Received: 9 June 2020 | Accepted: 8 July 2020
DOI: 10.1002/sctm.20-0247

CONCISE REVIEW

STEM CELLS TRANSLATIONAL MEDICINE

Hematopoietic stem cells from pluripotent stem cells: Clinical potential, challenges, and future perspectives

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Abstract

The generation of hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) is an active and promising area of research; however, generating engraftable HSCs remains a major obstacle. Ex vivo HSC derivation from renewable sources such as iPSCs offers an experimental tool for studying developmental hematopoiesis, disease modeling, and drug discovery, and yields tremendous therapeutic potential for malignant and nonmalignant hematological disorders. Although initial attempts mostly recapitulated yolk sac primitive/definitive hematopoiesis with inability to engraft, recent advances suggest the feasibility of engraftable HSC derivation from iPSCs utilizing ectopic transcription factor expression. Strategic development for de novo HSC generation includes further investigations of HSC ontogeny, and elucidation of critical signaling pathways, epigenetic modulations, HSC and iPSC microenvironment, and cell-cell interactions that contribute to stem cell biology and function.

KEYWORDS
embryo, hematopoiesis, hematopoietic differentiation, hemogenic endothelium, induced pluripotent stem cells, reprogramming

1 | INTRODUCTION

Hematopoietic stem cells (HSCs) are immature multipotent adult stem cells that give rise to all mature blood cell lineages, demonstrate long-term engraftment, and are able to hierarchically reconstitute the entire hematopoietic system in a conditioned recipient after infusion. Allogeneic HSC transplantation (AHSCT) with immunocompatible donors has been successfully used for more than 60 years for the treatment of various malignant and nonmalignant diseases. However, challenges including host-dependent variability of response to therapy, scarce availability of human leukocyte antigen (HLA)-matched donors, limited ex vivo proliferation and expansion of engraftable HSCs, and transplant-related morbidity and mortality restrict broad use of this approach.[1-3] The paucity of HLA-matched donors, especially for ethnic minorities who are underrepresented in the donor registries worldwide, are a major hurdle for curative options for patients with malignant and nonmalignant hematologic disorders.[4,5] In sickle cell disease (SCD), for instance, HLA-matched sibling AHSCT demonstrates high overall survival and event-free survival[6]; however, greater than 85% of the patients lack an HLA-matched sibling donor and alternative HSC sources such as unrelated, cord blood, or mismatched AHSCT are more variable in their success.[7] AHSCT is limited by donor choice, graft rejection, and graft-vs-host disease (GVHD); therefore, autologous HSCT after genetic engineering

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and correction of the pathologic genotype is an attractive option for patients that would theoretically be available to all.

Striking advances in gene therapy and genome editing tools, particularly lentiviral-based gene therapies and CRISPR/Cas9 editing, hold tremendous power to cure hematological diseases. Chimeric antigen receptor-T cell therapies are emerging as powerful treatments in hematologic malignancies, whereas autologous HSCT after gene correction is an ideal curative strategy in nonmalignant hemoglobinopathies where allogenic graft-vs-tumor effect does not apply. Initial results from ongoing gene therapy trials in hemoglobinopathies are encouraging[3,8]; however, a lack of long-term follow-up data from gene addition studies, the generally low efficiency of genome editing tools, and cost concerns raise safety, applicability, and practicality concerns that must be addressed before there is widespread clinical use. Simultaneous research into alternative strategies is, therefore, important and necessary to continue in pursuit of disease-modifying and curative strategies in blood disorders.

Ex vivo culture of embryonic stem cells (ESCs) that can produce all cell types in the adult body was established 40 years ago[9] and has provided an important understanding of developmental biology. Differentiated cells can be reprogrammed to an embryonic-like state with the groundbreaking discovery in 2006 of inducing pluripotent stem cells (PSCs) from mouse stromal cells after introducing four transcription factors (*OCT4*, *SOX2*, *KLF4*, and *c-Myc*) using integrating viral vectors under ES cell culture conditions.[10] This discovery brought the possibility of using this modality to produce patient-specific production of adult stem/mature cells or HSCs in the context of SCD that can be produced and corrected, for an efficient autologous stem cell transplantation modality. This system was quickly adapted for human cells,[11] and various starting cells and manufacturing techniques have been introduced (reviewed in Reference 12). Although distinct gene expression signature is a reality for induced PSCs (iPSCs) (reviewed in Reference 13), and there is donor age- and epigenetic-signature-dependent variability in the iPSCs (reviewed in References 14, 15, respectively), iPSC models serve as a powerful tool to unravel the progression of malignant diseases, screen potential drug candidates for particular diseases, and have significant potential therapeutic applications in multiple hematologic and nonhematologic disorders.[16]

Considering the remarkable therapeutic value of HSCs for regenerative medicine applications and their limited availability, significant experimental efforts have been devoted to generating true HSCs from PSCs. The simultaneous research into genome editing techniques offers a clinically relevant, therapeutic bridge when combined with iPSC generated HSCs, particularly in nonmalignant hematologic disorders where HLA-matched sibling donors are lacking, GVHD is detrimental, and the generation of definitive HSCs from patient-derived iPSCs has the potential to be curative when combined with genetic correction. While there remains significant potential, attempts to demonstrate engraftable HSC generation from PSCs have been largely unsuccessful ex vivo without teratoma formation or exogenous transcription factor expression (Table 1). Here, we review the dynamic regulations of developmental hematopoiesis as required for the establishment of bona fide HSC derivation protocols from PSCs, and discuss the unmet scientific needs and challenges required to overcome in order to translate this technology into successful therapeutic application in human diseases.

Significance statement

Patient-specific hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) offer possibility for the treatment of hematological diseases, particularly when no other options are available. This review highlights the current advances in HSC derivation from PSCs and discusses the obstacles that need to be overcome for future autologous PSC-derived HSC transplantation as a therapeutic modality. The feasibility of HSC generation from transcription factor engineered PSCs has been demonstrated in laboratory conditions and is suggestive of clinically relevant application.

2 | DEVELOPMENTAL HEMATOPOIESIS: WAVES OF PRIMITIVE AND DEFINITIVE HEMATOPOIESIS ARE NECESSARY FOR LIFELONG FUNCTIONAL HEMATOPOIESIS

Our understanding of mammalian hematopoiesis has primarily been derived from small animal models given ethical concerns surrounding research with human fetal tissue. Our current understanding of developmental hematopoiesis involves tightly regulated sequential events occurring in multiple, partially overlapping but spatiotemporally separated waves (Figure 1). The first wave, which appears in the yolk sac, lacks lymphoid potential but produces transitory primitive blood cells (erythrocytes, megakaryocytes, and macrophages) to provide the growth needs and first innate defense mechanism for the embryo.[28-30] The second wave also emerges in the yolk sac and generates lympho-myeloid progenitors (LMPs) and erythro-myeloid progenitors (EMPs) which subsequently migrate to the fetal liver as a distinct source of hematopoietic progenitors that precede HSC detection.[31,32] Although yolk-sac progenitor cells cannot reconstitute hematopoiesis as HSCs do, they remain functional after birth and most likely throughout the lifespan as a common origin for tissue macrophages.[33] The first true HSCs are generated by the definitive third wave of hematopoiesis. This complex process involves generation of the aorta-gonad-mesonephros (AGM) region, a potent hematopoietic site within the mammalian embryo body, and the first place from which HSCs emerge. HSCs first appear in the dorsal aorta of AGM, with high frequencies of HSCs emerging from the vitelline and umbilical arteries suggesting a close relationship between the developing hematopoietic and major arterial vascular system of the vertebrate embryo.[34,35] After the specification of the HSCs, they localize the fetal liver via circulation where they propagate before they seed bone marrow that is capable of lifelong support of HSC self-renewal.[36]

TABLE 1 Selected reports for engraftable hematopoietic stem and progenitor cells (HSPCs) derived from PSCs

Cellular source	Approach	Engraftment	Reference
HoxB4 engineered mouse yolk sac embryonic cells	Monolayer culture on OP9 cells	Myeloid biased and low lymphocyte engraftment (primary and secondary recipients, 10-20 wk)	17
Human ESCs or iPSCs	Subcutaneous transplantation of PSCs with or without OP9 feeder cells and cytokines to derive HSPCs from PSCs induced teratomas	B and T cells and myeloid engraftment (primary and secondary recipients, 4-12 wk)	18,19
Human ESCs	Monolayer culture on stromal cells derived from mouse aorta-gonad-mesonephros (AGM) region	Myeloid and lymphoid engraftment (primary and secondary recipients, 8-12 wk)	20
Human ESCs- and monkey iPSCs-derived CD34+ cells	Embryoid body formation followed by sorting of CD34+ cells cultured on *E4ORF1* engineered primary endothelial cells from human umbilical cords	Myeloid, lymphoid, and erythroid engraftment (primary and secondary recipients, 12-16 wk)	21
HOXA9, *ERG*, *RORA*, *SOX4* and *MYB* engineered CD34+CD45+ from human iPSCs	Embryoid Body formation followed by sorting of CD34+CD45+ cells to transduce with the transcription factors	Erythroid and myeloid engraftment (primary recipient, 4-5 wk)	22
ERG, *HOXA5*, *HOXA9*, *HOXA10*, *LCOR*, *RUNX1* and *SPI1* engineered hemogenic endothelium cells derived from human ESCs and iPSCs	Embryoid body formation followed by sorting of hemogenic endothelium cell to transduce with the transcription factors	B and T cells and myeloid (primary and secondary recipient, 12-16 wk)	23
MLL-AF4 engineered HSPCs derived from human iPSCs	Monolayer differentiation followed by a collection of HSPCs to transduce with the transcription factor	B and T cells and myeloid (primary and secondary recipient, 8 wk)	24
Inducible *Runx1* and *Hoxa9* engineered mouse iPSCs and ESCs	Embryoid body formation followed by sorting of inducible hemogenic endothelium cells to culture on OP9-DL1 cells	T cells (primary and secondary recipients, 4-6 wk)	25
Mouse PSCs	Embryoid body formation followed by hematopoietic specification in 3D hydrogel	Myeloid and B cells (primary recipient, 3 wk)	26
Gfi1b, *c-Fos*, and *Gata2* Mouse iPSCs cells	Subcutaneous transplantation of engineered iPSCs to derive HSPCs from iPSCs induced teratomas	B and T cells and myeloid engraftment (primary and secondary recipients, 16 wk)	27

The terminology of "primitive" and "definitive" embryonic blood cells does not refer to engraftment potential, rather is dependent on the characteristics of the cell. The first primitive embryonic erythroblasts, detected at embryonic day 7.25 to 8.5 (E7.25-E8.5) in the mouse embryo[37] and at 3 to 4 weeks in the human embryo,[38] mostly express embryonic globins, are nucleated, and larger compared to their definitive counterparts. In the mouse, these nucleated cells continue to divide and enter the embryo proper after the onset of cardiac contractions at E8.25.[39] Circulating primitive erythrocytes lose the ability to proliferate and eventually enucleate, remaining in the neonatal mouse circulation several days after birth.[40] Similarly, primitive megakaryocytes are detected in the yolk sac of the mouse embryo at E7.25 and are smaller in size compared to adult megakaryocytes with limited capacity to endoreplicate (reviewed in Reference 41). Compared to adult platelets, fetal platelets are relatively larger, have small alpha granules, respond to collagen inefficiently, and display low coagulation potential.[29,42] Primitive macrophages, first detected in the yolk sac after E9.5,[29] mature rapidly without peroxidase activity as otherwise observed in adult canonical monocyte-derived macrophages from the bone marrow.[43]

Although it is not known that whether definitive hematopoiesis emerges as a continuation of primitive hematopoiesis, increasing pluripotency in later hematopoietic waves is a hallmark of the developing embryo. The transient second wave in the yolk sac produces definitive LMPs that can differentiate into B and T cells but lack engraftment potential,[31] and EMPs that colonize the fetal liver as progenitors of erythrocytes, macrophages, megakaryocytes and neutrophils.[32] These early fetal liver erythroblasts express the definitive erythroid-specific transcriptional modifiers *c-myb*, *Sox6*, and *Bcl11a*, and produce erythrocytes that enucleate similar to adult RBCs; however, express γ-globin with low levels of ε-globin in contrast to adult RBCs expressing β-globin and negligible levels of γ-globin.[44] EMP-derived megakaryocytes display relatively lower ploidy and generate fewer platelets compared to the HSC-derived megakaryocytes.[45] Intriguingly, EMP-derived macrophages become tissue-resident macrophages that continue to remain in the adult body with a self-renewal capacity similar to ESCs.[46]

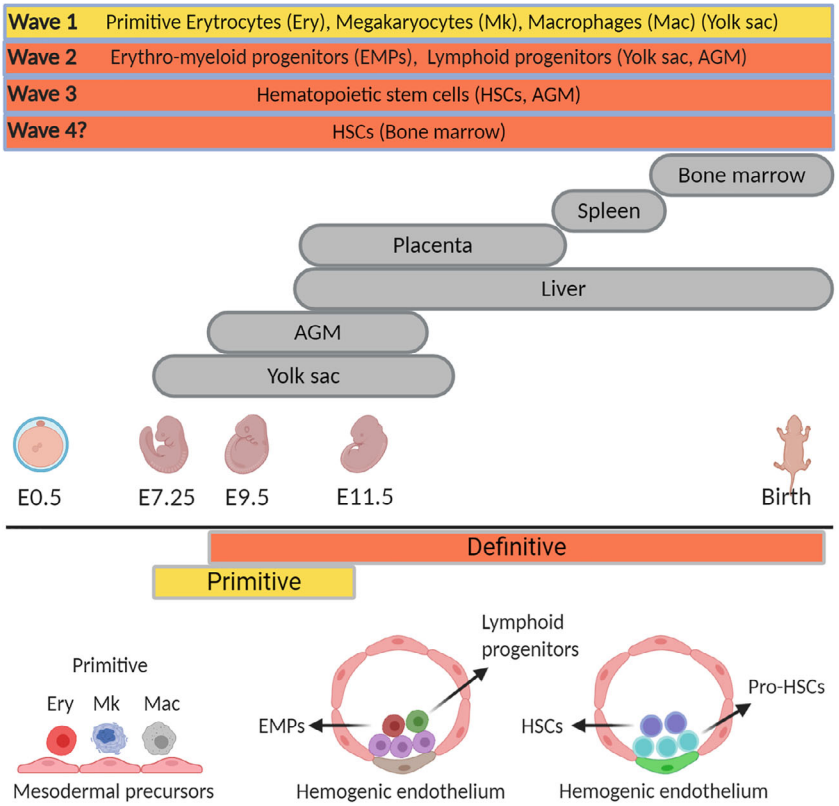

FIGURE 1 Schematic representations of mouse embryonic hematopoietic development. AGM, aorta-gonad-mesonephros

Definitive HSCs with extreme proliferation capacity give rise to all blood cell lineages and reconstitute the blood system of the adult body, and are detected at around E11 in the AGM region, and thereafter in the placenta and extraembryonic tissues.[34,47,48] Shortly after emerging in the vascular region of the embryo, HSCs migrate to the fetal liver where they produce blood lineages necessary to support the growing embryo, then initiate migration to the bone marrow shortly before birth to supply life-long hematopoiesis.[49] This derivation of true HSCs capable of supporting lifelong hematopoiesis begins with strict dependence on the niche environment given stromal cell support from AGM allows for the maintenance, expansion, and multilineage potential of murine and human HSCs.[50,51] Specifically for definitive HSC-derived RBCs, two major properties are required; efficient enucleation and activation of developmentally regulated machinery, referred to as globin switching, to switch the globin profile from fetal to adult globin after birth.[52]

Recently, a previously unappreciated transient fourth wave of definitive HSC generation has been reported arising from hemogenic endothelium (HE) cells in the bone marrow in chickens and mice in the late fetus and young adult stage,[53] indicating HSCs can be generated de novo after developmental stages. It is well established that HSCs are generated from a transient subset of specialized endothelial cells termed hemogenic, present in the yolk sac, placenta, and aorta, through an endothelial-to-hematopoietic transition (EHT). HSC generation via EHT was thought to be restricted to the early stages of development; however, the discovery of bone marrow HE revealed that HSCs can be generated past embryonic stages. This transient wave might support the neonatal bone marrow environment until fetal liver amplified HSCs take up their adult-type niches.

Definitive hematopoietic cells are derived through a process that does not involve cell division but instead results from a strong bending and rounding up of a small specialized form of endothelial cells with hemogenic potential (HE cells).[54] The process by which HE cells emerge from the aortic ventral wall into the subaortic space is called EHT. Many transcription factors (such as *Runx1*, *Sox17*, *Scl*, *Gfi1*/*Gfi1b*, and *Gata2*) and signaling factors (including Hedgehog, Bpm4, Notch ligands, and Wnt) are associated with either HE specification or EHT.[55] The *Gata2* transcription factor is essential for HSC generation and function; therefore, recent index-sorting of *Gata2*-expressing intra-aortic hematopoietic cluster cells combined with single-cell RNA-seq revealed a unique transcriptome for definitive HSCs with specific expression levels of *CD31*, *Gata2*, *cKit*, and *CD27* from endothelium within the aortic clusters.[56] Further, *Runx1* increases during HE specification,[53] while a *Yes-activated protein*-mediated mechanical signaling by way of a Rho-GTPase-mediated mechanism was shown to result in the commitment of HE to HSCs.[57] Here, although hemodynamic forces seem critical for in vivo HSC formation, the exact molecular mechanism is not known and adaptation of biomechanical forces to ex vivo differentiation protocols for successful de novo HSC generation is lacking. A recent study demonstrated primitive vascular endothelial cells (at E8) follow a two-step fate choice to become HSC-primed HE cells; specification into arterial phenotype followed by a subsequent hemogenic conversion.[58] This transition from pre-HE to HE stage was further demonstrated through trajectory analyses and genetic perturbation experiments.[59] Here, two distinct subpopulations from intra-arterial clusters, namely precursors of HSCs and CD45+ lympho-myeloid-biased progenitors, were reported, although it is not

known whether these subpopulations differentiate from an equivalent or distinct population within the HE.

Understanding the complexity of developmental hematopoiesis and the connection among the hematopoietic waves aides in the establishment of ex vivo differentiation protocols, with definitive HSC production dependent on cell-cell interactions, close regulation of growth factors, and identifying the various biomechanical interactions within the AGM. Further clarification of these complex interactions is required for the establishment of more sophisticated ex vivo protocols to derive HSCs from PSCs. For any therapeutic potential, however, definitive HSCs must have long-term self-renewal and engraftment capabilities.

3 | HSC GENERATION FROM PSCs: TRANSCRIPTION FACTORS SPECIFY THE GENERATION OF MULTIPOTENT PROGENITORS AND ENGRAFTABLE CELLS

Other than understanding and modeling developmental hematopoiesis, the long-sought goal in the field of HSC generation from PSCs is to develop well-established protocols that generate bona fide HSCs with long-term engraftment potential. Early attempts could only recapitulate primitive first wave and inefficient definitive second wave hematopoiesis, similar to yolk sac hematopoiesis.[60-62] Since then, investigations using fetal stromal cells as feeder cells for PSCs, fine-tuning growth factor supplementation, and developing tightly orchestrated differentiation protocols have allowed the generation of mostly phenotypically definitive PSC-derived HSCs with multipotent differentiation potential but without engraftment ability. In general, RBC globin expression is a useful general assessment tool to define primitive/definitive characteristics of any HSC-like cells from PSCs given differences in globin expression profiles. Alternatively, T lymphocyte potential has been shown to track the onset of definitive hematopoiesis in human pluripotent cultures.[63,64]

Initially, RBCs derived from PSCs were reported to enucleate inefficiently and express mostly ε-globin/γ-globin with no or negligible levels of adult β-globin.[65,66] Insights into developmental hematopoiesis and understanding of the regulatory mechanisms of globin switching allowed the derivation of relatively higher levels of β-globin expressing RBCs from PSCs; however, engraftment has again not been demonstrated.[67-70] Although the improvement in β-globin expression assumes successful activation of the definitive third wave-like HSC generation from PSCs, single-cell analysis in iPSC-RBCs revealed that one cell can express all globin types to a certain degree.[71] This single-cell analysis reflects a possibility of recapitulating engraftment deficient definitive yolk sac hematopoiesis for most cases rather than creation of a heterogeneous population with cells from distinct waves.

EMP-RBCs and AGM-RBCs in the embryo are phenotypically indistinguishable as both predominantly express γ-globin; therefore, engraftment studies are the main hallmark for true HSC assessment. A plethora of the published protocols only generated HSC-like cells with inefficient self-renewal capacity without long-term engraftment potential in conditioned immunodeficient mouse models, indicating a lack of definitive HSC generation. Although it is not the focus of this review, terminally differentiated blood cells from iPSCs could be used for transfusion purposes (although this topic is also debated), including generation of myeloid cells, RBCs (given normal oxygen transport for both fetal and adult hemoglobins), and megakaryocytes (for a deeper review, see Reference 72). Such therapeutic application would have implications to address issues surrounding resources and shortages, and would eliminate alloimmunization and transfusion-transmitted infectious diseases from transfusion.

A long-standing question has been whether inadequate engraftable HSC generation from PSCs is a technical or biological inefficiency. Ectopic expression of *HoxB4* in mouse primitive yolk sac embryonic cells combined with culture on bone marrow stromal cells (OP9 cells) provided modest engraftment and multilineage differentiation in primary and secondary irradiated recipients with mostly myeloid bias and low lymphocyte (particularly T cells) reconstitution.[17] A follow-up study demonstrated tetracycline-inducible *Cdx4* and *HoxB4* engineered mouse ESC engraftment (with again low T-cell chimerism), possibly due to activation of the *Hox* pathway. However, the results could not be confirmed for human ESCs.[73] On the other hand, subcutaneously transplanted human iPSCs into immunocompromised mice could provide long-term repopulating intra-teratoma HSC derivation, indicating the theoretical possibility of true HSC generation from PSCs under proper conditions without the need for ectopic gene expression.[18,19] Here, iPSC-derived HSCs migrated from teratomas into the bone marrow, and resulted in multilineage and long-term reconstitution of the hematolymphopoietic system in serial transfers. Ultimately, true engraftable HSCs from PSCs without teratoma formation would be required for any translatable clinical application.

Significant effort has been devoted to transcription factor screening for ex vivo hematopoiesis from PSCs on the basis of the finding that specific transcription factor expression (*Hlf*, *Runx1t1*, *Pbx1*, *Lmo2*, *Zfp37*, *Prdm5*, *Meis1*, and *Mycn*) in pro-B cells and common myeloid progenitors were sufficient to derive serially transplantable HSCs with multilineage differentiation potential,[74] and that lineage-restricted CD34+CD45+ myeloid precursors derived from iPSCs modified with five transcription factors (*HOXA9*, *ERG*, *RORA*, *SOX4*, and *MYB*) conferred short-term engraftment with myeloid and erythroid lineages.[22] Such transcription factor screening studies provide proof-of-principle data for in vivo modeling using donor-derived iPSCs for genetic blood diseases. In one such example, hematopoietic progenitors from Diamond-Blackfan anemia (DBA) patient-derived iPSCs engineered with aforementioned five transcription factors engrafted immunocompromised mice with impaired erythropoiesis[75]; however, screening of small molecules identified autophagy inducer SMER28 as a potential therapeutic candidate for DBA that rescued impaired erythroid differentiation in chimeric mice. Additional transcription factor screening eventually resulted in the derivation of long-term and serially transplantable HSCs from human iPSCs. In a separate study modeling hematopoietic disease in humanized mice for therapeutic strategies in genetic blood disorders, a larger cohort of transcription factors (*ERG*,

HOXA5, *HOXA9*, *HOXA10*, *LCOR*, *RUNX1*, and *SPI1*) was sufficient to convert HE cells into HSCs that engrafted myeloid, B and T cells in primary and secondary mouse recipients (33 out of 76 mice engrafted, 9 multilineage reconstitution).[23] Here, characterization of differentiated cells revealed RBC enucleation potential and a hemoglobin profile similar to human umbilical cord blood, antigen-specific responses in lymphocytes, and T-cell receptor rearrangement, indicating recapitulation of developmental hematopoiesis for definitive HSC generation in ex vivo conditions. One of the rare human embryo studies recently reported that fetal liver HSPCs are specifically associated with HOX family transcription factors such as *HOXA6* and *HOXA10*, confirming the importance of the HOX pathway for bona fide HSCs.[76] A recent report showed transient expression of a single transcription factor, *MLL-AF4*, was sufficient to specify engraftable HSCs from PSCs; however, engrafted cells displayed a tendency for leukemic transformation during the long-term follow-up.[24] Here, the authors also tried to replicate five transcription factor engineering that was shown to result in myeloid and erythroid biased short-term engraftment in mice,[22] and evaluate their potentially synergistic activity with *MLL-AF4* expression; however, their results revealed successful engraftment in only *MLL-AF4* engineered groups. These results indicate that complex transcription factor engineering approaches might be limited in their potential clinical use due to being hard to adapt in standard laboratory conditions, being time-consuming and costly, and requires significant expertise.

Ex vivo HSC derivation from PSCs offers an experimental tool for studying developmental hematopoiesis, disease modeling, and drug discovery, but engraftable HSCs are currently only possible with ectopic transcription factor expression or teratoma formation, thereby limiting clinical applications.

4 | CHALLENGES AND OUTLOOK

iPSC-derived cellular therapy has tremendous therapeutic potential for a wide breadth of disorders, ranging from regenerative needs to correction of hematologic disorders. Various diseases have been modeled in ex vivo conditions, now with the discovery of genome editing tools such as CRISPR/Cas9 contributing to the possibility of genetic correction of underlying disease mutations in patient-derived iPSCs.

To date, ex vivo true HSC generation without genetic intervention remains theoretic and can only be achieved by ectopic expression of at least one transcription factor. *MLL-AF4* engineering has already been shown for possible leukemia transformation of long-term engrafted iPSC-HSCs,[24] and dysregulation of *HOX* genes is strongly associated with acute myeloid leukemia and acute lymphoid leukemia.[77] Therefore, to generate therapeutic-grade HSCs from iPSCs, safety evaluation is required from both small and large animal models particularly if genetic manipulations are involved in order to avoid secondary mutations and possible carcinogenesis. For regenerative approaches, contamination of undifferentiated PSC during HSC generation causing teratoma formation is of concern. In addition to safety profiles, efficient purification/selection strategies are necessary to eliminate this possibility. To this end, it has been demonstrated that two drug-inducible safeguards, which have separate functionalities, can eliminate undifferentiated PSCs to prevent teratoma formation and deplete all PSC-derived cells in vivo if adverse events arise.[78]

Although most of the current protocols can derive blood cells with a definitive phenotype, immunocompromised mice models are the only available tools to differentiate definitive yolk sac and AGM HSCs, and determine the functionality of HSC-like cells derived from PSC. Mouse models are a costly and time-consuming investment, and yet the success of these methods in larger animal models is eventually required for meaningful therapeutic translation. Establishment of ex vivo protocols with a proper HSC functionality evaluation would, therefore, enhance the speed of methodology screening, potentially reduce cost, and shorten the overall time for success in the animal model. To do such an evaluation, defining discrimination markers for human cells at different developmental stages is required. Currently, known cell surface markers to distinguish primitive/definitive hematopoiesis are similar; therefore, identifying globin profiles in RBCs and lymphoid potential of HSC-like cells from PSCs are the generally applied methods. To improve this, for instance, a recent report demonstrated CD44 as a novel cell surface marker for HSC-primed HE cells in the AGM region.[79] Besides, hepatic leukemia factor (*Hlf*) expression was recently reported as a discriminating factor for mouse liver HSCs from EMPs or hematopoietic clusters in the yolk sac (E9).[80] Although these findings are waiting to be confirmed for human, HSC-specific reporter ES/iPS cell lines (ie, *RUNX1c* reporter lines[81]) would be a versatile tool for ex vivo differentiation method optimizations. Devotion to the full characterization of progenitor blood cells, HSCs, and progenitors of HSCs would contribute to our understanding of engraftable HSCs while greatly reducing the need for animal engraftment studies in all protocol designs.

The microenvironment and interactions between various cell types play a critical role in true HSC derivation from PSCs given biological sufficiency of PSCs without a need for genetic manipulation shown in teratoma formation.[18,19] Currently, transcription factor engineered PSCs can compensate for the absence of microenvironmental signals; therefore, a detailed description of the crucial activated signaling pathways in fetal hematopoiesis would realize ex vivo bona fide HSC generation without genetic engineering where critical growth factors or small molecules could be provided in fine-tuned differentiation protocols. Another critical consideration is the HSC niche in patients. Although the bone marrow microenvironment is difficult to recapitulate ex vivo, the establishment of nontoxic conditioning regimens that maintain critical function for homing and engraftment of iPSC-HSCs to the bone marrow niche is important, for a less potent and supportive bone marrow environment of hematology patients (ie, SCD patients[82]).

iPSC production is labor-intensive; therefore, cost and maintenance of the generation and differentiation of patient-specific iPSCs is a major concern, particularly for the developing countries. The high cost and labor necessary for clinical-grade iPSC production requiring significant expertise would hamper the wide-use of iPSC-HSCs.

To translate iPSC-derived HSCs into clinical applications, cell products must be made under Good Manufacturing Practice (GMP)-grade practices which further increases the cost. One possible approach to leverage the success of iPSC-based regenerative therapies would be the generation of "universal" iPSCs by genetic inactivation of HLAs and overexpression of specific cell surface markers such as CD47 to mask them from immune cells.[83] A possible disadvantage of HLAs inactivation would be missing self-response to natural killer cell (NK) activity. Knock-in HLA-E genes at the *Beta-2 Microglobulin* locus in human iPSCs without surface expression of HLA-A, B, or C were resistant to NK cell-dependent lysis.[84] Another solution includes banking of particular iPSCs that serve the majority of the population. For instance, 12 iPSC lines retaining HLA-C combined with HLA-class II knockout are immune compatible and estimated to match >90% of the world's population,[85] proposing a drastic reduction in the costs and variability for PSC-HSC therapy. However, HLA-C expressing cells can still be detected by T-cells, and suppression of other immune cells should also be considered for the long-term survival of engrafted cells. In this manner, HLA-A/-B/-C and CIITA molecules were simultaneously deleted in a recent study along with knock-in of immune regulatory factors PD-L1, HLA-G, and CD47 to the safe harbor *AAVS1* locus to evade immune surveillance by T cells, NK-cells, and macrophages, respectively.[86] Although the upfront cost may ultimately be high for such therapies, the ability to potentially cure diseases that otherwise has high lifetime economic and quality-of-life costs may ultimately be advantageous in the long run.[87]

5 | CONCLUSION

Establishment of ex vivo protocols for iPSC derived HSCs contributes to the understanding of developmental hematopoiesis, is a methodology for high-throughput drug screening and gene therapy development, and offers a promising potential therapeutic option for malignant and nonmalignant heritable blood disorders. Although the biological capacity of PSCs to generate HSCs have been presented through teratoma formation, ex vivo differentiation protocols without ectopic transcription factor expression have not been established and require further investigation. Further research must approximate all of the properties of true HSC generation, including but not limited by critical gene regulation, epigenetic modulations, microenvironment interactions, and signaling pathways in order to become a realistic, applicable, and ideally cost-effective therapeutic option.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

S.D.: conception/design, manuscript writing, and final approval of the manuscript; A.L., J.F.T.: manuscript writing, final approval of the 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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How to cite this article: Demirci S, Leonard A, Tisdale JF. Hematopoietic stem cells from pluripotent stem cells: Clinical potential, challenges, and future perspectives. *STEM CELLS Transl Med*. 2020;9:1549–1557. https://doi.org/10.1002/sctm.20-0247