Effects of Trifunctional anti-CD123 NK cell engager (NKCE) on Natural Killer (NK) cell subsets at Single Cell level

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INTRODUCTION

In Humans, circulating Natural Killer (NK) cytotoxic lymphocytes are represented by distinct subsets which differ in cytokine production, cytotoxicity and homing properties. CD56^{dim} (NK1) cells are more cytotoxic and CD56^{bright} (NK2) cells are more “cytokinic”[1]. Single-cell RNA sequencing of circulating NK cells from healthy individuals recapitulated these distinctions.

We previously reported the development of a trifunctional NK Cell Engager (NKCE), SAR443579, that targets CD123 on leukemic cells and co-engages NKp46 and CD16a activating receptors on NK cells, inducing NK cell activation and CD123-positive tumor cell killing [2]. Here, we utilize single cell transcript and protein profiling to further characterize the effects of the co-engagement of NKp46 and CD16a on NK cells and further document the mechanism of action and characterize the key NK activation markers induced by this novel NKCE.

TRI-FUNCTIONAL ANTI-CD123 NKCE IN AML

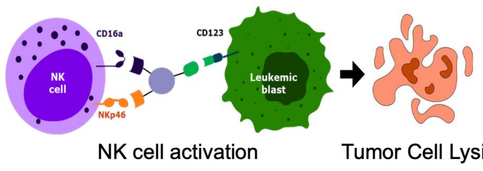
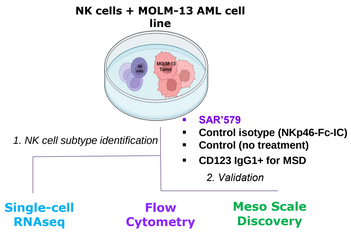


Figure 1: SAR443579 Mechanism of Action
SAR443579 (SAR’579) targets CD123 on cancer cells and engages NK cells via both NKp46 and CD16a activating receptors

METHODS

NK cells were characterized by a Cellular Indexing of Transcriptomes and Epitopes by Next Generation Sequencing (CITE-seq) profiling of NK cells purified from peripheral blood from 4 independent healthy donors. Profiling was performed at baseline and after treatment with anti-CD123 NKCE or control compounds *in vitro,* in presence of target cells. Validation of Single-cell findings was performed by Flow Cytometry using a panel of 26 cell surface proteins and by the Meso Scale Discovery (MSD) technology for secreted proteins, measured in supernatant of co-cultured cells.



4h time point; Compounds tested at EC_{max} (100 ng/mL)

Figure 2: Experimental Workflow
1. NK cell after isolation were treated for 4h in presence of MOLM-13 cell line (1:1 ratio). Following the treatment, NK cells were sorted and profiled by Single-cell.
2. Flow Cytometry and MSD were performed in similar conditions in an independent experiment, using NK cells purified from peripheral blood from 4 independent healthy donors.

EFFECT OF SAR’579 ON NK CELL STATE

SAR’579 induces a decrease in CD56^{dim} cells and an increase in the Memory - Like (ML) NK cells

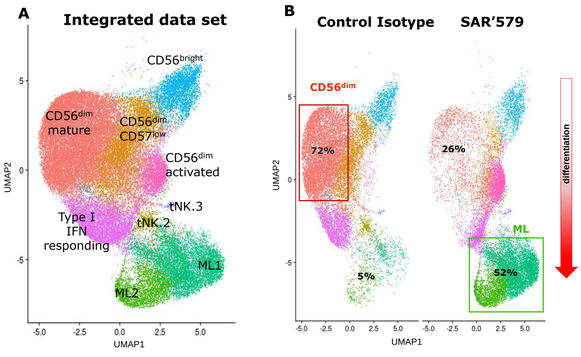


Figure 3: Changes in NK cell state following SAR’579 treatment
A. Six major and two small NK cell populations were identified by single-cell RNA-seq using marker genes from Smith et al [3].
B. SAR’579-treated NK cells show a decrease in CD56^{dim} cells (red) and an increase in ML cells (green) compared to control isotype-treated cells.

SAR’579 ENHANCES NK CELL DIFFERENTIATION TO ML

Trajectory analysis of gene expression showed that MLs are the most differentiated NK cell type in this experiment

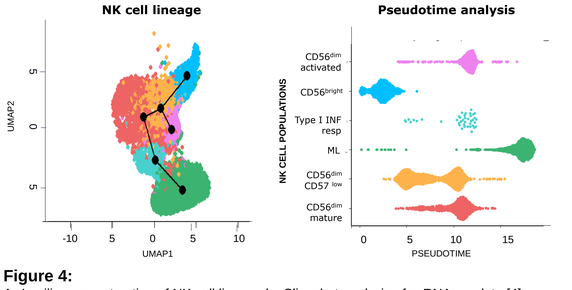


Figure 4:
A. *In-silico* reconstruction of NK cell lineage by Slingshot analysis of scRNAseq data [4].
B. NK cell populations ranked by infered pseudotime, reflecting their differentiation state.

TWO DISTINCT STATES OF ML NK CELLS

Archetype analysis identified two distinct states of ML cells. ML2 cells express higher levels of cytokines genes than ML1 (at RNA level)

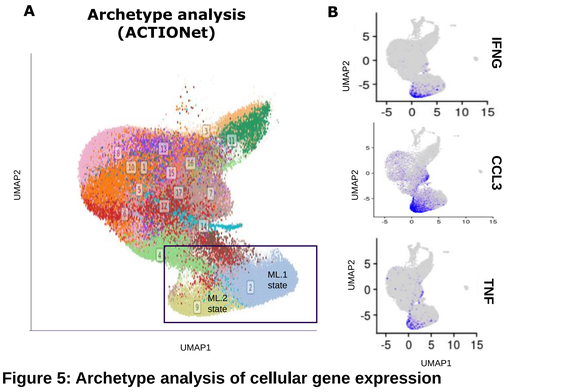


Figure 5: Archetype analysis of cellular gene expression
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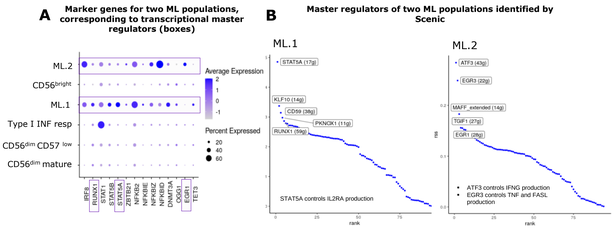
A. Cell archetype analysis using ACTIONet identified two distinct states within the ML population (box) [5].
B. ML2 express higher level of genes coding for cytokines compared to ML1 (at RNA level).

EFFECT OF SAR’579 ON ML TRANSCRIPTOMIC PROGRAMS

Regulatory network analysis identified distinct master regulators of ML1 and ML2 cells



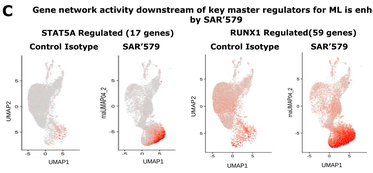
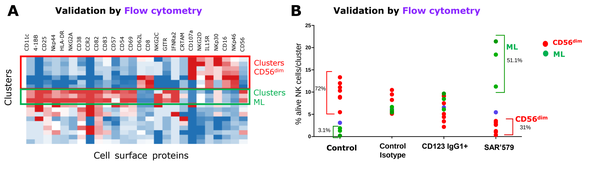


Figure 6: Expression of transcriptional regulators and target signatures
A. Marker genes for both ML populations are enriched in transcription factors and genes, coding for epigenetic modulators, defining their distinct transcriptomic profiles.
B. Distinct master regulators identified by Scenic for ML.1 and ML.2 populations, corresponding to some marker genes identified in independent analysis (Fig.5A) [6].
C. Regulatory network activity, defined by Scenic, predicted to be increased by SAR’579.

ORTHOGONAL VALIDATION OF SURFACE AND SECRETED PROTEIN CHANGES

Flow cytometry confirmed increased ML frequency and enhanced secretion of cytokines after treatment with SAR’579



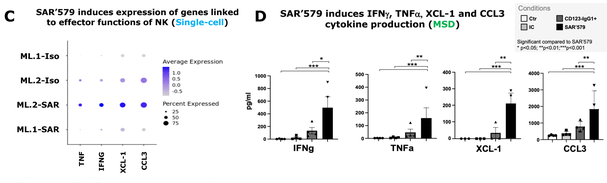


Figure 7: Changes in surface and secreted protein levels after SAR’579 treatment

A. Identification of NK cell populations by unsupervised analysis of 26 cell surface markers selected from single-cell analysis. Validation at protein level of multiple states for CD56dim population and presence of at least 2 different ML populations.
B. Flow cytometry confirmed ML NK subset increase and CD56^{dim} NK subset decrease upon treatment by SAR’579.
C. SAR’579 increased expression of effector cytokines in NK cells by single cell profiling.
D. Measurement of secreted cytokine/chemokines confirmed the increase in effector cytokine/chemokine at protein level, following SAR’579 treatment compared to reference CD123 antibody and isotype control-treated cells.

Conclusions

Together, our work helped characterize the NK cell changes induced by SAR443579 anti-CD123 NKCE in presence of target cells at the RNA and protein level:

- We identified 6 major and two minor NK cell subpopulations
- We observed that SAR’579 treatment induced an increase in ML NK cell subset, and a decrease in CD56dim NK cells
- Pseudotime analysis identified ML as the most differentiated NK subpopulation.
- We confirmed our findings at the protein level using flow cytometry and secreted cytokine/chemokine profiling in an independent experiment.

DISCLOSURES:
- This study was funded by Sanofi.
- Sanofi employees and may hold shares and/or stock options in the company.

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