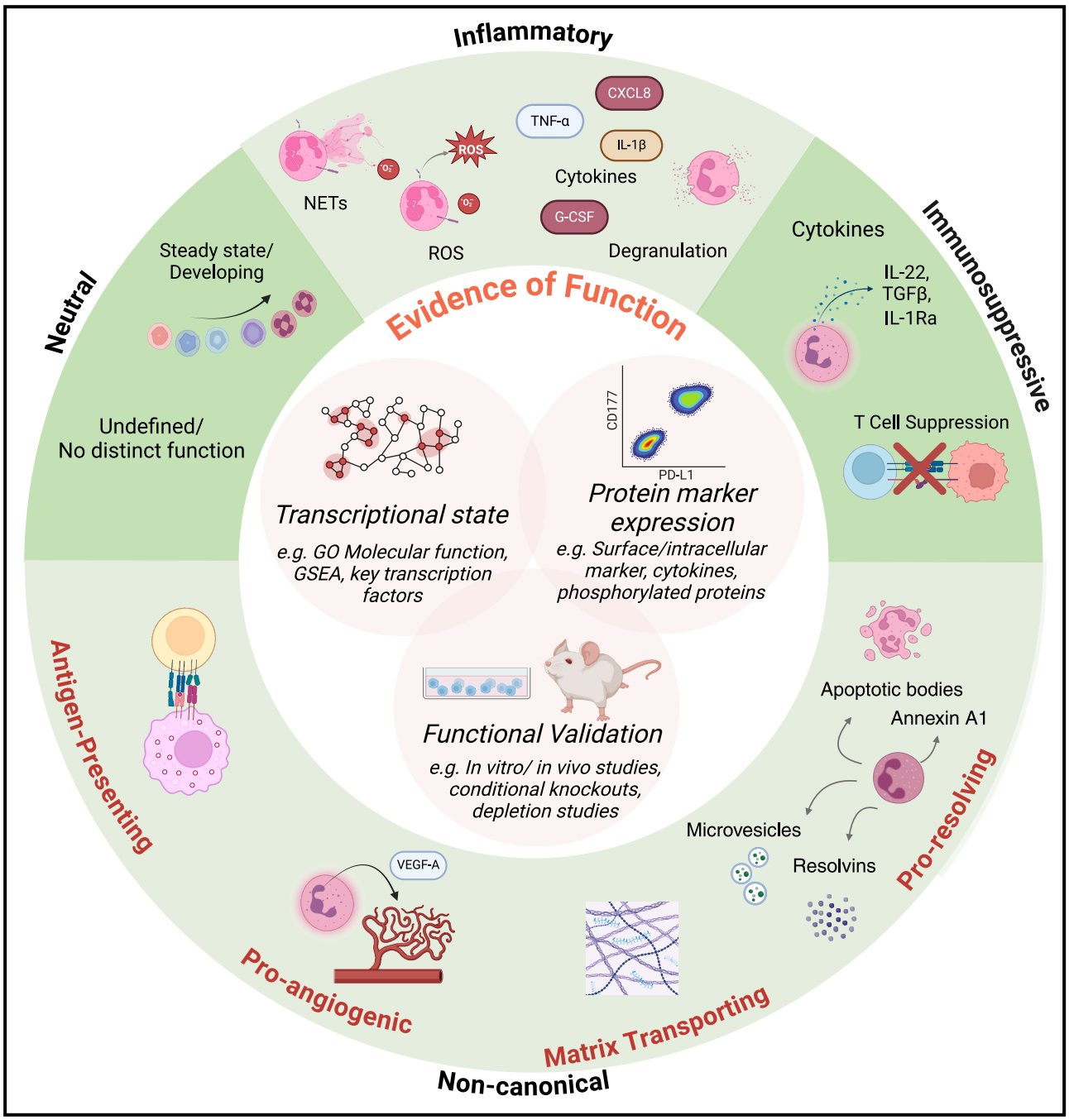

Figure 3. Functional categories of neutrophils

This attribute is likely the most variable and exciting part of neutrophils, considering their plasticity to adopt an assortment of functions. Categorizing functions into four groups allows easier communication, grouping overlapping discoveries that can be treated and targeted in a similar manner. Likely, future discoveries may reveal neutrophil subpopulations with functions corresponding to more than a single group. Such possible conundrums demand clarity in the specific contexts of disease severity these functions were demonstrated. As evidence accumulates, these categories can expand and further add to the vocabulary of terms used to describe neutrophils. Notably, subgroups currently placed in the non-canonical category may eventually warrant recognition as distinct categories, reflecting their well-established functional roles. Created with Biorender.com.

optional, the inclusion of such identifiers as descriptors adds biological depth to the classification, capturing meaningful features that contribute to neutrophil heterogeneity. An example is SiglecF, expressed by neutrophils in lung and liver tumors, peritonitis, and myocardial infarction.[33,53,84–86] Intracellular markers can be similarly used, such as interferon-induced protein with tetratricopeptide repeats 1 (IFIT1),[87,88] denoting a subpopulation of interferon-stimulated neutrophils.

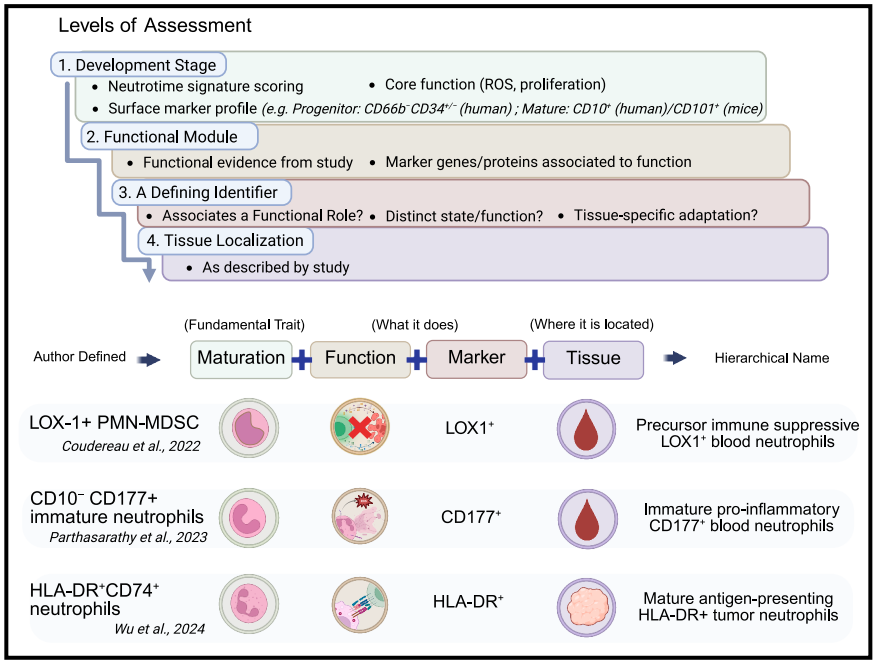

Figure 4. Proposed framework for neutrophil classification

Cell population identities are first assessed through a series of criteria associated with the minimal evidence required to ascertain the attributes of the population in question. These attributes collectively describe the neutrophil subpopulation, standardizing a naming convention for neutrophils in health and disease. Importantly, utilizing this nomenclature will reveal the similarity between existing subpopulations, allowing streamlined efforts to implement targeted approaches. Examples demonstrating the framework include neutrophil precursors,[89] immature neutrophils,[90] and mature neutrophil subpopulations.[55] It is likely that multiple existing discoveries can be classified under one hierarchical name, while some subpopulations can be further subdivided. Regardless, this nomenclature will provide a clearer communication of neutrophil states as they relate to each other within and beyond the field of neutrophil biology. Created with Biorender.com.

Level 4—Tissue localization

At this level, the phenotypic and functional diversity is further refined by incorporating their specific tissue localization. Distinct microenvironments shape neutrophil phenotypes, functional programs, and adaptations, making tissue context a critical factor in classification. Within this hierarchical framework, it is important to recognize that decisions made at each level—maturation, function, defining identifiers, and localization—are interconnected. Each level contributes to the overall biological relevance of the classification; therefore, the relationships between levels should be carefully considered. This is especially important when defining neutrophil subtypes that share maturation states and functions but reside in distinct tissue environments, where local cues may drive distinctive adaptations.

To illustrate the applicability of the proposed naming system within this framework, we applied it to a mature neutrophil subpopulation from pancreatic tumors identified through single-cell transcriptomic datasets. This population is enriched with GO pathways associated with hypoxia (GO:0001666), regulation of angiogenesis (GO:0045766), and glycolysis (GO:0061621).[30] These GO pathway enrichments indicate a specialized role in tissue adaptation and immune regulation. Applying the hierarchical classification system, these neutrophils fall under level 1: mature, level 2: non-canonical, level 3: currently undefined, and level 4: pancreatic tumor. Thus, under our proposed framework, these neutrophils would be classified as mature non-canonical pancreatic tumor neutrophils. Recognizing that this subpopulation exhibits a specialized function enables researchers to further investigate its specific role in the tumor microenvironment. In this case, we found that these cells possessed pro-angiogenic properties, suggesting that they can be more precisely classified as mature pro-angiogenic pancreatic tumor neutrophils.[30] This refined classification enhances clarity in neutrophil subtyping and provides a foundation for exploring their biological significance and guides the development of microenvironment-tailored interventions. For cases where transcriptomic data are unavailable, classification can be based on protein marker-based phenotypes. As an example, a neutrophil subpopulation identified in septic patients expresses LOX1$^+$ CD10$^-$ CD16$^-$ CD15$^+$.[89] Applying the hierarchical classification system, these neutrophils fall under level 1: precursor, level 2: immune suppressive activity, level 3: LOX1$^+$, and level 4: blood. Thus, within this framework, these neutrophils from septic patients would be classified as precursor immunosuppressive LOX1$^+$ blood neutrophils (Figure 4).

While our proposed four-tiered hierarchical system is structured to be inclusive and flexible, we foresee potential challenges during its implementation. Classification at each level will inevitably involve subjective decisions, such as choosing GO terms or defining markers, which could lead to potential inconsistencies across. To mitigate this, we propose that the assignment of presumed functional modules (level 2) be supported by *in vitro* or *in vivo* validation studies should be carried out to ascertain the correct overarching functional category (Figure 3). In the absence of such supporting evidence, this level should be left unassigned, allowing future studies to complete it. Similarly, identifiers (level 3) should only be done when clear supporting evidence is available (Table 1). By encouraging researchers to provide robust functional validation, this system promotes the rigorous characterization of bona fide neutrophil subpopulations, ensuring that additional classifications are grounded in biological relevance.

Other important descriptors, such as species and disease conditions, can be included alongside this framework to provide additional context without altering the core characteristics essential for nomenclature. While these details enhance comparability across studies and improve translational insights, incorporating them directly into the framework risks over-specification and adds complexity. Therefore, it is important to explicitly reference such descriptors in the text to provide necessary context without mandating their inclusion within the four-tiered naming system itself.

Table 1. Examples of implementing the proposed nomenclature

Current name	Maturation	Functional module	Marker	Tissue
Cancers				
Ly6E$^{(hi)}$ neutrophils[91]	mature	pro-inflammatory	Ly6E^{hi}	tumor
CCL4^{+} TANs[52]	mature	pro-inflammatory	CCL4^{+}	liver tumor
HLA-DR^{+}CD74^{+} neutrophils[55]	mature	antigen-presenting	HLA-DR^{+}	tumor
PMN-MDSCs[67]	mature	immunosuppressive	CD84^{+}	blood
Sepsis				
IL-1R2+ Immature neutrophils[75]	immature	immunosuppressive	IL-1R2^{+}	blood
CD10^{-}CD177^{+} immature neutrophils[90]	immature	pro-inflammatory	CD177^{+}	blood
LOX-1 PMN-MDSC[89]	precursor	immunosuppressive	LOX-1^{+}	blood
Autoimmune				
LDGs[68]	immature	pro-inflammatory	–	blood
LDGs[68]	mature	pro-inflammatory	–	blood
Viral				
Immature subset[92]	immature	pro-inflammatory	–	blood
CD16^{Hi} LDNs[93]	mature	immunosuppressive	–	blood

By combining these four levels, this proposed hierarchical framework integrates the core attributes of neutrophil subpopulations across both physiological and pathological states, offering a structured yet adaptable neutrophil classification scheme that balances biological relevance with practical usability. Furthermore, the framework maintains continuity with previously characterized neutrophil populations within the context of existing knowledge. We anticipate that fostering greater consistency in neutrophil nomenclature will enhance reproducibility and improve communication across the field and ultimately facilitate both fundamental discoveries and translational insights into neutrophil biology.

REFRAMING NEUTROPHIL HETEROGENEITY AS A CLINICALLY ACTIONABLE TRAIT

Given the increasing recognition of neutrophil heterogeneity, a robust classification system holds major clinical relevance, as the identification of disease-specific neutrophil phenotypes could aid clinicians in diagnostics, prognostics, and therapeutic decision-making. Because neutrophils are the most abundant circulating immune cells, their specialized physiological properties make them ideal biomarkers for blood-based tests, enabling early disease detection and monitoring of disease progression. Several studies have demonstrated the utility of detailed neutrophil characterization in correlating disease severity, often surpassing traditional metrics such as the neutrophil-to-lymphocyte ratio (NLR),[94] which is used in conditions such as inflammatory diseases and cancers. While NLR provides a general measure of inflammation and disease severity, it lacks the specificity needed to distinguish between functionally distinct neutrophil subpopulations that may drive disease progression or resolution. Therefore, a more refined classification approach enables a deeper understanding of neutrophil involvement in disease. For instance, the appearance and frequency of immature CD10-negative neutrophils in circulation are highly associated with COVID-19 disease severity,[41,95] while specific subsets like CXCR4^{high}CD62L^{low} neutrophils are linked to worse outcomes in ischemic stroke.[96] Similarly, specific neutrophil phenotypes, such as CD177^{+}, CD64^{+}, and OLFM4^{+}, serve as early biomarkers for sepsis and asthma.[97] By contrast, high receptor tyrosine kinase MET-expressing neutrophils exhibit anti-tumor effects in lung cancer, highlighting their prognostic value.[98] These findings highlight the importance of moving beyond broad neutrophil metrics, like NLR, toward a biologically meaningful classification framework that can enhance diagnostic precision, risk stratification, and therapeutic targeting across a range of diseases.

Due to their short lifespan, investigating the biology of neutrophils can be technically challenging. However, this characteristic can provide a distinct advantage: neutrophils can serve as real-time indicators of ongoing immune responses. Their rapid turnover and responsiveness to environmental cues enable dynamic monitoring of acute inflammation, disease progression, treatment responses, and immune homeostasis. By integrating a standardized neutrophil characterization framework, clinicians can profile neutrophil subpopulations with a structured approach. Moreover, it offers guidelines for interpreting neutrophil phenotypes in clinical settings, allowing timely insights into a patient's immune status and facilitating more personalized treatment strategies in conditions such as cancer, autoimmune diseases, and infections. Its adoption can encourage further technical standardization in sample handling in the clinical setting, increasing comparability between patient cohorts. Ultimately, a refined neutrophil profiling methodology bridges the gap between basic research and clinical applications, providing a standardized method for utilizing neutrophil-based biomarkers in personalized medicine, early intervention, and therapeutic monitoring.

CONCLUDING REMARKS

In our view, adopting the classification presented here represents an important step toward fostering a unified understanding of the dynamism and plasticity of neutrophils. While we

acknowledge that its implementation will require some effort, we hope it will provide a much-needed, structured, and biologically meaningful approach to neutrophil categorization. Notably, this system captures the trajectory of neutrophils, from their ontogeny and release into circulation to their infiltration into tissues and adaptation in response to environmental cues. While this framework may not capture all aspects of neutrophil diversity and lifespan, it provides a foundational structure for documenting neutrophil heterogeneity, ensuring that emerging discoveries can be systematically classified and integrated into future research. By serving as a scaffold for classification, this system allows for continuous refinement, enabling researchers to expand upon existing categorizations as advanced technologies and insights emerge. Furthermore, efforts to construct detailed atlases of neutrophils, such as recent resources focused on neutrophil development and cancer, will be instrumental in harmonizing discoveries and annotations across diseases and physiological states. This includes the use of machine learning and AI approaches to systematically characterize neutrophils by integrating information across all four classification levels. By adopting a standardized framework, we can improve reproducibility, enhance collaboration, refine our understanding of neutrophil heterogeneity, and ultimately facilitate innovation in research and clinical applications.

ACKNOWLEDGMENTS

We regret that we are unable to include and reference many other important studies and concepts covering the expanding roles of neutrophils. All authors contributed ideas and assisted with writing, editing, and revising the manuscript. Authors are ordered alphabetically by their last name. L.G.N. is supported by the National Natural Science Foundation of China (grant number 92374205), the Chinese Ministry of Science and Technology (grant numbers 2023YFC2306300 and 2023YFC2506400), and the Research Fund for International Senior Scientist (grant number W2431020). This study was supported by the Basic-Clinical Collaborative Innovation Project from the Shanghai Immune Therapy Institute. M.A.C. receives funding from Associazione Italiana per la Ricerca sul Cancro (AIRC IG-27613). M.E. is supported by the International Neutrophil Institute/Ainslie Foundation and the National Cancer Institute (R01CA297338). Z.G.F. acknowledges funding from the Israel Science Foundation (grant number 1708/20) and the Sasson and Luisa Naor Fund. R.G.-B. was supported by the Deutsche Forschungsgemeinschaft (DFG) through GR 5979/2-1, the Else Kröner-Fresenius-Stiftung through 2022_EKEA.72, the IZKF Erlangen (grant N10), and the Lupus Research Alliance. C.C.H. was supported by NIH P01 HL152958. R.O. is supported by grants from the Italian Telethon Foundation (SR-Tiget Grant Award F04), the European Research Council (ERC) (ERC-CoG 101088887 and ERC-PoC 101158288), AIRC (AIRC 5×1000 22737, IG 31053), the Italian Ministry of Health (GR-2021-12374094), and the Lustgarten Foundation-Swim Across America-AACR (24-60-67-OSTU). M.J.P. is supported in part by the ISREC Foundation and Ludwig Cancer Research. D.F.Q. acknowledges funding from the Canadian Institutes of Health Research (PJT-195760) and Cancer Research Society (PT-103132). O.S. receives funding from the DFG (TRR332, CFU342, and SO876/16-1). C.S.-R. receives support from the Deutsche Forschungsgemeinschaft (CRC TRR332 project A1, CRC1123 project A6) and the IZKF of the University of Münster. M.N. is supported by the Singapore Ministry of Health's National Medical Research Council Young Individual Research Grant (MOH-OFYIRG24-jan-0012). N.M.M. is funded in part by the intramural program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). M.J.K. was supported by the intramural research program at NIAMS/NIH(ZIA AR041199). P.A.N. was supported by NIH/NIAMS 2R01AR065538, P30AR070253, R01AR075906, P30AR070253, and P30AR072577; the Lupus Research Alliance; the Rheumatology Research Foundation; an investigator-initiated research grant from Pfizer, Inc.; and the Peabody Foundation. R.X. is supported by the National Natural Science Foundation of China (82322047 and 82173035), the Noncommunicable Chronic Diseases-National Science and Technology Major Project (2024ZD0525000), the National Key Research and Development Project of China (2022YFC3400902), the Beijing Municipal Natural Science Foundation (Z240016), and the Beijing Nova Program (20230434854). I. K. is supported by the Singapore Ministry of Health's National Medical Research Council Young Individual Research Grant (MOH-OFYIRG24jan-0032) and the Skin Research Institute of Singapore Joint Research Grant (SRIS_JRG_2003). M.J.K. and N.M.M. are employees of NIH. This research was supported [in part] by the Intramural Research Program of the National Institutes of Health (NIH). The contributions of the NIH authors were made as part of their official duties as NIH federal employees, are in compliance with agency policy requirements, and are considered Works of the United States Government. However, the findings and conclusions presented in this paper are those of the authors and do not reflect the views of the NIH or the U.S. Department of Health and Human Services.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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