Journal of
Stem Cell Research & Therapy

Al-Anazi, J Stem Cell Res Ther 2015, 5:1
DOI: 10.4172/2157-7633.1000258

Review Article Open Access

Induced Pluripotent Stem Cells and Their Future Therapeutic Applications in Hematology

Khalid Ahmed Al-Anazi*

Section of Adult Hematology and Oncology, Department of Medicine, College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia

Abstract

Induced pluripotent stem cells have recently acquired particular attention because of the following reasons: they can be derived from a wide variety of cells and tissues, their use can avoid many of the obstacles and ethical issues that limit the use of other stem cell lines and their rapidly advancing therapeutic and clinical applications. These cells can be efficiently utilized in regenerative medicine, tissue engineering, disease modeling, drug development and discovery, genetic therapies as well as various types of cell treatments.

Plenty of human and animal studies have already shown that these cells have great potentials for treating benign as well as malignant hematological disorders and that they can be utilized to generate several blood cell lines which may be used in clinical practice. In this review, several aspects of induced pluripotent stem cells will be discussed with the main focus on their future clinical applications in the field of hematology.

Keywords: Induced pluripotent stem cells; Embryonic stem cells; Fanconi anemia; Hemophilia; Sickle cell disease

Introduction

Based on their potency and their sources, stem cells can generally be classified into totipotent, multipotent and pluripotent types (Table 1) [1,2]. Induced pluripotent stem cells (iPSCs) have certain identification properties and specific stringency criteria both in human as well as in mouse model systems (Table 2) [3-5]. IPSCs have been obtained from humans in addition to various animal species and they have been generated from various cell sources that belong to the three germ layers: ectoderm, mesoderm and endoderm (Table 3) [4-9]. Human iPSCs resemble human embryonic stem cells (ESCs) in many aspects including: morphology, proliferation, pluripotency markers, gene expression profiles, epigenetic status and differentiation potential. Hence, human iPSCs have the capacity to replace human ESCS and they can provide the correct direction of addressing the ethical disputes over stem cell sources and immunological rejection in cell therapy [7].

Totipotent stem cells; zygote + 2 to 4 cell embryo	These cells are capable of giving rise to the entire organism (both embryonic and non-embryonic tissues)
Multipotent stem cells; adult stem cells	
Pluripotent stem cells; embryonic stem cells and embryonic germ cells	These cells can give rise to derivatives of all 3 germ layers [embroynic tissue only and not extraembryonic tissues] Embryonic stem cells (ESCs) have the potential to form any cell type in the body. Pluripotent stem cells (PSCs) can be divided into 2 types [a] Embryonic stem cells (ESCS): derived from the inner cell mass of the mammalian blastocyte. [b] Induced pluripotent stem cells (iPSCs): somatic cells reprogrammed to a pluripotent state by exogenous expression of transcription factors responsible for conferring pluripotency on ESCs.

Table 1: Classification of stem cells based on their potency and their sources.

Pluripotency stringency criteria	
Mouse model systems	** In vitro morphology and gene expression
	** In vitro teratoma formation
	** In utero chimeric embryogenesis
	** Tetraploid aggregation and germline transmission
Human model systems	** In vitro morphology and gene expression
	** In vitro teratoma formation
Identification properties of iPSCs	
Cellular biological properties	Morphology, Growth, Telomere activity, Stem cell markers, Stem cell genes
Pluripotency	Neural differentiation, Cardiac differentiation, Teratoma formation, Embryoid body, Chimeric mice, Tetraploid complementation
Epigenetic programming	Promoter demethylation, DNA methylation globally, Histone demethylation

Table 2: Properties of iPSCs and their stringency criteria.

Reprogramming of iPSCs should have the following requirements: (1) species such as humans or mice, (2) cell type such as fibroblasts or blood cells, (3) factor or chemical such as protein, gene or valproic acid, (4) vector such as retrovirus or lentivirus, and (5) diseases with specific genetic mutations [6,7,9]. DNA and non-DNA methods have been employed in induction and programming of iPSCs (Table 4) [4-9]. The following genes have been used in inducing PSCs: Oct 3/4, Sox2, Klf4, C-Myc, Nanog, LIN 28, Glis 1 and REX 1 [3,4,7]. Also, a long list of agents or vectors have been used in iPSC programming and these include: single or multiple transient transfections, excisable and non-integrating vectors, proteins and direct protein transduction, plasmid and episomal vectors, minicircle DNA, modified RNA, RNA-based Sendai viruses, mRNA-based transcription factor delivery, microRNA transfections, artificial chromosome vectors, chemical compounds and small molecule compounds. These agents have been reported to improve safety and efficacy of the reprogramming process [5,7]. Small molecule compounds have recently been utilized to generate mouse iPSCs from

***Corresponding author:** Dr. Khalid Ahmed Al-Anazi, Consultant Hemato-Oncologist, Section of Adult Hematology and Oncology, Department of Medicine, College of Medicine and King Khalid University Hospital, King Saud University, P.O. Box: 2925, Riyadh 11461, Saudi Arabia, Tel: 966- 011- 4671546; Fax: 966 - 011- 4671546; E-mail: kaa_alanazi@yahoo.com

Received December 03, 2014; **Accepted** December 31, 2014; **Published** january 02, 2015

Citation: Al-Anazi KA (2015) Induced Pluripotent Stem Cells and Their Future Therapeutic Applications in Hematology. J Stem Cell Res Ther 5: 258. doi:10.4172/2157-7633.1000258

Species	Cell Type	
- Humans	* Fibroblasts	* Cord blood cells
- Mice	* Keratinocytes	* Neural stem cells
- Rats	* Melanocytes	* Adipose stromal cells
- Pigs	* Hepatocytes	* Gastric epithelial cells
- Rabbits	* Thymocytes	* Lymphocytes:
- Marmosets	* Amniotic fluid	** B - cell type
- Rhesus Monkeys	* Peripheral blood cells	** T - cell type
Germ Layer		
(1) Ectoderm:	** Fibroblasts	** Neural stem cells
	** Keratinocytes	** Meningiocytes
	** Melanocytes	** Dermal papilla cells
	** Other cell sources	
(2) Mesoderm:		
- Synoviocytes	- Granulocyte-colony stimulating factor (G-CSF) mobilized human CD34+ peripheral blood cells	
- Adipose stem cells	- Mesenchymal-like cells from umbilical cord matrix	
- Periosteal cells	- Amniotic membrane mesenchymal cells.	
- Bone marrow progenitor cells	- Peripheral mononuclear blood cells [T-and B-lymphocytes]	
- CD 133 + cord blood cells	- Other cell sources	
(3) Endoderm:		
** Liver cells; hepatocytes	** Nasal epithelial cells	
** Stomach cells, gastric cells	** Urine - derived cells	
** Pancreatic β- cells	** Other cells	
** Dental pulp cells		

Table 3: Sources of iPSCs classified according to species, cell type and germ layer.

Classes	Types	Examples
DNA Methods	(1) Viral delivery systems:	* Adenoviral transduction
		* Retroviral transduction
		* Lentiviral transduction
	(2) Non-viral systems:	** Episomal plasmids; Plasmid and episomal vectors
	(3) Conditional systems and transposons:	* Cre -loxP recombination
		* Piggyback transposon
Non-DNA Methods	(a) Protein - mediated:	* Direct delivery of programming proteins onto the cell
		* Wnt 3a conditioned medium
	(b) Manipulation of cell culture condition:	- Lower amount of oxygen
	(c) Use of histone deacetylase inhibitors:	** Valproic acid enhances programming of iPSCs
	(d) Sindae virus transduction	
	(e) Direct fusion with embryonic stem cell; cell fusion	
	(f) Extraction of embryonic stem cell; cell extract	

Table 4: Methods used in induction and programming of iPSCs.

somatic cells. Small molecule compounds have the following advantages over other inducers: (1) cell-permeability, (2) non-immunogenicity, (3) easily synthesis, (4) cost-effectiveness and (5) the often reversible effects on inhibition or activation of specific proteins [7].

History and Landmarks in the Evolution of iPSCs

The history of iPSCs is rich in discoveries, landmarks and achievements as shown in table 5 [6,7,9-11]. Modern technology has advanced the industry of iPSCs [4]. The background of iPSC technology includes the following stages and historical landmarks:

First generation technology

In 2006, Shinya Yamanaka, Kyoto University in Japan, successfully reprogrammed adult mouse fibroblasts into iPSCs. The approach employed a retrovirus to transduce mouse fibroblasts with the selected genes and cells were isolated by antibiotic selection of Fbx15 positive cells. Unfortunately this iPSC line showed DNA-methylation errors compared to the original patterns in ESC lines and failed to produce viable chimeras when injected into developing embryos [4].

Second generation technology

In 2007, Yamanaka group and 2 other independent groups from the universities of Harvard and California in the United States of America (USA) showed reprogramming of mouse fibroblasts from which viable chimeras were produced. These cell lines were derived from mouse fibroblasts by retroviral mediated reactivation of the same 4 pluripotent factors [Oct 4, Sox 2, Klf 4 and c-Myc] but a different marker for detection was utilized [4].

Human iPSCs

Late in 2007, Thomson and Yu (university of Wisconsin-Madison, USA) and Yamanaka et al. (university of Kyoto, Japan) independently reported successful creation of iPSCs from adult human cells. The same 4 genes were used with retroviral system. However, Thomson and Yu revealed the use of 2 alternative factors [Nanog and Lin 28] to facilitate the programming process using a lentiviral system. These

1.	C. Waddington proposed the epigenetic landscape: cell differentiation was considered a one-way street with traffic flowing from an immature cell to stem or progenitor cell then to a more mature differentiated state (1957).
2.	Sir J. Gurdon succeeded in generating cloned dogs by transferring the nucleus of a tadpole's somatic cell into an oocyte (1962).
3.	E. McCulloch and J. Till reported the presence of self-renewing cells in the stroma of mouse bone marrow [stem cells] (1963).
4.	Evans et al. and Kaufman et al. established mouse ESCs (1981).
5.	Davies et al. first demonstrated direct cell fate conversion by a defined transcription factor (1987).
6.	Thomson et al. established human ESCs (1998).
7.	Takahashi and Yamanaka generated iPSCs (2006).
8.	Totipotent IPSCs were established by Maherali et al. (2007).
9.	Yamanaka and Thomson reprogrammed human somatic cells into iPSCs (2007).
10.	Peripheral blood cell programming started with research on mice by Hanna (2008).
11.	Hong et al. reported generation of iPSCs from mouse T lymphocytes by the introduction of Oct 3/4, Sox 2, Klf4 and c-Myc in a P53 - null background (2009).
12.	Generation of human iPSCs from cord blood by Haase et al. (2010).
13.	Ye et al. derived human iPSCs from previously frozen cord blood and CD34+ cells from healthy adult donors (2010).
14.	Loh et al. reprogrammed human blood cells into iPSCs (2010).
15.	Chou reprogrammed newborn cord blood and adult blood mononuclear cells into iPSCs (2011).
16.	Lei et al. made human iPSCs to differentiate into both conventional and antigen-specific T lymphocytes for T cell-based immunotherapy by in vitro or in vivo induction systems (2012).
17.	Ebihara et al. established human iPSCs that represent the potentially unlimited safe sources of donor-free red blood cells for blood transfusion [without potential of infectious disease via transfusion] (2012).
18.	Hou et al. used small molecule compounds to generate mouse iPSCs (2013).
19.	The Scottish National Bank Transfusion Service announced synthesis of blood group O RBCs that can hopefully be used by the year 2016 (2014).

Table 5: Landmarks in the history of iPSC evolution.

human ESC-like cells also expressed markers of ESCs and were capable of differentiating into cell types of all 3 germ layers [4].

Rationale to Study Hematopoiesis from Human PSCs

It is essential to study human PSCs due to the following reasons: (1) they provide an optimal model to study basic human developmental biology, (2) they provide a model for human genetics and a platform for gene therapy, (3) they can provide a cell source for transfusion medicine including mature red blood cells (RBCs) and platelets, (4) they can provide a novel source of immune therapies such as natural killer cells, T-cells and dendritic cells, (5) they can provide an alternative source of cells for hematopoietic stem cell transplantation (HSCT) in order to promote engraftment, and (6) pharmaceutical testing with human PSC-derived cells can be used to evaluate efficacy of novel therapies [12].

Clinical Applications of iPSCs

The therapeutic applications of iPSCs are rapidly expanding. These stem cells can be efficiently utilized in regenerative medicine, tissue engineering, disease modeling, drug development and discovery, genetic therapies, various types of cell treatments in addition to synthesis of several blood components (Table 6) [3,4,6,9,13,14]. Although their early clinical effects are promising, their use in the clinical arena may not be entirely safe as utilization of iPSCs has several disadvantages in addition to its known advantages (Table 7) [8,15-17]. Fortunately, several disease models have recently been successfully treated with iPSC-based interventions (Table 8) [3].

iPSCs in Drug Development and Regenerative Medicine

Despite facing numerous challenges and being in its infancy, the potential of iPSC technology in drug development and discovery is enormous [18]. IPSC-based models of cardiovascular disease, such as long QT interval and arrhythmias, have been generated and in these disease models, a number of drugs have been tested including: nefidipine, flecainide, dantrolene, isoproterenol, nicorandil and roscovitine [19].

1.Disease modeling	
2. Regenerative medicine: tissue engineering and organ repair	
3. Drug screening for toxicity, drug development and drug discovery	
4. Genetic therapy; treatment of intractable and genetic disorders:	
** Sickle cell anemia	** Cystic fibrosis
** β- Thalassemia	** Lysch - Nyhan syndrome
** Hemophilia	** Hurler syndrome
** Fanconi anemia	** Pompe disease
** Dyskeratosis congenita	** Wilson disease
** Schwachman-Bodian-Diamond syndrome	** Down's syndrome
** Primary myelofibrosis	** Huntington's disease
** Polycythemia rubra vera	** Parkinson's disease
** Epidemolysis bulosae	** Alzheimer's disease
** Retinitis pigmentosa	** Schizophrenia
** Spinal muscular atrophy	** Ducchene muscular dystropy
	** Amyotrophic lateral sclerosis
5. Cell-based therapies.	
6. Synthesis of blood components: the following blood components have been generated from embryonic and iPSCs:	
[a] Red blood cells [definitive and primitive erythroid cells]; can be used in severe anemia or blood loss.	
[b] Megakaryocytes and platelets; transfusion in critical thrombocytopenia	
[c] Dendritic cells: antigen-specific vaccines for cancer or human immunodeficiency virus (HIV)	
[d] Natural killer cells: natural or antibody-assisted anticancer cytotoxicity	
[e] T- lymphocytes: antigen-specific anti-cancer or anti- HIV adoptive cell transfer.	
[f] B- lymphocytes	
[g] Neutrophils	
[h] Monocytes and macrophages	

Table 6: Clinical applications of iPSCs.

Advantages	Disadvantages
Avoidance of using human embryos	Use of integrative DNA methodology
Capacity to induce stem cell like phenotype	Genomic instability and aberrations
New promises to cellular therapy	Increased risk of cancer development due to the use of oncogenes for the induction of iPSCs phenotype.
Possibility of studying several diseases including cancer	Immunogenicity of iPSCs and rejection potential.

Table 7: Advantages and disadvantages related to the utilization of iPSCs.

Disease Condition	Therapeutic outcome achieved
Sickle cell disease	Improvement of hematopoiesis at functional and physiological levels
Hemophilia A	- Survival benefit obtained - Decreased clotting time achieved
Parkinson's disease	Dopamine production enhanced, leading to symptomatic improvement
Ischemic heart disease	In situ tissue repair leading to improved cardiac performance

Table 8: Models of diseases treated with iPSC-based interventions.

Regenerative medicine is an expanding field that aims to replace damaged tissues in human body by either endogenous repair or cellular transplantation [20]. Infusion of stem cells into the circulation is taking the lead in regenerative medicine, but survival of these cells in patients, after infusion, is limited to few weeks. Various stem cell types such as mesenchymal cells, cord blood cells, adipose tissue cells and adult stem cells have been utilized in wound repair and tissue regeneration with variable degrees of success [21]. The use of iPSCs in cardiac repair has been extensively investigated and it has shown success, while in wound healing the use of multipotent stem cells has been more beneficial than the use of iPSCs [22,23].

iPSCs in the Treatment of Hematological Disorders

Although iPSCs are remarkably similar to ESCs in pluripotent function, many studies have identified subtle but potentially significant molecular differences showing the need to continue to study and improve reprogramming methods to achieve the closest facsimile to the naturally derived ESCs which remain the gold standard [2]. With regard to their applications for hematologic diseases, there appear to be differences in the differentiation potential of human PSC lines to form various tissues of clinical interest, particularly blood components [2]. The blood differentiation potential of 22 human PSC lines generated at multiple institutions was examined [2]. The 14 human ESC lines tested showed higher hematopoietic colony forming unit (CFU) activity and 100-fold increase in erythroid differentiation efficacy, whereas the other 8 human iPSC lines displayed limited growth potential and decreased CFU activity [2].

To date, no successful protocol exists for differentiation of PSCs into definitive HSCs. PSCs can be directly differentiated into primitive RBCs, but not in sufficient numbers to enable treating patients and the cost of clinical scale differentiation is prohibitively expensive with current differentiation methods and efficiencies [2]. Although the advent of PSCs offers an appealing theoretical platform for treating not

only hemoglobinopathies but also various genetic and malignant bone marrow disorders, many hurdles must first be overcome [2]. Therefore, advances are needed to: (1) improve the efficiency of transgene-free human IPSC derivation, (2) enhance direct differentiation toward engraftable HSCs or transfusable RBCs, and (3) reduce the cost of maintaining and differentiating human PSCs in order to make cell therapies feasibly affordable [2].

Fanconi Anemia

Fanconi anemia (FA) is a recessive disease characterized by: genomic instability, congenital malformations, predisposition to cancer and bone marrow failure [23,24]. FA is the most common inherited bone marrow failure syndrome [25]. The etiology of bone marrow failure in FA remains unclear, but limited studies on patient bone marrow cells indicate that intrinsic cell defects may be etiologically related [25]. Also, the pathogenesis of FA is not fully understood due to limitations of current disease models [24]. Defective DNA repair is caused by mutations in the genes involved in replication-dependent repair and removal of DNA cross-links [26,27]. The underlying genetic defect in FA can reside in any of the 16 FANC genes which function in a common DNA damage repair pathway and loss of function of any of the FA pathway genes which coordinate cellular DNA damage repair mechanisms, particularly those involved in protection from DNA cross-liking agents may produce the disease [24,28].

Allogeneic HSCT remains the only proven curative therapy for the hematological manifestations of FA. Over the last 2 decades, major advances have been achieved and outcomes of HSCT have improved remarkably [29]. The development of *in vitro* fertilization, pre-implantation genetic diagnosis and human leucocyte antigen (HLA)-matched sibling donor umbilical cord blood transplantation resulted in an increase in the available therapeutic options of patients with FA. PSCs can also provide a potential source of autologous stem cells that can be genetically manipulated and utilized to generate hematopoietic progenitor cells [29]. Upon correction of the genetic defect, somatic cells from FA patients can be reprogrammed to generate patient-specific iPSCs [30]. Consequently, corrected FA-specific iPSCs can give rise to hematopoietic progenitor cells of the myeloid and erythroid lineages that are phenotypically normal and disease-free [30].

In contrast to many other FA proteins, BRCA2 participates downstream in the FA pathway and has critical role in homologous recombination (HR), which is critical for cell reprogramming [26]. Reprogramming defects characteristic of BRCA2 mutant cells can be efficiently overcome by gene complementation [26]. Gene complementation has facilitated the generation of iPSCs from genetically corrected, disease-free, BRCA2 cells which could be differentiated *in vitro* toward hematopoietic lineage [26]. Somatic cells harboring mutations that render FA pathway defective are resistant, but not refractory, to programming into iPSCs [23]. To resolve this issue, 2 suggestions have been proposed: (1) to perform genetic complementation prior to reprogramming as this will: restore DNA repair, rescue reprogramming and yield iPSCs less likely to contain genetic alterations, and (2) to correct and reprogram simultaneously and then screen at the end of the process to rule out genotoxicity caused by both genetic modification and reprogramming of cells [28].

Despite the recent progress, establishment of an iPSC-based FA model still faces the following challenges: (1) studying FA in primary patient cells is often impractical due to the following reasons: the rarity of the disease, the low bone marrow cellularity of patients, and the inaccessibility to certain tissues, (2) reprogramming FA cells into iPSCs has proven to be highly inefficient, (3) the established FA-iPSCs often fail to be maintained in culture, and (4) lentiviral gene complementation has remained the sole method of correction of FA defects [24]. However, 2 recently published studies suggested solutions and hence hopes to overcome these challenges have increased [24,31]. In the first study, isogenic FA-iPSC lines free of FANCA mutation were generated by HR. These cell lines represent a multifaceted platform to: understand FA pathogenesis, discover novel therapeutic agents and develop cell replacement therapies for FA [24]. In the second study, high-risk human papillomavirus E6 oncogenic protein was found to enhance reprogramming of FA patient cells through repression of P53 but did not allow sustained growth of FA-iPSCs [31].

Hemophilia A

Hemophilia A (HA) is one of the most common congenital coagulation disorders, caused by various mutations in the X-linked factor VIII gene leading to depleted protein production and inefficient blood clotting [32,33]. It occurs in 1 or 2 per 10,000 individuals and represents 90% of all hemophilia cases [32]. The clinical manifestations of HA vary considerably according to the phenotype [33]. Severity of HA can be divided into 3 grades depending on the relative amount of factor VIII activity in patient's plasma: severe: <1% activity; moderate: 1-5% activity and mild: 5-30% activity [28]. Current therapies of HA include fixed-dose factor VIII prophylaxis and factor VIII replacement therapy [32-34]. Prophylaxis and replacement therapy are limited by: incomplete efficacy, high cost, restricted availability and the possible development of neutralizing antibodies in chronically treated individuals [32,33].

Novel therapeutic modalities include: (1) gene therapy: replacement of a deficient gene by a healthy one so that it generates a certain functional, structural or transport protein, (2) cell therapy: incorporation of a full array of healthy genes and proteins through perfusion or transplantation of healthy cells, (3) tissue engineering: tissue transplantation and formation of healthy organs, and (4) utilization of advanced iPSC technology [34,35]. Currently, there is no established curative therapy for HA [33]. However, novel therapeutic modalities have increased treatment options for HA patients and can potentially provide a cure in the near future [34,35]. HA has long been recognized as a condition amenable to gene therapy [32,33]. Gene therapy can be administered by means of: (1) lentiviral or adenoviral-associated vectors applied to adult stem cells, autologous fibroblasts, platelets or HSCs, (2) non-viral vectors, or (3) through the repair of mutations by chimeric oligonucleotides [35]. Although gene therapy appears to be a promising option to cure patients with HA, several attempts at gene therapy have failed for various reasons including immune rejection [32,33]. In HA, cell therapy approaches have been based mainly on transplantation of healthy cells, adult stem cells or iPSC-derived progenitor cells, in order to restore alterations in coagulation factor expression [36].

Recently, two major studies using iPSCs to treat animal models of HA were published [32,33]. In the first study, endothelial progenitor cells derived from iPSCs were used. Murine iPSCs were obtained from tail-tip fibroblasts then cells were differentiated into progenitor cells that expressed factor VIII both *in vivo* and *in vitro*. Later on, progenitor cells were injected or transplanted into liver of irradiated HA mice. The following therapeutic benefits were obtained: (1) transplanted mice survived longer than 3 months as endothelial progenitor cells engrafted within the liver parenchyma, (2) effective expression of factor VIII protein was obtained as plasma factor VIII levels increased to 8-12%, and (3) HA phenotype was corrected [32]. In the second study, iPSCs were generated in a mouse model of HA using non-integrating vectors

(Sendai virus and human artificial chromosomes) under the control of platelet factor 4-promoter for development. Results of this study showed successful expansion of factor VIII [36].

Also, recently, two human studies using iPSCs to treat HA patients were published [33,37]. In the first study, urine samples were collected from HA patients. Using, non-viral, integration-free episomal vectors, iPSCs were generated then they were differentiated into hepatocyte-like cells. HA-specific iPSCs exhibited pluripotent properties but HA-iPSCs-derived hepatocytes failed to produce factor VIII [37]. In the second study, wild-type iPSCs were obtained from human dermal fibroblasts using episomal vectors, then differentiated into epithelial cells and cultured iPSCs were treated with collagenases [33]. Engineered, programmable nucleases such as zinc finger nucleases (ZFNs) and transcription activation-like effector nucleases (TALENs) were used to rearrange large genomic segments in iPSCs and isolate clones harboring genomic rearrangements. The defective gene was corrected with targeted inversion and reversion in human iPSCs using engineered nucleases [33]. Thus, patient-derived iPSCs are likely to provide another promising option to cure patients with HA [32,33,36].

Hemoglobinopathies

Attempts to cure thalassemia and SCD using gene therapy have been hampered by the large globin gene and globin promoters that are difficult to accumulate within vector systems [38]. Amelioration or even cure of mouse models of SCD and β-thalassemia major has been achieved using lentivirus vectors that contain complex and regulatory sequences from the locus control region [38].

Prenatal screening and diagnosis of congenital hemoglobinopathies are available in many countries, thus making such approaches an attractive option for an in utero therapeutic intervention [38]. Prenatal diagnosis can be achieved from 15 weeks of gestation onwards using amniocentesis and from 11 weeks of gestation using chorionic villus samples [38]. There are advances in non-invasive prenatal diagnosis and screening using circulating fetal DNA detected in the maternal plasma thus allowing the diagnosis of congenital disorders as early as 7 weeks of gestation [38]. In comparison to the relative success of postnatal HSCT for blood disorders, results of the clinical cases of in utero-HSCT to cure blood disorders have been disappointing [38].

PSCs hold great promise for not only research but also treatment of hemoglobinopathies. In principle, patient-specific iPSCs could be derived from a blood sample, genetically corrected to repair the disease-causing mutations, differentiated into HSCs and returned to the patient to provide cure through autologous gene and cell therapy [2]. However, there are many challenges at each step of this complex treatment paradigm. Gene repair is currently insufficient in stem cells, but use of ZFNs and TALENs appear to be a major advance [2]. TALENs can be used for the treatment of genetic disorders such as sickle cell disease (SCD). The future use of TALENs in the treatment of genetic disorders represents a significant advance toward human iPSC-based cell and gene therapies [39]. Disease-specific patient-derived human iPSCs have great potential for developing novel cell and gene therapies. With the disease-causing mutations corrected in situ, patient-derived human iPSCs can restore normal cell function and serve as a renewable autologous cell source for the treatment of genetic disorders [39]. TALENs represent a recently emerging novel gene editing tool. Highly specific, engineered TALENs have recently been successfully utilized to correct SCD mutation in patient-derived human iPSCs. In combination with piggyBac transposon, TALEN-mediated gene targeting left no residual ectopic sequences at the site of correction and the corrected human iPSCs retained full pluripotency and a normal karyotype [39].

Thalassemia

β-thalassemias are caused by approximately 300 genetic mutations involving the β-globin gene leading to absent or decreased production of adult hemoglobin [40]. Current therapies of β-thalassemia include: blood transfusion and iron chelation therapy [41]. Allogeneic HSCT performed in the postnatal period is the only potentially curative therapeutic modality and is associated with 90% overall survival and 80% disease free survival [38,41]. Unfortunately, this therapeutic option is only available for approximately 30% of patients due to the lack of HLA matched donors [38,41].

Gene therapy, although promising, has shown limited success so far [41]. The major breakthrough in β-thalassemia gene therapy took place a decade ago with the development of globin lentiviral vectors [41]. Since then researchers have focused on designing safe and efficient vectors that can successfully deliver the therapeutic transgene without demonstrating insertional mutagenesis [41]. Improvement in lentivirus vector design has enabled successful introduction of transgenes into both murine and human HSCs leading to amelioration of β-thalassemia in murine models and restoration of erythropoiesis *in vitro* [41]. Extensive studies in this field have led to success of the first clinical trial in France in June 2007, where a young patient with HbE/β° thalassemia was treated with β-globin vector and one year after HSCT, the patient managed to become transfusion-independent [41].

Prenatal diagnosis and selective abortion have been shown to be effective in decreasing the number of β-thalassemia births in countries which have adopted genetic counselling and screening for thalassemia carriers [42]. However, the cells taken for prenatal diagnosis can be converted into iPSCs that can be utilized for treatment in the perinatal period. Early treatment has the advantage of much fewer cells than adult treatment and can also prevent organ damage that can develop in utero or at an early age [42].

Modification of β-globin gene expression in β-thalassemia cells can be achieved by gene therapy combined with induction of Hb F production or correction of the mutated β-globin gene [40]. The generation of patient-specific iPSCs and subsequent correction of the disease-causing mutations offer an ideal therapeutic solution for β-thalassemia [43]. Realizing the therapeutic potential of iPSCs requires robust, precise and safe strategies for genetic modification as randomly integrated transgenes pose risks of oncogenesis [44]. The generation and identification of transgene-expressing iPSC clones are feasible by screening a limited number of single-copy clones and applying safe harbor criteria for their selection [44].

In one study, gene-corrected iPSCs were generated from two patients carrying different types of homozygous mutations. The generated cells showed features of pluripotency and had normal karyotype [43]. The gene-corrected β-thalassemia-iPSC lines from each patient were capable of differentiation into not only hematopoietic progenitor cells but also erythroblasts expressing normal β-globin [43]. In another study, iPSCs were programmed from skin fibroblasts of a patient with homozygous β-thalassemia [42]. Human iPSCs obtained from this patient were able to differentiate into hematopoietic cells that synthesized fetal Hb *in vitro*, thus raising the possibility of using iPSCs to treat patients with homozygous β-thalassemia [42].

Successful treatment of β-thalassemia based on transplantation of genetically modified autologous hematopoietic cells using lentiviral

vectors set the stage for its application in clinical settings [45]. Cavazzano et al. reported one effective case in a phase I/II clinical trial, but several recently published studies showed that these approaches remained rather inefficient [45]. Therapies using iPSCs combined with transgenes can potentially cure thalassemia in the future, but obstacles such as safety, efficacy and cell stability remain to be solved. A state of mixed chimerism is usually detected after transplantation and often progresses towards complete chimerism after HSCT for thalassemia. This implies that thalassemia can be controlled with appropriate doses of HSCs [45]. However, effective postnatal therapy with autologous modified iPSCs may require engineered expression of normal β-globin and chimerism levels exceeding 20% [45].

Two mouse models for β-thalassemia and one mouse model for α-thalassemia have been reported. Use of these mouse models in gene transfer studies revealed Hb tetramers containing human β-globin at levels high enough to ameliorate the disease phenotype [2]. Several human PSC models for thalassemia have also been reported. Human ESC models for β-thalassemia have also been generated through nuclear transfer using the nucleus from a fibroblast of a β-thalassemia patient which could also be used for cell or gene therapy studies. Thalassemia human iPSCs have also been derived from a pre-implantation genetic diagnosis embryo [2].

Sickle Cell Disease

SCD is caused by a genetic mutation, where amino acid valine is substituted with glutamic acid in the 6th codon of β-globin chain [2,46]. This genetic mutation produces an abnormal type of hemoglobin (Hb) called HbS which induces rigidity and structural deformation of RBCs [2,46]. Sickled RBCs cause complications in patients with SCD including: veno-occlusive crises (VOCs), organ damage and premature death. Current therapeutic modalities for SCD are limited. Analgesia, hydration, oxygen supplementation and blood transfusions have classically been administered to treat VOCs and other complications of SCD [2,46]. Hydroxyurea induces Hb F formation and decreases incidence of VOCs and other complications of SCD [2,46]. Various forms of HSCT are being utilized. However, allogeneic HSCT from HLA-matched sibling donor has the best yield. Despite being the only curative therapy for patients with SCD, HSCT carries considerable risks of morbidity and mortality due to graft versus host disease, infections and drug toxicity [2,46].

Various modalities of gene therapy are being developed and these include: gene addition, gene replacement and gene repair [46]. The combination of IPSC technology and targeted gene modification by HR represents a promising approach to generate genetically corrected, patient-derived cells that could be used for autologous transplantation therapies [47]. This strategy has several potential advantages over conventional gene therapy including: (1) eliminating the need for immunosuppression, (2) avoiding the risk of insertional mutagenesis by therapeutic vectors, and (3) maintaining expression of the corrected gene by endogenous control elements rather than a constitutive promoter. However, gene targeting in human pluripotent cells has remained challenging but inefficient [47].

Engineered ZFNs have been shown to substantially increase HR frequencies in human iPSCs raising the prospect of employing this technology to control disease-causing mutations. IPSC lines from SCD patients have been generated using 3 ZNF pairs made by the oligomerized pool engineering (OPEN) method [47]. HR events in human iPSCs can occur as far as 82 bps from a ZFN-induced break. These results provide a proof of principle that ZFNs can be used to produce gene-corrected human iPSCs that could be used for therapeutic applications indicating that ZFN technology can be used to correct a disease-causing mutation by HR [47].

There are 2 humanized mouse models of SCD: (1) the Berkeley mouse which is an engineered transgenic strain, and (2) the Townes mouse which is a knock-in model. The 2 humanized mouse models have proven valuable in studying disease mechanisms and testing therapeutic strategies such as gene therapy [2]. In 2002, the first proof of principle experiment of combined gene and cell therapy for blood disorders in a murine model was performed using an immunodeficient Rag $2^{-/-}$ mouse strain. Results of the study showed that engrafted mice revealed mature lymphoid cells [2]. In 2006, a similar experiment was performed using the Townes humanized SCD mouse model. Autologous ESCs were genetically corrected by HR, differentiated toward the hematopoietic lineage and transplanted into the Townes mice. Subsequent analysis showed: (1) high levels of adults Hb A, (2) reduced RBC sickling, and (3) corrected pathology in animals [2].

Mouse and human fibroblasts can be programmed into an embryonic stem cell-like state by introducing combinations of 4 transcription factors. However, the therapeutic potential of such iPSCs remained undefined [48]. By using a humanized SCD mouse model, it was shown that mice can be rescued after transplantation of hematopoietic progenitors obtained *in vitro* from autologous iPSCs. This was achieved after correction of the human sickle Hb allele by gene-specific targeting. These results provided proof of principle that transcription factor-induced reprogramming combined with gene and cell therapy can be employed for treatment of the disease in mice [48]. However, the problems associated with using retroviruses and oncogenes for reprogramming need to be resolved before considering iPSCs for human therapy [48]. Although reprogramming of human somatic cells into iPSCs has been achieved, future therapeutic application of iPSCs in humans requires overcoming the following obstacles: (1) bypassing the use of harmful oncogenes as part of the reprogramming factors, (2) avoiding the use of gene delivery of retroviral vectors that carry the risk of insertional mutagenesis, and (3) developing robust and reliable differentiation protocols for human iPSCs [48]. Current advances in molecular reprogramming set the stage for devising alternative strategies such as: (1) transient gene expression vectors, (2) engineered membrane-permeable transcription factor proteins, and (3) small molecules that can replace potentially hazardous factors and lessen the risk of cancer associated with the current reprogramming approach [48].

In 2007, with the advent of iPSCs, the same experiment was performed with autologous iPSCs made from the Townes mice. The results impressively demonstrated the potential for harnessing personalized stem cells for the treatment and potential cure of SCD [2]. Later on, iPSCs were derived from murine models of human SCD, restored to a normal allele of human β-globin through HR, then engraftable hematopoietic precursors were derived through directed *in vitro* differentiation and finally mice were treated by hematopoietic transplant [2]. The peripheral blood of engrafted mice showed remarkable restoration of normal erythroid indices and compelling evidence for the potential therapeutic use of iPSCs in hemoglobinopathies [2]. Subsequently, two groups reported the generation of human iPSC models of SCD and ZFNs were used to correct the sickle cell mutation. However, neither of the reports differentiated the cells and functionally tested them to show sickling or a reduction in sickling after correction [2].

Human iPSCs are capable of: (1) achieving complete terminal

erythroid maturation in terms of enucleation [nucleus expansion] *in vitro* and in terms of Hb maturation [synthesis of adult Hb] *in vivo* and (2) opening the way to generation of functionally corrected RBCs for SCD-iPSCs without any genetic modification or drug treatment [49]. Since iPSCs can proliferate indefinitely and can be selected for a phenotype of interest, they are potential candidates to organize complementary sources of RBCs for transfusion. Before use in clinical practice, the procedures need to be optimized [49]. The crucial points that remain to be resolved are optimization of erythroid proliferation and differentiation, and definition of good manufacturing practice conditions for industrial production [49].

iPSCs in other Hematological Disorders and Cancer

Severe congenital neutropenia (SCN)

The autosomal recessive type of SCN is caused by deficiency of HAX1 gene [50]. iPSC lines were generated from a patient with SCN having HAX1 gene deficiency. Genetic correction of iPSCs was made by a novel *in vitro* neutrophil differentiation system. The study resulted in improvement of defective granulopoiesis [50].

Congenital amegakaryocytic thrombocytopenia (CAMT)

Myeloproliferative leukemia virus oncogene (MPL), encoding for thrombopoietin receptor, is deleted or nonfunctional in patients with CAMT. CAMT is caused by loss of thrombopoietin-related MPL-mediated signaling which induces severe pancytopenia leading to bone marrow failure with the onset of thrombocytopenia and anemia prior to leucopenia [51]. Generated CAMT-iPSCs exhibited defective MPL signaling. Excessive MPL signaling in both normal and CAMT-IPSCs led to deleterious megakaryopoiesis and production of $CD41a^{+}$, $CD42a^{-}$ and $CD42b^{-}$ megakaryocytes and platelets [51].

Myelofibrosis

Recently, iPSCs were successfully generated from patients with: (1) primary myelofibrosis with chromosome 13 deletion, and (2) secondary myelofibrosis with JAK 2 V617 F mutation [52]. These disease specific iPSCs provide a research tool for studying the disease and potentially providing targeted therapy ultimately [52].

Myelodysplasia and acute myeloid leukemia (AML)

MonoMAC syndrome is caused by GATA 2 deficiency and patients are predisposed to myelodysplastic syndrome and AML transformation [53]. In a pre-clinical study, iPSCs were derived from a patient with GATA 2 syndrome harboring R361H mutation. These iPSCs displayed severe reduction in hematopoietic differentiation potential and absent clonogenic capacity [53]. Familial platelet disorder (FPD) is an autosomal dominant disease of hematopoietic system that is caused by heterogenous mutations in RUNX1 [54,55]. In another study, iPSC lines were successfully generated from fibroblasts of a patient with FPD. After correction of RUNX1 mutation, megakaryopoiesis was restored [55]. In a third pre-clinical study, iPSCs were successfully established from 3 distinct FPD/AML pedigrees [54]. These FPD-iPSCs were shown to be uniformly defective in emergence of hematopoietic progenitor cells and megakaryocytic differentiation. In addition, the phenotypes of FPD-iPSCs were found to be a consequence of haploinsufficiency of RUNX1 [54].

Juvenile myelomonocytec leukemia (JMML)

iPSCs were generated from malignant cells belonging to 2 children with JMML having p.E76K mutations in the PTPN11 gene [56]. Pharmacological inhibition of MEK (PD0325901) kinase in IPSCs-derived JMML cells caused normalization or reduction of granulocyte monocyte-colony stimulating factor (GM-CSF) independence and hypersensitivity in myeloid precursors of JMML-iPSCs. These results provide a rationale for a potential targeted therapy for JMML [56].

Chronic myeloid leukemia

iPSCs were generated from primary CML patient samples [56,57]. The methylation pattern and the gene expression of CML-iPSCs were very similar to those with normal iPSCs [56]. CML-iPSCs provide a novel platform to investigate CML pathogenesis on the basis of patient derived samples [56].

Lymphomas

The concept of cancer stem cell (CSC) in AML has yielded a new understanding of carcinogenesis and relapses [58]. These leukemia-originating stem cells are critical for the initiation and maintenance of leukemias [59]. However, the existence of a similar cell population that may generate B-cell lymphocytic malignancies remains uncertain [59]. Detailed selection and molecular characterization of the specific cells of origin of each B-cell lymphoma entity are essential steps to better understanding of lymphomagenesis and to develop effective and potentially curative therapeutic modalities [59].

Solid tumors

iPSCs have also been generated from malignant cells belonging to patients with gastrointestinal cancers [8]. Antitumor immunotherapy using T cell and natural killer (NK) cell-based therapies has demonstrated promising therapeutic potentials in patients with renal cell carcinoma, malignant melanoma and chemotherapy refractory AML [60]. The ability to modify human ESC and iPSC-derived NK cells with tumor-specific receptors may be utilized against a wide range of malignancies in the near future after having the appropriate pre-clinical and clinical trials performed [60]. Cancer-derived iPSCs are expected to provide a novel experimental opportunity to establish disease models [61].

Generation of Engraftable HSCs by Teratoma Formation

Injection of iPSCs into immunodeficient mice caused subcutaneous reproduction of HSC niche by means of teratoma formation and this led to successful induction of engraftable HSCs [62]. These HSCs can practically be used in the treatment of hematological disorders including bone marrow failure syndromes and immunological disorders such as X-linked severe combined immunodeficiency with risks of graft rejection or graft versus host disease [62].

Banking of iPSCs

Blood transfusion is a common procedure in modern medicine and it is being practiced throughout the world. However, blood supply is usually strained during natural disasters and wars [63]. Alternative substitutes such as synthetic oxygen radical carriers have been proven to be unsuccessful [63]. iPSCs may be utilized in the near future for RBC and platelet transfusions as well as other cell therapies [64]. The main obstacle in organ and tissue transplantation is rejection by the recipient due to the 3 main immunological barriers: HLA, ABO blood group and minor antigens [65]. The problem of rejection in transplantation can be circumvented by using autologous stem cells such as iPSCs derived directly from the patient as these cells have the advantage of theoretical absence of immune rejection by the recipient [66]. Establishment of stem cell banks comprising HLA-typed human ESCs and iPSCs is

a strategy that is proposed to overcome the immunological barriers of transplantation by providing HLA-matched tissues for the target population [67]. A tissue bank from 150 healthy homologous HLA-matched volunteers could match 93% of the population of the United Kingdom with minimal requirement for immunosuppression [68].

Tissues or organs derived from human PSCs could be the best solution to cure several human diseases that do not respond to standard therapies [65]. However, iPSCs have certain limitations, particularly the disease of the recipient and the possible difficulties in generation, expansion and manipulation of autologous preparations of iPSCs [66]. An alternative to using autologous iPSCs in the establishment of banks of well-characterized human adult cells that could be used to generate iPSCs and their derivatives has recently emerged [66]. However, current initiatives to establish banks for human iPSCs face real challenges in recruiting large numbers of donors with diverse diseases as well as genetic and phenotypic representations [69]. Also, reaching standards of good clinical manufacturing practices and quality controlled stem cell lines could be challenging [65,70]. A recent study showed that single-drop volumes of finger-prick blood samples are sufficient to perform cellular reprogramming, DNA sequencing and blood serotyping in parallel. This novel strategy has the potential to facilitate development of large-scale human iPSC banking worldwide [69]. Additionally, a consortium of 26 partners has been formed to establish a European bank for iPSCs that will act as a central storage and distribution facility for human iPSCs to be used by researchers in order to study intractable disease and to develop new therapeutic strategies [71].

The discovery of deriving iPSCs from cord blood combined with the presence of non-hematopoietic stem and progenitor cells in cord blood may lead to enhanced therapeutic applications of these cells as well as enhanced use of cord blood banking [72]. The genetic diversity related to ancestry backgrounds of populations living in certain communities is another limitation for the establishment of iPSC banks [73]. However, a recent study performed in USA suggested that establishment of multiethnic haplobank of iPSC lines is a possible solution, but this can only be achieved by a large scale concerted worldwide collaboration [73].

Imaging of iPSCs

Tracking iPSCs and investigating the feasibility of their utilization require the development of novel technology to monitor their location, proliferation, integration and differentiation in human recipients [74]. The ideal imaging technique should be non-toxic, biocompatible and highly specific. Plenty of progress has been achieved in this field and hopefully this will become a reality in clinical practice in the near future [74].

Challenges Limiting Clinical Applications of iPSCs

The following concerns and future challenges may limit the clinical applications of iPSCs: (1) the consistent and efficient routine generation of iPSCs without DNA integration into patient's genome, (2) inferior efficacy of human iPSCs manipulation relative to mature derivatives, (3) biased generation of certain and not all desired cell types through directed differentiation of iPSCs, (4) the heterogeneity of cells derived from iPSCs, and (5) lack of routine high throughput comprehensive characterization of human iPSCs and their cellular derivatives for quality control purposes [4].

Ethical Issues Related to the Use of PSCs

Regenerative medicine, which is currently on the stage of research, implies important ethical, legal and social issues that need to be addressed [75]. The use of human embryonic cells in experimental research and clinical medicine is ethically unacceptable as obtaining such cells requires the destruction of human embryos produced by *in vitro* fertilization techniques [76]. New advances in cell reprogramming, particularly generation of iPSCs represents a promising possibility of avoiding the use of human embryonic cells [76]. Although destruction of human embryos is avoided, ethical debate on cell reprogramming, particularly the experimental and clinical utilization of iPSCs remains open [75-77].

Conclusions

iPSCs are pluripotent cells that can be can be derived from various cell lines and can be reprogrammed to give rise to any cell type found in the body. Their clinical applications are rapidly expanding and these include: disease modeling, regenerative medicine, drug discovery, cell and genetic therapy as well as synthesis of several blood components. The future role of iPSCs in hematology is promising as they can be used to treat several benign and malignant hematological disorders. Additionally, generation of various blood components from iPSCs will enhance their future utilization in providing a more comprehensive management for critically ill patients.

References

1. Liras A, Segovia C, Gaban AS (2013) Induced pluripotent stem cells: therapeutic applications in monogenic and metabolic diseases and regulatory and biochemical considerations. In Pluripotent Stem Cells; In Tech.
2. Arora N, Daley GQ (2012) Pluripotent stem cells in research and treatment of hemoglobinopathies. Cold Spring Harb Perspect Med 2: a011841. [PubMed]
3. Nelson TJ, Martinez-Fernandez A, Yamad S, Ikeda Y, Perez-Terzic C, et al. (2010) Induced pluripotent stem cells: advances to applications. Stem Cell Clon 3: 29-37. [PubMed]
4. Nordin N, Lai MI, Veerakumarasivam A, Ramasamy R, Abdullah S, (2011) Induced pluripotent stem cells: history, properties and potential applications. Med J Malaysia 66: 4-9. [PubMed]
5. Li J, Song W, Song W, Pan G, Zhou J (2014) Advances in understanding the cell types and approaches used for generating induced pluripotent stem cells. J Hematol Oncol 7: 50. [PubMed]
6. Jiang W, Zhang J, Yang X, Gao J (2012) Induced pluripotent stem cells: origins and directions. Human Genet Embryol.
7. Zhao J, Jiang WJ, Sun C, Hou CZ, Yang XM, et al. (2013) Induced pluripotent stem cells: origins, applications, and future directions. J Zhejiang Univ Sci B 14: 1059-1069. [PubMed]
8. de Souza Fernandez T, de Souza Fernandez C, Luiz Mencalha A (2013) Human induced pluripotent stem cells from basic research to potential clinical applications in cancer. Biomed Res Int 2013: 430290. [PubMed]
9. Teoh HK, Cheong SK (2012) Induced pluripotent stem cells in research and therapy. Malaysian J Pathol 34: 1-13. [PubMed]
10. Takahashi K, Yamanaka S (2013) Induced pluripotent stem cell in medicine and biology. Development 140: 2457-2461. [PubMed]
11. Ben Coxworth (2014) First transfusion of manufactured blood planned for 2016. Wellcome Trust, University of Glasgow via The Telegraph.
12. Kaufman DS (2009) Toward clinical therapies using hematopoietic cells derived from human pluripotent stem cells. Blood 114: 3513-3523. [PubMed]
13. Kimbrel EA, Lu SJ (2011) Potential clinical applications for human pluripotent stem cell-derived blood components. Stem Cells Int 2011: 273076. [PubMed]
14. Lengerke C, Daley GQ (2010) Autologous blood therapies from pluripotent stem cells. Blood Rev 24: 27-37. [PubMed]
15. Gutierrez-Aranda I, Ramos-Mejia V, Bueno C, Munoz-Lopez M, Real PJ, et al. (2010) Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embroyonic stem cells regardless the site of injection. Stem Cells 28: 1568-1570. [PubMed]

16. Zhao T, Zhang ZN, Rong Z, Xu Y (2011) Immunogenecity of induced pluripotent stem cells. Nature 474: 212-215. [PubMed]

17. Fu X (2014) The immunogenecity of cells derived from induced pluripotent stem cells. Cell Mol Immunol 11: 14-16. [PubMed]

18. Inoue H, Yamanaka S (2011) The use of induced pluripotent stem cells in drug development. Clin Pharmacol Ther 89: 655-661. [PubMed]

19. Mercola M, Colas A, Willems E (2013) Induced pluripotent stem cells in cardiovascular drug discovery. Circ Res 112: 534-548. [PubMed]

20. Svendsen CN (2013) Back to the future: how human induced pluripotent stem cells will transform regenerative medicine. Hum Mol Genet 22: R32-38. [PubMed]

21. Liu Z, Zhou J, Wang H, Zhao M, Wang C (2013) Current status of induced pluripotent stem cells in cardiac tissue regeneration and engineering. Regen Med Res 1:6.

22. Falanga V (2012) Stem cells in tissue repair and regeneration. J Invest Dermatol 132: 1538-1541. [PubMed]

23. Muller LUW, Milsom MD, Harris CE, Vyas R, Brumme K, et al. (2012) Overcoming reprogramming resistance of fanconi anemia cells. Blood 119: 5449-5457. [PubMed]

24. Liu GH, Suzuki K, Li M, Qu J, Montserrat N, et al. (2013) Modelling fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. Nature Commun 5: 4330. [PubMed]

25. Muller LUW, Schlaeger TM, DeVine AL, Williams DA (2012) Induced pluripotent stem cells as a tool for gaining new insights into fanconi anemia. Cell Cycle 11: 2985-2990. [PubMed]

26. Navarro S, Moleiro V, Molina-Estevez FJ, Lozano ML, Chinchon R, et al. (2014) Generation of iPSCs from genetically corrected Brca 2 hypomorphic cells: implications in cell reprogramming and stem cell therapy. Stem Cells 32: 436-446. [PubMed]

27. Yung SK, Tilgner K, Ledran MH, Habibollah S, Neganova I, et al. (2013) Brief report: human pluripotent stem cell models of fanconi anemia deficiency reveal an important role for fanconi anemia proteins in cellular reprogramming and survival of hematopoietic progenitors. Stem Cells 31: 1022-1029. [PubMed]

28. Papapetrou EP (2012) FA iPS: Correction or reprogramming first? Blood 119: 5341-5342. [PubMed]

29. MacMillan ML, Hughes MR, Argawal S, Daley GQ (2011) Cellular therapy for fanconi anemia: the past, present and future. Biol Blood Marrow Transplant 17: S109-114. [PubMed]

30. Raya A, Rodriguez-Piza I, Guenechea G, Vassena R, Navarro S, et al. (2009) Disease-corrected hematopoietic progenitors from fanconi anemia induced pluripotent stem cells. Nature 460: 53-59. [PubMed]

31. Chlon TM, Hoskin EE, Mayhew CN, Wikenheiser-Brokamp KA, Davies SM, et al. (2014) High risk human papillomavirus E6 protein promotes reprogramming of fanconi anemia patients cells through repression of P53 but does not allow for sustained growth of induced pluripotent stem cells. J Virol 88: 11315. [PubMed]

32. Xu D, Alipio Z, Fink LM, Adock DM, Yang J, et al. (2009) Phenotypic correction of marine hemophilia A using an iPS cell-based therapy. Proc Natl Acad Sci USA 106: 808-813. [PubMed]

33. Park CY, Kim J, Kweon J, Son JS, Lee JS, et al. (2014) Targeted inversion and reversion of the blood coagulation factor 8 gene in human iPS cells using TALENS. Proc Natl Acad Sci USA 111: 9253-9258. [PubMed]

34. Liras A (2011) Induced human pluripotent stem cells and advanced therapies: future perspectives for the treatment of hemophilia. Thromb Res 128: 8-13. [PubMed]

35. Liras A, Segovia C, Gaban AS (2012) Advanced therapies for the treatment of hemophilia: future perspectives. Orphanet J Rare Dis 7: 97. [PubMed]

36. Yakura Y, Ishihara C, Kurosaki H, Kazuki Y, Komatsu N, et al. (2013) An induced pluripotent stem cell-mediated and integration-free factor VIII expression system. Biochem Biophys Res Common 431: 336-341. [PubMed]

37. Jia B, Chen S, Zhao Z, Liu P, Cai J, et al. (2014) Modeling of hemophilia A using patients-specific induced pluripotent stem cells derived from urine cells. Life Sci 108: 22-29. [PubMed]

38. Ramachandra DL, Shaw SSW, Shangaris P, Loukogeorgakis S, Guillot PV, et al. (2014) In utero therapy for congenital disorders using amniotic fluid stem cells. Front Pharmacol 5: 270.

39. Sun N, Zhao H (2014) Seamless correction of the sickle cell disease mutation of the HBB gene in human induced pluripotent stem cells using TALENs. Biotechnol Bioeng 111: 1048-1053. [PubMed]

40. Finotti A, Gambari R (2014) Recent trends for novel options in experimental biological therapy of β-thalassemia. Expert Opin Biol Ther 14: 1443-1454. [PubMed]

41. Drakopoulou E, Papanikolaou E, Anagnou NP (2011) The ongoing challenge of hematopoietic stem cell-based gene therapy for β-thalassemia. Stem Cells Int 2011: 987980. [PubMed]

42. Ye L, Chang JC, Lin C, Sun X, Yu J, et al. (2009) 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 106: 9826-9830. [PubMed]

43. Ma N, Liao B, Zhang H, Wang L, Shan Y, et al. (2013) Transcription activator-like effector nuclease (TALEN)-mediated gene correction in integration-free β-thalassemia induced pluripotent stem cells. J Biol Chem 288: 34671-34679. [PubMed]

44. Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, et al. (2011) Genomic safe harbors permit high β-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol 29: 73-78. [PubMed]

45. Yang G, Shi W, Hu X, Zhang J, Gong Z, et al. (2014) Therapeutic effects of induced pluripotent stem cells in chimeric mice with thalassemia. Haematologica 99: 1304-1311. [PubMed]

46. Harley MN (2013) Current treatment and perspective therapies to manage sickle cell disease. Lab Med 44: e 92-e96.

47. Sebastiano V, Maeder ML, Angstman JF, Haddad B, Khayter C, et al. (2011) In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. Stem Cells 29: 1717-1726. [PubMed]

48. Hanna J, Werning M, Markoulaki S, Sun CW, Meissner A, et al. (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 318: 1920-1923. [PubMed]

49. Kobari L, Yates F, Oudrhiri N, Francina A, Kiger L, et al. (2012) Human induced pluripotent stem cells can reach complete terminal maturation: in vivo and in vitro evidence in erythropoietic differentiation model. Haematologica 97: 1795-1803. [PubMed]

50. Morishima T, Watanabe KI, Inwa A, Hirai H, Saida S, et al. (2014) Genetic correction of HAX1 in induced pluripotent stem cells from patient with severe congenital neutropenia improves defective granulopoiesis. Haematologica 99: 19-27. [PubMed]

51. Hirata S, Takayama N, Jono-Ohnishi R, Endo H, Nakamura S, et al. (2013) Congenital amegakaryocytic thrombocytopenia iPS cells exhibit defective MPL-mediated signaling. J Clin Investig 123: 3802-3814. [PubMed]

52. Hosai M, Kumano K, Taoka K, Arai S, Katoaka K, et al. (2014) Generation of induced pluripotent stem cells derived from primary and secondary myelofibrosis patient samples. Exp Hematol 42: 816-825. [PubMed]

53. Jung M, Winkler T, Donaires FS, Hong SG, Hsu AP, et al. (2014) Induced pluripotent stem cells from a patient with GATA2 deficiency display a block in hematopoietic differentiation. 17th Annual Meeting of the American Society of Gene & Cell Therapy, Washington.

54. Sakurai M, Kunimoto H, Watanabe N, Fukuchi Y, Yuasa S, et al. (2014) Impaired hemtopoietic differentiation of RUNX1-mutated induced pluripotent stem cells derived from FPD/AML patients. Leukemia 28: 2344-2354. [PubMed]

55. Connelly JP, Kwon EM, Gao Y, Trivedi NS, Elkahloun AG, et al. (2014) Targeted correction of RUNX1 mutation in FPD patient-specific induced pluripotent stem cells rescues megakaryopoietic defects. Blood 124:1926-1930. [PubMed]

56. Gandre-Babbe S, Paluru P, Aribeana C, Chou ST, Bresolin S, et al. (2013) Patient-derived induced pluripotent stem cells recapitulate hematopoietic abnormalities of juvenile myelomonocytic leukemia. Blood 121: 4925-4929. [PubMed]

57. Bedel A, Pasquet JM, Lippert E, Taillepierre M, Lagarde V, et al. (2013) Variable behavior of iPSCs derived from CML patients for response to TKI and hematopoietic differentiation. PLoS One 8: e71596. [PubMed]

58. Gross E, Quillet-Mary A, Ysebaert L, Laurent G, Fournie JJ (2011) Cancer stem cells of differentiated B-cell malignancies: models and consequences. Cancer 3: 1566-1579. [PubMed]

59. Martinez-Climent JA, Fontan L, Goscoyne RD, Siebert R, Prosper F (2010) Lymphoma stem cells: enough evidence to support their existence? Haematologica 95: 293-302. [PubMed]

60. Knorr DA, Kaufman DS (2010) Pluripotent stem cell-derived natural killer cells for cancer therapy. Transl Res 156: 147-154. [PubMed]

61. Kumano K, Arai S, Hosoi M, Taoka K, Otsu M, et al. (2012) Generation of induced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. Blood 119: 6234-6242. [PubMed]

62. Suzuki N, Yamazaki S, Yamaguchi T, Okabe M, Masaki H, et al. (2013) Generation of engraftable hematopoietic stem cells from induced pluripotent stem cells by way of teratoma formation. Mol Ther 21: 1424-1431. [PubMed]

63. Daley GQ (2014) Deriving blood stem cells from pluripotent cells for research and therapy. Best Pract Res Clin Haematol 27: 293-297. [PubMed]

64. Shah S, Huang X, Cheng L (2014) Concise review: stem cell-based approaches to red blood cell production for transfusion. Stem Cells Transl Med 3: 346-355. [PubMed]

65. de Rham C, Villard J (2014) Potential and limitation of HLA-based banking of human pluripotent stem cells for cell therapy. J Immunol Res 2014: 518135. [PubMed]

66. Zimmerman A, Preynat-Seauve O, Tiercy JM, Krause KH, Villard J (2012) Haplotype-based banking of human pluripotent stem cells for transplantation: potential and limitations. Stem Cells Dev 21: 2364-2373. [PubMed]

67. Tayler CJ, Bolton EM, Bradley JA (2011) Immunological considerations for embryonic and induced pluripotent stem cell banking. Phil Trans R Soc Lond B Biol Sci 366: 2312-2322. [PubMed]

68. Tayler CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM (2012) Generating an IPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell 11: 147-152. [PubMed]

69. Tan HK, Delon Toh CX, Ma D, Yang B, Liu TM, et al. (2014) Human finger-prick induced pluripotent stem cells fascilitate the development of stem cell banking. Stem Cell Transl Med 3: 586-598. [PubMed]

70. Stacey GN, Crook JM, Hei D, Ludwig T (2013) Banking human induced pluripotent stem cells: lessons learned from embryonic stem cells? Cell Stem Cell 13: 385-388. [PubMed]

71. Roslin Cells-Latest News (2014) EBISC-the first Europian bank for induced pluripotent stem cells, Edinburgh.

72. Broxmeyer HE (2010) Will iPS cells change therapeutic applicability of cord blood cells and banking. Cell Stem Cell 6: 21-24. [PubMed]

73. Gourraud PA, Gilson L, Girard M, Peschanski M (2012) The role of human leucocyte antigen matching in the development of multiethnic "haplobank" of induced pluripotent stem cell lines. Stem Cells 30: 180-186. [PubMed]

74. Cai W, Zhang Y, Kamp TJ (2011) Imaging of induced pluripotent stem cells: from cellular reprogramming to translation. Am J Nucl Med Mol Imaging 1: 18-28. [PubMed]

75. Ida R (2008) Ethical, legal and social issues in regenerative medicine. Nihon Rinsho 66: 991-996. [PubMed]

76. Aznar Lucea J, Martinez M (2012) Ethical reflections on cell reprogramming. Caud Bioet 23: 287-299. [PubMed]

77. Gamez-Escalona JA (2013) IPS an ethical paradigm for biomedical research. Caud Bioet 24: 419-442. [PubMed]