CONCISE REVIEW

Therapeutic use of red blood cells and platelets derived from human cord blood stem cells

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Abstract
Red blood cells (RBCs) and platelets derived from stem cells are possible solutions to the increasing demand for blood transfusion. Based on the availability of stem cells, their relatively defined differentiation mechanisms, and the massive exploration of induction systems, the generation of RBCs or platelets in vitro from cord blood hematopoietic stem/progenitor cells (CB-HSPCs) has potential for clinical applications. However, information on the clinical translation of stem cell-derived RBCs and platelets in the literature and at the ClinicalTrials.gov website is very limited. The only clinical trial on cultured RBCs, which aimed to assess the lifespan of RBCs cultured in vivo, was reported by Luc Douay and colleagues. Of note, the cultured RBCs they used were derived from autologous peripheral blood HSPCs, and no cultured platelets have been applied clinically to date. However, CB-HSPC-derived megakaryocytes, platelet precursors, have been used in the treatment of thrombocytopenia. A successful phase I trial was reported, followed by phase II and III clinical trials conducted in China. In this review, the gap between the many basic studies and limited clinical trials on stem cell-derived RBCs and platelets is summarized. The possible reasons and solutions for this gap are discussed. Further technological improvements for blood cell expansion and maturation ex vivo and the establishment of biological standards for stem cell derivatives might help to facilitate the therapeutic applications of cultured RBCs and platelets derived from CB-HSPCs in the near future.

KEYWORDS
clinical translation, cord blood hematopoietic stem/progenitor cells, differentiation, megakaryocytes, platelets, red blood cells

Significance statement
This article reviews the progress in producing red blood cells (RBCs) and platelets from human cord blood hematopoietic stem/progenitor cells (HSPCs) and relatively limited reports on the clinical trials with cultured RBCs and megakaryocytic progenitors. The goal is to figure out the hurdles in clinical translation of CB-HSPC-derived RBCs and platelets, as well as to propose biological standards for these stem cell derivations in clinical applications. Technical improvement together with the establishment of biological standards might help to achieve therapeutic applications of cultured RBCs and platelets.
1 | INTRODUCTION

Cord blood hematopoietic stem/progenitor cells (CB-HSPCs) have been utilized in hematological disease management and malignant cancer therapies. Transplanted HSPCs can develop into all kinds of blood cells, including red blood cells (RBCs), platelets and immune cells. Correspondingly, blood cells derived from HSPCs in vitro may be an alternative to blood donation when regular blood supplies are low due to numerous reasons.

The major limitation of standard blood transfusion is inadequate blood resources. According to an investigation of global blood safety and availability, the unmet need for transfusion in 2017 was 102 million blood product units. Standard blood product transfusions are also susceptible to infectious pathogens and leucocyte transfer, causing a series of refractory reactions. Immunologic incompatibility, owing to ambiguous phenotype identification, is another factor underlying the reverse reactions to standard transfusion. Therefore, innovative blood resources, such as CB-HSPC-derived functional blood cells, are needed to further improve transfusion therapies.

As a specific type of stem cell that has been indicated to produce blood cells for therapeutic use, CB-HSPCs hold some advantages in blood cell manufacturing over other types of stem cells. (a) The hematopoietic hierarchy is clear, and the factors that dominate the lineage differentiation of these stem cells are largely well defined after decades of research, thereby making lineage-specific induction easily accessible. (b) CB is relatively rich in HSPCs (approximately 1% of CD34+ cells can be obtained from CB MNCs), and the convenient and noninvasive harvesting of CB makes CB-HSPCs readily available. (c) Extensive CB banking makes seed cells with matched special blood types or human leukocyte antigens (HLAs) available. Of note, their limited proliferation ability and remaining bloodborne pathogens should be considered when CB-HSPCs are utilized as a blood cell source.

In this review, we discuss the clinical application of RBCs and platelets derived from human CB stem cells; we specifically discuss the development of criteria for promoting the translation of these cultured blood cells.

2 | ACHIEVEMENTS OF BASIC STUDIES ON THE IN VITRO DERIVATION OF RBCs AND PLATELETS FROM CB-HSPCs

2.1 | Cultured RBCs from CB-HSPCs

To generate special blood cells, stepwise induction protocols are commonly applied to imitate microenvironment alterations during development. For RBC generation, protocols have mostly been developed from studies reported by Neleidez-Nguyen et al in 2002 and include HSPC expansion, erythroid lineage commitment and full maturation and enucleation. A stepwise cytokine cocktail supplement is the key feature of the protocol, and feed cells are indispensable for the final enucleation step. However, Miharada et al utilized a feeder-free method and successfully obtained enucleated erythrocytes with the aid of human serum with the AB blood type and mifepristone in the final step. To date, complete enucleation with CB-HSPC-derived erythrocytes remains difficult without the addition of feeder cells or serum. To improve the outcome of cultured RBCs (cRBCs), poloxamer 188 (Pluronic F-68) was added to protect enucleated cells; melanocortins, such as ACTH39, ACTH24 and α-MSH, were proven to induce efficient erythroid expansion, and a roller bottle bioreactor was reported to yield up to 2.5 × 10^8 erythrocytes from one CB CD34+ cell.

2.2 | Deriving platelets from CB-HSPCs

The strategies for megakaryocyte differentiation are similar to those for RBC induction. Using a 2-step protocol, as an HSPC expansion step followed by megakaryocyte induction and maturation, Guan et al reported that one CB CD34+ cell could produce up to 1 × 10^6 megakaryocytes. The main evolution of platelet derivation in vitro is the platelet production stage. Ito et al utilized a turbulence-produced bioreactor to imitate shear stress from sinusoidal blood vessels, which constitutes the native microenvironment for megakaryocyte maturation and platelet release. Approximately 70-80 platelets per megakaryocyte can be generated by this system, which has thus far yielded the best platelet outcome in vitro. Of note, these megakaryocytes were derivatives of human induced pluripotent stem cells (iPSCs). Furthermore, optimization of the platelet induction system also includes an extracellular matrix-mimicking component-coated scaffold, such as Matrigel, composed of von Willebrand factor (VWF) and fibrinogen, which act to capture megakaryocytes and produce platelets. However, the quantity and quality of platelet production must be further improved to meet clinical requirements. The challenges of separating platelets from megakaryocytes and inducing contact platelet activation are additional factors that must be considered in the future.

2.3 | Lessons learnt from blood cell induction with pluripotent stem cells

The generation of RBCs and megakaryocytes/platelets in vitro can also be fulfilled using pluripotent stem cells (PSCs), which include embryonic stem cells (ESCs) and iPSCs. As it is lined out previously, compared with CB-HSPCs, PSCs are superior in expansion potential and gene editing feasibility. The iPSC-derivations can even avoid immune rejection since these cells are autologous origin. However, to derive blood cells from PSCs is relatively low efficacy and less mature. People are still endeavoring to figure out the best stem cell source for blood cell induction. A parallel compare of the intrinsic difference of stem cells and different induction approaches might help in generation of clinical-scale blood cells. Besides, cord blood
bank may give benefit to the establishment of iPSCs with defined genetic profiles and further hematopoietic cell deriving.15

2.4 Novel strategies for special transfusion demands

Although CB-HSPCs have substantially more amplification potential than peripheral blood (PB)-HSPCs, their products still do not meet the dramatic requirements of transfusion medicine. Numerous approaches are currently under investigation, including providing a microenvironment for CB-HSPC self-renewal and targeting defined molecules to achieve ex vivo expansion. Natural conditions for HSPC growth can be imitated by adding soluble cytokines, culturing feeder cells and using biomaterials as a matrix.16,17 Gene modification of the key regulators of stem cell self-renewal and cell fate decisions, such as DUSP16 and HOXB4, has also been utilized in the context of HSPC expansion.18 Recent progress on the optimization of HSPC expansion was based on the advantage of small molecules that function in stem cell self-renewal, such as StemRegenin 1 (SR1) and UM171.19 Researchers are also devoted to generating immortalized erythroid and megakaryocytic progenitors for continuous blood cell expansion and generation.20,21 Patients harboring minor blood group antigens or alloimmunity against HLA class I molecules are usually refractory to standard blood products. The screening, expansion and direct lineage differentiation of CB-HSPCs from CB banks might be a solution for these patients. On the other hand, with the aid of gene-editing techniques, blood group gene knockout for the generation of universal RBCs and HLA I-related gene modification for better platelet transfusion response have been achieved in induced pluripotent stem cells (iPSCs) and immortalized erythroid progenitors, and these might be applicable for CB-HSPCs.22-24

2.5 Animal models for preclinical stem cell product evaluation

The survival, functionality, and safety of cultured RBCs and platelets should be evaluated in animal models before being investigated in clinical trials. To date, immunodeficient mouse models, including chronically anemic SCID, NOD/LtSz-SCID and IL-2Rγc null (NSG) mice, are commonly used.25 However, achieving long-term survival of human RBCs and platelets in heterologous models remains difficult, especially due to rejection from macrophages. Clodronate liposomes for macrophage depletion were shown to improve the recovery of human RBCs and platelets in mouse circulation.26,27 A recent study also reported the establishment of a humanized mouse model, by knocking in five human cytokine genes into Rag2−/−Il2rg−/− mice, and replacing mouse liver with human hepatocytes by the aid of fumarylacetoacetase hydrolase (Fah) gene knock out. The duration of human RBCs circulation was improved in these MISTRGFah mice.28

3 | CLINICAL TRANSLATION OF CULTURED RBCs AND PLATELETS

3.1 Clinical trial with cRBCs

Blood transfusion is the conventional method for the treatment of thalassemia, chronic aplastic anemia and radiotherapy- or chemotherapy-derived anemia. Blood transfusion is also broadly applicable as a supportive treatment for genetic and autoimmune diseases. Compared with the high demand for RBCs, only one clinical trial on stem cell-derived RBCs has been reported to date. Considering the massive concentration of RBCs in the human body [males: (4.5-5.5) × 1012/L; females: (4.0-5.0) × 1012/L], the substantial difficulty of cultivating these cells in vitro hampers the application of cRBCs.

A pioneer study was reported in 2011 that indicated the possible therapeutic usage of cRBCs (registration number NCT0929266 at ClinicalTrials.gov).29 In this study, the researchers used HSPCs isolated from autologous PB premobilized with granulocyte colony-stimulating factor (G-CSF). RBCs were developed from HSPCs in the culture system, which included only basic medium and cytokine cocktails, and a 61 500 ± 7600-fold expansion was achieved from CD34+ HSPCs. Subsequently, 1010 cRBCs labeled with 51Cr were transfused back into the donor and detected in circulation for several weeks. The half-life of the infused cRBCs was approximately 26 days, which is very close to the reported half-life of 28 ± 2 days for native RBCs.

HSPCs isolated from mobilized PB were used as the seed cells in this study. However, the author noted that CB-HSPCs might be better for manufacturing RBCs since they can generate 5- to 10-fold more RBCs and proliferate better than PB-HSPCs, while their enucleation capacities are similar. PB-HSPC-derived RBCs may have been utilized in the first clinical trial instead of CB-HSPC-derived RBCs because of the possible rejection risk of CB derivatives during allogenic transplantation. This limitation can be overcome by using fully enucleated RBCs, from which genetic material has been eliminated for infusion, and only a regular blood-type matching assay is required under such circumstances.

3.2 Clinical trials with cultured megakaryocytes

Thrombocytopenia caused by chemotherapy or radiotherapy commonly occurs and is potentially fatal, and platelet transfusion is one of the only available treatments. Megakaryocytes are progenitors of platelets and are much easier to handle than platelets at the progenitor cell stage. Using an alteration of the platelet transfusion protocol, Xi et al explored the feasibility and safety of CB-HSPC-derived megakaryocytic progenitor cells (MPs) for the treatment of thrombocytopenia.30 In this phase I study, mononuclear cells (MNCs) were isolated from CB and incubated in cytokine-defined megakaryocytic differentiation medium. Based on surface marker expression, 8% to 77% CD41+ MPs were obtained from 14 days of culture, and the median cell number was increased by 73.5-fold. Twenty-four patients received an infusion of 5.45 × 10⁹ MPs/kg approximately 14 days
after the completion of chemotherapy, and a positive effect was preliminarily observed, especially in patients who received multiple rounds of high-dose radiotherapy and chemotherapy. The 1-year follow-up examinations showed that patients had no adverse effects after infusion and did not experience acute/chronic graft-vs-host-disease (GVHD) during the follow-up period, even without ABO blood group and HLA type matching, suggesting that infusion of CB-derived MPs is safe and feasible for the treatment of thrombocytopenia.

In this phase I study, standardized protocols were developed for cell collection, separation, large-scale preparation, transportation and infusion to make cultured MPs clinically applicable. In addition, a quality control protocol was established for cultured MPs that included the evaluation of apoptosis-related gene expression, telomerase activity, chromosome karyotypes, cell viability and tumorigenicity. Functional-related items were also evaluated by the colony forming unit-MK assay, and the MP content in the injection was determined by surface marker expression.

The MP injection was certified by the Chinese Institute of Pharmaceutical and Biological Products. The clinical trial was approved by the State Food and Drug Administration (SFDA, approval number: 2006100711), and a supplementary approval document (approval number: 2009b000528) was obtained in 2009. In accordance with the new policy requirements, follow-up phase II and III studies were conducted under a multicenter cooperation agreement and with the class III medical device license issued by the Ministry of Health of the General Logistics Department in 2011. And in 2014, this advanced-stage trial is registered under NCT02241031 at ClinicalTrials.gov.

4 | HURDLES AND SOLUTIONS REGARDING THE TRANSLATION OF CULTURED RBCs AND PLATELETS

By analyzing the progress made on the clinical translation of CB-HSPC-derived RBCs and platelets (or their progenitors), we found a gap between benchtop studies and bedside applications that is widely prevalent throughout the entire field of stem cell translational medicine. To date, nearly 8000 clinical trials on stem cells have been registered and implemented internationally, but more than 80% of these trials are still in phase I/II, and only a small portion have entered phase III/IV. The lack of standards for stem cell and stem cell derivative sources, isolation, operation, quality control, administration, in vivo tracing, safety and efficacy evaluation have created technical problems for stem cell therapies. Reliable stem cell therapies require the implementation of policies regarding the regulation of stem cell clinical trial management, approval and access. Cell product quantity and quality are technically the major problems regarding the culturing of RBCs and platelets. RBCs represent a quarter of the total body cell count, and an RBC transfusion unit contains 2.5 x 10¹² cells. Although the protocols for inducing the differentiation of RBCs from HSPCs are well established, the blood cell culture density does not exceed 10⁶/mL, and approximately 2500 L of medium are therefore needed to culture a unit of RBCs. The substantial cost and labor required to produce such a large number of RBCs make their manufacture very difficult, and technique breakthroughs are required for the final clinical translation of RBCs. In fact, the development of hollow-fiber perfusion systems, fed-batch systems or WAVE bioreactors with enhanced gas exchange, metabolic waste removal and growth factor supplementation has shed light on erythrocyte culture at a high density. Additionally, RBCs are genetically safe because they lack nuclei, and enucleation enables their flexibility during transport in capillary vessels. Complete cRBC enucleation is very valuable but difficult to achieve without feeder cells or serum. A component-defined culture system is required to avoid unknown antigenicity from the infusion. To this end, an improved culture system or purification method for enucleated RBCs is required. Alternatively, erythroid progenitors may also be applicable when critical cell quality control and blood-type matching are performed before transfusion.

Aside from innovative basic research on cRBC and platelet applications that resolves technical hurdles, the policies and regulations of stem cell therapy are lagging. Similar to other stem cell therapies, regulations of the following aspects should be established: safety (tumorigenicity, immune rejection, etc), effectiveness (stem cell source, indication selection, differentiation protocols, tracing, etc), controllability (technical standards, quality control standards, process flow, operator qualification, access management, hardware standards, third-party quality control, etc) and ethical issues (informed consent for stem cell therapy, clinical trial grouping, etc). Hopefully, the maturation of stem cell society and improvement of stem cell therapeutic policies will lead to the generation of stem cell products.

5 | SUGGESTED STANDARDS FOR THE CLINICAL APPLICATION OF cRBCs AND PLATELETS

The quality control system for RBCs and platelets generated from human CB stem cells remains in its infancy, probably due to the limited consensus, flexible in vitro manufacturing processes, inadequate characterization of products and lack of a validated quantitative control assay for these manipulated cell types before they are administered to patients. Although an approved consensus has not been reached and quality specifications or standards have not been established for the regulation of stem cell-based blood cell products, quality management protocols should be followed regarding donor screening, interprocess monitoring, and comprehensive quality evaluations of cell products at the end of the manipulation process to ensure the safety and efficacy of these manufactured cell products. In principle, the protocol for manufacturing stem cell-
derived RBCs and platelets for human use should comply with good manufacturing practice (GMP). Furthermore, as cell therapy products, RBCs and platelets generated from stem cells should be of sufficient quality to comply with the general quality control guidelines of cell and gene therapies,\textsuperscript{36,37} including those regarding cell identification, purity, safety and efficacy.

Cell identification and purity: Special attention should be paid to the flexibility and heterogeneity of stem cell-derived blood cell products. The possible contamination of irrelevant cells during the tissue culture and purification of CD34\textsuperscript{+} HSCs should be assessed after MNC separation and HSC purification, respectively. During the expansion and induction process, cell identification assays, including polyploidization and phenotyping analysis, are performed to determine the proportions of cells at different developmental stages at different induction times, which can help to optimize the manufacturing process. In the final products, in addition to the intended cell type, the proportions of unintended cell contents, such as HSCs, monocytes, granulocytes, lymphocytes, erythroid cells (for platelet products) and megakaryocytic cells, should be defined for RBC products.

Safety: It is strongly recommended that the following critical quality attributes of product safety be addressed. Microbial contaminants, including bacteria, fungi, mycoplasma, and exogenous and endogenous viruses of human and nonhuman origin, should be routinely monitored throughout the manufacturing process. CB should be screened and subjected to microbiological safety validation before being separated and purified. MNC separation and HSC purification and induction are recommended to be conducted in automatic facilities to eliminate the risk of contamination from frequent operations. Tumorigenicity should be evaluated based on tumor formation in susceptible animals in vivo and supplemented with several in vitro assays, such as tumor-related gene screening, colony formation assays and telomerase activity assays. Owing to the sustained use of recombinant cytokines and small-molecule chemicals during the stem cell-based blood cell product manufacturing process, acceptable levels of these residual cytokines and chemicals, especially additives that pose a risk to humans, should be quantitatively defined and determined in each final product lot.

Efficacy: The functional properties of stem cell-derived RBCs and platelets should be characterized sufficiently in both preclinical studies and clinical applications. In terms of RBC products, their functions posttransfusion are affected by the hemoglobin type (HbA or HbF), hemoglobin concentrations in the recipient, enucleation potential, and cell maturation level.\textsuperscript{13} To evaluate the functions of stem cell-generated megakaryocytic precursors and platelet products, the platelet-shedding potency of megakaryocytes and the activation and aggregation capacity of platelets need to be assessed in vitro. The therapeutic efficacy of stem cell-generated RBCs and platelets should be validated in relevant animal models.

In the final products of these manipulated cell formulations that are ready for human use, other quality parameters, including the cell number, cell viability, components, cytokine and supplement residues, osmotic pressure, and pH, should be monitored, and endotoxin tests should be performed.

Preclinical safety assessments should be incorporated that comprise evaluations of acute and chronic toxicity, survival/engraftment, biodistribution and ectopic differentiation, immunogenicity and immunotoxicity. If the final cell products are intended for allogeneic applications, HLA matching for platelets and blood-type matching for RBCs should be considered before clinical use. All the above quality control strategies will help to further guarantee the safety and efficacy of stem cell-based blood cell products in clinical applications.

6 \hspace{1cm} CONCLUSION

The clinical application of RBCs and platelets derived from CB hematopoietic stem progenitor cells is promising for the replacement of donated blood in the future. Two pioneering clinical trials that assessed cRBCs and cultured megakaryocytic progenitors uncovered the translation of stem cell derivatives. However, technical hurdles remain that must be overcome to ensure the optimal quality and quantity of cultured RBCs and platelets. Additional policy development with regard to stem cell therapy together with strict criteria for stem cell derivations will further advance the clinical application of stem cell products.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

X.X., H.Y., X.H.: manuscript writing, final approval of manuscript; W.Y. and X.P.: conception and design, manuscript writing, final approval of manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Roberts N, James S, Delaney M, et al. The global need and availability of blood products: a modelling study. Lancet Haematol. 2019;6(12): e606–e615.
2. Suddock JT, Crookston KP. Transfusion Reactions. Treasure Island, FL: StatPearls Publishing; 2021.
3. Mayani H, Lansdorp PM. Biology of human umbilical cord blood-derived hematopoietic stem/progenitor cells. Stem Cells. 1998;16(3): 153-165.
4. Neildez-Nguyen T, Wajcman H, Marden MC, et al. Human erythroid cells produced ex vivo at large scale differentiate into red blood cells in vivo. *Nat Biotechnol*. 2002;20(5):467-472.

5. Mihara K, Hiroyama T, Sudo K, et al. Efficient enucleation of erythroblasts differentiated in vitro from hematopoietic stem and progenitor cells. *Nat Biotechnol*. 2006;24(10):1255-1256.

6. Lee E, Sivalingham J, Lim ZR, et al. Review: in vitro generation of red blood cells for transfusion medicine: progress, prospects and challenges. *Biotechnol Adv*. 2018;36(8):2118-2128.

7. Baek EJ, Kim HS, Kim JH, et al. Stroma-free mass production of clinical-grade red blood cells (RBCs) by using poloxamer 188 as an RBC survival enhancer. *Transfusion*. 2009;49(11):2285-2295.

8. Simamura E, Arikawa T, Ikeda T, et al. Melanocortins contribute to sequential differentiation and enucleation of human erythroblasts via melanocortin receptors 1, 2 and 5. *PLoS One*. 2015;10(4):e0123232.

9. Zhang Y, Wang C, Wang L, et al. Large-scale ex vivo generation of human red blood cells from cord blood CD34+ cells. *STEM CELLS TRANSLATIONAL MEDICINE*. 2017;6(8):1698-1709.

10. Guan X, Qin M, Zhang Y, et al. Safety and efficacy of megakaryocytes induced from hematopoietic stem cells in murine and non-human primate models. *STEM CELLS TRANSLATIONAL MEDICINE*. 2017;6(3):897-909.

11. Ito Y, Nakamura S, Sugimoto N, et al. Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. *Cell*. 2018;174(3):636-648.e18.

12. Seo Y, Shin K, Kim H, et al. Current advances in red blood cell generation using stem cells from diverse sources. *Stem Cells Int*. 2019;2019:9281329.

13. Hansen M, von Lindern M, van den Akker E, et al. Human-induced pluripotent stem cell-derived blood products: state of the art and future directions. *FEBS Lett*. 2019;593(23):3288-3303.

14. Esposito MT. Blood factory: which stem cells? *BMJ*. 2018;18:10-10.

15. Rao M, Ahrlund-Richter L, Kaufman DS. Concise review: cord blood banking, transplantation and induced pluripotent stem cell: success and opportunities. *STEM CELLS*. 2012;30(1):55-60.

16. Ferreira MS, Jahnen-Dechent W, Labude N, et al. Cord blood-hematopoietic stem cell expansion in 3D fibrin scaffolds with stromal support. *Biomaterials*. 2012;33(29):6987-6997.

17. Pino S, Frenette PS. Haematopoietic stem cell activity and interactions with the niche. *Nat Rev Mol Cell Biol*. 2019;20(5):303-320.

18. Wang X, Brommeyer HE. DUSP16 is a regulator of human hematopoietic stem and progenitor cells and promotes their expansion ex vivo. *Leukemia*. 2020;35(5):1516-1520.

19. Huang X, Guo B, Capitano M, et al. Past, present, and future efforts to enhance the efficacy of cord blood hematopoietic cell transplantation. *F1000Res*. 2019;8:1833.

20. Kurita R, Suda N, Sudo K, et al. Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells. *PLoS One*. 2013;8(3):e59890.

21. Nakamura S, Takayama N, Hirata S, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell*. 2014;14(4):535-548.

22. Kim YH, Kim HO, Baek EJ, et al. Rh D blood group conversion using transcription activator-like effector nucleases. *Nat Commun*. 2015;6:7451.

23. Park YJ, Jeon SH, Kim HK, et al. Human induced pluripotent stem cell line banking for the production of rare blood type erythrocytes. *J Transl Med*. 2020;18(1):236.

24. Norbnap P, Ingrungruanglert P, Israsena N, et al. Generation and characterization of HLA-universal platelets derived from induced pluripotent stem cells. *Sci Rep*. 2020;10(1):8472.

25. Shah SN, Gelderman MP, Lewis EM, et al. Evaluation of stem cell-derived red blood cells as a transfusion product using a novel animal model. *PLoS One*. 2016;11(12):e0166657.

26. Hu Z, Yang YG. Full reconstitution of human platelets in humanized mice after macrophage depletion. *Blood*. 2012;120(8):1713-1716.

27. Hu Z, Van Rooijen N, Yang YG. Macrophages prevent human red blood cell reconstitution in immunodeficient mice. *Blood*. 2011;118(22):5938-5946.

28. Song Y, Shan L, Gbryl R, et al. Combined liver-cytokine humanization comes to the rescue of circulating human red blood cells. *Science*. 2021;371(6533):1019-1025.

29. Giarratana MC, Rouard H, Dumont A, et al. Proof of principle for transduction of in vitro-generated red blood cells. *Blood*. 2011;118(19):5071-5079.

30. Xi J, Zhu H, Liu D, et al. Infusion of megakaryocytic progenitor products generated from cord blood hematopoietic stem/progenitor cells: results of the phase 1 study. *PLoS One*. 2013;8(2):e54941.

31. Biancon E, Piovesan A, Facchin F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol*. 2013;40(6):463-471.

32. Zeuner A, Martelli F, Vaglio S, et al. Concise review: stem cell-derived erythrocytes as upcoming players in blood transfusion. *STEM CELLS*. 2012;30(8):1587-1596.

33. Housler GJ, Miki T, Schmelzer E, et al. Compartmental hollow fiber capillary membrane-based bioreactor technology for in vitro studies on red blood cell lineage direction of hematopoietic stem cells. *Tissue Eng Part C Methods*. 2012;18(2):133-142.

34. Csaszar E, Kirouac DC, Yu M, et al. Rapid expansion of human hematopoietic stem cells by automated control of inhibitory feedback signaling. *Cell Stem Cell*. 2012;10(2):218-229.

35. Chen L, Yue w, Xie XY, et al. The role of poloxamer 188 for cord blood mononuclear cells into megakaryocytes cultivation and induction in three-dimensional WAVE Bioreactor. *Zhonghua Xue Ye Xue Za Zhi*. 2018;39(1):28-31.

36. F.D.A. Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products. MD: U.S. Department of Health and Human Services; 2013 [November].

37. C.P. Commission. Chinese Pharmacopoeia. Part III. Beijing: China Medical Science Press; 2020.

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