Stem Cell Research

Differentiation of PSC suspension cultures into cytotoxic NK cells at scale

Marcus Bunn, Lindsey Bailey Steinitz, Vivek Chandra, Michael Akenhead, Mark Kennedy, David Kuninger
Thermo Fisher Scientific, 7335 Executive Way, Frederick, MD, USA, 21704

Abstract

The field of cell therapy has experienced significant growth as a prominent therapeutic option for various diseases. However, there are still significant challenges to overcome, including the ability to obtain large quantities of the appropriate cell type. Natural killer (NK) cells, which are cytotoxic innate lymphoid immune cells, have shown promise in cancer cell therapies as they can effectively target malignant cells without the need for HLA matching. Clinical trials involving NK cell therapy typically require a substantial number of NK cells, ranging from ~ $5x10^6$ to $1x10^8$ cells per kilogram of body weight per dose.

Existing methods for obtaining NK cells, such as donor sourcing and 2D differentiation, have protocols involving co-culture or embryoid body formation steps. These methods often yield varying results and fail to generate the necessary quantities of functional NK cells. To address these limitations, we have developed a 3D pluripotent stem cell (PSC) protocol that focuses on scaling up the generation of hematopoietic stem cells (iHSCs), which are multipotent stem cells capable of producing induced PSC derived iNK cells. This protocol highlights the use of CTS™ StemScale™ PSC culture medium, which has proven to be an efficient platform for cultivating large quantities of undifferentiated PSCs as cell aggregates, known as spheroids. The iNK cells generated with the protocol can be cryopreserved and recovered, are expandable, and show cancer cell killing potential.

Introduction

We have developed and optimized a working protocol in which iNK cells can be generated at scale from PSCs cultured in CTS™ StemScale™, transitioned to StemPro™-34 SFM then matured/expanded in a CTS™ NK-Xpander™ Medium/MEMα nucleosides blend. Parameters such as orbital shake speed, cytokine cocktail, seeding densities etc., have been refined, creating an ~40-day, feeder-free PSC to iNK differentiation protocol. This method has been evaluated in cultures up to 100mL producing 5-10x10^6 $CD56^+CD3^-$ iNK cells/mL.

Methodology

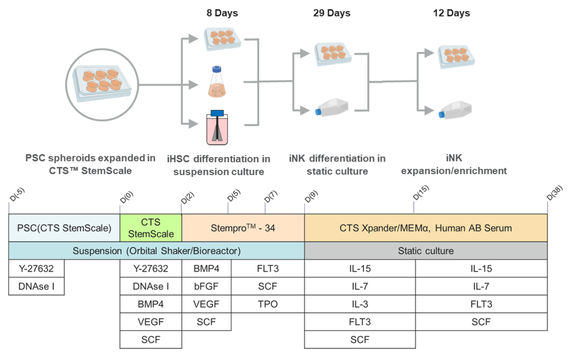

Figure 1. Overview of the PSC to iNK differentiation protocol. Media systems, proteins, and small molecules used for differentiation.

Prior to differentiation, iPSCs were cultured in CTS™ StemScale™ PSC Suspension Medium. The iPSCs were adapted to suspension culture for at least three passages. After three passages, differentiation induction started following Figure 1.

- The initial PSC seeding density $3x10^5$ cells/mL in all vessels(6 well plate- 100 mL vertical wheel bioreactor.
- Days 0-9 are performed under constant agitation on an orbital shake platform at 60 rpm.
  - For larger vessels i.e. vertical wheel bioreactors, 35 rpm.
- Once iHSC differentiation is complete(~day 9) the culture is transferred to a 6 well plate or T-75 culture flask for static iNK culture.

After ~5 weeks The cells can either be cryopreserved in CTS™ PSC Cryomedium or transferred to CTS™ NK-Xpander for enrichment.

Results

We have developed and optimized a working protocol in which iNK cells can be generated at scale from PSCs cultured in CTS™ StemScale™, transitioned to StemPro™-34 SFM then matured/expanded in a CTS™ NK-Xpander™ Medium/MEMα nucleosides blend. Parameters such as orbital shake speed, cytokine cocktail, seeding densities etc., have been refined, creating an ~40-day, feeder-free PSC to iNK differentiation protocol. This method has been evaluated in cultures up to 100mL producing 5-10x10^6 $CD56^+CD3^-$ iNK cells/mL.

Morphological observations during iNK differentiation

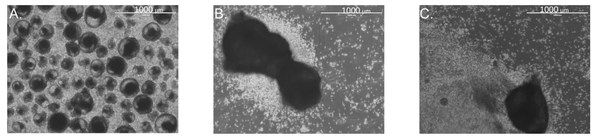

Figure 2. Morphological observations during iNK differentiation. A. Day 12: Individual iHSC and spheroids begin attaching to vessel floor. B. Day 21: Spheroids form a "feeder" like monolayer for suspension cells to adhere and further mature the iNK cells. C. Day 30: Further expansion of the spheroid monolayer and expansion of iNKs.

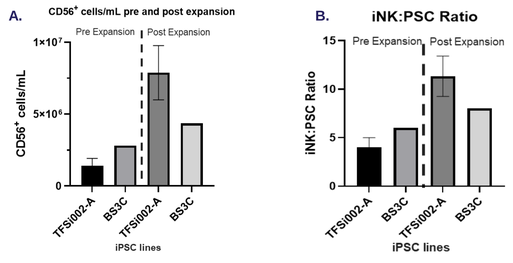

Figure 3. iNK cell yields before and after expansion in CTS™ NK-Xpander medium after 10 days of culture in 2 different iPSC lines.

iNKs maintain surface marker expression post thaw

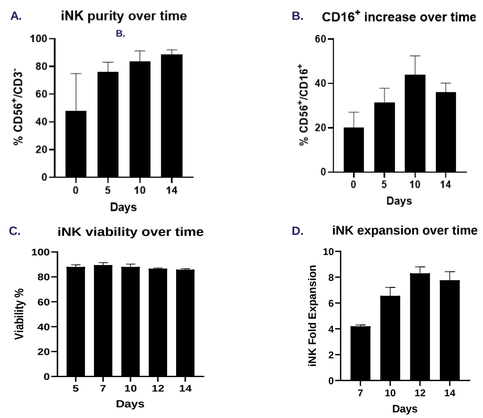

Figure 4. Post cryopreservation characterization of iNK cells enriched in CTS™ NK-Xpander culture medium. A. Surface marker expression of $CD56^+CD3^-$ B. $CD56^+/CD16^+$ increase over a 14 day period. C. Viability of iNKs remained over 80% two weeks post thaw. D. iNKs proliferate post cryopreservation, expanding ~8 fold within 14 days.

iNKs are useful in immuno-oncology applications

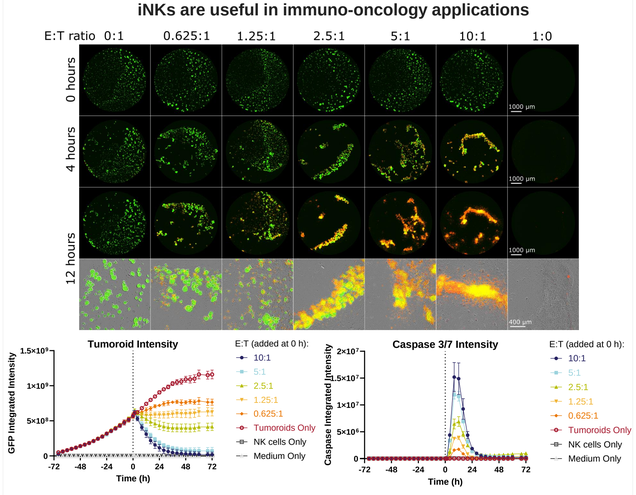

Figure 5. Demonstration of iNKs targeting and killing patient-derived GFP-tagged tumoroid cell line grown in OncoPro™ tumoroid culture media system. The top images, captured from the IncuCyte, show colorectal tumoroids (green) and caspase-based signaling indicating cell death (red). Optimal tumoroid targeting was observed at 5:1 and 10:1 ratios with iNKs, which further correlated with an increase in caspase activity.

Generation of function iPSC-derived natural killer cells

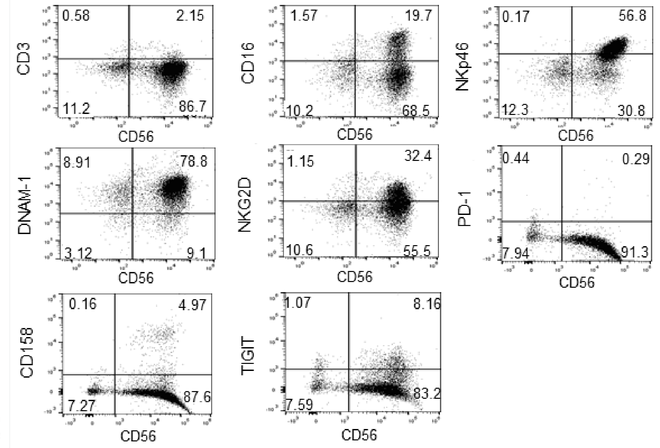

Figure 6. Activation/Inhibition Surface Marker Expression. iNKs express key activation markers post expansion in CTS™ NK-Xpander.

Conclusions

- Human PSCs are expanded in CTS™ StemScale™ PSC suspension medium for efficient scale-up.
- This expansion method allows generation of large quantities of differentiated cells, such as iNK cells.
- Cytokine/small molecule cocktails drive differentiation of PSC spheroids into iNK cells, avoiding co-cultures.
- The iNK cells generated are functional in immuno-oncology applications.
- These cells can be expanded further to increase yields and purity.
- Cryopreservation and recovery of functional iNKs simplifies characterization and downstream assay timelines.

Acknowledgements

We would like to thank Logan Wilson and Erica Heipertz for assistance with expansion studies.

Trademarks/licensing

Learn more at **thermofisher.com/ctsstemscale**