De Novo Generation of HSCs from Somatic and Pluripotent Stem Cell Sources

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Abstract
Generating human hematopoietic stem cells (HSCs) from autologous tissues, when coupled to genome editing technologies, is a promising approach for cellular transplantation therapy, and for in vitro disease modeling, drug discovery and toxicology studies. Human pluripotent stem cells (hPSCs) represent a potentially inexhaustible supply of autologous tissue, yet to date directed differentiation from hPSCs has yielded hematopoietic cells that lack robust and sustained multilineage potential. Cellular reprogramming technologies represent an alternative platform for the de novo generation of HSCs via direct conversion from heterologous cell types. In this review, we discuss the latest advancements in HSC generation by directed differentiation from hPSCs or direct conversion from somatic cells, and highlight their applications in research and prospects for therapy.

Introduction
Bone marrow transplantation (BMT) is the most established cellular replacement therapy, dating back to 1951 when Lorenz et al. first described protection from the lethal effects of X-irradiation by bone marrow injection in mice and guinea pigs. Thomas and colleagues later infused patients receiving radiation and chemotherapy with bone marrow from fetal and adult cadavers. BMT remains the only curative treatment for patients suffering from a variety of hematological disorders including sickle cell anemia, leukemia, lymphoma, and in at least one case, HIV infection. The functional unit of a bone marrow transplant is the hematopoietic stem cell (HSC), which resides at the apex of a complex cellular hierarchy and replenishes blood development throughout life. Primary bone marrow, umbilical cord blood or mobilized peripheral blood are the only sources of HSCs presently available. The scarcity of HLA-matched HSCs severely limits the ability to carry out transplantation, disease modeling and drug screening. HSC expansion represents one potential source of additional transplantable units. Considerable progress has been made in defining molecular determinants that can expand HSCs in culture. However, even the most robust current protocols achieve only a modest expansion of long-term repopulating (LT)-HSCs, and the expanded stem cells often have reduced multilineage and migratory potential compared to fresh HSCs. Furthermore, for a wide range of conditions, such as bone marrow failure syndromes, too few functional HSCs are available for autologous expansion of gene correction strategies. Thus, in parallel with the efforts to expand HSCs, many studies have aimed to generate HSCs from alternative sources. This review will consider the latest advancements in the efforts to generate HSCs either by directed differentiation from pluripotent stem cells or direct conversion from somatic cell types.

Directed differentiation of hematopoietic cells from pluripotent stem cells
During mammalian embryogenesis, blood development occurs in at least two waves. Primitive
Hematopoietic differentiation from pluripotent stem cells

Many directed differentiation protocols from pluripotent stem cells have been established but these protocols invariably produce short-lived progenitors without bona fide HSC functionality (Table 1). Chadwick and colleagues showed that hematopoietic growth factors and bone morphogenetic protein-4 (BMP-4) – a ventral mesoderm inducer, promoted hematopoietic development in the context of EBs. The isolated CD45+ hematopoietic progenitors were capable of normal lineage maturation when plated into colony-forming assays. Other groups have developed co-culture methods with bone marrow stromal cells, such as OP9, and reported the emergence of CD34+ cells. These protocols demonstrate that hematopoietic cells with clonogenic capacity can be generated from human pluripotent stem cells (hPSCs), though these hematopoietic progenitors lack robust lymphoid differentiation and engraftment potential. An in-depth kinetic analysis of blood differentiation from ESCs by Keller and colleagues revealed that blood development in EBs largely recapitulated primitive hematopoiesis. Like yolk sac progenitors, EB-derived hematopoietic cells lacked robust and durable repopulating capability in lethally irradiated adult recipients, hindering their therapeutic potential. This deficiency is believed to reflect an immature developmental program. In support of this belief, when yolk sac progenitors are transplanted into neonates, or cultured on stroma derived from the blood-forming aorta-gonad-mesonephros (AGM) region, they engraft in the adult. Although these data suggested that primitive embryonic blood progenitors can acquire definitive potential, it is unclear whether the embryonic program can only be matured with proper microenvironmental cues or if the definitive program can be induced with cell-intrinsic regulators.
While the molecular mechanisms that distinguish primitive yolk sac and definitive hematopoiesis remain largely unknown, investigation of the homeobox (Hox) genes in primitive and definitive hematopoietic populations has revealed that several of the Hox A and B clusters are highly expressed in definitive, but not yolk sac, cells. Building on these studies, Kyba and colleagues demonstrated remarkably that ectopic expression of HoxB4 endowed both yolk sac and mouse ESC-derived hematopoietic cells with multilineage engraftment potential. However, the donor grafts exhibited marked myeloid skewing, indicating that overexpression of HoxB4 alone is insufficient to fully convert primitive ESC-derived progenitors into bona fide HSCs. Nonetheless, these seminal experiments with HoxB4 set the precedent for the induction of HSCs using defined transcriptional regulators of ontogeny. However, induction of HOXB4 in hPSCs did not generate engraftable cells, suggesting species-specific differences. Molecular analysis of human ESC (hESC)-derived hematopoietic cells compared to somatic HSCs revealed that these cells express high levels of HOX B genes but only low level of the HOX A cluster. Ramos-Mejía et al. demonstrated that overexpression of HOXA9 enhanced hematopoietic commitment from hESCs but was not sufficient to confer engraftment. Taken together, these data suggest that HOX A genes may be more important in augmenting definitive hematopoietic potential in human cells than HOX B genes.

**Derivation of hemogenic endothelium from human pluripotent stem cells**

The marked deficiency of stem cell properties in hPSC-derived progenitors has prompted the need to develop refined hematopoietic differentiation protocols. Achieving this goal requires a detailed understanding of the cellular and molecular pathways that lead to blood formation – specifically, the pathways that distinguish primitive and definitive hematopoiesis. During embryogenesis, endothelial and hematopoietic cells develop in parallel, and the close developmental association has led to the hypothesis that they arise from a common precursor – the hemangioblast. In the embryo, hemangioblast development is followed by a transient wave of primitive hematopoiesis that gives rise to primitive erythroid, macrophage and megakaryocyte lineages, which is then replaced by definitive hematopoiesis bearing true HSCs with lymphoid potential. During hESC differentiation, Wang et al. used cell surface markers associated with endothelial cells capable of early hematopoietic potential in the human embryo to identify a subpopulation of endothelial-like cells that possess hemangioblastic properties. Until recently, it was unclear whether a parallel definitive program existed in pluripotent stem cell differentiation cultures.

It is now widely accepted that definitive HSCs arise from hemogenic endothelium (HE) rather than a hemangioblast. During mammalian embryogenesis, definitive HSCs originate in the vascular microenvironment of the AGM and arterial endothelium in other anatomical sites. Emergent HSCs have been observed to bud directly from HE cells lining the dorsal aorta. Accordingly, hPSC differentiation protocols must include stepwise addition of morphogens to specify a definitive HE. Choi and colleagues identified a VE-cadherin^+^CD73^−^CD235a/CD43^−^ HE population distinct from hemangioblasts that were capable of forming endothelium and primitive blood cells. In an attempt to define the signaling pathways that distinguish primitive and definitive hematopoietic development from hPSC differentiation cultures, Kennedy et al. defined a CD34^+^CD43^−^ HE population that gave rise to of T lymphoid, myeloid and erythroid lineages. Primitive hematopoiesis was found to be temporally dependent on Activin/Nodal signaling, a pathway previously used to predict hPSC lines prone to hematopoietic specification. The definitive wave, marked by T lymphopoiesis, however, was not dependent on this signaling...
pathway. The same group sought to separate primitive and definitive progenitors during early hPSC differentiation, particularly during the stage of Activin/Nodal dependence. Sturgeon et al. found that T cell potential was restricted to the KDR⁺CD235α⁻ mesoderm-derived HE population. Furthermore, inhibition of Wnt-β-catenin signaling during the mesoderm specification stage negatively affected the definitive potential of KDR⁺CD235⁺ HE, whereas stimulation with a Wnt agonist restored T lymphoid potential. Collectively, these studies established a system where primitive hematopoiesis is specified by the combination of Activin/Nodal signaling and Wnt inhibition, whereas activation of Wnt-β-catenin and Activin/Nodal inhibition promotes definitive hematopoiesis from hPSC. However, a major limitation of these studies is that T cell potential alone may not accurately define definitive potential. Böiers et al. recently detected restricted lymphomyeloid progenitors that emerged before definitive HSCs during murine development. Since HSC-independent lymphopoiesis can arise in the developing embryo, these results imply that T lymphoid potential might not unequivocally indicate conditions permissive to the emergence of HSCs. True HSCs are defined by multilineage potential and engraftment capability, therefore, a more accurate in vitro read-out would encompass multi-lymphoid (B and T cell) potential. Nonetheless, these experiments have provided valuable insights into the signaling pathways that control the emergence of primitive or definitive populations. Although no verifiable engraftment potential has yet been reported from hPSC-derived HE, these protocols have provided a unique opportunity to better understand the complexities of hematopoietic ontogeny.

HSC generation from teratomas in vivo
Two recent reports suggest that HSCs with long-term, multilineage repopulating potential can be derived from hPSCs under experimental conditions. Amabile et al. reported that direct injection of human iPSCs (hiPSCs) into NOD-scid IL2rγnull (NSG) mice produced human CD45⁺ cells capable of mobilization and engraftment via teratoma formation. The flow-sorted CD34⁺CD45⁺ teratoma-derived cells were capable of multilineage engraftment and could engraft secondary recipients though at very limited levels. In a similar report, Suzuki and colleagues co-injected either mouse- or human-derived iPSCs with OP9 and subcutaneously implanted a micro-osmotic pump to supply hematopoietic cytokines in vivo. They reported iPSC-derived HSCs migrate from teratomas to the mouse bone marrow. Transplantation of murine teratoma-derived cells into irradiated recipients resulted in long-term multilineage reconstitution in serial transfers. Isolation of teratoma-derived human CD34⁺CD45dull cells showed multilineage differentiation in colony assays. Extensive molecular analysis of these teratoma-derived cells was not performed, so it remains unclear how they compare to primary HSCs. Tumor formation from injecting pluripotent stem cells is an obvious limitation of this differentiation system. Moreover, generation of HSCs by way of teratoma formation is inherently stochastic and variable. Still, these studies suggest that human hematopoietic cells with long-term multilineage repopulating activity can in principle be derived from hPSCs. The in vitro system is devoid of supportive cues from the physiological microenvironment, but without precise knowledge of the cell intrinsic and extrinsic factors that contribute to the efficient production of HSCs, it remains a significant challenge to generate HSCs from hPSCs for any therapeutic application.

Direct conversion: reprogramming somatic cells into hematopoietic lineages
The concept of direct conversion, or directly switching cellular fate without reverting to a pluripotent state, was first demonstrated with myocytes. Taylor and Jones provided the initial
evidence that somatic cells exhibit cell fate plasticity by treating fibroblasts with 5-azacytidine, an inhibitor of DNA methylation, and observing spontaneous differentiation into muscle and fat cells. This finding suggested that DNA methylation restricted expression of genes that regulate differentiation into alternative lineages. Davis, Weintraub and Lassar later showed that the transcription factor MyoD was sufficient to convert fibroblasts into contracting myocytes. This seminal finding demonstrated that trans-acting factors have the powerful capacity to regulate the epigenome and reprogram cell identity. In the decades that followed, many direct conversions between somatic cells have been reported, including many therapeutically relevant cell types such as induced motor neurons, pancreatic islet cells, β-cells and cardiomyocytes.

Direct conversions between hematopoietic lineages
Direct conversions between hematopoietic cell types have also been described, owing to the fact that lineage-specific transcription factors are relatively well-defined in hematopoietic lineages. GATA1, a critical erythroid transcription factor, directs GMPs and lymphoid progenitors to erythroid and megakaryocytic cell fates. B and T lymphocytes can be reprogrammed to macrophages through overexpression of C/EBPα. GATA3, an essential transcription factor for early T cell development, can drive T lineage precursors to acquire mast cell identity. These studies demonstrate that cells of the hematopoietic system are amenable to reprogramming and highlight the instructive role of transcription factors in fate conversions.

Induced hemogenesis from fibroblasts and endothelial cells
Successes in transcription factor-mediated reprogramming has encouraged several groups to adopt the strategy of directly converting somatic cells to HSCs. Szabo et al. reported that ectopic expression of OCT4 combined with hematopoietic promoting cytokines could induce restricted myeloerythroid progenitors with limited self-renewal capacity from human fibroblasts. The conversion likely relies on partial reprogramming and a recent follow-up study with molecular analysis revealed that OCT4-transduced fibroblasts display elevated levels of developmentally related genes associated with multiple cell types but not those associated with pluripotency. More recently, Pereira et al. employed an H2BGFP fibroblast reporter line to identify a combination of transcription factors (TFs) capable of inducing a hemogenic program from murine fibroblasts. They found that the hematopoietic transcription factors Gata2, Gfi1b, cFos and Etv6 were sufficient to convert murine fibroblasts to hematopoietic progenitors. Extensive molecular analysis of factor-induced intermediates showed sequential downregulation of a fibroblast gene expression signature, followed by induction of an endothelial program and finally, the activation of a hematopoietic gene expression profile. The TF-induced fibroblasts transitioned through an HE-like intermediate mimicking the in vivo progression of blood development. Although emergent hematopoietic cells possessed a LT-HSC gene expression profile and surface antigen phenotype, their functional potential remains to be fully interrogated. In a similar report, Batta et al. converted embryonic and adult murine fibroblasts into multilineage hematopoietic progenitors with short-term engraftment potential by ectopic expression of Erg, Gata2, Lmo2, Runx1c and Scl. Interestingly, p53-deficient fibroblasts were more efficiently reprogrammed to hematopoietic progenitors with erythroid, myeloid, megakaryocytic and lymphoid potential than wild-type fibroblasts, suggesting that p53 expression may be a barrier for reprogramming to blood.

The close ontogenetic relationship between endothelium and hematopoietic cells makes
endothelial cells an attractive starting cell type for reprogramming. In an attempt to recreate a vascular microenvironment, Sandler and colleagues co-cultured transduced human umbilical vein endothelial cells and adult dermal microvascular endothelial cells with E4ECs – engineered endothelium-derived stromal cells that support HSC maintenance by producing physiological levels of paracrine factors. Using this co-culture system, introduction of FOSB, GFI1, RUNX1 and SPI1 (FGRS) into primary vascular endothelial cells induced multilineage hematopoietic progenitors with durable repopulating potential\(^6\). FGRS-reprogrammed cells expressed HSC markers and importantly, maintained serial engraftment in NSG mice. However, the engrafted cells had limited lymphoid contribution. Sustained expression of some of the transgenes could interfere with lymphoid differentiation. The authors addressed this possibility using an inducible SPI1 transgene, which modestly augmented T cell potential. Further optimization of transcription factor combinations is required to achieve a balanced multilineage output. In addition, without a full molecular comparison of reprogrammed cells to primary HSCs and progenitors to establish cell identity relationships in an unbiased fashion, it is difficult to ascertain the true fidelity of fate conversion. Nonetheless, these experiments have established a novel approach to HSC reprogramming underscoring the important role of the vascular niche in providing instructive and supportive signals for reprogramming.

**Reprogramming committed murine hematopoietic cells to induced HSCs**

Although master regulators such as GATA1 and C/EBP\(\alpha\) are capable of inducing conversions between mature hematopoietic cell types, no TF combination had been reported to interconvert mature cells to HSC-like cells until a recent study by Riddell and colleagues\(^6\,2\). The authors identified 36 factors specific to HSCs and systematically investigated their capacity to convert B cell or myeloid progenitors into long-term engrafting stem cells. Using immunoglobulin rearrangement as a genetic marker to track the clonal origin of donor-derived cells, they found that pro-B cells transduced with the 36 TFs could engraft recipients. Retrospective analysis of transgene integration reduced the 36 factor cocktail to just 6 necessary factors: Hlf, Lmo2, Pbx1, Prdm5, Runx1t1 and Zfp37. The addition of Meis1 and Mycn and delivery of factors in polycistronic cassettes markedly enhanced reprogramming efficiency. The transduction of these 8 TFs as polycistronic constructs imparted long-term, multilineage differentiation capacity with serial transplantation capability. At the single cell level, these induced HSCs (iHSCs) clustered closely to bona fide HSCs based on quantitative analysis of 151 genes. This was the first study to report the de novo generation of functional murine HSCs; however, translation to human cells is complicated by several factors. First, the same set of TFs may not function similarly to reprogram human cells. Second, it is unclear whether the in vivo microenvironment is essential for reprogramming or merely to sustain HSC potential once the stem cell program has been established by the TFs. If the in vivo microenvironment is indeed necessary, the critical issue is how the same experimental procedure could be applied in a more translational setting. Since there is little control over the reprogramming process once the cells are transplanted in vivo, it is difficult to predict efficiency of conversion and success rate of multilineage reconstitution, if this procedure were attempted directly in patients. A better understanding of the cell intrinsic and extrinsic reprogramming factors that can establish an HSC transcriptional network will provide cues necessary for the de novo generation of human HSCs.

**Direct conversions starting from hPSCs**

Direct conversions between somatic and hematopoietic cells have been shown to be a promising
alternative to classical directed differentiation for HSC generation but primary cells ultimately lack the scalability that pluripotent cells offer. Elcheva et al. recently identified two transcriptional combinations capable of directly converting hPSCs to endothelium, which subsequently gave rise to blood cells with restricted pan-myeloid (ETV2 and GATA2) or erythro-megakaryocytic (GATA2 and TAL1) potential. The authors did not detect meaningful engraftment of these cells, suggesting that the TF-induced cells have similar properties to hematopoietic progenitors differentiated from hPSCs using more conventional protocols. A more detailed molecular comparison between TF-induced cells and hematopoietic progenitors differentiated from hPSCs, as well as primary human HSC and progenitor populations, may be useful to identify potential regulators capable of inducing a definitive program directly from hPSCs.

Using a hybrid approach between directed differentiation and reprogramming, Doulatov and colleagues differentiated hPSCs to CD34⁺CD45⁺ myeloid precursor cells and screened for a combination of transcription factors to reprogram to an HSC-like state – a process termed “respecification”. The rationale for this approach is that first differentiating hPSCs to CD34⁺CD45⁺ precursor cells would minimize the “epigenetic distance” to a true HSC. While the endothelial-to-hematopoietic transition in humans remains poorly understood, the molecular differences between primary human HSCs and progenitors have been well-characterized by gene expression profiling providing a rational approach to introduce stem cell genes back into progenitors. Previous attempts involving overexpression of single transcription factors such as HOXB4, SCL and RUNX1 in hPSCs have failed to yield durably engrafting cells. A combination of HOXA9, ERG and RORA endowed myeloid precursors with self-renewal capacity. The addition of SOX4 and MYB to this cocktail of transcription factors conferred engraftment potential with myeloid and erythroid lineages. Notably, the engrafted human erythrocytes underwent definitive maturation and hemoglobin switching to express β-hemoglobin in vivo. Although lymphoid lineages were absent in vivo, this is the first report of robust TF-mediated hematopoietic engraftment from hPSCs. However, reported engraftment was transient and restricted to myeloid and erythroid lineages. Further optimization of transcription factor combinations will be necessary to generate HSC-like cells.

Applications of hematopoietic cells derived from hPSCs

Disease modeling and drug screening

Despite the recent progress, fully functional human HSCs are yet to be successfully generated by direct conversion or differentiation. Existing derivation protocols and TF combinations must be refined and the converted cells further characterized. However, they are nonetheless invaluable model systems for hematological diseases, since primary patient samples are often scarce, for instance in bone marrow failure syndromes. Easily obtainable tissues such as fibroblasts and endothelial cells can be converted to engraftable hematopoietic progenitors to model diseases in vitro and in vivo. Patient-derived iPSCs can help elucidate the molecular pathways involved in disease through the establishment of “disease in a dish.” To date, numerous pluripotent lines have been generated from patients with hematological diseases, including Fanconi anemia, sickle cell anemia, Diamond Blackfan anemia, Shwachman-Diamond syndrome, chronic myelogenous leukemia, JAK2 V617F myelo-proliferative disorder, dyskeratosis congenita, Pearson syndrome and others. Hematopoietic differentiation of these iPSCs have recapitulated some aspects of these disorders, and uncovered mechanistic links between multiple tissue
deficits. However, hematopoietic differentiation of hPSCs yield short-lived progenitors and mature cells and without the possibility of generating large numbers of hematopoietic stem and progenitor cells, these protocols limit the scope of experiments that can be performed in vitro and preclude disease modeling in vivo.

The various transgene-driven systems outlined above present new platforms for chemical screening and in vivo modeling of hematological disease. For example, in iPSC respecification, transcription factor-mediated expansion of CD34+CD38− cells followed by lineage-inductive culture conditions provides a large number of target cells available for chemical screening. A similar drug screening platform has been applied to iPSC-derived motor neurons to identify therapeutic compounds for amyotrophic lateral sclerosis. The near-unlimited capacity to produce erythroid precursors may be suitable for transfusion-related applications, disease modeling of red blood cell disorders and chemical screening in vitro. Importantly, the disease phenotypes can now be recapitulated in vivo permitting greater insight into the etiology and a platform for drug testing. For instance, in vivo maturation of erythrocytes may offer a unique opportunity to perform screens for new therapies for the β-globin disorders.

**Generation of clinically relevant cell types**

**Red blood cell transfusion**

There is an enormous demand for transfusable blood products. Chronically transfused patients often develop erythrocyte alloimmunization or suffer from iron overload complications. The in vitro generation of red blood cells (RBCs) of a defined antigen profile from pluripotent stem cells represents an alternative to classic transfusion products. Nakamura and colleagues established methods for generating RBCs from murine ESCs and demonstrated that transfusion of in vitro differentiated RBCs protected mice from lethal hemolytic anemia. Several groups have reported protocols for differentiating RBCs from hESCs; however, blood group polymorphisms of different hESC lines may not be suitable for generating universal donor RBCs. iPSCs, on the other hand, in principle could represent an inexhaustible supply of autologous RBCs for transfusion. Chang et al. and Papapetrou et al. have described methods for generating RBCs from hiPSCs, but these RBCs express embryonic and fetal hemoglobin. Interestingly, patients who retain expression of fetal hemoglobin do not manifest any RBC abnormalities in adult life so the fetal phenotype of iPSC-derived RBCs does not necessarily present a barrier to transfusion, especially in patients with hemoglobinopathies. However, similar to primitive yolk sac erythrocytes, iPSC-derived RBCs do not enucleate efficiently, and ongoing efforts are aimed at promoting terminal erythroid maturation.

**Immortalized megakaryocytes for platelet production**

Platelets are a central component of the blood-clotting cascade. As with RBCs, current human donor-derived platelets are limited in supply and have a short shelf life. The in vitro hematopoietic differentiation of pluripotent stem cells to megakaryocytes, the precursors to platelets, represents a potentially unlimited supply of platelets for transfusion. Previous efforts to increase megakaryocyte yield from hESCs have had limited success. In a novel strategy, Nakumura et al. generated immortalized self-replicating megakaryocyte progenitor cell lines from hiPSCs using BCL-XL, c-MYC and BMI1. These immortalized lines could be matured into CD42b+ platelets with in vivo functionality in a thrombocytopenic mouse model upon transgene
silencing\textsuperscript{91}. The clinical application of this system could provide a plentiful supply of platelets from screened and selected immortalized megakaryocytic clones.

**Engineered T cells with chimeric antigen receptor for immunotherapy**

Adoptive T cell transfer for cancer immunotherapy is often limited by the number of antigen-specific T cells that can be expanded from individual donors. Previous studies have demonstrated the feasibility of generating T lymphocytes from hPSCs, although the yield has been low\textsuperscript{92,39}. Themeli and colleagues combined iPSC and chimeric antigen receptor (CAR) technologies to produce antigen-specific T cells. CARs are engineered antibody-based receptors comprised of an extracellular domain of chosen specificity fused with cytosolic domains with signal transduction and co-stimulatory signaling capabilities\textsuperscript{93}. Themeli et al. generated iPSC lines from donor T cells and engineered them to express CAR targeting CD19 showing that the engineered T cells elicited strong anti-tumor responses in a xenogenic Burkitt lymphoma mouse model\textsuperscript{94}. CAR-engineered iPSCs are amenable to further genetic modification to remove potentially unwanted recognition from the native T cell receptor locus. These studies highlight the therapeutic value of combined hematopoietic differentiation from iPSCs and CAR technologies to yield clinically useful cell types for cancer immunotherapy.

**Conclusion**

De novo generation of human HSCs remains a “Holy Grail” of regenerative stem cell biology. For many years, the concept of direct conversion has been an attractive alternative approach to hematopoietic differentiation from pluripotent stem cells for the de novo generation of HSCs. In the past year, a number of papers have demonstrated the proof of principle that hematopoietic cells with varying degrees of stem cell potential can be induced from somatic cells. The critical issue with all these cell fate conversions, however, is the relationship of derived hematopoietic cells to their native counterparts. HSCs are functionally defined by their ability to reconstitute the entire hematopoietic system in transplant recipients, and this metric remains the gold standard for HSC engineering. However, functional interrogation must be combined with extensive molecular analysis of the transcriptome and epigenome to assess the relatedness of derived HSC-like cells to their native counterparts. This may be accomplished using advanced bioinformatics algorithms such as CellNet, a network biology platform for quantifying how closely engineered cell populations resemble their target cell type\textsuperscript{95,96}. We would argue that all of the direct conversion and differentiation strategies aimed at generating HSCs remain to be more fully characterized and improved before any consideration of clinical application. However, the goal of obtaining de novo HSCs now seems attainable. In addition, differentiation protocols have produced other useful blood cell types, including RBCs, platelets and T cells with the potential to transform the practice of blood transfusion and immunotherapy. The efforts devoted to HSC derivation have significantly advanced our understanding of early human hematopoietic commitment, endothelial-to-hematopoietic transition, critical HSC gene networks and hematological disease mechanisms. Moving forward, network biology must be combined with rigorous functional interrogation to provide a comprehensive strategy to reconstruct gene regulatory networks that control HSC identity and ultimately enable conversion of heterologous cells into clinical grade HSCs.

**Authorship**

Contribution: L.T.V. reviewed the literature; L.T.V. and G.Q.D. wrote the paper.
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20. Müller AM, Dzierzak EA. ES cells have only a limited lymphopoietic potential after adoptive transfer into mouse recipients. *Development.* 1993;118(4):1343-1351.


Table 1. Summary of parameters for HSC generation reported to date.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Species</th>
<th>Starting cell type</th>
<th>Factors or conditions</th>
<th>Hematopoietic differentiation potential</th>
<th>Engraftment in vivo</th>
<th>Reference(s)</th>
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<tr>
<td>Directed differentiation</td>
<td>human</td>
<td>ES cells</td>
<td>S17 or C166 co-culture</td>
<td>Myeloid/erythroid/megakaryocytic</td>
<td>No</td>
<td>Kaufman et al. 2001(^{17})</td>
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<tr>
<td>Directed differentiation</td>
<td>human</td>
<td>ES cells</td>
<td>BMP4, hematopoietic cytokines</td>
<td>Myeloid/erythroid</td>
<td>No</td>
<td>Chadwick et al. 2003(^{16})</td>
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<tr>
<td>Directed differentiation</td>
<td>human</td>
<td>ES cells</td>
<td>OP9 co-culture</td>
<td>Myeloid/erythroid/NK cell/B lymphoid</td>
<td>No</td>
<td>Vodyanik et al. 2005(^{18})</td>
</tr>
<tr>
<td>Directed differentiation</td>
<td>human</td>
<td>ES and iPS cells</td>
<td>Activin/Nodal; Wnt-(\beta)-catenin signaling manipulation</td>
<td>Myeloid/erythroid/T lymphoid</td>
<td>No</td>
<td>Kennedy et al. 2012(^{36}); Sturgeon et al. 2014(^{40})</td>
</tr>
<tr>
<td>Directed differentiation</td>
<td>human, mouse</td>
<td>iPS cells</td>
<td>In vivo teratoma formation</td>
<td>Myeloid/erythroid/B and T lymphoid</td>
<td>Long-term</td>
<td>Amabile et al. 2013(^{42}), Suzuki et al. 2013(^{43})</td>
</tr>
<tr>
<td>Directed differentiation, direct conversion</td>
<td>mouse</td>
<td>ES cells, yolk sac progenitors</td>
<td>HoxB4</td>
<td>Myeloid/erythroid/megakaryocytic /B and T lymphoid</td>
<td>Long-term</td>
<td>Kyba et al. 2002(^{25})</td>
</tr>
<tr>
<td>Direct conversion</td>
<td>mouse</td>
<td>fibroblast</td>
<td>Gata2, Gfi1b, cFos, Etv6; Erg, Gata2, Lmo2, Runx1c, Scl</td>
<td>Myeloid</td>
<td>No</td>
<td>Pereira et al. 2013(^{59})</td>
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<tr>
<td>Direct conversion</td>
<td>human</td>
<td>Umbilical vein and adult endothelial cells</td>
<td>FOSB, GFI1, RUNX1 and SPI1; E4EC co-culture</td>
<td>Myeloid/erythroid/megakaryocytic /NK cell/B lymphoid</td>
<td>Long-term</td>
<td>Sandler et al. 2014(^{61})</td>
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<tr>
<td>Direct conversion</td>
<td>mouse</td>
<td>Pro-B cells, myeloid cells</td>
<td>Hlf, Lmo2, Pbx1, Prdm5, Runx1t1, Zfp37</td>
<td>Myeloid/erythroid/megakaryocytic /B and T lymphoid</td>
<td>Long-term</td>
<td>Riddell et al. 2014(^{62})</td>
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<tr>
<td>Direct conversion</td>
<td>human</td>
<td>ES and iPS cells</td>
<td>ETV2 and GATA2; GATA2 and TAL1</td>
<td>Myeloid; Erythroid/megakaryocytic</td>
<td>No</td>
<td>Elcheva et al. 2014(^{43})</td>
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<td>Directed differentiation, direct conversion</td>
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<td>ES and iPS cells</td>
<td>HOXA9, ERG, RORA, SOX4, MYB</td>
<td>Myeloid/erythroid</td>
<td>Short-term</td>
<td>Doulatov et al. 2013(^{64})</td>
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Figure 1. Routes for HSC engineering. Directed differentiation of ES and iPS cells relies on morphogens and growth factors to recapitulate hematopoietic development in vitro. Direct conversion utilizes transcription factors to force somatic cells to switch cell fate without transitioning through normal developmental intermediates. Transcription factor combinations employed to convert heterologous cell types to hematopoietic cells are listed. Abbreviations: ES/iPS - embryonic stem/induced pluripotent stem cells; HE - hemogenic endothelium; HSC - hematopoietic stem cell; MPP - multipotent progenitor; MLP - multilymphoid progenitor; CMP - common myeloid progenitor; ES- and YS-HPC - embryonic stem- and yolk sac-derived hematopoietic progenitor cells. Combinations in lower case indicate conversions in mouse cells and those in upper case represent conversions in human cells. Conversions from pluripotent stem cells comprise a distinct approach; blue arrows show direct hematopoietic induction from pluripotent stem cells using transcription factor combinations. The green arrow represents a hybrid strategy of directed differentiation and direct conversion, termed “respecification.” Extensive molecular analysis must be combined with functional interrogation to assess the relatedness of the engineered cell types to their native counterparts.
FOSB, GFI1, RUNX1, SPI1
ETV2, GATA2
Hlf, Lmo2, O2, Pbx1, Prdm5, GATA2, TAL1
ERG, HOXA9, RORA, SOX4, MYB
Gata2, Gfi1b, cFos, Etv6
or Erg, Gata2, Lmo2, Runx1c, Scl
HIF, Lmo2, Pbx1, Prdm5, Runx1c, Zfp37, Meis1, Mcyn
ES/HPC
HE
OCT4
Activin/Nodal
Wnt/β-catenin
HSC
MPP
MLP
CMP
ES/IPS
ES-HPC
YS-HPC
CD34+
CD45+
ERG, HOX9, RORA, SOX4, MYB
GATA2, TAL1
Fibroblast
Endothelial cell
B cell
T cell
NK cell
Dendritic cell
Erythrocyte
Megakaryocyte
Granulocytes
Macrophage
Activin/Nodal
Wnt/β-catenin
HoxB4
De novo generation of HSCs from somatic and pluripotent stem cell sources

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