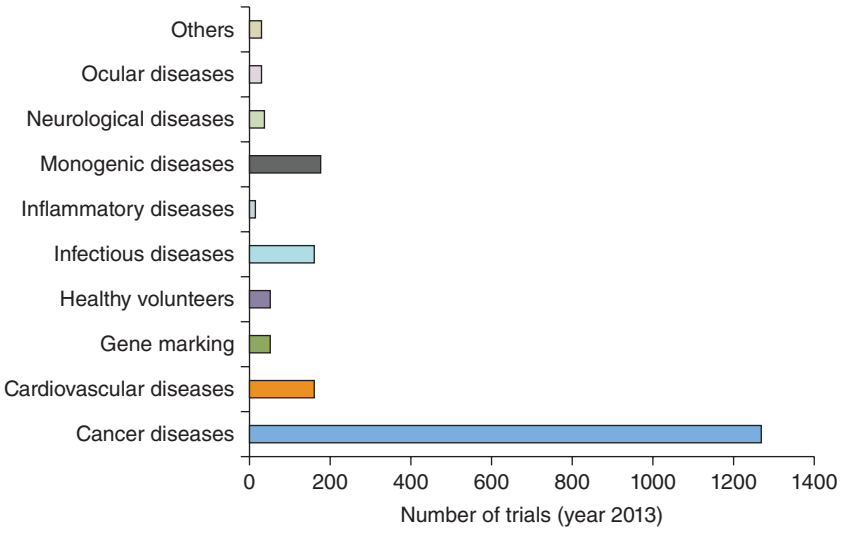

FIGURE 91e-1 Indications in gene therapy clinical trials. The bar graph classifies clinical gene transfer studies by disease. A majority of trials have addressed cancer, with monogenic disorders, infectious diseases, and cardiovascular diseases the next largest categories. *(Adapted from SL Ginn et al: J Gene Med 15:65-77, 2013. Published online in Wiley Online.)*

T and natural killer (NK) cells (Chap. 374). 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 the transduced cells, even if few in number, would have a proliferative advantage compared to the nontransduced cells, which lack receptors for the cytokines required for lymphocyte development and maturation. Complete reconstitution of the immune system, including documented responses to standard childhood vaccinations, clearing of infections, and remarkable gains in growth occurred in most of the treated children. However, among 20 children treated in two separate 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 increases 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; of the 20 children eventually treated in these trials, 18 achieved correction of the immunodeficiency disorder. Unfortunately, 5 of the 20 patients later developed a leukemia-like disorder, and one died of this complication; the rest are alive and free of complications at time periods ranging up to 14 years after initial treatment. These studies demonstrated that insertional mutagenesis leading to cancer was more than a theoretical possibility (Table 91e-2). 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 insertion and clonal proliferation. Strategies to overcome this complication have included using a "suicide" gene cassette in the vector, so that errant clones can be quickly ablated, or using "insulator" elements in the cassette, which can limit the activation of genes surrounding the insertion site. Lentiviral vectors, which can efficiently transduce nondividing target cells, are also likely to be safer than retroviral vectors, based on patterns of integration; the field is thus gradually moving toward these to replace retroviral vectors.

TABLE 91e-2 POTENTIAL COMPLICATIONS OF GENE THERAPY

Gene silencing – repression of promoter
Genotoxicity – complications arising from insertional mutagenesis
Phenotoxicity – complications arising from overexpression or ectopic expression of the transgene
Immunotoxicity – harmful immune response to either the vector or transgene
Risks of horizontal transmission – shedding of infectious vector into environment
Risks of vertical transmission – germline transmission of donated DNA

More clear-cut success has been achieved in a gene therapy trial for another form of SCID, adenosine deaminase (ADA) deficiency (Chap. 374). ADA-SCID is clinically similar to X-linked SCID, although it can be treated by enzyme replacement therapy with a pegylated form of the enzyme (PEG-ADA), which leads to immune reconstitution but not always to normal T cell counts. Enzyme replacement therapy is expensive (annual costs: $200,000–$300,000 in U.S. dollars). The initial trials of gene therapy for ADA-SCID were unsuccessful, but modifications of this protocol to include the use of HSCs rather than T cells as the target for transduction; discontinuation of PEG-ADA at the time of vector infusion, so that the transduced cells have a proliferative advantage over the nontransduced; and the use of a mild conditioning regimen to facilitate engraftment of the transduced cells have led to success without the complications seen in the X-linked SCID trials. There have been no complications in the 10 children treated on the Milan protocol, with a median follow-up of >8 years. ADA-SCID, then, is an example where gene therapy has changed therapeutic options for patients. For those with a human leukocyte antigen (HLA)-identical sibling, bone marrow transplantation is still the best treatment option, but this includes only a minority of those affected. For those without an HLA-identical match, gene therapy has comparable efficacy to PEG-ADA, does not require repetitive injections, and does not run the risk of neutralizing antibodies to the bovine enzyme.

NEURODEGENERATIVE DISEASES: EXTENSION 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 modified autologous cells carried several advantages including no risk of graft-versus-host disease, guaranteed availability of a "donor" (unless the disease itself damages the stem cell population of the patient), and low likelihood of failure of engraftment. Cartier and Aubourg capitalized on this realization to conduct the first trial of lentiviral vector transduction of HSCs for a neurodegenerative disorder, X-linked adrenoleukodystrophy (ALD). X-linked ALD is a fatal demyelinating disease of the central nervous system caused by mutations in the gene encoding an adenosine triphosphate–binding cassette transporter. Deficiency of this protein leads to accumulation of very-long-chain fatty acids in oligodendrocytes and microglia, disrupting myelin maintenance by these cells. Affected boys present with clinical and neuroradiographic evidence of disease at age 6–8 and usually die before adolescence. 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 neurodegenerative disorder that has 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 accumulation 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, Naldini and colleagues engineered a lentiviral vector that directed supraphysiologic