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ORAL ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

A Multiomic Single-Cell Atlas of Human Myelopoiesis Reveals Cellular and Molecular Drivers of Immunomodulatory Drug-Induced Neutropenia

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Neutrophils are crucial innate immune cells which protect the host by killing infectious pathogens. Single cell RNA-sequencing analyses have previously described transcriptional heterogeneity of neutrophils and precursors, however the genome regulatory events underlying the transcriptional changes are less well characterised. Understanding this is important as neutropenia can be caused by dysregulation of the genome regulatory events that orchestrate neutrophil maturation. A good example is the use of immunomodulatory drugs (IMiDs, lenalidomide [LEN] & pomalidomide [POM]) for the treatment of multiple myeloma (MM) which are now standard-of-care, but their use is frequently complicated by neutropenia because of neutrophil maturation impairment. This is due to Cereblon (CRBN)-driven substrate ubiquitination and Ikaros degradation, causing PU.1 downregulation. However, restoration of Ikaros levels cannot fully alleviate the IMiD-induced myeloid differentiation block suggesting other mechanisms might also contribute.

Our aim was to build a multiomic atlas of human myelopoiesis and use this to interrogate the molecular drivers of IMiD-induced neutropenia.

We developed an *ex vivo* myeloid cell differentiation assay using human mobilized peripheral blood CD34 $^{+}$ cells from healthy donors and optimised methods for single cell multiomic (combined RNA-seq & ATAC-seq) analysis. This platform was validated orthogonally (immunophenotype, morphology & functional) and employed to construct a multi-omic single-cell landscape of normal human neutrophil differentiation and it's perturbation by IMiDs.

Single cell transcriptomes from >110,000 cells from untreated and IMiD-treated samples were analyzed, capturing the full spectrum of human myelopoiesis spanning from early myeloid progenitors through to neutrophil populations (band, segmented, mature) (Fig.1A-left). *Ex vivo* differentiated neutrophils retained transcriptional signatures reflective of effector functions (granule biogenesis, chemotactic activity, respiratory burst) and mapped to previously described human neutrophil populations *in vivo*. *Ex vivo* generated neutrophils showed expected functional properties of phagocytosis and neutrophil extracellular trap formation upon stimulation.

Exposure to IMiDs caused a maturation arrest with a 40-50% ($p<0.001$) decrease in abundance of differentiating metamyelocytes, and neutrophil populations. Crucially, this maturation arrest was due to an accumulation of transcriptionally distinct myeloid precursors that differentiated though an aberrant pathway (population 6, Fig 1A). Furthermore, immature erythroid clusters representing 10% of live cells accumulated specifically in IMiD-treated samples ($p<0.001$) in absence of erythropoiesis-stimulating cytokines.

Simultaneous profiling of gene expression and chromatin accessibility in individual cells confirmed the presence of distinct phenotypes for untreated and IMiD-treated myeloid cells (Fig.1A) enabling the characterisation of the genome landscape of

myelopoiesis, which has previously proven challenging. As expected, we observed dysregulation of Ikaros targets including PU.1 upon IMiD treatment (Fig. 1B-frame). The ability to study distinct and aberrant cellular populations at transcriptional and genome regulation level in parallel allowed us to identify several novel regulators of normal and perturbed neutrophil development. For example, we observed perturbed activation patterns of the transcription factors (TFs) zinc finger protein (ZNF467) and interferon regulatory factor 5 (IRF5) and the expression of their downstream targets as illustrated in Fig.1B. ZNF467 orchestrates a transcriptional program supporting terminal neutrophil differentiation in untreated cells (Fig.1B-top right), whilst the regulatory repertoire of IRF5, a hallmark of neutrophil activation is confined primarily to treatment-specific immature populations (Fig.1B-bottom right).

We present a multiomic atlas of normal myelopoiesis which we believe will provide an important resource for the study or normal and perturbed neutrophil development. We use this platform to study IMiD-induced neutropenia and identify two aberrant cellular progenitor populations (aberrant myeloid precursors & erythroid progenitors) with distinct molecular properties as the likely cause of myelosuppression.

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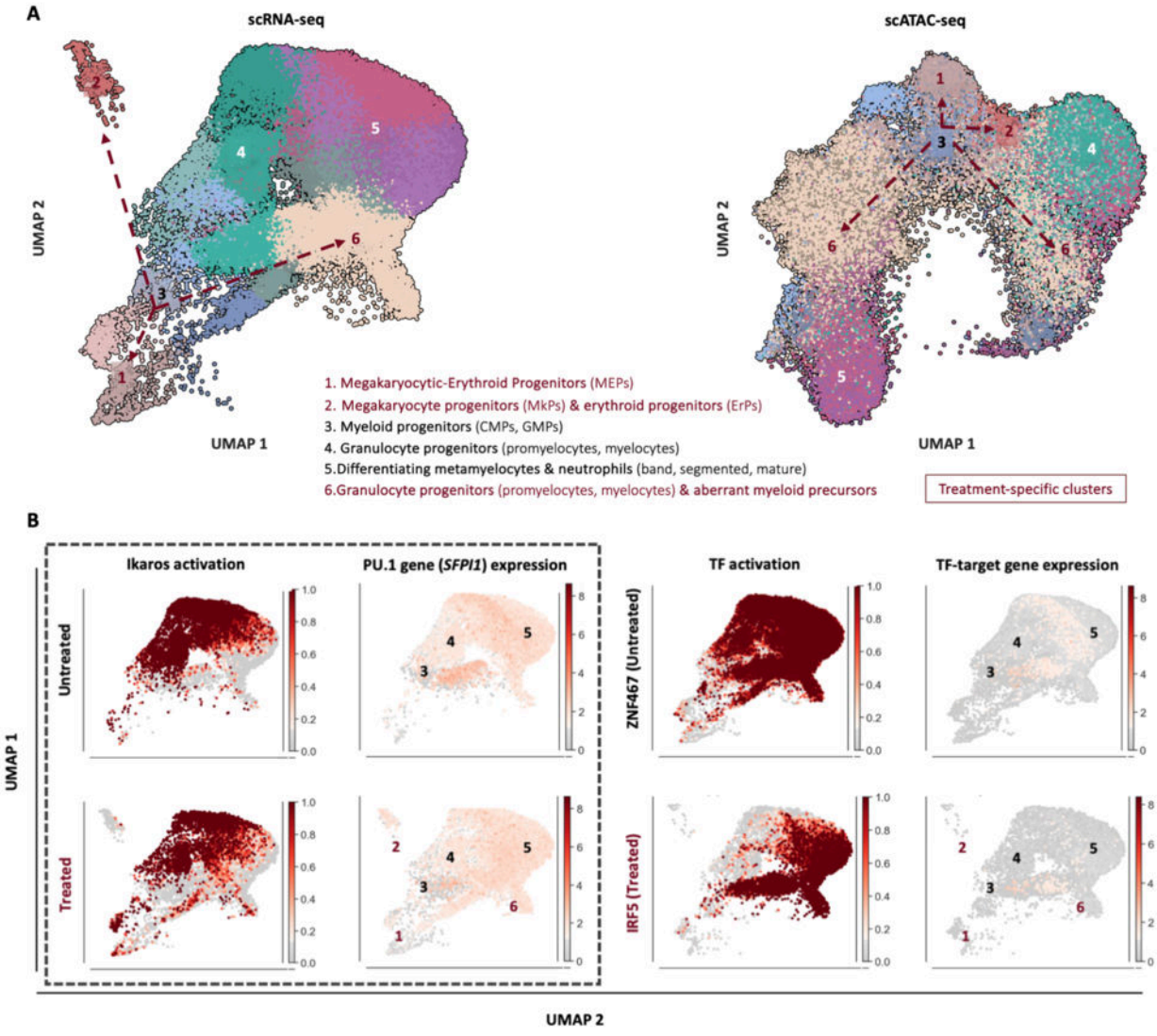

Figure captions

Figure 1A Uniform Manifold Approximation and Projection (UMAP) dimension reduction plot of human *ex vivo* differentiated CD34⁺ cells based on single cell multiomic (combined RNA-seq & ATAC-seq) analysis showing distinct profiles reflective of maturation arrest for aberrant myeloid precursors *(population 6)* in IMiD-treated samples.

Figure 1B Representation of the diverging activation and regulatory dynamics of molecular drivers of neutrophil differentiation and IMiD-induced differentiation block. Exposure to IMiDs leads to Ikaros degradation and the ensuing SFPI1 downregulation *(control-left, framed columns)*. ZNF467 (zinc finger protein 467) activation in untreated cells *(top right)* is followed by upregulation of target genes in granulocyte precursors *(populations 3,4),* whist IRF5 (interferon regulatory factor 5) activation in IMiD-treated cells *(bottom right)* is followed by enhanced transcriptional activity at the aberrant myeloid precursors *(population 6)*.

Figure 1

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