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Gene Therapy

Current Applications and Future Possibilities

David H.M. Steffin, MD[a,b], Emily M. Hsieh, MD[a,b], Rayne H. Rouce, MD[a,b,*]

[a]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston Methodist Hospital and Texas Children's Hospital, Houston, TX, USA; [b]Texas Children's Cancer and Hematology Centers/Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Keywords

• Gene therapy • Immunotherapy • CAR T cell • Human genome editing
• CRISPR-Cas9

Key points

- Early clinical trials of gene therapy, despite mixed outcomes, solidified the use of viral vectors to transmit genetic material and paved the way for current clinical applications.
- Gene therapy has demonstrated success in inherited and acquired pediatric diseases including immunodeficiencies, inherited retinal disorders, neurologic disorders, blood disorders, and cancer.
- Chimeric antigen receptor (CAR) T cells targeting CD19 expressed on B-ALL, the most common childhood cancer, have induced complete remissions in refractory patients, leading to recent FDA approval.
- Novel gene-editing techniques such as CRISPR-Cas9 offer precise mechanisms of human genome modification, a technique that will be tested in upcoming clinical trials.

INTRODUCTION

Gene therapy encompasses multiple approaches to manipulate genetic material in an effort to treat specific diseases, including replacing a mutated or defective gene with a healthy one, introducing a new gene to help fight disease, or editing an existing gene to change its function [1]. From a clinical perspective, gene therapy has the potential to reverse the clinical sequelae of a condition, a

*Corresponding author. Center for Cell and Gene Therapy, Feigin Center, 1102 Bates Street, 17th Floor, Houston, TX 77030, USA. *E-mail address:* rhrouce@gmail.com

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concept that was long met with understandable skepticism. In pediatrics, these strategies hold special promise, because many childhood diseases were considered incurable before recent innovations in gene therapy.

Although gene therapy has been studied for decades, the last few years have yielded some of the most exciting clinical advances in the treatment of inherited and acquired diseases. The road to translating these therapies to the clinic has been a perilous one, although positive results in some rare diseases have contributed to optimism in the field. Nevertheless, concerns over safety and appropriate clinical applications have arisen, highlighting the importance of careful regulation and monitoring of in-human use.

Here, we will briefly review types of gene therapy and their potential uses, before highlighting recent advances in gene therapy for pediatric disorders. We will focus on therapies that have been translated to the clinic. We will also discuss current safety and ethical guidelines that must be considered when using gene therapy, as well as what the future holds for this promising therapy.

SUCCESSFUL EXAMPLES OF GENE THERAPY USED TO TREAT DISEASES

Gene therapy is typically divided into 2 main types: germ line and somatic. In somatic gene therapy, genes are introduced into somatic cells or patients, and thus therapy is limited to the individual patient and cannot be inherited or passed on. Germ line gene therapy involves introducing a new gene into sperm or egg cells, which allows the introduced changes to be heritable and therefore passed on [1]. Germ line therapy is less common, and more technically and ethically challenging because of the ability of therapeutic effects to be passed on. Thus, somatic gene therapy has had the most clinical success to date and is the main focus of this review.

Despite years of preclinical work, it was not until the early 1990s that the first gene therapies were studied in humans, albeit with mixed results. The first clinical trial to gain approval for transfer of a foreign gene into humans was conducted at the National Cancer Institute in Bethesda, Maryland, in 1990. This trial used a retroviral vector to insert the gene encoding the cytokine, inteleukin-2 (IL-2) into patient-derived tumor-infiltrating lymphocytes (TILs). The genetically modified TILs were then infused back into patients with advanced melanoma. Although the efficacy results were disappointing–all 5 patients treated ultimately died of progressive disease–TILs from 4 of 5 patients displayed in vivo expansion without significant toxicity [2].

That same year, the first gene therapy trial targeting an inherited immunodeficiency, severe combined immunodeficiency (SCID), was performed. Again, a retroviral vector was used to insert the gene encoding adenosine deaminase (ADA), the enzyme deficient in patients with SCID, into patient T cells. These genetically modified T cells were then given back to 2 patients with SCID, one of whom demonstrated an increase in ADA activity that may have been attributable to the therapy, although the patient was concurrently receiving an enzyme replacement therapy, thus confounding results [3].

The early clinical applications of cell therapy, while demonstrating safety and feasibility, unfortunately resulted in limited efficacy. More than 100 clinical trials of various gene therapies were developed in the 1990s. Despite their mixed outcomes, one concept solidified by these early gene therapy trials was the use of viral vectors to transfer genetic material. Therefore the promise of adoptively transferring cells genetically modified in the laboratory using viral vectors, once considered myth, became reality [4].

Despite a plateau in clinical progress in the 1990s, over the last 20 years there has been a resurgence of research committed to improving cell therapies, with significant progress (Fig. 1). Since the 2000s, there have been successes in the manipulation and use of a variety of vectors, namely retro and lentiviral ones, which has directly correlated with enhanced clinical translation.

GENE THERAPY FOR HEREDITARY DISEASES

Gene therapy has been particularly useful in the treatment of inherited diseases whereby there is often a nonfunctional or dysfunctional gene product. These approaches also provide an alternative, less-toxic, but potentially curative therapy to hematopoietic stem cell transplant (HSCT), which has historically been the only therapeutic option to reverse some inherited diseases.

Inherited immunodeficiencies

As mentioned in the preceding section, one of the first diseases successfully treated with gene therapy was SCID, an inherited combined immunodeficiency resulting from several gene deletions. Patients with these gene deletions lack functional B, T, and natural killer (NK) cells, and thus are very susceptible to life-threatening bacterial, viral, and fungal infections [5]. HSCT is the only known cure, but is associated with its own short- and long-term toxicities. Following the initial gene therapy trials for SCID in the 1990s, a significant breakthrough in gene therapy for SCID due to ADA deficiency was reported

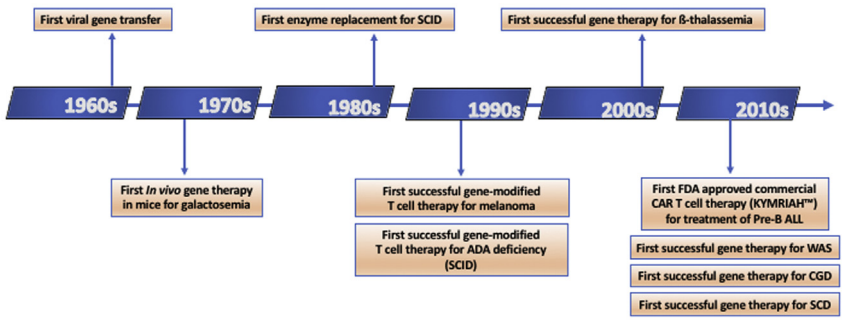

Fig. 1. Timeline depicting key milestones in preclinical research and clinical trials in gene therapy from the 1960s to the present day. CGD, Chronic granulomatous disease; SCD, sickle cell disease; WAS, Wiskott-Aldrich syndrome.

in 2002. The first clinical study using gene transfer into HSCs was completed at Hadassah Hospital in Israel (NCT00598481) [6]. This study inserted a functional ADA gene into a retroviral vector, then transferred this vector into HSCs of patients with SCID. The study evaluated 18 patients from 2000 to 2014 with a primary endpoint of safety and secondary endpoint of efficacy. All 18 patients underwent initial conditioning treatment with busulfan, followed by infusion of the genetically modified therapy. Overall, the intervention was safe, with all patients surviving to the last follow-up in 2014. The treatment was well tolerated with most adverse events being cytopenias, increased transaminases, and hypertension, symptoms that resolved in all patients and were thought to be related to busulfan conditioning rather than the gene therapy itself. Most importantly, 15 of the 18 patients developed increased ADA levels, increased lymphocyte counts, sustained immune reconstitution, and sustained humoral and cellular immune responses [6,7], indicating the efficacy of this therapeutic approach. None of these patients developed evidence of myelodysplasia or leukemia at any point during follow-up, which is notable given that this long-term toxicity has occurred as a result of other gene therapies, as discussed in the safety section below.

The success in SCID paved the way for attempts to correct genetic impairments in other inherited immunodeficiencies. Chronic granulomatous disease (CGD) is a debilitating immunodeficiency that occurs as a result of mutations in the CYBB gene. CGD is characterized by a decrease in oxidase-positive neutrophils, resulting in an inability to respond to bacterial and fungal infections [8]. The identified isolated CYBB mutation in CGD makes it an ideal target for gene therapy.

There has been extensive preclinical research demonstrating success for gene therapy in CGD [9,10]. Based on these promising data, a phase 1 clinical trial (NCT02234934) was opened in January 2015 in 3 locations: Boston Children's Hospital, University of California, LA, and the NIH [11]. The study used a lentiviral vector to insert a functional CYBB gene into patient HSCs. Recently reported interim data on 7 pediatric patients with a 12-month follow-up showed patients have tolerated the therapy well so far. Aside from one infusion-related inflammatory syndrome that resolved with steroid supplementation, there have been no reported significant adverse events. Six of 7 patients had an increased percentage of oxidase-positive neutrophils (range 16%–46%) from baseline (notably, at least 10% oxidase-positive neutrophils are necessary for normal neutrophil function). All 6 responding patients demonstrated persistent peripheral blood copies of the lentiviral vector expressing the CYBB gene, even at 12 months postinfusion [12]. Further, all patients were able to discontinue prophylactic antimicrobials.

Gene therapy for Wiskott-Aldrich syndrome (WAS) has also demonstrated promising results. WAS is an X-linked disorder characterized by a mutation in the WAS protein (WASP) gene leading to thrombocytopenia and immunodeficiency. As a result, patients suffer from eczema, inability to fight infections, and have a significant risk of bleeding [13]. Recently, a phase 1/2 clinical trial

(NCT01347242) started in March 2019, at the Great Ormond Street Hospital for Children in London, demonstrated promising interim results of 7 pediatric patients treated with ex vivo gene-modified CD34+ stem cells encoding a lentiviral vector with a functional WASP gene. One patient died from a preexisting drug-resistant herpes infection. Of the remaining 6 patients, all had immune constitution with corrected lymphoid cells, and all 6 patients had resolution of bleeding episodes and eczema [14].

Leber congenital amaurosis

One of the earliest successes in gene therapy came in the treatment of Leber congenital amaurosis, which is an early and irreversible cause of blindness. There are no supportive treatments for the disease, which typically occurs in the first 2 decades of life. Once researchers, in 1988, discovered hereditary mitochondrial DNA mutations as the primary defect leading to congenital amaurosis, the possibility arose of reversing this devastating cause of blindness with targeted gene therapy [15]. In 2007, 3 groups treated patients in early clinical trials in the United States and United Kingdom. In 1 phase 1 clinical trial, 3 patients (21–24 years) underwent unilateral intraretinal injections of an adenoviral-associated vector (AAV) carrying a functional copy of RPE65, known to be deficient in Leber congenital amaurosis. Treatment was well tolerated with no significant adverse events reported. All 3 patients noted significant improvement in visual acuity in the treated eye, prompting a cascade of gene therapy clinical trials in the field [16]. The exciting results ultimately led to US Food and Drug Administration (FDA) approval of the gene therapy, Luxturna, in 2017 [17].

Neurologic disorders

Identification of the precise causative mutations for most neurologic disorders has aided in the ability of researchers to develop targeted gene therapies to combat them. AAV gene therapy enables both gene replacement to address loss-of-function mutations and gene silencing to address gain-of-function mutations. Both strategies have been used in preclinical and clinical studies for neurologic diseases. For example, survival motor neuron (SMN) protein has been replaced to treat spinal muscular atrophy (SMA) [1,2,3], while superoxide dismutase 1 has been silenced to treat amyotrophic lateral sclerosis [4,5,6], as has Huntington for Huntington disease [7,8,9] [18]. The FDA recently approved the drug Spinraza, which increases production of SMN protein, after it showed promising results in patients with SMA. In an expanded access program in Europe, 61 children with SMA were treated with Spinraza and experienced substantial improvement in motor function after only 6 months [19]. Another gene therapy drug for SMA type 1 called Zolgensma, which provides a normal copy of the SMA1 gene, also showed great promise in early clinical trials and is currently pending FDA approval [20].

X-linked myotubular myopathy is another rare neuromuscular disorder caused by a mutation in the myotubularin gene and characterized by progressive muscle weakness and respiratory failure [21]. A recent clinical trial testing a

gene therapy called AT132, composed of an AAV encoding the functional copy of the myotubularin gene has reported early success (NCT03199469). Interim results from 7 patients enrolled in an AT123 trial showed that 3 patients attained ventilator independence at 48 weeks follow-up, demonstrating their rapid improvement in muscle function [22].

Lysosomal storage diseases

Targeting causative gene mutations in lysosomal storage diseases may also provide promising therapeutic options. Fabry disease is caused by a mutation in the α-galactosidase A gene, which produces galactosidase A. A recent phase 1 clinical trial (NCT02800070) performed ex vivo transduction of CD34+ stem cells with a lentiviral vector containing a normal copy of the human α-Gal A gene with the intention to determine the safety and toxicity, as well as any potential benefit in adult patients. The trial began in 2017, operating at 3 centers in Canada. Six patients have been enrolled thus far. Interim data report no adverse effects, and 1 patient demonstrated engraftment and generation of galactosidase A activity at 2 years follow-up [23]. Promising data also have been reported for ex vivo clinical gene therapies as treatment of mucopolysaccharoidosis (MPS), Gaucher disease, and Hurler syndrome. In 2018, a phase 1 clinical trial (NCT02702115) was opened also using ex vivo transduction of CD34+ stem cells with an AAV to insert a corrected gene copy expressing iduronidase, a liver enzyme deficient in MPS1. The first patient was recently enrolled with, results pending [24,25].

Inherited hematologic diseases

Gene therapy has become a viable therapeutic option for several inherited hematologic diseases. β-Thalassemia major, characterized by loss of 3 copies of the β-globin gene, leads to severe anemia, growth and developmental delay, and complications of transfusion dependence often leading to liver and heart failure. The incidence of β-thalassemia is 1/100,000 worldwide and HSCT is the only curative therapy [26]. Recently, 2 phase 1/2 gene therapy trials have entered the spotlight after demonstrating success [25,27]. In both trials, CD34-mobilized stem cells were transduced in vitro with a lentiviral vector encoding HbA, the normal hemoglobin that is missing in patients with β-thalassemia major. Twenty-two patients (aged 12–35 years of age) received busulfan as preconditioning chemotherapy, followed by infusion of the gene-modified stem cell product. Thirteen of the patients had a mild phenotype characterized by residual function of the β-globin gene, while 9 had a severe phenotype. Twelve of the 13 patients with the mild phenotype stopped requiring red blood cell (RBC) transfusions at a median of 26 months after treatment. Moreover, of the 9 patients with the completely nonfunctional phenotype, the mean transfusion volume per year was reduced by 73%, allowing discontinuation in 3 of the patients. Neither study resulted in significant toxicities, although 9 of 22 patients suffered minor adverse events consistent with side effects related to busulfan [28].

The exciting results in β-thalassemia paved the way for the use of gene therapy in other hemoglobinopathies. Worldwide, approximately 250,000 babies are born every year with sickle cell disease (SCD) and 95,000 people are living with SCD in the United States [29]. SCD is the result of a single-nucleotide mutation in the SCD β-globin chain that leads to a substitution of glutamic acid to valine. This mutation results in an unstable hemoglobin, producing the classic "sickled" RBCs, which, despite modern treatment modalities can become trapped, leading to several complications including significant anemia, veno-occlussive crisis, infection, stroke, organ failure, and decreased lifespan [30]. Conventional treatment has consisted of supportive care including prophylactic antibiotics to protect from encapsulated bacteria. More recently, therapeutic options such as hydroxyurea have emerged. Hydroxyurea stimulates the production of fetal hemoglobin (HbF), thus minimizing the production of sickled RBCs.

Despite recent advances, SCD remains one of the most challenging inherited disorders to treat, with HSCT being the only curative option. However, the less than ideal outcomes following HSCT for SCD largely related to lack of ideal donor sources, have resulted in significant focus being placed on gene therapy [31]. Clinical trials conducted at 3 different centers have reported exciting results to date. Bluebird Bio (Cambridge, MA) is treating patients with SCD on an ongoing phase 1/2 trial (NCT02140554) that is enrolling patients at multiple sites in the United States. In this trial, patient CD34+ stem cells are collected and undergo lentiglobin gene therapy to insert a normal β-globin gene. Before infusion of these gene-modified HSCs, patients are treated with busulfan conditioning therapy [32]. Early results from 7 patients treated with this therapy revealed improvements in hemoglobin in 4 of them after 6 months of follow-up. No veno-occlusive crises were reported, and laboratory values commonly used to follow SCD were improved compared with baseline levels in all patients. These clinical improvements correlated with a quantitative reduction in mutated globin chain β^S in all treated patients [32].

Other groups have explored mechanisms to increase HbF production, as opposed to repairing the β-globin gene. In a phase 1/2 clinical trial at Cincinnati Children's (NCT02186418), CD34+ stem cells were collected by way of marrow harvest or pheresis, followed by in vitro transduction of HSCs with a lentiviral vector encoding a modified γ-globin chain encoding for HbF. Patients subsequently underwent conditioning with melphalan before product infusion. Preliminary results demonstrate an acceptable safety profile and prompt count recovery. Two patients have been treated thus far, both experiencing significant improvement in chronic pain and reduction in sickling events [33].

With regard to the inherited bleeding disorders hemophilias A and B (Factor VIII and IX deficiency, respectively), there are several phase 1 and 2 clinical trials that are ongoing or were recently completed. Despite recombinant factor replacement and vigilant treatment, patients with hemophilia often experience

significant and repeated bleeding into joints, muscles, or intracranially, leading to considerable morbidity and mortality [34]. Several groups have explored the use of AAV as a means of correcting the deficiencies in hemophilia A and B. Because the mutations associated with the hemophilias are complex–due to either inversions, missense, or small/large deletions [35]–researchers have focused on providing a functional copy of the gene encoding the absent factor.

Despite promising preclinical results, the clinical translation of gene therapy for the hemophilias remains challenging. For one, there are constraints limiting the use of viral vectors as modes of gene transfer: gene insertions must be relatively small to ensure that the gene is inserted properly, efficiently transferred to the patient, and that its expression is maintained on infusion. As the size of the insert increases, successful gene therapy becomes more challenging. This concept is especially relevant for factor VIII, which is quite large [36]. Factor IX (FIX) gene therapy has yielded more success. Recently, a phase 1 clinical trial (NCT03307980) conducted by Pfizer at multiple centers in the United States and Australia treated 10 patients with severe FIX deficiency with an AAV-transduced FIX gene product. The product was well tolerated, yielding a mean increase in FIX activity of 33.8% $\pm$ 18.1% (mean $\pm$ SD) [37]. Table 1 summarizes gene therapies for heritable conditions to date [38].

NONVIRAL GENE THERAPIES: CYSTIC FIBROSIS AS A MODEL

Early attempts at gene therapy in cystic fibrosis (CF) were hindered by the limited ability of viral vectors to effectively deliver gene-modified therapies from the luminal surface to the respiratory epithelium. More recently, nonviral gene transfer (using plasmids, for example) has arisen as a potential therapeutic option for patients with CF.

CF is a heritable disease due to a mutation in the CFTR gene that leads to worsening respiratory function, infections, and high rates of mortality at an early age. Most current treatments are primarily supportive, focused on respiratory support and antimicrobial therapy [39]. One challenge distinct to gene therapy products for CF is the requirement to localize to the respiratory epithelium to ensure maximal therapeutic effect while minimizing inflammation, which is an already prominent feature of the disease [40]. Recently, a phase 1/2 trial (NCT01621867) across 18 CF centers evaluated directed lung delivery (via nebulization) of a plasmid DNA encoding the functional CFTR gene. One hundred and forty patients $\geq$12 years old were randomized to receive either placebo or the nebulized product monthly over a 1-year period (n = 78 in treatment group). Using a primary endpoint of percentage predicted forced expiratory volume, the treatment group was noted to have significantly improved lung function compared with the placebo group [41]. This important study provided the first proof-of-concept of nonviral gene therapy specifically designed to improve lung disease.

GENE THERAPY FOR ACQUIRED DISEASES

A genetic engineering strategy that has gained substantial attention over the past 10 years because of its widespread success, predominantly in treating

Table 1
Summary of recently completed and/or recruiting clinical trials (as indicated by NCT no.) for patients with heritable diseases

Gene therapies for heritable disease				
Disease Treated	Intervention	Location/Year Initiated	Clinicaltrials.gov ID	Outcomes Reported
X-linked SCID	Retroviral gene transfer to insert a functional copy of ADA	NIH, Bethesda, USA/2001	NCT00028236	n/a
MPS type III A; Sanfilippo disease type A	AAV transfer of human SGSH and SUMF1 cDNAs	Hôpital Bicêtre – Assistance Publique, Paris, France/2011	NCT01474343	n/a
WAS	Lentiviral-mediated HSC gene transfer of a functional WAS gene	Children's Hospital Boston, Boston, USA/2011	NCT01410825	n/a
CGD, X-linked	Lentiviral-mediated viral transfer of the human CGD gene (G1XCGD)	UCLA, LA; NIH, Bethesda, Boston Children's, Boston, USA/2018	NCT02234934	n/a
MPS II (Hunter syndrome)	AAV transfer of iduronate-2-sulfatase (IDS) gene	UPMC, Pittsburgh, USA/2018	NCT03566043	n/a
CF	Liposomal transfer of pGT-1 gene lipid complex	University of Alabama, Birmingham, AL, USA/1999	NCT00004471	n/a
CALD	Lentiviral transfer of gene encoding the human ALD protein	Boston Children's Hospital, Boston, USA/2013	NCT01896102	n/a
LCA, hereditary retinal diseases	AAV gene transfer of RPE65	Moorfields Eye Hospital, London, UK/2016	NCT02946879	n/a
SCD	Lentiviral-transduced CD34+ HSC targeting BCL11a	Boston Children's Hospital, Boston, USA/2017	NCT03282656	n/a
SMA1-1	AAV transfer to insert functional copy of SMN (AVXS-101)	Nationwide Children's, Columbus, USA/2014	NCT02122952	15/15 patients alive and event free at 20 mo out, compared with 8% in control

Abbreviations: AAV, adenovirus-associated vector; CALD, cerebral adrenoleukodystrophy; CGD, chronic granulomatous disease; NCT, Clinicaltrials.gov identifier; CF, cystic fibrosis; LCA, Leber congenital amaurosis; MPS, mucopolysaccharidosis; n/a, not applicable; RPE65, retinal pigment epithelium 65; SCID, severe combined immunodeficiency; SCD, sickle cell disease; SMA, spinal muscular atrophy; WAS, Wiskott-Aldrich syndrome.

cancer, is the combination of cellular and gene therapy. This combination, often known simply as "cellular immunotherapy," typically involves immune effector cells collected from a patient and genetically manipulated in the laboratory. These cells are then expanded to a specified dose and returned to the patient. Similar to other forms of gene therapy, the most commonly used gene transfer vectors include adenoviral vectors, retroviral and lentiviral vectors, and naked plasmids. Although most trials to date have used T cells, genetically modified NK and NK-T cells have also been used in clinical trials.

Success in treating cancer

Cellular immunotherapies have been most successful against cancer. In fact, cancer is the most common disease treated by gene therapy, comprising 66% of all ongoing clinical gene therapy trials worldwide [42]. The most successful cellular immunotherapy to date are T cells genetically modified to express chimeric antigen receptors that target cancer antigens (CAR T cells). CAR T cells are manufactured in the laboratory to combine the antigen-recognition capabilities of a monoclonal antibody with the signaling and killing capacity of the T cell. CAR T cells typically contain 4 major components: the antigen-recognition component that is derived from a single-chain variable fragment of a monoclonal antibody; a linker or transmembrane domain; an endodomain that is, responsible for providing the T-cell activation signal; an additional costimulatory domain responsible for enhanced in vivo persistence. These artificial CAR receptors can therefore be designed to recognize almost any antigen on the surface of a cancer cell, then introduced into the patient's own T cells, typically using a retroviral or lentiviral vector. One of the primary advantages of CAR T cells is that they bypass the requirement of antigen presentation by major histocompatibility complex molecules and thus have human leukocyte antigen-independent killing [43].

Whereas CAR T-cell therapy has been clinically studied in a variety of malignancies, it has been most effective in hematologic malignancies, presumably because of the accessibility of leukemic blasts in bone marrow/blood of affected patients and widespread antigen availability. The most successful CAR T cell by far has been the CD19-CAR T cell, targeting the CD19 antigen expressed on the vast majority of B-cell malignancies. Lymphodepleting chemotherapy followed by a single infusion of autologous CD19-CAR T cells to patients with refractory acute lymphoblastic leukemia ([ALL], the most common childhood cancer) has had resounding success (>70% responses) in heavily pretreated patients with limited treatment options [44]. This groundbreaking therapy led to recent FDA approval of the first cellular gene therapy, a CD19-CAR T cell, by the company Novartis (Kymriah [tisagenlecleucel]), approved for the treatment of pediatric B-ALL that is refractory or in second or greater relapse [43]. There are numerous ongoing early-phase clinical trials of CAR T cells targeting other childhood and adult hematologic malignancies, some of which are highlighted in Table 2.

Table 2
Summary of recently completed and/or recruiting clinical trials (as indicated by NCT no.) for children with hematologic malignancies, solid tumors, and brain tumors

Cancer type	Target Antigen	Clinicaltrials.gov ID
T-cell malignancies	CD5	NCT03081910
AML, blastic plasmacytoid dendritic cell neoplasm		NCT02159495
Lymphoma	CD30	NCT02663297
Liver neoplasms	Glypican 3	NCT02932956
Neuroblastoma	GD2	NCT03294954
GD2+ sarcoma and neuroblastoma	GD2	NCT01953900
Sarcoma and central nervous system tumors	Human epidermal growth factor receptor 2 + tumor cells	NCT00902044 NCT02442297
Malignant glioma	Tumor-associated antigen interleukin-13 receptor $\alpha 2$	NCT02208362

Extending the success of CAR T cells to the treatment of solid and brain tumors has proven more difficult because of difficulties in navigating the hostile tumor microenvironment. Several studies have shown that CAR T cells targeting solid tumors, once infused into patients, face inhibitory signals, immunosuppressive cell populations, and a hypoxic, nutrition-depleted environment that can impair T-cell function [45]. These factors limit T-cell activation, expansion, and persistence, which translates to limited antitumor activity [46,47]. Box 1 shows actively enrolling clinical trials of CAR T cells for solid and brain tumors registered on clinicialtrials.gov as of February 2019.

GENE EDITING AND GENE THERAPY OF THE FUTURE

As gene therapy efforts have become more sophisticated over the past few years, much of the field has focused on refining tools to offer more elegant and precise methods of treating genetic diseases. Gene editing has generated enthusiasm for its promise in achieving these goals. In genome editing, a double-strand break is created using engineered nucleases that modify the genome in a site-specific manner. Whereas several artificial nuclease systems have been developed for genome editing, including protein-guided DNA cleavage systems of zinc-finger nucleases (ZFNs) and transcription activatorlike effector nucleases, the CRISPR/CRISPR-Cas9 system has shown most promise and earned most media attention of late. CRISPR relies on small RNA for sequence-specific cleavage, enabling researchers to precisely manipulate specific genomic elements to achieve a therapeutic effect [48] A schematic of CRISPR-Cas9 gene editing is shown in Fig. 2.

The first clinical trial showing feasibility of gene editing was published in 2014. In an effort to render CD4 T cells from patients with human immunodeficiency virus (HIV) resistant to infection with the virus, researchers used ZFNs to disrupt or "knock out" the gene that encodes a cellular co-receptor

Box 1: List of indications and potential ethical considerations for heritable genome editing.

Regulatory Framework for Clinical Trials That Use Heritable Genome Editing

1. Absence of reasonable alternatives
2. Restriction to preventing a serious disease or condition
3. Restriction to editing genes that have been convincingly demonstrated to cause or strongly predispose to the disease or condition
4. Restriction to converting such genes to version that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects
5. Availability of credible preclinical and/or clinical data on risks and potential health benefits of the procedures
6. Ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants
7. Comprehensive plans for long-term, multigenerational follow-up that still respect personal autonomy
8. Maximum transparency consistent with patient privacy
9. Continued reassessment of health and societal benefits and risks
10. Reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition

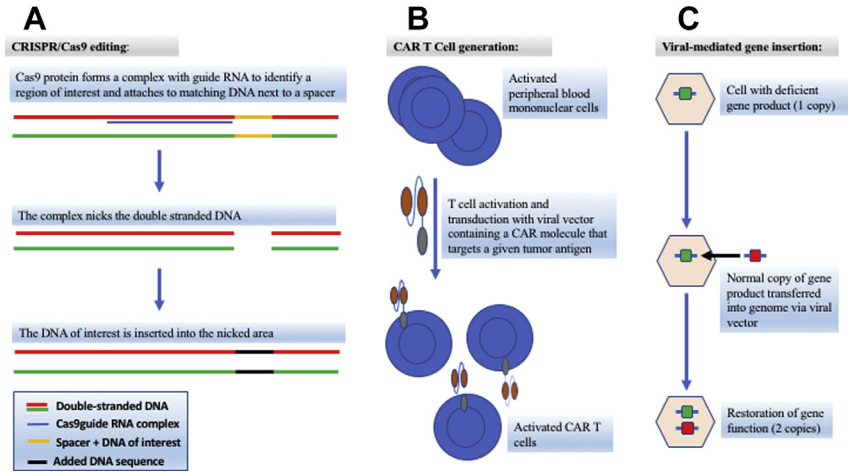

Fig. 2. Schematic of gene therapy techniques. (*A*) CRISPR-Cas9 gene editing. (*B*) Transduction of T cells with chimeric antigen receptor (CAR) to create CAR T cells. (*C*) Example of ex vivo viral insertion of a functional gene copy before insertion in a patient.

for HIV, CCR5 [49]. These patient-specific T cells were manipulated in the laboratory before re-infusion. The study revealed the safety, tolerability, and persistence of CCR5-modified CD4-T cells on treatment. During periods of viremia, the decline in circulating CCR5-modified cells was significantly less than the decline in unmodified cells, indicating that the modified cells were protected from infection. Despite the limited therapeutic effect of this treatment, the trial paved the way for the clinical application of ex vivo gene-editing strategies [50,51].

As mentioned above, much of the gene-editing field recently has shifted to exploring the use of CRISPR-Cas9 because of its ability to precisely manipulate genes. There are currently 11 actively recruiting clinical trials that use CRISPR-Cas9 to address several hereditary and acquired diseases, including β-thalassemia, SCD, solid tumors, and multiple myeloma [51–54]. Our group is exploring methods to use CRISPR-Cas9 to edit T cells engineered with CAR molecules targeting a pan-T cell antigen. This approach (NCT03690011) should protect the CAR T cells from targeting themselves, allowing them to expand before infusion into patients with refractory T-cell malignancies [55].

In addition, upcoming clinical trials using CRISPR-Cas9 to treat β-thalassemia have gained recent approval in the United States (NCT03655678, NCT03728322). Based on demonstration of therapeutic benefit in mouse models for Duchenne muscular dystrophy [56] and hereditary tyrosinemia [57], clinical trials using genetic-editing techniques have been submitted for regulatory approval for these and many other diseases.

SAFETY

As with most innovative strategies, gene therapy is not without safety concerns. As the feasibility and applicability of gene therapy has grown over the past few years, more data on real and theoretic safety concerns have come to light. As illustrated in the previous sections, most clinical gene therapy strategies use viral, specifically retroviral, vectors.

Genotoxicity due to retroviral vectors

Several reports of T-cell leukemia occurring in patients with immunodeficiencies receiving genetically modified stem cell products in the initial trials rocked the field in the late 1990s and shed important light on the necessity of long-term follow-up. In these early trials, retroviral vectors were transferred to HSCs to correct inherited immunodeficiencies. However, despite the clinical activity discussed in preceding sections, several years after treatment, a subset of patients receiving gene therapy for SCID, CGD, and WAS developed acute leukemias owing to activation of proto-oncogenes adjacent to proviral insertions [58,59]. These toxicities led to the adoption of enhancer-deleted lentiviral or retroviral vectors for HSC clinical gene therapies. Up to now, these newer vectors have ameliorated disease without causing genotoxicity [60].

Retroviral transduction results in new, random integrations in host cell DNA, which infrequently may cause abnormal or uncontrolled proliferation. Moreover, this effect is much more common with replication-competent retrovirus (RCR), whereby each cell receives multiple additions. Clinical trials regulated by the FDA are required to test transduced cells and postinfusion samples for RCR, and to exclude any RCR-containing products from clinical use. More recently, several publications have reviewed the extensive and long-term data on RCR monitoring in gene-modified T cells immediately after gamma retroviral transduction and in follow-up patient samples. Importantly, in over 1000 patients treated with gene-modified T cells, no evidence for RCR has been identified, demonstrating the safety of genetic modification of T cells [61–63].

A separate concern that is discussed in the Ethics section below is the modification of germline genetic material, which is potentially heritable [9,10].

Inflammatory responses to chimeric antigen receptor T cells

The promising clinical responses observed with CAR T cells in pediatric leukemia have not come without concern. In fact, in all CD19 CAR T-cell trials demonstrating clinical responses, serious toxicities have also been reported. The most frequent toxicity occurring after infusion of CD19 CAR T cells is cytokine release syndrome (CRS). Cytokine release syndrome is a commonly occurring systemic inflammatory response that can range from a mild syndrome requiring supportive care alone (antipyretics, IV fluids, close monitoring) to symptoms resembling sepsis, systemic inflammatory response syndrome, and hemophagocytic lymphohistiocytosis and requiring intensive care intervention [64]. The symptoms of CRS correspond with peripheral expansion of the infused CAR T cells, and are typically more severe in patients with larger disease burden. Identification of IL-6 as the most prominent cytokine responsible for the symptomatology led to adoption of the anti-IL-6R antibody tocilizumab as the treatment of choice in patients with more severe symptoms. Standardized grading algorithms based on clinical and laboratory features have streamlined CRS identification and management, ensuring that patients treated with this promising therapy are managed safely [64,65].

Neurologic toxicity has also occurred following infusion of CD19 CAR T cells, and can range from somnolence to seizure activity and cerebral edema. Whereas the cause of CAR-related neurotoxicity, sometimes known as cytokine release encephalopathy syndrome, is less clear than that of CRS, endothelial activation and disruption of the blood-brain barrier seems to play a role, and symptoms typically are self-limited requiring supportive care, including empiric antiepileptics, although therapy with steroids is sometimes required [64,65].

ETHICS

As developments in gene therapy and human genome editing continue to evolve, so have ethical concerns surrounding these advancements. Many of

these concerns are focused specifically on newer genome-editing tools, which have yet to be used in humans, thus safety and efficacy data do not exist.

In response to these growing ethical concerns, The US National Academy of Sciences and the National Academy of Medicine convened a multidisciplinary, international committee to review the current status of human genome editing and make recommendations. The final report was published in February of 2017 [66], with specific recommendations regarding regulation of clinical trials using heritable genome editing as detailed in Box 1.

Ethical concerns regarding genome editing largely surround its use in correcting germ line mutations. Germ line editing is highly contentious because the resulting genetic changes could be inherited by offspring, thus broadening the scope of concern beyond the individual being treated. The result is a complex situation whereby the appropriateness of genome-editing interventions and their potential effects on individuals and society must be considered.

Aside from obvious concerns that a heritable gene edit could do harm (to the individual and their progeny), some have cited potential for misuse of this technology for state-imposed eugenic applications [67]. As gene therapy continues to push the boundaries of science and technology, bioethics experts continue to struggle to ensure that the ethical use of these procedures occurs and that ethical concerns are taken into account.

FUTURE DIRECTIONS

In summary, this is an exciting time for the ever-growing field of gene therapy. Successful translation of preclinical discoveries to clinical trials has yielded promising results in several historically devastating diseases. Although some techniques are in their third decade of use and require only optimization, others such as genome editing are in their infancy. Like many novel therapies, the future of gene therapy requires a revisit of the past—attention to safety, efficacy, cost, and ethical concerns must be addressed in order for the promise of this therapy to become reality.

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