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Disease modeling of bone marrow failure syndromes using iPSC-derived hematopoietic stem progenitor cells

Mahmoud I. Elbadry[a,b], J. Luis Espinoza[c], and Shinji Nakao[a]

[a]*Hematology/Respiratory Medicine, Faculty of Medicine, Institute of Medical Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan;* [b]*Department of Internal Medicine, Division of Hematology, Faculty of Medicine, Sohag University, Egypt;* [c]*Department of Hematology and Rheumatology, Faculty of Medicine, Kindai University, Osaka, Japan*

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The plasticity of induced pluripotent stem cells (iPSCs) with the potential to differentiate into virtually any type of cells and the feasibility of generating hematopoietic stem progenitor cells (HSPCs) from patient-derived iPSCs (iPSC-HSPCs) has many potential applications in hematology. For example, iPSC-HSPCs are being used for leukemogenesis studies and their application in various cell replacement therapies is being evaluated. The use of iPSC-HSPCs can now provide an invaluable resource for the study of diseases associated with the destruction of HSPCs, such as bone marrow failure syndromes (BMFSs). Recent studies have shown that generating iPSC-HSPCs from patients with acquired aplastic anemia and other BMFSs is not only feasible, but is also a powerful tool for understanding the pathogenesis of these disorders. In this article, we highlight recent advances in the application of iPSCs for disease modeling of BMFSs and discuss the discoveries of these studies that provide new insights in the pathophysiology of these conditions. © 2019 Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Aplastic anemia (AA) is a life-threatening bone marrow failure (BMF) disorder, resulting in bone marrow hypoplasia, infection and hemorrhage, and severe peripheral pancytopenia. Although the most cases of AA are acquired and associated with the autoimmune destruction of hematopoietic stem progenitor cells (HSPCs) in the BM, in some cases, the BMF is caused by genetic or inherited anomalies that impair hematopoiesis [1]. The destruction or dysfunction of HSPCs in the BM of patients with BMF syndromes (BMFSs) limits the study of these disorders because the use of conventional in vitro HSPC culture or in vivo animal models for creating patient-specific disease modeling is technically impossible due to the unavailability of patient-derived HSPCs. With the development of induced pluripotent stem cells (iPSCs) [2], a promising venue for the study of rare diseases such as BMFSs has been opened. The generation of patient-derived iPSCs and their subsequent differentiation into iPSC-HSPCs offer a unique opportunity for generating disease models to study several genetic and immune backgrounds of BMFSs, facilitating the investigation of human rare diseases based on individual patients' phenotypes (Figure 1). Previously, we summarized some aspects of using iPSCs for understanding AA pathogenesis and the methods of establishing animal models for acquired AA (aAA) using iPSCs [3]. To achieve the goal of this review, we will focus on the previous successful trials to generate iPSCs from patients with different BMFSs. By drawing upon the broad experimental expertise of the previous published works, we will try to summarize the possible future application of this technology in understanding the pathogenesis of BMFSs and the potential challenges encountered using iPSC-based models of these disorders.

Generation of iPSC clones from patients with inherited BMFSs

Inherited BMFSs are a rare group of disorders often developing in childhood that are characterized by BMF with a marked reduction of all hematopoietic lineages or a single-cell lineage usually in association with one or more physical abnormalities [4]. Although the genetic lesions linked with most inherited BMFSs have been identified, some of the cellular events resulting from such genetic aberrations have not been clarified [5]. The utilization of iPSC-derived hematopoietic cells

Offprint requests to: Shinji Nakao, MD, PhD, Hematology/Respiratory Medicine, Faculty of Medicine, Institute of Medical Pharmaceutical and Health Sciences, Kanazawa University, Takara-machi 13-1, 920-8641 Kanazawa, Japan; E-mail: snakao8205@staff.kanazawa-u.ac.jp

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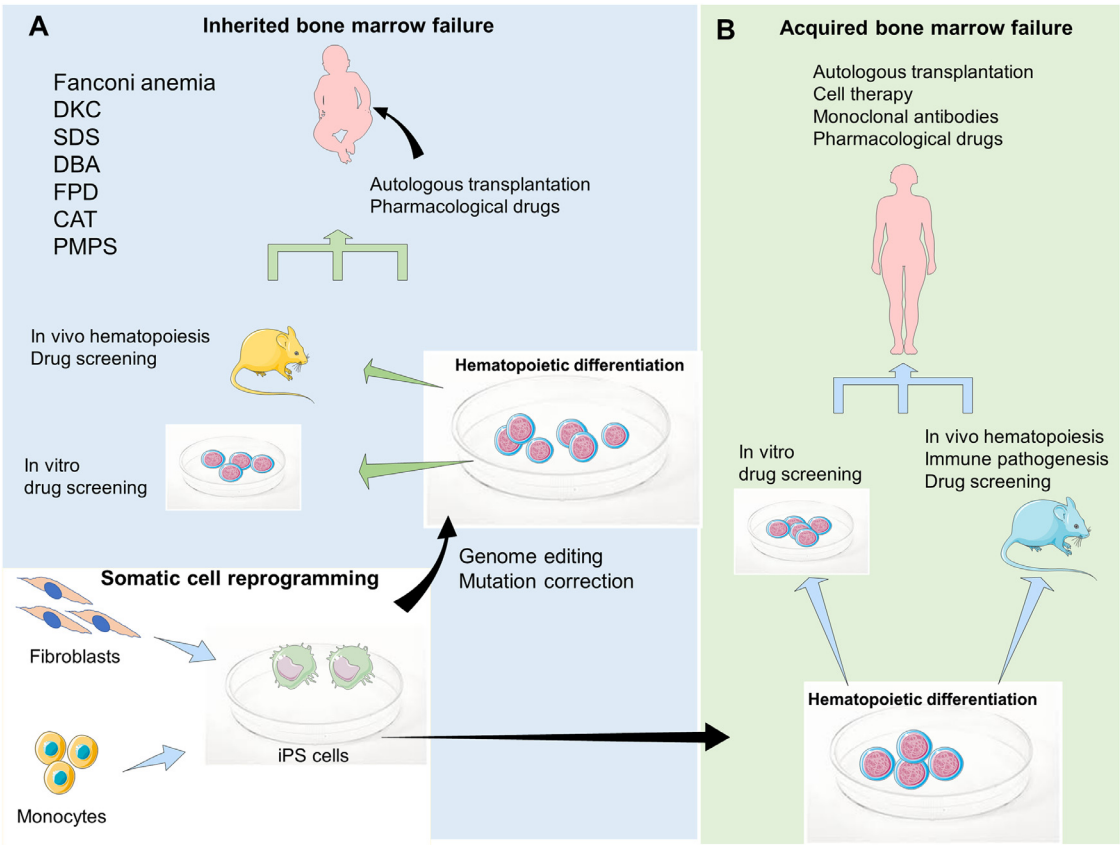

Figure 1. Disease model of BMFSs. Reprograming of somatic cells (fibroblasts or monocytes) to generate iPSCs opens new opportunities for the study of BMFSs. **(A)** Differentiation of iPSCs into hematopoietic stem cells coupled with genome editing and mutation correction has been tested to verify the pathogenesis of inherited BMFSs including FA, DKC, SDS, DBA, FPD, congenital megakaryocytic thrombocytopenia (CMT), and PMPS. **(B)** The hematopoietic differentiation of iPSCs derived from patients with acquired BMFS (AA) has been used to study in vitro and in vivo hematopoiesis with an emphasis on experiments aimed at unraveling the autoimmune mechanisms involved in the pathogenesis of this disorder. These studies may result in the establishment of various therapeutic approaches such as autologous transplantation, cell therapy, the use of blocking monoclonal antibodies, or the utilization of pharmaceuticals with inhibitory or stimulatory activities.

has emerged as a promising tool for the study of the somatic and germline mutations linked to inherited BMFSs and various studies, including the use of in vitro cell culture or in vivo animal models, have been reported.

Fanconi anemia

Fanconi anemia (FA) is the most common type of BMFS and is characterized by a spectrum of congenital physical abnormalities, coupled with progressive fatal BMF, chromosomal instability, and cancer predisposition [6]. At a molecular level, 21 FA genes encoding proteins involved in multiple DNA damage repair pathway essential for maintaining genomic stability have been identified [6,7]. The complete functional characterization of FA genes is crucial for understanding the molecular pathogenesis of this disease; however, this has been hindered by the scarcity of FA patient samples and the difficulty of generating bona fide disease models. Due to the inherent defects in the DNA repair pathway, the effective reprogramming and maintenance of pluripotency of FA-derived somatic cells has been challenging. Not surprisingly, the first attempts to generate iPSCs from patients with FA reported very low efficiency in iPSC induction [8–10]. In addition, FA-derived iPSCs (FA-iPSCs) showed reduced potential to differentiate into the hematopoietic cells, producing fewer HSPCs and impaired erythroid and megakaryocytic differentiation capacity than normal iPSCs [11,12]. Liu et al. [11] generated iPSCs from a patient with FA and succeeded in generating isogenic control iPSC lines using helper-dependent adenoviral vector (HDAdV)–mediated in situ targeted correction of the FANCA mutation. This study confirmed that genome stability was preserved in FA-iPSCs (normal karyotype at passage 13) and their differentiated progeny. Compared with control iPSCs, FA-iPSCs generated a significantly lower percentage of hematopoietic progenitor cells (HPCs) defined as a $CD34^{high}/CD43^{low}$ population and those FA-HPCs were restricted to colony-forming unit-

granulocyte macrophage and failed to generate erythroblast or megakaryocyte colonies. This phenotype was completely rescued by in situ FANCA gene correction [11]. A higher reprogramming efficiency of FA fibroblasts was reported in a model of conditional reprogramming combined with the introduction of a FANCA transgene. In this study, the inhibition of CHK1 effectively restored the growth of FA-deficient iPSCs at a level comparable to that of normal iPSCs by bypassing the cell cycle at the G2-M checkpoint [13]. The findings that targeted gene correction rescued the phenotypic abnormalities in FA-iPSCs [11] indicate that iPSC technology has a therapeutic potential for this disease; however, given the defects in the DNA repair pathway, further studies will be required not only to find ways to enhance the reprogramming efficiency of FA somatic cells and their differentiation into different lineages, but also to exclude the possible emergence of mutations that may occur during cell reprogramming.

Dyskeratosis congenita

Dyskeratosis congenita (DKC) is a rare progressive congenital disorder with a highly variable phenotype. Clinically, DKC has been characterized by the triad of abnormal nails, reticular skin pigmentation, and oral leukoplakia, with some manifestations that resemble premature aging (similar to progeria), although this phenotype is not always present [14]. In addition to developing BMFS, which occurs in more than 80% of cases, DKC patients have an increased risk of malignant transformation. At the molecular level, DKC is a disorder of poor telomere maintenance, in which one or more mutations directly or indirectly affect the vertebrate telomerase RNA component (TERC), giving rise to an abnormal ribosome function.

To study the disease mechanisms in humans, Agarwal et al. [15] generated iPSCs from DKC patients harboring DKC1 mutations and observed that the reprogrammed DKC somatic cells were able to overcome TERC levels, restoring telomere maintenance and cell self-renewal despite the underlying genetic lesions affecting telomerase. In contrast, other studies reported that iPSC clones derived from fibroblasts of five DKC patients [16] and four patients with heterozygous mutations in either *TERT* or *TERC* and hypocellular BM [17] exhibited some telomere abnormalities, including impaired telomere elongation or progressive telomere attrition with markedly reduced accumulation of TERC, dyskerin, and TCAB1 protein levels in Cajal bodies compared with wild-type (WT) iPSCs reprogrammed in parallel, which could be related to mislocalization of the telomerase complex. These observations substantiate the therapeutic potential of methods aimed at increasing TERC expression in DKC. At the same time, the ability to generate DKC-iPSCs provide limitless cells with which to study functional roles of telomeres in maintaining the pluripotency and to investigate the phenotypic variability among individuals with telomerase mutations; however, the dynamic role of telomere and telomerase during the process of cell reprogramming needs to be clarified by further investigations.

Shwachman–Diamond syndrome

Shwachman–Diamond syndrome (SDS) is a rare, autosomal-recessive congenital disorder characterized by BMF, exocrine pancreatic insufficiency, skeletal abnormalities, and short stature [18]. At the molecular level, the genetic defect in this syndrome lies on the long arm of 7 position 7q11. The Shwachman–Bodian–Diamond syndrome (*SBDS*) gene is expressed in all tissues and encodes a protein of 250 amino acid residues [18]. However, the function of this protein is not known, so many aspects of SDS pathogenesis are not completely understood. The generation of iPSCs derived from SDS patients showed a significant heterogeneity among iPSC clones because one patient's iPSC lines could not differentiate into the hematopoietic lineage at all, whereas another patient's iPSC lines showed impaired hematopoietic differentiation. Although, under the appropriate culture conditions, these iPSC lines differentiated into pancreatic progenitor cells, they showed impaired ductal formations and deficient synthetic functions. Intracellular granules containing proteases were observed in iPSC-derived myeloid and pancreatic cells, suggesting that adding protease inhibitors during hematopoietic and pancreatic differentiation could be helpful to investigate the pathogenesis of SDS and may contribute to identify new drug targets for SDS [19]. Recently, Ruiz-Gutierrez et al. [20] established SDS-iPSCs with homozygous IVS2+2 T>C *SBDS* mutations that expressed low levels of SBDS protein with reduced production of HSPCs, similar to levels noted in the primary patient samples. Interestingly, they also modeled SDS with IVS2+2 T>C *SBDS* mutations and deletion of 7q at locus (11.2) by generating SDS (del7q) iPSCs using a modified Cre-Lox approach. The SDS-iPSCs with del(7q) demonstrated a marked reduction in proliferation without an increase in cell death in comparison with isogenic SDS-iPSCs. These isogenic SDS-iPSCs with or without del(7q) models revealed a novel strategy to determine the effects of del 7q on hematopoiesis and new drug targets for this cryptic clinical disorder.

Diamond–Blackfan anemia

Diamond–Blackfan anemia (DBA) is an inherited BMFS characterized by severely decreased erythroid precursors coupled with progress to overt BMF and leukemia predisposition. The genetic aberrations observed in this disease

mainly affect different ribosomal gene loci, leading to altered ribosomal functions [21]. However, the exact mechanism by which haploinsufficiency results in erythroid failure and other clinical manifestations remains uncertain. The generation of iPSC lines from fibroblasts of DBA patients showed reprogramming inefficiently, although stable clones similar to those generated from healthy individuals were obtained. The mutant clones exhibited globally defective hematopoiesis upon multipotent hematopoietic progenitor differentiation and erythroid potential with ability to be rescued using zinc finger nuclease (ZFN) by site-directed gene correction [22]. Recently, Doulatov et al. [23] succeeded in generating DBA-iPSC clones recapitulating the defects in erythroid differentiation that are typically observed in the disease. Interestingly, they also identified small-molecule enhancers of rapamycin 28 (SMER28), a small-molecule inducer of autophagy, which rescued the erythropoiesis in in vitro models as well as in vivo models of DBA. These findings suggest that autophagy may be dysregulated in DBA and provide an explanation for enhancing erythropoiesis by upregulating the expression of globin genes using SMER28, which acted through autophagy factor ATG5 in RPS19-deficient cells. These findings demonstrate the power of iPSC models in drug screening strategies for hematological diseases.

Congenital amegakaryocytic thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare, inherited, autosomal-recessive disorder that presents with BMF characterized by an isolated and severe decrease in the number of platelets and megakaryocytes during the first years of life that can progress to pancytopenia later in childhood [24]. The cause of this disorder appears to be a mutation in the gene for the thrombopoietin (TPO) receptor, c-Mpl. Hirata et al. [25] succeeded in establishing iPSCs from a patient with CAMT due to compound heterozygous mutations in the myeloproliferative leukemia (*MPL*) virus oncogene 1499delT from the father and *MPL* Q186X from the mother. The CAMT-iPSCs lines showed fewer megakaryocytes upon hematopoietic differentiation and this defect was not rescued by TPO treatment. Interestingly, retrovirus-mediated MPL gene transfer rescued the hematopoietic phenotype and restored MPL expression [25]. This CAMT-iPSC model suggested a novel role for MPL signaling in erythropoiesis because it showed that increased expression of MPL in MPL^{low} CAMT iPSCs led to comparable erythroid and megakaryocyte output. In contrast, the CAMT iPSC with MPL^{high} expression showed increased megakaryocytic differentiation but displayed blocked erythropoiesis. TPO and MPL signaling controls megakaryocytic and erythroid bifurcation through the cross-antagonism of the transcriptional factors Friend leukemia virus integration 1 (FLI1) toward megakaryopoiesis and Kruppel-like factor 1 (KLF1) toward erythropoiesis [26]. Conversely, Kuvardina et al. [27] found that, during megakaryopoiesis, runt-related transcription factor 1 (RUNX1) inhibited erythroid differentiation of primary human $CD34^+$ progenitor cells and murine megakaryocytic and erythroid progenitors through its blockage of the KLF1-dependent erythroid gene expression. These findings with CAMT-iPSCs models could lead to the identification of new roles for *TPO* and *MPL* signaling in megakaryocytic and erythroid bifurcation.

Familial platelet disorder

Familial platelet disorder (FPD) with predisposition to acute myelogenous leukemia (AML) is a very rare disorder characterized by mild to moderate thrombocytopenia, an abnormal platelet function, and an increased risk of developing blood malignancies especially myelodysplastic syndrome (MDS) and AML [28]. The disease is an inherited, autosomal-dominant disorder caused by heterozygous loss-of-function mutations in *RUNX1* [29]. Impaired hematopoiesis and defects in megakaryocytic differentiation were reported in iPSC lines derived from patients with FPD. In these studies, FPD-iPSCs displayed normal early erythroid –megakaryocyte progenitor formation, but the megakaryocytic differentiation and maturation from FPD-iPSCs were profoundly impaired. The FPD-HSPC phenotype was rescued by overexpression of WT *RUNX1* in FPD-iPSCs with megakaryocyte differentiation [30–32], supporting the notion that *RUNX1* mutations are responsible for defects in megakaryocytic differentiation in FPD patients. Furthermore, the facts that genomic instability was consistently observed in the *RUNX1*-mutated iPSCs and that the *RUNX1*-iPSC phenotype depended on the regulation of NR4A3 in *RUNX1*-mutated cells [33] suggest that a marked loss of *RUNX1* expression increases the risk of malignant transformation observed in this disease

Pearson marrow pancreas syndrome

Pearson marrow pancreas syndrome (PMPS) is a fatal BMFS caused by heteroplasmic deletions in mitochondrial DNA (mtDNA) that translate to pancreatic insufficiency and other systemic organ dysfunction, transfusion-dependent sideroblastic anemia, and other cytopenias [34,35]. The cause of the hematopoietic failure in PMPS is unknown and there are no experimental models to reproduce the specific defects in this disorders [35]. A distinctive characteristic of PMPS is the varying levels of mutant mtDNA in different cells, which ultimately determines the severity of the clinical manifestations in individuals with the disease. Cherry et al. [36] generated iPSCs from three patients with PMPS (PMPS-iPSCs) and, despite technical challenges, were able to isolate PMPS-iPSCs that had no mutant mtDNA. All PMPS-iPS lines were capable of forming functional HSPCs; however, compared with isogenic

PMPS-iPSC lines without mitochondrial mutations, samples carrying deleted mtDNA showed a trend toward reduced numbers of colonies. In addition, colonies carrying a high burden of mutant mtDNA, yielded high numbers of erythroid precursors with pathologic iron granule deposition compared with PMPS-iPSCs, with less mutant mtDNA [36]. These data demonstrate clonal variation in changes in mtDNA heteroplasmy during culture and recapitulate a tissue-specific phenotype by directed differentiation of iPSCs carrying mutant mtDNA. Although the pathogenic mechanisms underlying this disorder remain largely unknown, iPSC-derived models for PMPS and other disorders with mtDNA mutations open up new ways to use patient cells in research. This is particularly important considering the fact that targeted modification of the mitochondrial genome is technically challenging [37].

Generation of iPSC clones from patients with acquired BMFS

Paroxysmal nocturnal hemoglobinuria

Human phosphatidylinositol glycan class A (*PIGA*)-null iPSCs were successfully engineered using plasmids expressing ZFN-mediated homologous recombination [38] or a knockout model [39], allowing for the production of glycosylphosphatidylinositol-anchored protein (GPI-AP)-deficient iPSCs for testing the importance of GPI-APs in hematopoiesis. First, Yuan et al. [39] examined the potential GPI-AP role in the generation of hematopoietic cells by using human iPSC lines derived from adult male dermal fibroblasts (hFib2-iPS5), a PIGA gene knockout model of the hFib2-iPS5 cells (*PIGA*-null iPSCs), and *PIGA*-null iPSCs reconstituted with *PIGA* transgene expression (iPSCs-*PIGA*). These *PIGA*-null iPSCs were unable to form embryonic bodies or to generate HSPCs or any cells expressing the CD59, CD34, and CD45 markers and were defective in generating mesodermal cells expressing kinase insert domain receptor [39]. Their biological and phenotypic defects were rescued by *PIGA* transgene expression. Interestingly, the investigators succeeded in establishing a paroxysmal nocturnal hemoglobinuria (PNH) disease model that could provide a limitless source of GPI-AP-deficient blood cells by transducing the *PIGA*-null iPSCs with a doxycycline (Dox)-inducible *PIGA* expression system. Culturing these $CD59^+$ iPSCs in medium with Dox for up to 14 days followed by further culturing without Dox in medium supplemented with myeloid or erythroid-inducing cytokines allowed for the generation of both myeloid and erythroid lineages that were CD59 deficient [39]. These results confirmed that GPI-APs are critical for primitive hematopoiesis. Phondeechareon et al. [40] succeeded in establishing iPSCs derived from a PNH patient's dermal fibroblasts that capable of producing normal autologous HSPCs that could be used to treat PNH patients by autologous transplantation.

Acquired aplastic anemia

Recently, three iPSC lines were successfully generated from three severe AA (SAA) patients by Melguizo-Sanchis et al. [41]. The iPSCs showed failure to elongate their telomeres during the reprogramming process and reduction in hematopoietic differentiation to erythroid and myeloid cells, which was not rescued by eltrombopag. This study suggests that some (not all) SAA cases could be related to an underlying genetic predisposition with negative effects on the proliferation or differentiation of myeloid and erythroid and cells.

We previously reported that leukocytes lacking HLA class I alleles are frequently detectable in patients with aAA due to copy number neutral loss of heterozygosity of the short arm of chromosome 6 (6pLOH) or other allelic mutations [42]. Whereas pediatric SAA can often be attributable to genetic causes, the HLA-lacking cells support the widely accepted immunological nature of aAA pathogenesis in both children and adults. Recently, we succeeded in reprogramming monocytes from a patient with aAA in remission to generate three different iPSC lines with unique *HLA-B*40:02* mutations, including the start−loss mutation, in addition to WT and 6pLOH(+) iPSC clones. Monocytes from another aAA patient (non-SAA) were also successfully reprogrammed to generate three different iPSC clones with unique *HLA-B*54:01* mutations, including nonsense and start codon mutations, in addition to WT and 6pLOH(+) iPSC clones [43,44]. The generation of iPSC clones from monocytes lacking specific HLA alleles that are produced by the patients' HSPCs during the development or the progression of this disease is consistent with the notion that a strong immune pressure directed toward HSPCs is involved in the pathogenesis of this disease.

HSPCs derived from HLA-lacking iPSCs showed a unique HLA-gene expression pattern and different mutation types similar to the primary monocytes from the aAA patients. We observed an apparent higher expression of the retained HLA-A alleles in 6pLOH clones (probably as a compensatory response to the missing alleles) and no expression of HLA-A alleles in iPSC-HSPCs with 6pLOH. When these iPSC-HSPCs were transplanted to immunodeficient mice, the engraftment and blood-repopulating capacity were similar, regardless of the HLA expression, to WT, 6pLOH, and HLA-A(+)B(−) clones. Importantly, hematopoietic cells generated in the transplanted mice retained the phenotype of the original iPSC clones, indicating that this method could be an excellent tool for the study of aAA in vivo. Table 1 summarizes the successful reports of iPSC generation from patients with different

Table 1. Induction of iPSC-HSPCs from patients with inherited and acquired BMFSs

BMFS type	Cells used	Genes affected	Reprogramming efficiency	Functionality assay	Pathogenesis	Blood repopulating capacity	Genome-editing method	Gene correction effect	Reference
FA	FA patients' fibroblasts	FANCA, and D2	Decreased	In vitro and in vivo	Defect in DNA damage repair	No reduction in E/M	Lentiviruses vectors	Maintain a fully functional FA pathway	[8]
	FA patients' fibroblasts FA-mouse tail-tip fibroblasts	FANCA, C, G, and D2 FANCA, D2 and I	Decreased Decreased	In vitro In vitro		No reduction in E/M	No No	No No	[9]
	FA patients' fibroblasts	FANCA, C, and D2	Decreased	In vitro		Reduced E/M & increased HSPC apoptosis	No	No	[10]
	FA patients' fibroblasts	FANCA	Decreased	In vitro		Reduced E/M, neural and MSC differentiation	HDAdV	Rescued the cell cycle and clonogenicity defects	[11]
	FA patient's fibroblast	FANCA	Decreased	In vitro		Reduced E/M & endothelial lineages	No	No	[12]
	FA patient's fibroblast	FANCA	Decreased	In vitro and in vivo		Generation of squamous epithelial rafts and intestinal organoids but increase HSPC apoptosis	Activated DNA damage response signaling through ATR and CHK1. by inhibition of CHK1 1	Completely restored the growth of FA-deficient iPSCs through a remarkable rapid bypass of the G2-M checkpoint	[13]
DKC	DKC patients' fibroblasts	TERC 821 bp deletion	Slow reprogramming capacity	In vitro	Telomere attrition	Impaired hematopoietic differentiation	Retroviral vector and telomerase activation	Increased pluripotency, TERT and TERC upregulation	[15]
	DKC patients' fibroblasts	TCAB1 mutation	Decreased	In vitro and in vivo	Progressive telomere attrition	Impaired hematopoietic differentiation	Reprogramming and telomerase activation	No TERT and TERC upregulation	[16]
	DKC patients' fibroblasts	TERT and TERC	Decreased	In vitro	Progressive telomere attrition	Impaired hematopoietic differentiation	Reprogramming and telomerase activation	No TERT and TERC upregulation	[17]

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Table 1 (*Continued*)

BMFS type	Cells used	Genes affected	Reprogramming efficiency	Functionality assay	Pathogenesis	Blood repopulating capacity	Genome-editing method	Gene correction effect	Reference
SDS	SDS patients' fibroblasts	SBDS	Decreased	In vitro	Ribosomopathy	Impaired hematopoietic, pancreatic and ductal development	lentiviral vector expressing human SBDS cDNA	Restored pancreatic structures, not enhance hematopoietic development	[19]
	SDS patients' bone marrow mononuclear cells	IVS2+2 T>C SBDS mutations IVS2+2 T>C SBDS mutations with deletion of 7q at locus (11.2)	Decreased Markedly decreased	In vitro In vitro	Ribosomopathy Clonal evolution to MDS and AML	The number of CD34+ cells were limiting Decreased hematopoietic differentiation and clonogenic capacity	No	No	[20]
DBA	DBA patients' fibroblasts	*RPS19* and *RPL5* mutations	Inefficiently for some clones and normally for others	In vitro	Ribosomopathy	Reduced hematopoiesis	ZEN	Restored ribosome assembly and hematopoiesis	[22]
	DBA patients' fibroblasts	*RPS19* and *RPL5* mutations	Normal as healthy cells	In vitro and in vivo		Reduced differentiate into $CD71^{+}$glycophorin A erythroid cells	CRISPR/Cas9 and SMER28	Restored erythroid differentiation	[23]
CAMT	CAMT patients' fibroblasts	MPL	Decreased	In vitro	Loss of MPL-mediated signaling	Fewer megakaryocytes, impaired MPP to MEP transition	TPO Retroviral-mediated gene transfer	No rescue of megakaryopoiesis Rescue of the hematopoietic phenotype	[25]
FPD	Peripheral FPD patients' T cells	*RUNX1*	Normal as healthy cells	In vitro	Transcription factors	Early E/M was not affected with megakaryocytic differentiation was decreased	Vector expressing Flag-tagged WT-RUNX1 transfection	Recovered the capacity to differentiate into hematopoietic lineage	[30]
	FPD patients' fibroblasts	*RUNX1* (*AML1 or (CBFA2)*	Decreased	In vitro		Impaired E/M progenitor	ZENs	Rescued megakaryocytes differentiation	[31]

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Table 1 (*Continued*)

BMFS type	Cells used	Genes affected	Reprogramming efficiency	Functionality assay	Pathogenesis	Blood repopulating capacity	Genome-editing method	Gene correction effect	Reference
PMPS	PMPS patients' bone marrow −derived fibroblasts	Mitochondrial genetic (mtDNA) deletion	Decreased	In vitro	Mitochondrial dysfunction	Give rise to sideroblastic erythroid progenitors	No	No	[36]
PNH	*PIGA* knockout model in human iPSCs	*PIGA*	Decreased	In vitro and in vivo	CD55 and CD59 lacking leading to complement-mediated intravascular hemolysis	Failure of Teratomas formation and production of $CD34^+$ or $CD45^+$ cells upon hematopoietic differentiation	*PIG-A* ZEN transgene expression	Rescued phenotypic and biological defects and teratomas formation containing cells representing all three embryonic germ layers	[39]
AA	Severe AA patients' dermal fibroblasts	CN-LOH (3q11.2) CN-LOH (7q22.1) CN-LOH (11p11.12-q11) Deletion (15q13.3) Duplication (16p13.11)	Less than normal cells	In vitro	All iPSC-derived-HSPCs showed significantly shorter telomeres than undifferentiated iPSCs	Impaired E/M differentiation	TPO	E/M proliferation and/or differentiation not rescued by TPO	[41]
aAA	Peripheral very SAA patients' monocytes	Start codon mutation of *HLA-B*40:02* allele 6pLOH	Variable	In vitro and in vivo	Immune alterations (CTL capable of killing WT $iCD34^+$ cells but not B4002 (−) $iCD34^+$ cells	E/M differentiation	No	No	[43]

aAA=Acquired aplastic anemia; AML=acute meloid leukemia; ATR=ataxia telangiectasia mutated and Rad3 related serine/threonine kinase; BMFS=bone marrow failure syndrome; CAMT=congenial amegakaryocytic thrombocytopenia; CHK1=checkpoint kinase 1; CN-LOH=copy number-neutral loss of heterozygosity; CRISPR=clustered regularly interspaced short palindromic repeats/Cas9 enzymes that can be used to edit genes within organisms; CTL=cytotoxic T cell; DBA=Diamond−Blackfan anemia; DKC=dyskeratosis congenital; E/M=erythroid and myeloid progenitor cells; FA=Fanconi anemia; HDAdVs=Helper-dependent adenoviral vectors; HSPCs=hematopoietic stem and progenitor cells; MDS=myelodysplastic syndrome; MPL=myeloproliferative leukemia virus oncogene; PIGA=phosphatidylinositol glycan class A; 6pLOH=loss of heterozygosity of the short arm of chromosome 6; PNH=paroxysmal nocturnal hemoglobinuria; RUNX1=Runt-related transcription factor 1; SAA=severe aplastic anemia; SBDS=Shwachman−Bodian−Diamond syndrome; SMER28=small-molecule enhancers of rapamycin; TERT=telomerase reverse transcriptase; TPO=thrombopoietin; WT=wild-type cells; ZFN=zinc finger nuclease; MSC=Mesenchymal stem cells.

BMFSs and the effects of the gene defect correction on the proliferation and hematopoietic differentiation of their iPSCs.

HLA lacking iPSC-derived CD34$^+$ (iCD34$^+$) cells from patients with aAA evade attack by autologous cytotoxic T cells specific to WT HSPCs

HLA-lacking iCD34$^+$ cells enabled us to study the phenotype of HSPCs and the influence of the immune system by examining the ability of patient-derived cytotoxic T lymphocytes (CTLs) to kill autologous HSPCs and to discriminate between HLA-retaining [HLA(+)] and HLA-lacking [HLA(−)] iCD34$^+$ cells in coculture experiments (Figure 2). Stimulation of the patient's CD8$^+$ T cells with the WT iCD34$^+$ cells generated a CTL line capable of killing WT iCD34$^+$ cells, but not B4002(−) iCD34$^+$ cells [43]. These data suggest that B4002(−) iCD34$^+$ cells show a repopulating ability similar to WT iCD34$^+$ cells when autologous T cells are absent and CTL precursors capable of selectively killing WT HSPCs are present in the patient's peripheral blood. Experiments using HLA-B5401(−) iCD34$^+$ cells confirmed the ability of these cells to escape from autologous CTLs specific to WT HSPCs when the four T-cell receptor transfectants were tested for their response to WT and B5401-lacking iCD34$^+$ cells [44]. These findings strongly indicate that HSPCs that deleted HLA alleles evade CTL attack and support the patient's hematopoiesis.

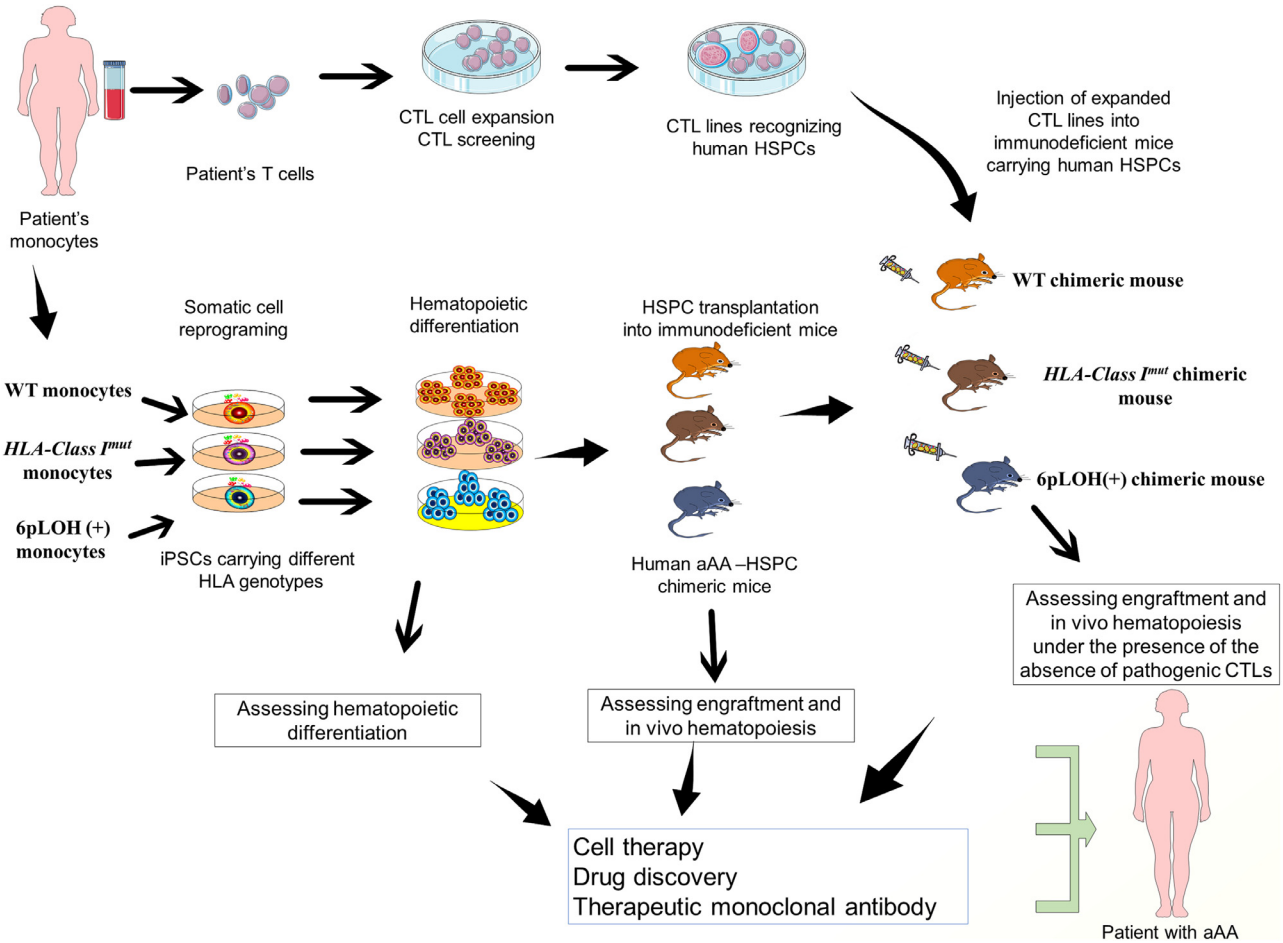

Figure 2. Disease model of aAA. Reprograming of somatic cells (monocytes) from patients with aAA harboring various HLA genotypes (WT-HLA, 6pLOH (+), or HLA-Class I ^{mutant}) to generate iPSCs with the corresponding genotypes has opened new opportunities for the study of the pathogenesis of this disorder. The differentiation of iPSCs into HSPCs and the transplantation of HSPCs into immunodeficient mice has been tested to verify the impact of HLA loss in the hematopoietic potential of HSPCs. The enrichment of CTLs from patients with aAA was used to verify that pathogenic CTLs are able to kill WT HSPCs but fail to recognize the HLA-lacking counterparts in vitro and the identification of the TCR spectrotypes likely involved in the recognition and killing of HSPCs. This model can be tested in vivo by injecting pathogenic CTLs into mice harboring iPSC-HSPCs. It is expected that this disease model would lead to better understating the pathogenesis of aAA, which eventually may lead to the development of novel therapeutic approaches such as cell therapies, therapeutic monoclonal antibodies, or therapeutic drugs.

Role of iPSCs in studying clonal hematopoiesis in BMFSs

DNMT3A, ASXL1, and *BCOR/BCORL1* mutations, as well as 6pLOH and *PIGA*, are genetic abnormalities found in <40% of patients with aAA and the first three mutations involve genes commonly mutated in myeloid malignancies [45]. In addition, up to 20% of patients with aAA who do not receive allogeneic hematopoietic stem cell transplantation (HSCT) eventually develop MDS or AML [46]. This indicates that the early identification of patients who will evolve to MDS could help make decision regarding the use of immunosuppressive therapy (IST) or HSCT; however, until now, the risk factors associated with progression to MDS or acute AML remain poorly defined. The generation of iPSCs carrying the previously mentioned mutations may be helpful for identifying the factors predisposing patients to MDS and AML progression. These include the chronological profiles, clone size, and role of autoimmunity in clonal selection. Although MDS with monosomy 7/del(7q) is a frequent clonal abnormality that emerges in the context of inherited BMFSs such as SDS, a critical question remains as to whether monosomy 7/del(7q) acts as a driver cause of MDS and leukemic risk or whether it is just an marker associated with clonal progression in BMFS. Kotini et al. [47] reported that del(7q) iPSCs derived from BM cells of two patients with del(7q) MDS showed increased apoptosis with decreased proliferation and ineffective hematopoiesis, with markedly decreased clonogenic capacity. Ruiz-Gutierrez et al. [20] succeeded in generating SDS del7q^{+} iPSCs with del(7q) models, which also showed markedly decreased hematopoietic differentiation compared with isogenic SDS-iPSCs without an increase in cell death. The previous approach highlights the usefulness of human iPSC-based disease modeling for studying the role of 7q loss in clonal evolution from BMFSs in addition to facilitating a functional mapping of large-scale chromosomal deletions linked to BMFSs. In addition, the use of in vitro cell cultures or in vivo animal models with iPSCs carrying these mutations would also be suitable to investigate the role of those genetic abnormalities in response to IST. Therefore, these mouse models engrafted with iPSCs carrying distinct mutations have a broad range of applications for the study of BMFSs and numerous benefits could be obtained from these models in investigations about the relationship among BMFSs, clonal evolution, and leukemic risk.

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Conflict of interest disclosure

The authors declare no competing financial interests.

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