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PART 16 Genes, the Environment, and Disease FIGURE 482-5 Pathologic manifestations of telomere diseases. A. In the bone marrow, telomere erosion predisposes to aplastic anemia, characterized by an empty hematopoietic marrow replaced by fat (hematoxylin and eosin). B. In the liver, telomere attrition predisposes to cirrhosis (hematoxylin and eosin). C. Telomere shortening may also result in nodular regenerative hyperplasia of the liver (reticulin stain). D. In the lungs, telomere dysfunction predisposes to pulmonary fibrosis mainly in the subpleural regions, which may be detected by high-resolution computed tomography scan. Genetic counseling is necessary after screening, as the inheritance pattern may be autosomal dominant, mutation penetrance is highly variable, and phenotypes may be diverse even within a pedigree. Poten tial family stem cell donors must be screened before transplantation to ensure that they do not have mutations. TREATMENT Telomere Disease Patients with severe aplastic anemia due to telomere disease may undergo allogeneic hematopoietic stem cell transplant when a suitable donor is available. Treatment-related mortality may be increased due to pulmonary and hepatic complications, for which reduced intensity conditioning regimens appear advantageous. Lung transplant for pulmonary fibrosis is feasible but often not performed due to coexisting cytopenias and other comorbidities. Patients with pulmonary fibrosis associated with telomere disease have a poorer outcome after lung transplant and with nontransplant therapies. Similarly, there is no specific treatment for the liver in telomere disease; liver transplant has been performed in several cases with good outcome and without excessive posttransplant mortality and improvement in the respiratory status. Telomeropa thy patients should be advised to avoid toxins (metal dust, busulfan, amiodarone), ionizing radiation, cigarette smoke, and alcohol, as these can be possibly harmful. Long-term therapy with androgens may mitigate telomere attri tion and even elongate leukocyte telomere length in humans. In research trials, danazol and nandrolone improved blood counts in marrow failure patients and reduced transfusion requirements. ■ ■FURTHER READING Blackburn EH et al: Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection.

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Gene and Cell-Based

Therapy in Clinical

Medicine Gene therapy is a novel area of therapeutics in which the active agent is a nucleic acid sequence rather than a protein or small molecule. One of the most powerful concepts in modern molecular medicine, gene therapy has the potential to address a host of diseases for which there are currently no available treatments. Because delivery of naked DNA or RNA to a cell is an inefficient process, most gene therapy is carried out using a vector, or gene delivery vehicle, typically engineered from viruses by deleting some or all of the viral genome and replacing it

TABLE 483-1 Characteristics of Commonly Used Gene Delivery Vehicles VIRAL BASE NONVIRAL FEATURES RETROVIRAL/LENTIVIRAL ADENOVIRAL AAV LIPID NANOPARTICLES Genome RNA DNA DNA RNA Cell division requirement G1 phase No No No Packaging limitation 8 kb 8–30 kb 5 kb 10 kb or more Immune responses to vector Extensive Few Few Genome integration Yes Poor Poor May be used to package either RNA or DNA Long-term expression Yes No Yes Transient for RNA Main advantages Persistent gene transfer in transduced tissues Highly effective in transducing various tissues Main disadvantages Might induce oncogenesis in some cases; only used ex vivo Viral capsid elicits strong immune responses with the therapeutic gene of interest under the control of a suitable promoter. Nonviral delivery vehicles such as lipid nanoparticles are increasingly being used (Table 483-1). Gene therapy strategies can thus be described in terms of three essential elements: (1) a gene delivery vehicle; (2) a gene to be delivered, sometimes called the transgene; and (3) a physiologically relevant target cell to which the DNA or RNA is delivered. The series of steps in which the vector and donated DNA or RNA enter the target cell and express the transgene is referred to as transduction. Gene delivery can take place in vivo, in which the vector is directly injected into the patient, or, in the case of hematopoietic, liver, immune, and some other target cells, ex vivo, with removal of the target cells from the patient, followed by return of the gene-modified autologous cells to the patient after manipulation in the laboratory. The latter approach effectively combines gene transfer techniques with cellular therapies. In the past few years, gene therapy for genetic disease has moved from addressing ultra-rare inherited diseases to more common ones including sickle cell disease and hemophilia. Similarly, chimeric anti gen receptor (CAR) T cells have expanded beyond hematologic malig nancies to address solid tumors and

autoimmune diseases (currently investigational products). Therapeutic approaches have expanded from gene transfer to gene editing, and RNA-based therapies have gained ground rapidly, partly as a key component of CRISPR-based gene edit ing (guide RNAs and mRNAs encoding editing enzymes) and as the active agent in multiple SARS-CoV-2 vaccines. This chapter will focus primarily on approved therapies (Table 483-2), with some discussion of investigational therapies in late-phase development and earlier trials critical to the development of the field. Clinical trials of gene therapy have been under way since 1990; the first gene therapy product to be licensed in the United States or Europe was approved in 2012 (see below). Given that vector-mediated gene therapy is arguably one of the most complex therapeutics yet developed, typically consisting of both a nucleic acid and a protein component, this time course from first clinical trial to licensed product is noteworthy for being similar to those seen with other novel classes of therapeutics, i.e., monoclonal antibodies or bone marrow transplan tation. Thousands of people have now received approved products or participated in investigational studies of gene transfer. Potential adverse events, predicted based on first principles (Table 483-3), have occurred but have been rare. Some of the initial trials were character ized by an overabundance of optimism and a failure to be appropriately critical of preclinical studies in animals; in addition, it was sometimes not fully appreciated that animal studies are only a partial guide to safety profiles of products in humans (e.g., in the setting of insertional mutagenesis or human immune responses to the vector). Clinical experience and laboratory research led to a more nuanced understand ing of the actual risks (Table 483-4) and dramatic benefits of these new therapies and to more sophisticated selection of disease targets. Currently, gene therapies are being developed for a variety of disease entities. Critical aspects of the history to be assessed when evaluating

Elicits few inflammatory responses, nonpathogenic RNA expressed transiently Limited packaging capacity Immunogenic; predominantly targets the liver; challenging manufacturing process a patient who has received a gene therapy product (or investigational agent) are outlined in Table 483-5. GENE TRANSFER AND GENE EDITING

FOR GENETIC DISEASES Most approved gene therapies for genetic diseases involve gene addi tion therapy. Recently the first gene editing products, for sickle cell dis ease and β-thalassemia, have been approved. Gene therapy strategies generally involve transfer of the missing gene to a physiologically rel evant target cell. However, other strategies are possible, including sup plying a truncated form of the gene with comparable biological activity (e.g., a gene encoding B domain-deleted FVIII for hemophilia A or microdystrophin for Duchenne muscular dystrophy); supplying a gene that achieves a similar biologic effect through an alternative pathway (e.g., utrophin in place of dystrophin for Duchenne muscular dystro phy); or downregulating a harmful effect through a small interfering or short hairpin RNA. From a therapeutic standpoint, gene therapies for genetic diseases fall into two distinct categories: (1) they may provide treatment for diseases that have hitherto lacked any pharmacologic therapies; or (2) they may provide an alternative to complex medical regimens that are frequently characterized by significant nonadherence due to the burden of treatment (e.g., monthly red blood cell transfu sions and iron chelation in transfusion-dependent β-thalassemia). Gene therapy for genetic disease requires long-term expression of the transgene. Two distinct strategies are available to achieve this goal: one is to transduce stem cells with an integrating vector, so that all progeny cells will carry the donated gene; and the other is to transduce long-lived, postmitotic cells, such as skeletal muscle or neurons. In the case of long-lived

cells, integration into the target cell genome is unnecessary. Instead, because the cells are nondividing, the donated DNA, even if stabilized predominantly in an episomal form, will give rise to expression for the life of the cell. This latter approach mitigates risks related to integration and insertional mutagenesis. CRISPR/Cas9-based gene editing differs from gene therapy in that the therapeutic moiety utilizes a bacterial Cas9 enzyme and a guide RNA to introduce a double strand break (DSB) at a specific site in the DNA. When co-delivered with an appropriately designed gene-target ing vector, gene insertion can take place at the site of the DSB, resulting in a corrected sequence at the endogenous locus of a gene. This perma nent correction in the genome is thus under the control of endogenous regulatory signals and will be passed to every daughter cell. In practice, the Cas9 enzyme is complexed with the guide RNA to form a ribonu cleoprotein (RNP), which is then delivered to the cell, where the guide RNA directs the RNP complex to the genome target, introducing the DSB at a precise locus. In the absence of a gene-targeting vector, the DSB will be repaired using nonhomologous end joining (NHEJ), which typically results in small deletions or insertions, disrupting the expres sion of the target gene. In the presence of a targeting vector, repair at the DSB may occur by homology-directed repair, resulting in gene replacement at the targeted locus. With currently available systems, the

TABLE 483-2 Currently Approved Gene and Cell Therapy Products in North America and/or Europe YEAR FIRST APPROVED PRODUCT INDICATION AGE GROUP Strimvelis®a ADA-SCID Pediatric

Europe Retroviral ADA (adenosine deaminase) PART 16 Genes, the Environment, and Disease Kymriah® (tisagenlecleucel) Relapsed or refractory (R/R) B-cell acute lymphoblastic leukemia (pediatric); R/R large B-cell lymphoma (adult); third-line follicular lymphoma Pediatric and adult, different disease indications

United States, Europe, China, Japan Yescarta® (axicabtagene ciloleucel) R/R and second-line large B-cell lymphomas; third-line follicular lymphoma Adult

United States, Europe, Japan Luxturna® (voretigene neparvovec) Confirmed biallelic RPE65 mutation–associated retinal dystrophy Pediatric and adult

United States and Europe Zolgensma® (onasemnogene abeparvovec) Spinal muscular atrophy type 1 due to biallelic mutations in the SMN1 gene Pediatric

<2 years of age

United States and Europe Zynteglo® (betibeglogene autotemcel) Adults and pediatric ≥12 years of age

Europe and United States Transfusion-dependent β thalassemia; sickle cell disease Libmeldy®b (aditarsagene autotemcel) Metachromatic leukodystrophy due to biallelic mutations in the arylsulfatase A gene Pediatric

Europe, United States Tecartus® (brexucabtagene autoleucel) R/R mantle cell lymphoma; R/R B-cell acute lymphoblastic leukemia Adults

United States and Europe Breyanzi® (lisocabtagene maraleucel) R/R and second-line large B-cell lymphoma Adult

United States, Europe, and Japan Abecma® (idecabtagene vicleucel) Fifth-line treatment for multiple myeloma Adult

United States and Europe Carvykti® (ciltacabtagene autoleucel) Fifth-line treatment for multiple myeloma Adult

United States and Europe Skysona® (elivaldogene autotemcel) Early active cerebral adrenoleukodystrophy Boys age 4–17

Europe and United States Upstaza® (eladocagene exuparvovec) Confirmed AADC deficiency with severe phenotype Children 18 months and older

Europe AAV2 Human aromatic L-amino acid decarboxylase (AADC) Roctavian® (valoctocogene roxaparvovec) Severe hemophilia A and no history of inhibitors Adults

Europe and United States Hemgenix® (etranacogene dezaparvovec) Severe or moderately severe hemophilia B Adults

Europe and United States Vyjuvek® (beremagene geperpavec) Dystrophic epidermolysis bullosa due to mutations in COL7A1 Age 6 months and older

United States Herpes simplex viral vector Elevidys® (delandistrogene moxeparvovec) Duchenne muscular dystrophy Ages 4–5

United States AAVrh74 cDNA encoding microdystrophin Casgevy® (exagamglogene autotemcel) Sickle cell anemia and

β thalassemia Ages 12 and up

United States and Europe Beqvez® (fidanacogene elaparvovec) Severe or moderately severe hemophilia B Adults

Canada,

United States and Europe aAutologous CD34+-enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence. bAutologous CD34+ cells encoding arylsulfatase A. Abbreviations: AAV, adeno-associated virus; ADA-SCID, adenosine deaminase severe combined immunodeficiency; CAR, chimeric antigen receptor; RBC, red blood cell.

WHERE APPROVED VECTOR TRANSGENE TARGET TISSUE Autologous hematopoietic stem cells (HSCs) Lentiviral CAR directed to CD19 with 4-1BB signaling domain Autologous T cells Retroviral CAR directed to CD19 with CD28 signaling domain Autologous T cells AAV2 RPE65 (retinal pigment epithelial 65 kD protein) Retinal pigment epithelial cells by single subretinal injection AAV9 SMN1

(survival motor neuron 1) Spinal motor neurons by single IV infusion Lentiviral βA-T87Q globin gene Autologous HSCs Lentiviral ARSA (arylsulfatase A) Autologous HSCs Retroviral Same molecular construct as axicabtagene Autologous T cells Lentiviral CAR directed at CD19 with 4-1BB signaling domain; CD4 and CD8 T-cell products manufactured and infused separately Autologous T cells Lentiviral CAR directed to B-cell maturation antigen (BCMA); 4-1BB signaling domain Autologous T cells Lentiviral CAR directed to BCMA with two single-domain antibodies; 4-1BB signaling domain Autologous T cells Lentiviral Adenosine triphosphate– binding cassette, subfamily D, member 1 (ABCD1) Autologous HSCs Cells in putamen via single neurosurgical procedure AAV5 cDNA encoding human factor VIII, B domaindeleted, SQ form Hepatocytes via single IV infusion AAV5 cDNA encoding factor IX Padua Hepatocytes via single IV infusion Collagen type VII alpha 1 chain (COL7A1) Keratinocytes and fibroblasts at sites of lesions Skeletal muscle via single IV infusion Gene editing Inactivates BCL11a in RBCs Autologous HSCs AAVrh74variant cDNA encoding factor IX Hepatocytes via single IV infusion Padua

TABLE 483-3 Potential Complications of Gene Therapy Gene silencing—repression of promoter Genotoxicity—complications arising from insertional mutagenesis, or acceleration of malignant transformation in a cell on the path to oncogenesis before transduction (i.e., CAR introduced into a premalignant T cell) Phenotoxicity—complications arising from overexpression or ectopic expression of the transgene Immunotoxicity—harmful immune response to either the vector or transgene, or a harmful immune response of the vector (e.g., CAR T cells) Risks of horizontal transmission—shedding of infectious vector into environment Risks of vertical transmission—germline transmission of donated DNA Abbreviation: CAR, chimeric antigen receptor. first (cleavage) step is much more efficient than the second (targeting) step. The only approved gene editing product requires only the cleavage step (vide infra), and the same is true for most investigational products that have been published as clinical studies. ■ ■EX VIVO GENE TRANSFER Early attempts to effect gene replacement into hematopoietic stem cells (HSCs) were stymied by the relatively low transduction efficiency of TABLE 483-4 Adverse Events in Gene Therapy and Gene Editing VECTOR OR TREATMENT MODALITY SYMPTOM OR LABORATORY FINDING MECHANISM DOSE DEPENDENCE MITIGATION STRATEGIES Retroviral or lentiviral vectors Malignancya Insertional mutagenesisa Yes for retroviral vectors AAV Vector sequences in semen, risk of germline transmission Based on animal studies, present in prostatic fluid but not in gametes Immune responses directed to capsid, sometimes accompanied by loss of expression Memory T cells directed to vector capsid in humans, who are natural hosts for wild-type AAV Thrombotic microangiopathy with high-dose systemic infusion Rapid rise in antibodies to AAV, formation of antigen-antibody complexes, triggering of complement activation Ex vivo and in vivo genome editing Off-target cleavage resulting in unintended gene silencing Guide RNA lacks requisite specificity In vivo genome editing Liver-directed in vivo editing has shown mild and transient transaminase elevations but excellent efficacy at doses studied clinically Possibly immune responses to bacterial proteins in editing machinery, potentially resulting in loss of edited cells CAR-T therapy Cytokine release syndrome: fever, hypotension, tachycardia, hypoxia, multiorgan failure Systemic inflammatory response caused by cytokines released by CAR T cells Neurotoxicity-cerebral edema and encephalopathy Peripheral immune overactivation, endothelial activation-induced blood-brain barrier dysfunction, CNS inflammation Immunodeficiency (hypogammaglobulinemia and susceptibility to viral injections) On-target effect against B cells and/or plasma cells Preparatory lymphodepleting chemotherapy regimen also contributes New T-cell malignancy Insertional mutagenesis or potentially chronic activation due to new transgene aIn

target cells, either hematopoietic stem cells or T cells. Abbreviations: AAV, adeno-associated virus; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CNS, central nervous system; FDA, U.S. Food and Drug Administration; PJP, Pneumocystis jirovecii pneumonia; VZV, varicella-zoster virus.

retroviral vectors, which require dividing target cells for integration. Because HSCs are normally quiescent, they are a formidable trans duction target. However, identification of cytokines that induce cell division without promoting differentiation of stem cells, along with technical improvements in the isolation and transduction of HSCs, led to modest but real gains in transduction efficiency.

CHAPTER 483 Immunodeficiency Disorders: Proof of Principle The first convincing therapeutic effect from gene transfer occurred in children with X-linked severe combined immunodeficiency disease (SCID), which results from mutations in the gene (IL2RG) encoding the γc sub unit of cytokine receptors required for normal development of T and natural killer (NK) cells (Chap. 362). Affected infants present in the first few months of life with overwhelming infections and/or failure to thrive. In this disorder, it was recognized that successfully transduced cells, even if few in number, would have a proliferative advantage com pared to nontransduced cells, which lack receptors for the cytokines required for lymphocyte development and maturation. Isolation of autologous CD34+ cells, followed by transduction with a retroviral vec tor encoding the γc subunit and transplantation of the gene-modified autologous cells, led to complete reconstitution of the immune system, including documented responses to standard childhood vaccinations, clearing of infections, and remarkable gains in growth in most treated Gene and Cell-Based Therapy in Clinical Medicine Less frequent with lentiviral vectors, likely because of differences in integration patterns Yes Barrier birth control until three sequential semen samples are negative for vector DNA Yes Reduce doses Administer immunomodulatory agents

(short-term) to reduce or ablate response Yes Has responded to therapy with complement inhibitors including eculizumab Likely Preclinical assessment for off-target effects Long-term follow-up of trial participants and patients Yes Careful dose-ranging studies in early-phase testing Consider short-term immunomodulatory agents if needed at higher doses Possibly Tocilizumab/corticosteroids Possibly Avoid seizure-threshold-lowering medications in early phase of treatment Treat with dexamethasone as early as possible (use specific management guidelines) No Prophylaxis for opportunistic infections (PJP, VZV) for at least 1 year; vaccination schedule (specific guidelines) No Report to FDA and manufacturer Consider activation of suicide gene if present in the transgene expression cassette Treat per standard guidelines

TABLE 483-5 Taking History from Patients Who Have Received Gene Therapies or Gene Editing Elements of History for Patients Who Received Gene Therapy (or Have Participated in Trials)

1. What vector was administered? Is it predominantly integrating (retroviral, PART 16 Genes, the Environment, and Disease lentiviral, herpesvirus, or gene editing) or nonintegrating (plasmid, adenoviral, adeno-associated viral)?
2. What were the dose and the route of administration of the vector?
3. What was the target tissue?

4. What gene was transferred in? The gene that is defective in the patient’s disease? A truncated version? A gene encoding a different protein with similar properties? A knockdown approach?
5. Were there any adverse events noted after gene transfer? Screening Questions for Long-Term Follow-Up in Gene Transfer Subjectsa
6. Has a new malignancy been diagnosed? If so, clinicians should contact the manufacturer to report the event and obtain instructions on the collection of patient samples for testing.
7. Has a new neurologic/ophthalmologic disorder, or exacerbation of a preexisting disorder, been diagnosed?
8. Has a new autoimmune or rheumatologic disorder been diagnosed?
9. Has a new hematologic disorder been diagnosed? aFactors influencing long-term risk include integration of the vector into the genome, vector persistence without integration, and transgene-specific effects. children. However, among 20 children treated in the initial trials, five eventually developed a syndrome similar to T-cell acute lymphocytic leukemia, with splenomegaly, rising white counts, and the emergence of a single clone of T cells. Molecular studies revealed that, in most of these children, the retroviral vector had integrated within a gene, LMO-2 (LIM only-2), which encodes a component of a transcription factor complex involved in hematopoietic development. The retroviral long terminal repeat acted as a promoter to increase the expression of LMO-2, resulting in T-cell leukemia. The X-linked SCID studies were a watershed event in the evolution of gene therapy. They demonstrated conclusively that gene therapy could cure disease, with dramatic and durable clinical results. However, they also demonstrated that insertional mutagenesis leading to cancer was more than a theoretical possibility (Table 483-3). As a result of the experience in these trials, all protocols using integrating vectors in hematopoietic cells must include a plan for monitoring sites of inser tion and clonal proliferation for 15 years after treatment. Initial strate gies to overcome the possible complication of insertional mutagenesis included using a “suicide” gene cassette in the vector, so that errant clones can be quickly ablated, or using “insulator” elements in the cas sette, which can limit the activation of genes surrounding the insertion site. However, the occurrence of malignancy in the X-linked SCID trials led to a transition to lentiviral vectors. These vectors efficiently transduce nondividing target cells and are characterized by a different pattern of integration into the genome that appears to be safer than retroviral vectors. However, recent developments in the field of CAR-T therapy, notably reports of development of T-cell lymphoma, have underscored the need for caution (vide infra). Transfusion-Dependent Thalassemia: Extension of Principle

Therapeutic success for inherited immunodeficiencies, though a clear unmet medical need, affects only a very small population. The success of gene therapy in β thalassemia, one of the most common genetic dis eases in Asian and Mediterranean populations, and one that provided the foundation for success in sickle cell disease, the most common genetic disease in Africans, demonstrated conclusively the therapeu tic impact of gene therapy. The red cell disorders β thalassemia and sickle cell disease are more challenging targets for gene therapy than the immunodeficiencies for several reasons. First, in immunodefi ciency disorders, the transduced stem cells have a survival advantage over nontransduced cells, which is not the case in red cell disorders (although the fully differentiated gene-modified red blood cells [RBCs] have a survival advantage compared to thalassemic or sickle RBCs).

Second, in order to achieve transfusion independence or freedom from vaso-occlusive crises, one must achieve higher transduction efficiency as well as engraftment of higher numbers of stem cells. There are now two approved products, one a lentiviral-based gene therapy and the other a gene editing approach, for both of these conditions (Table 483-2). Standard of care for transfusion-dependent β thalassemia (TDT) consists of lifelong regular RBC transfusions, typically monthly, to support hemoglobin (Hgb) levels >9 g/dL, coupled with an intensive regimen of iron chelation to minimize iron overload to the liver, heart, and endocrine system (Chap. 103). Allogeneic stem cell transplanta tion addresses the underlying cause of the disease but carries risks of myeloablation, graft-versus-host disease (GVHD), and graft rejection and thus is reserved primarily for those with an human leukocyte antigen (HLA)-matched sibling donor (<25% of patients). The first approved gene therapy for β thalassemia consists of a lentiviral vec tor driving expression of an antisickling variant of β-globin (βT87Q, the same product used for sickle cell disease), introduced into autologous HSCs, which are then transplanted back into the patient after mye loablation. Results of clinical trials for both β0/β0 genotype (the most severe) and for non-β0/β0 showed durable transfusion independence, defined as Hgb ≥9 g/dL and no transfusion for ≥12 months, in 20 of 22 evaluable participants in one phase 3 study and 12 of 14 patients in a second study. The remaining subjects all demonstrated reduction in the transfusion requirement, enabling iron removal therapy by either phlebotomy or iron chelation and removing risks related to iron over load. Gene therapy with lentiviral transduction of autologous cells thus dramatically simplifies the medical regimen for these patients, since it eliminates the need for ongoing transfusion and iron chelation and car ries no risk of GVHD or graft rejection because it is generated from the patient's own cells. Similarly, since the transduced cells are autologous, there is no requirement for an HLA-matched donor, expanding the numbers of patients who can be treated. Safety in the initial trials has been excellent, with most adverse events related to the known risks of the myeloablative conditioning regimen. The same lentiviral vector is also now approved for sickle cell dis ease; a single-arm, 24-month, open-label study assessed 36 participants who underwent apheresis followed by myeloablative conditioning and transplantation of gene-modified autologous cells. Of the 32 evaluable patients, 30 achieved complete elimination of vaso-occlusive crises between 6 and 18 months after infusion, a key efficacy endpoint in the study, and 31 of 36 achieved a globin response defined as hemoglo bin AT87Q (the transgene product) of at least 30% of total Hgb and an increase in total Hgb of ≥3 g/dL. Using an earlier version of this product that was prepared using a different manufacturing process and a dif ferent transplant procedure, two patients died following development of acute myeloid leukemia. Interpretation of these data is not straight forward, since patients with sickle cell disease have an increased risk of hematologic malignancy compared to the general population. The product carries a boxed warning summarizing this risk; twice yearly monitoring of a complete blood count is recommended. Neurodegenerative Disease: Broadening of Principle The SCID trials gave support to the hypothesis that gene transfer into HSCs could be used to treat any disease for which allogeneic bone marrow transplantation was therapeutic. Moreover, the use of genetically modi fied autologous cells carried the advantages noted above, i.e., no risk of GVHD, guaranteed availability of a "donor" (unless the disease itself damages the stem cell population of the patient), and low likelihood of failure of engraftment. Investigators in Paris capitalized on this realiza tion to conduct the first trial of lentiviral vector transduction of HSCs for a neurodegenerative disorder, X-linked adrenoleukodystrophy (ALD). The key to the mechanism of action is that a subpopulation of the gene-modified cells gives rise to myeloid cells that cross the bloodbrain barrier and engraft as central nervous system (CNS)-resident microglia and perivascular CNS macrophages. The transduced cells carry the gene encoding the missing protein, in this case

an adenosine triphosphate–binding cassette transporter (Table 483-2). Following lentiviral transduction of autologous HSCs in young boys with the disease, dramatic stabilization of disease occurred, demonstrating that

stem cell transduction could work for neurodegenerative as well as immunologic disorders. Investigators in Milan carried this observation one step further to develop a treatment for another pediatric neurodegenerative disorder that had previously responded poorly to bone marrow transplantation. Metachromatic leukodystrophy is a lysosomal storage disorder caused by mutations in the gene encoding arylsulfatase A (ARSA). The late infantile form of the disease is characterized by progressive motor and cognitive impairment and death within a few years of onset, due to accu mulation of the ARSA substrate sulfatide in oligodendrocytes, microglia, and some neurons. Recognizing that endogenous levels of production of ARSA were too low to provide cross-correction by allogeneic transplant, a lentiviral vector was used to create supraphysiologic levels of ARSA expression in transduced cells. Transduction of autologous HSCs from children born with the disease, at a point when they were still presymp tomatic, has led to preservation and continued acquisition of motor and cognitive milestones at time periods as long as 8 years after treatment, with observation ongoing. This product is approved in Europe and the United States for those with late infantile or early juvenile forms of the disease (Table 483-2). These results illustrate that the ability to engineer levels of expression can allow gene therapy approaches to succeed where allogeneic bone marrow transplantation cannot. A similar approach may be useful in other neurodegenerative conditions. ■ ■EX VIVO GENOME EDITING The first approved genome editing product, and those furthest along in clinical development, all use strategies that require only a cleavage event, rather than both a cleavage and a targeting event. For sickle cell disease (Chap. 103), the genome editing strategy is carried out ex vivo in HSCs. A Cas9/guide RNA ribonucleoprotein complex targeting the erythroid enhancer of the BCL11A gene, which normally represses γ-globin (the fetal β-like globin) during the fetal-to-adult β-globin switch, is introduced into autologous CD34+ cells of patients with TDT or sickle cell disease. Reduced BCL11A expression results in increased γ-globin expression and Hgb F production in erythroid cells, reducing the Hgb S levels and preventing sickling. The product was approved based on a study of 44 children and adults (age range 12–34 years) with sickle cell disease; of these, 31 had been followed for at least 16 months after gene editing, myeloablation, and engraftment. Of the 31 partici pants with adequate follow-up, 29 achieved the primary efficacy end point of at least 12 consecutive months without any protocol-defined vaso-occlusive crisis. The mean total Hgb at month 18 was 13.3 g/dL, compared to a baseline mean of 7.5 g/dL. This product is now approved for children (12 and older) and adults with sickle cell disease and recur rent vaso-occlusive crisis. Other genome editing strategies, in earlier stages of clinical develop ment, use Cas9/guide RNA to introduce a cleavage within the β-globin locus near the site of the sickle mutation and simultaneously supply a targeting vector (in this case, an AAV6 vector) encoding a short sequence of the wild-type β-globin gene. In a process dependent on the cellular homology-directed repair pathway, this sequence is used as a DNA repair template at the site of the break, resulting in replacement of the mutant βs sequence with the wild-type sequence. ■ ■LONG-TERM EXPRESSION IN GENETIC DISEASE: IN VIVO GENE TRANSFER WITH RECOMBINANT ADENO-ASSOCIATED VIRAL VECTORS Recombinant adeno-associated viral (AAV) vectors have emerged as attractive gene delivery vehicles for genetic disease. Engineered from a small replication-defective DNA virus, they are devoid of viral coding sequences and trigger very little immune response in experimental ani mals. They are capable of transducing nondividing target cells, and the donated DNA is stabilized

primarily in an episomal form, thus mini mizing risks arising from insertional mutagenesis. Because the vector has a tropism for certain long-lived cell types, such as skeletal muscle, neurons, and hepatocytes, long-term expression can be achieved even in the absence of integration. Of note, because the donated DNA is predominantly nonintegrated, long-term expression requires targeting of nondividing or slowly dividing cells; otherwise, expression is lost as

cells divide. The other shortcoming of AAV as a gene delivery vehicle is that it cannot package inserts of more than ~5 kb, owing to the fact that the wild-type viral genome is only ~4.7 kb; fortunately, with some notable exceptions, most cDNAs fall below this limit.

CHAPTER 483 First Approved Products for Ultra-Rare Diseases As was the case with ex vivo gene transfer, the first approved products for in vivo gene therapy were for ultra-rare disorders. In the Western world, the first approved gene therapy product for genetic disease was an AAV vector, conditionally approved in Europe in 2012, for treatment of the autosomal recessive disorder lipoprotein lipase deficiency. The sponsor allowed the approval to expire in 2017, without completing the postmarketing requirements, but the initial approval was a crucial catalyst for the current robust activity in gene therapy, and AAV vectors are now the largest category of approved products for genetic disease, including products for spinal muscular atrophy type 1, a rare form of genetic blindness, hemophilia A and B, and Duchenne muscular dys trophy (Table 483-2). Gene and Cell-Based Therapy in Clinical Medicine
The first approved AAV therapy for genetic disease in the United States was also for an ultra-rare disease, an inherited retinal dystrophy due to mutations in the gene encoding retinal pigment epithelialassociated 65-kDa protein (RPE65). The retina is an attractive target for AAV-mediated gene transfer. It is a relatively immunoprivileged space, obviating problems related to immune responses, and the photorecep tors, retinal ganglion cells, and retinal pigment epithelial cells are all long-lived postmitotic cells. Routes of administration for these cell types—either intravitreal or by subretinal injection—involve standard procedures in ophthalmology. Given the small space, doses required are relatively low, lessening the manufacturing burden. Finally, canine mod els of a number of inherited retinal dystrophies have been well-charac terized and faithfully model the human diseases. Work carried out in the 1990s had demonstrated that the canine disease could be reversed, with durable restoration of visual behavior, by subretinal injection of an AAV vector in dogs with a mutation in the gene encoding RPE65, an enzyme key to the visual cycle. Like the canine disease, the human disease is characterized by early-onset visual impairment, with most patients pro gressing to blindness over time. Phase 1 clinical trials by multiple groups established the safety of subretinal injections of an AAV vector express ing RPE65. A single phase 3 trial, the first randomized controlled trial in human gene therapy, demonstrated improvement in multiple measures of retinal and visual function. Of note, and likely to be a recurring theme as gene therapies address diseases for which there are no existing treat ments, successful clinical development required the development and validation of a novel clinical endpoint that could measure improvements in functional vision. This product, the first licensed AAV gene therapy product in the United States, is now approved worldwide (Table 483-2). Trials for both inherited and complex acquired retinal disorders such as age-related macular degeneration, affecting millions worldwide, are now underway. Hemophilia B: Addressing More Common Genetic Disorders and Unraveling the Human Immune Response to Systemi cally Administered AAV Hemophilia is the X-linked bleeding diathesis caused by mutations in the genes encoding factor VIII (hemo philia A) or factor IX (hemophilia B) (Chap. 121). Current treatment relies on intravenous infusion of clotting

factor concentrates or, as an alternative in hemophilia A, administration of a bispecific antibody that replaces the cofactor factor VIII by binding to the enzyme (fac tor IXa) and its substrate (factor X), resulting in a biologically active conformation. Gene therapy for hemophilia began with hemophilia B, a smaller patient population compared to hemophilia A, but the F9 cDNA is smaller (2.8 kb) and is more easily accommodated in an AAV vector. The early vectors were successful at demonstrating long-term expression of factor IX at therapeutic levels in hemophilic mice and dogs when vector was delivered to the liver (hepatocytes are the normal site of synthesis of factor IX), but the initial clinical trials uncovered a plethora of problems not predicted by animal models that had to be addressed to allow successful development to proceed. Fortunately, the solutions to these problems were generalizable across multiple

therapeutic indications that rely on systemic delivery of AAV vector (vide infra) (Table 483-4). Most complex among these was working out the human immune responses, both innate and adaptive, to the AAV vector. With systemic administration, the presence of neutralizing antibodies, harbored in a substantial portion of children and even more prevalent in adults, can prevent transduction before the target cells are reached, and the cellular immune response, not predicted by animal models, which are not natural hosts for AAV and thus lack memory T cells directed to the capsid, can result in the loss of the transduced cells in a matter of weeks after successful initial transduction. The preexisting antibodies can be screened for in advance, assuring that only those likely to benefit receive the therapy. The cellular immune response typically presents as asymptomatic transaminase elevation and concomitant loss of factor IX (or the transgene product) in the circulation. This response is dose-dependent and can be mitigated by the use of immunomodulatory agents such as glucocorticoids or by strategies that reduce the vector dose. The use of appropriately timed steroid treatment to dampen the cellular immune response resulted in the first report of durable factor IX expression in men with severe hemophilia B. The eventual widely adopted solution for hemophilia B gene therapy came from human genetics, specifically the use of a high specific activity variant of factor IX, factor IX Padua, that allowed a substantial reduction in vector dose and/or higher levels of circulating factor IX; this strategy has shown durable expression. The two cur rently approved hemophilia B gene therapies show an increase in mean factor IX levels well into the mild hemophilia range; these levels result in annualized bleeding rates that are noninferior to clotting factor pro phylaxis in men with the disease.

PART 16 Genes, the Environment, and Disease Successful extension to hemophilia A required the use of a truncated version of the factor VIII cDNA. The only approved product drives therapeutic levels of expression, but durability has been less than that seen in gene therapy for hemophilia B. Spinal Muscular Atrophy Type 1 Spinal muscular atrophy type 1 is the most common genetic cause of death in infancy and affects about 1 in 11,000 births. The disease is caused by autosomal reces sive mutations in the SMN1 gene, encoding survival motor neuron 1; affected infants undergo degeneration and loss of lower motor neurons, presenting as hypotonia, severe weakness, and failure to sit without support. In a large natural history study of untreated infants with the disease, by age 20 months, only 8% of patients with the disease were alive and free of ventilator support. In a phase 1 gene therapy study, intravenous infusion of an AAV vector (one with tropism for the nervous system [AAV9]) expressing SMN1 showed survival without ventilator support in 100% of participants (n = 15) at 20 months of age. A phase 3 trial was initiated, but the treatment was approved in the United States based on the efficacy data from the first 21 participants enrolled in that study, coupled with the safety data from the ongoing phase 3 and the completed phase 1

study (Table 483-2). The major safety concern was the risk of acute serious liver injury; because the vector is infused intravenously, there is considerable biodistribution to the liver (and to the spinal motor neurons, the therapeutic target). The approved dose of 1.1 × 1014 vector genomes/kg is quite high, and results in marked elevation of liver transaminases if untreated. Leveraging the results in the early hemophilia trials, the phase 1 study showed that the liver toxicity could be controlled using a course of corticosteroids begun 1 day before the vector infusion and continued for 30 days, with tapering begun at that point and carried out with monitoring of liver transaminases. Postmarketing studies revealed an additional rare adverse reaction, thrombotic microangiopathy (TMA). Presenting as thrombocytopenia, microangiopathic hemolytic anemia, and acute kidney injury, this constellation of findings in the setting of AAV gene therapy was first described in the AAV gene therapy trials for Duch enne muscular dystrophy, which also use very high doses of vector. When it occurs, TMA appears early after vector infusion, is complementmediated, and has responded to complement inhibitors, i.e., eculi zumab. Patients receiving onasemnogene abeparvovec are currently followed in a registry designed to assess effectiveness, long-term safety, and overall survival of patients with Spinal muscular atrophy.

■ ■IN VIVO GENOME EDITING Clinical trials using ex vivo genome editing of HSCs by CRISPR/Cas9 systems have been ongoing for several years and have now led to the first approved genome editing product (vide supra). More recently, investigators have begun to explore the feasibility of in vivo genome editing, with intriguing results. As has been the case with ex vivo editing, these initial trials all center on strategies that require a cleav age step only, i.e., not a cleavage event followed by a targeting event. For in vivo editing of hepatocytes, lipid nanoparticles containing an mRNA encoding the Cas9 protein and a single guide RNA directed to the gene of interest are infused intravenously. The major safety concern involves the risk of off-target editing; an additional concern prior to clinical trials was whether a robust immune response to the bacterial Cas9 enzyme would result in unacceptable toxicity or loss of efficacy. Published experience to date does show mild, transient, dose-dependent rises in liver transaminases, but also robust effi cacy in reducing circulating levels of transgene products of interest, including transthyretin in transthyretin amyloidosis and plasma kallikrein B1 in hereditary angioedema. Additional in vivo genome editing trials are underway, notably for cardiovascular conditions, to reduce circulating levels of lipoprotein(a), proprotein convertase subtilisin/kexin type 9 (PCSK9), or angiopoietin-like protein 3, all associated with atherosclerotic cardiovascular disease. An advantage of genome editing approaches is that the edit will be passed to every daughter cell; thus, changes should persist over time, and this treat ment can be used even in children, without fear that the edit will be lost as the liver grows in size. GENE THERAPY FOR CANCER The majority of clinical gene transfer experience has been in subjects with cancer. The intent has been to increase the precision of cancer therapies and thereby make them less toxic and more effective. Most approaches have either modified the tumor directly or altered the host’s response to the malignancy to produce immune effector cells that are precisely targeted to the tumor phenotype. ■ ■MODIFYING THE CANCER Since cancer is an (acquired) genetic disorder, initial efforts were directed at correcting the genetic deficits of the tumor or introducing lethal genes. Two major and persisting obstacles, however, are the poor biodistribution and transduction efficiency of all currently available vectors, and the heterogeneity and genetic instability of the tumor tar gets themselves, so that correction of single driver mutations does not preclude the evolution of a resistant population. Tumor Correction One widely used direct intratumoral approach was adenoviral-mediated expression of the tumor suppressor p53, which is mutated in many different cancers. Initial studies showed some complete and partial

responses in squamous cell carcinoma of the head and neck, esophageal cancer, and non-small cell lung cancer, but as yet, there have been no successful product licensing studies for this approach except in China. Prodrug Metabolizing Genes Efforts to overcome the above limitations have included the introduction of a prodrug or a suicide gene that would increase sensitivity of tumor cells to cytotoxic drugs. A strategy used early on was intratumoral injection of an adenoviral vector expressing the thymidine kinase (TK) gene. Cells that take up and express the TK gene can be killed after the administration of ganciclovir, which is phosphorylated to a toxic nucleoside by TK. The advantage of this approach is that the effects of transducing even a lim ited number of tumor cells are amplified by the spread of active drug to adjacent tumor cells. Although the approach continues to be examined in aggressive brain tumors and locally recurrent prostate, breast, and colon tumors, progress remains slow, and systemic benefits against metastatic disease have not been established. ■ ■MODIFYING THE HOST Recruiting the Immune System The successful use of mono clonal antibodies that produce antitumor activity by activating the

immune response has demonstrated the feasibility of manipulating the immune system to recognize the abnormal pattern of antigen expres sion on tumor cells. Immune cells are capable of almost unlimited expansion and persistence and can provide long-term tumor control. They can also traffic to tumor sites irrespective of location and, in prin ciple, have the potential to evolve with the changing pattern of tumor cell phenotype and function. Antibodies targeting “checkpoint” mol ecules, particularly CTLA-4 and the PD-1/PD-L1 axis, which naturally limit T-cell responses and maintain tolerance, have been particularly successful. Vaccination This strategy promotes more efficient recognition of tumor cells by the immune system, but the development of a therapeutic as opposed to the preventative vaccines required to combat infectious diseases has proved to be a considerable chal lenge. Approaches have included direct injection of tumor or tumor-antigen–derived RNA or DNA; transduction of tumor cells with immune-enhancing genes encoding cytokines, chemokines, or co-stimulatory molecules; and the ex vivo manipulation of dendritic cells to enhance the presentation of tumor antigens. A dendritic cell vaccine for treatment of recurrent prostate cancer has received approval in the United States, but its limited potency and high cost have constrained commercial success. Adoptive Cell Transfer Host immune cells such as T cells, NK cells, and others can be modified to express new transgenic recep tors intended to recognize tumor cells and their microenvironment (Fig. 483-1). Retargeting may use a modification of the cells’ own receptor or a molecularly synthesized CAR that is usually composed of the antigen recognition portion of an antibody and the signaling components of the cell’s native antigen receptor along with one or more additional signaling domains that boost T-cell activation. Both approaches have been successful, with significant responses reported with native receptors targeted to melanoma and synovial cell sarcoma and—most dramatically—with CARs targeted to CD19, an antigen expressed at high levels on normal and many malignant B cells, or B-cell maturation antigen (BCMA), an antigen expressed at high levels in normal and multiple myeloma plasma cells. Infused CAR T cells can expand many thousand-fold in vivo, persist long term, and have pro duced >80% complete response rates when targeting intractable B-cell acute lymphoblastic leukemia; approximately half of those patients have remained in remission for many years afterward without further CAR T cells Native T cells VL CL Target cell VL VH CH MHC I β2 VH CH2 CH3 Antigenic peptide Spacer TCR Monoclonal antibody α β TM CD3ζ COSTIM α β CAR ζ δ ε ε γ ζ ζ TCR complex γ ε ε δ ζ ζ TCR complex FIGURE 483-1 T-cell receptors. A native T-cell receptor (TCR) recognizes processed peptide antigens bound to major histocompatibility (MHC) molecules through its αβ chains. Signaling then occurs through a multichain intracellular CD3 complex. A chimeric antigen

receptor (CAR) usually contains an extracellular receptor component derived from the antigen binding portion (VH and VL) of a monoclonal antibody. This produces a receptor that can recognize either protein or nonprotein antigens independent of the MHC. A transmembrane (TM) domain then connects this receptor to the ζ chain of the CD3 complex derived from the native TCR. A costimulation domain (COSTIM), such as CD28 or 4-1BB, is also present.

cancer therapies (i.e., have been “cured”). This approach has also been successful in adult patients with relapsed or chemotherapy-refractory B-cell–derived large cell lymphoma, mantle cell lymphoma, and mul tiple myeloma. Many responses are sustained long term, and several of these CAR T-cell products have been approved by the U.S. Food and Drug Administration (FDA), as well as international regulatory agen cies, and adopted as standard of care. In 2021, the first reports of T-cell lymphoma were reported with the use of piggyBac-modified (transpo son) CAR-T therapy; 2 of 10 patients developed CAR-T lymphoma, but this may have been a result of widespread copy number variations and multiple insertions, and there was no apparent evidence of insertional mutagenesis. In November 2023, the FDA issued a warning for most approved CAR-T therapies, all of which use retroviral or lentiviral vec tors for gene delivery of the CAR, suggesting that there was a higherthan-expected rate of T-cell lymphoma and that patients treated with CAR-T therapy should be monitored for secondary malignancies for life. Nevertheless, the overall benefits of these products continued to outweigh their potential risks for their approved uses. Although the FDA did not list the frequency, a flurry of subsequent reports noted that the rate of T-cell malignancies after CAR-T treatment for patients with relapsed or refractory hematologic malignances was ~20 in 34,000 patients (~0.06%); however, because reporting to the FDA on the inci dence of T-cell malignancies is voluntary once products are approved, it is possibly an underestimated frequency. A boxed warning listing the possibility of secondary malignancies was added to the label of most CAR-T products in January 2024, and the FDA published instructions on reporting secondary malignancies to the community in order to gather more information. It is important to keep in mind that the vast majority of secondary malignancies in patients treated with approved CAR-T therapies are unrelated to the presence of the CAR transgene, including solid tumors and myeloid malignancies; such secondary malignancies may be related to age, the presence of clonal hemato poiesis, and exposure to prior chemotherapy or other antineoplastic treatments.

The general approach of CAR-T therapy is under rapid develop ment, including trials with CAR T cells targeting different antigens for solid tumors and other hematologic malignancies and CAR T cells with different molecular structures and different gene transfer vectors. Remaining challenges in the field and application of adoptive T-cell approaches include the following: (1) the immune inhibitory micro environment associated with most solid tumors, and recent studies further modify the T cells with countermeasures to tumor inhibitory signals; (2) acute and severe (though rarely fatal) systemic inflamma tory and neurologic toxicities during the phase of T-cell expansion and tumor killing, which typically require access to intensive care for clini cal management; (3) the off-target or on-target but off-tumor effects that may damage normal host tissues (e.g., normal B cells following CD19 CAR therapy); and (4) the cost, time, and complexity of manu facture, which are particular problems when antigens unique to each tumor’s individual mutations are targeted (neoantigens), rather than widely shared tumor-associated antigens. Nonimmunologic Modifications to Host Gene transfer can be used to protect normal cells from the toxicities of chemotherapy and thereby increase the therapeutic index of these drugs. The most extensively studied approach has been to transduce hematopoietic cells with genes encoding resistance to

chemotherapeutic agents, including the multidrug resistance gene MDRI or the gene encod ing O6-methylguanine DNA methyltransferase (MGMT). Although such approaches reduce hematologic toxicity, cytotoxic dose escala tion quickly reveals dose-limiting toxicities to other organ systems. Chemotherapy resistance can also be engineered into immune cells redirected to target cancer, to enable combination treatments with cells and chemotherapy. T cells Finally, gene transfer can be used to inhibit the host angiogenesis required for tumor support, for example by constitutive expression of inhibitors such as angiostatin and endostatin, or the transfer of T cells genetically modified to recognize antigens specific to newly forming vasculature. These studies are in early phases.

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