# Generating Autologous Hematopoietic Cells from Human Induced Pluripotent Stem Cells through Ectopic Expression of Transcription Factors

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**Purpose of review**

Hematopoietic cell transplantation (HCT) is a successful treatment modality for patients with malignant and non-malignant disorders, usually when no other treatment option is available. The cells supporting long-term reconstitution after HCT are the hematopoietic stem cells (HSCs), which can be limited in numbers. Moreover, finding an appropriate HLA-matched donor can be problematic. If HSCs can be stably produced in large numbers from autologous or allogeneic cell sources, it would benefit HCT. Induced pluripotent stem cells (iPSCs) established from patients' own somatic cells can be differentiated into hematopoietic cells in vitro. This review will highlight recent methods for regulating human (h) iPSC production of HSCs and more mature blood cells.

**Recent findings**

Advancements in transcription factor (TF)-mediated regulation of the developmental stages of in vivo hematopoietic lineage commitment have begun to provide an understanding of the molecular mechanism of hematopoiesis. Such studies involve not only directed differentiation in which TFs, specifically expressed in hematopoietic lineage-specific cells are over-expressed in iPSCs, but also direct conversion in which TFs are introduced into patient-derived somatic cells which are dedifferentiated to hematopoietic cells. Since iPSCs derived from patients suffering from genetically mutated diseases would express the same mutated genetic information, CRISPR-Cas9 gene editing has been utilized to differentiate genetically corrected iPSCs into normal hematopoietic cells.

**Summary**

IPSCs provide a model for molecular understanding of disease, and also may function as a cell population for therapy. Efficient differentiation of patient-specific iPSCs into HSCs and progenitor cells is a potential means to overcome limitations of such cells for HCT, as well as for providing in vitro drug screening templates as tissue-on-a-chip models.

**Keywords**

Induced pluripotent stem cells (iPSCs), hematopoietic stem cells (HSCs), direct differentiation, direct conversion, CRISPR/Cas9

## INTRODUCTION

There are three main sources of transplantable cells for HCT: bone marrow (BM), mobilized peripheral blood, and umbilical cord blood (CB) [1, 2], but it can be very difficult finding an appropriate donor, and numbers of HSCs isolated from these sources could be limited, such as for CB HCT [3, 4▪]. Various ways to expand HSCs ex vivo have been evaluated, but there are limitations to these efforts [5, 6]. To develop a patient-specific cell therapeutic agent, investigators have been evaluating pluripotent stem cells (PSCs; especially reprogrammed iPSCs) [7] and their capacity to differentiate into HSCs/hematopoietic progenitor cells (HPCs) [3]. We highlight recent approaches to achieve functional HSCs and other hematopoietic cells from PSCs, ranging from ectopic expression of transcription factors, to use of gene editing technology for correcting mutated genes in PSCs (Figure 1).

***Human embryonic (ESCs) and iPSCs.*** hESCs were established from the inner cell mass of the human embryo [8]. Since these cells can undergo differentiation into most cell types in the body, they have been explored for cell-based therapy. However, ethical controversies of using embryos have in part prevented our realizing the full potential of these cells for clinical use. A breakthrough overcoming some ethical concerns of utilizing hESCs has been discovery of iPSCs [7]. By introducing four reprogramming genes (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) into mature somatic cells, a new type of PSCs was established. To establish clinically applicable iPSCs, reprogramming efficiency has been increased [9▪, 10], a non-genome integrating gene delivery system has been devised [11], and efforts have been made to differentiate these cells into tissue-specific transplantable cells. As an example, iPSC-derived retinal pigment epithelium cell sheets generated from autologous fibroblasts were successfully transplanted into patients suffering from age-related macular degeneration [12]. However, generating functional HSCs with high engraftment efficiency remains an ongoing target, the promise of which is suggested by the following interesting studies.

***Direct differentiation of hematopoietic cells from hPSCs using ectopic expression of TFs.*** Hematopoietic lineage differentiation of hiPSCs has been most commonly carried out by two conventional methods: 1) a two-dimensional differentiation protocol utilizing co-culture of PSCs with stromal cells [13, 14], or 2) embryoid body (EB)–mediated three-dimensional differentiation [15]. $CD34^+$ hematopoietic precursor cells expressing hematopoietic transcription factors were derived from iPSCs using a co-culture method in the presence of either primary human BM-derived stromal cells or an assortment of stromal cell lines, including OP9, OP9-DL4, M2-10B4, and FH-B-hTERT [16-18]. As an alternative to this approach, EB–mediated hematopoietic lineage differentiation of hPSCs was developed [15, 19]. The first such studies incorporated a mixture of defined cytokines and Fetal Bovine Serum. The most widely used differentiation methods of hPSCs into hematopoietic cells have involved addition of cytokines or induction of spontaneous differentiation during EB formation [20]. To achieve higher differentiation efficiency, there is a need for identifying specific intrinsic signals that regulate production of hematopoietic cells in vitro. To generate more HSCs/HPCs from hPSCs, unlike classic differentiation methods (co-culture and EB formation), hematopoietic cells derived were during in vivo teratoma formation [21, 22]. Human PSCs were transplanted into immunodeficient mice to form teratomas (typically comprising three germ layer derivatives, including endodermal and neuronal lineage cells) from which $CD34^+CD45^+$ cells were isolated, suggesting that *in vivo* microenvironmental cues are important for directed differentiation and development of immature blood cells [4]. It is unclear if such derived cells would be a viable option for clinical use.

***Enhancing directed differentiation by ectopic expression of a single TF.*** Direct differentiation by introducing ectopic expression of TFs represents an alternative strategy over classical differentiation methods. This approach involving candidate TFs such as TAL1 (SCL), RUNX1, SOX17, and HOXB4 as key regulators of mesodermal and HSC developments from hPSCs has been recently introduced to control stem cell fate determination [23-27, 28▪▪].

TAL1 (T cell acute lymphocytic leukemia 1), also known as SCL (stem cell leukemia), is a transcript in the emerging hemangioblast during hESC hematopoietic differentiation [23, 29]. Since TAL1-overexpressing hESC-derived EBs was used to accelerate formation of erythro-megakaryocytic progenitors [29], TAL1 has been shown to support an hematopoietic program in hPSCs. Overexpression of TAL1 in hPSC enhanced emergence of megakaryocytic precursors, mature megakaryocytes, and platelets in vitro [30▪], however these cells failed to engraft in vivo [30].

RUNX1 (Runt-related transcription factor) is a key TF regulating differentiation of hematopoietic stem cell into mature blood cells [31]. Overexpression of RUNX1A in hESC-/ hiPSC-derived EBs significantly enhances hematopoietic differentiation and accelerates generation of hemato-endothelial cells [25]. RUNX1 controls lineage specification of hPSCs into mesoderm and specifically enhances hemogenic differentiation as well as production of definitive HSCs. However, ectopic RUNX1 expression may entail risk of mediating transformation of hPSC-derived cells, since it is known to contribute to leukaemogenesis [32].

Two types of inducible fusion proteins have been developed, including HOXB4-$ER^{T2}$ and KLF1-$ER^{T2}$ that can be induced at a defined time point during differentiation of hPSC to red blood cells (RBCs) [33▪, 34▪]. Activation of HOXB4 increases progenitor cell (CD43+/CD34+) populations and proportions of immature CD235a+/CD71+ erythroid cells from hPSC [33]. HOXB4 activity promotes generation of embryonic (ε)/fetal (γ) globins rather than more mature adult (β) globin, a definitive phenotype, suggesting that HOXB4 induces production of progenitors. But, it does not overcome the transitioning barrier for production of enucleated red blood cells. Activation of KLF1 at day 10 of differentiation of hiPSCs enhanced erythroid commitment and differentiation. Extended in vitro culture resulted in generation of more enucleated cells, but not expression of adult (β)globin [34▪]. Thus, HOXB4 or KLF1 plays important roles in hematopoietic development, but there are still limitations to producing mature RBC from hPSC.

***Enhancing directed differentiation using ectopic expression of multiple TFs.*** Overexpression of a single TF alone in hPSCs is not enough to yield hematopoietic cells that engraft *in vivo*. Concomitant ectopic expression of multiple TFs has been employed to overcome this barrier [24, 26]. The most noticeable findings suggest that HOXA9, ERG, and RORA confer self-renewal capacity of myeloid precursor cells in vitro, and addition of SOX4 and MYB to these TFs confers short-term engraftment of myeloid and erythroid lineages in vivo [24]. GATA2 and ETV2 promoted pan myeloid differentiation, whereas GATA2 and TAL1 enhanced erythro-megakaryocytic differentiation from hPSCs [26].

Large-scale production of megakaryocytes and platelets from PSCs has been achieved by simultaneously overexpressing GATA1, FLI1, and TAL1 during early stages of differentiation in chemically defined conditions [35]. Functional platelets were generated throughout the culture, allowing prospective collection of several transfusion units from as few as 1 million starting hPSCs. It remains to be determined how closely these three TFs recapitulate normal hematopoietic development from hPSCs.

Ectopic overexpression of TFs in hiPSCs is a relatively quick and efficient tool for induction of HSCs hematopoietic cells, and this system can be used for identifying specific TFs that are required for endothelial and hematopoietic specification. However, current protocols have limited utility for studies of extracellular signaling involved in hematopoietic development, as it uses TFs to bypass surface receptor-mediated signaling. If we are to produce HSCs in vitro with the capacity for efficacious engrafting short- and long-term, we must understand the exact orchestration of both intrinsic and extrinsic signals which mimic the complex environment of the human embryo. Understanding TF programming is in its infancy. We are still a ways from achieving large-scale production of engraftable HSCs and functional mature blood cells that can be used safely in the clinic [28].

***Direct conversion of somatic cells into hematopoietic cells.*** Direct conversion methods that introduce lineage-restricted transcription factors into somatic cells and induce them into tissue-specific cells has generated attention [36-38]. This method bypasses intermediate stages where cells acquire pluripotency; these directly induced cells are not able to form teratomas. Attempts have been made to directly induce hematopoietic cell production by overexpressing early developmental hematopoietic associated TFs along with TFs controlling cell fate in somatic cells.

Studies succeeded in directly converting human fibroblasts into immature blood cells by overexpressing the transcription factor, OCT4 [39]. Although succeeding in directly converting fibroblasts into cells capable of differentiating into granulocytic, erythrocytic, monocytic and megakaryotic colonies, they had limited in self-renewal potential, hematopoietic reconstitution, and differentiation capacity into lymphoid cells. Some have used a parallel strategy [40-43]. Over-expression of GATA1, TAL1, LMO2, and c-MYC (GTLM) genes in mouse / human fibroblast cells de-differentiated them into primitive erythroid progenitors [42▪▪]. An induced erythroid progenitor was established with an adult-type globin expression pattern by additionally overexpressing KLF1 and MYB with GTLM [42]. GATA2 and RUNX1 were used with GTLM gene to produce megakaryocytes and platelets cells from patients with Fanconi anemia [43▪▪]. These studies [42, 43] exemplify how direct conversion is emerging as a promising tool to generate blood cells in vitro. Efforts are needed to obtain therapeutic-scale production and to design other gene introduction methods, possibly using non-viral systems. Although direct conversion methods may not have a high risk of producing cells that form teratomas, mechanisms of direct conversion have not been clarified yet, and there is a possibility of mutation due to the introduced gene; safety validations are required for their clinical application.

***Improving Hematopoietic Differentiation of iPSCs by CRISPR/Cas9.*** Since development of the CRISPR-Cas9 gene editing system [44], this technology evaluated for its potential to treat various genetic diseases [45▪]. A number of groups have successfully applied CRISPR-Cas9 technology to

correct β-thalassemia mutations in patient-derived iPSCs [46-49]. A disease-causing mutation in the β-globin gene (HBB) was corrected using CRISPR-Cas9 in iPSCs derived from a β-thablassemia patient with minimal off-target effects [48]. HBB mutations in the patient-derived iPSCs were completely corrected, with increased production of HPCs. In another study, mutations of CD41/42 (-CTTT) in iPSCs from a β-thalassemia patient were successfully corrected using a combination of single-strand oligodeoxynucleotides with CRISPR/Cas9 [45, 49]. The corrected iPSCs were selected for erythroblast differentiation and manifested restored expression of HBB protein. Sickle cell disease, which has a homozygous missense point mutation in the HBB gene encoding adult β-globin protein, is a severe incurable chronic anemia. HBB 20 bp downstream to the beta mutation was corrected in human iPSCs using CRSPR/Cas9, with the 16-kDa β-globin protein expressed from the corrected HBB allele in erythrocytes that were differentiated from the genome-edited iPSCs [46▪].

Hemophilia, which affects about 400,000 people worldwide, is a hemorrhagic disease caused by a lack of protein that hardens blood due to a genetic mutation. The inverted FVIII gene in hemophilia patient-derived iPSCs was corrected using CRISPR/Cas9, without detectable off-target mutations in other genome locations. The corrected cells were then induced to differentiate into vascular endothelial cells, producing blood coagulation factors. These cells were transplanted into hemophilia mice, with symptom improvement [47▪].

The CRISPR/Cas9 system for precise genome editing may be a useful tool for removing and correcting genes or mutations involved in inherited hematological disorders. However, before use of CRISPR/Cas9-mediated gene correction in humans, appropriate delivery systems with higher efficiency and specificity must be identified [50, 51].

## CONCLUSION

Some believe that hiPSCs hold better promise than hESCs for clinical translation [52]. Human iPSCs may in the future help to generate larger numbers of histocompatible cells for HCT and other organ transplants, possibly as a customized cell therapeutic agent. We are not there yet, but if iPSCs established from patient somatic cells can be differentiated into functional somatic cells after correcting the mutant genes with CRISPR/Cas9 or other technologies, this would be a major health advance. This is especially of interest if hematopoietic differentiation of iPSCs and production of engrafting HSCs and HPCs, is successfully demonstrated by using a combination of intrinsic and extrinsic influences. Presently, transplantation of iPSC-derived hematopoietic cells is still limited to animal testing models. Low hematopoietic differentiation efficiency with low engrafting capability, and the potential to form cancer in vivo are limiting factors that must be overcome for this field to progress.

### Key Points

- IPSC is a pluripotent stem cell that can differentiate into an autologous hematopoietic lineage-specific cell.

- IPSCs and somatic cells with introduced TFs associated with hematopoietic cells, can produce blood cells.

- Gene-corrected iPSCs from patients can generate normal hematopoietic lineage-specific cells in vitro.

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**Conflicts of Interest**

**The authors have no competing financial interests potential conflicts of interest.**

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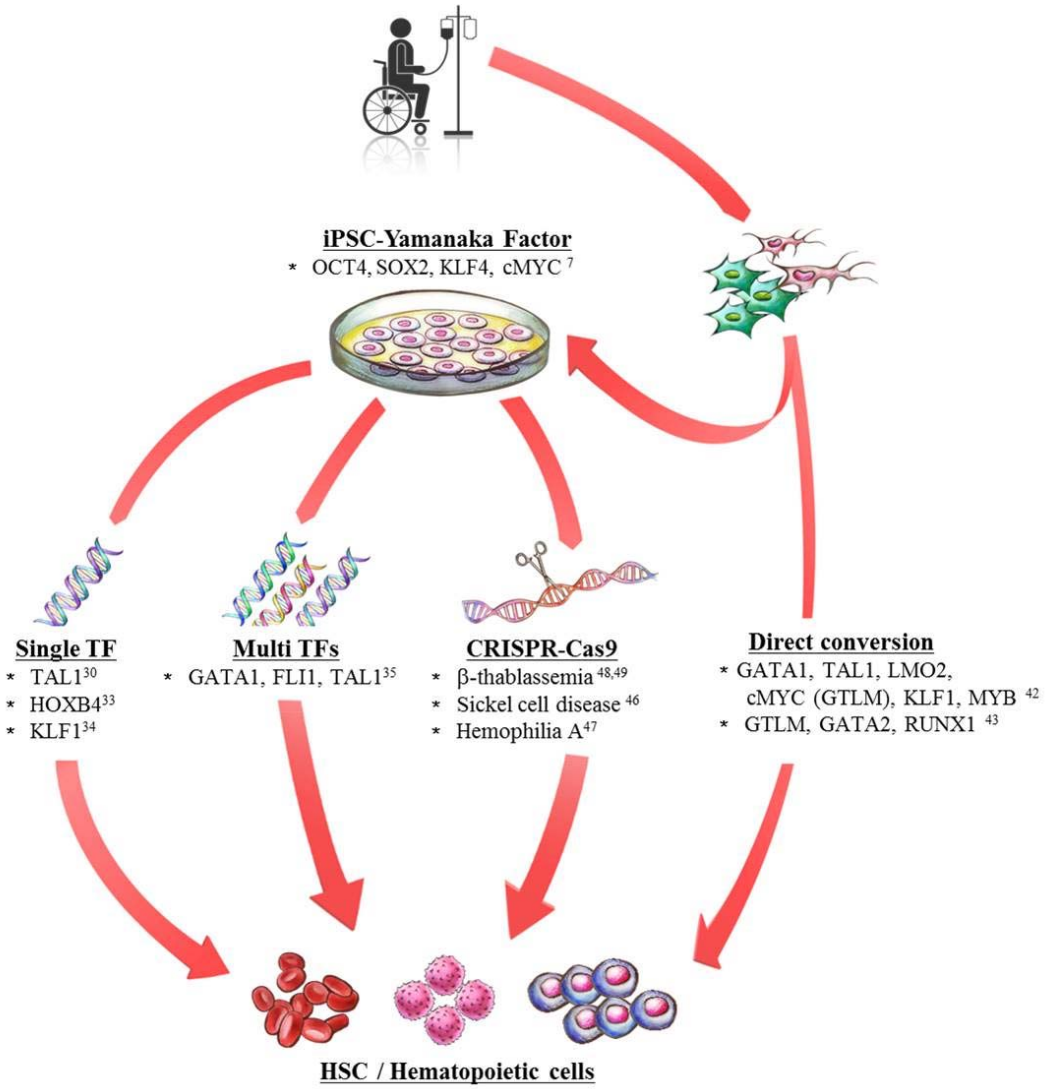
**Figure legend**

Figure 1. Schematic representation of the generation of hematopoietic cells from hiPSC using ectopic gene expression of TFs. Differentiation from hPSC or direct conversion from somatic cells rely on specific TFs to force cell fate switches. Mutations in patient derived iPSCs can be corrected using CRISPR/Cas9 technology to generate functional hematopoietic cells. iPSC; Induced pluripotent stem cells, HSC; hematopoietic stem cells, and TF; transcription factor


iPSC-Yamanaka Factor
* OCT4, SOX2, KLF4, cMYC [7]
Single TF
* TAL1[30]
* HOXB4[33]
* KLF1[34]
Multi TFs
* GATA1, FLI1, TAL1[35]
CRISPR-Cas9
* β-thablassemia [48,49]
* Sickel cell disease [46]
* Hemophilia A[47]
Direct conversion
* GATA1, TAL1, LMO2, cMYC (GTLM), KLF1, MYB [42]
* GTLM, GATA2, RUNX1 [43]
HSC / Hematopoietic cells