New Blood for Gene Therapy

The two 3-year-olds were very, very sick. One was bleeding internally, suffered from severe eczema and anemia, and had multiple infections in his lungs and colon. The other had a dangerously low platelet count, recurring respiratory tract infections, and a life-threatening salmonella infection in his blood. Both turned up in pediatric hematologist Christoph Klein's office in 2006, their parents clutching tightly to the hope that Klein might be able to save their children's lives.

Klein, then at the Hannover Medical School in Germany, was running the first-ever gene therapy trial for Wiskott-Aldrich Syndrome, a rare and life-threatening disease caused by mutations in *WAS*, a gene whose protein product normally regulates the formation of actin polymers in hematopoietic cells. A dearth of functional WAS protein causes blood and immune system disorders, susceptibility to infections, and an increased risk of lymphoma. The only effective therapy for the disease had been a bone marrow transplant from a closely matched donor, and in case of a mismatch, patients survived only 50 percent of the time.

The parents of the sick boys were hoping instead that Klein could use experimental gene therapy to insert healthy copies of the WAS gene into their children's genomes.

But gene therapy trials haven't had a good track record: there have been few successes and many failures since the first, an experimental treatment for a rare genetic form of severe combined immunodeficiency, was initiated in 1990, and the Food and Drug Administration has yet to approve a gene therapy treatment in the United States. But in 2003, Klein and colleagues at Children's Hospital in Boston successfully rescued *WAS*-deficient mice by inserting a retrovirus with a healthy copy of the gene into their hematopoietic stem cells (*Blood*, 101:2159-66, 2003). Bolstered by these results, they continued the work in human cells and finally, in 2005, began a clinical trial in Germany.

Klein working with five-year old Felix Ott, who was diagnosed with Wiskott-Aldrich Syndrome at age three. When he was four, Felix received stem-cell gene therapy, and the now seven-year-old has since been able to live a normal life.

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In 2006 the two boys' blood stem cells were isolated, purified, and grown in culture with retrovirus vectors designed to insert a healthy copy of *WAS* into the stem cells' genome. "This trial has been very successful in transferring the vector into patients' cells, some of them even [inserting] multiple copies into the same cell," says Christof von Kalle, director of the National Center for Tumor Diseases, Heidelberg, who helped conduct the trial.

The treatment was a resounding success. The team reported the results last November (*NEJM*, 363:1918-27, 2010), and today, the two 7-year-old boys are in "excellent condition," says Klein, now at University Children's Hospital in Munich. Both boys have robust immune systems, have stopped bleeding and bruising, and attend school, he says. "It's just fantastic."

Since 2005, eight more patients from around the world have been enrolled in the trial, and Klein presented the latest results at the American Society of Hematology meeting in Florida in December. Most of the children demonstrated the same positive effects as the first two: increased platelet counts, followed by evidence of normal WAS protein expression. Klein's team has also documented the presence of new functional immune cells, including T cells and B cells.

But despite its successes, the trial had its downsides.

In 2007, the team struggled to harvest enough stem cells from one young Lebanese boy, and when transplanted back into his body, the cells did not engraft successfully. His illness continued, and the team resorted to a bone marrow transplant from the boy's father. The last-ditch treatment was successful, says Klein, who recently visited the boy in Lebanon.

Then, last autumn, a boy involved in the trial developed leukemia. Retroviral vectors have strong enhancer elements that switch on the replacement gene once it is inserted into the genome, but these enhancers can also activate nearby genes involved in cell growth and differentiation. "Any retroviral gene transfer carries this inherent risk," says Klein.

Although this child was treated with chemotherapy and is now in remission,

the diagnosis was enough to put the trial on hold. The team is developing a new generation of viral vectors designed to decrease the likelihood that they will activate nearby genes and cause cancer. "We probably will be able to reduce the risk, though we may never completely abolish it," says Klein. The team is testing these new vectors in mouse models and human cells and hopes to resume the trial by the end of this year or early in 2012.

"There is optimism that this vector issue can be addressed," says von Kalle. "Then gene therapy can be used more successfully, with less side effects, and on a more regular basis. There's a whole lot of excitement in the field."

—Megan Scudellari