Leveraging advanced analytics for detailed process characterisation in iPSC-NK cell therapy generation

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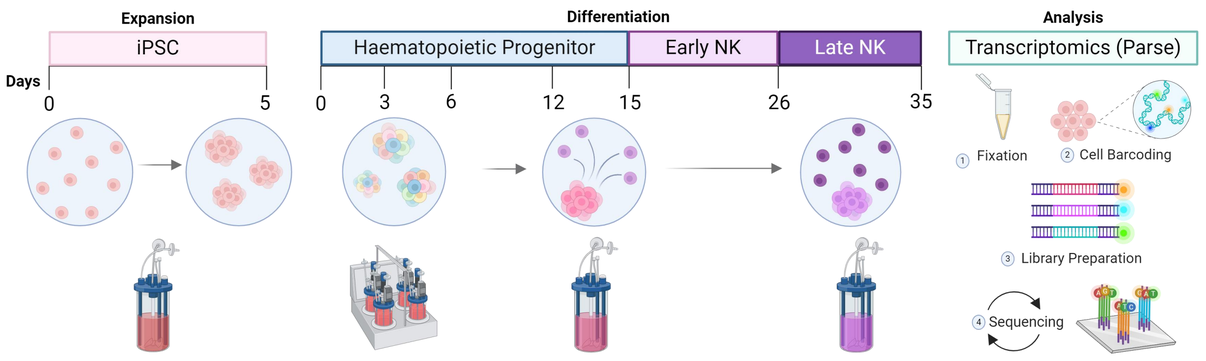
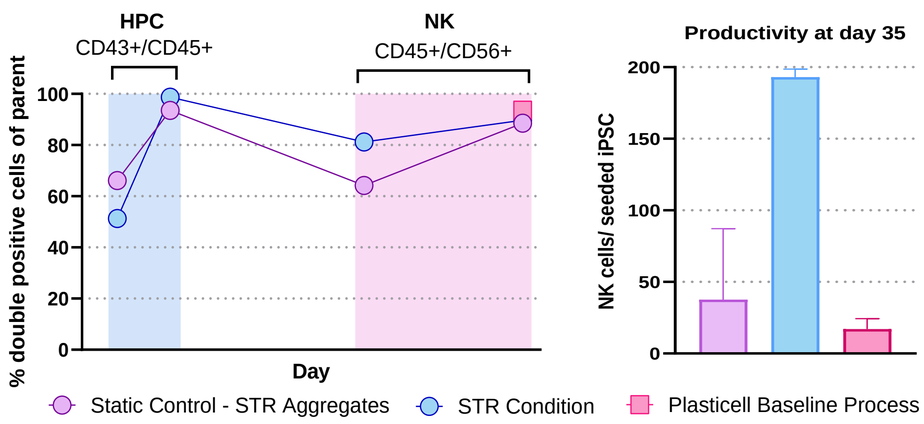
Utilisation of large-scale bioprocessing for allogeneic iPSC-derived cell therapy development requires comprehensive process understanding for the implementation of robust, biologically relevant controls during long-term cell differentiation. Following the successful demonstration of seamless end-to-end iPSC-derived natural killer cell (iNK) generation in a stirred-tank reactor (STR) system in collaboration with Plasticell, single cell transcriptomics (employing Parse Evercode™ technology) was used to elucidate the dynamic gene expression profiles associated with critical stages of differentiation and map the cell trajectory over a 35-day process. This was coupled with extensive flow cytometry and cancer cell cytotoxicity outputs to provide a detailed overview of cell phenotype, maturity and end-product functionality, which can help to infer the potency and quality of cells produced by this process. The data presented here highlights an intrinsic relationship between bioprocessing parameters and the yield of functional iNK cells, with a direct impact of cell density on key biological characteristics.

SCALED iPSC-NK DIFFERENTIATION



- **Integrated iPSC expansion** (5 days) **and NK differentiation** (35 days) was performed in an STR system. Cell samples were collected and fixed throughout the time course.
- Single cells were barcoded, and libraries were prepared using the Parse Evercode kit for **multiplexed single cell RNA sequencing**.

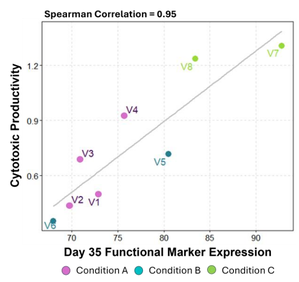
- **First evidence** that baseline iNK differentiation protocol is amenable to large-scale bioprocessing
- **24x improvement** in iNK cells produced per seeded iPSC.

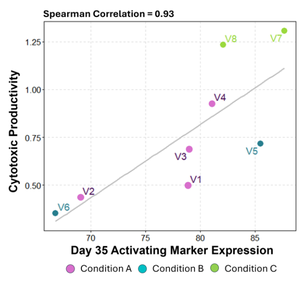


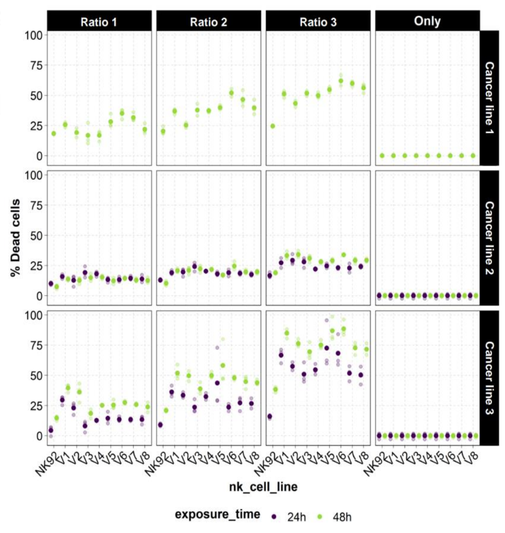
PROCESS CHARACTERISATION USING A MULTIOMIC APPROACH

NK Identity and Function

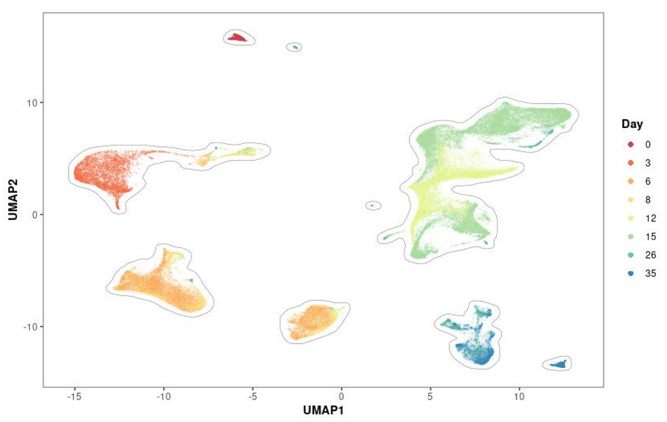
- STR-generated iNKs showed cytotoxic function **against 3 cancer cell lines.**
- Correlating these outcomes with FCM data, revealed different phenotypes associated with:
  - iNKs from processes that were highly **productive.**
  - iNKs that were highly **functional.**





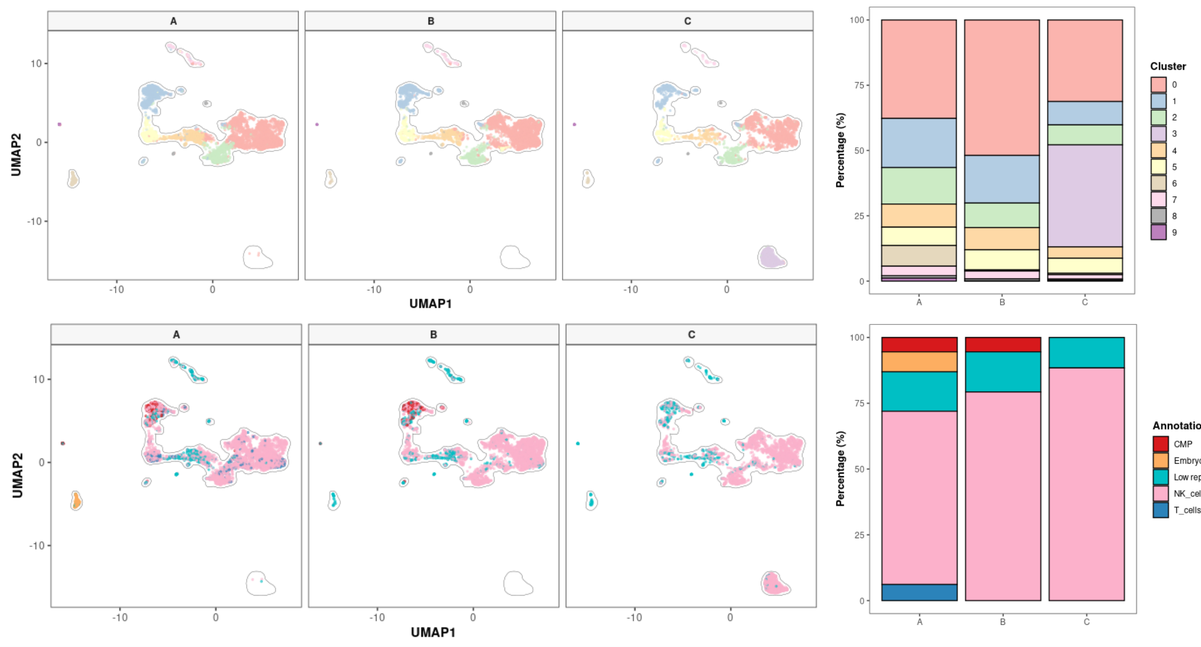


Pathway of Differentiation



- Mapping the development pathway in UMAP space showed distinct transcriptomic profiles for key timepoints.

Influence of Bioprocessing Parameters on Differentiated Populations



- Louvain clustering at day 35 indicated the presence of different cell populations per condition, with emergence of unique clusters in condition C.
- Modulation of cell density at a key transitional timepoint influenced the proportion of these differentiated cell populations, suggesting this may be a critical process parameter (CPP) for iNK bioprocessing.
- Cluster annotation (Human Cell Atlas) showed the largest population in all STR conditions consisted of target iNK cells, with small proportions of off-target cells.
- Differential gene expression will be used to characterise the phenotype of these unique populations for biomarker discovery and future development of in-process controls.

High-content analytical methods including transcriptomics, proteomics and metabolomics have been applied to identify key biomarkers and critical process parameters (CPPs). By integrating these multiomics technologies, indicators of high-quality iNK differentiation may be identified and utilised for future in-process monitoring, including novel non-destructive methods such as miRNA and metabolite analysis, to ensure consistent cell quality and functionality during manufacture. Using these insights, with deeper investigations into the mechanisms that drive high productivity, future work can look to optimise production of highly cytotoxic iNKs at clinically relevant doses. Offline analytics can additionally be used to inform future development of online methodologies for providing real-time monitoring of differentiation.

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