Ham-Wasserman Lecture

Hemophilia and Related Bleeding Disorders:
A Story of Dismay and Success

Pier M. Mannucci, MD*

Known since the beginning of the first millennium, the hemophilias are among the most frequent inherited disorders of blood coagulation and definitely the most severe. In the 1970s, with the availability of concentrated preparations of the deficient coagulation factors VIII and IX and with the large-scale adoption of home treatment, hemophilia care became one of the most gratifying examples of successful secondary prevention of a chronic disease. Unfortunately, in the early 1980s it was recognized that factor concentrates prepared from plasma pooled from thousands of donors transmitted the hepatitis and the human immunodeficiency viruses. The scientific community reacted promptly to the devastation brought about by hepatitis and AIDS. The last 15 years of the second millennium have witnessed the development of methods that, when applied during concentrate manufacturing, inactivate viruses escaping the screening procedures. The adoption of these measures has reduced dramatically the risk of transmission of bloodborne infections. The production of recombinant factors and their availability for patients’ treatment epitomize progress in hemophilia care through DNA technology. Methods based on the polymerase chain reaction (PCR) have unraveled an array of gene lesions associated with hemophilia, permitting improved secondary control of the disease through carrier detection in women from affected families and prenatal termination of their affected male infants. This article will review the aforementioned areas of progress and discuss unresolved problems (such as treatment of patients with antibodies, the risk of new infectious complications, and the issue of secondary tumors). Hopes and expectations for further improvement in the third millennium and particularly the prospects of hemophilia cure through gene replacement therapy will also be mentioned.

. . . and there shall be no more death, neither sorrow, nor crying; neither shall be any more pain: because the former things are passed away.

Revelation 21:4

The inherited deficiency of blood coagulation factors leads to lifelong bleeding disorders commonly called hemophilias. The factors most frequently found deficient in hemophilias are factors VIII (FVIII) and IX (FIX), whose genes are located on the X chromosome and, when mutated, cause the X-linked recessive traits hemophilia A and B. The reported incidence of hemophilia A is 1 in 10,000 births and that of hemophilia B is 1 in 60,0001 (Table 1). Deficiencies of other coagulation factors, which are transmitted as autosomal recessive traits and affect both sexes, are much rarer (1 in 500,000 or less)2 (Table 1).

Hemophilias occur in mild, moderate, and severe forms (corresponding to plasma factor levels of 6-30%, 1-5%, and less than 1%, respectively). Although patients with mild hemophilia usually bleed only after trauma or surgery, those with severe hemophilia A or B bleed spontaneously or after trivial trauma, particularly into joints and muscles, on average 20 to 30 times per year but sometimes more frequently. Coagulation defects other than FVIII and FIX are inherited as autosomal recessive traits (afibrinogenemia, hypoprothrombinemia, factor V deficiency, factor VII deficiency, factor X deficiency, factor XI deficiency, and factor XIII deficiency) and are usually less severe than FVIII and FIX deficiency, with the

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exception of factor X and factor XIII deficiencies. The number of patients with autosomal coagulation defects is increasing in several countries characterized by large immigration of Islamic populations, which have an approximately 10-fold higher incidence of these recessive disorders as a consequence of the frequent custom of consanguineous marriages. The modern management of hemophilia started in the 1970s, when the increased availability of plasma concentrates of coagulation factors and the widespread adoption of home replacement therapy led to the early control of hemorrhages and to the reduction or prevention of the musculoskeletal damage typical of untreated or poorly treated patients. Prophylactic treatment was successfully implemented in Sweden and other countries, achieving the goal of preventing the majority of bleeding episodes and further reducing the impact of arthropathy. On the whole, during these years hemophilia care became one of the most gratifying examples of successful secondary prevention of a chronic disease. This optimistic perception of hemophilia changed dramatically in the early 1980s, when 60-70% of persons with severe disease became infected with the human immunodeficiency virus (HIV) that had contaminated concentrates. Practically all treated hemophiliacs had also been infected with the hepatitis C virus (HCV) transmitted by factor concentrates manufactured from plasma pooled from thousands of donors.

The scientific community reacted promptly to the devastation caused by acquired immunodeficiency syndrome (AIDS) and hepatitis. The last 15 years witnessed the production of safer plasma concentrates of coagulation factors. Availability of recombinant factors has been the result of progress in DNA technology. Analysis of patients’ DNA has permitted identification of the great majority of the gene lesions that cause hemophilia and has allowed secondary control of the disease through carrier detection and antenatal diagnosis. New treatments have substantially improved the previously unfavorable prognosis of patients who develop alloantibodies (inhibitors) to FVIII or FIX (10-20% in hemophilia A and 3-5% in hemophilia B). Finally, in the past few years the first experiments of somatic gene therapy started in persons with hemophilia have had promising results. This article will review those aspects of hemophilia where progress has recently taken place, with reference to previous articles of Hoyer and Mannucci and Tuddenham for information not available in this review.

### Molecular Defects in Hemophilia

A database of hemophilia A mutations can be found on the Internet at http://europium.csc.mrc.ac.uk. There are two prevalent mutations in severe hemophilia A. About 40% of the cases are caused by an inversion involving a gene within intron 22 of the FVIII gene, of which 2 further copies exist distal to the FVIII locus on Xq28. Recently, an inversion in intron 1 has also been detected, accounting for approximately 1% of cases. Even though, in general, null mutations predicting no or truncated protein production (stop codons, deletions, insertions, splicing abnormalities) are associated with severe factor deficiencies and clinical phenotypes, there are a number of severe deficiencies associated with missense mutations. It is thought that impaired folding and/or altered conformation of the mutant FVIII lead to both intra- and extracellular instability, which in turn causes severe factor deficiency in plasma. The pattern of gene mutations in hemophilia B (see http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html) and autosomal recessive coagulation defects is substantially different from that of hemophilia A. For each of these defects there is an array of mutations, and the majority of them are unique for each kindred, with relatively few instances in which a founder effect can be traced. Additional Internet sites on various aspects of hemophilia and other inherited coagulation disorders are listed in Table 2.

### Antenatal and carrier diagnosis using specific mutation analysis

### Table 1. General features of inherited deficiencies of coagulation factor associated with bleeding disorders.

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Incidence in General Population</th>
<th>Gene on Chromosome</th>
<th>Mode of Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>1:1 million</td>
<td>4</td>
<td>AR</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>1:2 million</td>
<td>11</td>
<td>AR</td>
</tr>
<tr>
<td>Factor V</td>
<td>1:1 million</td>
<td>1</td>
<td>AR</td>
</tr>
<tr>
<td>Factor VII</td>
<td>1:500,000</td>
<td>13</td>
<td>AR</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>1:10,000</td>
<td>X</td>
<td>XLR</td>
</tr>
<tr>
<td>Factor IX</td>
<td>1:60,000</td>
<td>X</td>
<td>XLR</td>
</tr>
<tr>
<td>Factor X</td>
<td>1:1 million</td>
<td>13</td>
<td>AR</td>
</tr>
<tr>
<td>Factor XI</td>
<td>1:1 million</td>
<td>4</td>
<td>AR</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>1:2 million</td>
<td>A subunit: 6</td>
<td>AR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B subunit: 1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AR, autosomal recessive; XLR, X-linked recessive.
production of truncated FVIII or no factor at all, whereas in carriers of missense mutations and small deletions, production of factor even in minute amounts makes it more likely that the exogenous factor introduced with replacement therapy will be recognized as self. In hemophilia B, inhibitors are less frequent than in hemophilia A (3-6% versus 10-20%) and are almost always associated with large deletions and nonsense mutations. Another approach to evaluate vulnerability to inhibitor development, HLA typing, has shown a weak correlation between the complication and HLA classes I and II.8 Hence, both approaches are of limited clinical sensitivity and specificity at the moment. In view of recent advances in the definition of recognition motifs for HLA class II molecules, studies to correlate HLA type and FVIII gene defects might be expected to provide new information that could be of use to more accurately predict inhibitor formation. If we had methods to antenatally predict the likelihood of inhibitor formation, then in utero administration of exogenous FVIII or FIX during fetal development prior to the completion of self-recognition would result in tolerance of the administered clotting factor and potentially avoid the development of inhibitors.

**Choice of Replacement Therapy**

**Recombinant factors**

Two preparations of recombinant FVIII formulated in human albumin were licensed in the early 1990s. Clinical studies have since demonstrated their excellent efficacy, approximately 80% of bleeding episodes being controlled by a single dose.9,12 Very high levels of safety in terms of viral transmission and immunological reactions against animal proteins used in cell culture and manufacturing processes have been observed. The current view is that recombinant FVIII triggers no more inhibitors than do plasma-derived factors.13,14

Second-generation recombinant FVIII products are

### Table 2. Medical and scientific information on hemophilia accessible through the Internet.

<table>
<thead>
<tr>
<th>Site</th>
<th>URL</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>The hemophilia A database</td>
<td><a href="http://europium.csc.mrc.ac.uk/">http://europium.csc.mrc.ac.uk/</a></td>
<td>Known as HAMSteRS (Hemophilia A Mutation Structure Resource Site), it contains, among other features, the following: 1. A fully searchable listing of all published mutations (and many unpublished ones) with phenotype data. 2. A molecular model of the A domains of factor VIII with links to tools for modeling any point mutation. 3. Detailed methods for mutation screening. 4. A comprehensive review of the molecular pathology of hemophilia A. 5. A list of polymorphisms with methods for detection.</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td><a href="http://www.kcl.ac.uk/lp/petergreen/haemBdatabase.html">http://www.kcl.ac.uk/lp/petergreen/haemBdatabase.html</a></td>
<td>A list of mutations compiled by an international consortium. The entire hemophilia B populations of several countries have been analyzed as the basis for mutation-specific carrier testing and antenatal diagnosis.</td>
</tr>
<tr>
<td>Von Willebrand disease</td>
<td><a href="http://mmg2.im.med.umich.edu/VWF/">http://mmg2.im.med.umich.edu/VWF/</a></td>
<td>Lists the published mutations found in types 1, 2A, 2B, 2M, 2N, and 5 von Willebrand disease.</td>
</tr>
<tr>
<td>Factor VII deficiency</td>
<td><a href="http://193.60.222.13/index.htm">http://193.60.222.13/index.htm</a></td>
<td>Tabulates the reported naturally occurring mutations and their associated phenotypes.</td>
</tr>
<tr>
<td>Human Gene Mutation Database-Cardiff</td>
<td><a href="http://www.cf.ac.uk/biosci/research/bioinfo/nuc.html">http://www.cf.ac.uk/biosci/research/bioinfo/nuc.html</a></td>
<td>Maintains an up-to-date listing of all published mutations excluding large deletions. Contains useful search functions and generates gene maps with locations of point mutations. Limited phenotype information.</td>
</tr>
<tr>
<td>European Bioinformatics Institute</td>
<td><a href="http://www.ebi.ac.uk/databases/">http://www.ebi.ac.uk/databases/</a></td>
<td>A project to link all mutation databases in a single unified relational database that enables integrated searching for features that, for example, might give clues to mechanism or phenotype correlation.</td>
</tr>
<tr>
<td>Haemophilia Forum</td>
<td><a href="http://www.haemophilia-forum.org/">http://www.haemophilia-forum.org/</a></td>
<td>Site for health care professionals who have specific questions about management of hemophilia generally or of a particular difficult case. A panel of experts answers posted questions.</td>
</tr>
<tr>
<td>Gene Therapy</td>
<td><a href="http://www4.od.nih.gov/oba">http://www4.od.nih.gov/oba</a></td>
<td>Established by the Recombinant DNA Advisory Committee of the Office of Biotechnology Activities at the National Institutes of Health, it contains a database of ongoing human gene transfer clinical trials, including hemophilia.</td>
</tr>
</tbody>
</table>
now licensed in the United States and Europe. One is formulated in sucrose instead of human albumin;\textsuperscript{15} another lacks the large B domain of the full-length protein but still retains coagulant activity.\textsuperscript{16,17} No human albumin is added in the final formulation of this product, and its manufacturing process includes a virus inactivation step (solvent/detergent). Third-generation preparations of FVIII manufactured and formulated without any exposure to human or animal proteins, with the exception of murine monoclonal antibodies, are currently in clinical trials. On the whole, newer recombinant FVIII products are perceived as an improvement because there is no other human protein in the final formulation but they are more expensive than albumin-formulated products (on average, 20\% more). No human or animal protein is used during the purification steps of recombinant FIX nor is any added to the final product for formulation. Pharmacokinetic and efficacy studies in previously treated patients gave satisfactory results,\textsuperscript{18} even though the in vivo recovery is substantially lower than that of plasma-derived factor, probably because of minor differences in the structure of the recombinant protein.

What further developments of recombinant coagulation factors can be expected? There are efforts to engineer forms of FVIII that preserve the functions of the molecule in coagulation but may be less likely to trigger the onset of inhibitors because the strongest and more frequent antigenic determinants are removed. Another potential approach is to produce molecules with a longer plasma half-life and/or greater coagulant activity. This approach should help to increase the intervals between doses, particularly for continuous prophylaxis. For instance, one might envisage the possibility of disrupting the sites in the FVIII molecule involved in its binding to the low-density lipoprotein receptor, the major hepatic mechanism for FVIII clearance from the circulation.\textsuperscript{19}

**Plasma-derived factors**

The current safety of these products depends on improved methods of virus inactivation and of screening plasma for the presence of viruses.\textsuperscript{20} The solvent/detergent mixture, widely used because it is highly efficacious in inactivating hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV, does not inactivate non-lipid-enveloped viruses like the hepatitis A virus (HAV)\textsuperscript{21} and B19 parvovirus.\textsuperscript{22} As a result, concentrate manufacturers now adopt at least two virus-inactivation procedures. To tackle the other determinant of safety, viral plasma load, a recent further step is based on screening plasma, pooled or as single units, using nucleic acid amplification assays for HIV, HCV and HBV. Some manufacturers also test for HAV and B19 parvovirus.

The outbreak of variant Creutzfeldt-Jakob disease (VCJD) in the United Kingdom has raised the fear that prion proteins might be contained in and transmitted by plasma coagulation factors and by human albumin used for manufacturing and formulation of first-generation recombinant factors.\textsuperscript{23,24} Several studies, carried out also in multitransfused hemophiliacs, have conclusively indicated that sporadic Creutzfeldt-Jakob disease (CJD) has not been transmitted by blood or its derivatives.\textsuperscript{25-28} However, these data on sporadic CJD cannot be taken as complete reassurance, because the agent of VCJD is a new emerging agent with limited epidemiological information. The tissue distribution of the agent of VCJD is different; the incubation period is different; and the number of blood donors potentially incubating the disease may be much higher than that of donors incubating sporadic CJD. No test to detect the presence of prions in blood or plasma has yet been developed, and currently used virucidal methods are definitely ineffective.\textsuperscript{29} However, the Cohn fractionation process used to purify plasma proteins, including albumin and coagulation factors, contributes significantly to clear prion strains, making it unlikely that this agent, even if present in plasma, would be carried into the final product at levels capable of causing VCJD.\textsuperscript{29} Nanofiltration, a process recently used to remove infectious agents in the production of factor concentrates, appears to be able to remove VCJD strains.\textsuperscript{29}

**Which one to choose**

The choice of the product for replacement therapy has to take into account three facts: plasma-derived factors are becoming ever safer; recombinant factors cost 2 to 3 times more than plasma-derived factors; and, most important, the production capacity for recombinant factors is still limited, and there was recently a period of dramatic shortage. On the other hand, recombinant factors are inevitably perceived as safer than plasma-derived factors. There are countries, like Canada and Ireland, that chose to switch practically all hemophiliacs to recombinant products. In these countries the recent shortage of recombinant FVIII has generated emotion and anxiety in persons with hemophilia because some of them have been obliged to return to plasma-derived factors. In the United States, approximately 70\% of severe hemophiliacs use recombinant products. European countries like Italy and the United Kingdom give priority to newly diagnosed, previously untreated hemophiliacs and then to those who have not developed bloodborne infections despite previous exposure to plasma-derived factors. These policies may change soon, as production increases and cost decreases. Finally, it should be reiterated that desmopressin is the treatment of choice in responsive patients with mild hemophilia A (and von
Willebrand disease). Its early adoption in Italy in the late 1970s to early 1980s, at the time of the onset of the HIV epidemic, has minimized the number of patients with mild hemophilia A who became infected. This number was much smaller than that of Italian patients with mild hemophilia B, who are unresponsive to desmopressin and therefore could be treated only with plasma-derived FIX.

Treatment of Patients with Inhibitors

Until the 1980s, the risk of death due to uncontrollable bleeding was high in hemophiliacs with inhibitors, particularly when emergency surgery was needed, and limb-threatening hemorrhages such as hemarthroses and muscle hematomas could not be treated effectively. Treatments that bypass the need for FVIII and FIX in muscle hematomas could not be treated effectively. SOS. This success rate is definitely lower than the trials have shown that both types of products are efficacious in controlling 50-60% of spontaneous bleeding episodes. This success rate is definitely lower than the 85-90% rate obtained with one or two doses of FVIII or FIX in hemophiliacs without inhibitors.

In the past few years a new bypassing product, recombinant activated factor VII (rFVIIa), has been licensed. It is thought to ensure hemostasis by binding, directly or in complex with tissue factor, to negatively charged phospholipids exposed on the surface of activated platelets. According to an alternative theory, the therapeutic effect is due to increasing the ratio of FVIIa to FVII. Cell localization of the enzymatic reactions that lead to the generation of factor Xa and ultimately of thrombin should reduce the risk of systemic coagulation activation and of thrombotic complications. However, thrombosis has been reported after the administration of rFVIIa, although it is infrequent. Infused as bolus at the recommended doses of 90-120 µg/kg, to be repeated 2 to 4 times at 2- to 3-hour intervals, rFVIIa is claimed to stop approximately 80% of spontaneous hemorrhages and prevent excessive bleeding during major surgical procedures. There are also experiences with continuous intravenous infusion, which is particularly useful for prolonged treatments and surgery, and with home therapy, which makes early intervention possible. On the whole, it would appear from the published reports that rFVIIa is nearly as effective as factor concentrates in patients without inhibitors, with a success rate close to 80%. Results of randomized controlled trials comparing the clinical efficacy and safety of this product with that of the less expensive plasma-derived products are still lacking; however, 2 studies are ongoing. The concept of suppressing the production of FVIII inhibitors by building tolerance in patients through repeated exposure to the antigen was first implemented by Brackmann, who administered FVIII, 100 U/kg, twice a day. Schedules of immune tolerance based on lower doses (25-50 U/kg) given at less frequent intervals are also apparently successful. These treatments do not eliminate the development of FVIII inhibitors but rather induce the production of neutralizing anti-idiotypic antibodies. Not all patients respond to immune tolerance, and outcome cannot be easily predicted. More than 20 years after the first study on immune tolerance, there is still little ground for choosing between high- and low-dose regimens, but an international randomized study is now addressing this issue.

A few newer approaches to the treatment and prevention of inhibitors are emerging. Experiments carried out as yet only in animal models indicate that idiotypic regulation may form the basis of new methods for the induction of long-term immune tolerance in patients with anti-FVIII antibodies (reviewed by Lacroix-Desmazes et al). Because anti-FVIII antibodies with inhibitory activity can be neutralized by anti-idiotypic antibodies, active immunization with idiotypic antibodies or with polypeptides that mimic idiotypes of anti-FVIII antibodies may generate anti-idiotypes capable of neutralizing the neutralizing activity of inhibitors. Another approach, so far examined only in animal models, is based upon the disruption of T-cell help by antigen-independent blockade of the interaction between B and T cells. Antibodies that quench the CD40-CD40L interaction or strategies targeting the B7-CDC 28 pathway are potential approaches to the neutralization of ongoing anti-FVIII immune responses. The use of these approaches to prevent the primary onset of inhibitors is less promising, because as mentioned earlier our capacity to identify patients at high risk is limited. Another potential approach may be the design of molecules that mimic the prevalent epitopes recognized by inhibitors in the FVIII molecule and that function as “inhibitor inhibitors.”

Secondary Diseases in Hemophilia

The hepatitis viruses and HIV can cause long-term consequences other than the development of cirrhosis or immunodeficiency, of which the most prominent is the development of tumors. In HIV-infected hemophiliac patients, Kaposi’s sarcoma is 200 times and non-Hodgkin’s...
Lymphoma 29 times more frequent. As lymphomas are associated with the increasing duration of HIV infection, they might become a prominent problem with the increased survival of HIV-infected patients. However, the advent of highly active antiretroviral therapy has profoundly improved the natural history and prognosis of HIV infection in patients with hemophilia, and it is hoped that this treatment may reduce the incidence of secondary cancers.

Cirrhosis of the liver, which develops in 10% to 20% of patients chronically infected with HCV and HBV, does increase the risk of hepatocellular carcinoma, the incidence of which is 30 times higher in hemophiliacs than in the general population. The combination of interferon and ribavirin has doubled the effectiveness of interferon alone in HCV infection and should be implemented in viremic hemophilic patients before they develop cirrhosis. The favorable experience with the combination of interferon and ribavirin is limited mainly to HIV-uninfected hemophiliacs. Perhaps it should now be studied in patients coinfected with HCV and HIV in whom antiretroviral treatment has stabilized HIV infection.

Gene Replacement Therapy

The hemophiliacs represent probably the best possible combination of features for a favorable response to gene replacement therapy in the whole of human genetic diseases. Clinical manifestations are entirely attributable to the lack of a single specific gene product; the gene product circulates in minute amounts in plasma; levels of FVIII and FIX do not require regulation; a minor increase in plasma levels will markedly ameliorate the symptoms of severe cases; murine and canine models of hemophilia are available; and FVIII and FIX do not have to be expressed in their normal tissue of synthesis but can be produced by any cell type provided the proteins can gain access to blood.

Early efforts focused on retroviral vectors, which proved to have many problems including the following: efficient insertion of vector occurs only in actively dividing cells; the inserted gene tends to be silenced, leading to rapid falloff in circulating factor levels; poor expression levels; the development of antibodies to foreign protein; concerns about insertional mutagenesis with vectors related to tumor-inducing viruses; and difficulty in obtaining sufficiently high titers of virus. These and other constraints have been gradually overcome, partly by switching to adenovirus, adeno-associated virus, and other vectors and partly by redesigning the inserts and promoters and by using novel delivery systems. Achievements in animal studies include sustained correction of hemophilia B in mice and dogs and of hemophilia A in mice and dogs.

Three gene therapy trials in humans with hemophilia A and B are now completed and 2 are ongoing (reviewed by Kelley et al57). The first Avigen study in patients with hemophilia B was a dose-escalating safety trial based upon the intramuscular injection in 8 adults of an adeno-associated-virus-based vector. A favorable effect on plasma levels of FIX (up to 3.7%) and/or concentrate usage was evident in 3 of 8 patients. Muscle biopsy demonstrated the presence of vector genome and expression of FIX in muscle fibers. The Chiron study treated patients with severe hemophilia A by peripheral vein injection of a vector based on a complement resistant murine leukemia retrovirus containing B-domainless FVIII cDNA. Levels of FVIII up to 6% lasting as long as 250 days were obtained in 6 of 13 patients, accompanied by reduced requirements for replacement factor. The study was stopped because small amounts of the transgene were detected in the sperm of 2 patients. Transkaryotic Therapies have removed fibroblasts from the skin of severe hemophilia A patients, grown them in culture, transfected them with B-domainless FVIII cDNA in a plasmid by electroporation, and reimplanted the autologous cells back into the omentum laparoscopically. Six patients with severe hemophilia A (all with HCV and 4 with HIV infection) have been treated; 3 of them had measurable levels of FVIII varying between 0.2% and 2%, with a maximum of 4% in 1 patient. The increase in FVIII was accompanied by a decreased bleeding frequency or transfusion requirements.

One of the two ongoing studies is that of GenStar and is based on the peripheral vein injection of a minimal (gutless) adenovirus containing B domainless FVIII cDNA and an albumin promoter to drive liver-specific expression. The treatment of the first patient was stopped because he developed systemic side effects probably related to the increased production of inflammatory cytokines, with FVIII levels of approximately 1%. A second patient has been recently put on treatment with a 10-fold lower dose. The second trial is that of Avigen and is based on the nonsurgical, radiological guided infusion into the hepatic artery of a recombinant adeno-associated virus vector driving a FIX minigene using an α-antitrypsin promoter and an apolipoprotein enhancer. The trial is on hold because the transgene was found in the sperm of the first 2 patients enrolled.

The preliminary data available on gene therapy for hemophiliacs, summarized in Table 3, are moderately encouraging, but several questions and issues remain. In terms of efficacy, the plasma levels of FVIII or FIX reached so far are insufficient to free patients from the need for infusion of exogenous factors. Sustained levels of at least 5% are needed largely to ameliorate the clinical phenotype and to guarantee that supplementary fac-
tors are required only for trauma or surgery. Promising new approaches currently in the preclinical phase are based on the insertion of a lentivirus vector similar to HIV but devoid of the genes involved in viral replication into CD34 stem cells, and on the use of homologous blood outgrowth endothelial cells as the target of gene transfer.

In terms of safety, no inhibitor developed in gene replacement trials, but this risk is still of concern, particularly in previously untreated patients, even though one can hope that the continued antigen presentation that occurs in gene therapy may decrease the likelihood of this complication. Small amounts of the viral genome detected in the semen of a few patients suggest that the risk of germline integration and passage of the vector to descendants cannot be ruled out, even though it appears that the rate of spontaneous mutations in the human germline is greater than the apparent rate of factor germline integration. From the latter standpoint, the ex vivo approach using transfected autologous fibroblasts or perhaps circulating endothelial cells seems to be potentially safer.

Conclusions
In the past two decades, hemophilia has moved from the status of a neglected and often fatal hereditary disorder to that of a defined group of molecular-pathological entities for which safe and effective treatment is available. Hemophilia is likely to be the first widespread severe genetic condition to be cured by gene therapy. Apart from the long-term consequences of viral infections caused by infected blood products and the still unknown and unquantifiable risk of transmission of VCJD, there seem to be two remaining major unsolved problems. In the clinical area there is the still difficult problem of the patient who develops high-titer antibody to FVIII or FIX. Perhaps the production of less antigenic forms of recombinant factors lacking the most common epitopes for inhibitors will in the future prevent this dire complication in newly diagnosed cases. In the socioeconomic arena it remains a challenge to humanity to know that 80% of the world’s hemophiliacs still receive no treatment at all. Production of FVIII and FIX in the milk of transgenic farmyard animals could provide a source of less expensive replacement therapy for developing countries. Affordable gene therapy will be the ultimate solution for hemophilia. Thus it may be confidently predicted that the early new millennium will see an end to this ancient scourge.

REFERENCES
4. Mannucci PM, Tuddenham EDG. The hemophiliacs—from royal


