991 EFFECTS OF SAR443579, A TRIFUNCTIONAL ANTI-CD123 NK CELL ENGAGER (NKCE), ON NATURAL KILLER (NK) CELL SUBSETS AT THE SINGLE CELL LEVEL

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Background In Humans, circulating Natural Killer (NK) cytotoxic lymphocytes are represented by distinct subsets, differing in cytokine production, cytotoxicity and homing properties. The two major NK cell subsets correspond to CD56dim (NK1) cells, which are more cytotoxic and CD56bright (NK2) cells, which are more 'cytokinic'.[1] Single cell RNA sequencing of circulating NK cells from healthy individuals allowed to identify some additional NK cell sub-populations, which need to be further characterized.[2]

Given the promising results of anti-cancer NK therapeutics, a deeper understanding of the regulatory modules controlling the activation and phenotypic changes of NK subsets is warranted. We previously reported the development of SAR443579, a trifunctional NKCE targeting the CD123 antigen on leukemic cells and co-engaging NK cells through NKp46 and CD16a activating receptors, which preclinically induces NK cell activation and antitumor activity with no signs of toxicity, preclinically.[3] We performed Single-cell RNA-seq coupled to Cellular Indexing of Transcriptomes and Epitopes by next-generation sequencing (CITE-seq) on circulating NK cells from healthy donors after treatment with the trifunctional anti-CD123 NKCE *in vitro* in presence of target cells by to uncover the mechanism of action and the effect of SAR443579 on different NK cell subsets.

Methods The NK cell landscape was characterized by multiomic profiling of NK cells from peripheral blood of 4 healthy donors by CITE-seq.

Results We identified both at the transcriptomic and proteomic level, 7 major NK cell subpopulations including: $CD56^{bright}$(NK2), 3 populations of $CD56^{dim}CD16^{+}$ (NK1) present in different states, Type I IFNs responding, and 2 populations of Cytokine-Induced Memory-Like (CIML) NK cells. Uncovered NK cell population heterogeneity was then further validated using ActionNet, an archetypal analysis method, capturing both fine- and coarse-grain patterns of cell diversity.[4] Looking at the effects of anti-CD123 NKCE in presence of target cells we observed a change in the proportion of two NK cell subsets: an increase in CIML NK cells, and a decrease in $CD56^{dim}$ NK cells. *In-silico* differentiation trajectory analysis using Slingshot pseudotime algorithm identified CIML as the most differentiated NK subpopulation.[5] Network analysis using Scenic revealed distinct transcriptomic programs and corresponding master regulators for each NK cell subpopulation.[6] Finally, we confirmed our findings at the protein level using flow cytometry and Meso-Scale Discovery (MSD) analyses.

Conclusions Together, our work characterized peripheral blood NK cell compartments and explained the NK changes induced by SAR443579 in the presence of target cells at Single-Cell RNA and protein levels.

REFERENCES

1. Crinier A, Milpied P, Escaliere B, Piperoglou C, Galluso J, Balsamo A, Spinelli L, Cervera-Marzal I, Ebbo M, Girard-Madoux M, Jaeger S. High-Dimensional Single-Cell Analysis Identifies Organ-Specific Signatures and Conserved NK Cell Subsets in Humans and Mice. *Immunity*. 2018;**49**:971–986.
2. L Smith, PR Kennedy, KB Stacey, JD Worboys, A Yarwood, S Seo, E Hegewisch Solloa, B Mistretta, SS Chatterjee, P Gunaratne, K Allette, YC Wang, M Laird Smith, R Sebra, EM Mace, A Horowitz, W Thomson, P Martin, S Eyre, DM Davis. Diversity of peripheral blood human NK cells identified by single-cell RNA sequencing. *Blood Adv*. **4**(7):1388–1406.
3. Gauthier L, Virone-Oddos A, *et al.* Control of acute myeloid leukemia by a trifunctional NKp46-CD16a-NK cell engager targeting CD123. *Nature Biotechnol*. 2023 https://doi.org/10.1038/s41587–022-01626–2.
4. Mohammadi S, Davila-Velderrain J, Kellis M. A multiresolution framework to characterize single-cell state landscapes. *Nat Commun*. 2020. https://doi.org/10.1038/s41467–020-18416–6.
5. Street K, Risso D, Fletcher RB, Das D, Ngai J, Yosef N, Purdom E, Dudoit S. Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics. *BMC Genomics*. 2018. https://doi.org/10.1186/s12864-018-4772-0.
6. Aibar S, González-Blas CB, Moerman T, Huynh-Thu VA, Imrichova H, Hulselmans G, Rambow F, Marine JC, Geurts P, Aerts J, van den Oord J . Scenic: single-cell regulatory network inference and clustering. *NatMethods*. 2017;**14**:1083–1086

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