



Gene Therapy

The Cutting Edge of PI Treatment

Some PIs are currently being treated and cured with gene therapy, and as advancements with this life-saving technology progress, there is hope for many others.

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Gene therapy, or the use of genetic material such as DNA or RNA to treat disease, has received growing attention in recent years and is becoming a recognized form of medical therapy. Mere fanciful imagination a few decades ago, the idea of manipulating or repairing genes has evolved from science fiction to a real hope for those with serious incurable diseases, including primary immunodeficiencies.

History of Gene Therapy

The concept of gene therapy developed during the 1960s, when scientists discovered enzymes that could essentially cut and paste DNA, allowing them to manipulate and potentially repair sequences of genetic material in a test tube. Around the same time, immortal cell lines were also developed that permitted testing of these DNA sequences, demonstrating that modified foreign DNA could be reintroduced into an individual in a stable manner. In addition, manipulated, or “transformed,” cells could continue to grow while permanently maintaining the change in their genome. This set the stage for realizing that gene manipulation and repair was indeed possible.

Although the transfer of genetic material into cells was met initially by frustrating technical difficulties, pioneering researchers observed that virally infected cells stably inherited small amounts of genetic information from the virus. This led to the hypothesis that it might be possible to modify viruses to transfer therapeutic genes instead of the virus’ own genes. Since then, many gene therapy studies have utilized these modified viral carriers (adenovirus, retrovirus, lentivirus) to insert corrected gene sequences, thus creating a transgene. These modified viruses are no longer infectious, but they retain their inherent ability to enter cells and desirably insert the “corrected gene” in the right location. These discoveries set the stage for future experiments in gene therapy to address human disease, allowing scientists to correct disease-causing DNA mutations in the laboratory, package them into viral vectors (the tools used to deliver genetic material into cells), and deliver the corrected transgene into diseased cells.

Over time, it has been learned that a number of factors need to be taken into consideration before attempting gene therapy: 1) The defective gene(s) need to be identified and their location carefully mapped out, which is not as simple as it sounds. 2) In many diseases in which there is a defect in manufacturing a functional protein (proteins within a cell determines its health and function), there may be multiple genes regulating different portions of a large

protein. What this means is that even if it were possible to repair the defective gene, the final protein product may not be fully functional. For instance, in X-linked lymphoproliferative syndrome characterized in males by fatal susceptibility to the Epstein Barr virus, there may be missense (an incorrect genetic code sequence) or insertional mutations (permanent transmissible changes in the genetic material) that impair the function of a vital protein or prevent its manufacturing altogether. Therefore, not only is there the enormous problem of identifying one of potentially many mutations in a gene, but there also is the problem of inserting it in the correct position in the chromosome and ensuring that it is stable and functions correctly. At best, the procedure might work, thus resulting in manufacturing proteins that function correctly. At worst, the gene might be inserted in a wrong location or incorrectly, resulting in malignant or defective cells.

Although the first attempt at gene therapy in humans did not result in a permanent cure, it established the foundation that using DNA as a form of treatment could be safe and that the potential to cure disease existed.

Therefore, it became clear that the ideal target disease for initial gene therapy is one in which blood cells can be used (rather than heart, liver or brain cells), and one that has one and not multiple gene mutations, and in which insertion is “technically simple.” Severe combined immunodeficiency (SCID) is one such disease. Thus, an initial trial was attempted in a 4-year-old girl with adenosine-deaminase deficiency (a variant of the “bubble boy” disease known as SCID), which is characterized by profound T-cell deficiency and early death from overwhelming infection. Without the ADA enzyme, she could not detoxify DNA byproducts that damaged her T cells, causing profound

immunodeficiency. In 1990, physician scientists at the National Heart, Lung and Blood Institute and the National Cancer Institute inserted the gene into defective white blood cells collected from the patient and re-infused these “corrected cells,” which then produced ADA, thus detoxifying fatal byproducts. The treatment was safe and the corrected cells were able to produce ADA enzyme, but the cells were not long-lived, and the patient continued to require intermittent gene therapy, as well as additional treatment with exogenous ADA enzyme replacement.

Although the first attempt at gene therapy in humans did not result in a permanent cure, it established the foundation that using DNA as a form of treatment could be safe and that the potential to cure disease existed. Since that time, there have been significant advances in understanding gene delivery methods and the ability to stably correct genetic defects in multiple cell types such as bone marrow stem cells, tumor killing T cells, muscle cells and many others.

Safety of Gene Therapy

Work in gene therapy boomed after the initial clinical trial for ADA deficiency. However, in 1999, an 18-year-old male who enrolled in a protocol for treatment of ornithine

Although the general concept of gene therapy is simple — replace or fix a mutated gene to allow proper protein expression — carrying out this process is highly complex. While viral vectors can efficiently transfer modified DNA, certain viruses can insert the genetic material at actively expressed sites that may result in abnormal proliferation (the growth of cells), called insertional oncogenesis. Therefore, efforts in using a more targeted approach for gene therapy and additional modifications to viral vectors have made gene transplantation safer, but not necessarily failsafe.

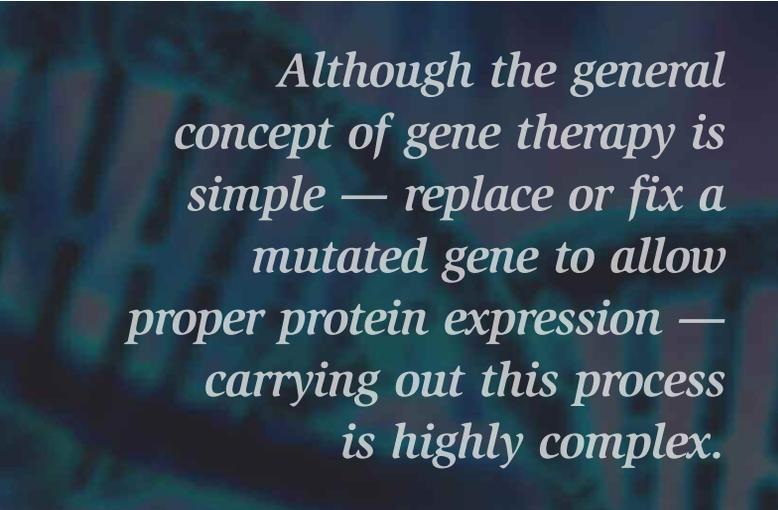
While it is understandable that patients and doctors are desperate for a genetic cure for potentially fatal diseases, many years of pre-clinical work are required to ensure and confirm the safety profile of gene therapy agents before they are allowed in clinical trials. Additionally, there has been heightened monitoring for malignant (deadly) events. Adequate safeguards are in place to control gene delivery and hopefully pre-empt gene therapy-related cancers. The Food and Drug Administration (FDA) and Recombinant DNA Advisory Committee (RAC) are intimately involved in trials involving gene therapy and play important roles in maintaining rigorous scientific, as well as ethical, standards in the field.

Clinical Trials Worldwide

More than 1,800 gene therapy clinical trials are either ongoing or have been completed worldwide involving 31 countries. The large majority of trials have been conducted in the United States, followed by Europe and, increasingly, Asia. The diseases most commonly addressed are cancer, cardiovascular disease and monogenic diseases that are due to a single, identifiable gene defect.

Thus far, most clinical trials (60 percent) have revolved around treating cancer, including those that affect the pulmonary, neurologic, gastrointestinal, hematologic and dermatologic systems. Multiple strategies have been employed in this approach such as inserting genes that are known to suppress tumors or engineering immune cells to specifically recognize and kill an individual's malignant cells. These clinical trials have shown early promise.

Advancements have also occurred in monogenic diseases, with more than 160 clinical trials conducted to date. Cystic fibrosis, which is commonly inherited in the U.S. and Europe and carries a life expectancy of less than 40 years, as well as SCID, which is generally fatal within the first year of life if untreated, are examples of gene therapy attempts that are actively being pursued and show



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transcarbamylase (OTC) deficiency died due to a severe immune reaction to the adenovirus carrier used to insert the correct gene. This was the first time death could be directly associated with the viral vector, and patient safety issues remain a priority for any gene therapy research or clinical trial to this day, accounting for what seems to be slow progress clinically.



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*Mary Elizabeth M. Younger,
CRNP, PhD*

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Important Safety Information

Hizentra treats various forms of primary immunodeficiency (PI) in patients age 2 and over.

WARNING: Thrombosis (blood clotting) can occur with immune globulin products, including Hizentra. Risk factors can include: advanced age, prolonged immobilization, a history of blood clotting or hyperviscosity (blood thickness), use of estrogens, installed vascular catheters, and cardiovascular risk factors.

If you are at high risk of thrombosis, your doctor will prescribe Hizentra at the minimum dose and infusion rate practicable and will monitor you for signs of thrombosis and hyperviscosity. Always drink sufficient fluids before administration.

Please see additional Important Safety Information on reverse side and brief summary of full prescribing information for Hizentra, including boxed warning, on adjacent page.

Hizentra[®]
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Steady State / Low Volume:

Hizentra delivers steady-state levels in half the volume of 10% solutions*

Confidence:

More than 10,000 patients and providers put their confidence in Hizentra 20%.¹ Hizentra has demonstrated safety and tolerability in pediatric through geriatric patients (age 2 years and older)

Individualized Dosing:

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**Based on an equivalent dose in grams.*



Hizentra Co-Pay Relief Program

The Hizentra Co-Pay Relief program, created to assist Hizentra patients, reflects CSL Behring's commitment to patient care. We are proud to offer this assistance to eligible patients as part of our continuing effort to provide patients with the best care possible.

To see if you are eligible for the Hizentra Co-Pay Relief program, please call IgIQ® Support Services:

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Important Safety Information (continued)

Tell your doctor if you have had a serious reaction to other immune globulin medicines or have been told you also have a deficiency of the immunoglobulin called IgA, as you might not be able to take Hizentra. You should not take Hizentra if you know you have hyperprolinemia (too much proline in your blood).

Infuse Hizentra under your skin *only*; do not inject into a blood vessel.

Allergic reactions can occur with Hizentra. If your doctor suspects you are having a bad allergic reaction or are going into shock, treatment will be discontinued. Immediately tell your doctor or go to the emergency room if you have signs of such a reaction, including hives, trouble breathing, wheezing, dizziness, or fainting.

Tell your doctor about any side effects that concern you. Immediately report symptoms that could indicate a blood clot, including pain and/or swelling of an arm or leg, with warmth over affected area; discoloration in arm or leg; unexplained shortness of breath; chest pain or discomfort that worsens with deep breathing; unexplained rapid pulse; and numbness or weakness on one side of the body. Your doctor will also monitor symptoms that could indicate hemolysis (destruction of red blood cells), and other potentially serious reactions that have been seen with Ig treatment, including aseptic meningitis syndrome (brain swelling); kidney problems; and transfusion-related acute lung injury.

Reference: 1. Data on File. Available from CSL Behring as DOF HIZ-003

The most common drug-related adverse reactions in the clinical trial for Hizentra were swelling, pain, redness, heat or itching at the site of injection; headache; back pain; diarrhea; tiredness; cough; rash; itching; nausea and vomiting.

Hizentra is made from components of human blood. The risk of transmission of infectious agents, including viruses and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent, cannot be completely eliminated.

Before being treated with Hizentra, inform your doctor if you are pregnant, nursing or plan to become pregnant. Vaccines (such as measles, mumps and rubella) might not work well if you are using Hizentra. Before receiving any vaccine, tell the healthcare professional you are being treated with Hizentra.

Please see brief summary of full prescribing information for Hizentra, including boxed warning, on adjacent page.

You are encouraged to report negative side effects of prescription drugs to the FDA. Visit www.fda.gov/medwatch, or call 1-800-FDA-1088.

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HIZENTRA®, Immune Globulin Subcutaneous (Human), 20% Liquid
Initial U.S. Approval: 2010

BRIEF SUMMARY OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use HIZENTRA safely and effectively. See full prescribing information for HIZENTRA.

WARNING: THROMBOSIS

See full prescribing information for complete boxed warning.

- Thrombosis may occur with immune globulin products, including Hizentra. Risk factors may include: advanced age, prolonged immobilization, hypercoagulable conditions, history of venous or arterial thrombosis, use of estrogens, indwelling vascular catheters, hyperviscosity, and cardiovascular risk factors.
- For patients at risk of thrombosis, administer Hizentra at the minimum dose and infusion rate practicable. Ensure adequate hydration in patients before administration. Monitor for signs and symptoms of thrombosis and assess blood viscosity in patients at risk for hyperviscosity.

INDICATIONS AND USAGE

Hizentra is an Immune Globulin Subcutaneous (Human) (IGSC), 20% Liquid indicated for the treatment of primary immunodeficiency (PI) in adults and pediatric patients 2 years of age and older.

DOSAGE AND ADMINISTRATION

For subcutaneous infusion only. Do not inject into a blood vessel.

Administer weekly or biweekly (every two weeks).

Dosage

Before switching to Hizentra, obtain the patient's serum IgG trough level to guide subsequent dose adjustments.

Weekly: Start Hizentra 1 week after last IGIV infusion

$$\text{Initial weekly dose} = \frac{\text{Previous IGIV dose (in grams)}}{\text{No. of weeks between IGIV doses}} \times 1.53$$

- Biweekly: Start Hizentra 1 or 2 weeks after the last IGIV infusion or 1 week after the last weekly Hizentra infusion. Administer twice the calculated weekly dose.
- Adjust the dose based on clinical response and serum IgG trough levels (see *Dose Adjustment*).

Administration

- Infusion sites – 1 to 4 injection sites simultaneously, with at least 2 inches between sites.
- Infusion volume – First infusion, up to 15 mL per site. Fifth infusion, up to 20 mL per site, then to 25 mL per site as tolerated.
- Infusion rate – Up to 15 mL per hr per site. Increase to 25 mL per hr per site as tolerated.

DOSAGE FORMS AND STRENGTHS

0.2 g per mL (20%) protein solution for subcutaneous injection

CONTRAINDICATIONS

- Anaphylactic or severe systemic reaction to human immune globulin or components of Hizentra, such as polysorbate 80
- Hyperprolinemia (type I or II) (Hizentra contains the stabilizer L-proline)
- IgA-deficient patients with antibodies against IgA and a history of hypersensitivity

WARNINGS AND PRECAUTIONS

- IgA-deficient patients with anti-IgA antibodies are at greater risk of severe hypersensitivity and anaphylactic reactions.
- Thrombosis may occur following treatment with immune globulin products, including Hizentra.
- Aseptic meningitis syndrome has been reported with IGIV or IGSC treatment.
- Monitor renal function, including blood urea nitrogen, serum creatinine, and urine output in patients at risk of acute renal failure.
- Monitor for clinical signs and symptoms of hemolysis.
- Monitor for pulmonary adverse reactions (transfusion-related acute lung injury [TRALI]).
- May carry a risk of transmitting infectious agents, e.g., viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent.

ADVERSE REACTIONS

The most common adverse reactions observed in ≥5% of study subjects were local reactions (i.e., swelling, redness, heat, pain, and itching at the injection site), headache, diarrhea, fatigue, back pain, nausea, pain in extremity, cough, rash, pruritus, vomiting, abdominal pain (upper), migraine, and pain.

To report SUSPECTED ADVERSE REACTIONS, contact CSL Behring Pharmacovigilance at 1-866-915-6958 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

The passive transfer of antibodies may interfere with the response to live virus vaccines, and lead to misinterpretation of the results of serological testing.

USE IN SPECIFIC POPULATIONS

- Pregnancy: No human or animal data. Use only if clearly needed.
- Pediatric: No specific dose requirements are necessary to achieve the desired serum IgG levels.

Based on September 2013 version

promise. The general aim of gene therapy with monogenic disorders is to deliver the functional gene to stem cells, which are long-lived and can continue to give rise to cells that contain the corrected gene. This would result in a permanent cure since corrected stem cells can divide and ultimately differentiate into different cell types that serve various functions in the body. Significant progress also has been made in the field of primary immunodeficiency diseases (PIs), and new trials continue to improve upon previous work (see Table 1).

There are still many more indications too long to list. Briefly, they include treatment of infections, notably the human immunodeficiency virus, neurological diseases such as Alzheimer's disease and multiple sclerosis, ophthalmologic

diseases such as glaucoma and retinitis pigmentosa, inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease, and blood diseases such as sickle cell disease.

Advancements in Gene Therapy

Traditional forms of gene therapy have focused on the delivery and incorporation of functional genes to diseased cells without targeting them to their natural location in the genome (an individual's complete DNA set). However, genes in their endogenous locations are surrounded by intricate control elements that affect their expression (their ability to produce functional genes). Random incorporation of corrected genes into cells is acceptable only when

Table 1.
Gene Therapy in Primary Immunodeficiency

Primary Immunodeficiency	Overall Outcomes	Future Directions
ADA-SCID	<ul style="list-style-type: none"> • 100% survival with most achieving protective immune function • No complications due to viral vector 	<ul style="list-style-type: none"> • Can soon be considered standard of care for patients without matched sibling bone marrow donors
X-Linked SCID	<ul style="list-style-type: none"> • 95% engraftment with immune reconstitution in infants. Four of five older patients age 10 years to 20 years did not engraft with gene modified stem cells, although several of these patients also failed traditional bone marrow transplant • 5 patients developed acute T cell leukemia, 4 of whom were treated successfully 	<ul style="list-style-type: none"> • New trial underway using a new generation of self-inactivating gene delivery vectors lacking elements that can result in insertional oncogenesis
Chronic Granulomatous Disease (CGD)	<ul style="list-style-type: none"> • Early trials were not able to achieve enough gene correction or resulted in insertional oncogenesis • Clinical trial ongoing in Europe may be followed by similar trials in the U.S. 	<ul style="list-style-type: none"> • Newer modified viral vectors are being used to decrease/eliminate the risk of insertional oncogenesis
Wiskott-Aldrich Syndrome (WAS)	<ul style="list-style-type: none"> • Majority of patients achieved permanent correction of their immunodeficiency • Leukemia occurred in 7 out of 10 patients due to the retroviral vector 	<ul style="list-style-type: none"> • Newer modified viral vectors are being used to decrease/eliminate the risk of insertional oncogenesis
Leukocyte Adhesion Deficiency (LAD)	<ul style="list-style-type: none"> • A few patients were treated with a retroviral vector, but there were no corrected cells found in circulation • No adverse events resulted from the gene therapy 	<ul style="list-style-type: none"> • Promising results in canine models represents potential for human clinical trials in the near future

expression of the gene is not tightly regulated. Therefore, a targeted approach can significantly decrease the risk of insertional oncogenesis that can potentially occur with random integration of genetic material by a viral vector.

In recent years, there has been a strong focus on site-specific gene therapy using engineered genome editing tools to locate specific sequences of DNA, create a cut in the DNA near the location of a mutation, and make appropriate changes such as inserting or cutting out a sequence of DNA that affects expression of the gene. TALENs, or Transcription Activator-Like Effector Nucleases, are one such tool discovered in the 2000s from a type of plant bacteria. They can be designed and assembled in the laboratory to bind specific gene sequences and create a double-stranded break in the DNA. When a break in DNA occurs, natural repair mechanisms are activated that can use a provided DNA template to fix the break and incorporate a corrected gene sequence to override the diseased gene.

Another genome editing platform that has gained significant momentum in the last few years is the CRISPR/Cas-9 system, or Clustered Regularly Interspaced Short Palindromic Repeats. It is derived from a form of bacterial immunity, and it has been engineered so that researchers can utilize it to target almost any gene. Similar to TALENs, CRISPRs also create breaks in the DNA that can be repaired using a template containing the correct DNA sequence. However, both techniques can still result in off-target effects, cutting unintended sequences of DNA, so efforts are ongoing to further understand and decrease this complication.

Ongoing research has begun to gain a deeper understanding of which specific cells are best treated with gene therapy to provide a more permanent source of corrected cells to patients. These cells can be sorted out of the general population of cells through modern advancements in technology such as flow cytometry (which provides rapid analysis of multiple characteristics of single cells). In terms of DNA delivery, modifications have been made to viral systems to decrease the risk of infection and insertional oncogenesis. Non-viral methods of gene delivery are also underway, including the permeabilization of cell membranes with an electric pulse (electroporation), ultrasound-mediated transfer and chemical delivery, among many more.

The Future of Gene Therapy

Clinical trial and research activity in gene therapy continue to grow, and the future of the field is even brighter than before. It offers the promise of a cure for those with serious

illnesses, and it may be only a matter of time before it becomes the standard of care for certain diseases.

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In the area of immunodeficiency, recent results in utilizing gene therapy have shown promise in diseases such as X-linked hyper-IgM syndrome (XHIM), X-linked agammaglobulinemia (XLA), X-linked lymphoproliferative disease (XLP) and X-linked agammaglobulinemia (XLA). The goal is to translate this work into clinical trials. Although the path from the research bench to the clinic has not been entirely smooth, and the process to bring efforts to fruition takes years and significant investment, the knowledge that there is a realistic potential to cure serious, life-threatening diseases makes this effort entirely worthwhile. ■

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