

# GMP-compliant iPSC lines and immuno-oncology platform for allogeneic cell therapy

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## INTRODUCTION

Human induced pluripotent stem cells (iPSCs) are emerging as a potent starting material for allogeneic cell therapies. As a prerequisite, we have established a GMP manufacturing process to give rise to clinical-grade iPSC lines and we continue to produce new cell lines from US donors that are both commercially exploitable and FDA-compliant from a donor eligibility perspective. The cells display full acquisition of pluripotency and a low mutational burden reflecting their neonatal origin, cord blood.

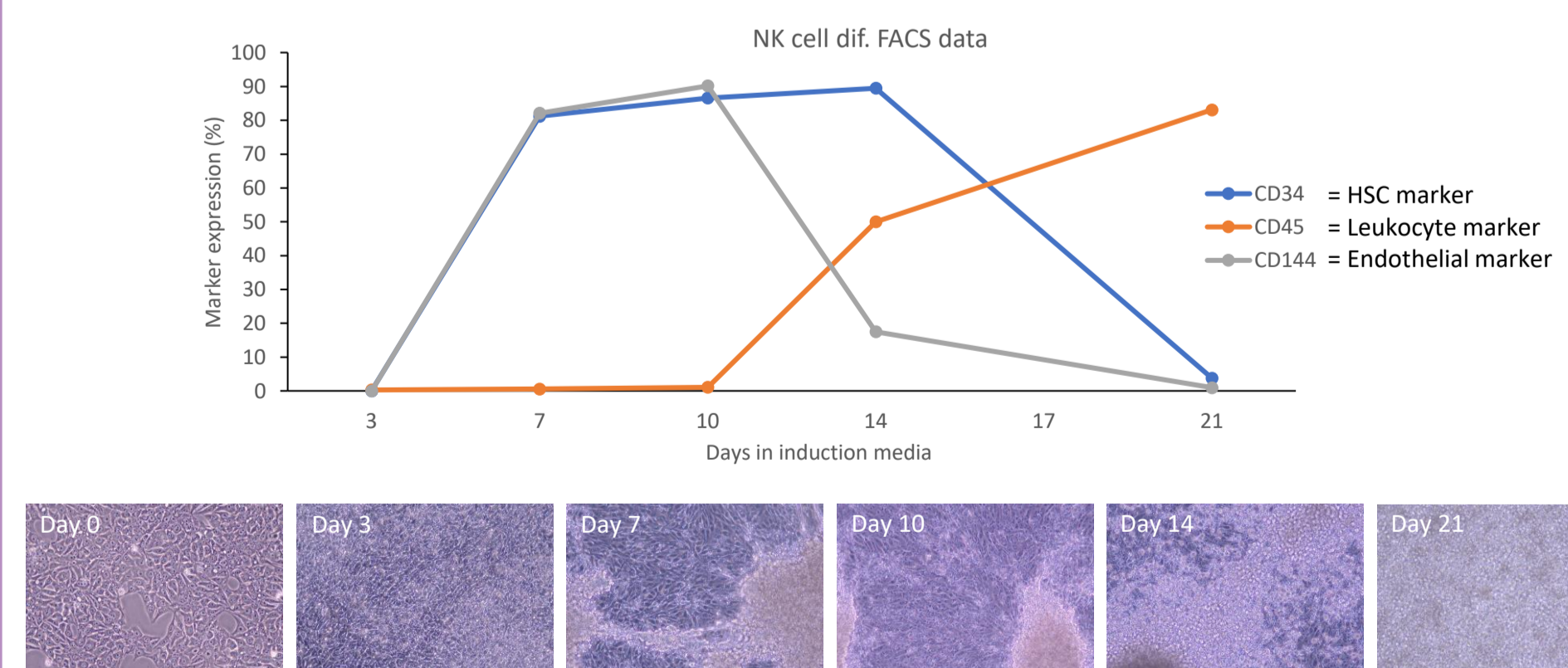
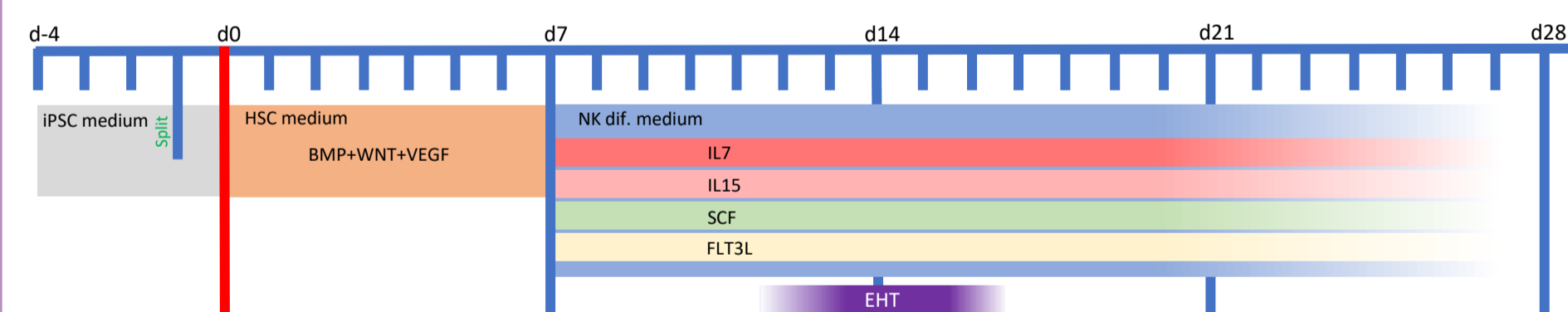
In parallel efforts, beyond cell types for replacement therapy, we have developed a feeder cell-independent immune cell differentiation platform starting from these iPSC lines. In the first stage, iPSCs are converted to hematopoietic precursor cells sharing characteristics of *bona fide* hematopoietic stem cells (HSCs). Using a newly established protocol, these HSC-like cells are generated at high purity in a defined manner and may be cryopreserved as intermediate cell banks. Following recovery, the HSC-like precursors may, in a flexible manner, be further differentiated to different immune cell types - T cells, macrophages, or natural killer (NK) cells. This approach via HSC-like intermediates has key advantages for GMP manufacturing and upscaling as it significantly simplifies and shortens the process.

## OBJECTIVES

Here we show our defined iPSC-derived HSC platform generate two different kinds of NK cells depending on the basal media used. The first cell type represents cytotoxic NK cells, with potent killing ability against target cancer cells and strong expression of markers for NK activation. The second cell type halts NK development at a proliferative, immature stage with no activation markers and a distinct lack of cytotoxicity. After a desired expansion duration, these immature NK cells can be rapidly matured into cytotoxic NK cells through exposure to an activating agent, providing an end-to-end feeder-free solution for upscaling NK therapy.

## METHODS

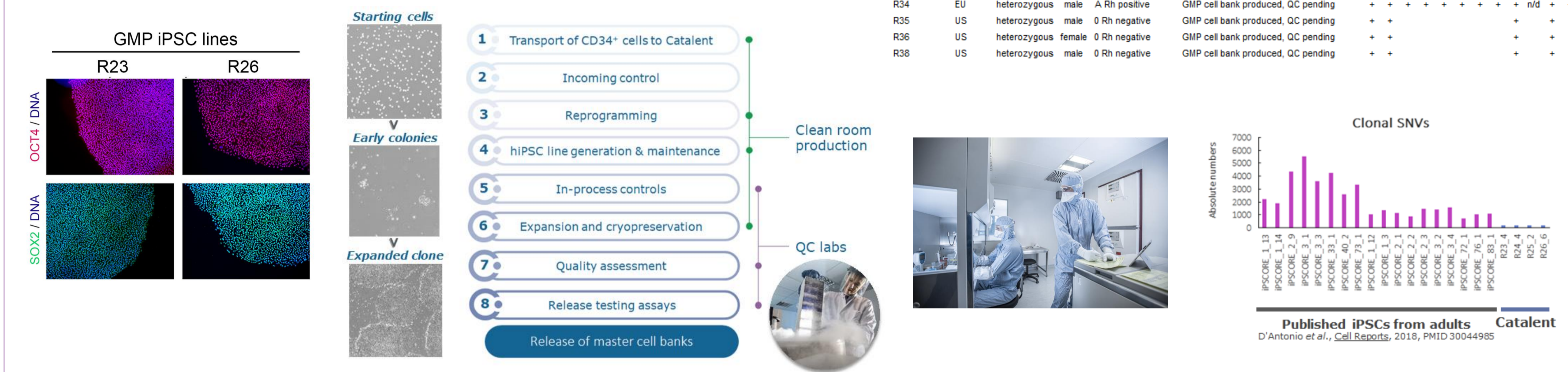
- **iPSC culture** - iPSCs were maintained by weekly passage of 10,000 iPSCs onto Laminin 511-coated 6-well plates. Cells are grown in Miltenyi iPSC brew XF, with cells reaching 70-90% confluence after 7 days of growth.
- **NK differentiation** - iPSCs are seeded in a 12-well format one day prior to starting differentiation. From days 0-7, iPSCs are first differentiated into hemogenic endothelium to generate CD34/CD144+ cells. From days 7-14, NK differentiation media excluding IL3 is applied as described by Miller & McCullar<sup>1</sup>. From day 14 onwards, the same media is used with half media changes twice per week.



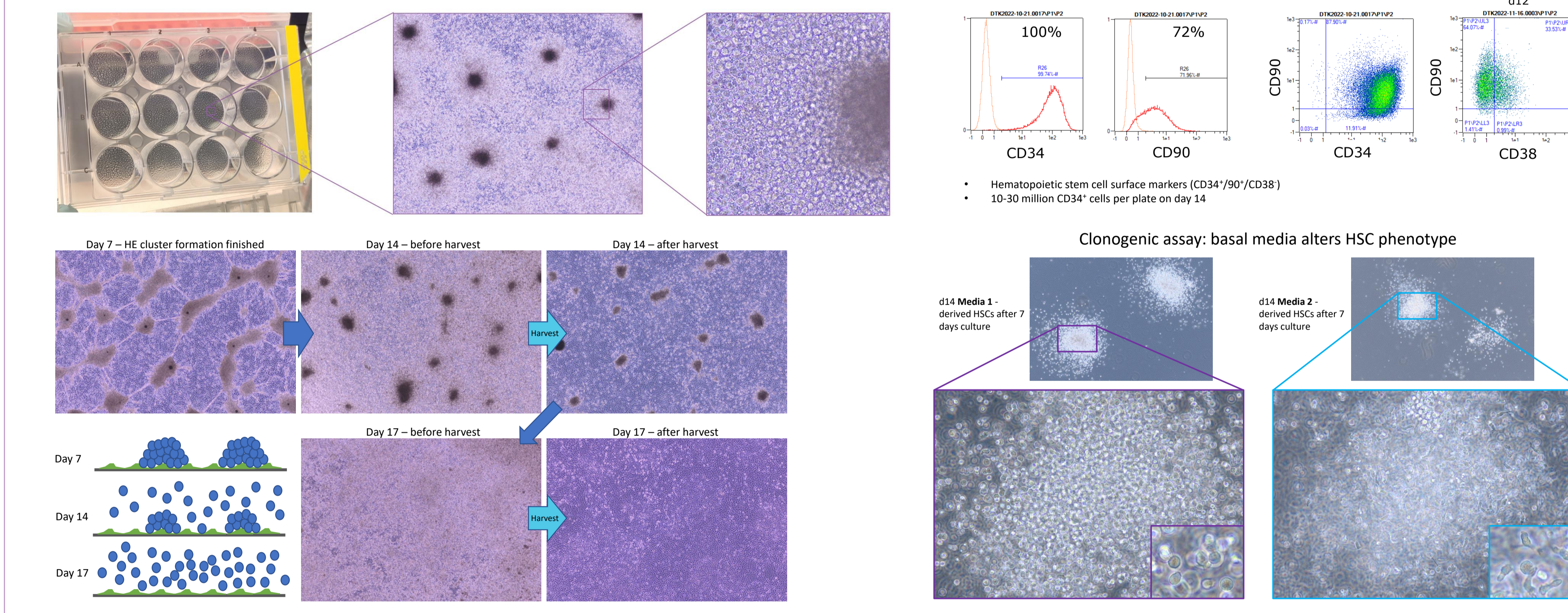
- **Flow Cytometry** - Performed using a Miltenyi MACS Quant10 flow cytometer. Cells were stained using Miltenyi 1:50 FACS antibodies, washed, and then immediately analysed. Gating: P1: lymphocyte region, P2: doublet exclusion, P3: CD56+ cells (NK marker panels only). In each case, 30,000 cells were measured at the final gate. Isotype control antibodies were used as negative controls.
- **Clonogenic assay** - 3000 cells were mixed with 2ml of complete methylcellulose medium (EPO, IL3, GM-CSF and SCF) and distributed into two wells of a 6-well plate (1ml = 1500 cells per well). After 7 days incubation (37°C, 5% CO<sub>2</sub>), colonies (defined as clusters of >20 cells) were quantified.
- **Killing assay** - K562 target cells (strain: ACC10) were stained with cell trace violet. These cells were mixed with NK cells at the indicated ratios. They were incubated for 4 hours (37°C, 5% CO<sub>2</sub>). 30 mins before the end, cell event caspase 3/7 green was added. 5 mins before the end, Sytox 7AAD living/dead stain was added. The cells were analysed by flow cytometry. Gating: P1: cell fragment exclusion, P2: doublet exclusion, P3: Only cell trace violet positive cells (K562 target cells). Stop gate: 30,000 events in the P3 gate.

## RESULTS

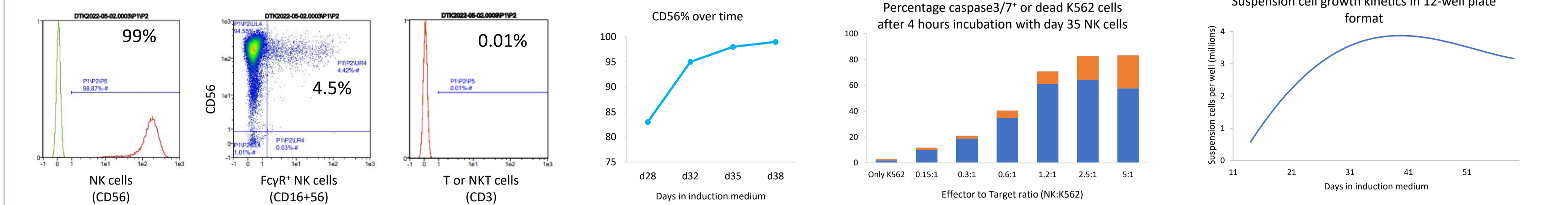
### GMP-compliant iPSC lines



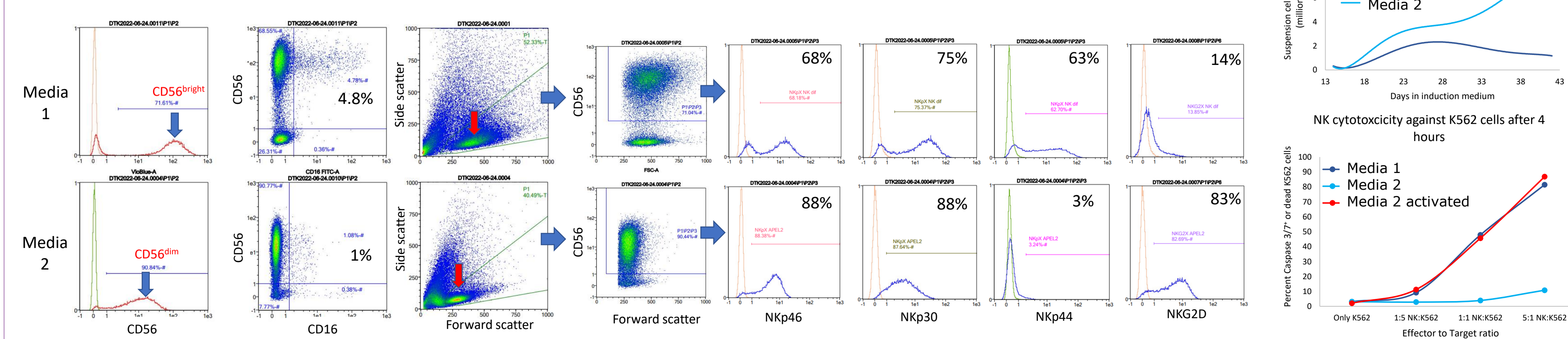
### iPSC-derived HSCs



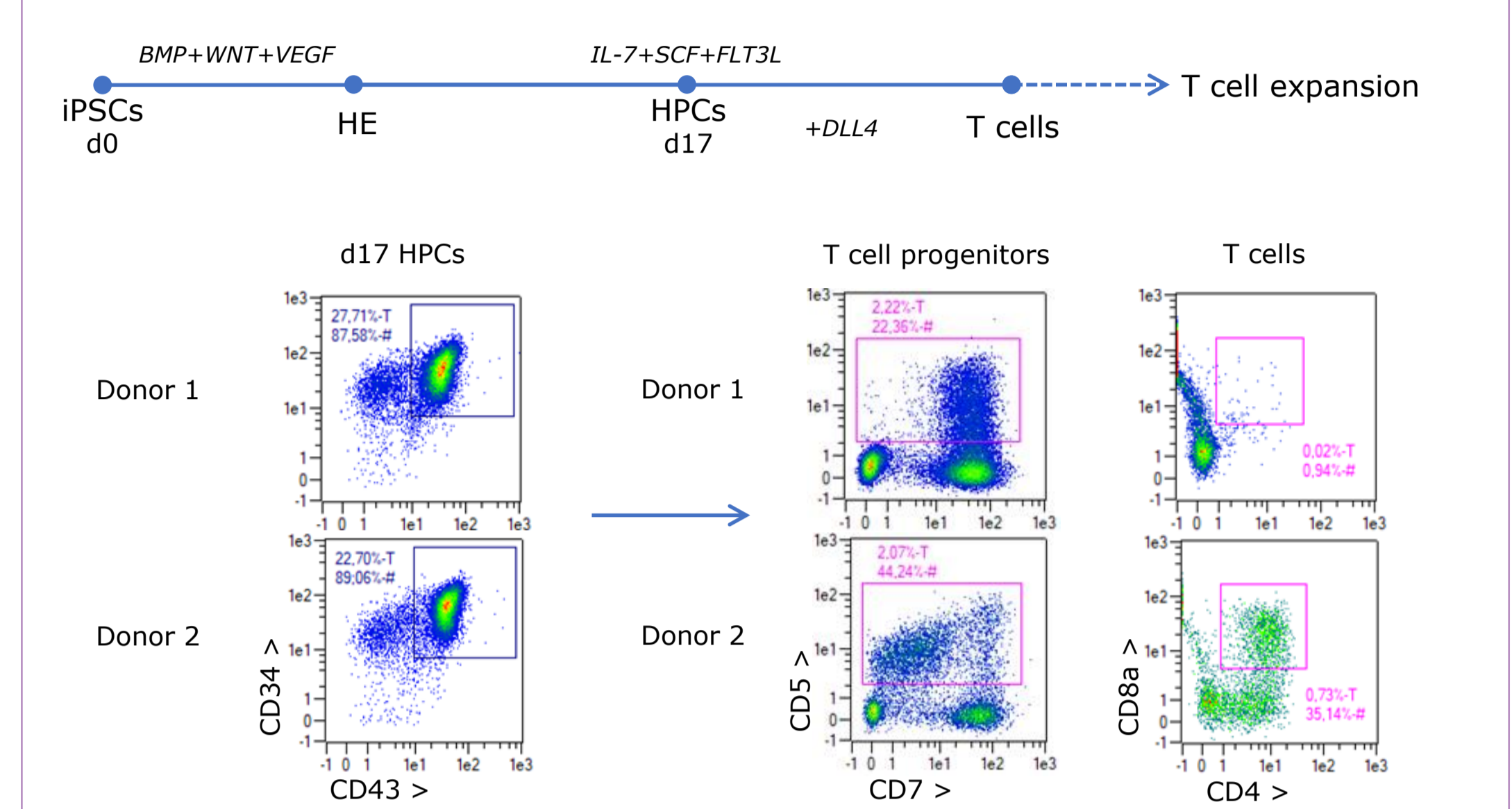
### iPSC-derived NK cells



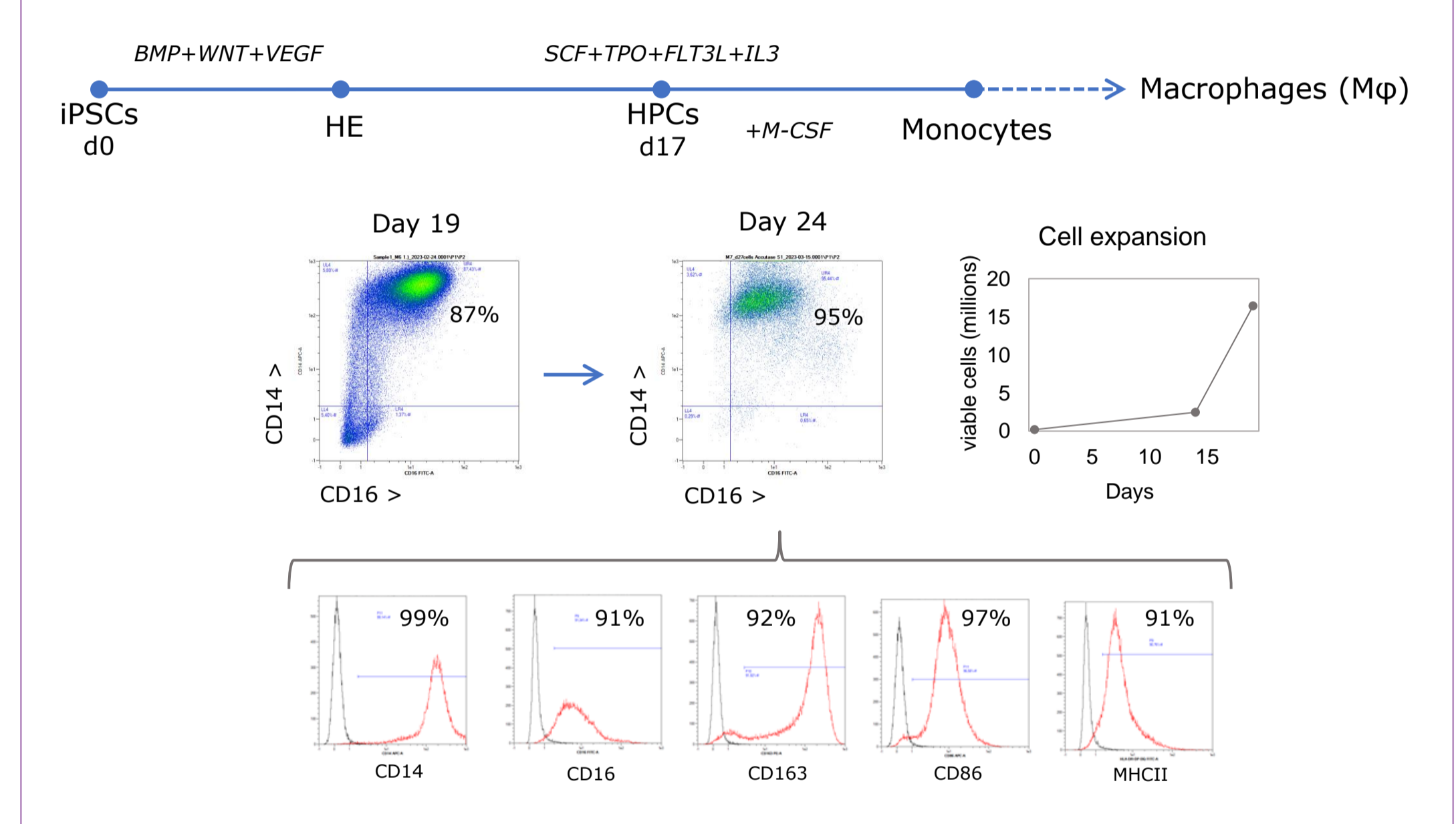
### Generating *in vitro* CD56<sup>bright</sup> or CD56<sup>dim</sup> NK cells from iPSCs



### iPSC-derived T-cells



### iPSC-derived Macrophages



## CONCLUSIONS

- GMP iPSC cells display a fully reprogrammed state and acquired pluripotency.
- As compared to adult tissue-derived iPSCs, these lines show a low mutation load, attributable to their neonatal origin.
- All tested iPSC lines were capable of HSC differentiation and subsequent NK differentiation, demonstrating the robustness of the HSC platform.
- Avoids spontaneous differentiation seen in embryoid body-based immuno differentiation strategies, reducing run to run variability.
- Intermediate HSCs can be cryopreserved as a process intermediate.
- Basal media has a huge impact on both the HSCs generated and their subsequent ability to differentiate.
- We have identified two specific basal media formulations which result in two phenotypically different types of NK cell: Cytotoxic and non-cytotoxic/immature.
- HSCs generated with this platform have been shown to be capable of directed differentiation into both myeloid (macrophage) and lymphoid (T-cell) lineages.

## REFERENCES

1. Jeffrey S. Miller and Valerie McCullar (2001) Human natural killer cells with polyclonal lectin and immunoglobulinlike receptors develop from single hematopoietic stem cells with preferential expression of NKG2A and KIR2DL2/L3/S2, Blood, Vol 98; 3, p705-713.

## ACKNOWLEDGEMENTS

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