REVIEW SUMMARY

MEDICINE

Gene therapy comes of age

Cynthia E. Dunbar,* Katherine A. High, J. Keith Joung, Donald B. Kohn, Keiya Ozawa, Michel Sadelain*

BACKGROUND: Nearly five decades ago, visionary scientists hypothesized that genetic modification by exogenous DNA might be an effective treatment for inherited human diseases. This "gene therapy" strategy offered the theoretical advantage that a durable and possibly curative clinical benefit would be achieved by a single treatment. Although the journey from concept to clinical application has been long and tortuous, gene therapy is now bringing new treatment options to multiple fields of medicine. We review critical discoveries leading to the development of successful gene therapies, focusing on direct in vivo administration of viral vectors, adoptive transfer of genetically engineered T cells or hematopoietic stem cells, and emerging genome editing technologies.

ADVANCES: The development of gene delivery vectors such as replication-defective retro viruses and adeno-associated virus (AAV), coupled with encouraging results in preclinical disease models, led to the initiation of clinical trials in the early 1990s. Unfortunately, these early trials exposed serious therapy-related toxicities, including inflammatory responses to the vectors and malignancies caused by vector-mediated insertional activation of proto-oncogenes. These setbacks fueled more basic research in virology, immunology, cell biology, model development, and target disease, which ultimately led to successful clinical translation of gene therapies in the 2000s. Lentiviral vectors improved efficiency of gene transfer to nondividing cells. In early-phase clinical trials, these safer and more efficient vectors were used for transduction of autologous hematopoietic stem cells, leading to clinical benefit in patients with immunodeficiencies, hemoglobinopathies, and metabolic and storage disorders. T cells engineered to express CD19-specific chimeric antigen receptors were shown to have potent antitumor activity in patients with lymphoid malignancies. In vivo delivery of therapeutic AAV vectors to the retina, liver, and nervous system resulted in clinical improvement in patients with congenital blindness, hemophilia B, and spinal muscular atrophy, respectively. In the United States, Food and Drug Administration (FDA) approvals of the first gene therapy products occurred in 2017, including chimeric antigen receptor (CAR)–T cells to treat B cell malignancies and AAV vectors for in vivo treatment of congenital blindness. Promising clinical trial results in neuromuscular diseases and hemophilia will likely result in additional approvals in the near future.

In recent years, genome editing technologies have been developed that are based on engineered or bacterial nucleases. In contrast to viral vectors, which can mediate only gene addition, genome editing approaches offer a precise scalpel for gene addition, gene ablation, and gene "correction." Genome editing can be performed on cells ex vivo or the editing machinery can be delivered in vivo to effect in situ genome editing. Translation of these technologies to patient care is in its infancy in comparison to viral gene addition therapies, but multiple clinical genome editing trials are expected to open over the next decade.

OUTLOOK: Building on decades of scientific, clinical, and manufacturing advances, gene therapies have begun to improve the lives of patients with cancer and a variety of inherited genetic diseases. Partnerships with biotechnology and pharmaceutical companies with expertise in manufacturing and scale-up will be required for these therapies to have a broad impact on human disease. Many challenges remain, including understanding and preventing genotoxicity from integrating vectors or off-target genome editing, improving gene transfer or editing efficiency to levels necessary for treatment of many target diseases, preventing immune responses that limit in vivo administration of vectors or genome editing complexes, and overcoming manufacturing and regulatory hurdles. Importantly, a societal consensus must be reached on the ethics of germline genome editing in light of rapid scientific advances that have made this a real, rather than hypothetical, issue. Finally, payers and gene therapy clinicians and companies will need to work together to design and test new payment models to facilitate delivery of expensive but potentially curative therapies to patients in need. The ability of gene therapies to provide durable benefits to human health, exemplified by the scientific advances and clinical successes over the past several years, justifies continued optimism and increasing efforts toward making these therapies part of our standard treatment armamentarium for human disease. ■

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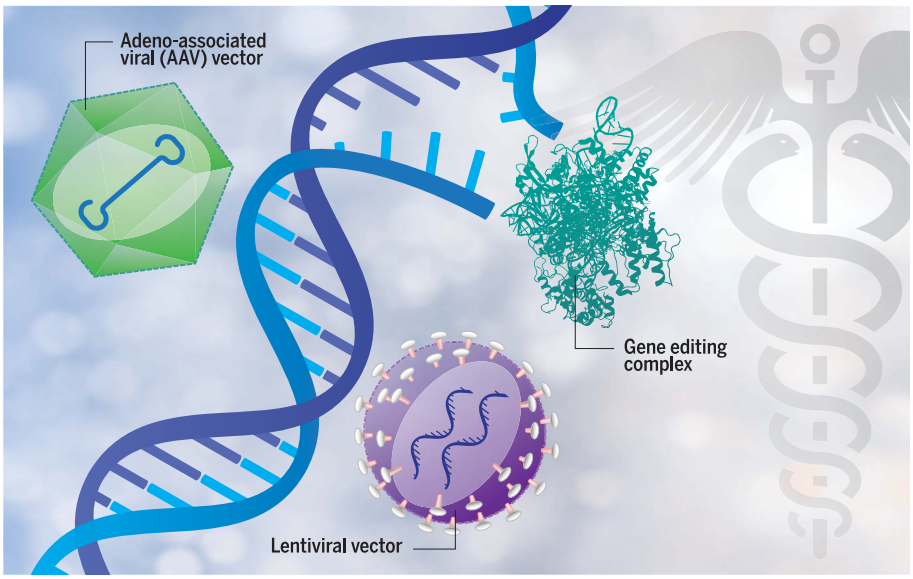

Three essential tools for human gene therapy. AAV and lentiviral vectors are the basis of several recently approved gene therapies. Gene editing technologies are in their translational and clinical infancy but are expected to play an increasing role in the field.

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REVIEW

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Gene therapy comes of age

Cynthia E. Dunbar,[1]* Katherine A. High,[2] J. Keith Joung,[3] Donald B. Kohn,[4] Keiya Ozawa,[5] Michel Sadelain[6]*

After almost 30 years of promise tempered by setbacks, gene therapies are rapidly becoming a critical component of the therapeutic armamentarium for a variety of inherited and acquired human diseases. Gene therapies for inherited immune disorders, hemophilia, eye and neurodegenerative disorders, and lymphoid cancers recently progressed to approved drug status in the United States and Europe, or are anticipated to receive approval in the near future. In this Review, we discuss milestones in the development of gene therapies, focusing on direct in vivo administration of viral vectors and adoptive transfer of genetically engineered T cells or hematopoietic stem cells. We also discuss emerging genome editing technologies that should further advance the scope and efficacy of gene therapy approaches.

Gene therapies are bringing new treatment options to multiple fields of medicine. Forty-five years ago, Theodore Friedmann provided a prophetic account of the potential and challenges of using gene therapy to treat inherited monogenic disorders (*1*). Growing interest in gene therapy was inspired by the recognition that—at least in principle—a single treatment might achieve durable, potentially curative clinical benefit. Investigators hypothesized that in contrast to protein-based drugs that may require repeated infusion, gene-based therapies delivered to long-lived cells might afford sustained production of endogenous proteins, such as clotting factors in hemophilia (*2*). Long-term cell replacement afforded by genetically engineered hematopoietic stem cells (HSCs) may durably alleviate a range of conditions, obviating, for example, the need for lifelong enzyme administration or transfusion therapy (*3*, *4*). Originally envisioned as a treatment solely for inherited disorders, gene therapy is now being applied to acquired conditions, a concept best illustrated by genetic engineering of T cells for cancer immunotherapy. Recent clinical studies have found that single infusions of T cells engineered with synthetic genes encoding a chimeric antigen receptor can produce durable responses in a subset of patients (*5*).

Translation of gene therapy concepts to patient care began in the early 1990s but was plagued by repeated cycles of optimism followed by disappointing clinical trial results. A number of these early experimental therapies were found to provide no clinical benefit or produce unexpected toxicities that in some cases led to widely publicized patient deaths (*6*). In 1996, a National Institutes of Health (NIH) advisory panel concluded that these disappointing clinical results were due to insufficient knowledge of the biology of the viral vectors, the target cells and tissues, and the diseases. The panel recommended that investigators return to the laboratory and focus on the basic science underlying gene therapy approaches (*7*). Development of new vectors and a better understanding of target cells sparked a second generation of clinical trials in the late 1990s and early 2000s. These trials produced evidence of sustained genetic modification of target tissues and, in some instances, evidence for clinical benefit. However, progress was slowed by the emergence of serious toxicities related to high gene transfer efficiency; for instance: insertional genotoxicity, immune destruction of genetically modified cells, and immune reactions related to administration of certain vectors (*6*, *8*, *9*).

Over the past 10 years, further maturation of the "science" of gene therapy, safety modifications, and improvements in gene transfer efficiency and delivery have finally resulted in substantial clinical progress. Several gene and gene-modified cell-based therapies are already approved drugs, and over a dozen others have earned "breakthrough therapy" designation by regulators in the United States and around the world. In this Review, we highlight key developments in the gene therapy field that form the foundation for these recent successes and examine recent advances in targeted genome editing likely to transform gene therapies in the future.

Genetic engineering from viral vectors to genome editing

Recombinant, replication-defective viral vectors were the first molecular tool enabling efficient, nontoxic gene transfer into human somatic cells (*10*). Retroviruses and adeno-associated virus (AAV) have shown the most clinical promise, and we will limit our discussions to these vectors.

Retroviral vectors

The identification of a genome packaging signal (*11*) and the creation of a producer cell line (*12*) paved the way for design and facile production of vectors capable of undergoing reverse transcription and DNA integration but lacking replication potential (*13*, *14*). The γ-retroviral vectors developed in the 1980s and early 1990s were the first to be shown to deliver genes into repopulating HSCs (*15*–*17*). C-type retroviruses were also adapted for efficient gene transfer into primary T lymphocytes (*18*–*21*). These vectors were used in first-generation clinical trials designed to deliver a normal copy of a specific defective gene into the genome of T cells or HSCs from patients with immunodeficiencies or cancer [reviewed in (*22*)] (Fig. 1).

Two other genera of the retroviruses were subsequently added to this armamentarium: the lentiviruses (*23*) and spumaviruses (*24*). In contrast to γ-retroviral vectors, lentiviral vectors enabled gene transfer into nondividing cells but still left quiescent G_0 cells out of reach (*25*). Lentiviral vectors can carry larger and more complex gene cassettes than γ-retroviral vectors and thus their development provided a critical advance for hemoglobinopathies (*26*). Lentiviral and spumavirus vectors have another advantage over γ-retroviral vectors in that they preferentially integrate into the coding regions of genes. The γ-retroviral vectors, by contrast, can integrate into the 5′-untranslated region of genes (*27*), a feature that increases the risk of potentially oncogenic insertional mutagenesis in hematopoietic cells (*28*). Lentiviral vectors are currently the tools of choice for most HSC applications, but γ-retroviral vectors are still used for certain applications in T cell engineering and HSC gene therapy (Table 1). Removal of endogenous strong enhancer elements from lentiviral and γ-retroviral vectors using a "self-inactivating" SIN design (*29*) is another approach that decreases the risk of genotoxicity (*30*); this design is used in most current clinical trials (Table 1). Integrating retroviral vectors are reviewed in more detail in (*31*, *32*).

Adeno-associated viral (AAV) vectors

AAV vectors are engineered from a nonpathogenic, nonenveloped parvovirus that is naturally replication-defective. Wild-type AAV requires another virus such as an adenovirus or a herpesvirus to replicate (*33*, *34*). All viral coding sequences in AAVs are replaced with a gene expression cassette of interest. One limitation of AAV vectors is that they cannot package more than ~5.0 kb of DNA (in contrast to γ-retroviral or lentiviral vectors, which can accommodate up to 8 kb). AAV vectors are predominantly nonintegrating; the transferred DNA is stabilized as an episome. This feature lessens risks related to integration but also limits long-term expression from AAV vectors to long-lived postmitotic cells.

In the mid-1990s, two groups demonstrated long-term expression of a transgene following

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in vivo muscle administration of AAV vectors to mice (35, 36). This seminal work led to the demonstration that AAV vectors could also efficiently transduce a variety of target tissues in animal models, including liver, retina, cardiac muscle, and central nervous system, with specific tissue tropisms discovered for several naturally occurring AAV serotypes and AAV engineered with optimized capsids (37). Improved manufacturing techniques [reviewed in (38)] increased both yield and purity of AAV vector product, allowing proof-of-concept studies in large-animal models of disease (Fig. 2). Pioneering AAV gene therapy clinical trials for hemophilia B were initiated in the late 1990s, first testing delivery of AAV vectors to muscle via injection (39) and then moving to intravenous administration, taking advantage of AAV2 liver tropism (40). These early trials established safety but were limited by insufficient dosing, and anti-AAV immune responses, most likely because many people carry neutralizing antibodies and memory T cells directed against the AAV capsid. The full exploitation of the therapeutic potential of AAV vectors, as described below, required rigorous analysis of anti-AAV immune responses (41), including both cellular and humoral responses to a range of serotypes (42).

Genome editing

In contrast to viral vectors, which can mediate only one type of gene modification ("gene addition"), new genome editing technologies can mediate gene addition, gene ablation, "gene correction," and other highly targeted genome modifications in cells. Genome editing can be performed on cells ex vivo or the editing machinery can be delivered in vivo to effect in situ genome editing. A targeted DNA alteration is initiated by creation of a nuclease-induced double-stranded break (DSB), which stimulates highly efficient recombination in mammalian cells (43). Nonhomologous end-joining (NHEJ)–mediated repair results in the efficient creation of variable-length insertion or deletion mutations (indels) at the site of the DSB, which generally inactivates gene function. Homology-directed repair (HDR) can be used to create specific sequence alterations in the presence of a homologous donor DNA template, which following recombination results in correction of a mutation or insertion of new sequences in a site-specific manner (44).

Early genome editing studies relied on engineering of specific zinc finger nucleases (ZFNs) (45) or meganucleases (46) for each individual DNA target site to induce the required DSBs. These nuclease platforms required specialized expertise to customize the DNA binding nuclease effector proteins for each cleavage target, which limited their broader use and application. The demonstration in 2009 that the DNA binding domain of bacterial proteins called transcription activator–like effectors (TALEs) can be readily altered (47, 48) opened the door to the creation of TALE nucleases (TALENs) (49, 50). These enzymes can efficiently cleave essentially any DNA sequence of interest (51). However, TALEN approaches still require design of a specific pair of nucleases for each new DNA target.

The genome editing landscape changed in 2012 with a seminal discovery by Doudna and Charpentier, who showed that a bacterial defense system composed of clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated 9 (Cas9) nucleases can be efficiently programmed to cleave DNA at sites of interest, simply by designing a specific short guide RNA (gRNA) complementary to the target site of interest (52). The CRISPR-Cas9 nuclease technology was rapidly extended to mammalian cells (53, 54), thereby simplifying the process of genome editing (55). TALENs and CRISPR-Cas9 nucleases, which can be easily reprogrammed to cleave specific target DNA sequences, are now widely used for a myriad of applications in basic research (56–58). A number of clever strategies that could eventually be applied clinically involve the use of RNA-guided catalytically inactive Cas9 ("dead Cas9" or dCas9) to turn genes on and off by blocking transcriptional machinery or recruiting epigenetic regulators (59, 60). Correction of mutations at a single-base level via Cas9-based targeting of "base editors" has recently been reported (61, 62).

Genome editing approaches offer a precise scalpel for correcting or altering the genome and can overcome many of the drawbacks of strategies that rely on viral vector–mediated semi-random genomic insertion. For instance, genotoxicity due to ectopic activation of nearby proto-oncogenes, knockout of tumor suppressor genes, or perturbation of normal splicing should not occur with on-target editing. In addition, the regulation of an introduced or corrected gene will be controlled by the endogenous promoter, resulting in more physiologic and appropriately regulated gene expression (63). Targeted introduction of clotting factor genes downstream of the highly active albumin promoter in hepatocytes has shown promise in animal models (64). The potential of genome editing strategies to bypass pathology in muscular dystrophy by altering splicing of the mutated dystrophin gene or by directly correcting the dystrophin mutation has been demonstrated in preclinical models (65–67). Finally, disease due to dominant negative mutations, which cannot be treated by gene addition therapy, should be amenable to gene correction strategies.

There are challenges in delivering all the components required for editing into target cells. Genome mutation by NHEJ is simplest, requiring just targeted nucleases for meganuclease, ZFN, or TALEN techniques, or a nuclease plus gRNA

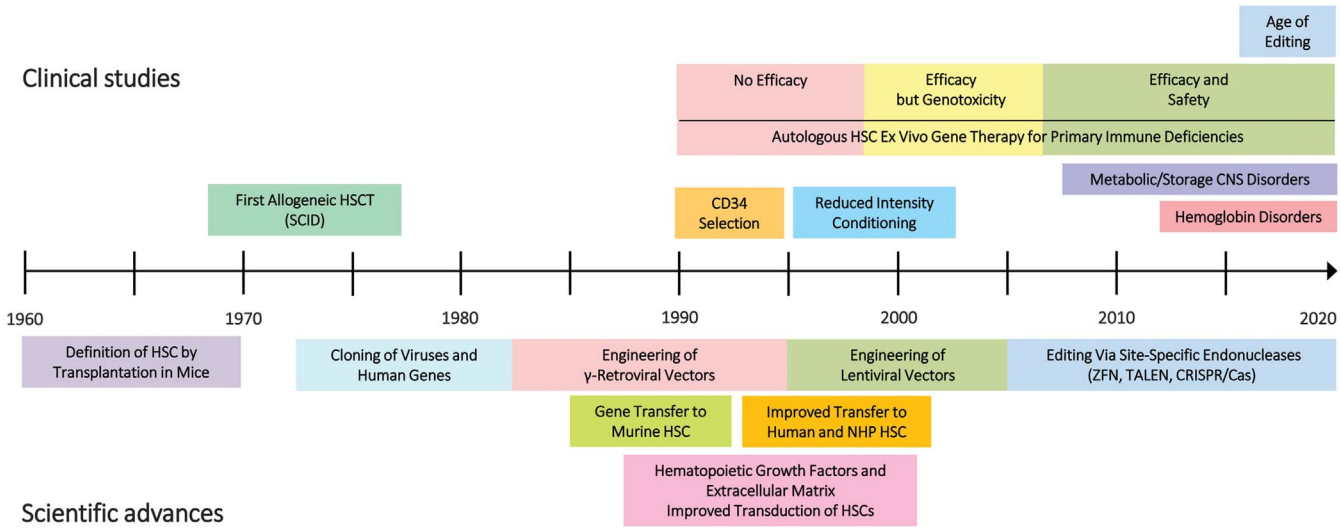

Fig. 1. Historical overview of HSC gene therapy. HSCT: hematopoietic stem cell transplantation; HSC: Hematopoietic stem cell; SCID: severe combined immunodeficiency; NHP: nonhuman primate; ZFN: zinc finger nuclease; TALEN: transcription activator–like effector nuclease; CRISPR/Cas9: clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated 9 (Cas9) nucleases.

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Table 1. Clinical and product development landmarks for ex vivo gene therapies.

Cell type	Disease	Vector/transgene	Key publication(s) or clinicaltrials.gov no.	Primary institution and/or company	Breakthrough designation or product approval
T cells	Adult ALL*	γRV CD19 (CD28) CAR-T	(134, 143, 144)	Memorial Sloan Kettering Cancer Center	FDA 2014
	Pediatric ALL	LV CD19 (4-1BB) CAR-T	(145)	University of Pennsylvania/Novartis	FDA Oncology Advisory Committee recommended approval 2017; EMA 2016
		γRV CD19 (CD28) CAR-T	(146)	National Cancer Institute/Kite	
		LV CD19 CAR-T, TALEN knockout of TCR and CD52	(74) NCT02808442	Cellectis/Servier/Pfizer	
	Diffuse large B cell lymphoma	γRV CD19 (CD28) CAR-T	(147) NCT00924326	National Cancer Institute/Kite	FDA 2014
		γRV CD19 (CD28) CAR	NCT02348216	Multiple academic sites/Kite	FDA 2015; EMA 2016
		LV CD19 (4-1BB) CAR-T	(148) NCT02631044	Multiple academic sites/Juno	FDA 2016; EMA 2016
		LV CD19 (4-1BB) CAR-T	NCT02445248	Multiple academic sites/Novartis	FDA 2017
	CLL/indolent lymphoma	LV CD19 (4-1BB) CAR-T	(149)	University of Pennsylvania/Novartis	
		γRV CD19 (CD28) CAR-T	(150)	National Cancer Institute	
	Multiple myeloma	γRV BCMA (CD28) CAR-T	(136) NCT02215967	National Cancer Institute/Kite	
		γRV BCMA (4-1BB) CAR T	NCT03070327	Memorial Sloan Kettering Cancer Center/Juno	
		LV-BCMA CAR-T	NCT03090659	Nanjing Legend Biotech	
	Synovial sarcoma	γRV -NY-ESO-TCR	(151)	National Cancer Institute	
		LV-NY-ESO-TCR	NCT03090659	Multiple academic sites/Adaptimmune	FDA 2016; EMA 2016
	Human immunodeficiency virus	ZFN CCR5 electroporation	(73)	University of Pennsylvania/Sangamo	
HSPCs	β-Thalassemia	LV anti-sickling β-hemoglobin	(120) NCT01745120 NCT02151526 NCT03207009	Hopitaux de Paris/academic centers worldwide/Bluebird Bio	FDA 2015; EMA 2016
		LV β-hemoglobin	NCT02453477	San Raffaele Telethon Institute of Gene Therapy/GlaxoSmithKline	
		LV β-hemoglobin	NCT01639690	Memorial Sloan Kettering Cancer Center	
	Sickle cell anemia	LV anti-sickling β-hemoglobin	(121) NCT02151526, NCT02140554	Hopitaux de Paris/US academic sites/ Bluebird Bio	
		LV anti-sickling β-hemoglobin	NCT02247843	UCLA/California Institute of Regenerative Medicine	
	Wiskott-Aldrich syndrome	LV WAS	(114)	San Raffaele Telethon Institute of Gene Therapy/GlaxoSmithKline	
		LV WAS	(152)	Hopital Necker-Enfants/ University College/Genethon	
	Adenosine deaminase deficiency	γRV ADA	(116)	San Raffaele Telethon Institute of Gene Therapy/GlaxoSmithKline	EMA 2016 approved "Strimvelis"
		LV ADA	NCT02999984	University College/UCLA/ Orchard Therapeutics	FDA 2015
	IL2Rγ-deficient X-SCID	γRV SIN IL2Rγ	(115)	Hopital Necker-Enfants/Great Ormond Street	
		LV IL2Rγ	(153)	National Institute of Allergy and Infectious Diseases	
	Adrenoleukodystrophy	LV ABCD1	(118)	St. Vincent de Paul, Paris	
		LV ABCD1	(119)	Multiple academic sites/Bluebird Bio	
	Metachromatic leukodystrophy	LV ARSA	(117, 154)	San Raffaele Telethon Institute of Gene Therapy/GlaxoSmithKline	EU Orphan Drug 2007
	Human Immunodeficiency virus	ZFN CCR5 electroporation	NCT02500849	City of Hope/Sangamo	

*Abbreviations: FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; γRV, murine γ-retrovirus; LV, lentivirus; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; HSPC, hematopoietic stem and progenitor cells; X-SCID, X-linked severe combined immunodeficiency; ZFN, zinc finger nuclease; BCMA, B cell maturation antigen; ARSA, arylsulfatase A; ABCD1, transporter gene mutated in adrenoleukodystrophy.

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for CRISPR-based approaches; these components can be delivered by nonintegrating viral vectors or transfected as mRNA or RNA-protein complexes into target cells such as HSCs ex vivo. However, gene correction by HDR requires donor DNA, which is more difficult to deliver, and HDR appears to be particularly inefficient in certain quiescent cell types such as long-term repopulating HSCs (68, 69), although progress is being made (70).

Genome editing as a therapeutic modality is rapidly advancing into the clinic (Table 1). Engineered ZFNs have been used to disrupt CCR5 (C-C motif chemokine receptor type 5) expression in human T cells (71) and HSCs (72) to render these cells resistant to HIV infection. A phase I/II (73) study of T cell CCR5 editing has been completed, and a phase I trial of HSC editing is ongoing (NCT02500849). TALENs have been used to make "off-the-shelf" third-party anti-CD19 chimeric antigen receptor (CAR) T cells less likely to cause graft-versus-host disease (GVHD). This was done by T cell receptor gene deletion. These modified cells were administered to two patients with refractory B cell acute leukemia on a compassionate basis, with evidence for tumor response (74), and are in phase I clinical trials (NCT02808442). In addition, early trials have begun for allogeneic TALEN-edited CAR T cells targeting CD123 in acute myeloid leukemias and blastic plasmacytoid dendritic cell neoplasms (NCT03190278). The U.S. Food and Drug Administration (FDA) has approved the launch of three clinical trials for ZFN-mediated in vivo insertion of therapeutic genes into the albumin locus of hepatocytes, delivering the factor IX gene for hemophilia B (NCT02695160), the α-L-iduronidase gene for mucopolysaccharidosis I (NCT02702115), and the iduronidate-2-sulfatase gene for mucopolysaccharidosis II (MPS II) (NCT03041324). The first patient to be treated by in vivo genome editing was recently enrolled in the MPS II trial, with delivery of editing components to the liver via AAV intravenous infusion. At least nine trials using CRISPR-Cas nucleases have been approved by regulatory agencies in China, primarily to knockout PD1 expression in tumor-targeted T cells, and several have reportedly enrolled patients.

It is important to stress that in comparison to standard gene transfer approaches, genome editing—particularly that based on CRISPR-Cas nucleases—is in its translational and clinical infancy. A number of potential feasibility and safety hurdles exist that may affect clinical applications; these will require further preclinical studies in appropriate models and carefully designed clinical trials. For example, the extent of "off-target" mutations, due to nuclease-mediated NHEJ or even HDR at alternative sites, is under intense investigation. Related questions under study are how best to design nucleases or CRISPR gRNAs to avoid off-target cutting and how to predict, screen for, and detect on- versus off-target genome alterations before or during clinical applications (75). Notably, high-fidelity CRISPR-Cas9 nuclease variants with no or very few detectable off-target effects have recently been developed (76–78). Questions remain regarding immunogenicity of nucleases for in vivo genome editing (79) and ensuring targeted delivery of editing machinery to the desired target tissue.

The rapid technological advances in genome editing have made heritable germline editing [defined as manipulation of germ cells, gametes, zygotes, or embryos with the intent to generate a new human being with the ability to pass on the edited gene(s) to future generations] a realistic possibility. In 2015, scientists in China published results from experiments using CRISPR-Cas9 to attempt to modify the hemoglobin gene in "nonviable" preimplantation human embryos, demonstrating low efficiency and reportedly frequent off-target mutations (80). This publication prompted statements of concern from professional societies around the world (81) and a series of meetings sponsored by the U.S. National Academies of Sciences, Engineering, and Medicine that brought together an international group of scientists, clinicians, ethicists, patient advocates, and government officials. This group published a report in 2017 laying out principles of governance and oversight for human genome editing, and presenting a possible pathway for eventual use of genome editing technologies to correct germline mutations for certain serious diseases (82). In the United States, federal government funds presently cannot be used for research on germline editing and clinical trials cannot be considered for approval by the FDA. Similar restrictions exist in many other countries. Clearly most countries are far from a societal consensus on germline editing. The acquisition of more efficacy and safety data from studies of genome editing in somatic cells is critical before implementation of human germline editing can be considered.

Gene delivery in vivo

Targeting organs in vivo is very attractive because it avoids the practical and regulatory hurdles of ex vivo cell-based gene therapies, which require cell collection, culture, and manipulation and transplantion. However, in vivo approaches depend on tissue-specific targeting or local delivery and/or target cell–specific gene expression. Inadvertent germline modification is of concern, and immune responses to vector components can occur. Some of these challenges have been overcome, with encouraging clinical outcomes in trials delivering genes to the liver or the retinal pigment epithelium of the eye, paving the way for further advances targeting other tissues, including the brain and muscle.

The liver

Studies beginning in 1997 showed that AAV vectors introduced into skeletal muscle or liver ameliorated disease in animal models of hemophilia B (83, 84) (Fig. 2). In the initial clinical trial of hemophilia B (40), factor IX was found to be

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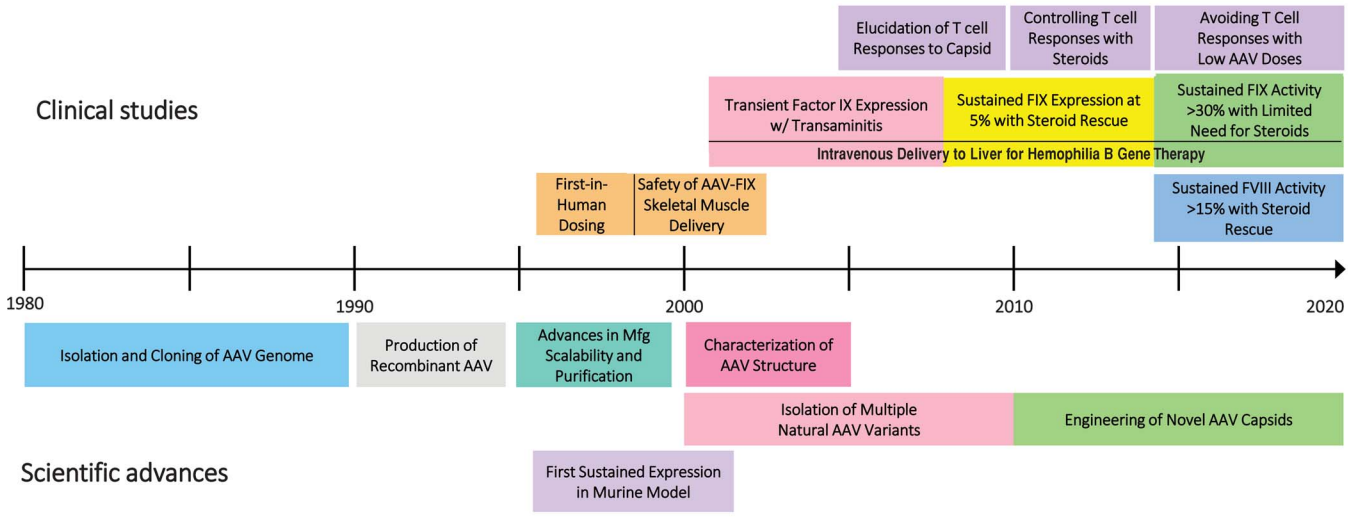

Fig. 2. Historical overview of AAV gene therapy for hemophilia. AAV: adeno-associated viral vector; FVIII: factor VIII; FIX: factor IX; Mfg: Manufacturing.

produced at therapeutic levels, but the expression persisted for only several weeks, in contrast to the stable expression observed in preclinical studies of hemophilic dogs (85). Subsequent studies revealed that expression of the factor IX transgene in humans was of short duration because of an immune response to the AAV capsid (42). In a later clinical trial that incorporated short-term immunosuppression (86, 87) (Table 2), transgene expression persisted for years, resulting in circulating factor IX levels that were 2 to 7% of normal; this was sufficient to reduce bleeding and lessen the need for recombinant factor IX infusions. A subsequent trial involved the transfer of a transgene encoding factor IX Padua, a naturally variant of factor IX with high specific activity. The transgene was carried by a vector containing an optimized AAV capsid and a liver-specific expression cassette. Patients showed a mean sustained factor IX activity level of more than 30%, resulting in complete cessation of factor IX infusions in 8 of 10 treated patients (88). These levels of factor IX activity are well above the threshold of 12% associated with a greatly reduced risk of bleeding in natural history studies. Use of this high–specific activity factor IX allowed delivery of 4 to 120 times lower doses of AAV particles to achieve therapeutic levels of factor IX, likely accounting for the low rate and severity of antivector immune responses in this trial. Results from the first positive early-phase clinical trial for hemophilia A were recently reported, demonstrating increases in factor VIII activity into or even above the normal range in six of seven patients, accompanied by decreased bleeding (89).

Additional trials of AAV-mediated gene therapy for both hemophilia B and the more common hemophilia A are ongoing (Table 2). Problems that still need to be addressed include the delayed $CD8^+$ T cell response to the capsid, which has been well controlled with a short course of steroids with some AAV vectors but not with others, and the prevalence—particularly in the adult population—of preexisting neutralizing antibodies to AAV (90, 91). At present, most clinical trials circumvent the antibody problem by excluding subjects who carry them, but other strategies will be required going forward.

The eye

Phase 1/2 clinical trials conducted by multiple groups have demonstrated improvement in visual function following subretinal injection of AAV2 vectors expressing retinal pigment epithelium–specific 65 kDa protein (RPE65) in patients with inherited blindness caused by mutations in the *RPE65* gene (92–94) (Table 2). A cohort of phase III–eligible subjects from one of the phase I/II trials continues to demonstrate clinical benefits lasting a minimum of 3 years after injection, with observation ongoing (95); however, patients in the other two original trials have experienced regression of visual function over similar follow-up periods (96, 97). At present, there is no clear explanation for the differences in outcome because all used AAV2-based vectors. Subtle differences in manufacturing process, final formulation, design of expression cassette, or adjuvant immunomodulatory regimens could potentially affect long-term efficacy (98). In the only randomized controlled phase 3 gene therapy trial completed to date, visually impaired patients who carried RPE65 mutations were randomized to undergo sequential bilateral injection of AAV2-RPE65 or to undergo the same series of evaluations without the intervention (99). One year after randomization, patient mobility, a measure of functional vision, as well as certain tests of visual function were significantly improved in the treatment group. Based on this pivotal study, an FDA advisory panel recently unanimously recommended drug approval. Direct injection of AAV is now being pursued in clinical trials for other inherited forms of blindness, including achromatopsia, choroideremia, Leber's hereditary optic neuropathy, X-linked retinoschisis, and X-linked retinitis pigmentosa.

Neuromuscular targets

The common, clinically devastating degenerative neurologic disorders are a focus of gene therapy efforts; however, these multigenic, pathophysiologically complex and incompletely understood disorders are much more challenging targets than Mendelian inherited disorders. Parkinson's disease (PD), which is characterized by loss of dopaminergic neurons in the substantia nigra and a decrease in dopamine in the striatum, has been an intensely pursued target. Gene therapy–mediated transfer of dopamine-synthesizing enzymes into striatal neurons has been found to normalize movement in a nonhuman primate PD model (100). Early-phase clinical trials have established the safety of AAV vector–mediated gene delivery of aromatic L-amino acid decarboxylase (AADC), an enzyme that converts L-dopa to dopamine; glutamic acid decarboxylase (GAD), an enzyme that modulates production of the neurotransmitter GABA (γ-aminobutyric acid); and neurturin, a neurotrophic factor (101–104). Promising results were obtained with AADC gene therapy, with additional early-phase clinical trials ongoing (Table 2). An early-phase trial of AAV2 vector injection into the brain has also been conducted in patients with AADC deficiency, a monogenic movement disorder characterized by compromised dopamine and serotonin synthesis, and some improvement was noted (105).

The treatment of childhood-onset spinal muscular atrophy (SMA), a rapidly fatal neuromuscular disorder due to loss-of-function mutations in the survival motor neuron 1 (*SMN*) gene, has been revolutionized by antisense oligonucleotide drug nusinersen, a triumph of nucleoside-based gene therapies, as reviewed in (106). Intrathecal delivery of nusinersen modulates alternative splicing of the intact *SMN2* gene in spinal motor neurons, resulting in higher expression of a functional form of the gene product able to compensate for SMN1 loss. An alternative type of gene therapy for SMA is also showing great promise. A serotype of AAV that efficiently crosses the blood-brain barrier was engineered to carry the *SMN1* gene, and given as a single intravenous infusion to 15 infants and young children. Compared with historical control subjects, survival of the trial participants was extended, with all alive to date, and motor function improved to the extent that some children could sit up and even walk (107)

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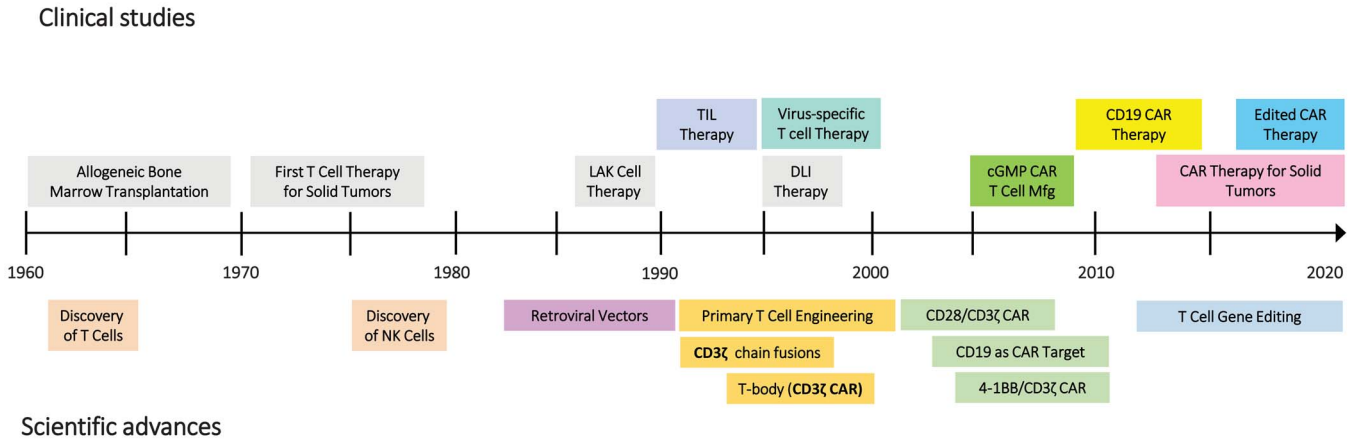

Fig. 3. Historical overview of CAR-T cell therapy. CAR: chimeric antigen receptor; cGMP: current good manufacturing practices; DLI: donor leukocyte infusion; LAK: lymphokine-activated killer; Mfg: Manufacturing; NK: natural killer; TIL: tumor-infiltrating lymphocytes.

(Table 2). This approach may be preferable to repeated intrathecal injections of an oligonucleotide drug.

Ex vivo gene delivery via cell engineering: Monogenic blood disorders and cancer immunotherapies

Hematopoietic stem cells

The clinical applications of gene therapies targeting HSCs derive from the success of allogeneic bone marrow transplantation for many genetic immunodeficiencies and blood cell diseases. These ex vivo approaches entail the transplantation of autologous stem cells in which an underlying genetic defect is alleviated or corrected [e.g., adenosine deaminase deficit in severe combined immunodeficiency (SCID)], β-hemoglobin deficit or structural alteration in hemoglobinopathies) (Table 1). Autologous transplants have an advantage over allogeneic transplants in that they do not require a histocompatible donor, they avoid the immune complications of GVHD, and they eliminate the need for administration of immune suppressants.

Clinical trials based at academic medical centers have piloted these approaches in successive technological eras (Fig. 1). Trials using γ-retroviral vectors in the late 1990s and early 2000s demonstrated unequivocal improvement in immune function in patients with SCID caused by loss-of-function mutations in the genes encoding interleukin-2 receptor γ or adenosine deaminase (Table 1). Despite relatively low HSC transduction efficiencies with γ-retroviral vectors, gene-modified T-lineage cells were able to expand and fill the empty T cell compartment, improving immune function despite minimal levels of gene-corrected cells in other lineages (*108*, *109*). However, several years after treatment, patients in the X-SCID trials, as well as those for chronic granulomatous disease and Wiskott-Aldrich syndrome, developed acute myeloid and lymphoid leukemias due to activation of proto-oncogenes adjacent to proviral insertions, linked to strong enhancers present in γ-retroviral vectors and the propensity of these vectors to insert near promoters (*110*–*113*). These serious toxicities led to accelerated adoption of enhancer-deleted lentiviral or γ-retroviral vectors for HSC clinical gene therapies of immunodeficiency disorders. Encouraging clinical results with these newer vectors have been reported in Wiskott-Aldrich syndrome (*114*) and in X-SCID (*115*), demonstrating disease amelioration without leukemia or uncontrolled expansion of individual transduced clones.

The high HSC transduction efficiency achieved with lentiviral vectors allowed a broader application of this gene therapy approach to diseases where corrected cells do not have a survival advantage. In addition, optimization of methods for vector production, ex vivo HSC manipulation, and pretransplant cytoreductive conditioning (*116*) all contributed to clinical benefit in several more recent trials. The metabolic disorder adrenoleukodystrophy and the lysosomal storage disorder metachromatic leukodystrophy result in profound neurologic degeneration and death in childhood. Lentiviral gene therapy clinical trials in both disorders have been encouraging, with high-level production of the missing enzymes from hematopoietic cells, including in the central nervous system, and a slowing of neurodegeneration (*117*–*119*). The clinical trial in adrenoleukodystrophy was the first reported using lentiviral vectors for HSC gene therapy.

The hemoglobin disorders β-thalassemia and sickle cell disease, which affect millions of patients worldwide, have historically been an intense focus of gene therapy research, but require high efficiency and substantial hemoglobin expression to correct the underlying pathophysiology (*3*). Lentiviral vectors harboring multiple regulatory elements to direct high-level, erythroid-specific hemoglobin expression have been developed (*26*) and in case reports have shown promise in patients with β-thalassemia or sickle cell disease (*120*, *121*), with larger multicenter clinical trials ongoing (Table 1). Also likely to move forward in the near future are clinical trials of genome editing approaches to treat sickle cell

Table 2. Clinical and product development landmarks for in vivo gene therapies.

Cell type	Disease	Vector/transgene	Key publication(s) or clinicaltrials.gov no.	Institutional and/or industry partners	FDA breakthrough/EMA* PRIME designation or product approval
CNS	Parkinson's disease	AAV2-AADC	(*101*, *102*)	Jichi Medical University/ UCSF/Voyager	
	Aromatic l-amino acid decarboxylase deficiency	AAV2-AADC	(*105*)	Jichi Medical University/ National Taiwan University	
	Spinal muscular atrophy	AAV9-SMN	(*107*)	Nationwide Children's Hospital/AveXis	FDA 2016; EMA 2017
Liver	Hemophilia B	AAV8-Factor IX	(*86*, *87*)	Royal Free Hospital/St. Jude	FDA 2014; EMA 2017
		AAV100-FIX Padua	(*88*)	Spark Therapeutics	FDA 2016; EMA 2017
		AAV5-Factor IX	NCT02396342	uniQure	FDA 2017; EMA 2017
		AAV2/6-Factor IX and ZFNs	NCT02695160	Sangamo Therapeutics	FDA 2017
	Hemophilia A	AAV5-Factor VIII	NCT02576795	Multiple academic sites/ Biomarin	EMA 2017
		AAV200-Factor VIII	NCT03003533	Spark Therapeutics	
		AAV2/6-B domain-deleted Factor VIII and ZFNs	NCT03061201	Sangamo Therapeutics	
	Mucopolysaccharidosis type II (Hunter's syndrome)	AAV2/6-IDA and ZFNs	NCT03041324	Sangamo Therapeutics	
Muscle	Lipoprotein lipase deficiency	AAV1-LPL	(*155*)	uniQure	EMA 2012 approval of "Glybera", company will not renew license as of 2017
Retina	Inherited retinal dystrophy due to utosomal recessive mutations in *RPE65*	AAV2-RPE65	(*93*, *95*, *99*)	Children's Hospital of Philadelphia/Spark	FDA approval 2017
		AAV2-RPE65	(*92*, *97*)	University College London/ MeiraGTx	
		AAV2-RPE65	(*94*, *96*)	University of Florida	

*Abbreviations: CNS, central nervous system; FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; AAV, adeno-associated virus; AADC, amino acid decarboxylase; ZFNs, zinc finger nucleases; IDA, iduronate-2-sulfatase.

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anemia via reactivation of endogenous fetal hemoglobin (HbF) expression. NHEJ-mediated disruption of the erythroid-specific enhancer element responsible for expression of the *BCL11A* gene results in high-level HbF in animal models and in human sickle cell erythroid cells in vitro (*122*). Similarly, disruption of a genomic locus to mimic a genetic variant associated with hereditary persistence of the fetal hemoglobin locus also shows promise as a target for HSC genome editing to treat sickle cell anemia (*123*).

CAR therapy

Engineered T cells are emerging as powerful medicines for cancer (Fig. 3) (*5*). Chimeric antigen receptors (CARs) are synthetic engineered receptors for antigen, which, in a single molecule, reprogram the specificity, function, and metabolism of T lymphocytes (*124*, *125*). They consist of an antigen-binding domain, either from an immunoglobin molecule or a T cell receptor, fused to an intracellular signaling domain that mediates activation and costimulation to enhance T cell function and persistence. Unlike the physiological receptor for antigen, CARs can be engineered to recognize proteins and carbohydrate glycolipids, as well as HLA-peptide complexes (*126*, *127*). CARs are transduced into T cells ex vivo, creating expandable antigen-specific T cells that bypass the barriers and incremental kinetics of active immunization used to prime endogenous T cells. The generation of CAR-T cells requires stable gene transfer to enable sustained CAR expression in dividing and persisting T cells.

γ-retroviral vectors were originally used to demonstrate that CARs targeting CD19, a cell surface antigen found on most B lineage lymphomas and leukemias, can eradicate systemic cancer in immunodeficient mice (*128*). CD19 is at present the most common CAR target and serves as a paradigm for CAR therapy (Fig. 3). Durable responses have been obtained in patients with refractory diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia, and adult and pediatric acute lymphoblastic leukemia (ALL) (see references in Table 1). Collectively, the preclinical and clinical studies on CD19 CARs, using different vector systems (lentiviral vectors, transposons, mRNA, CRISPR-Cas9) (*129*), CAR designs (*130*, *131*), and T cell subsets (*132*, *133*), have validated the CAR concept (*5*, *126*). Notably, CAR-T cell administration has been associated with serious systemic toxicities that often require intensive care and in some instances have caused patient deaths. Investigators are intensely focused on better understanding, mitigating, and treating these complications, which include off-tumor effects and the cytokine release syndrome (CRS), as well as poorly understood neurotoxicities (*134*, *135*).

The clinical benefit conferred by CD19 CARs in refractory ALL and DLBCL resulted in 2017 FDA approvals for two genetically engineered cell products, the first to be approved in the United States. Several other CARs have obtained FDA breakthrough designation for treatment of B cell malignancies (Table 1). Promising, early clinical data bode well for CAR therapy of multiple myeloma (*136*).

Current research aims to expand CAR therapy to myeloid malignancies and solid tumors (*137*, *138*). These diseases present challenges because reliable tumor-specific cell surface antigens have not yet been validated. In addition, there is a need for methodologies that facilitate CAR-T cell entry into large tumors or immune-privileged sites and that overcome tumor microenvironment signals that disarm T cells. Universal third-party CAR-T cells that can be used "off the shelf" would allow more rapid and cheaper treatment compared to autologous patient-specific T cells. T cells lacking endogenous T cell receptors and/or major histocompatibility complex molecules to decrease the risk of GVHD and rejection are in preclinical or early clinical development as first steps toward this goal (*62*, *73*). CAR-T cells have had a large impact on the treatment of certain cancers (*139*), and this success provides a foundation for future T cell–based therapies for other cancers and other diseases such as autoimmune disorders and AIDS (*5*, *140*).

Conclusions

Gene therapies may well be the most complex "drugs" ever developed. Building on the demonstration of therapeutic efficacy in proof-of-concept clinical studies conducted in the academic setting, gene therapies are now undergoing accelerated clinical and commercial development. They are in transition from an academic-based "cottage industry" to an industrial drug development pathway, relying on partnerships with biotechnology and pharmaceutical companies whose expertise in manufacturing and scale-up will be required for these therapies to have a broader impact on human disease. Investigators in academia and industry are working with regulators and entities such as the National Institute of Standards and Technology (NIST) to develop and standardize assays used to characterize potency and safety of vector preparations and criteria for product release. Similar efforts for genome editing are underway. These intiatives should speed future clinical development, commercialization, and utilization of these multifaceted treatment modalities.

Various models for delivery of ex vivo gene therapies to patients are being explored—for example, centralized versus hospital-based cGMP (current good manufacturing practices) facilities. In addition, it will be critical to engage with health reimbursement entities, including governments and insurers, to develop new models for reimbursement applied to one-time gene therapies with high up-front costs but likely long-term benefits in patients with serious diseases, many of whom have no other options, or poor quality of life and/or lifelong high medical costs on currently available therapies (*141*). Reimbursement must be addressed for the field to advance, as illustrated by recent events in Europe, where two gene therapy products were approved by regulators but have either been withdrawn from the market, in the case of uniQure's Alipogene tiparvovec (an AAV1 vector for treating patients with a rare inherited lipoprotein lipase deficiency), or are at risk of discontinuation of the program by the parent pharmaceutical company, as in the case of Strimvelis, a γ-retroviral vector HSC gene therapy treatment for adenosine deaminase–deficient SCID (*142*). The U.S. Centers for Medicare and Medicaid Services announced a collaboration with the manufacturer of the first approved CAR therapy to provide the product under an "outcomes-based" approach, with payment collected only if patients initially respond to the treatment.

The past year has been marked by a flurry of scientific advances in genome editing, the publication of mature data from multiple clinical trials demonstrating the efficacy and safety of gene therapies for a wide variety of serious human diseases, and regulatory approvals of the first gene therapies in the United States. Scientists and clinicians engaged in basic, translational, and clinical research, supported by government and philanthropies, will continue to innovate and provide new or improved technologies. The increasing involvement of the biotechnology and pharmaceutical sectors in gene therapy efforts demonstrates the maturation of the field and is necessary to accelerate delivery of these treatments to patients. Many challenges remain, including addressing genotoxicity from integrating gene delivery vectors or off-target genome editing, improving gene transfer or editing efficiency to levels necessary for effective treatment of many diseases, addressing immune responses to repeated in vivo administration of vectors, and reaching a societal consensus regarding contentious issues such as the ethics of germline editing and payment for expensive curative therapies. The potential for gene therapy to provide durable benefits to human health, exemplified by the scientific advances and clinical successes over the past several years, justifies continued optimism and increasing efforts toward making this therapy part of our standard armamentarium for treatment of serious human diseases.

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Gene therapy comes of age

Cynthia E. Dunbar, Katherine A. High, J. Keith Joung, Donald B. Kohn, Keiya Ozawa and Michel Sadelain

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Gene therapy: The power of persistence

Nearly 50 years after the concept was first proposed, gene therapy is now considered a promising treatment option for several human diseases. The path to success has been long and tortuous. Serious adverse effects were encountered in early clinical studies, but this fueled basic research that led to safer and more efficient gene transfer vectors. Gene therapy in various forms has produced clinical benefits in patients with blindness, neuromuscular disease, hemophilia, immunodeficiencies, and cancer. Dunbar *et al.* review the pioneering work that led the gene therapy field to its current state, describe gene-editing technologies that are expected to play a major role in the field's future, and discuss practical challenges in getting these therapies to patients who need them.

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