Trends and targets of various types of stem cell derived transfusable RBC substitution therapy: Obstacles that need to be converted to opportunity

Francesxco Lanza [a], Jerard Seghatchian [b],*

[a] Romagna Transplant Network, Hematology Unit, Ravenna Hospital & University of Ferrara-Italy, Via Randi 5, Ravenna, Italy
[b] International Consultancy in Strategic Safety/Quality Innovations of Blood-Derived Bioproducts and Quality Audit/ Inspection, London, England, UK

ARTICLE INFO

ABSTRACT

A shortage of blood during the pandemic outbreak of COVID-19 is a typical example in which the maintenance of a safe and adequate blood supply becomes difficult and highly demanding. So far, human RBCs have been produced in vitro using diverse sources: hematopoietic stem cells (SCs), embryonic SCs and induced pluripotent SCs. The existing, even safest core of conventional cellular bioproducts destined for transfusion have some shortcoming in respects to: donor –dependency variability in terms of hematological /immunological and process/ storage period issues. SCs–derived transfusable RBC bioproducts, as one blood group type for all, were highly complex to work out. Moreover, the strategies for their successful production are often dependent upon the right selection of starting source materials and the composition and the stability of the right expansion media and the strict compliance to GMP regulatory processes. In this mini-review we highlight some model studies, which showed that the efficiency and the functionality of RBCs that could be produced by the various types of SCs, in relation to the in-vitro culture procedures are such that they may, potentially, be used at an industrial level. However, all cultured products do not have an unlimited life due to the critical metabolic pathways or the metabolites produced. New bioreactors are needed to remove these shortcomings and the development of a new mouse model is required. Modern clinical trials based on the employment of regenerative medicine approaches in combination with novel large-scale bioengineering tools, could overcome the current obstacles in artificial RBC substitution, possibly allowing an efficient RBC industrial production.

1. The choice of starting source materials and the contributing role of non-hematopoietic cell types

The main source materials for the production of RBCs in vitro are:

a) Haematopoietic stem cells (HSCs) obtained from the bone marrow (BM) [[1,2],[1,2]]

b) Umbilical cord blood (UCB)-derived SC [2,3]

c) Peripheral blood (PB)-derived SC (either steady state or mobilized) (3)

d) Pluripotent stem cells (PSCs) (3)

e) Embryonic stem cells (ESCs) (3)

f) Induced pluripotent stem cells (iPSCs) [3,4].

These types of SCs can, differentiate into RBCs via culture protocols consisting of three main stages: commitment, expansion and maturation [[1–5],[1–5]].The RBCs produced should be evaluated in vivo for survival, functionality, and safety [6]. The reported studies, so far, have been performed in mouse models. To ensure survival of the cultured RBCs, the animal should be a cross-species permissive host. This is achieved by inducing a state of immunodeficiency. The most common and novel animal models used are the chronically anemic SCID, the NOD/LtSz-SCID and the IL-2Rγc null (NSG) mouse a strain of a NOD/SCID derived line that is among the most immunodeficient animal models described to date. These are used for evaluating whether the ex-vivo generated RBCs are able to recapitulate the conditions of clinical transfusion settings. For example, an anemic mouse is most appropriate to study the oxygen delivery of cultured RBCs, whereas a deeply immunodeficient mouse is better suited for studies that question the survival of transplanted cells [7]. Clearly, human trials are far more complicated, and to date, there is only one report of testing manufactured RBCs on a single human recipient [8–13].

1.1. Ex- vivo generation of RBCs from HSCs

One of the most well-characterized somatic SC in adult humans is the

---

* Corresponding author.
E-mail addresses: francesco.lanza@auslromagna.it (F. Lanza), jsegnatchian@btopenworld.com (J. Seghatchian).

F. Lanza and J. Seghatchian

HSC, which resides in the bone marrow [5]. Somatic SCs are present in most tissues of the adult body and they control the continuous production of differentiated cell types in highly regenerative tissues. The self-renewal and maturation characteristic of SCs is controlled by their surrounding microenvironment, which is composed of various cell types including MSCs as well as stromal cells which contribute to the formation of the HSC niche [14]. From an immunophenotypic point of view, HSCs express the CD34 antigen. CD34+ cells can also be isolated in smaller amounts from peripheral blood (PB), and cord blood (CB) [5]. The ability of those cells to promote ex-vivo erythropoiesis has been studied by many research teams. The protocols include addition of specific combinations of growth factors to the cell culture medium in a sequential fashion [9,10,15].The role played by MSCs and other cell types from the BM microenvironment has been investigated in several reports [14].

Initially, Neildez-Nguyen et al. reported the generation of RBCs from HSPCs isolated from CB. The culture method involved the cytokines Flt3 ligand, thrombopoietin, stem cell factor, erythropoietin, and insulin like growth factor I, added at three sequential culturing steps. The production of erythroid precursors was characterized by low enucleation efficiency (4 %) and expression of foetal haemoglobin (HbF). The cells could further differentiate into enucleated RBCs in vivo by injection into NOD/SCID mice [16]. Three years later, Giarratana et al. used a similar three-step culture protocol that included feeder stromal cells of murine origin (MS-5 cell line) in an attempt to recapitulate the BM milieu. Under these conditions, either CB or PB HSPCs produced enucleated RBCs at high rates (up to 100 %), which survived normally post infusion in NOD/SCID mice. The amplification was tenfold higher for CB HSCPs (10^6) compared to PB HSPCs (10^5). However, this promising method is not clinically applicable due to the enormous demand in culture area (approximately 150 km^2) and the consequent lack of cost-effectiveness [8,15]. In 2011 the same group reported the proof of principle for transfusion of ex- vivo manufactured RBCs starting from PB CD34+HSPCs in a human. Their survival in- vivo was comparable to that of native RBCs (half-life of 26 days versus 28 $\pm$ 2 days for the native RBCs). Despite the small number of RBCs produced by this method (equivalent to 2 mL of packed RBCs), the study remains a major step forward since it was the first case of a clinical grade cultured product that was tested successfully in human [8]. Satisfactory RBCs expansion from CB HSPCs has also been shown following co-culture with BM- or CB-derived MSCs. The enucleation levels were approximately 64 %. Despite the fact that the feeder cell co-culture system provided high expansion and enucleation rates in cultured cells, it has many drawbacks such as difficulties in isolating pure, non-contaminated RBCs, the presence of xenogeneic cell types (when cells of murine origin are used), variability in CD34+ expansion and issues of allogenicity when human MSCs are used. Undoubtedly, the adaptation of feeder-free protocols could reduce both cost and the complexity involved. Towards that end, many protocols have been developed and optimized, with a production level of up to 2 × 108 RBC in bioreactors. Moreover, high-yield results were recapitulated by using an FBS-containing culture medium in a roller bottle culture system. By similar robust expansion processes, it could be feasible to produce over 500 units of RBCs starting from one CB unit (5 × 10^6 CD34+cells). Finally, in an attempt to develop a feeder layer- and FBS-free culture process, Poloxamer 188 (a polymer with cytoprotective function against hydrodynamic stress) has been applied, which showed a high enucleation rate (95 %) and enhanced survival of mature RBCs [17]. What has become clear from those studies was that CD34+from different sources exhibit different erythropoietic potential. Interestingly, coculture with stromal cells proved to stimulate the proliferation of HSCs, as well as to improve the efficiency of RBC maturation.

The utilization of circulating HSCs from the PB is challenging, since it is easier and less labor intensive than BM. HSC can be collected either in a steady state condition or after G-CSF priming [10–12]. Researchers have developed an in-vitro RBCs production protocol from PB and proved the therapeutic potential of generated RBCs both in the preclinical and clinical settings [18].

However, although PB CD34+would be an ideal source for manufacturing autologous RBCs (especially in cases with alloimmunization or rare blood groups) the low number of circulating HSPCs (without GSCF mobilization) and their lower expansion potential pose limitations for routine clinical adaptation.

1.2. Ex- vivo generated RBCs from ESCs

Embryonic stem cells (ESCs) are derived from the inner cell mass of blastocysts, namely, of mammalian embryos at an early developmental stage [3]. ESCs, when kept in culture under certain conditions, have the potential of indefinite self-renewal without differentiation. However, when allowed to differentiate back into a host blastocyst, they maintain the ability to generate cells of all three germ layers. ESCs from human sources (hESCs) are generated after in-vitro fertilization; the major disadvantage of using these ESCs is the ethical issues that arise from their origin, and hence, limitations of integrating them into clinical applications. HESCs have the advantage of being able to divide approximately 300 times while retaining their normal karyotype, pluripotency and full-length telomeres [3]. Differentiation of ESCs begins when factors that specifically contribute to the maintenance of the SC state, are withdrawn. Three basic methods are used to trigger differentiation: (i) formation of three-dimensional colonies, the embryoid bodies (EBs), (ii) co-culturing with stromal feeder cells and (iii) culturing on a layer of extracellular matrix proteins [3].

The ability of hESCs to differentiate into haematopoietic cells and thus, to the erythroid cell line, was studied with protocols involving EB formations and a co-culture system [9–12]. The stromal cells used in most experiments were derived from non-human cell lines. The EBs-based systems resulted in erythropoiesis that morphologically resembled the stage of definitive erythropoiesis, except for Hb that was of embryonic or foetal stage. Initially, the authors managed to isolate haemangioblasts, namely bipotential precursors of haemopoietic and endothelial cells, which could differentiate into multiple haemopoietic lineages including erythroid. Two limitations were observed, namely the lack of enucleation and the expression of foetal Hb. An improved protocol by the same group led to enucleation of 65 % of cells and expression of adult Hb after prolonged culture in a proportion of cells (15 %). By applying this protocol, however, only 10^{10}-10^{11} 108 RBC were developed, compared to the unit of packed RBCs that contains approximately 2 × 10^{12}cells [8,9].

Definite erythropoiesis (enucleation along with adult Hb expression) and robust RBC expansion remain an issue when ESCs have been used as the primary source of cells. According to many studies, culture time lengthening or forcing the expression of transcription factors involved in erythropoiesis, e.g. RUNX1a or HOXB4, would enhance the expression of beta-globin genes [19]. Finally, a more efficient, serum- and feeders-free potential system for differentiation has also been validated.

1.3. Ex- vivo generated RBCs from iPSCs

Generation of induced pluripotent stem cells (iPSCs) was based on the methodology developed by Yamanaka's laboratory in 2006; iPSCs were produced by reprogramming somatic cells back to inner cell mass-like cells through forced experimental expression of a set of four genes (Oct3/4, Sox2, c-Myc and Klf4) [4]. Human iPSCs may derive from any somatic cell and behave similarly to hESCs; they are capable of self-renewal, large-scale expansion and differentiation into all cell types of the three germ layers in vitro. The major advantage of iPSCs over ESCs, however, is that they can be produced from any cell type thus, allowing selection of the donor's phenotype without posing the ethical dilemmas related to using ESCs. On the other side, the main disadvantage of iPSCs is that in efficient reprogramming may lead to a mix of fully and partially reprogrammed cells and thus, to a far from perfect

technology to pluripotency. Several iPSC generation protocols that involved strategies for avoiding viral transfer of genes have been developed, the most recent and encouraging of which refer to entering key genes into the cell via episomal carriers, synthetic RNA transcripts and through recombinant proteins. Protocols used for ex -vivo expansion are similar to those of ESCs and include formation of EBs or use of co-cultures with feeder cells. Establishment of iPSCs from dermal fibroblasts of a Bombay blood type individual was first reported in 2009, by applying the Yamanaka's technique [3]. The pluripotent SC had the characteristics of hESCs: they could differentiate into all three haemopoietic cell lineages and expressed HbF. In 2010 Lapillonne et al. designed, for the first time, a two-step cell culture protocol for the direct commitment of foetal and adult fibroblast-derived human iPSCs to definitive erythropoiesis. Despite success in reprogramming, the amplification and enucleation rates differed significantly compared to those involving ESCs (10 % vs 66 %). Moreover, Hb synthesis was blocked at the stage of HbF, independently of the origin of the reprogrammed cells [20]. As shown later, iPSCs can achieve terminal maturation in terms of enucleation in-vitro, but complete maturation, in terms of both enucleation and the Hb switch happens only in-vivo, that is, in an adult haematopoietic microenvironment, following injection and maturation of progenitors into NOD/SCID mice in situ. Thus, the iPSCs can walk all the erythroid way ahead toward full maturation but not under the current in vitro differentiation context. Mature RBCs were kept in circulation for four days and the total cell number generated from 1×10^6 iPSCs was $15-28,3 \times 10^8$ RBCs. To improve enucleation rate, inhibition of specific miRNAs (i.e. miR30 A) was proposed. Recent advances in the scale-up production include: a) development of a good manufacturing practice (GMP)-compatible, feeder-free and serum-free method that takes advantage of small molecule effectors to specifically promote erythroid differentiation of hPSCs (with a potential to generate 50.000–200.000 erythroid cells from one HSPC) and, b) application of expanded cultures of iPSCs and ESCs in spinner flasks. ESCs seem to be superior to iPSCs in terms of expansion and enucleation rate, but still, both sources are inferior to the potential of CB and PB CD34$^+$cells, posing thus limitations for scale up production necessary for clinical applications.

1.4. Immortalized cell lines

Robust and reproducible erythroid precursor immortalization techniques may finally provide efficient numbers of viable and functional RBCs for clinical use in-vitro. The first demonstration of the feasibility of using immortalized human erythroid progenitor cell lines as an ex -vivo source for producing RBCs came in 2013, by using the HPV16-E6/E7 oncogene and forced expression of the transcription factor TaL-1 that is essential for early haemopoiesis. Nakamura's laboratory developed cell lines able to produce enucleated RBCs (though at a low efficiency) with functional Hb after differentiation in vitro [21]. An immortalized erythrocyte progenitor cell line was also developed by the transduction of c-MYC and BCL-XL into multipotent HSPC-derived from pluripotent SCs. Differentiation was achieved by turning off the overexpression of those factors. The cells expressed HbF and showed high rates of enucleation following injection into NOD/SCID mice.

In 2017, Trakarnsanga et al. generated the first human immortalized adult erythroid line (BEL-A) by introducing the HPV16 E6/E7 oncogenes into BM CD34$^+$cells [22]. BEL-A RBCs had biochemical and structural features of normal erythropoiesis and developmental potential to functional, enucleated reticulocytes that survived in vivo expressing mainly HbA. In 2020, the first proof of principle for the feasibility of scaling up erythroblast expansion in controlled bioreactors by using the ImEry cell line was reported [23]. These cells were derived from immortalizing CD71$^+$ CD235a$^+$ erythroblasts isolated from adult PB. The generated RBCs seemed to share common metabolic and functional characteristics with those of adult RBCs [23].

2. The obstacles that need to be overcome

The erythroid cells that are intended for use in clinical applications must be produced at a large scale and at a terminal differentiation state. It is well established that the culture conditions affect the proliferation potential of the cultured progenitor erythroid cells as well as their enucleation capability. Conditions such as the kind and the concentration of growth factors or the timing of their administration in the three-step culture system mentioned above, seem to have a significant impact on the efficiency of enucleation; these conditions need to be optimized and further defined.

Deep knowledge of peptides and molecules that play an integral role in definite erythropoiesis and their supply exogenously (or counterpart imitators, such as the erythroid macrophage peptide or VCAM-1) could induce enucleation through ligand-receptor interactions. Additionally, for an optimization of the differentiation process and stable enucleation in vitro, mifepristone (an antagonist of glycocorticoid action), or factors involved in vesicle trafficking have been applied in the culture systems. In the same context, histone acetyltransferases or histone deacetylases involved in chromatin remodeling, and caspases involved in apoptosis could also serve as possible targets for the in vitro modification of the enucleation process. However, chromatin remodeling factors are non-specific, affect many genomic regions and might be able to either downregulate genes which are vital to cell functions or activate silenced oncogenes. The long-term consequences of using such agents require further assessment.

Beyond culture conditions, usage of feeder cells has the potential to enhance maturation and optimize cell population expansion, but the presence of foreign material sets essential restrictions on clinical applications since (i) the cost is higher due to the huge number of stromal cells required, (ii) the technique is more complicated, and (iii) the stromal cell lines carry the risk of contamination by xenogeneic pathogens [7,8,12]. In addition, the common static culture conditions have failed to reach the required expansion of ex vivo generated RBCs. A major step forward has been materialized with bioreactors that allow three-dimension cell growth and consequent increments in production yields [24]. It seems that the best way to increase the production of RBCs would be the generation of committed cell lines derived from the ideal stem cell source, that is, a cell population able to provide unlimited proliferation. Genetic manipulation of these cell lines is unlikely to be an obstacle to their clinical application, provided that the cells are enucleated. This can be ensured by filtration or by irradiation. Moreover, in order to maintain genetic and epigenetic stability of the cell lines, periodic controls should be performed on their genotype [21].

2.1. Functional issues

During the ex vivo production of RBCs, additional controls are required for testing the multiple physical and biochemical factors that characterize the final product. The manufactured RBCs mostly resemble the native RBCs that are generated under stress conditions, since they are slightly macrocytic and express greater amounts of HbF, a fact that clearly affects the oxygen dissociation curve. However, transfusing HbF-containing RBCs may not be contraindicated taking into account the fact that subjects with hereditary persistence of foetal Hb (HbFH) are asymptomatic and moreover, an increase in HbF expression is the aim of targeted therapies in patients with haemoglobinopathies, such as sickle cell disease. In contrast, the metabolic pathways and the proteome of the produced RBCs show a high degree of similarity to the native RBCs, indicating that the manufactured cells have a closer affinity with their native counterparts at those functional levels. Nevertheless, differences have been reported depending on the stem cell sources used. Another consideration is the high heterogeneity of the expansion and enucleation potentials, as well as of the Hb type that is expressed. Indeed, both hESC- and iPSC-derived cells have low enucleation capability and express mainly embryonic and foetal globin genes. Additionally, the reported

yields from these sources are lower in comparison to those reported from HPSCs.

2.2. Scaling issues

The use of bioreactor systems has enabled scale up culture of HSCs and erythroid cells. The clinical application of RBCs generated ex-vivo requires mass production (10^{12} cells/unit) of a safe and functional product at an acceptable cost. The calculated cost ranges from 8000–15,000 USD if hPSCs serve as a source [25]. Use of low-cost media and reagents, replacement of culture media with small molecules, omission of the feeder cells and generation of erythropoietic cell lines, may result in a more cost-effective product. Safety issues, such as accidental transmission of harmful agents and immunogenic reactions, may be overcome if the protocols used for the production of ex vivo RBCs comply with Good Manufacturing Practice (GMP) [11,12,15].

2.3. Clinical applications

Manufactured RBCs from selected donors are currently applied as reagent RBCs for antibody identification in alloimmunized patients, and as a vehicle for drug delivery. The design of clinical trials for testing transfusion of ex-vivo generated RBCs should be careful since there are still several inconsistencies in the procedures and the protocols. In vitro generated RBCs should be compatible with ABO and Rhesus antigens (a sum of eight antigens), suggesting that eight different erythroid cell lines would be sufficient to supply the transfusion demands of the majority of the world's population [22]. It is anticipated that ten clones of human iPSCs representing the most common RBC phenotype combinations could meet the need for transfusion of 99.43 % of alloimmunized patients [20]. Alternatively, RBCs can be produced by using healthy O Rh negative or Bombay blood type donors or by applying innovative techniques which omitting blood group antigens (enzymatic cleavage or antigen masking), thus providing universal RBCs for all recipients [15]. It is a fact that this innovative medical product has stimulated scientific interest, and as research continues in this direction, it is anticipated that RBCs production ex vivo will definitely become a significant tool in the field of transfusion therapy in the near future. The first clinical application of in vitro generated RBCs would be in rare blood groups and chronic transfusion dependent patients.

3. Concluding remarks

Major advances in haematopoiesis in general and of erythropoiesis in particular, and the continual evolution in the scientific and technological tools, have made the in-vitro generation of RBCs for transfusion feasible in the near future. However, further improvements are still necessary in order to achieve this goal in full as a proof-of-principle, ex vivo production of RBCs with the functional characteristics of native RBCs has been achieved. It is a matter of cost-efficient scaling to allow this technology to reach clinical applications, and in this leap forward, industrial development will have a major role for successful production and therapeutic substitution of transfusable SC- derived RBCs.

Clearly, clinical trials are far more complicated and to date there is only one report of testing manufactured RBCs on a single human recipient. With regard to future perspectives, we believe that the functionality/ safety aspects of cultured products still need a step by step, in process validation of several factors, i.e. the influence of filtration, pathogen reduction treatment, closed system centrifugation, and storability of the final product. It is conceivable that modern quality tools such as molecular (OMICS) may provide further insights, possibly contributing to implementing the short- and long-term safety of artificial cultured transfu sable cells. It is our hope that artificial intelligence combined with human intelligence, may have major impact in big data analysis [26]. Finally, we believe that modern clinical trials based on

novel regenerative medicine approaches in combination with innovative large-scale bioengineering tools as a part of an industrial production could overcome the current obstacles to artificial RBCs substitution.

References

[1] Lanza F, Seghatchian J. New horizon on stem cell cryopreservation through the artificial eyes of CD34+, using modern flow cytometry tools. Transfus Apher Sci 2020. https://doi.org/10.1016/j.transci.2020.102785.

[2] Lanza F, Mangianti S, Accorsi P, Lombardini L, Martino M, Saccardi R, et al. Manipulation, and cryopreservation of autologous peripheral blood stem cell products in Italy: a survey by GITMO, SIDEM and GIIMA societies. Transfus Apher Sci 2020. https://doi.org/10.1016/j.transci.2020.102753.

[3] Bonsi L, Alviano F. Stem cells. Book, Edited by Società Editrice Esculapio s.r.l.. 2020.

[4] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76.

[5] Lanza F, Healy L, Sutherland R. Structural and functional features of the CD34 antigen: an update. J Biol Regul Homeost Agents 2001;15:1–13.

[6] Tzounakas VL, Valsami SI, Kriebardis AG, Papassideri IS, Seghatchian J, Antonelou MH. Red cell transfusion in paediatric patients with thalassaemia and sickle cell disease: current status, challenges and perspectives. Transfus Apher Sci 2018;57:347–57.

[7] Shah SN, Gelderman MP, Lewis EM, Farrel J, Wood F, Strader MB, et al. Evaluation of stem cell-derived red blood cells as a transfusion product using a novel animal model. PLoS One 2016;11:e0166657.

[8] Giarratana MC, Rouard H, Dumont A, Kiger L, Safeukui I, Le Pennec PY, et al. Proof of principle for transfusion of in vitro-generated red blood cells. Blood 2011;118:5071–9.

[9] Migliaccio AR, Whitsett C, Migliaccio G. Erythroid cells in vitro: from developmental biology to blood transfusion products. Curr Opin Hematol 2009;16:259–68.

[10] Seo Y, Shin KH, Kim HH, Kim HHS. Current advances in red blood cell generation using stem cells from diverse sources. Stem Cells Int 2019. https://doi.org/10.1155/2019/9281329.

[11] Douay L. Why industrial production of red blood cells from stem cells is essential for tomorrow's blood transfusion. Regen Med 2018;13:627–32.

[12] Rousseau GF, Giarratana MC, Douay L. Large-scale production of red blood cells from stem cells: what are the technical challenges ahead? Biotechnol J 2014;9:28–38.

[13] Bessos H, Fraser R, Seghatchian J, Scotblood A. Tackling local and global issues in transfusion medicine - donor recruitment, effective use of blood, stem cell plasticity, and vCJD. Transfus Apher Sci 2007;38(2008):77–83.

[14] Campioni D, Rizzo R, Stignani M, Melchiorri L, Ferrari L, Moretti S, et al. A decreased positivity for CD90 on human mesenchymal stromal cells (MSCs) is associated with a loss of immunosuppressive activity by MSCs. Cytometry B Clin Cytom 2009;76B:225–30.

[15] Migliaccio AR, Masselli E, Varricchio L, Whitsett C. Ex-vivo expansion of red blood cells: how real for transfusion in humans? Blood Rev 2012;26:81–95.

[16] Neildez-Nguyen TM, Wajcman H, Marden MC, Moncollin V, Giarratana MC, et al. Human erythroid cells produced ex vivo at large scale differentiate into red blood cells in vivo. Nat Biotechnol 2002;20:467–72.

[17] Zhang Y, Wang C, Wang L, Shen B, Guan X, Tian J, et al. Large-scale ex vivo generation of human red blood cells from cord blood CD34(+) cells. Stem Cells Transl Med 2017;6:1698–709.

[18] Jin H, Kim HOS, Kim S, Kim HO. Erythropoietic potential of CD34+ hematopoietic stem cells from human cord blood and G-CSF-mobilized peripheral blood. Biomed Res Int 2014;2014:435215.

[19] Nazareth EJP, Rahman N, Yin T, Zandstra PW. A multi-lineage screen reveals mTORC1 inhibition enhances human pluripotent stem cell mesendoderm and blood progenitor production. Stem Cell Rep. 2016;6:679–91.

[20] Lapillonne H, Kobari L, Mazurier C, Tropel P, Giarratana MC, Zanella-Cleon I, et al. Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine. Haematol 2010;95:1651–9.

[21] Kurita R, Suda N, Sudo K, Miharada K, Hiroyama T, Miyoshi H, et al. Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells. PLoS One 2013. 8e59890.

[22] Trakarnsanga K, Griffiths RE, Wilson MC, Blair A, Satchwell TJ, Meinders M, et al. An immortalized adult human erythroid line facilitates sustainable and scalable generation of functional red cells. Nat Commun 2017;8:14750.

[23] Lee E, Lim ZR, Chen HY, Yang BX, Lam AT, Chen AK, et al. Defined serum-free medium for bioreactor culture of an immortalized human erythroblast cell line. Biotechnol J 2018;13. e1700567.

[24] Zhang Y, Wang C, Wang L, Shen B, Guan X, Tian J, et al. Large-scale ex vivo generation of human red blood cells from cord blood CD34(+) cells. Stem Cells Transl Med 2017;6:1698–709.

[25] Timmins NE, Athanasas S, Gunther M, Buntine P, Nielsen LK. Ultra-high-yield manufacture of red blood cells from hematopoietic stem cells. Tissue Eng Part C Methods 2011;17:1131–7.

[26] Sniecinski I, Seghatchian J. Artificial Intelligence: A Joint Narrative on Potential Use in Pediatric Stem and Immune Cell Therapies and Regenerative Medicine. Transfus Apher Sci 2018;57(3). https://doi.org/10.1016/j.transci.2018.05.004. May.