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Advances in gene therapy for hematologic disease and considerations for transfusion medicine

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ABSTRACT

As the list of regulatory agency-approved gene therapies grows, these products are now in the therapeutic spotlight with the potential to cure or dramatically alleviate several benign and malignant hematologic diseases. The mechanisms for gene manipulation are diverse, and include the use of a variety of cell sources and both viral vector- and nuclease-based targeted approaches. Gene editing has also reached the realm of blood component therapy and testing, where cultured products are being developed to improve transfusion support for individuals with rare blood types. In this review, we summarize the milestones in the development of gene therapies for hematologic diseases, mechanisms for gene manipulation, and implications for transfusion medicine and blood centers as these therapies continue to advance and grow.

Introduction

After decades of research advances tempered by scientific and regulatory hurdles, the direct manipulation of human cells and genes to cure disease is now at therapeutic center stage, representing yet another milestone in modern medicine. In a recent historic action, the United States Food and Drug Administration (FDA) approved 2 gene therapy agents for the treatment of hematologic malignancies, ushering in a new era of cancer therapeutics [1,2]. Gene supplementation strategies utilizing viral vectors have also moved from promise to reality for the treatment of benign hematologic conditions, including hemoglobinopathies [3-5] and hemophilia [6]. Further, novel gene editing technologies capable of precise, sequence-specific alterations in DNA are now poised for clinical application for the treatment of both benign and malignant hematologic disease [7,8].

The hematopoietic system has represented a dominant area of interest for gene therapy researchers. This is due, in part, to the relative ease of collection, ex vivo culture, manipulation, reinfusion, and homing of these cells. Based on recent data compiled by the *Journal of Gene Medicine*, gene therapy clinical trials for hematologic disease and cancer constitute more than 70% of approved, ongoing, or completed gene therapy studies worldwide [9]. Even for nonhematologic indications utilizing cellular and immune therapies, the starting material is often obtained from cells in the hematopoietic compartment, such as hematopoietic stem and progenitor cells (HSPCs), marrow-derived mesenchymal stromal cells, and peripheral blood cells for the generation of induced pluripotent stem cells (iPSC).

Further, hematologists have focused on the subcellular origin of disease since the 1950s. Sickle cell disease (SCD) was the first condition to be characterized at a molecular level, by the Nobel-prize winning scientist, Linus Pauling in 1949 [10,11]. Over the last 5 decades, cell-based therapies (eg, blood component transfusions and HSPC grafts) and protein-based therapies (eg, erythropoietin, granulocyte colony-stimulating factor (G-CSF), immunoglobulins, and clotting factors) have constituted the main and/or adjunct forms of treatment for benign hematologic diseases and cancer, serving as forerunners for the clinical development of newer gene therapies. Gene therapy has become an important part of hematology since it is being used to treat many hematologic disorders, the clinical application of many gene therapies is similar to hematopoietic stem cell therapy, and gene therapy and transplantation are alternative forms of therapy for many diseases.

This review will summarize advances in gene therapies as potential cures for certain benign hematologic diseases and cancer. Further, it will assess implications of these advances for blood component utilization, synthesis and testing. The role of blood centers as decentralized manufacturing facilities for gene therapies will be explored.

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Milestones in gene therapy for hematologic disease

Progress in gene manipulation for hematologic disease may be studied in 3 evolutionary eras: (i) Gene "replacement" therapy by hematopoietic stem cell transplantation (HSCT) (ii) Gene "supplementation" therapy using viral vectors, and (iii) Nuclease-based genome "editing."

In 1953, the discovery of the double helix structure of DNA by Watson and Crick paved the way for a new age of biotechnological advances, including gene sequencing and genetic engineering [12]. In the context of hematology, this led to the understanding of the genetic basis of disease, including hemoglobinopathies [13,14]. In the preceding years, a series of experiments in the transfer of heredity had also resulted in the understanding of gene transduction in prokaryotes by "carrier" viruses. This was then followed by the utilization of recombinant viruses for nucleic acid transfer into mammalian cells [15-18].

The development of HSCT has been important for the clinical application of gene therapy. In the 1950s, the first successful identical-twin HSCT was performed by E. Donnall Thomas in an effort to replace cancer-causing hematopoietic stem cells in the bone marrow of a patient suffering from leukemia [19]. Some have regarded this as the earliest clinical gene replacement therapy experiment, using a nonviral approach. Not long after, in 1968, an allogeneic bone marrow transplant from a sibling donor successfully cured a patient with adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID) [20].

In the early 90s, the first clinical gene therapy trial reported successful retroviral transfer of the ADA gene into the T cells of 2 children with SCID. However, T cell persistence was only transient [21]. Other investigators used the more durable HSPCs as the starting fraction. In 2000, Cavazzana-Calvo et al. reported the first gene therapy-based "cure" in 4 of 5 subjects with X-linked SCID [22]. Unfortunately, almost 3 years after therapy was completed, 2 of the subjects in the trial developed T-cell leukemia, likely owing to retroviral insertion-induced mutagenesis [23]. For hemophilia, gene therapy was developed with nonintegrating adeno-associated viruses (AAV), after a fatal immune reaction stalled further development of adenoviral gene transfer in clinical trials [24].

Optimized vector design and the introduction of nonmyeloablative conditioning prior to the infusion of gene-transduced cells improved safety and efficacy of these gene therapy clinical trials [25]. For example, the application of lentiviruses allowed for carriage of larger and more complex gene cassettes necessary for the treatment of hemoglobinopathies. Recently, lentiviral beta-globin gene and antisickling beta-globin gene-based treatments for patients with beta-thalassemia [3,5] and SCD [4] resulted in transfusion independence within a year of gene correction, in addition to the reversal of some disease-related co-morbidities. One of these studies, however, reported that about 5% of the circulating hematopoietic cells of a treated patient showed a significant increase in the levels of HMGA2, a protein implicated in potential oncogenic stimulation [3]. Nevertheless, there has been no evidence of a malignant or premalignant state in this patient [26].

Genetic enhancement of cells has also revolutionized cancer immunotherapy. Engineered chimeric antigen receptor (CAR)-T cells are designed by augmenting the killing capacity of T cells with co-stimulatory molecules and the antigen-targeting specificity of B cells. They have demonstrated tremendous success and are the first gene therapy products to be FDA approved for the treatment of leukemia and lymphoma [27].

In contrast to viral vector-based gene addition, gene editing utilizes endonucleases or "molecular scissors" and guide proteins or RNA molecules or "GPS systems" to create sequence-specific DNA breaks, followed by repair. Previous studies of individuals resistant to HIV demonstrated that a mutation in the T cell receptor gene, *CCR5,* conferred resistance to HIV infection, without impairing T-cell function. This discovery prompted a proof-of-concept human study of patients with HIV disease, wherein zinc finger nuclease (ZFN)-mediated disruption of the *CCR5* receptor gene on autologous T cells ex vivo resulted in a decrease in viral load, even in the absence of antiretroviral therapy. Although the response was transient, the safety and feasibility of gene editing was confirmed [28,29].

Alongside meteoric scientific advancements in genome editing, ethical and regulatory concerns were identified in a National Academies of Sciences report (2017) with the use of this technology for eugenics, wherein embryos were CRISPR edited to inactivate the CCR5 receptor to confer resistance to HIV infection [30].

Fig. 1 provides a summary of landmarks in gene therapy, in the context of pertinent advances in transfusion medicine. Notably, the last decade has seen a number of exciting breakthroughs in the field for both transfusion medicine and clinical gene therapy. Red cell genotyping has been increasingly used to enable improved transfusion support to allo-immunized, multiply-transfused patients with hemoglobinopathies. Rapid advances in preclinical development of the CRISPR technology have also impelled commercialization, and interestingly, one of the first clinical applications of this technology was for the generation of rare blood group red cell reagents for serologic testing [31]. Clinical trials making use of CRISPR technology in transfusion-dependent thalassemia and SCD are also now underway [32].

Table 1
Overview of gene therapy tools and techniques for hematologic diseases.

Target organ/cell type for gene transfection
Terminally differentiated dividing (T cells) or nondividing cells (hepatocytes)
Multipotent somatic cells (HSPC)
Induced pluripotent stem cells (iPSC)
Germline cells (blastocysts/embryonic stem cells)
Location of target cell manipulation
Ex vivo (HSPC, lymphocytes, iPSC, embryonic stem cells)
In vivo (hepatocytes, HSPC)
Method of gene manipulation
Cell and gene replacement (allogeneic/autologous transplantation)
Gene supplementation (viral vectors)
Sequence-specific gene editing (targeted nucleases, direct base editing)
Gene delivery strategies
Viral transfer (γ-retrovirus, lentivirus, adeno-associated virus)
Physical/mechanical methods (electroporation, nucleofection, sonoporation, microinjection)
Chemical methods (nanoparticles, liposomes)

Overview of gene therapy techniques

An ideal method of gene therapy would involve efficient, site-specific nucleic acid alterations that result in safe, durable responses and/or disease cure. In reality, however, formidable technical challenges hinder the achievement of adequate and sustained gene expression, and hence, the success of these therapies. Further, dysregulated transgene expression, off-target effects, and host immune responses to these foreign materials can result in life-threatening iatrogenic complications. As such, several technical questions emerge—What is the targeted organ or cell type for gene therapy? Where should gene manipulation occur? What is the optimal method of gene correction? And how should the transgene be delivered? Table 1 provides a summary of technical considerations for the implementation of gene therapies.

Location and target cell types for gene manipulation

The earliest clinical studies in gene therapy were performed using ex vivo techniques which allowed for preliminary safety and in

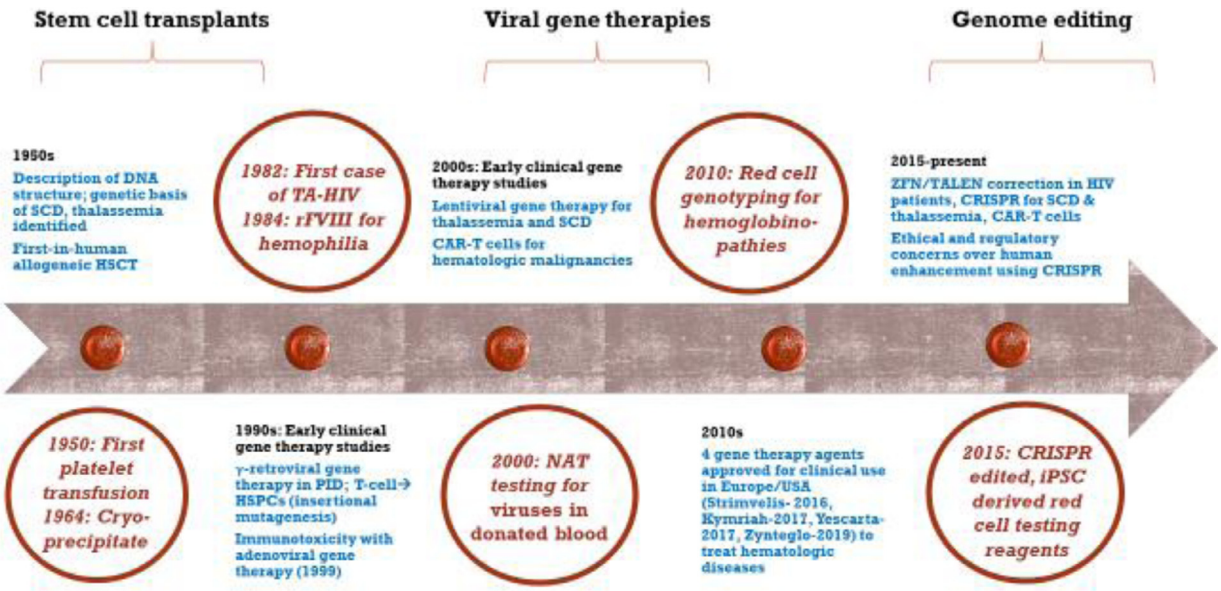

Fig. 1. Landmarks in gene therapy for hematologic disease are shown in the context of pertinent developments in transfusion medicine (in red, circled). Progress in gene manipulation for the treatment of these diseases has progressed from HSCT to gene therapy utilizing viral vectors as well as targeted gene-editing approaches. Advances in genome editing are now being utilized to enhance the safety of blood transfusion. (Milestones in gene therapies) CRISPR = clustered regularly interspaced short palindromic repeats; HSCT = hematopoietic stem cell transplantation; iPSC = induced pluripotent stem cells; NAT = nucleic acid testing; PID = primary immunodeficiency; rFVIII = recombinant factor VIII; SCD = sickle cell disease; TA-HIV = transfusion-associated HIV transmission; TALEN = transcription activator-like effector nuclease; ZFN = zinc finger nucleases.

vitro efficacy checks on the gene-transduced cells prior to reintroduction into subjects [21,33]. This strategy remains the preferred method for gene introduction in hematologic and nonhematologic conditions. Patient or donor cells, such as HSPC and lymphocytes, are collected by apheresis or simple venipuncture and transduced with a viral vector carrying the transgene. Alternately, gene editing tools may be introduced ex vivo by electroporation and other techniques, followed by transplantation of gene-modified cells into the recipient. Ex vivo gene therapy has been used successfully in the treatment of hemoglobinopathies, primary immunodeficiencies (PID), and hematologic malignancies [34]. More recently, ex vivo gene manipulation strategies have been applied to iPSCs in early-clinical [35-37].

In vivo gene therapy involves the direct introduction of a vector carrying the therapeutic gene into the patient. The vector is administered either into or near the target organ. This strategy has been used in the treatment of nonhematologic disease (ocular, neurologic disorders) [38-40]. Alternately, viral vectors that demonstrate tropism for certain tissues (eg, AAV preferentially infect hepatocytes) may be injected intravenously [41]. This method has been used successfully in inherited coagulation disorders [42]. More recently, murine studies have demonstrated the successful in vivo transduction of HSPCs through the intraosseous injection of viral vectors [43]. In addition, a newer approach that has shown promising results involves the mobilization of HSPCs from the marrow into the peripheral blood, where in vivo transduction occurs prior to the return of the HSPCs to the marrow for re-engraftment [44,45].

Gene manipulation methods

Hematopoietic stem cell transplantation

Allogeneic HSCT is a well-established form of curative gene replacement therapy for benign and malignant hematologic diseases. Matched sibling donor transplants result in survival rates of up to 90% in patients with beta-thalassemia when offered early, before complications related to iron overload or transfusion-transmitted infections set in. However, HLA-matched sibling donors are available for less than 10% of patients. Further, despite high overall cure rates for benign hematologic diseases requiring transplant, intensity of conditioning regimens have led to regimen-related toxicities (if too high) or to graft rejection (if too low), leading to event-free survival rates of about 50% for thalassemia [46]. For hematologic malignancies, umbilical cord donor transplants as well as matched related, unrelated, and haploidentical PBSC or bone marrow grafts and are in routine clinical use. Nevertheless, co-morbidities due to graft rejection, graft vs host disease and other long-term post-transplant sequelae present routine challenges to survival.

Transplantation of autologous HSPC following ablative or partially ablative chemotherapy has been used in autoimmune diseases to “reset” and remove autoreactive immune responses, and in certain malignant conditions (multiple myeloma, Hodgkin, and non-Hodgkin lymphomas [NHL]), wherein the patient receives intensive chemotherapy to wipe out malignant stem cells in the bone marrow followed by purged autologous stem cell rescue [47].

Allogeneic and autologous transplants form the platform for newer gene addition strategies using viral vectors. The collection of autologous HSPCs or lymphocytes for ex vivo gene therapy, as well as culture, manipulation and transplantation rely on techniques used in stem cell transplantation. For hematologic conditions amenable to viral gene therapies, such as SCD and thalassemia, stem cell collection may present certain additional challenges. For example, G-CSF is contraindicated in SCD patients, who may also have poor collection yields due to the ongoing use of hydroxyurea. In addition, patients with thalassemia may have inefficient stem cell collections due to an erythroid hematopoiesis bias from ineffective erythropoiesis. Recent studies have reported successful autologous stem cell collections in SCD patient subsets using plerixafor alone [48-50], and with a combination of G-CSF with plerixafor for thalassemia [51]. Following stem cell collection, expansion of the gene-corrected cell fraction may be required to obtain sufficient cells doses for transplantation. Several agents,

including the more recent nicotinamide, SR1 and UM171 small molecules, have shown promise [51,52]. Further, viral gene addition strategies require conditioning regimens to facilitate engraftment of gene-corrected cells. Preclinical and early clinical studies have demonstrated successful transient bone marrow niche HSPC clearance with certain antibody-drug conjugates [53-55], followed by gene-corrected cell engraftment. These non−genotoxic, highly-targeted conditioning agents may enable the safe and effective receipt of donor stem cells without chemotherapy or radiation.

Viral gene therapy

Early gene therapy vectors were constructed with integrating viruses such as γ-retroviruses. During development, these were found to have dysregulated gene integration with reported instances of insertional mutagenesis. Lentiviral vectors have been used as a substitute, and demonstrate safer designs with a lower predilection for oncogenicity. While retroviral vectors require active cell division for gene transfer, lentiviruses have the ability to transduce nondividing cells in a shorter culture period. However, they remain inefficient at quiescent cell transduction. Nonintegrating viral vectors, such as adenoviral and AAV vectors, can infect a variety of cell types including nondividing cells. They typically exhibit tissue tropism, as seen with the preference of AAV for hepatocytes. However these constructs are capable only of carrying smaller DNA inserts. Occasionally, they are able to integrate into the host genome, raising safety concerns similar to those for retroviruses and lentiviruses. AAV vectors also are capable of inducing an immune response against the viral envelope [56].

Gene editing

In contrast to gene supplementation and addition methods utilized with viral gene therapy, gene editing technologies theoretically offer the ability for precise, site-specific and elegant gene deletion, substitution and/or enhancement. Unlike dysregulated viral integrations, the introduction of programmable sequence-specific nucleases (ZFNs, transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 systems) allow for the correction of specific, disease-causing mutations (eg, as seen in SCD) or for the integration of an entire expression cassette into a genomic “safe harbor” site (eg, X-linked SCID with numerous mutations in an affected gene) [57,58]. These corrections are performed by nonhomologous end joining, wherein the gene disrupted by the nuclease relies on endogenous repair mechanisms to correct itself. Correction efficiencies exceeding 80% have been reported with this method [59]. On the other hand, homology-directed repair may also occur, though with lower efficiency (30%-70%), whereby a normal DNA strand acts as a template to help repair and correct the abnormal strand. During this process, large deletions may also be repaired by adding in replacement DNA into the template strand, followed by correction of the sister strand [60]. Recently, components from CRISPR system together with other enzymes have been used for gene editing approaches such as gene repression/activation, epigenetic modification, base editing and prime editing. These may be performed without making double-stranded DNA breaks [61,62]. While gene editing approaches can lead to transformative therapies in the field of hematology and in other areas of medicine, several safety and ethical concerns need to be addressed alongside the scientific and early clinical advancements [30,59,63].

Gene delivery methods

Both viral as well as nonviral methods offer the ability to carry material for gene-protein end-organ repair and cancer immunotherapies. Viral methods are as described above. Exogenous nucleic acids may also be transfected into host cells by a transient mechanical disruption of the cell/nuclear membrane, as can be achieved by ex vivo electroporation, nucleofection, and sonoporation. These physical permeabilizing methods represent the predominant nonviral means of gene conveyance, and are applied in up to a quarter of all ongoing phase I/II gene therapy trials worldwide [64]. Electroporation is the most popular method for enhancing cellular function for therapeutic purposes with high efficiency of nucleic acid loading, simplicity of operation, and nonreliance on chemical or biological reagents [65]. Other nonviral delivery systems including nanoparticles, liposomes and cell-derived vesicles are in preclinical stages of development.

Gene therapy for hematologic conditions

Hemoglobinopathies

Sickle cell disease and beta thalassemia are hematologic conditions that arise from hemoglobin beta globin chain mutations and more than half of affected individuals require chronic red cell transfusion support. The main curative option for both diseases is HLA-identical sibling HSCT, available only for 10% to 20% of patients. Haplo-identical transplantation provides an expanded donor pool, but is currently experimental and graft rejection occurs in up to 60% of recipients. Gene therapy approaches have used ex vivo HSPC transductions with modified lentiviral vectors. These vectors are optimized to carry large transgene cassettes containing the globin gene and the locus control region required for high-level, erythroid-restricted transcription. Clinical trials in beta thalassemia and SCD also utilize gene editing (ZFN, CRISPR/Cas9) approaches to decrease (cleave off) the expression of the B cell lymphoma/leukemia 11A (BCL11A) protein, a major modulator of fetal hemoglobin (HbF) expression. The consequent increase in HbF levels has been shown to decrease disease severity in both conditions [59]. About 30 patients treated in 15 early phase trials are transfusion independent thus far. However stem cell mobilizing regimens, gene transduction or editing efficiencies, vector costs, conditioning regimens, off-target effects, toxic immune responses, and vector genotoxicity risks need to be optimized further [59,66]. Despite these potential hurdles, a lentiviral gene therapy product (Zynteglo—autologous CD34+ cells encoding βA-T87Q-globin gene) received approval in Europe for the treatment of thalassemia in 2019 [67].

Hemophilia

Hemophilia A and B (coagulation factor VIII and IX deficiency) also require transfusion support in the form of red cells and fresh frozen plasma for life threatening bleeding complications. Further, in several parts of the world where recombinant factors and newer monoclonal antibody products are expensive or unavailable, cryoprecipitate and plasma-derived factor concentrates still remain the therapeutic mainstay. Following the cloning of factor VIII cDNA, gene therapy for hemophilia was pursued as a curative option. Nonintegrating AAVs were developed to transfer the genetic cargo to host DNA. Gene therapy development for hemophilia B preceded that of hemophilia A, as the factor IX gene was smaller in size and easier to fit into AAV vectors than was the factor VIII gene [68]. Clinical trials utilizing AAV vectors in hemophilia A and B with up to 3 years of follow-up have shown reduced annual bleeding rates to zero, as well as reduced factor concentrate usage by 94% to 98% [69,70]. Nevertheless, immunotoxicity to the AAV capsid remains a challenge that needs further study [71]. Nonviral vector based gene editing for hemophilia B has also entered testing in an early clinical phase study (NCT02695160).

Hematologic malignancies

Based on data from a recent study on global blood product availability and utilization, as much as 13% of blood products (predominantly red cells and platelets) utilized in North America were issued for patients with hematologic malignancies [72]. Further, in individuals undergoing HSCT, lower CD34 cell doses and major ABO incompatibility can lead to transfusion dependence even up to a year after transplantation [73].

HSCT continues to be standard of care for several hematologic cancers with more than 9000 allogeneic and 14,000 autologous transplants performed in the United States alone as of 2018. Based on the most recent Center for International Blood and Marrow Transplant Research data, postallogeneic HSCT, 3-year survival probabilities for adults with acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, and myeloproliferative disorders were 49%, 55%, 43%, and 54%, respectively. During the same period, 3-year survival following autologous HSCT for Hodgkin disease, NHL (diffuse large B-cell lymphoma) and multiple myeloma were 87%, 67%, and 66%, respectively [74].

Gene therapies such as oncolytic virotherapy or the direct transfer of genes encoding immunostimulatory cytokines (GM-CSF, IL-4, and TNF-a) into tumor cells, or the introduction of antisense oligonucleotides, short interfering RNAs (siRNA) and microRNA into cells to silence genes and suppress aberrant proteins remain at their infancy for use in hematologic malignancies [75,76]. Whereas, genetically modified adoptive cell transfer therapies, such as CARs, Transgenic MHC restricted T-cell receptors (TCR-T) [83] and well as NK CAR T cells [84] have demonstrated success in early phase clinical trials for hematologic malignancies.

CAR-T cells are usually manufactured by retroviral vector transduction of the CAR transgene into the genome of autologous T cells. In acute lymphoblastic leukemia and NHL (diffuse large B-cell lymphoma), complete response rates (CRR) of up to 81% and 40%, respectively, were observed with the use of tisagenlecleucel (anti-CD19 CAR-T cell) in heavily pretreated individuals [75,76]. In patients with refractory DLBCL who received axicabtagene ciloleucel (anti-CD19 CAR-T cell), the complete response rates (CRR) was 58% [77]. In relapsed refractory multiple myeloma, 2 BCMA CAR-T cell products (JNJ-4528, decabtagene vicleucel) have been granted breakthrough therapy designation with CRR of 68% and 45% in early phase clinical trials, respectively [78,79]. CD19 CAR-T cell indications may expand to include mantle cell lymphoma and chronic lymphocytic leukemia. CAR-T trials are ongoing in Hodgkin lymphoma and acute myeloid leukemia. CAR-T cells targeting dual antigens (eg, CD19/CD22) are also under investigation [80]. Nevertheless, loss of CAR-T cell efficacy due to antigen escape or low persistence, as well as the development of toxicities including cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome (ICANS) remain ongoing concerns.

Gene editing approaches have also been used to manufacture engineered T cells [81], allowing for transgene integration into specific sites such as the *TRAC* locus. This strategy not only appears to have improved T cell effector function but it also disrupts the endogenous TCR, permitting the development of allogeneic "off-the-shelf" products. Other locations including the CD52, B2M, and CD7 genes have also been disrupted to circumvent cell rejection, and self-killing of CARs [82]. In a study of 3 patients, including 2 with refractory multiple myeloma, CRISPR-Cas9 technology was used to edit autologous T cells to remove the endogenous TCR and programmed cell death protein 1 (PD-1), along with the viral transduction of a TCR transgene specific for NY-ESO-1, a tumor antigen, to induce effective engineered T cell persistence and a reduction in target antigens [83].

PID

These are a heterogeneous group of monogenic disorders characterized by life-threatening infections, autoimmunity, immune dysregulation, inflammation and/or malignancy which occur as a result of impairments in host immunity. These include severe combined immune deficiency (X-SCID, ADA-SCID), chronic granulomatous disease, and Wiskott–Aldrich syndrome (WAS). These individuals may need chronic transfusion support secondary to cytopenias or dysfunctional cells. Chronic gut inflammation can also lead to gastro-intestinal blood losses requiring RBC support.

HSCT offers cure only when a suitably matched donor is available. In addition, ex vivo gene therapy with autologous transplantation of genetically corrected cells offers a life-saving alternative and several clinical trials have been conducted for PIDs over the years [84]. In 2016, Strimvelis, a gene therapy product (autologous CD34+ cells transduced with γ-retrovirus carrying the adenosine deaminase gene) was approved in Europe for the treatment of patients with ADA-SCID [85]. Lastly, gene editing techniques for use in PID are still in the preclinical phase [86-88].

Inherited bone marrow failure syndromes

These are also a heterogeneous group of disorders characterized by bone marrow hypoplasia or aplasia resulting in cytopenias of one or more blood cell lineages in association with one or more somatic abnormalities. Syndromes include Diamond-Blackfan anemia, Shwachman-Diamond syndrome, dyskeratosis congenita, and Fanconi anemia. Many cases require chronic transfusion support. However, the incidence of these disorders is relatively low with less than 5 to 7 cases per million live births in the United States.

HSCT is the preferred therapy for most inherited bone marrow failure syndromes. However, many patients lack a suitable histocompatible HSPC donor or are excluded from transplantation due to comorbidities [89]. For Fanconi anemia, virus transduced gene therapy has been attempted over the last 2 decades with limited success [90]. This is in part due to damaged HSPCs in the bone marrow compartment that are difficult to mobilize for ex vivo gene correction, low vector transduction efficiencies and toxic conditioning regimens. Gene editing is also under investigation wherein recent proof-of-concept studies have demonstrated successful CRISPR/Cas9-based editing of patient-derived fibroblasts followed by autologous iPSC-to-HSPC reprogramming [91-95].

Gene therapy: future implications for transfusion medicine and blood centers

The development of gene therapies is likely to have broader implications for the field of transfusion medicine. All hematologic conditions described above utilize blood products (RBCs, platelets, plasma, granulocytes, IVIG, and clotting factors) routinely. The impact of gene therapy-based cures on blood utilization is thus important to evaluate. In addition, gene manipulation is now applicable to blood product generation and testing, particularly for recipients with rare blood types and those who are heavily alloimmunized. Further, blood centers may serve as decentralized manufacturing facilities for gene therapy products given their expertise in aseptic collection techniques, closed-system processing, and regulatory handling of these "living drugs."

Blood product utilization

Population level data from the AABB Blood Collection, Utilization, and Patient Blood Management Survey as well as the US Department of Health and Human Services National Blood Collec-

Table 2
Hematologic diseases with curative potential using gene therapies.

Hematologic disease	Annual US prevalence	Blood product used	Units/ month	Curative therapies	Viral gene therapy	Gene editing
Sickle cell disease	100,000	PRBC	1-2	MSD-HSCT	Phase I/II	Phase I/II
Beta-thalassemia	15,000	PRBC	1-2	MSD-HSCT	Phase I/II/III	Phase I/II
Hemophilia	20,000	PRBC, FFP, clotting factors	variable	--	Phase I/II	Phase I
Hematologic malignancies*	180,000	PRBC, platelets, grans, FFP, IVIG	variable	HSCT/Chem otherapy	Phase III/IV	Phase I/II
Primary immune deficiencies (CGD, SCID)	1/20,000-200,000	PRBC, granulocytes^, IVIG^	1-2	Allo-HSCT	Phase I/II/III	Pre-clinical
Inherited bone marrow failure syndromes	1/1,000,000	PRBC, platelets, granulocytes^, IVIG^	2-3; 4-5	Allo-HSCT	Phase I/II	Pre-clinical

*Includes myeloma, leukemia and lymphoma (https://www.fa.org/facts-and-statistics/facts-and-statistics-overview/facts-and-statistics)
^Number of units/doses used and indications are variable
CGD: chronic granulomatous disease; FFP: fresh frozen plasma; HSCT: hematopoietic stem cell transplantation; IVIG: intravenous immunoglobulin; MSD: matched sibling donor; PRBC: packed red blood cell; SCID: severe combined immunodeficiency

tion and Utilization Survey (NBCUS), show that the total number of red cell units transfused has steadily declined from 2008 to 2017, though with a slower decline in the last few surveyed years [96,97]. Another recent study by Goel et al. demonstrated similar trends for red cells and plasma from 2011 to 2014. However, platelet product usage remained unchanged. Decreases in red cell usage persisted after stratifying by elective vs nonelective admissions, likely due to the increasing use restrictive transfusion strategies and the implementation of patient blood management programs [98]. Roubinian, et al. also demonstrated similar trends amongst a large cohort of medical and surgical patients treated from 2009 to 2013, including a notable decrease in RBC utilization in patients with nadir hemoglobin levels in the range of 8.0 to 8.9 g/dL. In addition, decreased pretransfusion hemoglobin levels could be seen, including amongst the cohort of patients treated for malignancies. Nonmalignant blood disorders were not reported separately [99]. There is also evidence from a recent randomized controlled trial showing decreases in blood product utilization in patients with hematologic malignancies and those undergoing HSCT [100,101].

Interestingly, data from the Healthcare Costs and Utilization Project demonstrated an increase in blood product utilization for anemias of various etiologies from 51.7% in 2000 to 73.2% in 2013. The diagnosis of anemias excluded septicemia and gastrointestinal bleeding [102].

Together, these trends suggest that the improved understanding of transfusion triggers and an increasing awareness of transfusion-related complications amongst practitioners may be contributing to reduced blood product utilization. Blood product utilization trends are unlikely to be impacted in the near future by the small fraction of patients who have undergone transplant and/or curative gene therapy for cancer, hemoglobinopathies, or hemophilias and have become transfusion independent (Table 2).

Blood component synthesis and testing

Protocols are underway for scalable ex vivo generation of RBCs utilizing a variety of starting cell fractions, including HSPCs from both cord blood [103-105] and adult sources [106,107], embryonic stem cells [108,109], as well as iPSCs [110-112]. If successful, these products could potentially alleviate some of the issues of contemporary blood component therapy, including blood shortages and risks of transfusion-transmissible infections. Concurrent with the development of a scalable cultured RBC product has been the use of gene editing to generate RBCs and erythroid precursors deficient in higher prevalence antigens [113,114]. These combined strategies have the potential to produce compatible RBC units for patients with rare blood types, and, for alloimmunized patients with SCD or thalassemia, provide sufficient support during the cytopenic phase of HSCT or gene therapy.

The use of RBC genotyping has significantly enhanced pretransfusion testing for chronically-transfused patients, and can assist in the provision of units phenotypically matched for the notoriously immunogenic antigens. Still, antibody identification can be hindered when rare antigen RBC reagents are unavailable. Currently, the practice of many immunohematology reference laboratories is to freeze and store these reagents for thaw and use when needed, but reagent availability is still subject to limited supplies. Efforts to combat this in the realm of gene manipulation include the development of reagent RBCs from cultured cells. A key challenge is the expression of adequate antigen density for visible agglutination to occur when cells are reacted with antigen-specific antibodies [115]. Kikuchi, et al. explored the use of a human erythroid progenitor cell line derived from human iPSCs as a serologic testing reagent, and demonstrated sufficient antigen expression to detect anti-Di^a after genetic manipulation using a lentiviral vector carrying the *DI*A* allele [116]. Application of CRISPR/Cas9 technology to genetically edit clinically-important human platelet antigens also holds great potential for production of designer platelets for diagnostic, investigative, and, ultimately, therapeutic use [117].

Blood centers as manufacturing facilities for cellular therapies

As the development of gene therapy products continues to grow, the ideal framework for how and where their clinical versions will be manufactured is still being fine-tuned. Gene therapy products have the potential to reach patients on a national scale, and their production may occur by both centralized and decentralized models [118]. With centralized manufacturing, a single hub carries out production and holds the responsibility of ensuring regulatory oversight and manufacturing availability and success. While sophisticated transport mechanisms that include GPS-tracking and continuous temperature monitoring are now available to support centralized manufacturing, decentralization also has its advantages. Notably, as the starting material for gene therapies is most commonly obtained from that patient himself. A decentralized model where a single site such as blood center collects the starting material and manufactures the cellular therapy would reduce the logistical issues of transporting the starting material and final product back and forth, to and from the manufacturing facility. As such, the duration between the time of starting material collection to treatment may also be reduced.

Blood centers, in particular, are poised to adopt gene therapy manufacturing as a part of their capabilities. As gene therapies are highly personalized products, the close communication between blood centers and treating physicians can enhance the production and success of these therapies. Blood centers collecting the starting material by apheresis can tailor collection parameters to the clinical status of the patient as well as to production needs. Moreover, the technical skillset needed to manufacture gene therapies overlaps with those of clinical laboratory scientists and blood bank specialists, who are also familiar with aseptic techniques and cGMP practices. Lastly, blood centers are accustomed to the requirements of regulatory bodies and accrediting agencies, and can ensure compliance from a quality standpoint.

Conclusions

Since the first HSCT was performed over half a century ago, the development of gene therapies has come a long way to now incorporate highly targeted gene manipulation techniques. Strategies for gene manipulation began with gene replacement with HSCTs, and have since progressed to include gene supplementation using viral vectors as well as nuclease-based genome editing mechanisms. Gene therapies are now available to treat a number of benign and malignant hematologic diseases, either through clinical trials or as

FDA-approved therapies. As their manufacturing is scaled up, blood centers will represent particularly well-suited avenues for the decentralized manufacturing of these products.

The increasing use of gene therapies also has other implications for blood centers. Prior to the administration of many gene therapies, patients may require variable amounts of chemotherapeutic conditioning, and accordingly, develop variable levels of cytopenia requiring support with blood product transfusions. Following successful gene therapies, many patients with hematopoietic conditions have also become transfusion-independent, though changes in population level-blood utilization may not be seen for some time. Furthermore, the principles of gene manipulation have also been applied to blood component synthesis and testing, with recent studies now showing promising findings for donor-independent blood products and pretransfusion testing reagents.

Nevertheless, cost may be an insurmountable barrier to gene therapy for many individuals. An AAV-based gene therapy for a rare genetic disorder was priced at approximately $1 million, which has been cited as a reason for its low clinical use and the eventual decision not to pursue further approval for this therapy [119]. On the other hand, gene therapy for hemophilia B has been estimated to save over USD $200,000 annually for those who no longer need routine factor prophylaxis. Similarly, the lifetime cost of managing a patient with SCD is roughly over a million [120]. Commercial interests are likely to justify the cost of gene therapies based on their ability to effect permanent cure and add quality adjusted life years, unlike for the more morbid HSCTs. It is also possible that with the increasing number of clinical trials for these disorders in the United States, blood component utilization may witness a paradoxical increase due to “reverse medical tourism.” At this time, the wider implications of gene therapies for blood centers are difficult to predict, but exciting developments for both are in store.

Conflict of Interest

Authors declare that they have no conflict of interest.

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