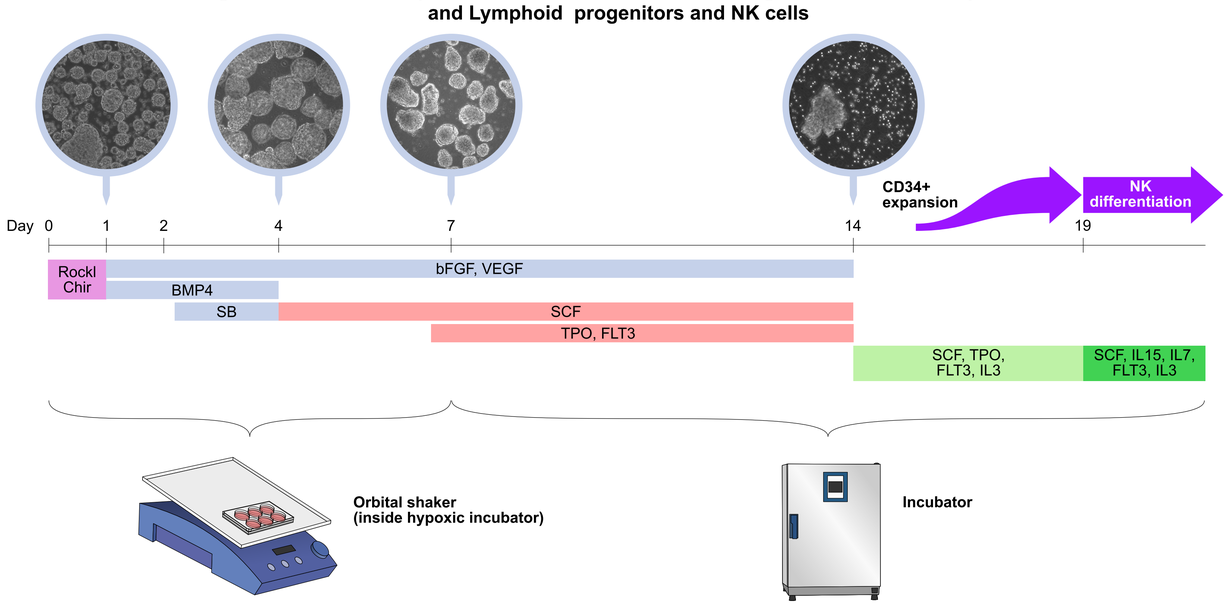
GENERATION OF HYPOIMMUNE HEMATOPOIETIC PROGENITORS FROM HLA CLASS-I AND CLASS-II NULL iPSC LINE

Idelman Gila, O'Driscoll Cliona, Sreenivasan Urmila, Chukwurah Evelyn, Ren Yongming Luke, Sakurada Yuko, Okuda Yuichi , Liu Litong, Kitajima Miha, Yoshimoto Shingo, Sugo Tsukasa, Richard Jean-Philippe, Seddon Michael, Modali Rama and Rao Mahendra

ABSTRACT

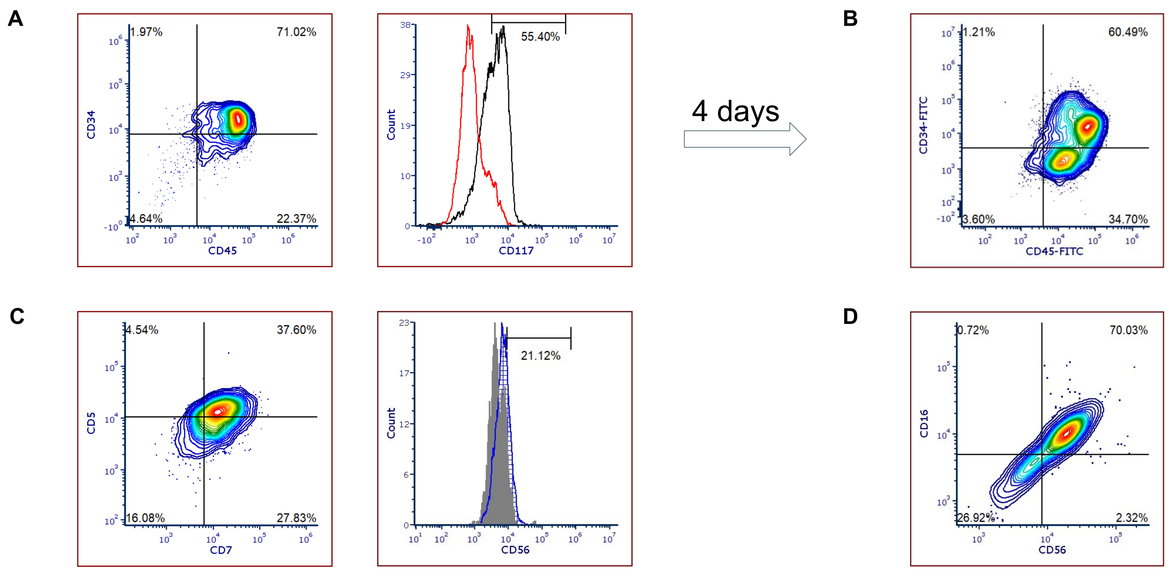
Over recent years, the field of regenerative medicine has witnessed a notable surge in the adoption of cell-based therapies. Among these innovative treatments are hematopoietic cell transplantation, CAR-T cell therapy, and CAR-NK cell therapy, which hold promise for addressing various diseases, particularly cancer. Nonetheless, a significant challenge in allogeneic transplantation is the potential development of graft-versus-host disease (GVHD), a serious condition where the transplanted immune cells attack the recipient's tissues. To mitigate the risk of GVHD there is a growing interest in utilizing hypoimmune cells. Induced pluripotent stem cells (iPSCs) have emerged as a versatile platform for generating ample quantities of the cells with the uniform genetic background. In this study, we present data on the generation of hypoimmune cells from iPSCs. Notably, we have previously utilized this line to generate HLA class-I and class-II null mesenchymal stem cells (MSCs). Specifically, we demonstrate the successful derivation of CD34+ hematopoietic progenitor cells and CD5+/CD7+ myeloid progenitor from HLA class-I and class -II null iPSC line. These progenitors exhibit the capability to further differentiate into T and NK cells. Additionally, we evaluated the differentiation efficiency of this genetically modified iPSC line in comparison to its parental counterpart. In conclusion, our findings underscore the promising prospects of utilizing HLA class-I and class-II null iPSC lines as a renewable and hypoimmune cell source for allogeneic transplantation, particularly in the realm of cancer treatment. By harnessing the regenerative power of iPSC technology, we endeavor to pave the way for safer and more effective therapies with the potential to revolutionize medical practice.

Figure 1. Schematic representation of the method used for generation of Hematopoietic and Lymphoid progenitors and NK cells



iPSCs were dissociated into a single-cell suspension and cultured in a hypoxic incubator at 5% oxygen on a rotating platform for 24 hours with Y27632 (Rock Inhibitor) and CHIR99021 (GSK3 inhibitor) to form embryoid bodies (EBs). After 24 hours, bFGF, VEGF, and BMP4 were added. Cells were cultured under hypoxic conditions for an additional 48 hours to induce mesoderm. On day four, SCF, TPO, and FLT3 ligand were introduced to the culture to stimulate hematopoiesis, maintaining hypoxic conditions. Starting on day seven, cells were incubated in normoxic conditions to allow the production of hematopoietic stem cells (HSCs) by EBs.

Figure 2. Differentiation of iPSC towards HSC and lymphoid progenitors



On day 13 of the differentiation protocol, cells were collected from the supernatant and analyzed using flow cytometry. The majority of these cells showed double positivity for CD34 and CD45 markers. Additionally, a significant percentage of these cells were positive for CD117 **(A)**. Notably, the CD34 marker was expressed on the majority of cells expanded for 4 days in SCGM media **(B)**. After 14 days in NK differentiation media, 37.6% of the cells were found to be double positive for CD5 and CD7, indicating myeloid progenitors. CD7-positive cells accounted for 27.8% of the total cell population, potentially representing progenitors of T or NK cells **(C)**. By day 21 in culture, over 70% of the cells expressed CD56 and CD16 markers **(D)**.

Figure 3: Expansion of iPSC derived myeloid progenitors and NK cells

In our iPSC-derived myeloid progenitor and NK cell expansion, we achieved an approximate yield of 53 NK cells per iPSC cell.

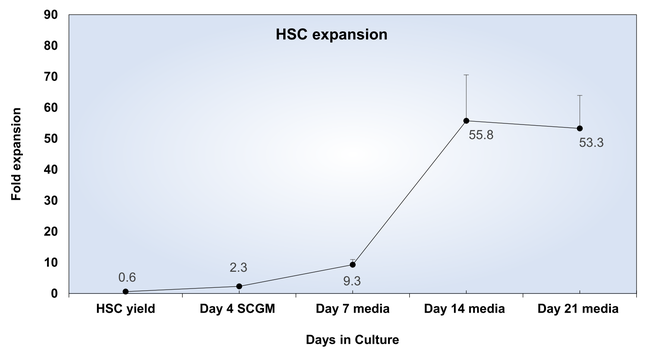
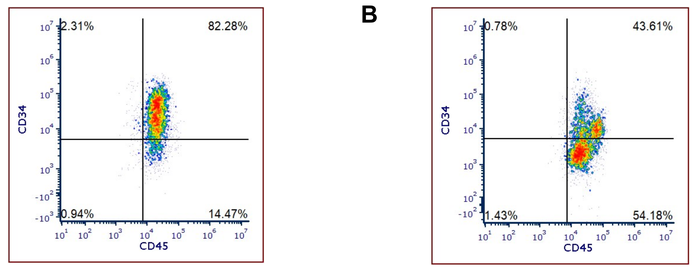


Table 1. Comparison with other cell lines (numbers, yield, percentage of differentiated cells.

Name	Yield of iHSC	CD4 Expansion (fold)	CD5+/CD7+ (%)	CD56+ CD16+ (%)
7713G, Clone G2	0.5-1	2.5-3.5	40%	50%-70%
7713G, Clone C4	0-1.6	2.6	25%	
7713, Parent Line	1.2-2.5	4-4.5	11%	40%, mostly CD56dim
D2-BCK	1.5-2	2-4	10-15%	
SK005.3 hTERT_SV40	0.5-1.5	2-4	30%	

Figure 4. Conditional immortalization increased proliferation capacity of CD34+ progenitors without losing CD34



We utilized vector hTERT_SV40 under the control of doxycycline to introduce conditional immortalization.

(A) Demonstrates higher expression of the CD34+ marker when hTERT_SV40 is induced compared to the wild-type **(B)**.

Table 2. Table illustrates the fold expansion of cells when cultured with or without doxycycline.

	Dox	No Dox
iPSC plates ($\times 10^5$)	8.7	8.7
CD34+ day 0 ($\times 10^5$)	5.3	4.2
Fold expansion of CD34+ (5 days)	6.7×	4.2×
Fold expansion of NK media (5 days)	4.0×	3.0×
Total expansion	26.8×	12.6×

SUMMARY

1. Here, we employed a feeder-free system to generate hematopoietic progenitors from iPSC lines lacking both HLA class-I and class-II expression.
2. These hypoimmune progenitors can be employed in regenerative medicine, cell therapy, and transplantation due to their lower risk of triggering immune rejection, which enhances their potential for successful therapeutic use.
3. Moreover, these hypoimmune cells retain the ability to differentiate into NK and T cells, thereby minimizing the risk of immune rejection in therapeutic applications.
4. By employing the hTERT_SV40 vector for conditional immortalization, we enhanced the proliferation capacity of CD34-positive cells and bolstered their ability to sustain CD34 expression even beyond 5 days in culture.

ACKNOWLEDGEMENTS

This project was funded, in part, by the Maryland Stem Cell Research Fund, through its Commercialization Program, award number: 2023-MSCRFCO-6157