A Solution for Robust and Reliable Single Cell RNA-Sequencing of Neutrophils

Neutrophils (Fig. 1A) are myeloid leukocytes that are a major component of the innate immune system. They are derived from myeloid progenitors, which reside in the bone marrow and extramedullary tissues, including the spleen (Fig. 1B). Due to their unique morphology, they can be identified easily by their round lobed nuclei and relatively dark cytoplasm. In human, neutrophils make up 50-70% of all circulating white blood cells. During acute inflammation, neutrophils belong to the major responders and localize to the site of infection. Thus, a deficiency of neutrophils in the blood can lead to severe immunodeficiency. As recently published, in cancer, tumor-associated neutrophils (TANs) are involved in shaping the tumor microenvironment. In this context, TANs might be part of the tumor-promoting inflammatory response by driving angiogenesis, metastasis, and immunosuppression or remodeling the extracellular matrix. Additionally, neutrophils mediate anti-tumor responses by directly killing tumor cells and participating in a network of cells with anti-tumoral activity. Therefore, neutrophils could be potential targets for cancer treatment and therapies for other diseases in the future.

However, up to now, single cell RNA-sequencing (scRNA-seq) of neutrophils remained challenging due to their low content of mRNA while expressing high levels of RNases and other inhibitory components. Additionally, neutrophils have a very short lifespan of only 15-20h requiring immediate processing of samples.

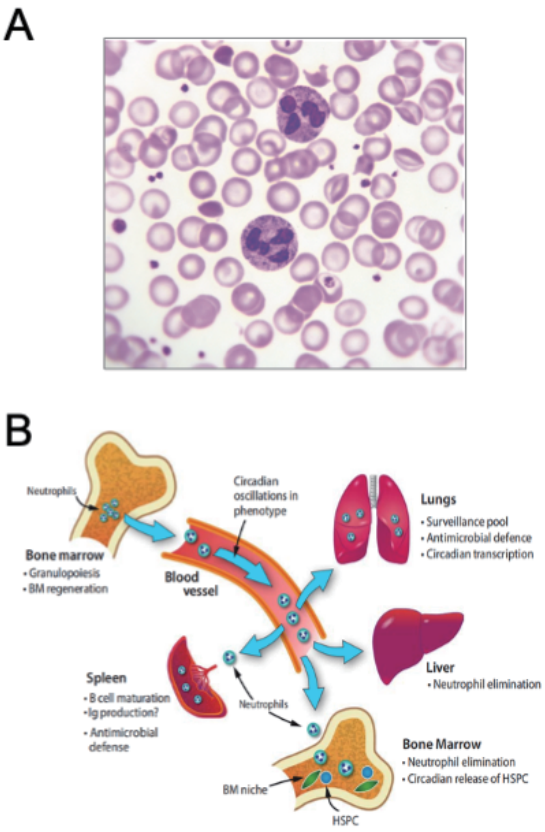

Figure 1: Neutrophil characteristics and development.

A. Morphology of neutrophils.

B. Origin and function of neutrophils (BM: bone marrow, HSPC: hematopoietic stem and progenitor cell) (Adapted from: Trends in Immunology, 2019, 40:584).

Singleron' s single-cell transcriptomics sequencing platform overcomes these obstacles and allows to identify neutrophils more reliably and reproducibly than ever before. Here, using Singleron' s GEXSCOPE® platform in comparison to another commercially available platform, four independent whole blood cell samples were analyzed. Overall, 11 cell types, including neutrophils, T cells, NK cells, B cells, classical and non-classical monocytes, dendritic and plasma dendritic cells, basophils, plasma cells, and platelets were detected (Fig. 2A). However, the side-by-side comparison to a different commercially available platform revealed that Singleron' s proprietary technology can maintain the composition of cellular subsets including neutrophils throughout processing, while neutrophils were largely lost using the other platform (Fig. 2B, C). This highlights Singleron' s accuracy in maintaining the original cellular composition and capture efficiency.

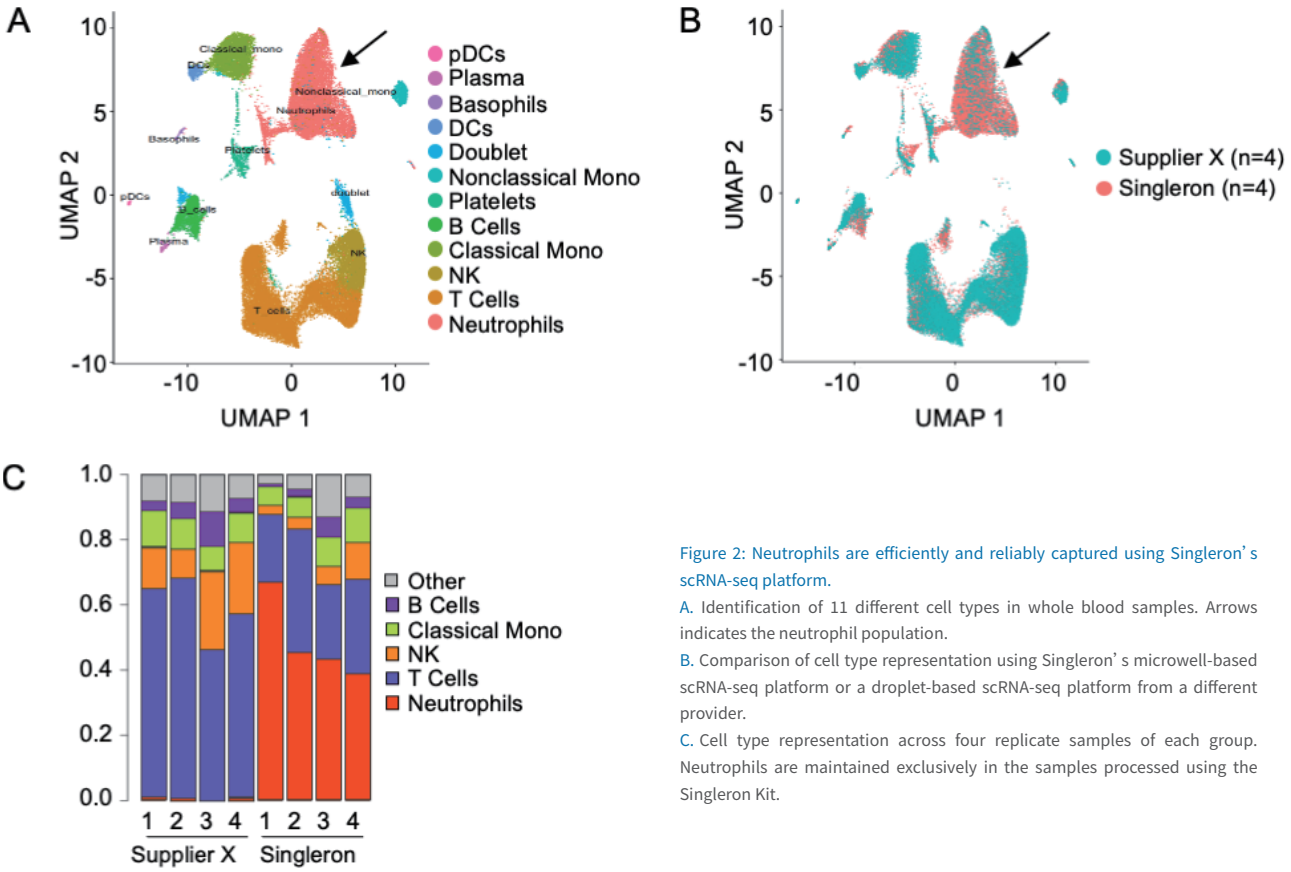

Figure 2: Neutrophils are efficiently and reliably captured using Singleron' s scRNA-seq platform.

A. Identification of 11 different cell types in whole blood samples. Arrows indicates the neutrophil population.

B. Comparison of cell type representation using Singleron' s microwell-based scRNA-seq platform or a droplet-based scRNA-seq platform from a different provider.

C. Cell type representation across four replicate samples of each group. Neutrophils are maintained exclusively in the samples processed using the Singleron Kit.

Next, low-density neutrophils (LDN), which unlike high-density neutrophils (HDN) have been associated with tumor-promoting activities in cancer, were investigated. First, LDN were enriched using discontinuous density gradient separation. While LDN were enriched in the PBMC layer, HDN were deposited together with the red blood cells. Similar results were obtained when using Ficoll density gradient separation (Fig. 3).

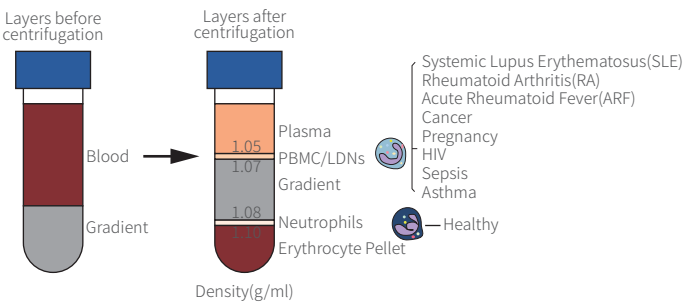

Figure 3: Separation of LDN and HDN using discontinuous density gradient separation.
(Adapted from: J Leukoc Biol. 2020; 107:809-818.)

To examine whether LDN are suitable biomarkers of disease, PBMCs from peripheral blood cells obtained from patients of four different disease groups (D1-4) were analyzed, including two types of cancer and two types of infectious disease, using the GEXSCOPE® Single-Cell Sequencing platform (Fig. 4). For each group, samples from four individual patients were collected. The cell type annotation confirmed that the PBMC samples contained LDN (Fig. 4A), indicating that these might be markers of disease as PBMCs from healthy patients usually do not contain this cell type. The frequency of neutrophils was highly disease-dependent ranging between 30-80% and could be identified reproducibly if present (Fig. 4B, C). This suggests that the presence of LDN could contribute to be an indicator and, thus, biomarker of specific disease types.

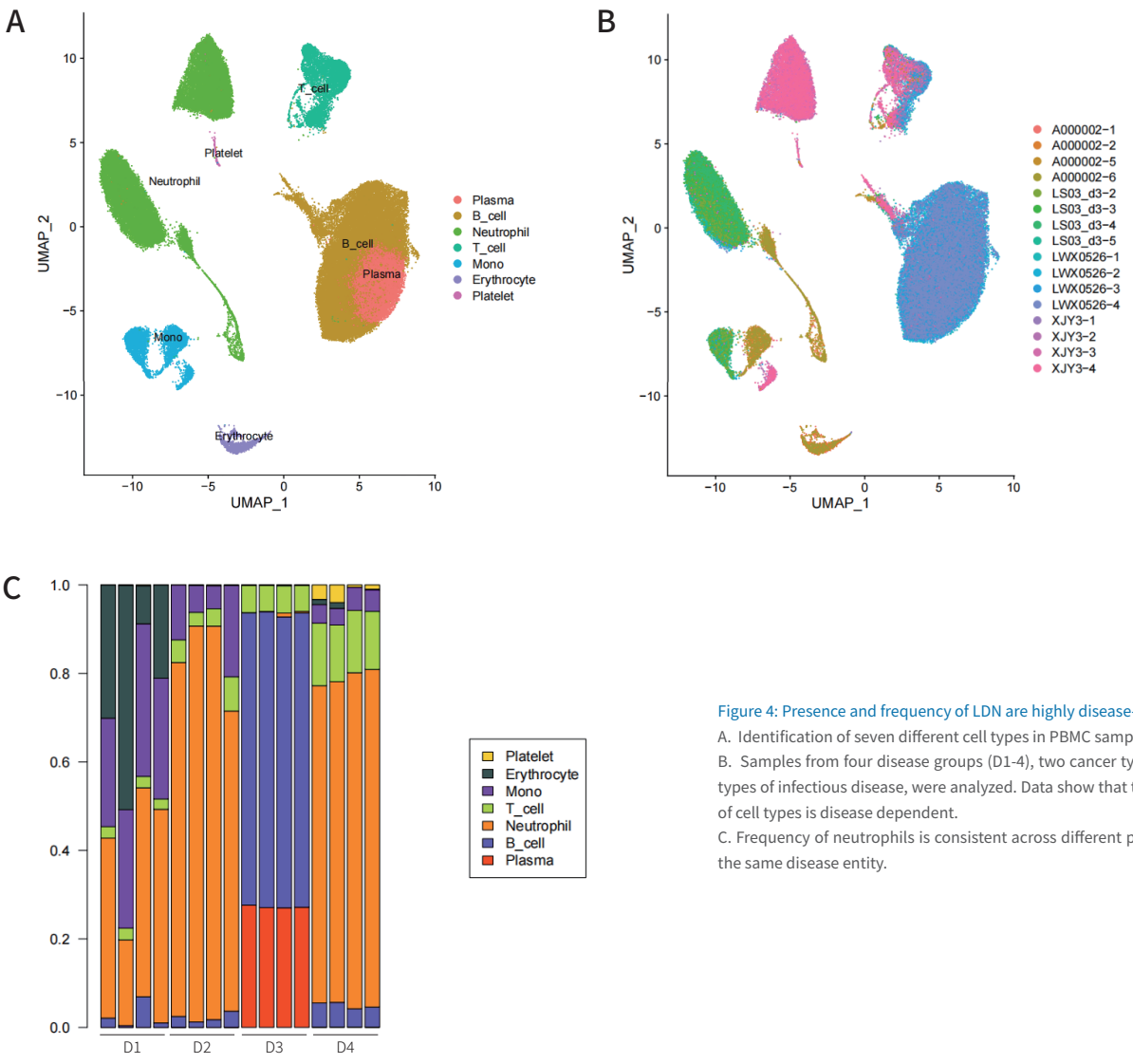

Figure 4: Presence and frequency of LDN are highly disease-dependent

A. Identification of seven different cell types in PBMC samples.

B. Samples from four disease groups (D1-4), two cancer types and two types of infectious disease, were analyzed. Data show that the presence of cell types is disease dependent.

C. Frequency of neutrophils is consistent across different patients from the same disease entity.

Neutrophils are not only present in blood but also infiltrate tumor tissues, and thus might play a role for the tumor microenvironment. To investigate this, Prof. Zhou's group from Shanghai Pulmonary Hospital, in collaboration with Prof. Buettner's group from the University Hospital Cologne, studied tumor heterogeneity and microenvironment of biopsies obtained from 42 patients with Advanced Non-Small Cell Lung Cancer (NSCLC) (Fig. 5). Taking advantage of Singleron's unique sCelLiVE solution that mimics physiological conditions to keep cells stable and alive for 72 hours, the researchers prevented loss of any cell types including the rather unstable neutrophil population. This data on the tumor cell heterogeneity, stromal and immune cell phenotypes, infiltration, and interaction microenvironment, was published in May 2021 in Nature Communications. The authors showed that tumors of different patients were heterogeneous in their cellular composition, chromosomal structure, differentiation trajectory, and intercellular signaling network. Furthermore, TANs were shown to be directly associated with lung squamous carcinoma, while absent in lung adenocarcinoma, reflecting tumor heterogeneity. This opens a new perspective on the function of neutrophils in NSCLC and their potential predictive role in efficacy of immunotherapy.

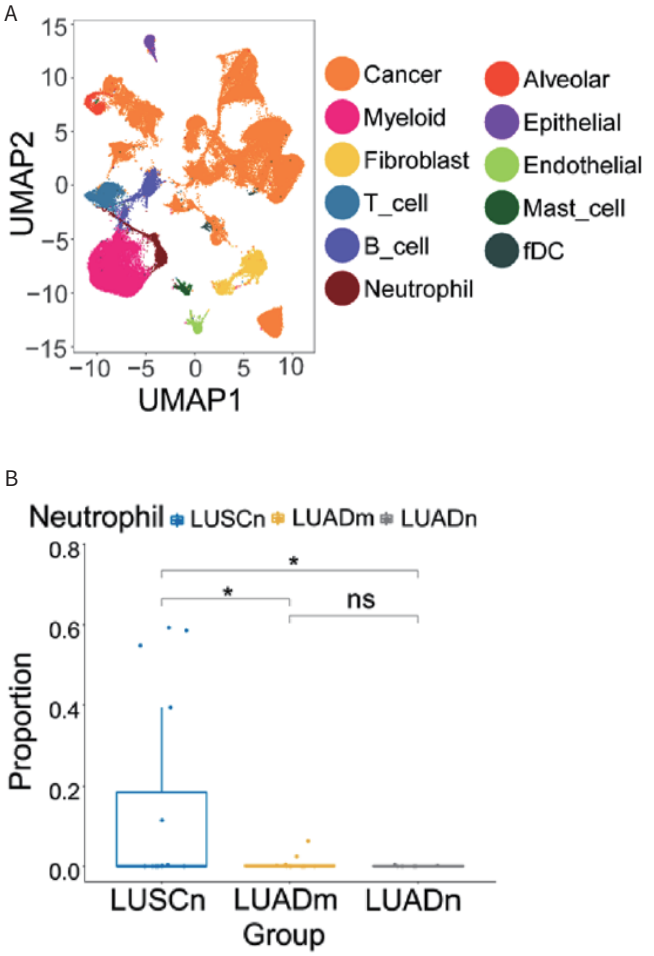

Figure 5: Neutrophils in Non-Small Cell Lung Cancer microenvironment and their association with tumor heterogeneity.

A. Cell type annotation of 90,000 NSCLC cells from 42 patients.

B. Frequency of neutrophils across different disease types. LUSC: lung squamous carcinoma, LUAD: lung adenocarcinoma. (Adapted from: Wu et al., Nature Communications (2021) 12:2540).

Singleron is committed to develop innovative single-cell sequencing technologies. We have accumulated a broad range of knowledge in analyzing different sample types while preserving their cellular composition including fragile cells like neutrophils. Investigating neutrophils at single cell resolution allows researchers to reveal even subtle changes of their transcriptome, identify new cellular subtypes and gain insight into tumor heterogeneity. The development of these technologies has greatly improved our understanding of neutrophil biology and associated diseases. Our goal is to keep providing valuable information regarding the role of all cell types in different diseases and further facilitate the progress of targeted therapy and data mining.

References:

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