Application of induced pluripotent stem cell technology for the investigation of hematological disorders

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Abstract

Induced pluripotent stem cells (iPSCs) were first described over a decade ago and are currently used in various basic biology and clinical research fields. Recent advances in the field of human iPSCs have opened the way to a better understanding of the biology of human diseases.

Disease-specific iPSCs provide an unparalleled opportunity to establish novel human cell-based disease models, with the potential to enhance our understanding of the molecular mechanisms underlying human malignancies, and to accelerate the identification of effective new drugs. When combined with genome editing technologies, iPSCs represent a new approach to study single or multiple disease-causing mutations and model specific diseases *in vitro*. In addition, genetically corrected patient-specific iPSCs could potentially be used for stem cell based therapy. Furthermore, the reprogrammed cells share patient-specific genetic background, offering a new platform to develop personalized therapy/medicine for patients.

In this review we discuss the recent advances in iPSC research technology and their potential applications in hematological diseases. Somatic cell reprogramming has presented new routes for generating patient-derived iPSCs, which can be differentiated to hematopoietic stem cells and the various downstream hematopoietic lineages. iPSC technology shows promise in the modeling of both inherited and acquired hematological disorders. A direct reprogramming and differentiation strategy is able to recapitulate hematological disorder progression and capture the earliest molecular alterations that underlie the initiation of hematological malignancies.

Keywords: induced pluripotent stem cells; hematological disorders; myelodysplastic syndromes; mutations; reprogramming; drug discovery

1. **Introduction**

Embryonic stem cells (ESCs) derived from the inner cell mass of mammalian blastocysts, have the potential to grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (ectoderm, endoderm and mesoderm) (Evans and Kaufman, 1981). The potential for their use in the study of human disease, drug discovery and transplantation medicine was first reported by Thomson and colleagues (Thomson et al., 1998). However, ethical issues associated with the use of human embryos, as well as the potential problems with tissue rejection, led researchers to look for ways to generate pluripotent cells directly from patient somatic cells.

Somatic cells were first reprogrammed by transferring their nuclear contents into oocytes (Gurdon, 1962a) and also by fusing their nuclear contents into ESCs (Tada et al., 2001), indicating that unfertilized oocytes and ESCs contain factors that can confer pluripotency to somatic cells.

In 2006, Takahashi and Yamanaka showed that terminally differentiated somatic cells (fibroblasts) can be reprogrammed to pluripotent stem cells that have a similar range of properties including gene expression profile and differentiation propensity (three germ layers) to ESCs, by expressing four transcription factors - *OCT4*, *SOX2*, *KLF4* and *cMYC* (OSKM factors) - in mouse cells (Takahashi and Yamanaka, 2006). Cells generated using this method were termed induced pluripotent stem cells (iPSCs). One year later, two research groups independently reported the generation of iPSCs from human somatic cells (Takahashi et al., 2007; Yu et al., 2007) by using different combinations of transcription factors.

Advances in the field of reprogramming somatic cells to human iPSCs have been numerous. These include reprogramming methods that do not require genomic integration of the reprogramming factors, such as episomal, mRNA and protein forms of the OSKM factors (Table 1), improvements in the reprogramming efficiency through the use of small molecules (Table 1) and progress in culture of viable iPSCs in xeno-free, serum-free and feeder-free conditions (Bell et al., 2018; Halaidych et al., 2018). Such technological advances have led to exciting research in the field of pluripotent stem cells, particularly for disease modeling, drug discovery and regenerative medicine/ stem cell based therapy. In addition, advances in the field of genetic engineering are enabling the generation of genetically defined human iPSCs to recapitulate disease model platforms *in vitro* as well as stem cell-based therapy.

The use of iPSC holds great promise for human disease modeling, drug discovery and stem cell-based therapy and the potential is only beginning to be realized with the advances in iPSC technology. We provide an overview of the progress in each of these applications and demonstrate how they have influenced the study of both inherited and acquired hematological disorders.

2. Reprogramming methods

Attempts to reprogram somatic cells date back to the 1960s, when Gurdon successfully transferred a nucleus from an endoderm cell to an enucleated *Xenopus laevis* oocyte in order to develop adult frogs (Gurdon, 1962a; Gurdon and Uehlinger, 1966). In 2001, Tada et al. established a new method to reprogram somatic cells by fusion with ESCs (Tada et al., 2001).

Both techniques were limited in their application potential for research and future therapy due to ethical issues associated with using human embryos (Li et al., 2017).

The development of iPSCs by Takahashi and Yamanaka in 2006 opened up new opportunities to overcome these issues. In their initial experiment of iPSC development, they used retrovirus for ectopic delivery of the OSKM transcription factors into somatic cells (Takahashi and Yamanaka, 2006). They reported about 20 retroviral integration sites which were unique for every iPSC clone (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). In addition, others have discovered that alternative combination of reprogramming factors such as, OCT4, SOX2, NANOG, and LIN28, can also generate iPSCs (Yu et al., 2007).

A number of reprogramming methods can be used to deliver reprogramming factors into cells. These methods are broadly divided into integrative systems and non-integrative systems. In light of the integrating vectors used in the original study, various reprogramming methods have been developed, including the use of integrating vectors with inducible promoters in order to control the expression of the iPSC reprogramming transcription factors, and of integrating vectors coupled with the Cre/loxP system to excise the transgene after the reprogramming has occurred (Soldner et al., 2009). These methods were developed in order to increase the safety, efficiency and quality of the iPSCs (Hussein and Nagy, 2012). However, integrative delivery systems come with an inherent risk of insertional mutagenesis which largely limits their therapeutic application.

Non-integrative methods have the advantage of lacking insertional mutagenesis, but show lower reprogramming efficiencies than integrating vectors (Gonzalez et al., 2011). Examples of non-integrative viral systems for reprogramming are adenovirus (Zhou and Freed, 2009) and Sendai virus (SeV) (Ban et al., 2011; Seki et al., 2012). However, the reprogramming efficiency with adenovirus is very low (Stadtfeld et al., 2008) and their production is labor intensive. SeV is a negative strand single-stranded RNA virus that does not enter the cell nucleus and therefore minimizes the risk of integration. Additionally, SeVs are very efficient in transferring genes into a wide range of somatic cells (Nakanishi and Otsu, 2012), making them an attractive method for reprogramming cells. Furthermore, use of non-integrative non-viral methods such as synthetic mRNA, recombinant protein (Kim et al., 2009) and synthetic miRNA has been shown to reprogram human somatic cells (Miyoshi et al., 2011). Recently, a novel CRISPR-based gene activation (CRISPRa) tool has been adapted as a reprogramming technique to induce activation of endogenous transcription factors. Use of CRISPRa enables direct targeting of endogenous loci of the reprogramming factors and has the benefit of high multiplexing capacity (Weltner et al., 2018).

Overall, the low risk of insertional mutagenesis makes non-integrative methods a more appealing option for iPSC generation, both in disease modeling and in therapeutic settings.

In addition to reprogramming methods, Huangfu et al. showed that small molecules such as valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, can enhance reprogramming efficiency by a 100-fold (Huangfu et al., 2008). Table 1 provides an overview of established and novel reprogramming methods.

3. Potential applications of iPSCs

Progress in the field of iPSCs has opened up new avenues for research in therapeutics. iPSCs are currently being used for disease modeling, drug screening and in regenerative medicine/ stem cell-based therapy.

3.1 Disease modeling

Conventional disease modeling using animal models and/or cell lines, has been widely used to study disease pathology and for therapeutic development (Hasegawa et al., 2018; Kim, 2015).

Transgenic animal models have been created and used in order to recapitulate human diseases for decades (Barre-Sinoussi and Montagutelli, 2015). However, not all results obtained from animal models can be directly translated to humans. There are a number of possible explanations for this lack of overlap. Firstly, differences exist between a given animal species and humans. For example, while mouse and human have homology of 95% in their genome, there are differences in the members of gene families, in gene redundancies and in the regulation of gene expression level (Barre-Sinoussi and Montagutelli, 2015). Secondly, each species or closely related species can have variation in their physiological responses owing to their genetic background. For example, laboratory mice are inbred strains with highly homogeneous genetic composition and hence only provide results in a given genetic background, limiting their generalizability (Barre-Sinoussi and Montagutelli, 2015).

Cell lines have also been used for decades in research to study human diseases in culture. Human cell lines are generated from individual patients with specific diseases (Risbridger, 2015). However, cell lines with a high passage number can acquire other genetic mutations in culture and no longer represent the true tumor genetic background (Wilding and Bodmer, 2014).

iPSC technology provides unprecedented possibilities to model human disease *in vitro*. Reprogramming patient cells into a pluripotent state followed by differentiation into disease-specific cell types can generate an unlimited source of human tissue carrying the genetic variations that caused or facilitated disease development (Soldner and Jaenisch, 2012).

iPSCs have been generated from a wide range of hematological diseases, both inherited (e.g. Fanconi anemia, Shwachman-Diamond syndrome, sickle cell anemia, and thalassemia) and acquired (e.g. myeloproliferative neoplasms and myelodysplastic syndromes) and have been used recently as disease models in culture (Arai et al., 2015; Kotini et al., 2017).

3.2 Drug screening

Human ESCs are considered to be a valuable *in vitro* model for drug screening due to their capacity for indefinite self-renewal and their pluripotent nature (Thomson et al., 1998). However, ethical issues surrounding the origin of these cells from pre-implantation human embryos have hindered their use in drug discovery (Robertson, 2001).

The breakthrough development of iPSCs (Takahashi et al., 2007; Takahashi and Yamanaka, 2006) and their similarities to ESCs at the molecular and genetic level, and in terms of their morphology and functions (Chin et al., 2010; Lowry et al., 2008) have provided a new platform for developing human disease models *in vitro* and for drug screening (Chang et al., 2018; Kotini et al., 2017; Yazawa et al., 2011). Disease-specific iPSCs from acquired or

inherited diseases have also been used for drug screening (Chang et al., 2018; Kotini et al., 2017).

Patient-derived iPSCs offer a unique vantage point for in-depth mechanistic understanding and dissection of specific genetic lesions that give rise to disease phenotypes. Owing to their pluripotency, they are an inexhaustible and a scalable source of cells that recapitulate the genetic environment of the cell of origin i.e., cells showing the disease phenotype (Papapetrou, 2016). Their large scale expansion can be harnessed to study disease mechanisms and iPSCs can be utilized as cellular material for high throughput drug screens.

The ESC-like characteristics of iPSCs allow them to be differentiated into various cell types in response to chemical cues. A number of studies have demonstrated that cell types related to the respective disorder can be derived from disease-specific iPSCs and have been shown to recapitulate disease phenotypes (Jang et al., 2012). Although some studies have shown differences between iPSCs and ESCs at the transcriptional and functional levels (Hanna et al., 2009; Ohi et al., 2011), iPSCs have been proven to be a valuable tool for *in vitro* drug discovery (Chang et al., 2018).

One of the first drug screening studies using iPSC was carried out in Rett syndrome (Marchetto et al., 2010). The research team developed a culture system using iPSC from patient fibroblasts, generating functional neurons showing similar phenotype (reduced spine density and smaller soma size) to Rett syndrome *in vivo*. They subsequently used iPSC-derived RTT neurons in culture to test the effects of drugs in rescuing synaptic defects (Marchetto et al., 2010). Nowadays scientists commonly screen disease-specific iPSCs (or derived cell lineages) with drug panels to discover new drugs and to demonstrate their efficacy (Avior et al., 2016; Chang et al., 2018; Lam and Wu, 2018; Ooi et al., 2013).

One of the most exciting ideas emerging is the use of iPSC technology in personalized medicine, where they may allow the use of drugs tailored according to each patient's genetic background, environment, and lifestyle (Sayed et al., 2016).

3.3 Regenerative medicine/ stem cell based therapy

Lack of donor availability, as well as immune rejections, limit the use of stem cell based therapy in its current form, raising the demand for new approaches. The potential use of iPSCs in regenerative medicine to replace damaged tissues or activate endogenous regenerative processes is widely considered to be a promising future application to overcome these problems (Singh et al., 2015).

Transplantation of hematopoietic stem cells (HSCs) has become the standard treatment for a number of hereditary diseases and blood disorders (Li et al., 2017). In autologous stem cell transplantation, patient cells are expanded *ex vivo* and reinjected into the same patient reducing the risk of immune rejection (Shi et al., 2017), unlike allogeneic stem cell transplantation, where stem cells from healthy donors are transplanted into patients (Singh et al., 2015).

When combined with genome editing technologies for the correction of disease-causing mutations, iPSCs offer a new possibility for therapy. Genetically corrected patient-specific iPSCs could be expanded, differentiated into disease cell type and re-introduced into patients (Paes et al., 2017). Hanna et al. were the first to show proof of principle for iPSC-based

regenerative medicine. They reprogrammed fibroblasts derived from a humanized sickle cell anemia mouse model, corrected the disease-causing mutation by homologous recombination, differentiated the iPSCs into hematopoietic progenitors and transplanted these back into the sickle cell anemia mouse model. A stable and significant increase in levels of human β globin protein A (HbA) and pronounced reduction in human β globin protein S (HbS) were observed, thus showing a reversal of sickle cell phenotype in treated mice (Hanna et al., 2007).

Another novel and exciting area for the use of iPSCs is immunotherapy with cytotoxic T cells. Limited amount of T cells have been produced *in vitro* for clinical application (Migliaccio et al., 2005). Use of iPSC-derived T cells could overcome this limitation since iPSCs can be expanded indefinitely (Paes et al., 2017). To avoid random genomic rearrangements of the T cell receptor (TCR) that occur during differentiation (Timmermans et al., 2009), iPSCs derived from T cells expressing a specific TCR can be used. Vizcardo et al. were the first to show that T cells which are derived from T cell-derived iPSCs express the same TCR as the donor T cell (Vizcardo et al., 2013). As TCRs only recognize antigens presented by a specific human leukocyte antigen (HLA), it is necessary for allogeneic clinical application that the iPSC-derived T cells match the recipient HLA. To overcome this problem and to ensure antigen specificity, Themeli et al. introduced a chimeric antigen receptor (CAR) acting independently from HLA into T cell-derived iPSCs (Themeli et al., 2013). These studies show that functional T cells can be derived from iPSCs and their use might be applicable more widely in immunotherapy in the near future (Kawamoto et al., 2018).

4. Clinical Trials of iPSCs

Despite numerous advances in iPSCs technology following its initial discovery in 2006, there are only a few clinical trials that have taken place. This is due to risks associated with using iPSCs in the treatment of human diseases (e.g. risk of tumorigenicity, genetic aberrations, and contamination with viruses and bacteria during the process of iPSC establishment) (Takashima et al., 2018).

In 2014, Takashima et al. used iPSCs in the first-in-human (FIH) trial in the form of a sheet of retinal pigment epithelial (RPE) cells that were transplanted into a patient suffering from neovascular age-related macular degeneration (AMD) (Takashima et al., 2018). One year after the surgery there were no signs of complications or proliferation and the graft had not shown any signs of rejection. In the same trial, however, a second patient was treated with allogenic iPSC-derived HLA-matched cells, as his own iPSC-derived retinal cells showed three copy number variations (Ilic et al., 2015; Martin, 2017).

In 2018, Professor Y Sawa (Osaka University) plans to begin treating heart disease patients by grafting iPSC derived cell sheet onto diseased myocardia. This method will be used for treating three patients over the next year (Cyranoski, 2018). Also in 2018, Professor J Takahashi (Kyoto University) announced the launch of a new clinical trial which involves injecting iPSC-derived dopaminergic progenitor cells into the substantia nigra of patients suffering from Parkinson's disease (Normile, 2018).

5. Genome editing

Over the past decade, a number of genome editing tools have emerged, including zinc finger nucleases (ZFNs), transcription activator like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9. All three technologies work by introducing double strand DNA breaks (DSBs) in the genome and the cellular repair mechanism initiates a repair of the DSBs. Genome editing in iPSCs plays an important role in disease modeling by the introduction of specific mutations in cells, and in regenerative medicine for the correction of mutations in patient-derived iPSCs.

5.1 ZFNs

ZFNs consist of a DNA binding domain and a DNA cleavage (nuclease) domain. The DNA binding domain is made of three to six zinc-fingers which bind specifically to three DNA base pairs each and these can be designed to bind to the desired target region of the genome. The nuclease domain is derived from the FokI restriction enzyme which dimerizes in order to introduce a DSB (Bitinaite et al., 1998; Pellagatti et al., 2016).

ZFNs have been used in iPSCs for disease modeling and cell-based therapy (Song and Ramakrishna, 2018). Various hematological disease-associated mutations including mutations in sickle cell anemia (Zou et al., 2011) have been corrected in iPSCs using ZFNs. Furthermore, ZFNs are also being used in an ongoing clinical trial to mutate the main co-receptor for HIV, *CCR5*, in T-cells to generate HIV resistance (Li et al., 2013; Tebas et al., 2014; Wang et al., 2015).

5.2 TALENs

TALENs works in a similar way to ZFNs. The DNA-binding domain is made of 33-35 amino acid repeats containing two variable amino acids at position 12 and 13 which render site-specificity. The DNA-binding domain is fused to a FokI nuclease domain which dimerizes in order to introduce a DSB (Pellagatti et al., 2016).

Like ZFNs, TALENs have been used widely in iPSCs to correct hematological disease-associated mutations such as mutations causing β-Thalassemia (Ma et al., 2013) and Hemophilia A (Wu et al., 2016).

5.3 CRISPR/Cas9

CRISPR/Cas9 technology is derived from a microbial adaptive immune system that uses RNA-guided nucleases to cleave foreign genetic elements with the aid of the Cas9 nuclease. The system uses a guide RNA to target the Cas9 nuclease to a specific 20nt genomic sequence loci upstream of a “protospacer adjacent motif” (PAM), which can take the form of NGG or NAG (Hsu et al., 2014). When it is bound, the Cas9 nuclease introduces a DSB which is repaired either by imperfect non-homologous end joining (NHEJ) to generate insertions or deletions (indels) (Barnes, 2001) or by homology directed repair (HDR) when a repair template is provided (Pellagatti et al., 2016; Ran et al., 2013).

The CRISPR/Cas9 system has been used for gene correction in β-Thalassemia patient iPSCs (Xie et al., 2014).

6. iPSCs in Hematological Disorders

Hematological disorders primarily affect the bone marrow. Bone marrow aspiration is an invasive procedure and in some diseases, the bone marrow may be hypoplastic at the time of diagnosis. Additionally, primary HSCs have a limited expansion potential and are often a heterogeneous population. These drawbacks severely limit access to primary tissue samples in quantities sufficient for functional studies. Therefore patient-derived iPSCs are an important alternative for the study of disease in culture. In particular, as iPSCs have the potential to differentiate into every cell of the hematopoietic lineage (Table 2), cell types relevant for a specific disease can be generated to recapitulate in culture a specific-disease environment to study. Below, we describe the role of iPSCs for the study of inherited and acquired hematological disorders.

6.1 iPSCs in inherited hematological disorders

There are several inherited hematological disorders, where HSC transplantation remains the only definitive treatment. However, HSC transplantation as a therapeutic modality is severely limited by availability of histocompatible donors and the risk associated with allogenic transplants. If HSCs can be routinely and safely derived from iPSCs *in vitro*, it is anticipated that these autologous HSCs could serve as the basis for curative treatment. Our focus here is aimed at red blood cell disorders and bone marrow failure syndromes where iPSC technology could serve as a viable therapeutic option, namely Sickle cell disease, β-thalassemia, Pyruvate kinase deficiency, Fanconi anemia and Shwachman-Diamond syndrome.

6.1.1 Red blood cell disorders

6.1.1.1 Sickle cell disease

Sickle cell disease (SCD) is one of the most common inherited monogenic diseases and results from a homozygous single base substitution i.e., A>T in the β-globin (*HBB*) gene, leading to a glutamic acid (polar amino acid) to a valine (non-polar amino acid) change in codon 6 of *HBB*. This shift leads to a defective form of adult hemoglobin (Malowany and Butany, 2012). The use of gene therapy to correct the disease phenotype has had limited efficacy and furthermore, hydroxyurea remains the only disease-altering and clinically approved drug for SCD, to date (Agrawal et al., 2014). Hence, there is room for novel therapeutic modalities that could alter the natural history of the disease.

A multitude of studies have been successful in deriving iPSCs from patients with SCD (Chou et al., 2011; Junqueira Reis et al., 2017; Li et al., 2011; Mali et al., 2010; Mali et al., 2008; Sebastiano et al., 2011; Ye et al., 2009) using a variety of starting cell types, that include skin fibroblasts (Sebastiano et al., 2011), bone marrow mesenchymal stem cells (Mali et al., 2010) and peripheral blood mononuclear cells (Chou et al., 2011), and a number of reprogramming methods, including lentiviral, retroviral, piggyback transposon and helper-dependent adenovirus/Epstein-Barr virus (HDAd/EBV) hybrid reprogramming episomal vectors (Chou et al., 2011; Li et al., 2016; Li et al., 2011; Mali et al., 2010; Sebastiano et al., 2011) .

The NIH-funded NextGen Consortium has created a comprehensive library of 54 iPSC cell lines from an ethnically diverse (African American, Brazilian, and Saudi Arabian) cohort of SCD patients, so as to capture novel haplotype-specific polymorphisms that affect disease severity. The study looked at the Senegal, Benin and Bantu haplotypes, and found that these correlated with fetal hemoglobin (HbF) levels, whose persistence is relevant to the severity of

the phenotype. As a proof of principle for gene therapy, they also demonstrated successful correction of the HBB locus using CRISPR/Cas9 nuclease and single-stranded oligodeoxynucleotide (ssODN) as donor template. Capacity for directed differentiation of these cell lines into erythrocytes was tested and all lines demonstrated robust co-expression of CD71 (transferrin receptor) and CD235 (glycophorin A) (Park et al., 2017). Erythroid-progenitor-derived iPSCs hold significant potential for development as an autologous therapeutic modality due to their intrinsic HbF expression and lack of progression to the adult globin phenotype (Smith et al., 2013).

In addition to CRISPR/Cas9, ZFNs (Zou et al., 2011) and helper-dependent adenoviral vectors (Li et al., 2011) have been previously used to correct disease mutation in SCD-derived iPSCs, restoring wild-type *HBB* expression *in vitro*.

6.1.1.2 Thalassemia

A diverse collection of mutations associated with the *HBB* gene can lead to either complete absence or partial quantitative reduction in synthesis of β-globin, giving rise to anemia (Cao and Kan, 2013). Homozygous *HBB* mutations are often clinically characterized by severe anemia that is managed with periodic blood transfusions and iron chelation therapy (Olivieri and Brittenham, 1997).

Ou et al. established β-thalassemia patient-specific iPSCs and subsequently, corrected the *HBB* gene using CRISPR/Cas9 nuclease system. Following this, they introduced HSCs derived from the corrected iPSCs into NOD-scid-IL2Rg-/-(NSI) mice and demonstrated successful engraftment which led to restored *HBB* expression *in vivo*. Additionally, the engrafted cells did not exhibit any tumorigenic potential for as long as 10 weeks of observation after injection (Ou et al., 2016). Similarly, others have shown restoration of *HBB* expression in HSCs derived from gene corrected patient-specific iPSCs (Huang et al., 2015; Ma et al., 2013; Niu et al., 2016; Song et al., 2015; Wang et al., 2012; Xie et al., 2014).

In addition to gene editing and gene therapy based correction of the *HBB* gene, modified U7 small nuclear (sn) RNA delivered through a lentivirus has been shown to restore correct aberrant splicing in patient-derived iPSCs with a splice site mutation (VS2-654) in intron 2 of the *HBB* gene. Erythroblasts derived from these transduced iPSCs showed restored levels of *HBB* mRNA transcripts and down-regulation of apoptosis related genes i.e., *CASP3* and *AIFM1*, suggesting normal splicing of the gene mediated by the U7 snRNA and subsequent decrease in apoptotic processes in the cell.

However, differential expression of globin chains in iPSCs derived erythrocytes, leads to increased expression of gamma chains, in comparison to adult erythroid cells, where an increased expression of beta chains is seen (Trakarnsanga et al., 2014). This is the results of embryonic-fetal-adult globin class switching that occurs in adult erythrocytes. Hence, it has been suggested that β-globin gene transfer into iPSCs is necessary, before they could be used as a source for blood transfusions in β-hemoglobinopathies (Tubsuwan et al., 2013).

6.1.1.3 Pyruvate kinase deficiency

Red cell pyruvate kinase deficiency (PKD) occurs due to mutation in the *PKLR* gene that codes for pyruvate kinase – an enzyme involved in the glycolytic process and responsible for production of 50% of the ATP stores in red blood cells (RBCs). RBC life span depends on

the availability of ATP and hence, a decrease in ATP leads to a shorter lifespan and thereby, it clinically manifests as anemia (Grace et al., 2015). The only available treatment for PKD is allogenic bone marrow transplantation. Garate and colleagues established iPSCs from peripheral blood mononuclear cells of PKD patients using non-integrating Sendai virus (Garate et al., 2015). Once the iPSC lines were established, TALEN-based knock-in gene-editing strategy was used to insert a partial codon-optimized (cDNA) *PKLR* gene. Erythroblasts derived from gene-edited clones demonstrated restored *PKLR* expression on Western blot and liquid chromatography mass spectrometry (LC-MS) studies. In addition, restoration of normal ATP levels was noted, indicating a reversal in disease phenotype (Garate et al., 2015).

6.1.2 Bone marrow failure syndromes

Inherited bone marrow failure syndromes (BMFS) are a group of heterogeneous disorders characterized by peripheral blood cytopenias and hypoplastic bone marrows. These cytopenias occur as a result of specific mutations or polymorphisms that impair HSC production (Shimamura and Alter, 2010). Patients with BMFS are predisposed to developing hematological malignancies. Allogenic transplantation remains the only therapeutic option at present (Smith and Wagner, 2012). Furthermore, functional studies on primary patient samples are often limited by the lack of availability of HSCs. Thus, patient-derived iPSC models can be used to generate HSCs for both studying the pathophysiology of the disease and as a potential therapeutic option.

6.1.2.1 Fanconi anemia

Fanconi anemia (FA) is an inherited BMFS arising as a result of mutations in the DNA repair complex, and with at least 17 genes (*FANCA* to *FANCS*), encoding proteins that form the FA core complex, implicated in the disease (Kottemann and Smogorzewska, 2013; Wang and Smogorzewska, 2015). The FA core complex is involved in interstrand cross-linked DNA repair and thus patients with FA show hypersensitivity to DNA cross-linking agents. Initial attempts by Raya *et al.* (Raya et al., 2009) to reprogram skin fibroblasts from FA patients using retrovirus were unsuccessful. However, when the authors used lentiviral transgenes of wild-type *FANCA* or *FANCD2* for genetic complementation in these fibroblasts, they were able to reprogram some of the fibroblasts into iPSCs successfully. The difficulty in reprogramming primary cells from FA patients and the need for gene complementation for efficient reprogramming suggest that a functional FA DNA repair complex is important during the reprogramming process. It has been shown that the process of reprogramming is often associated with cellular stress, thus leading to a possible increase in DNA damage and activation of cell cycle check point pathways such as *TP53* (Hong et al., 2009; Hong et al., 2013; Marion et al., 2009; Utikal et al., 2009). In addition, reprogramming-associated stress often leads to an accumulation of reactive oxygen species and activation of FA complex-associated DNA repair pathway. Drawing on these findings, Muller *et al.* (Muller et al., 2012) were able to derive iPSCs from two FA patients in hypoxic conditions, without the need for prior correction of the underlying lesion. Further evidence to link genotoxic stress to reprogramming resistance has emerged from the observations of Liu *et al.* (Liu et al., 2014) and Suzuki *et al.* (Suzuki et al., 2015) showing that shRNA knockdown of *TP53* leads to increased efficiency of reprogramming of FA patient cells.

In addition, Liu *et al.* have been successful in using FA patient-derived iPSC as a platform for drug screening (Liu et al., 2014). A panel of small molecules was tested and doramapimod (a

highly selective p38 MAPK inhibitor) and tremulacin (a natural anti-inflammatory compound) were shown to significantly improve the differentiation of FA-iPSCs into HSCs. Thus, FA patient derived iPSCs are an attractive platform to further understand disease pathology and for drug screening.

6.1.2.2 Shwachman-Diamond syndrome

Shwachman-Diamond syndrome (SDS) is an inherited BMFS, arising as a result of a mutation in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene, which is known to play a role in ribosome biogenesis (Burwick et al., 2012; Warren, 2018). SDS is characterized by cytopenias, pancreatic insufficiency and a predisposition to myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) (Shimamura, 2006). Given that SDS is a disorder with other systemic manifestations such as pancreatic insufficiency, iPSCs provide a unique opportunity to model hematopoietic, as well as extra-hematopoietic manifestations of the disease, at the same time. Tulpule *et al.* (Tulpule et al., 2013) derived iPSCs from SDS patients in this study that successfully recapitulated the disease phenotype. Ribosomal profiling of SDS iPSCs showed decreased 80S:40S and 60S:40S ratios, which were then reversed by transgene rescue. On hematopoietic differentiation, the SDS iPSCs showed a decreased percentage of $CD45^+$ cells, thus suggesting impaired differentiation. However, one of the two SDS iPSC lines did not differentiate into the hematopoietic lineage at all, even despite transgene rescue. This could possibly be explained by intrinsic heterogeneity of iPSC clones.

When the SDS iPSC lines were differentiated into pancreatic tissue, they displayed deficits in amylase positivity as measured by immunofluorescence and lower levels of amylase and carboxypeptidase gene expression, as measured by RT-PCR, relative to their gene-rescued counterparts. Both pancreatic cells and myeloid cells normally contain a large number of cytotoxic granules. Histological staining and electron microscopy of SDS iPSC cultures confirmed both increased granule size and number per cell compared to transgene-rescued counterparts. The authors posit that these differences may reflect immaturity of the granules and that the presence of such granules might correlate with abnormal enzymatic activation and autodigestion of both pancreatic and myeloid cells, thus explaining the SDS pathology. To test the functional consequences of these granules, the authors treated the SDS iPSCs with broad-spectrum protease inhibitors and found increased cell survival of HSCs. Overall, the study shows that iPSCs derived from SDS patients can successfully capture the phenotype of SDS and that they could be used to dissect the pathology of the disease (Tulpule et al., 2013).

6.2 iPSCs in acquired hematological disorders

Acquired hematological disorders such as myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPNs), paroxysmal nocturnal hemoglobinuria (PNH) and other forms of leukemia can be studied using iPSC models. However, the majority of disease-relevant mutations observed in acquired hematological malignancies are restricted to hematopoietic lineages. Therefore, in order to generate a disease model, iPSCs are commonly generated from bone marrow or peripheral blood cells.

iPSCs can be employed to investigate the impact of gene mutations, for the discovery of therapeutic targets and for the development of disease models in acquired hematological diseases. For the purpose of this review we have focused on the use of iPSCs for the study of MDS, MPN and PNH.

6.2.1 MDS

The MDS are myeloid malignancies originating in the HSC. These disorders are characterized by impaired hematopoiesis and anemia, with an increase in bone marrow blasts as the disease progresses. Approximately 30-40% of patients progress to AML (Jhanwar, 2015).

Kotini et al. successfully generated iPSCs from the hematopoietic cells of two MDS patients with del(7q) and isogenic karyotypically normal iPSCs (Kotini et al., 2015). All del(7q)-iPSCs exhibited reduced hematopoietic differentiation and clonogenic capacity affecting all myeloid lineages. These phenotypes of ineffective hematopoiesis and reduced clonogenicity were found to be consistent with the phenotypes observed in cultured primary MDS bone marrow cells (Kotini et al., 2015; Sato et al., 1998). Heterozygous chr7q loss was engineered in karyotypically normal iPSCs and a segment of the chr7q, 40Mb (7q 31.1–q36.1) that is involved in diminished hematopoietic differentiation potential and clonogenic capacity was pinpointed.

Kotini et al. also evaluated significantly reduced gene expression in isogenic iPSCs harboring one or two copies of chr 7q and found 64 genes to be reduced by at least 1.5 fold. They were able to demonstrate that four genes, *EZH2*, *ATP6V0E2*, *LUC7L2*, and, *HIPK2* are able to partially rescue the emergence of $CD45^+$ hematopoietic progenitors upon differentiation of iPSCs when overexpressed in del(7q)-iPSCs (Kotini et al., 2015). Interestingly, *EZH2*, a gene recurrently mutated in MDS (Ernst et al., 2010) was further studied by establishing two *EZH2* haploinsufficient clones in hESCs using the CRISPR/Cas9 genome engineering system and the hematopoietic differentiation and colony formation potential of both *EZH2* haploinsufficient clones were substantially reduced to levels intermediate between those of normal and del(7q)-iPSCs, suggesting that a model of haploinsufficiency of more than one gene might be required to generate the del(7q) hematopoietic phenotype (Kotini et al., 2015).

A more recent study by Kotini et al. used some of the iPSCs generated in an earlier study (mentioned above) and some additional iPSCs generated from other MDS patients to produce a panel of disease-stage-specific iPSCs (Kotini et al., 2017). A total of 4 patient samples were used to investigate low-risk MDS, high-risk MDS and MDS/secondary AML. The patient-derived iPSCs demonstrated a range of phenotypes that were stage specific. For example, while low-risk and high-risk MDS exhibited no *in vivo* engraftment potential, MDS/AML hematopoietic progenitor cells (HPCs) showed up to 80% engraftment in multiple mice (Kotini et al., 2017). Immature $hCD45^+$ cells with blast like morphology transplanted from primary recipient into secondary recipients showed AML-like disease within 21 days of transplantation (Kotini et al., 2017).

The group further demonstrated phenotypically the progression from pre-leukemic stage to low-risk MDS stage through CRISPR/Cas9 mediated monoallelic or biallelic *GATA2* inactivation and to high-risk MDS stage by loss of a copy of chromosome 7q. This was shown by the potential of iPSCs to differentiate to HPCs (Kotini et al., 2017). Different responses of HPCs differentiated from iPSCs to treatment with 5-AzaC and Rigosertib were also demonstrated. Treatment with 5-AzaC promoted differentiation in low-risk MDS iPSCs only in colony-forming assays, while in high-risk MDS-iPSC-derived HPCs an inhibitory effect in cell growth was observed in a competitive growth assay. A *KRAS* mutated subclone, in high-risk MDS stage iPSC showed marked sensitivity to Rigosertib (Kotini et al., 2017).

Chang et al. used patient-derived iPSCs and CRISPR/Cas9 genome editing to dissect the individual contributions of two recurrent genetic lesions, the splicing factor *SRSF2* P95L mutation and the chromosome 7q deletion, to the development of myeloid malignancy (Chang et al., 2018). *SRSF2* mutant iPSC derived HPCs were shown to be preferentially sensitive to splicing factor inhibitors (Chang et al., 2018). Furthermore, drugs preferentially targeting del7q cells were identified through an unbiased large-scale drug screen. Niflumic acid selectively inhibited the growth of HPCs from two del(7q) iPSC lines derived from different MDS patients. In addition, treatment of primary cells from patients with MDS and secondary AML with chr7/7q abnormalities with niflumic acid suppressed the growth of cells with monosomy 7 or del(7q). Expandable iPSC-derived HPCs (eHPCs) were obtained by transducing 9 factors with and without an addition of 4 factors. These eHPCs could be expanded in culture and may be used for drug discovery (Chang et al., 2018).

Overall, the generation of MDS patient iPSCs and introduction of mutation or chromosomal abnormalities in wild-type iPSCs demonstrates the utility of iPSCs as disease models.

6.2.2 MPN

Myeloproliferative neoplasms (MPNs) are clonal HSC disorders characterized by proliferation of cells from one or more of the myeloid lineages i.e. granulocytic, erythroid, and megakaryocytic (Skoda et al., 2015).

In a study by Saliba et al. iPSCs were generated from $CD34^+$ cells isolated from the blood of two MPN patients, one carrying a heterozygous *JAK2V617F* mutation and the other a homozygous *JAK2V617F* mutation with additional *ASXL1* mutation and abnormal chromosome 20 (Saliba et al., 2013). Hematopoietic differentiation of iPSCs with the *JAK2* mutation showed a significant increase in the production of the three myeloid lineages. Five different *JAK2* drug inhibitors were evaluated for growth response of erythroid colonies and they were all found to inhibit erythroid growth. This study suggests that iPSC lines are useful tools to study the clonal hierarchy, the impact of *JAK2V617F* burden on cytokine signaling and drug discovery (Saliba et al., 2013).

Another study by Smith et al. used iPSC lines obtained from patients with the myeloproliferative neoplasm polycythemia vera (PV) with *JAK2* and *SERPINA1* mutations, and used the CRISPR/Cas9 system to target disease-associated mutations (Smith et al., 2015). Using PV patient-derived iPSCs that harbor the acquired *JAK2V617F* point mutation, Cas9 was shown to target either the wild-type or the mutant allele with high specificity, demonstrating the feasibility of targeting a single-nucleotide mutation in human iPSCs using CRISPR/Cas9 technology (Smith et al., 2015).

In a more recent study by Takei et al. iPSCs were generated from an essential thrombocythemia (ET) patient harboring a *CALR*-Ins5 mutation. Megakaryopoiesis was found to be prominent in Ins5-hematopoietic progenitor cells implying that the iPSCs recapitulated megakaryocytosis caused by *CALR* mutation, as observed in the bone marrow of ET patients (Takei et al., 2018).

6.2.3 Paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal HSC disorder that manifests with hemolytic anemia, thrombosis, and peripheral blood cytopenias and is characterized by an acquired somatic mutation of the phosphatidylinositol glycan class A (*PIGA*) gene in a hematopoietic stem/progenitor cell. This mutation results in the complete absence or reduced expression of proteins anchored to the cell surface membrane through glycophosphatidylinositol (GPI), including CD55 and CD59 (Elbadry et al., 2017).

Zou et al. have successfully engineered PNH patient-derived iPSCs using plasmids expressing ZFNs, mediated by homologous recombination (HR), where the mutants of PIG-A result in a loss of glycosyl-phosphatidyl-inositol-anchored proteins (GPI-APs), such as CD59, which was rescued by *PIGA* transgene expression. The generation of these iPSC lines confirm the power of ZFNs for inducing specific genetic modifications in iPSCs (Zou et al., 2009).

7. Challenges

The discovery of iPSC technology has not only reanimated the field of regenerative medicine, but has also profoundly changed our perception of cellular identity. Since the discovery of the OSKM cocktail, iPSCs have been subjected to intense scrutiny and their therapeutic potential has been scrupulously discussed. Despite a large shift in our understanding of iPSCs, there remain a few roadblocks to their full potential of clinical translation. These obstacles mainly hinge on the following issues: tumorigenicity, immunogenicity, and genomic and epigenetic stability of iPSCs.

The pluripotent origin of iPSCs coupled with their incomplete differentiation make them intrinsically prone to teratoma formation (Okita et al., 2007). The overexpression of oncogenes, such as c-Myc to induce reprogramming, carries further uncertainty in terms of their tumorigenicity (Lee et al., 2013) . However, advances in differentiation protocols and development of footprint-free non integrating RNA based Sendai viral reprogramming methods mitigate the risk of tumor formation (Ban et al., 2011).

iPSCs carry a huge potential for use as cells for autologous transplantation. It is expected that patient-derived iPSCs will be tolerated by the immune system, thus avoiding the long-term immunosuppressive treatment associated with allogenic transplantation. Studies however show that immunogenicity of iPSCs is a function of the status of their differentiation and site of differentiation. Whereas undifferentiated cells are prone to immune rejection, transplantation of differentiated cells into mice evokes almost no immune response (Araki et al., 2013; Zhao et al., 2011). *In vitro* differentiated cells are also more prone to immune rejection in comparison to their *in vivo* chimeric counterparts (Guha et al., 2013; Tang and Drukker, 2011). These studies suggest that reprogramming, genetic instability, culture conditions, frequent passages and differentiation status could all alter iPSCs and alter the immune response to them. There is an additional uncertainty over the immune response to genetically corrected iPSCs due to the presence of a wild-type gene to which the immune system has not been previously exposed in inherited disorder (Wood et al., 2016).

The transcriptional and epigenetic state of an iPSC line is influenced by a host of factors. iPSCs have been shown to retain a transcriptional and an epigenetic memory of the donor cell type, with epigenetic variations mainly restricted to DNA methylation (Chin et al., 2009; Doi et al., 2009; Ghosh et al., 2010). The method of reprogramming is also known to account for

the differences in transcriptional states as transcriptional variation is known to diminish upon transgene excision (Soldner et al., 2009).

8. Conclusion

The reprogramming methods used to generate iPSCs have improved significantly since their discovery. The use of iPSCs in disease modeling, drug screening and regenerative medicine with a view of stem cell based therapy is currently building momentum. The combination of human iPSCs with other new technologies such as CRISPR/Cas9 genome engineering also provides a platform for the study of individual gene mutations observed in patients with specific disorders.

iPSCs have been derived from various types of blood cells and furthermore, they have been used to generate lineage specific hematopoietic cells improving our understanding of reprogramming and differentiation of iPSCs. In recent years, the study of inherited and acquired hematological disorders has picked up momentum with the use of new technologies such as iPSCs and CRISPR/Cas9 genome engineering. These technologies will not only provide a platform for modeling diseases by using patient derived somatic cells but also offer personalized medicine.

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Conflicts of interest

None.

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Table 1. Established and novel reprogramming methods and their advantages and disadvantages.

	Reprogramming method	**Advantages**	**Disadvantages**	**Example of application**	**References**
Formerly used methods	Somatic cell nuclear transfer	1- Complete epigenetic reprogramming 2- More ES-like than iPSCs	1-Ethical issues regarding use of embryonic cells 2-Limited source material	Cloning of frog	(Gurdon, 1962b; Kim et al., 2010; Li et al., 2017)
	Fusion of somatic cells with embryonic stem cells		1-Ethical issues regarding use of ES cells 2-Limited source material	Fusion of adult thymocytes with embryonic stem cells	(Li et al., 2017; Tada et al., 2001)
Integrating	Retrovirus	1-Efficient delivery and expression of reprogramming factors 2-Can be combined with inducible systems or Cre/loxP systems	1-Risk of insertion mutagenesis, viral transgene reactivation and cancer development 2-Transduction of proliferating cells only 3-Multiple vectors obligatory	Reprogramming of Beta-thalassemia patient-derived skin fibroblasts, these iPSCs were differentiated to hematopoietic cells	(Chhabra, 2017; Ye et al., 2009)
	lentivirus	1-Efficient delivery and expression of reprogramming factors 2-Can be combined with inducible systems or Cre/loxP systems 3-Transduction independent of cell cycle	1-Risk of insertion mutagenesis, viral transgene reactivation and cancer development 2-Poorly silenced after reprogramming if used without inducer	Reprogramming of Juvenile myelomonocytic leukemia patient-derived mononuclear bone marrow cells, these iPSCs were differentiated into hematopoietic lineages	(Chhabra, 2017; Gandre-Babbe et al., 2013; Li et al., 2017)

		4-Single vector suffices			
	PiggyBac (transposon-based)	Vector-free excision	1-Low efficiency compared to retrovirus 2-Multiple transposon integrations could cause mutations with unknown effect	Reprogramming of sickle cell disease patient-derived bone marrow stromal cells, gene correction in iPSC	(Chhabra, 2017; Hussein and Nagy, 2012; Zou et al., 2011)
Non-integrating	Sendai virus	1-No insertional mutagenesis 2-High efficiency compared to other non-integrating methods	1- Clinical grade SeV not (yet) available 2- Slow clearance of vector	Reprogramming of primary human B cells	(Bueno et al., 2016; Schlaeger et al., 2015)
	Adenovirus	Insertional mutagenesis unlikely	Low efficiency compared to integrating methods	Reprogramming of mouse adult hepatocytes	(Stadtfeld et al., 2008)
	Plasmid-based	No insertional mutagenesis	Low efficiency compared to integrating methods	Reprogramming of mouse adult hepatocytes	(Okita et al., 2008)
	Protein transduction	No genetic material used	Low efficiency compared to virus-based methods/ Technically challenging	Reprogramming of murine embryonic fibroblasts	(Malik and Rao, 2013; Zhou et al., 2009)
	CRISPRa	1-No insertional mutagenesis 2-No expression of foreign DNA	Sufficient efficiency only when additional factors are activated	Reprogramming of primary human skin fibroblasts	(Weltner et al., 2018)
	mRNA-based	1-No insertional mutagenesis 2-High efficiency	1-Technically challenging 2-Does not work with all cell types (e.g. blood	Reprogramming of human neonatal foreskin fibroblasts	(Warren et al., 2010)

			cells)		
	Episomal-based	No insertional mutagenesis/ Easy to produce under cGMP conditions	Low efficiency compared to virus-based methods	Reprogramming of CML patient-derived bone marrow cells	(Hu et al., 2011; Schlaeger et al., 2015)
	microRNA-mediated	1-No insertional mutagenesis 2-No expression of foreign DNA	Low efficiency compared to virus-based methods	Reprogramming of human dermal fibroblasts using mir-200c, mir-302 s, and mir-369 s	(Miyoshi et al., 2011)

Table 2. Methods for deriving specific hematopoietic lineages from human iPSCs.

iPS derived hematopoietic lineage	**Specific Lineage commitment**	**iPSC Source**	**Reprogramming method**	**TFs**	**Cytokines/ Small molecules**	**References**
Hematopoietic stem cells (HSCs)	$CD34^+CD45^+$	Mesenchymal stem cell (MSCs)/ Peripheral blood $CD34^+$ cells (PBCs)	Retrovirus/ Lentivirus	ERG, HOXA5, HOXA9, HOXA10, LCOR, RUNX1, SPI1	TPO, IL-3, SCF, IL-6, IL-11, IGF-1, VEGF, bFGF,BMP4, FLT3	(Sugimura et al., 2017)
		Keratinocytes	Retrovirus	*In vivo* differentiation		(Amabile et al., 2013)
		Keratinocytes/ Neonatal foreskin fibroblasts	Retrovirus		BMP-4, TGFβ1, VEGF, TPO, EPO, SCF, Flt3-L, PGE2	*(Woods et al., 2014)*
Myeloid	Erythrocytes	Human cord blood CD 34^+ cells (CBs)/ Fibroblasts	Lentivirus		SCF, TPO, Flt3-L, IL-3, IL-6, VEGF, EPO, BMP-4	(Dorn et al., 2015)
		Adult/Neonatal fibroblasts	Lentivirus		IL-3, IL-6, EPO, SCF, Flt3-L, TPO	(Dias et al., 2011)

	Megakaryocytes/Platelets	CB/ Peripheral blood T cells	Lentivirus	c-MYC, BMI1, BCL-XL	SCF, TA-316, KP-457, SR-1, GNF-351	(Ito et al., 2018)
		Fibroblasts	Retrovirus/ Sendai virus/ Episomal plasmids	GATA1, FLI1, TAL1	BMP-4, SCF, TPO, IL1-β	(Moreau et al., 2017)
	Granulocytes	PBCs	Lentivirus		IL-3, G-CSF, GM-CSF	(Lachmann et al., 2015)
		Fibroblasts from patients with chronic granulomatous disease	Episomal plasmids		SCF, Flt3-L, IL-6, IL-3, G-CSF	(Brault et al., 2014)
	Monocytes	Fibroblasts	Retrovirus		bFGF, BMP-4, SCF, VEGF, M-CSF, IL-3	(van Wilgenburg et al., 2013)
		Cord blood mononuclear cells	Episomal plasmids/ Retrovirus		BMP-4, VEGF, SCF, bFGF, Flt3-L, IL-3, TPO, M-CSF, GM-CSF	(Yanagimachi et al., 2013)
	Dendritic cells	Fibroblasts	Retrovirus		SCF, VEGF, BMP-4, GM-CSF, IFN-γ, TNF-α IL-1β, PG E2, IL-4, IL-10	(Sachamitr et al., 2018)
		Fibroblasts	Lentivirus		GM-CSF, M-CSF, IL-4, TNF-α	(Senju et al., 2011)
Lymphoid cells	NK cells	Peripheral blood mononuclear cells	Sendai virus		SCF, Flt3-L, IL-7, IL-15	(Zeng et al., 2017)
		MSCs	Lentivirus		bFGF, VEGF, BMP-4, IGF-1,	(Sturgeon et al., 2014)

					IL-3, IL-11, IL-6, SCF, TPO, EPO, IL-15, IL-13, Flt3-L	
	T cells	Fibroblasts from patients with RAG-1 dependent immune deficiency	Lentivirus		BMP-4, bFGF, SB-431542, VEGF, IL-6, IL-11, EPO, IGF1, SCF, IL-7, Flt3-L	(Brauer et al., 2016)
	B cells	CBs	Lentivirus/ Episomal plasmids		Il-7, IL-3, SCF, Flt3-L	(French et al., 2015)

Abbreviations: bFGF, basic fibroblast growth factor; BMP-4, bone morphogenetic protein-4; CB, human cord blood CD 34+ cells; EPO, erythropoietin;; FLT-3L, FMS-like tyrosine kinase 3 ligand; G-CSF, granulocyte colony–stimulating factor; GM-CSF, granulocyte monocyte colony-stimulating factor HSC, hematopoietic stem cell; cluster iPSC, induced pluripotent stem cell; IGF-1, insulin-like growth factor 1; IL, interleukin; IFN-γ, interferon- γ; MSC, mesenchymal stem cell; PBC, Peripheral blood CD34+ cells; PGE2, prostaglandin E2; SCF, stem cell factor; TNF-α, tumor necrosis factor – α; TF transcription factor; TPO, thrombopoietin; VEGF, vascular endothelial growth factor