levels of ARSA expression in transduced cells. Transduction of autologous HSCs from children born with the disease, at a point when they were still presymptomatic, led to preservation and continued acquisition of motor and cognitive milestones at time periods as long as 32 months after affected siblings had begun to lose milestones. These results illustrate that the ability to engineer levels of expression can allow gene therapy approaches to succeed where allogeneic bone marrow transplantation cannot. It is likely that a similar approach will be used in other neurodegenerative conditions.

Transduction of HSCs to treat the hemoglobinopathies is an obvious extension of studies already conducted but represents a higher hurdle in terms of the extent of transduction required to achieve a therapeutic effect. Trials are now under way for thalassemia and for a number of other hematologic disorders, including Wiskott-Aldrich syndrome, and chronic granulomatous disease.

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 animals. They are capable of transducing nondividing target cells, and the donated DNA is stabilized primarily in an episomal form, thus minimizing risks arising from insertional mutagenesis. Because the vector has a tropism for certain long-lived cell types, such as skeletal muscle, the central nervous system (CNS), and hepatocytes, long-term expression can be achieved even in the absence of integration.

FIRST LICENSED PRODUCT

These features of AAV were used to develop the first licensed gene therapy product in Europe, an AAV vector for treatment of the autosomal recessive disorder lipoprotein lipase (LPL) deficiency. This rare disorder (1–2/million) is due to loss-of-function mutations in the gene encoding LPL, an enzyme normally produced in skeletal muscle and required for the catabolism of triglyceride-rich lipoproteins and chylomicrons. Affected individuals have lipemic serum and may have eruptive xanthomas, hepatosplenomegaly, and in some cases, recurrent bouts of acute pancreatitis. Clinical trials demonstrated the safety of intramuscular injection of AAV-LPL and its efficacy in reducing frequency of pancreatitis episodes in affected individuals, leading to drug approval in Europe. Additional clinical trials currently under way that use AAV vectors in the setting of genetic disease include those for muscular dystrophies, α_1 antitrypsin deficiency, Parkinson's disease, Batten's disease, hemophilia B, and several forms of congenital blindness.

HEMOPHILIA

Hemophilia (Chap. 78) has long been considered a promising disease model for gene transfer, because the gene product does not require precise regulation of expression and biologically active clotting factors can be synthesized in a variety of tissue types, permitting latitude in the choice of target tissue. Moreover, raising circulating factor levels from <1% (levels seen in those severely affected) into the range of 5% greatly improves the phenotype of the disease. Preclinical studies with recombinant AAV vectors infused into skeletal muscle or liver have resulted in long-term (>5 years) expression of factor VIII or factor IX in the hemophilic dog model. Administration to skeletal muscle of an AAV vector expressing factor IX in patients with hemophilia B was safe and resulted in long-term expression as measured on muscle biopsy, but circulating levels never rose to >1% for sustained periods, and a large number of IM injections (>80–100) was required to access a large muscle mass. Intravascular vector delivery has been used to access large areas of skeletal muscle in animal models of hemophilia and will likely be tested for this and other disorders in upcoming trials.

The first trial of an AAV vector expressing factor IX delivered to the liver in humans with hemophilia B resulted in therapeutic circulating levels at the highest dose tested, but expression at these levels (>5%) lasted for only 6–10 weeks before declining to baseline (<1%). A memory T cell response to viral capsid, present in humans but not in other animal species (which are not natural hosts for the virus), likely led to the loss of expression (Table 91e-2). In response to these findings, a second trial included a short course of prednisolone, to be administered if factor IX levels began to decline. This approach resulted in long-term expression of factor IX, in the range of 2–5%, in men with severe hemophilia B. Current efforts are focused on expanding these trials, and extending the approach to hemophilia A.

RETINAL GENE THERAPY

A logical conclusion from the early experience with AAV in liver in the hemophilia trial was that avoidance of immune responses was key to long-term expression. Thus immunoprivileged sites such as the retina began to attract substantial interest as therapeutic targets. This inference has been elegantly confirmed in the setting of the retinal degenerative disease Leber's congenital amaurosis (LCA). Characterized by early-onset blindness, LCA is not currently treatable and is caused by mutations in several different genes; ~15% of cases of LCA are due to a mutation in a gene, *RPE65*, encoding a retinal pigment epithelial-associated 65-kDa protein. In dogs with a null mutation in *RPE65*, sight was restored after subretinal injection of an AAV vector expressing *RPE65*. Transgene expression appears to be stable, with the first animals treated >10 years ago continuing to manifest electroretinal and behavioral evidence of visual function. As is the case for X-linked SCID, gene transfer must occur relatively early in life to achieve optimal correction of the genetic disease, although the exact limitations imposed by age have not yet been defined. AAV-RPE65 trials carried out in both the United States and the United Kingdom have shown restoration of visual and retinal function in over 30 subjects, with the most marked improvement occurring in the younger subjects. Trials for other inherited retinal degenerative disorders such as choroideremia are under way, as are studies for certain complex acquired disorders such as age-related macular degeneration, which affects several million people worldwide. The neovascularization that occurs in age-related macular degeneration can be inhibited by expression of vascular endothelial growth factor (VEGF) inhibitors such as angiostatin or through the use of RNA interference (RNAi)-mediated knockdown of VEGF. Early-phase trials of siRNAs that target VEGF RNA are under way, but these require repeated intravitreal injection of the siRNAs; an AAV vector–mediated approach, which would allow long-term inhibition of the biological effects of VEGF through a soluble VEGF receptor, is now in clinical testing.

GENE THERAPY FOR CANCER

The majority of clinical gene transfer experience has been in subjects with cancer (Fig. 91e-1). As a general rule, a feature that distinguishes gene therapies from conventional cancer therapeutics is that the former are less toxic, in some cases because they are delivered locally (e.g., intratumoral injections), and in other cases because they are targeted specifically to elements of the tumor (immunotherapies, antiangiogenic approaches). Because cancer is a disease of aging, and many elderly are frail, the development of therapeutics with milder side effects is an important goal.

LOCAL APPROACHES

Cancer gene therapies can be divided into local and systemic approaches (Table 91e-3). Some of the earliest cancer gene therapy trials focused

TABLE 91e-3 GENE THERAPY STRATEGIES IN CANCER

Local/regional approaches
Suicide gene/prodrug
Suppressor oncogene
Oncolytic virus
Systemic approaches
Chemoprotection
Immunomodulation
Antiangiogenesis