Jesse Gelsinger

In the first 24 hours following infusion of the vector into the liver, all the patients (six groups of three) suffered minor fevers, aches, and pains--typical flulike side effects associated with adenoviral vector treatment. Encouragingly, liver toxicity indicators weren't increasing along with the dose amounts. "We were feeling very good because it looked like there wasn't a dose-response effect," Batshaw, the Phase I study's principal investigator, recalls.

The second day, Gelsinger inexplicably fell over the dose-response precipice. The 18-year-old Tucson, Ariz., man's bilirubin levels climbed rapidly. He soon lost ability to clot blood. Toxic ammonia built up in his liver. By the third day, he developed respiratory problems. He died the fourth day, after physicians were unable to rescue him from brain and kidney failure. Batshaw and colleagues in the gene therapy field have since been searching for what went wrong so quickly and so unexpectedly.

Gene Therapy

A Multi-Institutional Phase I/II Trial Evaluating the Treatment of SCID-X1 Patients with Retrovirus-mediated Gene Transfer

X-linked severe combined immunodeficiency (SCID-X) is a rare genetic immunodeficiency that prevents a child's bone marrow from producing infection-fighting white blood cells

- We have designed a novel self inactivating (SIN) vector, pSRS11.EFS. IL2RG. pre*, which lacks all enhancerpromoter elements of the LTR U3 region and is devoid of all gammaretroviral coding regions.
- Expression of the therapeutic gene is regulated by an internal housekeeping gene promoter derived from human elongation factor-1 (EF-1) gene.
- This vector, **which expresses the IL2RG gene**, has reduced mutagenic potential compared to LTR configuration in non-clinical studies.
- Based on experience in other transplant and gene therapy trials, we recently modified the protocol to include low dose Busulfan conditioning in patients without active infections, in order to enhance correction of humoral (B cell) immunity.

Prior Troubles in Gene Therapy

Of the 20 children enrolled in the previous two trials, one child did not have correction of the immune system, and died of complications after undergoing stem cell transplant. The second important reason why gene transfer is research is that we are still learning about the side effects of gene transfer and how to do gene transfer safely.

In the last two trials, 5 children have experienced a serious side effect. These children developed leukemia related to the gene transfer itself. Leukemia is a cancer of the white blood cells, a condition where a few white blood cells grow out of control. Of these children, 4 of the 5 have received chemotherapy (medication to treat cancer) and are currently in remission

(no leukemia can be found by sensitive testing), whereas one died of gene transfer-related leukemia.

Preliminary Results In SCID X-1 Trail Farber Institute
RESULTS:
All patients received bone marrow-derived CD34+ cells transduced with the SIN-γc vector, without preparative conditioning. After 12.1 to 38.7 months of follow-up, eight of the nine children were still alive. One patient died from an overwhelming adenoviral infection before reconstitution with genetically modified T cells. Of the remaining eight patients, seven had recovery of peripheral-blood T cells that were functional and led to resolution of infections. The patients remained healthy thereafter.

The kinetics of CD3+ T-cell recovery was not significantly different from that observed in previous trials. Assessment of insertion sites in peripheral blood from patients in the current trial as compared with those in previous trials revealed significantly less clustering of insertion sites within LMO2, MECOM, and other lymphoid proto-oncogenes in our patients.

Sickle Cell

Bone marrow was obtained twice from the patient to collect sufficient stem cells for gene transfer and backup (6.2×10^8 per kilogram and 5.4×10^8 per kilogram, respectively, of total nucleated cells obtained). Both procedures were preceded by exchange transfusion, and bone marrow was obtained without clinical sequelae. Anemia was the only grade 3 adverse event reported during these procedures. Bone marrow–enriched CD34+ cells were transduced with LentiGlobin BB305 vector (see the Methods section in the Supplementary Appendix).13 The mean vector copy numbers for the two batches of transduced cells were 1.0 and 1.2 copies per cell.

Outcomes in this patient provide further supportive evidence to our previously reported results of patients who underwent a similar ex vivo gene therapy procedure for β-thalassemia with the same BB305 vector22,23 or the previous HPV569 vector.23,24 In addition to the patient with sickle cell disease described here, under this same clinical protocol, 4 patients with transfusion-dependent β-thalassemia have received LentiGlobin BB305.

These participants had no clinically significant complications and no longer require regular transfusions.22 These findings are consistent with early results reported with 18 other patients with thalassemia who received LentiGlobin BB305 in clinical study HGB-204.23 Longer follow-up is required to confirm the durability of the efficacy and safety profile observed, and data from additional evaluations of gene therapy in a larger cohort of patients to confirm the promise of gene therapy for sickle cell disease are lacking.

A Phase 1/2a, Single Ascending IV Dose Clinical Trial Investigating **Human Low Density Lipoprotein Receptor (LDLR) Gene Therapy** in Subjects With Homozygous Familial Hypercholesterolemia (HoFH).

Homozygous Familial Hypercholesterolemia (HoFH) is a rare genetic metabolic disorder characterized by absent or severely reduced capacity to catabolize circulating LDL particles by the hepatic LDL receptor. As a consequence, HoFH subjects present abnormal total plasma cholesterol (LDL-C) levels, resulting in severe atherosclerosis often leading to early onset of cardiovascular disease. Early initiation of aggressive treatment for these patients is therefore essential. Unfortunately, despite existing therapies, treated LDL-C levels could remain well above acceptable levels.

Thus, the functional replacement of the defective LDLR via AAV-based liver-directed **gene therapy** may be a viable approach to treat this disease and improve response to current lipid-lowering treatments. This first-in-humans study is intended to evaluate the safety of this **gene therapy** investigational product and assess preliminary evidence of efficacy using plasma LDL-C levels as a surrogate biomarker for human LDLR transgene expression

Vector Concerns From OTC Trail

The clinical team took precautions in all three suspect areas: vector, screening, and delivery. The research group drew on previous experience to create what they thought was the safest, most appropriate vector to meet their goal--rescuing OTC patients from comas caused by excessive ammonia in the liver. Gelsinger had experienced such a coma last December and had to be temporarily placed on a ventilator. "We needed to have a vector that works very rapidly," Batshaw notes. "The adenovirus is the only vector available that does that." The slower-acting adeno-associated vector is better suited for maintenance and prevention.

They also decided to use a "third-generation" adenoviral vector. To create the first-generation adeno, they removed only the virus' E1 region, which scientists think causes viral pathogenesis. To engineer the second-generation vector, they also deleted the E2 region, which promotes involved viral replication. Making vectors replication-deficient ideally results in less liver inflammation. To make the third-generation vector used in this trial, scientists removed both E1 and E4, a stronger replication-promoting region than E3. That vector should have been "much safer" than the earlier models, Batshaw notes.

Adenoviral vectors have been used in about 1,000 of the approximately 4,000 gene therapy experiments done since 1989, Anderson estimates. However, RAC is revisiting adeno's track record by soliciting adverse effects of adenoviruses that may have gone un- or underreported in the scientific literature. "No one publishes adverse events in papers," notes Mickelson. Batshaw, too, suspects that the vector may be the underlying cause of Gelsinger's death. "It does not look like the principal problem was a response to the therapeutic material," Batshaw reflects. "It looked like he may have had an immune response to the viral protein itself." Still, Batshaw admits that other factors may also be at fault. "Was this some sort of idiosyncrasy where Jesse had some preexisting condition that was unrecognized? If so, can we screen for that?"

The clinical team administered extensive tests for known risk factors prior to admitting the young man into the trial. Gelsinger's family contacted Batshaw when Jesse was 17. The scientists told the family that only adults could participate in the trial. The family tried again this summer, after Jesse turned 18. The clinical team screened the young man for antibodies against the adenovirus, and Gelsinger responded normally. He also checked out in tests measuring liver

function, ammonia levels, and amino acids. "That young man did fit the criteria," notes Anderson. "There was no reason to exclude him." Still, scientists could do better to identify candidates for experimental procedures, notes Mickelson. "The linkage between genotype and phenotype may not be as strong as scientists may wish."

Systemic Delivery In OTC Trail.

RAC will also evaluate what role the method of delivery played. RAC originally recommended the protocol with the understanding that the vector be administered intravenously. The FDA approved the trial under condition that the vector be administered via an artery leading directly into the liver. The FDA recommended that approach because, theoretically, it could limit any toxicity in the liver to half of that organ and prevent toxicity from spreading to other organs, Batshaw notes. RAC was temporarily disbanded in 1995 before it could address the FDA's recommendations, and the trial went on as amended.

The FDA has since suspended enrollment in two cancer gene therapy trials sponsored by Schering-Plough, of Madison, N.J. Both trials use a different form of the adenoviral vector than the Penn-based trial. However, those vectors, which carry *p53* into tumor cells, are administered via intrahepatic artery--the same systemic delivery used in the OTC trial.

In the next several months, every decision of every involved party--from investigators to regulatory bodies--will likely be revisited and scrutinized. Just how much the whole gene therapy field will be reevaluated as a result of this death remains unclear. "I honestly don't think that this will damage the field," Mickelson notes. Still, it will have an impact. Anderson calls the death "a real jolt" to gene therapy researchers. "All of us are taking a look at our protocols," Anderson notes, adding that his plans to do in utero gene therapy to correct birth defects will likely be slowed.

Researchers should redouble their efforts to emphasize safety, recommends **Savio L. Woo,** president of the American Society of Gene Therapy (ASGT). "Based on this unexpected outcome, current clinical studies involving direct systemic delivery of this particular type of vector into patients should be reevaluated," notes Woo, a gene therapy researcher with the Institute for Gene Therapy and Molecular Medicine in New York. He adds that ASGT "challenges the scientific community to develop, through rigorous research efforts, better and safer vectors for clinical applications in the future."

For now, Batshaw notes that the clinical team is focusing on making as much information available as possible. He hopes that information will help scientists discover safer vectors, create new toxicity safety, and unearth less-risky delivery routes, so that Gelsinger, whom he calls "a hero and a pioneer," will not have died in vain.

The term *sickle cell disease* (SCD) describes a group of inherited red blood cell disorders. People with SCD have abnormal hemoglobin, called *hemoglobin S or* sickle hemoglobin, in their red blood cells.

Hemoglobin is a protein in red blood cells that carries oxygen throughout the body.

"Inherited" means that the disease is passed by genes from parents to their children. SCD is not contagious. A person cannot catch it, like a cold or infection, from someone else.

People who have SCD inherit two abnormal hemoglobin genes, one from each parent. In all forms of SCD, at least one of the two abnormal genes causes a person's body to make hemoglobin S. When a person has two hemoglobin S genes, Hemoglobin SS, the disease is called *sickle cell anemia*. This is the most common and often most severe kind of SCD.

Hemoglobin SC disease and hemoglobin Sβ thalassemia (thal-uh-SEE-me-uh) are two other common forms of SCD.

Some Forms of Sickle Cell Disease

- Hemoglobin SS
- Hemoglobin SC
- Hemoglobin $S\beta^0$ thalassemia
- Hemoglobin $S\beta^+$ thalassemia
- Hemoglobin SD
- Hemoglobin SE

Overview

Cells in tissues need a steady supply of oxygen to work well. Normally, hemoglobin in red blood cells takes up oxygen in the lungs and carries it to all the tissues of the body.

Red blood cells that contain normal hemoglobin are disc shaped (like a doughnut without a hole). This shape allows the cells to be flexible so that they can move through large and small blood vessels to deliver oxygen.

Sickle hemoglobin is not like normal hemoglobin. It can form stiff rods within the red cell, changing it into a crescent, or *sickle* shape.

Sickle-shaped cells are not flexible and can stick to vessel walls, causing a blockage that slows or stops the flow of blood. When this happens, oxygen can't reach nearby tissues.

Normal Red Cells and Sickle Red Cells

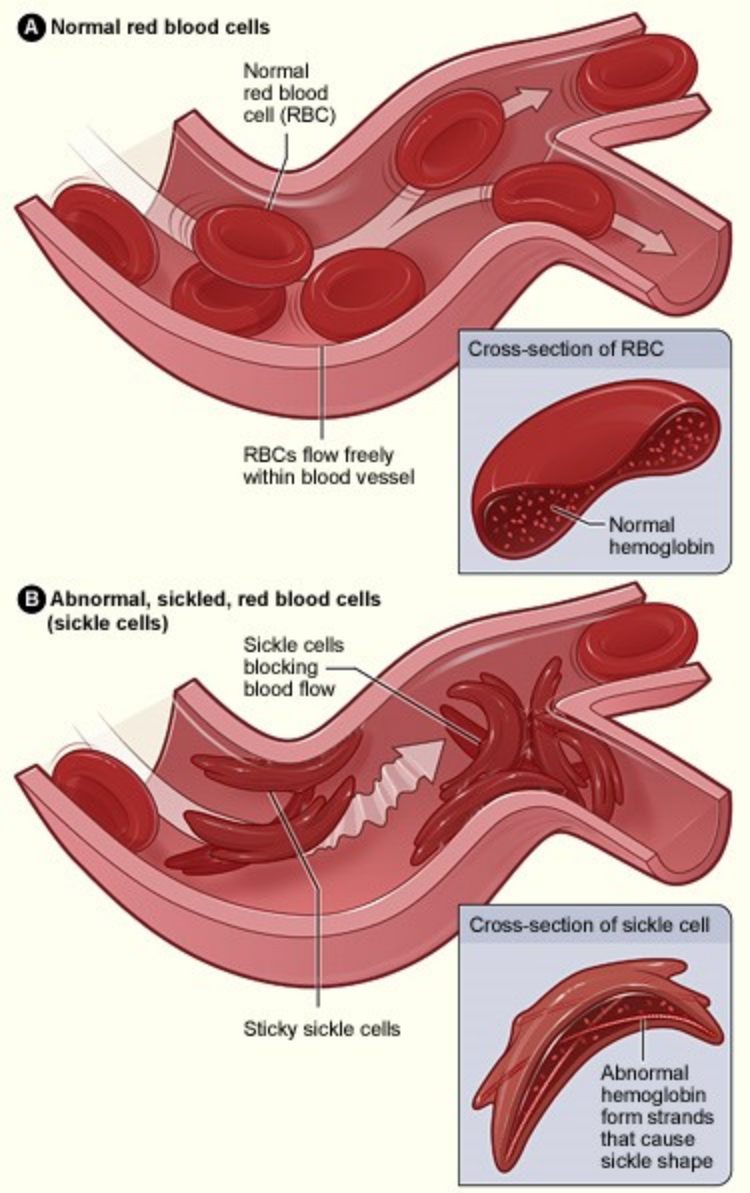


Figure A shows normal red blood cells flowing freely in a blood vessel. The inset image shows a cross-section of a normal red blood cell with normal hemoglobin. Figure B shows abnormal, sickled red blood cells blocking blood flow in a blood vessel. The inset image shows a cross-section of a sickle cell with abnormal (sickle) hemoglobin forming abnormal stiff rods.

The lack of tissue oxygen can cause attacks of sudden, severe pain, called *pain crises.* These pain attacks can occur without warning, and a person often needs to go to the hospital for effective treatment.

Most children with SCD are pain free between painful crises, but adolescents and adults may also suffer with chronic ongoing pain.

The red cell sickling and poor oxygen delivery can also cause organ damage. Over a lifetime, SCD can harm a person's spleen, brain, eyes, lungs, liver, heart, kidneys, penis, joints, bones, or skin.

Sickle cells can't change shape easily, so they tend to burst apart or *hemolyze*. Normal red blood cells live about 90 to 120 days, but sickle cells last only 10 to 20 days.

The body is always making new red blood cells to replace the old cells; however, in SCD the body may have trouble keeping up with how fast the cells are being destroyed. Because of this, the number of red blood cells is usually lower than normal. This condition, called *anemia*, can make a person have less energy.

Outlook

Sickle cell disease is a life-long illness. The severity of the disease varies widely from person to person.

In high-income countries like the United States, the life expectancy of a person with SCD is now about 40–60 years. In 1973, the average lifespan of a person with SCD in the United States was only 14 years. Advances in the diagnosis and care of SCD have made this improvement possible.

At the present time, hematopoietic stem cell transplantation (HSCT) is the only *cure* for SCD. Unfortunately, most people with SCD are either too old for a transplant or don't have a relative who is a good enough genetic match for them to act as a donor. A well-matched donor is needed to have the best chance for a successful transplant.

There are effective treatments that can reduce symptoms and prolong life. Early diagnosis and regular medical care to prevent complications also contribute to improved well-being.

Abnormal hemoglobin, called *hemoglobin S*, causes sickle cell disease (SCD).

The problem in hemoglobin S is caused by a small defect in the gene that directs the production of the *beta globin* part of hemoglobin. This small defect in the beta globin gene causes a problem in the beta globin part of hemoglobin, changing the way that hemoglobin works. *(See Overview.)*

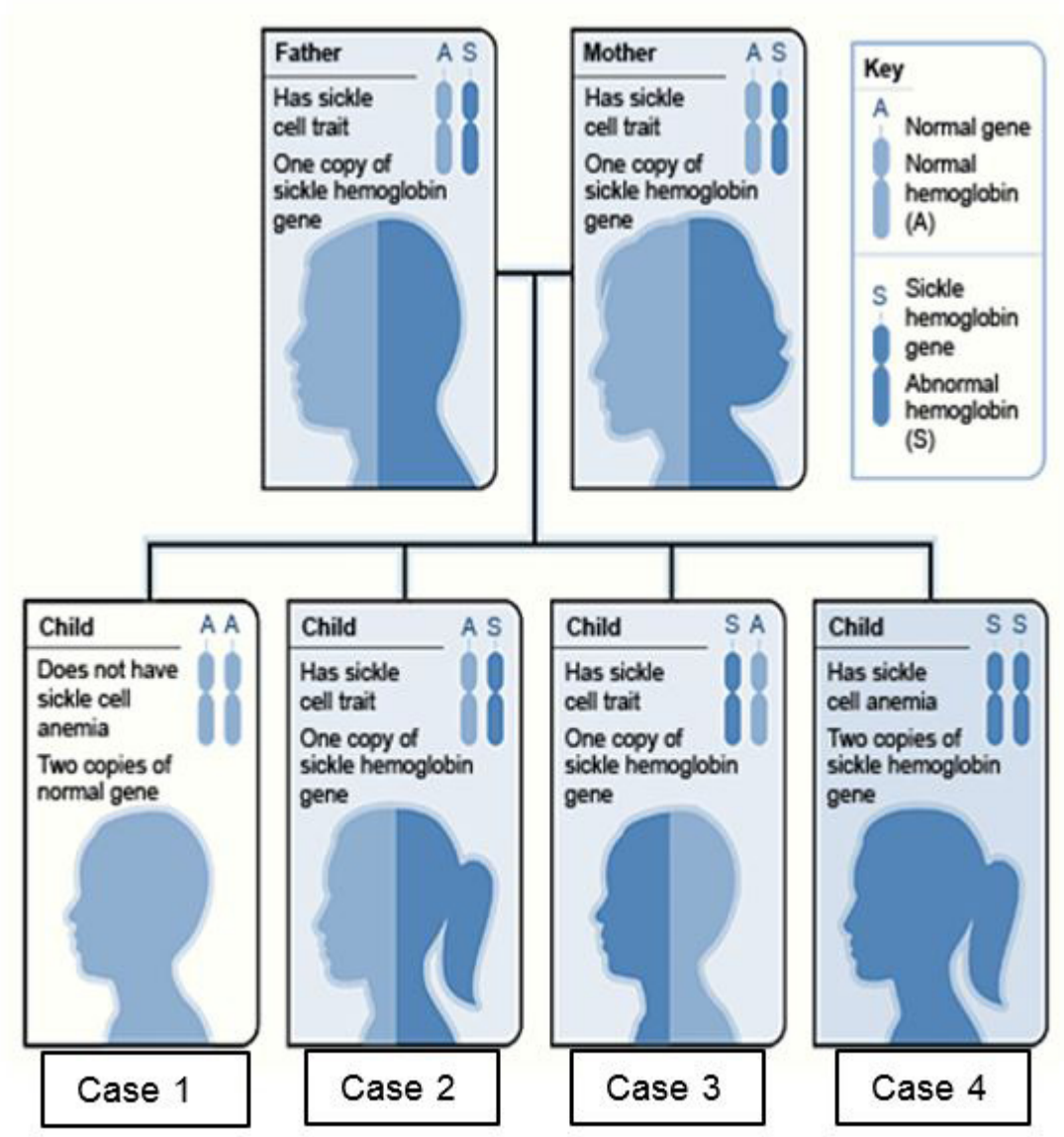
How Is Sickle Cell Disease Inherited?

When the hemoglobin S gene is inherited from only one parent and a normal hemoglobin gene is inherited from the other, a person will have *sickle cell trait*. People with sickle cell trait are generally healthy.

Only rarely do people with sickle cell trait have complications similar to those seen in people with SCD. But people with sickle cell trait are *carriers* of a defective hemoglobin S gene. So, they can pass it on when they have a child.

If the child's other parent also has sickle cell trait or another abnormal hemoglobin gene (like thalassemia, hemoglobin C, hemoglobin D, hemoglobin E), that child has a chance of having SCD.

Example of an Inheritance Pattern



The image shows how sickle hemoglobin genes are inherited. A person inherits two hemoglobin genes—one from each parent. A normal gene will make normal hemoglobin (A). A sickle hemoglobin gene will make abnormal hemoglobin (S).

In the image above, each parent has one hemoglobin A gene and one hemoglobin S gene, and each of their children has:

- A 25 percent chance of inheriting two normal genes: In this case the child does *not* have sickle cell trait or disease. (Case 1)
- A 50 percent chance of inheriting one hemoglobin A gene and one hemoglobin S gene: This child has *sickle cell trait*. (Cases 2 and 3)
- A 25 percent chance of inheriting two hemoglobin S genes: This child has *sickle cell disease.* (Case 4)

It is important to keep in mind that each time this couple has a child, the chances of that child having sickle cell disease remain the same. In other words, if the first-born child has *sickle cell disease*, there is still a 25 percent chance that the second child will also have the disease. Both boys and girls can inherit sickle cell trait, sickle cell disease, or normal hemoglobin.

If a person wants to know if he or she carries a sickle hemoglobin gene, a doctor can order a blood test to find out.