# Development of a large-scale, *ex-vivo* NK cell expansion protocol for use in assessments of NK-targeting therapeutics

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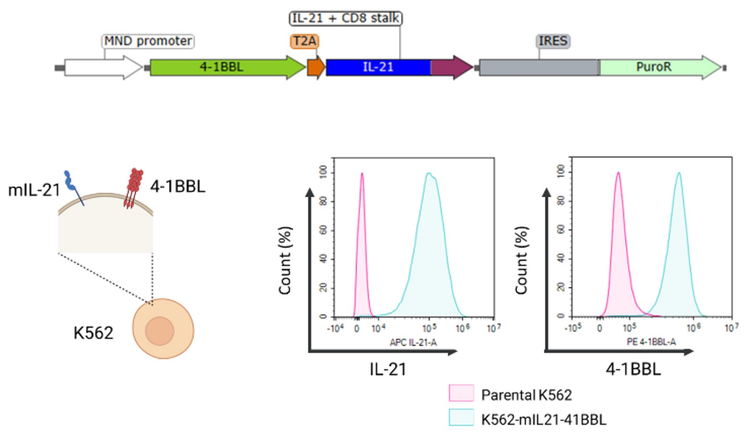
## Introduction

Natural killer (NK) cells play a crucial role in our innate immune defence system, primarily by targeting tumour or virus-infected cells. Immunology assays which assess the efficacy and safety of biotherapeutics require the need of primary immune cells, including NK cells. These cells are derived from blood products which rely on donations from healthy donors or patients. There exists a need for a solution that would increase the availability of primary donor material. Cryopreserved banks of expanded NK cells could improve assay quality by increased consistency and repeatability for functional assays. Using artificial antigen presenting cells (aAPCs), we aimed to expand NK cells isolated from cryopreserved PBMC.

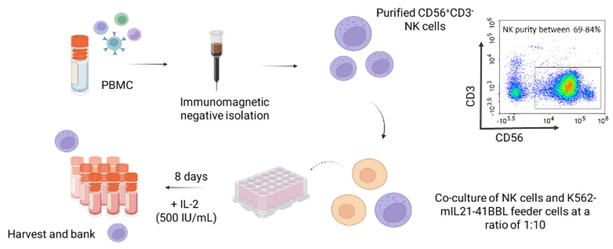
## Generation of aAPC

K562-mIL21-41BBL cells were generated by transducing parental cells with constructs encoding the membrane-bound form of IL-21 (IL-21 plus CD8a), and 4-1BB ligand. Selection was achieved via a puromycin resistance cassette. Surface expression was confirmed using flow cytometry.

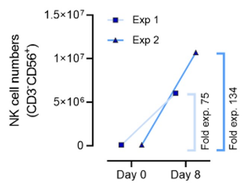



## Expansion of NK cells

NK cells were purified from cryopreserved peripheral blood mononuclear cells. Feeder cells were inactivated using Mitomycin C. Cells were co-cultured for 8 days in 24 well G-tex plates with media supplemented with IL-2. Following an incubation period of 8 days, expanded NK cells were harvested and cryopreserved.

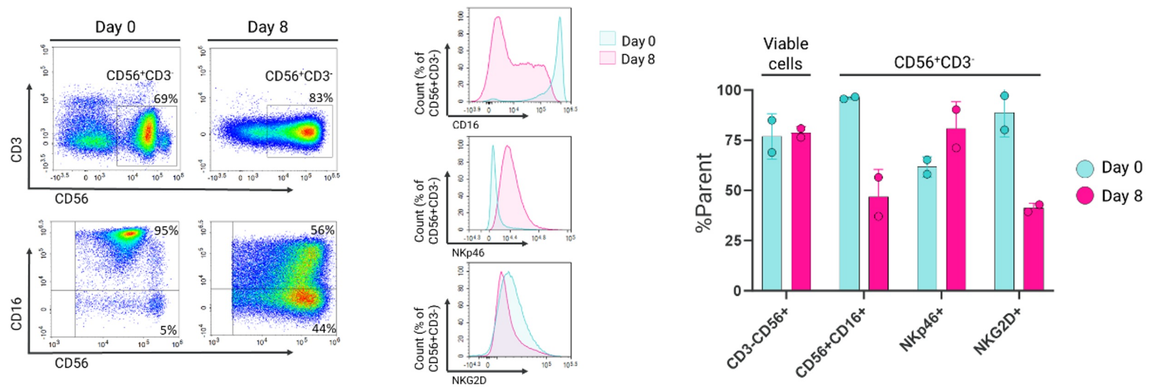



Fold increase of the expanded NK cells after incubation was 75 – 134.




## Expanded NK cell phenotype

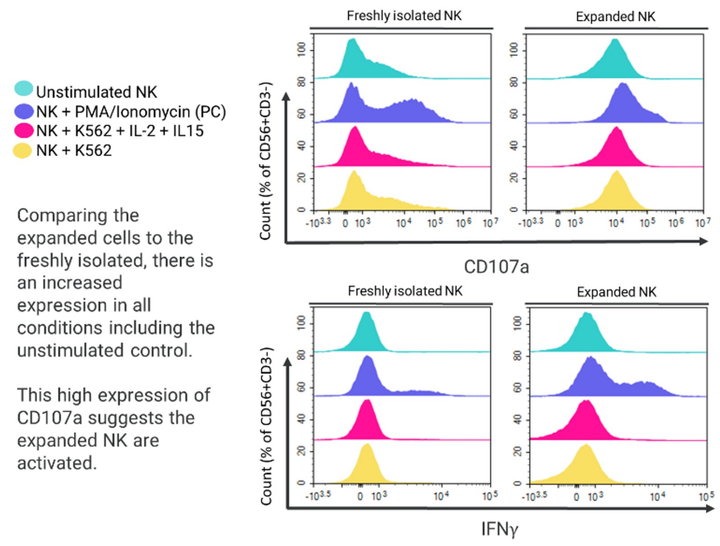
On Day 0 and Day 8, NK cells were stained to confirm expression of CD56, CD16 and their activation receptors NKp46 and NKG2D. Following the expansion period, we observed a reduction in CD16 and NKG2D whereas NKp46 increased slightly.




## Functional assessment

**Degranulation**

We sought out to assess the cytolytic activity and cytokine production of the expanded NK cells. Expanded NK cells were thawed for use on the day while freshly isolated NK cells were acquired from cryopreserved PBMC. CD107a, a membrane molecule on cytolytic granules, is used as an indicator of cytolytic function. IFN$\gamma$ is a cytokine produced by activated NK cells.

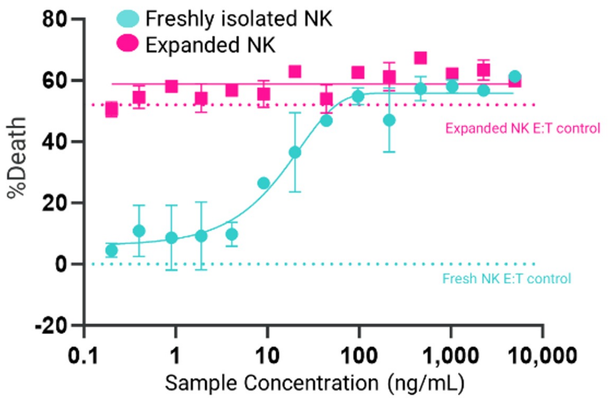



Comparing the expanded cells to the freshly isolated, there is an increased expression in all conditions including the unstimulated control.

This high expression of CD107a suggests the expanded NK are activated.

**Cytotoxicity**

Expanded NK cells were co-cultured with mTNFa-CHO target cells (effector target ratio of 10:1), and anti-TNFa (Remicade) to determine whether the expanded cells can exert cytotoxicity in an antibody-dependent fashion. Freshly isolated NK were able demonstrate ADCC in a dose-dependent manner. However, the expanded NK were shown to be highly cytotoxic at all sample concentrations including in its absence (effector target control, dotted line).




Taken together, the sustained upregulation of CD107a, and the cytotoxicity data demonstrate that the expanded NK have enhanced cytotoxicity.

## Summary

NK cells represent a small fraction of circulating peripheral blood cells. Acquiring a sufficient quantity of NK cells for *in vitro* assessments can pose challenging. Using the leukemia cell line K562 modified to express membrane bound IL-21 and 4-1BBL, we were able to generate NK cells with enhanced cytotoxicity *ex vivo*. These results suggest the potential of these NK cells to be utilised for *in vitro* assays to determine cytotoxicity against cancer cell lines in conjunction with immunotherapies, improving assay reproducibility and minimising inter-assay variability.