DEVELOPMENT OF GENE THERAPY FOR BLOOD DISORDERS: AN UPDATE

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Short Title: Gene Therapy for Blood Disorders

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ABSTRACT

This review addresses the current status of gene therapy for the immunodeficiencies, chronic granulomatous disease, suicide gene therapy for graft versus host disease, viral infections, malignant hematological disorders, hemophilia and the hemoglobin disorders. New developments in vector design have fostered improved expression as well as enhanced safety, particularly of integrating retroviral vectors. Several immunodeficiencies have been treated successfully by stem cell targeted, retroviral-mediated gene transfer with reconstitution of the immune system following infusion of the transduced cells. In a trial for hemophilia B, long-term expression of human FIX has been observed following rAAV vector-mediated gene transfer into the liver. This approach should be successful in treating any disorder in which liver production of a specific protein is therapeutic.
The field of gene therapy as applied to blood disorders has continued to advance rapidly since my review was completed in 2008. That review provided a historical prospective and described the initial successes in treating severe combined immunodeficiency and the first evidence in humans of the genotoxicity of integrating retroviruses. Further success in clinical trials, particularly in the immunodeficiencies, and also the development of a successful approach for hemophilia B are among the recent clinical advances.

VECTORS

Much of the effort to develop clinical gene therapy has focused on viral vector systems for gene transfer. Viruses have naturally evolved to insert genetic information into target cells and the field of gene therapy attempts to take advantage of this proclivity. The basic strategy is to express the individual viral proteins on independent expression cassettes within producer cells and introduce the vector genome with the packaging signals and transgene either by transfection or transduction. There are a number of vector systems that are in wide use both experimentally and clinically as summarized in Table I.

**Adenoviral Vectors:** Vectors based on adenovirus have the advantage of having a large transgene capacity allowing large or multiple gene expression cassettes to be included within the vector genome. Adenoviral vectors have had limited use in the treatment of blood disorders although they have been quite effective in facilitating the generation of viral-specific, T-cell populations as discussed in detail in a later section.

**Adeno-associated Viral Vectors:** Adeno-associated viral vectors (rAAV) are less immunogenic than adenoviral vectors and are capable of transducing quiescent cells and establishing a stable episome without vector integration in most cases. Only the ITRs are required as part of the vector genome and packaging can be achieved with various strategies to develop high titer vector preparations. Further, the incidence of pre-existing immunity is generally low particularly for certain serotypes such as serotype 8 and 9. However, rAAV
can carry only a transgene up to 4700 nucleotides. The AAV genome is single stranded and vector preparations are composed of a mixture of vector particles having one of the two strands of the virus. Upon transduction, the required annealing of the two strands delays gene expression. This limitation can be overcome by utilizing a self-complementary design in which the two strands of the transgene are on a single hair pin genome in an inverted orientation which allows quick assembly into a transcription unit following transduction.\textsuperscript{14-15}

**Retroviral Vectors:** The gamma-retroviral vectors initially used in clinical trials had an intact LTR. The LTR enhance-promoter combination was associated with genotoxicity. However, subsequently self-inactivating vectors in which the enhancer-promoter are not present in the integrated vector genome have been shown to be clinically efficacious in the treatment of certain immunodeficiencies.\textsuperscript{22} Lentiviral vectors based on HIV-1 were developed largely in response to the challenge of transducing non-mitotic, hematopoietic stem cells.\textsuperscript{9} Although cytokine activation to trigger the cells into the G1 phase of the cell cycle is still required, mitoses is not required as the pre-integration complex (PIC) of HIV vectors can cross the nuclear membrane without mitosis.\textsuperscript{9} Another advantage of the HIV PIC is that it persists in transduced cells for an extended period prior to integration thereby allowing a longer window of opportunity for integration to occur. Foamy viruses, another retrovirus, do not cause disease and have not infected humans.\textsuperscript{16} Its genome integrates diffusely in cellular DNA rather than within genes or at regulatory elements (Table I). A foamy viral vector has been used to achieve stem cell targeted gene transfer and resolution of leukocyte adhesion deficiency in a canine model.\textsuperscript{16}

Alpharetroviral vectors are being considered for therapeutic gene transfer as well.\textsuperscript{17-20} Such vectors integrate more uniformly within the genome, particularly within intergenic regions compared to gammaretroviral vectors which are found near transcriptional start sites and CpG islands and lentiviral vectors which tend to integrate within genes.\textsuperscript{19} This favorable integration pattern has been shown to result in lower genotoxicity compared to lentiviral and gammaretroviral vectors.\textsuperscript{19} A split-packaging design for self-inactivating alpharetroviral vectors has been developed and shown to be capable of generating high-titer vector particles.\textsuperscript{17} The therapeutic
potential for this class of vectors has been shown by functional correction of chronic granulomatous disease in a
cell line as well as within transduced human cells that engraft in the mouse model.20

**Genotoxicity of Integrating Vectors:** Integrating retroviruses are inherently mutagenic.21-22 Enhancer
mediated, proto-oncogene activation has resulted in leukemia in a number of participants in the trials for
immunodeficiencies.4-7,21-23 Myelodysplasia has also been observed in participants in a trial for chronic
granulomatous disease secondary to proto-oncogene activation.24 Various efforts have been made to improve
the safety of integrating retroviral vectors. These include elimination of the enhancer element in the context of
a self-inactivating vector design, the use of internal cellular promoters with lower activation potential25,26 and
the addition of insulator elements to the LTRs of the integrated vector.27 Historically, the chicken beta-globin
locus insulator28 has been used but more recent studies have identified a number of additional elements that
have insulating potential.29-31

Various assays have been used to evaluate the steps taken to improve vector safety although, as a noted in a
recent review, none of the assays is fully predictive.21 One factor which may affect safety is the relative
distribution of vector integrations (Table 1). For example, lentiviral vectors are less likely to integrate into
regulatory elements than gamma-retroviral vectors.32,33 However, recent studies suggest preferred regions of
integration for both types of vectors so the difference in integration pattern is presumably only relative with
respect to safety.32 Also, the methodology for determining vector distribution is still imperfect.34 Alternative
mechanisms of oncogenesis include alternative splicing, gene inactivation, truncation of cellular mRNA or
protein and miRNA activation.21 Each of these mechanisms has been observed in animal models. Clonal
dominance developed in one participant in a gene therapy trial for thalassemia was associated with interruption
of the HMGA2 gene by the vector genome resulting in the generation of a truncated mRNA with missing
regulatory elements.35
GENE THERAPY FOR IMMUNODEFICIENCIES

Generally bone marrow transplantation is used to treat severe immunodeficiencies. The outcome for individuals with a matched related donor is outstanding (> 90%) but much less satisfactory for individuals who have a matched unrelated donor or a partially mismatched, related donor. For those patients, gene transfer offers a potentially more satisfactory alternative form of treatment. In early trials, T-lymphocytes were transduced but much of the recent work has focused on transducing bone marrow hematopoietic stem cells expressing the CD34+ phenotype.

X-SCID: X-linked severe combined immunodeficiency (SCID) reflects the lack of the common gamma-chain that is part of several interleukin receptors including the interleukin 7 receptor which is required for T-cell development. Affected patients have deficient T and NK cells and poorly functional B-cells. As recently reviewed, between 1999 and 2006 twenty individuals with X-SCID were treated in two gene therapy trials, one in Paris and one in London (Table II). All subjects lacked an HLA-identical donor. Seventeen of the twenty treated participants are alive and display nearly full correction of the T-cell deficiency by genetically modified T-cells when evaluated between 5 and 12 years after the gene transfer procedure. However, half of the trial participants remain on immunoglobulin replacement. The NK cell deficiency also persisted. Older participants with hypomorphic mutations responded less well to the gene therapy procedure, possibly because of loss of thymic function with advancing age. Unfortunately, five of the participants developed T-cell leukemia within three to six years after the gene transfer procedure. Four were successfully treated with standard antileukemic therapy and one died from refractory leukemia. Vector integration analysis identified insertions near the LM02 proto-oncogene in four participants. The leukemogenic process was thought to be initiated by vector-mediated proto-oncogene activation but other mutations must have occurred over time before evolution to full neoplasia. The initial clinical trials closed after the development of leukemia in the five participants.

Subsequently, a self-inactivating gammaretroviral vector has been developed for treatment of X-SCID and patients are again being enrolled. A self-inactivating lentiviral vector with an insulator element has also been
designed and shown directly not to elevate LM02 expression in T-cell.\textsuperscript{27} Two clinical trials utilizing this vector have also been opened and are enrolling participants.

**ADA-SCID:** Deficiency in adenosine deaminase leads to SCID. Over the years there have been several attempts to perform gene therapy in such individuals (Table II). The early efforts were unsuccessful presumably because of inadequate transduction of target T-cells or primitive hematopoietic cells. However, since 2000, forty patients have been treated in Italy, the UK and the USA.\textsuperscript{40-43} CD34\(^+\) cells were transduced with a gammaretroviral vector encoding the ADA gene. Low intensity conditioning with either Busulfan or Melphalan was required to allow engraftment of primitive stem cells with subsequent recovery from the immunodeficiency. Integration site analysis demonstrated vector insertions near proto-oncogenes, including LM02, but none of these patients developed leukemia. Moreover, only minor changes in transcriptional activity was observed in T-cell clones harboring 1 or 2 copies of the vector genome.\textsuperscript{44}

**WAS:** Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by microthrombocytopenia, immunodeficiency, eczema and a proclivity to develop an autoimmune disorder and/or lymphoma secondary to a mutation in the WAS protein (WASp) gene.\textsuperscript{5,45} The disease is variably severe depending on the exact nature of the mutation in the WASp gene but individuals with early onset-severe forms have been identified and are the appropriate candidates for attempted gene therapy.\textsuperscript{46} An initial clinical trial was conducted in Hanover, Germany, using a gammaretroviral vector in which the LTR enhancer/promoter combination drove expression of the WASp gene (Table II).\textsuperscript{23} Gene transfer was targeted to bone marrow and/or peripheral blood CD34\(^+\) cells ex vivo and participants received myelosuppression prior to re-infusion of the transduced cells. Although nine of the ten participants that were enrolled did well clinically for several years, ultimately four developed leukemia secondary to insertional mutagenesis.\textsuperscript{4,7} Subsequently, self-inactivating lentiviral vectors were developed by a number of groups.\textsuperscript{25,47-50} Vectors have been tested in which the WASp coding sequences are under the control of various promoters.\textsuperscript{49} Full correction of the WAS phenotype was achieved in the murine model with the vector having a retroviral promoter whereas only partial correction was achieved with the vector.
having the 1.6 kb WAS promoter fragment. We have also found that the gammaretroviral promoter is much stronger than either the EF1α or the WAS 1.6 kb promoter in various model systems. Several other studies suggested that a 1.6 kb promoter fragment from the WASp gene achieves adequate levels for correction in the preclinical model as well as normal levels of expression in human CD34+ cells. Methodology for large scale manufacture of the lentiviral vector has permitted the initiation of a number of clinical trials. A recent report describes correction of the immune deficiency in three participants in a clinical trial who have now been followed up to 18 months after the gene therapy procedure.

**CGD:** Chronic granulomatous disease reflects a deficiency in neutrophil function due to mutations in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that result in chronic bacterial and fungal infections. The clinical characteristics of the disease have recently been reviewed in detail. NADPH is a complex of five separate proteins, each of which may be deficient and cause the CGS phenotype although the most common defect is in gp91phox. Early trials done without myeloablation or limited myeloablation resulted in only transient production of genetically modified neutrophils although a clinical benefit with clearance of a chronic infection was noted in several of the early patients. The initial clinical trials used gammaretroviral vectors with an intact LTR and, not surprisingly, proto-oncogene activation occurred resulting in myelodysplasia in all of the successfully treated participants (Table II). In contrast to X-SCID, ADA-SCID and WAS, the gene corrected cells in CGD do not have an engraftment advantage and thus, as treatment efforts evolve, regimens are likely to be more fully myeloablative. In an effort to achieve greater safety, lineage and stage restricted lentiviral vectors are being developed for treatment of CGD. Other vector systems are also being considered although they are in much earlier stages of development.

**LEUKODYSTROPHIES**

Many leukodystrophies are potential candidates for treatment by gene therapy. To date, two have been studied in detail, X-linked adrenoleukodystrophy (X-ALD) and metachromatic leukodystrophy (MLD).
ALD is caused by mutations in the ABCD1 gene that encodes a transporter protein. This deficiency leads to the accumulation of very long chain, fatty acids in plasma and tissue and progressive demyelination in the central nervous system. X-ALD has been successfully treated with hematopoietic stem cell transplantation although cessation of progression of the disorder occurs after 12-18 months after treatment. To date, four patients have received autologous hematopoietic stem cells transduced with the lentiviral vector encoding the transporter (Table II).\textsuperscript{64} Arrest of progression of the disorder occurred in the first two patients that were treated.\textsuperscript{62} Over time, their percentage of genetically modified myeloid and lymphoid cells stabilized at about 10%. The favorable outcome following stem cell transplantation, whether allogeneic or autologous and gene corrected, is thought to rely on the migration of monocytes/macrophages into the CNS and subsequent conversion to microglia that are long-lived and provide the therapeutic benefit. Integration site analysis in these two participants of the trial demonstrated the typical lentiviral pattern with no evidence of proto-oncogene activation.\textsuperscript{65}

MLD is an inherited autosomal recessive disorder secondary to a deficiency of the lysosomal enzyme, arylsulfatase A.\textsuperscript{62} Massive accumulation of non-metabolite sulfatides damages both the central and peripheral nervous systems. A mouse knock-out model of MLD has been developed and used for the exploration of gene therapy approaches.\textsuperscript{66} Stem cell targeted gene transfer followed by autologous transplantation is one approach.\textsuperscript{67} An alternative which is also being explored in the mouse model as is the direct injection of AAV vectors encoding ARSA into the central nervous system.\textsuperscript{68} Early correction is essential as the most common form of MLD develops in the second year of life with rapid, progressive CNS dysfunction. Thus, the introduction directly of AAV vectors into the brain seems preferable than stem cell targeted gene transfer in that correction of the phenotype with the latter approach requires several months during which the patients continue to deteriorate.
SUICIDE GENE THERAPY FOR GRAFT – VERSUS – HOST DISEASE

The basic strategy with this approach is to genetically modify donor T-cells ex vivo before infusion for enhancement of engraftment and prevention of viral infections. GVHD may develop in this context so that methodologies have been evolved to introduce a suicide gene into the T-lymphocytes before infusion into patients. By the time the T-cells are ablated, engraftment has been established and immunity is generally sufficient to protect from infections. A number of genetic systems have been used or explored experimentally.69 Two have been tested in clinical trials, one with a vector having the thymidine kinase gene and post-treatment is with ganciclovir.69 The other system involves Caspase-9.70 T-cells containing this gene can be abrogated using a dimerizing drug (AP1903). Overall, this approach shows promise for control of GVHD.

VIRAL INFECTIONS

Adoptive transfer of T-lymphocyte populations has been used as a strategy for preventing and treating viral infections in immunocompromised individuals.71 Adenoviral transduced, antigen-presenting cell lines were used to derive the T-cell specific populations to treat or prevent viral infections in stem cell transplant recipients.71,72 More recent studies rely on the generation of cytotoxic T-lymphocytes using immunostimulatory cells that have been transduced with peptide mixtures of the viral antigens.73,74 Current efforts to use gene transfer for prevention or treatment of viral infections are focused on AIDS.75-77 The goal in these efforts is to introduce one or more genetic elements into autologous hematopoietic stem cells that are then used to reconstitute the hematopoietic system of the AIDS patient to create a population of HIV resistant T-cells. Several studies have focused on the knock-down or knock-out of the CCR5 gene as it encodes a potent co-receptor for HIV infection. Inhibition of critical processes such as viral entry or replication reflects an alternative strategy of stem cell modification for HIV therapy.75

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MALIGNANT HEMATOLOGICAL DISORDERS

A number of creative approaches have been developed to use gene transfer in the context of treating cancer.\textsuperscript{1,78} These include the generating of T-cell populations in vitro with specificity for antigens expressed on tumor cells for reinfusion, the development of tumor vaccines by expressing genes that enhance the immune response to injected tumor cells and the creation of chimeric antigen receptors (CARs) on T-cells in which a single chain antibody with specificity for an antigen expressed on human tumor cells linked to internal domains which participate in cell activation.\textsuperscript{78,79} Remission has been achieved in patients with B-lymphoid malignancies who received chimeric antigen receptor-modified T-cells (Table II).\textsuperscript{80-82} Fortunately, no evidence of T-cell transformation has been observed in these patients although the period of observation is still rather short. Oncolytic adenoviruses with relative tumor cell specificity are being developed for a variety of cancers.\textsuperscript{83} Species D adenovirus oncolytics have been shown to infect and kill B-cancer cells but development of genetic variants to enhance activity has not yet been reported nor have clinical trials begun to date.\textsuperscript{84} Another strategy to use gene transfer to enhance cancer treatment involves the transfer of a drug resistance gene into autologous hematopoietic stem cells that are given to patients in the context of myelosuppression. Treatment is then given to amplify the genetically modified cell populations.\textsuperscript{85} This strategy has been tested in a small clinical trial that demonstrated that intensification of chemotherapy was feasible and there was an apparent improvement in outcome.\textsuperscript{86} Overall, gene transfer technology continues to offer many opportunities to improve the treatment of individuals with malignant hematopoietic disorders.

HEMOPHILIA

Several different approaches have been considered for using viral vectors to achieve therapy for hemophilia.\textsuperscript{1,87} Over the years, Amit Nathwani at University College London and Andrew Davidoff at St. Jude Children’s Research Hospital, have shown that intravenous injection of rAAV8 particles resulted in long-term hepatic production of human factor IX in the rhesus macaque model. These observations have now been translated into
a clinical trial (Table II). To date, ten participants have been enrolled and all show evidence of vector-derived FIX production. The length of follow-up ranged from 6.7 months to 3.3 years. The FIX levels varied from 1% to as much as 8 or 9% in the individual patients at different times with participants that received the highest dose having the higher levels. Seven have stopped routine prophylaxis with recombinant FIX and the remaining three individuals have reduced the frequency of recombinant protein infusion. The only complication in the trial was the development of transient elevation in transaminases in one and perhaps in two other participants which was abrogated with a short course of Prednisolone. Overall, these results are highly encouraging with respect to application of gene therapy for FIX deficiency. Efforts are underway to modify the AAV capsid to reduce its immunogenicity. Despite the larger size of the FVIII gene, an AAV vector has been constructed with a modified coding sequence capable of being packaged and transmitting an expressible FXIII gene. A clinical trial for hemophilia A is in the early stages of planning.

HEMOGLOBIN DISORDERS

The hemoglobin disorders, severe β-thalassemia and sickle cell anemia, were identified early as potential targets for therapeutic gene transfer. However, despite considerable effort over the years to develop vector systems and to test them in animal models, progress into the clinic has been limited and not fully successful. We have estimated that it would require 20% of the primitive hematopoietic cells to be genetically modified to achieve a definitive therapeutic benefit. Although levels of gene transfer of 7-14% were observed in two participants in a clinical trial for X-linked adrenoleukodystrophy, comparable levels have not been achieved in individuals with thalassemia. In one small trial, two patients failed to engraft with the transduced stem cells and had to receive backup bone marrow for hematopoietic rescue (Phillip Leboulch, personal communication). The other participant developed significant amounts of the transgene globin product but this apparently primarily reflected dominance by a large clone having a retroviral integration into the HMG2A gene. This participant remains transfusion independent for a period now up to 7 years.
There has been a long standing interest in reactivating the fetal gamma-globin gene in an effort to compensate for deficient beta-globin synthesis in thalassemia or to inhibit sickling in patients with sickle cell anemia.\textsuperscript{91,92} Efforts in this area were advanced with the discovery that BCL11A is a major regulator of developmentally stage-specific repression of the gamma-globin gene.\textsuperscript{93} Genetic deletion of BCL11A has been used to correct sickle cell disease in adult mice by interference with fetal hemoglobin silencing.\textsuperscript{94} In addition, therapeutic levels of fetal hemoglobin have been achieved in erythroid progeny of beta-thalassemic cells after lentiviral vector mediated gene transfer of an inhibitory BCL11A shRNA.\textsuperscript{95} Efforts to advance these preclinical observations to small scale clinical trials are of great interest.

FANCONI ANEMIA

Fanconi anemia is an inherited bone marrow failure disorder characterized by aplastic anemia and an enhanced risk for the development of leukemia. The syndrome may occur as a consequence of a defect in one of at least fifteen genes. The development of gene therapy approaches for this disorder have focused on the protein encoded by the FANCA gene\textsuperscript{96,97} which is most commonly mutated. An international working group has been established to chart the path and facilitate the development of gene therapy for Fanconi anemia.\textsuperscript{96,97} Gene therapy for patients with Fanconi anemia is particularly challenging because of low numbers of hematopoietic stem cells and sensitivity to myelosuppressive regimens.

Nuclease – mediated correction of blood disorders

Over the past decade, the use of chimeric nucleases to create specific double stranded breaks to alter specific sites in the genome has progressed significantly.\textsuperscript{98} Several classes of enzymes have been developed that enable a broad range of genetic modification by inducing DNA double strand breaks that stimulate error-prone non-homologous end joining or homology-directed repair at specific genomic locations.\textsuperscript{99} Various viral vectors
including AAV,\textsuperscript{100} lentiviral\textsuperscript{101} and adenoviral vectors\textsuperscript{102} have been adapted for various purposes to achieve gene editing in target cells. The methodology has been used to establish HIV-1 resistance in T-cells by disrupting the HIV co-receptor, CCR5.\textsuperscript{103} The mutation that results in synthesis of a sickle beta-globin has been corrected in human iPS cells providing proof of principal that this is a potentially viable approach.\textsuperscript{104-106} The nuclease methodology has also been used to achieve correction of alpha-thalassemia in iPS cells\textsuperscript{107} as well as genetic correction of beta-thalassemia mutations in such cells.\textsuperscript{108} Another potential application is the creation of genomic safe harbors that facilitate high beta-globin transgene expression. Genomic safe harbors are identified by screening of induced pluripotent stem cells from thalassemic individuals to identify clones with favorable integration sites.\textsuperscript{109} Basic research continues to increase the repertoire of target sites amenable to genetic modification.\textsuperscript{110} This technology could also be applicable to other blood disorders such as immunodeficiencies or chronic granulomatous disease.
ACKNOWLEDGEMENTS

I wish to thank Derek A. Persons and Brian P. Sorrentino for their review of the manuscript and for providing useful suggestions for improvement. I thank Pat Streich for her assistance in the preparation of this manuscript. This work was supported by the National Heart, Lung, and Blood Institute (grant P01HL 53749 and grant R01DK095169). We also received funding support from the Assisi Foundation (94-000 R12) and the American Lebanese Syrian Associated Charities.

AUTHORSHIP CONTRIBUTIONS

A.W.N. wrote and approved the manuscript.

CONFLICT-OF-INTEREST DISCLOSURE

No conflict of interest(s) to declare.
References


**Table I. Comparison Of The Most Commonly Used Vectors For Gene Therapy**

<table>
<thead>
<tr>
<th></th>
<th>Alpharetroviral Vector</th>
<th>Gammaretroviral Vector</th>
<th>Foamy Virus Vector</th>
<th>Adeno-associated Virus Vector</th>
<th>Lentivirus Vector</th>
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<tr>
<td><strong>Tropism</strong></td>
<td>Dividing &amp; non-dividing cells</td>
<td>Dividing cells</td>
<td>Dividing &amp; non-dividing cells</td>
<td>Dividing &amp; non-dividing cells</td>
<td>Dividing &amp; non-dividing cells</td>
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<tr>
<td><strong>Host Genome</strong></td>
<td>Neutral</td>
<td>Integration near regulatory elements</td>
<td>Integration nearly uniform</td>
<td>No integration</td>
<td>Integration in genes</td>
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<tr>
<td><strong>Transgene Expression</strong></td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable in non-dividing cells</td>
<td>Stable</td>
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<tr>
<td><strong>Packaging Capacity</strong></td>
<td>Not defined</td>
<td>~8 kb</td>
<td>~9 kb</td>
<td>~5 kb</td>
<td>~8 kb</td>
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<tr>
<td><strong>Advantages</strong></td>
<td>Neutral, integration, pattern high titer production</td>
<td>Large packaging capacity; long-term expression</td>
<td>Large packaging capacity; long-term expression</td>
<td>High production yields; low immunogenicity; long-term expression</td>
<td>Large packaging capacity; long-term expression</td>
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<tr>
<td><strong>Disadvantage</strong></td>
<td>None have emerged</td>
<td>High risk of insertional mutagenesis</td>
<td>Risk of insertional mutagenesis</td>
<td>Small packaging capacity</td>
<td>Risk of insertional mutagenesis</td>
</tr>
</tbody>
</table>
Table II. Ongoing Gene Therapy Studies for Blood Disorders*

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Vector, dose range, and number and ages of patients</th>
<th>Transgene and promoter</th>
<th>Route of administration and cell target</th>
<th>Scientific and clinical outcomes</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Hemophilia B</td>
<td>AAV8; $2 \times 10^{11}$, $6 \times 10^{11}$ or $2 \times 10^{12}$ vg per kg body weight; six patients (27–64 years old)</td>
<td>FIX gene, regulated by the human apolipoprotein hepatic control region and human α-1-antitrypsin promoter</td>
<td>Intravenous delivery targeting hepatocytes</td>
<td>Durable circulating Fix at 2–11% normal levels; decreased frequency (two of six patients) or cessation (eight of ten) of spontaneous hemorrhage</td>
<td>8</td>
</tr>
<tr>
<td>X-linked severe combined immunodeficiency (SCID-X1)</td>
<td>Gammaretrovirus; ten patients (4–36 months old); CD34+ cells were infused (without conditioning) at doses of $60 \times 10^6$ to $207 \times 10^6$ cells per patient</td>
<td>Interleukin-2 receptor common γ-chain, retroviral LTR</td>
<td>Ex vivo, CD34+ hematopoietic stem and progenitor cells</td>
<td>Functional polyclonal T-cell response restored in all patients; one patient developed acute T-cell lymphoblastic leukemia</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Gammaretrovirus; nine patients (1–11 months old); CD34+ cells were infused (without conditioning) at doses of $1 \times 10^6$ to $22 \times 10^6$ cells per kg</td>
<td>Interleukin-2 receptor common γ-chain, retroviral LTR</td>
<td>Ex vivo, CD34+ hematopoietic stem and progenitor cells</td>
<td>Functional T-cell numbers reached normal ranges. Transduced T cells were detected for up to 10.7 years after gene therapy. Four patients developed acute T-cell lymphoblastic leukemia, one died.</td>
<td>38</td>
</tr>
<tr>
<td>Condition</td>
<td>Route of gene delivery</td>
<td>Ex vivo stem cells</td>
<td>Details</td>
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<td><strong>Adenosine deaminase deficiency resulting in severe combined immunodeficiency (ADA-SCID)</strong></td>
<td>Gammaretrovirus; six patients (6–39 months old); CD34+ cells were infused (after non-myeloablative conditioning with melphalan (Alkeran), 140 mg per m² body surface area, or busulfan (Myleran), 4 mg per kg) at doses of &lt;0.5 × 10⁶ to 5.8 × 10⁶ cells per kg</td>
<td>Adenosine deaminase gene, retroviral LTR</td>
<td>Restoration of immune function in four of six patients; three of six taken off enzyme-replacement therapy; four of six remain free of infection</td>
<td>40</td>
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<tr>
<td><strong>Adenosine deaminase deficiency resulting in severe combined immunodeficiency (ADA-SCID)</strong></td>
<td>Gammaretrovirus; ten patients (1–5 months old); CD34+ cells were infused (after non-myeloablative conditioning with busulfan, 4 mg per kg) at doses of 3.1 × 10⁶ to 13.6 × 10⁶ cells per kg</td>
<td>Adenosine deaminase gene, retroviral LTR</td>
<td>Nine of ten patients had immune reconstitution with increases in T-cell counts (median count at 3 years, 1.07 × 10⁹ l⁻¹) and normalization of T-cell function. Eight of ten patients do not require enzyme-replacement therapy.</td>
<td>42</td>
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<tr>
<td><strong>Chronic granulomatous disorder</strong></td>
<td>A range of studies, using gammaretrovirus vectors pseudotyped either with gibbon ape leukemia virus envelope or with an amphotrophic envelope; various non-myeloablative conditioning strategies</td>
<td>Gp91phox, retroviral LTR</td>
<td>Twelve of twelve patients showed short-term functional correction of neutrophils with resolution of life-threatening infections. Three patients developed myeloproliferative disease.</td>
<td>57</td>
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<tr>
<td>Disorder</td>
<td>Treatment</td>
<td>Outcome</td>
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<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>Gammaretrovirus; ten patients; CD34+ cells were infused (after non-</td>
<td>Nine of ten patients showed improvement of immunological function and</td>
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<td>myeloablative conditioning with busulfan, 4 mg per kg)</td>
<td>platelet count. Four patients developed acute T-cell lymphoblastic</td>
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<td>WAS gene, retroviral LTR</td>
<td>leukemia.</td>
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<td>β-thalassemia</td>
<td>Self-inactivating HIV-1–derived lentivirus; one patient (18 years old)</td>
<td>Patient has been transfusion independent for 7 years. Blood hemoglobin</td>
<td>35</td>
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<td>received fully myeloablative conditioning with busulfan; 3.9 × 10^6</td>
<td>is maintained between 9 and 10 g dl⁻¹, of which one-third contains</td>
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<td>CD34+ cells per kg</td>
<td>vector-encoded β-globin.</td>
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<td>Mutated adult β-globin (β₄₁₋₈₀) with anti-sickling properties,</td>
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<td>LCR control</td>
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<td>Adrenoleukodystrophy</td>
<td>Self-inactivating HIV-1–derived lentivirus; two patients (7 and 7.5 years</td>
<td>9–14% of granulocytes, monocytes, and T and B lymphocytes expressing the</td>
<td>62-64</td>
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<td>old) received myeloablative conditioning with cyclophosphamide</td>
<td>ALD protein; beginning 14–16 months after infusion of the genetically</td>
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<td>(Cytoxan) and busulfan; transduced CD34+ cells, 4.6 × 10^⁶ and 7.2 ×</td>
<td>corrected cells, progressive cerebral demyelination in the two patients</td>
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<td>10^⁶ cells per kilogram, respectively. Two younger patients also</td>
<td>attenuated.</td>
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<td>treated with short-term follow-up.</td>
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<td>Wild-type ABCD1 cDNA under the control of the MND viral promoter</td>
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<td>Ex vivo, CD34+ hematopoietic stem and progenitor cells</td>
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<td>Gene therapy for cancer</td>
<td>B-cell leukemia and lymphoma</td>
<td>Self-inactivating lentivirus expressing a chimeric T cell receptor; a single patient was conditioned with pentostatin (Nipent; 4 mg per m²) and cyclophosphamide (600 mg per m²) before receiving $1.5 \times 10^5$ transduced T cells per kg (total $3 \times 10^8$ T cells, of which 5% were transduced)</td>
<td>Anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and transmembrane domain, and human 4-1BB and CD3ζ signaling domains</td>
<td>Ex vivo, autologous T cells, i.v. infusion, split over 3 d</td>
<td>Transduced T cells expanded more than 1,000 times in vivo, with delayed development of the tumor lysis syndrome and complete remission, ongoing 10 months after treatment. Engineered cells persisted at high levels for 6 months in the blood and bone marrow.</td>
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<td>Murine stem cell virus–based splice-gag (retroviral) vector expressing CD19 CAR; eight patients (47–63 years old) with progressive B-cell malignancies received cyclophosphamide and fludarabine (Fludara) before CAR-transduced autologous T cells and interleukin 2. Patients received $0.3 \times 10^7$ to $3.0 \times 10^7$ CAR+ T cells per kg, of which an average of 55% were transduced.</td>
<td>Anti-CD19 scFv derived from the FMC63 mouse hybridoma, a portion of the human CD28 molecule and the intracellular component of the human TCR-ζ molecule</td>
<td>Ex vivo, autologous T cells, single i.v. infusion, followed (3 h) by a course of IL2</td>
<td>Varied levels of anti–CD19-CAR–transduced T cells could be detected in the blood of all patients. One patient died on trial, with influenza A pneumonia, nonbacterial thrombotic endocarditis and cerebral infarction. Four patients had prominent elevations in serum levels of IFNg and TNF, correlating with severity of acute toxicities. Six of the eight patients treated obtained objective remissions.</td>
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<td>Acute leukemia</td>
<td>SFG retrovirus expressing an inducible suicide system for improved safety of stem cell transplantation to prevent graft-versus-host disease (GVHD); transduced haploidentical T cells (1 × 10⁶ to 1 × 10⁷ T cells per kg); five patients (3–17 years old)</td>
<td>FK506-binding protein linked to modified human caspase 9 with truncated CD19 as a selectable marker; in the presence of the drug, the iCasp9 promolecule dimerizes and activates apoptosis; retroviral LTR</td>
<td>Ex vivo, allodepleted haploidentical T cells, infused i.v. into recipients of allogeneic bone marrow transplants.</td>
<td>The genetically modified T cells were detected in peripheral blood from all five patients and increased in number over time. A single dose of dimerizing drug, given to four patients in whom GVHD developed, eliminated more than 90% of the modified T cells within 30 min after administration and ended the GVHD without recurrence.</td>
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* Adapted from Seymour and Thrasher."
Development of gene therapy for blood disorders: an update

Arthur W. Nienhuis