Published in final edited form as:

Regen Med. 2010 July ; 5(4): 521–530. doi:10.2217/rme.10.38.

Potential of human induced pluripotent stem cells derived from blood and other postnatal cell types

Zhaohui Ye¹ and Linzhao Cheng^{†,1}

¹Division of Hematology & Stem Cell Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Broadway Research Building, Room 747, 733 N. Broadway, Baltimore, MD 21205, USA

Abstract

Human induced pluripotent stem (iPS) cells have been generated from various cell types including blood cells, and offer certain advantages as a starting population for reprogramming postnatal somatic cells. Unlike adult stem cells, iPS cells can proliferate limitlessly in culture while retaining their potential to differentiate into any cell type, including hematopoietic lineages. Derivation of patient-specific iPS cells, in combination with improved hematopoietic differentiation protocols, provides an alternative to generate histocompatible stem cells for bone marrow transplantation. In addition, the ability to reprogram blood cells and redifferentiate iPS cells back to hematopoietic lineages provides opportunities to establish novel models for acquired and inherited blood diseases. This article will summarize recent progress in human iPS cells derived from blood cells and hematopoietic differentiation from iPS cells. Advantages of blood as a source for reprogramming and applications in regenerative medicine will be discussed.

Keywords

blood; hematopoietic differentiation; hematopoietic stem/progenitor cell; iPS cell; reprogramming

Recent development in induced pluripotent stem (iPS) cell technology provides powerful tools to generate patient-specific stem cells for both therapeutic and research purposes. Derivation of mouse iPS cells was first reported in 2006 by the Yamanaka group [1]. In this groundbreaking study, mouse embryonic fibroblasts and tail tip fibroblasts were transduced with combinations of retroviruses expressing candidate genes that are preferentially expressed in, or believed to be essential to the growth of, embryonic stem cells (ESCs). Using an elegant (albeit imperfect as the first version) genetic screen, they found that a few clones displayed mouse ESC morphology and proliferation capacity when cultured under mouse ESC culture conditions. These iPS cells could form teratomas that contained tissues of all three germ layers *in vivo*. They further narrowed down the list of genes that are required for efficient direct reprogramming of both embryonic and adult mouse fibroblasts

to a combination of four: Oct4, Sox2, Klf4 and c-Myc [1]. Later, several groups used the same four factors and improved reprogramming techniques to demonstrate that successfully reprogrammed mouse iPS cell lines are indeed pluripotent and germline transmission competent, similar to mouse ESCs [2,3].

Less than 18 months after the first report in mice [1], several groups independently reported the reprogramming of human cells using either the same set of factors as Yamanaka or with small modifications or additions [4–8]. Although the addition of NANOG, LIN28 and SV40 large T antigen further enhanced the pace and efficiency of reprogramming [5,8], it later became evident that the four Yamanaka factors are sufficient for reprogramming human fetal and adult fibroblasts. The iPS cell lines generated from human fibroblasts exhibit characteristics of human ESCs and can be expanded extensively under human ESC culture conditions. They express a set of cell surface markers such as TRA-1–60/TRA-1–81, and SSEA3/SSEA4, which are expressed in undifferentiated human ESCs and diminished after differentiation. The endogenous gene expression of transcriptional regulators that are important to human ESCs, such as NANOG and OCT4, is also upregulated in iPS cells. The successfully reprogrammed human iPS cells are capable of differentiating into various cell types by *in vitro* and *in vivo* assays, demonstrating their pluripotency. Subsequently, several groups have demonstrated that human iPS cells can be generated with a reduced number of factors, with the most success coming from reprogramming neural stem cells that already express SOX2, KLF4 and c-MYC [9–12]. Ultimately, human iPS cells need to be generated without permanent genomic alteration. Although it has been achieved using EBV OriP/EBNA1-based episomal plasmid transient transfection and by delivery of recombinant transcription factors to newborn dermal fibroblasts, the efficiency of integration-free reprogramming of fibroblasts is extremely low [13,14]. Nevertheless, these studies demonstrated the principle that genomic integration of retro-viruses is not essential for generating iPS cells. As an intermediate approach, *piggyBac* DNA transposition has been shown to be effective in the generation of iPS cell lines from mouse and human embryonic fibroblasts [15,16]. Transient *piggyBac* transposase expression can catalyze both integration and scar-free excision of the transposon vector expressing the reprogramming genes. This unique feature of *piggyBac* DNA transposition has been used to generate mouse iPS cells where the integrated transgenes were completely removed [15,16]. It has been shown that the same single *piggyBac* transposon vector expressing five transgenes [15] is able to reprogram human adult mesenchymal cells, including those from an adult patient with sickle cell disease [17]. The reprogramming efficiency by the *piggyBac* transposon vector is approximately 50-fold lower than that by four retroviral vectors. However, the reduction of reprogramming efficiency by the *piggyBac* transposon plasmid can be largely compensated by adding a small molecule such as butyrate that enhances the *piggyBac*-mediated iPS cell derivation by approximately 25-fold [17]. It remains to be determined how efficient the integrated *piggyBac* vector could be excised by the re-expression of *piggyBac* transposase in fully reprogrammed human iPS cells. With the increasing knowledge of reprogramming mechanisms and the continuing efforts on screening small molecules that can replace or enhance the effects of transcription factors, it is anticipated that efficient protocols for generating human iPS cells from adult somatic cells without genomic alteration can be achieved in the near future.

Blood cells as a source for reprogramming

The majority of earlier experiments for reprogramming adult human cells have been done using fibroblastic cells derived from skin biopsies (typically two $<5\text{ mm}^3$ full-thickness skin tissues) or from marrow aspirates (Figure 1A) [4–8]. However, it is highly desirable to generate iPS cells from easily accessible sources, such as peripheral blood, without an invasive procedure (Figure 1B). Generation of iPS cells from blood cells offer several advantages over other cell types. It is more convenient and less invasive to obtain blood than some other types of tissues, such as skin. Unlike skin fibroblasts and keratinocytes, which require several weeks to establish primary cell culture from biopsy, mononuclear hematopoietic cells isolated from blood can be utilized for reprogramming almost immediately, shortening the total time of iPS cell line establishment. The blood cell reprogramming process can also be applied to the large collections of umbilical cord blood (CB) that are stored in many CB banks. The diversified genetic backgrounds in CB banking, as well as the possibility that the neonatal CB cells are less likely to accumulate genetic mutations than some other adult tissues, make CB cells an attractive source for establishing iPS cell banks with a broad coverage of various human leukocyte antigen haplotypes. This provides the opportunity to generate a bank of histocompatible iPS cell lines for many individuals who need a matched and readily available allogeneic cell source sufficient for cell therapy.

Reprogramming of hematopoietic mono-nuclear cells, such as B cells, was first reported in the mouse [18]. Hanna *et al.* utilized a ‘secondary reprogramming system,’ in which the mouse embryonic fibroblasts were first reprogrammed to pluripotency using lentiviruses expressing Oct4, Sox2, Klf4 and c-Myc in a doxycyclin-dependent fashion. After chimeric animals were generated following injection of these iPS cells into blastocysts, B cells representing various developmental stages were isolated and subjected to reprogramming by doxycyclin-induced reactivation of the four reprogrammed genes. They demonstrated in this system that the cells committed to B lineage (indicated by genomic DNA rearrangement at the immunoglobulin loci) can be reprogrammed by this approach, although the reprogramming of more mature differentiated B cells may require additional factors (e.g., C/EBP- α) [18]. Nonetheless, it provided the proof-of-principle that differentiated cells, such as mouse B cells, can be directed to pluripotency through the overexpression of defined transcription factors.

Several laboratories subsequently reported the reprogramming of human blood cells [12,19–22]. Loh *et al.* reprogrammed CD34⁺ hematopoietic stem/progenitor cells (HSPCs) isolated from the peripheral blood of a healthy donor after granulocyte colony-stimulating factor mobilization, using the retroviruses expressing the four Yamanaka factors [20]. It has been subsequently reported that iPS cells were generated from CB and adult CD34⁺ or CD133⁺ HSPCs (~50% of CD34⁺ cells express CD133) [12,20–22] as well as from CB-derived endothelial cells [19]. We also assessed the feasibility of reprogramming adult bone marrow (BM) CD34⁺ cells that have been frozen for over 9 years. After retroviral transduction and further culture under human ESC culture conditions, iPS-like colonies emerged from blood and BM CD34⁺ cells. Upon further expansion, the iPS clones displayed typical human ESC morphology and pluripotency [21]. These reports of human blood cell-derived iPS cells used

HSPCs. HSPCs that can be enriched by CD34 or CD133 expression have the ability to proliferate and expand transiently in culture. Compared with some other lineage-committed cells (e.g., T or B cells), they can be transduced by retroviruses more efficiently, although the transduction efficiency may not be as high as fibroblastic cells. It is also conceivable that the epigenetic states of these cells are closer to a pluripotent state than other more differentiated lineages. These characteristics make HSPCs a more preferred source for reprogramming of adult cells. Indeed, all of these reports showed that HSPCs can be reprogrammed as efficiently as fibroblast cells. Giorgetti *et al.* have demonstrated that CB CD133⁺ cells can be reprogrammed even with a reduced number of factors (OCT4 and SOX2) [12]. In one study, we were able to generate human iPS cells from CB and adult blood CD34⁺ cells with the *piggyBac* DNA transposon vector that we previously used to reprogram human adult mesenchymal cells [17]. Currently, it is being tested if episomal plasmid vectors can efficiently reprogram human CD34⁺ HSPCs that appear more conducive to derive iPS cells than dermal fibroblasts.

In a separate study with mouse hematopoietic lineages, it has also been shown that HSPCs have a higher reprogramming efficiency than the more differentiated lineages, owing to either the intrinsic epigenetic differences or the fact that HSPCs can be better maintained and expanded in tissue culture conditions [23]. Although terminally differentiated blood cells have been reprogrammed back to iPS cells in the mouse secondary reprogramming system, it has not been demonstrated that more differentiated human blood cells can be reprogrammed at a reasonable efficiency. Similar to what was observed in fibroblast cell reprogramming [24–30], knocking down p53 significantly enhanced the efficiency of generating iPS cells from CD34⁺ CB cells [22], suggesting that the mechanisms underlying reprogramming processes from fibroblasts and blood cells are fundamentally similar. Improved gene delivery and small molecules that facilitate the reprogramming process are likely to be key in enhancing the efficiency of generating iPS cells from more differentiated or unfractionated blood mononuclear cells. Reprogramming blood cells at different stages of lineage commitment would also greatly facilitate basic research of elucidating mechanisms underlying reprogramming and pluripotency. The VD(J) recombination at the genomic DNA level in lymphoid cells provides a convenient marker for determining differentiation stages of the starting cell. Although it is a useful feature for research purposes, caution should be taken before applying iPS cells derived from B or T cells for cell replacement therapy involving blood and immune cells. It is reasonable to predict that immune cells generated from B- or T-derived iPS lines may have a limited repertoire for antigen recognition. Comprehensive studies will be needed to determine how these genomic alterations would affect the overall differentiation potential and safety of iPS cells.

Blood cells generated from iPS cells

Hematopoietic stem/progenitor cells, especially the more primitive HSCs, have been the leading model system to study stem cell biology in the past several decades. Knowledge obtained from the study of HSCs has led to the most successful clinical application of stem cells – BM transplantation (BMT). However, the limited supply of HSCs has significantly hindered the wider application of BMT. The frequency of human HSCs as measured by the only *in vivo* assay (*scid* mouse repopulating capability) is below one in every 500 CD34⁺

cells [31]. The richest sources for CD34⁺ cells that contain both HSCs and HSPCs are CB and BM with approximately 1% [32,33]. The percentage of CD34⁺ cells in the peripheral blood of healthy individuals is much lower than CB and BM. Mobilization (e.g., by granulocyte colony-stimulating factor treatment for 3–5 days) of CD34⁺ cells to peripheral blood is often used to enrich and obtain more HSCs and HSPCs [32]. In addition to the rarity of HSCs, HSC transplantation and research have been limited by the lack of a reproducible protocol to expand human HSCs in culture. However, we are still optimistic that someone will find a solution to achieve this elusive goal, aided by the advent of better understanding of stem cell biology. Ever since human ESC lines were established, it has been anticipated that pluripotent stem cells that can be expanded in culture indefinitely followed by differentiation into hematopoietic cells may serve as unlimited sources for BMT and blood transfusion purposes. The derivation of human iPS cells makes it possible to create patient-specific stem cell lines. This technology, in combination with further optimized hematopoietic differentiation methods, should provide a vital alternative way to store and amplify histocompatible blood stem cells for BMT purposes.

Human ESCs have been instrumental in studying human embryonic development, including blood formation [34,35]. Various hematopoietic cell types, including red blood cells [36–41], dendritic cells [42], monocytes [43], megakaryocytes [44] and lymphoid cells [45] have been reported to be generated from human ESCs and have been recently reviewed [46]. Since iPS cells show a similar pluripotency to ESCs, it is also anticipated that hematopoietic differentiation of iPS cells should replicate, at least to a certain degree, what is observed in ESCs. This was first demonstrated in the mouse system, where fibroblast cells from a sickle cell disease model were first reprogrammed to iPS cells [47]. These iPS cells were then differentiated to hematopoietic cells using a HoxB4 expressing EB formation protocol that has been successful in mouse ESC differentiation. It was shown that the hematopoietic progenitor cells generated from this process can engraft into recipient mice, similar to what is reported previously using mouse ESCs [47,48]. Although the human system is vastly different from the mouse in many ways, preliminary studies from our laboratory and others have shown that human iPS cells can be directed to hematopoietic lineages in a fashion similar to human ESC differentiation [6,21,49–52]. Park *et al.* first demonstrated the generation of hematopoietic colony-forming cells from iPS cells that were originally generated from skin fibroblast cells [6]. Using the serum-containing EB formation protocol, these authors also demonstrated in a separate study that BMP4 supports mesoderm induction and hematopoietic development from human fibroblast-derived iPS cells similar to human ESCs [49]. Choi *et al.* used the OP9 (mouse) stromal co-culture system to study hematopoietic and endothelial differentiation of multiple human iPS cell lines that were derived using slightly different reprogramming cocktails (OCT4, SOX2, NANOG and LIN28) [52]. Their results showed that hematopoietic cells can be generated from all of the iPS cell lines tested, although efficiency varied among iPS cell lines. The hematopoietic potential of the iPS cell lines derived from fetal and neonatal fibroblasts by the integrating viral vectors is similar to that of human ESCs as a group [48]. We assessed the possibility to differentiate iPS cells in serum-free, feeder-free conditions using a chemically defined medium. By sequentially treating iPS-derived EBs with growth factors BMP4, bFGF, VEGF and SCF, a significant percentage of cells expressing hematopoietic progenitor markers

CD34 and CD45 can be obtained. These cells can form both myeloid and erythroid colonies in methylcellulose assays [21]. More recently, Grigoriadis *et al.* reported a step-wise differentiation process in which hematopoietic precursors were generated from human ES and iPS cells following EB formation [50].

These studies have demonstrated in principle that hematopoietic cells can be generated from iPS cells, although no studies have demonstrated the ability of HSCs derived from human ES or iPS cells to reconstitute an immune-deficient mouse to any meaningful extent. The iPS technology, however, is still in its infancy and many questions remain. One key issue that needs to be addressed is the similarity and differences between iPS cells from different origins as well as between iPS cells and ESCs. Genome-wide transcriptome analyses indicated that human iPS cells display distinct gene expression patterns compared with blastocyst-derived human ESCs, and that human fibroblast-derived iPS cells can continue expressing some fibroblast-restricted genes that are inactive in neural stem cell-derived iPS cells and ESCs [53,54]. DNA methylation studies of multiple lines of human ES and iPS cells also showed distinct epigenetic features among these cell lines [55,56]. These distinct epigenetic features may be the result of incomplete reprogramming. It is therefore important to determine whether iPS cells derived from different origins possess epigenetic memories that are inherited from the starting cell types and, more importantly, how much would the origin of iPS cells affect differentiation propensity. We hypothesize that such epigenetic memories do exist and that blood-derived iPS cells may offer advantages for hematopoietic differentiation. Derivation of iPS cells from fibroblast cells as well as blood cells of the same individual would provide an ideal system to test the hypothesis. The information obtained from this type of study will not only improve our understanding of iPS biology but will also provide a foundation to improve current iPS cell differentiation protocols for disease modeling and cell therapy. Once a condition to expand human HSCs in culture is developed, we may be able to reprogram mature blood cells back to HSCs (dedifferentiation) or to reprogram readily available mesenchymal cells to HSCs (transdifferentiation) without the need to go all the way back to a pluripotent state of iPS cells/ESCs.

Human iPS cells & blood disease modeling

In addition to the potential cell replacement therapy, another interesting and more eminent application of iPS cells would be for disease modeling. Patient-specific iPS cells that carry disease-specific genetic information would be invaluable tools for delineating pathogenesis and for drug screening. For this reason, several patient-specific iPS cell lines have been reported shortly after the initial reports of human cell reprogramming [57–62]. We have derived human iPS cell lines containing the homozygous HbS mutation using fibroblasts, which could be used to develop a novel research model for the treatment of sickle cell disease [8]. However, for many other blood diseases, the underlying mutation is acquired and restricted to hematopoietic cells. Therefore, fibroblast- or keratinocyte-derived iPS cells would not contain the disease-related genomic abnormalities (Figure 2). Myeloproliferative disorders (MPDs) are such a group of diseases.

The classic Philadelphia-chromosome (BCR/ABL)-negative MPDs are a heterogeneous group of diseases characterized by increased proliferation of the erythroid, megakaryocyte or granulocyte lineages [63,64]. This group of diseases includes polycythemia vera (PV), essential thrombocytosis and primary myelofibrosis [65]. Unlike chronic myeloid leukemia, in which the Philadelphia chromosome or BCR/ABL's role in pathogenesis had been well studied, the underlying genetic defects in other MPDs were not well defined until the discovery of JAK2-V617F mutations in 2005 [66–71]. It has been reported that this mutation occurs at a frequency close to 95% in PV, 50% in essential thrombocytosis and 58% in primary myelofibrosis [66–73]. However, as with many other significant discoveries, it has raised more questions than it has answered. One of the top questions is why the same mutation attributes to three apparently different phenotypes in patients. There have been various hypotheses, such as that gene dosages (e.g., heterozygous vs homozygous V617F) may affect the stem/progenitor cell behavior [74–77]. In addition to JAK2-V617F, there are other types of mutations that also contribute to the MPD pathogenesis [78–85]. Related to the latter hypothesis are recent reports that support the idea that there are also pre-JAK2 lesion(s) in the patients that predispose them to the acquisition of a JAK2-V617F mutation at the multipotent stem/progenitor cell level [86–88]. Identifying this pre-JAK2 mutation(s) would have tremendous implications in MPD prevention and treatment. Mouse models that overexpress JAK2-V617F mutation by transgenic or adoptive BMT approaches reproduced some phenotypes seen in chronic MPDs [76,77,89–91]. These animal models are likely to be useful for testing anti-JAK2 drugs. However, it is unlikely that the current animal models are adequate for the identification of pre-JAK2-V617F lesions since overexpression of the JAK2-V617F mutant results in an acute MPD disease but lacks diseased clones. Similarly, overexpressing the JAK2-V617F mutated allele in human CD34⁺ cells would unlikely reveal the nature of pre-JAK2 lesions and their relationship to the JAK2 mutation. New approaches to establish a prospective model to study chronic MPDs at a clonal level are needed to understand the underlying pathophysiology and to develop an effective treatment. As the first step of modeling MPDs using pluripotent stem cells, we derived multiple iPS cell lines from the peripheral blood CD34⁺ cells of two MPD patients [21]. These patient-specific cell lines fulfill all the requirements for pluripotency by both *in vitro* and *in vivo* assays. They also carry the JAK2-V617F mutation, as the primary hematopoietic progenitor cells from the corresponding patients. Using the serum-free hematopoietic differentiation protocol, these iPS cell lines have been differentiated to CD34⁺CD45⁺ hematopoietic progenitor cells. Most importantly, the HSPCs generated from the PV patient-specific iPS cells showed enhanced erythropoiesis compared with healthy controls when they were placed into erythroid differentiation conditions, recapitulating the major clinical feature of PV [21]. It is anticipated that other blood diseases, such as paroxysmal nocturnal hemoglobinuria, which also has acquired somatic mutations in the *PIGA* gene, can be modeled by this approach. Combined with improved gene targeting technology that enables us to create or correct a genetic mutation in human iPS and ESCs [92], the iPS approach offers unique opportunities to study the roles of JAK2-V617F and other genetic mutations in acquired and inherited blood disorders.

For modeling other blood diseases affected by germline mutations, the choice of starting cells for reprogramming can be more flexible. However, certain genetic mutations may

negatively regulate the reprogramming process and alternative approaches need to be explored. Fanconi anemia (FA) is an example of such a disease. FA is a recessive chromosomal instability disorder caused by mutation(s) in genes in the FA pathway [93]. The major source of mortality in FA is complications associated with BM failure [94]. Mouse models with a gene knockout in the FA pathway failed to develop BM failure, although these mice do display sensitivity to DNA crosslinking reagents [95]. There is a need to create human disease models to study and treat FA. Raya *et al.* reported the attempt to reprogram skin fibroblast cells from FA patients to generate patient-specific iPS cells [60]. However, all reprogramming attempts from uncomplemented somatic cells failed, presumably caused by the accumulated genomic alterations. As an alternative approach, the authors were able to generate iPS cells from patient fibroblast cells that had been genetically corrected before reprogramming [60]. These FA iPS cells can give rise to hematopoietic progenitors that are phenotypically normal, providing a cell source for potential cell therapy applications. More recently, Tulpule *et al.* reported a different approach to create pluripotent stem cells to model FA [96]. Instead of reprogramming patient somatic cells, these authors used lentiviral RNAi technology to knockdown *FANCA* and *FANCD2*, two genes in the FA pathway. Reduced hematopoiesis from these modified human ESCs was observed and the reduction can be rescued by FA gene complementation [96]. These two studies provide examples of how to model or treat diseases using a pluripotent stem cell approach when the genetic mutation of interest is detrimental to reprogramming.

In addition to generating hematopoietic cells that can recapitulate blood disease features, iPS cells and their hematopoietic progeny will also facilitate the establishment of improved *in vivo* models for many other diseases, such as Type 1 diabetes and liver cirrhosis, in which the immune system plays important roles in disease progression. Currently, most *in vivo* models of human diseases involve immunodeficient mice that are suitable for the engraftment of human cells. However, they could not faithfully recapitulate disease progression or recovery in patients with a functional immune system. Although significant improvements are still needed, the technologies for iPS cell generation and hematopoietic differentiation provide the potential of creating humanized xenograft disease models with auto-logous immune cells and other cell types that are genetically identical.

Conclusion & future perspective

Recent progress in iPS technology has opened up unprecedented opportunities for the development of new strategies to model and treat diseases. The ability to reprogram blood cells and to differentiate iPS cells back to blood cells makes it possible to model blood diseases carrying acquired somatic mutations in hematopoietic systems. The differences between iPS cells generated from different origins are not clear. Future studies on the epigenetic features and differentiation propensities of these different iPS cells will provide valuable information on iPS cell biology. Knowledge obtained from these studies will facilitate the development of safer and more efficient cell therapy.

Acknowledgement

We thank the Cheng lab members and other colleagues for discussions. We regret that we are unable to cite other relevant studies because of space constraints.

The authors are supported by grants from the NIH (T32 HL007525, R01 HL073781, RC2 HL101582 and U01 HL099775) and Maryland Stem Cell Fund/TEDCO (MSCRFII-0047). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Bibliography

Papers of special note have been highlighted as:

■ of interest

■ of considerable interest

- 1 ■ ■. Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126(4):663–676. [PubMed: 16904174] [The landmark paper of induced pluripotent stem (iPS) cell generation from somatic cells.]
2. Okita K, Ichisaka T, Yamanaka S: Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007; 448(7151):313–317. [PubMed: 17554338]
3. Wernig M, Meissner A, Foreman R, et al. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*. 2007; 448(7151):318–324. [PubMed: 17554336]
- 4 ■ ■. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131(5):861–872. [PubMed: 18035408] [One of the first reports on human iPS cell derivation.]
- 5 ■ ■. Yu J, Vodyanik M, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318(5858):1917–1920. [PubMed: 18029452] [One of the first reports on human iPS cell derivation.]
- 6 ■ ■. Park I, Zhao R, West J, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 2008; 451(7175):141–146. [PubMed: 18157115] [One of the first reports on human iPS cell derivation.]
7. Lowry W, Richter L, Yachechko R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc. Natl Acad. Sci. USA*. 2008; 105(8):2883–2888. [PubMed: 18287077]
8. Mali P, Ye Z, Hommond H, et al. Improved efficiency and pace of generating induced pluripotent stem cells from human adult and fetal fibroblasts. *Stem Cells*. 2008; 26(8):1998–2005. [PubMed: 18511599]
9. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat. Biotechnol.* 2008; 26(11):1269–1275. [PubMed: 18849973]
10. Li W, Zhou H, Abujarour R, et al. Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. *Stem Cells*. 2009; 27(12):2992–3000. [PubMed: 19839055]
11. Kim J, Greber B, Araújo-Bravo M, et al. Direct reprogramming of human neural stem cells by OCT4. *Nature*. 2009; 461(7264):649–643. [PubMed: 19718018]
12. Giorgetti A, Montserrat N, Aasen T, et al. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell*. 2009; 5(4):353–357. [PubMed: 19796614]
- 13 ■. Yu J, Hu K, Smuga-Otto K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009; 324(5928):797–801. [PubMed: 19325077] [The first report of virus-free and integration-free human iPS cells.]
14. Kim D, Kim C, Moon J, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell*. 2009; 4(6):472–476. [PubMed: 19481515]
15. Woltjen K, Michael I, Mohseni P, et al. *piggyBac* transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*. 2009; 458(7239):766–770. [PubMed: 19252478]
16. Yusa K, Rad R, Takeda J, Bradley A. Generation of transgene-free induced pluripotent mouse stem cells by the *piggyBac* transposon. *Nat. Methods*. 2009; 6(5):363–369. [PubMed: 19337237]

17. Mali P, Chou B, Yen J, et al. Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. *Stem Cells*. 2010; 28(4):713–720. [PubMed: 20201064]
18. Hanna J, Markoulaki S, Schorderet P, et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell*. 2008; 133(2):250–264. [PubMed: 18423197] [A definitive proof on reprogramming of differentiated cell types using mouse B cells.]
19. Haase A, Olmer R, Schwanke K, et al. Generation of induced pluripotent stem cells from human cord blood. *Cell Stem Cell*. 2009; 5(4):434–441. [PubMed: 19796623]
20. Loh Y, Agarwal S, Park I, et al. Generation of induced pluripotent stem cells from human blood. *Blood*. 2009; 113(22):5476–5479. [PubMed: 19299331] [The first report of human iPS cell lines generated from blood CD34+ cells after granulocyte colony-stimulating factor mobilization.]
21. Ye Z, Zhan H, Mali P, et al. Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders. *Blood*. 2009; 114(27):5473–5480. [PubMed: 19797525] [The first demonstration of iPS cell potential for modeling acquired blood diseases.]
22. Takenaka C, Nishishita N, Takada N, Jakt L, Kawamata S. Effective generation of iPS cells from CD34+ cord blood cells by inhibition of p53. *Exp. Hematol*. 2010; 38(2):154–162. [PubMed: 19922768]
23. Eminli S, Foudi A, Stadtfeld M, et al. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat. Genet*. 2009; 41(9):968–976. [PubMed: 19668214] [A comprehensive study on reprogramming potentials of different types of mouse hematopoietic cells.]
24. Hong H, Takahashi K, Ichisaka T, et al. Suppression of induced pluripotent stem cell generation by the p53–p21 pathway. *Nature*. 2009; 460(7259):1132–1135. [PubMed: 19668191]
25. Kawamura T, Suzuki J, Wang Y, et al. Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature*. 2009; 460(7259):1140–1144. [PubMed: 19668186]
26. Li H, Collado M, Villasante A, et al. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature*. 2009; 460(7259):1136–1139. [PubMed: 19668188]
27. Marión R, Strati K, Li H, et al. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature*. 2009; 460(7259):1149–1153. [PubMed: 19668189]
28. Utikal J, Polo J, Stadtfeld M, et al. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature*. 2009; 460(7259):1145–1148. [PubMed: 19668190]
29. Banito A, Rashid S, Acosta J, et al. Senescence impairs successful reprogramming to pluripotent stem cells. *Genes Dev*. 2009; 23(18):2134–2139. [PubMed: 19696146]
30. Hanna J, Saha K, Pando B, et al. Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature*. 2009; 462(7273):595–601. [PubMed: 19898493]
31. Bhatia M, Bonnet D, Kapp U, Wang J, Murdoch B, Dick J. Quantitative analysis reveals expansion of human hematopoietic repopulating cells after short-term *ex vivo* culture. *J. Exp. Med*. 1997; 186(4):619–624. [PubMed: 9254660]
32. Steen R, Tjønnfjord G, Egeland T. Comparison of the phenotype and clonogenicity of normal CD34+ cells from umbilical cord blood, granulocyte colony-stimulating factor-mobilized peripheral blood, and adult human bone marrow. *J. Hematother*. 1994; 3(4):253–262. [PubMed: 7537606]
33. Traycoff C, Abboud M, Laver J, et al. Evaluation of the *in vitro* behavior of phenotypically defined populations of umbilical cord blood hematopoietic progenitor cells. *Exp. Hematol*. 1994; 22(2):215–222. [PubMed: 7507862]
34. Murry C, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*. 2008; 132(4):661–680. [PubMed: 18295582]
35. Orkin S, Zon L. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*. 2008; 132(4):631–644. [PubMed: 18295580]
36. Qiu C, Hanson E, Olivier E, et al. Differentiation of human embryonic stem cells into hematopoietic cells by coculture with human fetal liver cells recapitulates the globin switch that occurs early in development. *Exp. Hematol*. 2005; 33(12):1450–1458. [PubMed: 16338487]

37. Chang K, Nelson A, Cao H, et al. Definitive-like erythroid cells derived from human embryonic stem cells coexpress high levels of embryonic and fetal globins with little or no adult globin. *Blood*. 2006; 108(5):1515–1523. [PubMed: 16645170]
38. Olivier E, Qiu C, Velho M, Hirsch R, Bouhassira E. Large-scale production of embryonic red blood cells from human embryonic stem cells. *Exp. Hematol*. 2006; 34(12):1635–1642. [PubMed: 17157159]
39. Lu S, Feng Q, Park J, et al. Biologic properties and enucleation of red blood cells from human embryonic stem cells. *Blood*. 2008; 112(12):4475–4484. [PubMed: 18713948]
40. Ma F, Ebihara Y, Umeda K, et al. Generation of functional erythrocytes from human embryonic stem cell-derived definitive hematopoiesis. *Proc. Natl Acad. Sci. USA*. 2008; 105(35):13087–13092. [PubMed: 18755895]
41. Qiu C, Olivier E, Velho M, Bouhassira E. Globin switches in yolk sac-like primitive and fetal-like definitive red blood cells produced from human embryonic stem cells. *Blood*. 2008; 111(4):2400–2408. [PubMed: 18024790]
42. Zhan X, Dravid G, Ye Z, et al. Functional antigen-presenting leucocytes derived from human embryonic stem cells *in vitro*. *Lancet*. 364(9429):163–171. [PubMed: 15246729]
43. Karlsson K, Cowley S, Martinez F, Shaw M, Minger S, James W. Homogeneous monocytes and macrophages from human embryonic stem cells following coculture-free differentiation in M-CSF and IL-3. *Exp. Hematol*. 2008; 36(9):1167–1175. [PubMed: 18550257]
44. Takayama N, Nishikii H, Usui J, et al. Generation of functional platelets from human embryonic stem cells *in vitro* via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors. *Blood*. 2008; 111(11):5298–5306. [PubMed: 18388179]
45. Woll P, Grzywacz B, Tian X, et al. Human embryonic stem cells differentiate into a homogeneous population of natural killer cells with potent *in vivo* antitumor activity. *Blood*. 2009; 113(24):6094–6101. [PubMed: 19365083]
46. Kaufman D. Toward clinical therapies utilizing hematopoietic cells derived from human pluripotent stem cells. *Blood*. 2009; 114:3513–3523. [PubMed: 19652198]
47. Hanna J, Wernig M, Markoulaki S, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science*. 2007; 318(5858):1920–1923. [PubMed: 18063756]
48. Kyba M, Perlingeiro R, Daley G. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. *Cell*. 2002; 109(1):29–37. [PubMed: 11955444]
49. Lengerke C, Grauer M, Niebuhr N, et al. Hematopoietic development from human induced pluripotent stem cells. *Ann. NY Acad. Sci*. 2009; 1176:219–227. [PubMed: 19796250]
50. Grigoriadis A, Kennedy M, Bozec A, et al. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells. *Blood*. 2010; 115(14):2769–2776. [PubMed: 20065292]
51. Choi K, Vodyanik M, Slukvin I. Generation of mature human myelomonocytic cells through expansion and differentiation of pluripotent stem cell-derived lin-CD34⁺CD43⁺CD45⁺ progenitors. *J. Clin. Invest*. 2009; 119(9):2818–2829. [PubMed: 19726877]
52. Choi K, Yu J, Smuga-Otto K, et al. Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. *Stem Cells*. 2009; 27(3):559–567. [PubMed: 19259936]
53. Chin M, Mason M, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 2009; 5(1):111–123. [PubMed: 19570518] [One of the first gene expression profile studies comparing multiple lines of iPS cells to embryonic stem cells.]
54. Marchetto M, Yeo G, Kainohana O, Marsala M, Gage F, Muotri A. Transcriptional signature and memory retention of human-induced pluripotent stem cells. *PLoS One*. 2009; 4(9):e7076. [PubMed: 19763270]
55. Doi A, Park I, Wen B, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat. Genet*. 2009; 41(12):1350–1353. [PubMed: 19881528]
56. Lister R, Pelizzola M, Dowen R, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*. 2009; 462(7271):315–322. [PubMed: 19829295]

57. Dimos J, Rodolfa K, Niakan K, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008; 321(5893):1218–1221. [PubMed: 18669821]
58. Park I, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008; 134(5): 877–886. [PubMed: 18691744]
59. Ebert A, Yu J, Rose FJ, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*. 2009; 457(7227):277–280. [PubMed: 19098894]
60. Raya A, Rodríguez-Pizà I, Guenechea G, et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature*. 2009; 460(7251):53–59. [PubMed: 19483674]
61. Ye L, Chang J, Lin C, Sun X, Yu J, Kan Y. Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases. *Proc. Natl Acad. Sci. USA*. 2009; 106(24):9826–9830. [PubMed: 19482945]
62. Wang Y, Jiang Y, Liu S, Sun X, Gao S. Generation of induced pluripotent stem cells from human β -thalassemia fibroblast cells. *Cell Res*. 2009; 19(9):1120–1123. [PubMed: 19690515]
63. Skoda R. The genetic basis of myeloproliferative disorders. *Hematology Am. Soc. Hematol. Educ. Program*. 2007:1–10. [PubMed: 18024602]
64. Levine R, Gilliland D. Myeloproliferative disorders. *Blood*. 2008; 112(6):2190–2198. [PubMed: 18779404]
65. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood*. 1951; 6(4):372–375. [PubMed: 14820991]
66. Baxter E, Scott L, Campbell P, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 365(9464):1054–1061. [PubMed: 15781101]
67. James C, Ugo V, Le Couédic J, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005; 434(7037):1144–1148. [PubMed: 15793561]
68. Kralovics R, Passamonti F, Buser A, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N. Engl. J. Med*. 2005; 352(17):1779–1790. [PubMed: 15858187]
69. Levine R, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005; 7(4):387–397. [PubMed: 15837627]
70. Zhao R, Xing S, Li Z, et al. Identification of an acquired JAK2 mutation in polycythemia vera. *J. Biol. Chem*. 2005; 280(24):22788–22792. [PubMed: 15863514]
71. Tefferi A, Lasho T, Gilliland G. JAK2 mutations in myeloproliferative disorders. *N. Engl. J. Med*. 2005; 353(13):1416–1417. author reply 1416–1417. [PubMed: 16192494]
72. Moliterno A, Williams D, Rogers O, Spivak J. Molecular mimicry in the chronic myeloproliferative disorders: reciprocity between quantitative JAK2 V617F and Mpl expression. *Blood*. 2006; 108(12):3913–3915. [PubMed: 16912229]
73. Jones A, Kreil S, Zoi K, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood*. 2005; 106(6):2162–2168. [PubMed: 15920007]
74. Dupont S, Massé A, James C, et al. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. *Blood*. 2007; 110(3):1013–1021. [PubMed: 17389763]
75. Scott L, Scott M, Campbell P, Green A. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. *Blood*. 2006; 108(7): 2435–2437. [PubMed: 16772604]
76. Tiedt R, Hao-Shen H, Sobas M, et al. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood*. 2008; 111(8):3931–3940. [PubMed: 18160670]
77. Xing S, Wanting T, Zhao W, et al. Transgenic expression of JAK2V617F causes myeloproliferative disorders in mice. *Blood*. 2008; 111(10):5109–5117. [PubMed: 18334677]
78. Kralovics R, Stockton D, Prchal J. Clonal hematopoiesis in familial polycythemia vera suggests the involvement of multiple mutational events in the early pathogenesis of the disease. *Blood*. 2003; 102(10):3793–3796. [PubMed: 12829587]

79. Bellanné-Chantelot C, Chaumarel I, et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. *Blood*. 2006; 108(1):346–352. [PubMed: 16537803]
80. Campbell P, Baxter E, Beer P, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. *Blood*. 2006; 108(10):3548–3555. [PubMed: 16873677]
81. Kralovics R, Teo S, Li S, et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood*. 2006; 108(4):1377–1380. [PubMed: 16675710]
82. Levine R, Belisle C, Wadleigh M, et al. X-inactivation-based clonality analysis and quantitative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. *Blood*. 2006; 107(10):4139–4141. [PubMed: 16434490]
83. Pardanani A, Fridley B, Lasho T, Gilliland D, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. *Blood*. 2008; 111(5):2785–2789. [PubMed: 18006699]
84. Pietra D, Li S, Brisci A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. *Blood*. 2008; 111(3):1686–1689. [PubMed: 17984312]
85. Kiladjian J, Elkassar N, Cassinat B, et al. Essential thrombocythemias without V617F JAK2 mutation are clonal hematopoietic stem cell disorders. *Leukemia*. 2006; 20(6):1181–1183. [PubMed: 16598303]
86. Kilpivaara O, Mukherjee S, Schram A, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat. Genet.* 2009; 41(4):455–459. [PubMed: 19287384]
87. Olcaydu D, Harutyunyan A, Jäger R. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat. Genet.* 2009; 41(4):450–454. [PubMed: 19287385]
88. Jones A, Chase A, Silver R, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat. Genet.* 2009; 41(4):446–449. [PubMed: 19287382]
89. Wernig G, Mercher T, Okabe R, Levine R, Lee B, Gilliland D. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. *Blood*. 2006; 107(11):4274–4281. [PubMed: 16478879]
90. Lacout C, Pisani D, Tulliez M, Gachelin F, Vainchenker W, Villeval J. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood*. 2006; 108(5):1652–1660. [PubMed: 16670266]
91. Zaleskas V, Krause D, Lazarides K, et al. Molecular pathogenesis and therapy of polycythemia induced in mice by JAK2 V617F. *PLoS One*. 2006; 1:e18. [PubMed: 17183644]
92. Zou J, Maeder M, Mali P, et al. Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell Stem Cell*. 2009; 5(1):97–110. [PubMed: 19540188] [The first report of gene targeting in human iPS cells.]
93. D'Andrea A, Grompe M. The Fanconi anaemia/BRCA pathway. *Nat. Rev. Cancer*. 2003; 3(1):23–34. [PubMed: 12509764]
94. Tischkowitz M, Dokal I. Fanconi anaemia and leukaemia – clinical and molecular aspects. *Br. J. Haematol.* 2004; 126(2):176–191. [PubMed: 15238138]
95. Parmar K, D'Andrea A, Niedernhofer L. Mouse models of Fanconi anemia. *Mutat. Res.* 2009; 668(1–2):133–140. [PubMed: 19427003]
96. Tulpule A, Lensch M, Miller J, et al. Knockdown of Fanconi anemia genes in human embryonic stem cells reveals early developmental defects in the hematopoietic lineage. *Blood*. 2010; 115(17):3453–3462. [PubMed: 20089964]

Executive summary

Blood cells as a source for reprogramming

- Human blood CD34⁺ cells can be reprogrammed to pluripotency at compatible efficiencies to fibroblast reprogramming.
- It is less invasive to obtain blood and easy to prepare blood cells for reprogramming.
- Cord blood cells represent potential sources for establishing induced pluripotent stem (iPS) cell banks.

Blood cells generated from iPS cells

- Several protocols have been established to direct human iPS cells to hematopoietic cell types.
- Patient-specific iPS cells may provide alternative sources for histocompatible blood stem cells for bone marrow transplantation.
- Blood-derived iPS cells may offer advantages for hematopoietic differentiation owing to potential epigenetic memory.

Human iPS cells & blood disease modeling

- Acquired blood disorders containing hematopoietic-restricted somatic mutations can be modeled by blood cell-derived iPS cells.
- Fibroblast or other cell types can be used to derive blood disease-specific iPS cells if the mutation is inherited, such as in sickle cell anemia and Fanconi anemia.
- Certain genetic abnormalities may interfere with the reprogramming process; alternative approaches need to be explored.

Figure 1. Derivation of induced pluripotent stem cells from (A) skin biopsy or (B) peripheral blood

When the skin biopsy approach is used (A), fibroblastic and other adherent cells are established in culture after several weeks. Proliferating cells are then used as a source of iPS cell derivation. (B) Nucleated cells from blood or marrow after fractionation are cultured for 2–4 days and then used directly for reprogramming. The selected iPS cell clones can be expanded indefinitely while retaining their pluripotency. Upon induction of differentiation, iPS cells can generate any cell types such as neurons, cardiomyocytes, hepatocytes as well as white and red blood cells. They will likely be useful for disease modeling, toxicology tests and drug screening. After safety validation, patient-specific iPS cell-derived functional progeny could be used for novel cell therapies. iPS: Induced pluripotent stem.

Figure 2. Derivation of induced pluripotent stem cells from peripheral blood and other tissues of the same patients with an acquired blood disorder

(A) Fibroblastic cells are established from skin biopsy or bone marrow stromal samples. (B) Mononuclear hematopoietic cells from blood or bone marrow after fractionation are cultured for reprogramming. If a genetic mutation is present in the blood cells (illustrated by a small circle at the dsDNA), the derived iPS cells will contain the same mutation. Upon induction of differentiation, iPS cells can generate white and red blood cells. The paired iPS cell lines and their progeny (one with and one without the mutation) may provide novel research systems for disease modeling to investigate pathogenesis and to screen new drugs. iPS: Induced pluripotent stem.