

# Stem Cell Therapy and Gene Therapy



## Chapter 25

*Hematopoietic stem cell transplantation (HSCT) represents the mainstay of treatment for several severe forms of primary immunodeficiency diseases. Progress in cell manipulation, donor selection, the use of chemotherapeutic agents, and prevention and management of transplant-related complications has resulted in significant improvement in survival and quality of life after HSCT. In some forms of severe primary immunodeficiency diseases, gene therapy may represent a valid alternative for patients who lack acceptable stem cell donors.*

## Hematopoietic Stem Cell Transplantation

A “stem cell” is a type of cell that can divide over and over and produce more stem cells as well as descendant cells that turn into different types of cells. Embryonic stem cells, for instance, can make descendants that turn into any tissue in the body, like skin cells, brain cells, heart cells etc. For each organ in the mature body, there are specific stem cells that can make all the different kinds of cells in that organ. For example, in the blood system, hematopoietic (“blood-forming”) stem cells (HSC) give rise to each of the different types of blood cells such as red blood cells (RBC), white blood cells (WBC) and platelets.

Traditionally, HSCs were obtained from the bone marrow. This process was called “bone marrow transplantation.” However, new methods now obtain HSC from peripheral blood, or blood taken from the placenta at birth (“cord blood”). Cord blood, in particular, provides an excellent alternative source of HSC for the immune and blood systems. The process of taking HSCs from one person and transfusing them into another is called hematopoietic stem cell transplantation, or HSCT. Unlike transplantation of a solid organ (such as a kidney or liver), HSCT does not involve surgery. It is more similar to a blood transfusion. But instead of just blood, the fluid transfused contains HSCs.

The primary immunodeficiency diseases for which HSCT is most commonly performed include Severe Combined Immune Deficiency (SCID), Wiskott-Aldrich Syndrome (WAS), IPEX Syndrome, Hemophagocytic Lymphohistiocytosis (HLH) and X-linked Lymphoproliferative Disease (XLP). It can also be used in the treatment of Chronic Granulomatous Disease (CGD) and many other severe primary immunodeficiency diseases. The transplantation of HSCs from a “normal” individual to an individual with a primary immunodeficiency disease has the potential to replace the deficient immune system of the patient with a normal immune system and, thereby, affect a cure.

There are two potential obstacles that must be overcome for HSCT to be successful. The first obstacle is that the patient (known as the recipient or host) may have enough immune function remaining after the transplant to recognize the transplanted stem cells as something foreign. The immune system is programmed to react against things perceived as foreign and tries to reject them. This is called graft rejection. In order to prevent rejection, most patients require chemotherapy and/or radiation therapy to weaken their own residual immune system enough to prevent it from rejecting the transplanted HSCs. This is called “conditioning” before transplantation. Many patients with SCID have so little

## (Hematopoietic Stem Cell Transplantation continued)

immune function that they are incapable of rejecting a graft and do not require conditioning before HSCT.

A similar situation occurs when the recipient's bone marrow is full of its own, defective stem cells and the HSC cannot find anyplace to establish themselves. This is called "failure of engraftment." To prevent this, chemotherapy may be given to reduce the number of defective HSC in the recipient's bone marrow in order to "make room" for the new HSC to engraft.

Although the chemotherapy treatment prevents the host from rejecting the transplanted HSCs, it may cause serious side effects. These include transient loss of all of the cells of the bone marrow so the patient is very susceptible infections, anemia (low RBC) and bleeding problems due to low platelets. Chemotherapy also may cause severe blistering of the mouth or other mucous

membranes that makes getting adequate hydration and nutrition very difficult. It is because of these serious complications that HSCT is reserved for those patients with the most severe immune defects.

The second obstacle that must be overcome for the transplant to be successful is Graft versus Host Disease (GVHD). This occurs when the mature T-cells from the donor or which develop after the transplant, perceive the host's tissues as foreign and attack these tissues. To prevent GVHD, medications to suppress inflammation and T-cell activation are used. These medications may include steroids, cyclosporine and other drugs.

In order to prevent some of these potential obstacles, it is important to try to identify a "matched" donor. A matched donor is one whose Human Leukocyte Antigens (HLA) are the same as those of the recipient.

## Selecting a Donor

HLA are tissue types. Each of us has our own collection of HLA antigens on our cells including the cells of our immune system and bone marrow, as well as on cells in most other tissues and organs. The exact structure of these HLA antigens is determined by a series of genes clustered on the sixth (6th) human chromosome. Compatibility of HLA is very important to determine the chance of successful engraftment while keeping the risk of GVHD low.

There are many different variants for each of these HLA genes in humans. The combination of HLA alleles of each individual is relatively unique. However, since the HLA genes are closely clustered on chromosome 6, they are usually inherited as a single unit. Therefore, the chance that an individual's brother or sister shares the same HLA alleles is relatively high.

There is a 1 in 4 chance that any sibling could be a perfect match for the patient. Unfortunately, due to the laws of probability and the fact that most families have a limited number of children, fewer than 25% of patients have a sibling who is a "match." Therefore, there has been a major effort to develop alternative methods to offer the possibility of a transplant to patients who do not have a matched donor in their own family.

One alternative is to try to find a suitable matched donor through one of the worldwide computer-based registries of individuals who have volunteered to serve as bone marrow donors. The National Marrow Donor Program in the U.S. has listings of hundreds of thousands of individuals who have provided a blood sample to have their HLA type measured. Similar registries are present in many countries around the world.

(Selecting a Donor continued)

Information on the combination of HLA alleles of more than 19 million volunteer donors is collected in Bone Marrow Donors Worldwide (BMDW). This database can be easily accessed by authorized healthcare professionals to explore the possibility that there is a matched unrelated donor (MUD) available for a patient who needs HSCT and does not have an HLA-matched donor in the family.

Successful transplants for patients with a primary immunodeficiency disease using donors found through this worldwide registry have saved the lives of many patients over the past 20 years. Results of transplantation using fully matched unrelated donors for some diseases now approaches the success rate for transplants using sibling matches.

Another source of HSC used for transplantation in patients with primary immunodeficiency diseases is umbilical cord blood. In the growing fetus, HSC frequently leave the marrow and are found circulating in high numbers in the blood. At the time of birth, the placenta can be recovered, the blood that is remaining removed and the HSC isolated and banked. These cord blood HSC may then be HLA typed and used for transplantation. Since cord blood contains fewer mature T-lymphocytes than the marrow or blood of adult donors, sometimes cord blood transplants have been successful even though the degree of match between donor and patient was not very good. One limitation of cord blood HSC transplantation is that because of the limited volume of umbilical cord blood, there may not be a sufficient numbers of HSC to treat a larger child or adult.

If a perfect match cannot be identified, it is sometimes possible to use one of the parents as a donor. Either parent has half of the same alleles as the patient; the parent is said to be “haploidentical” to the patient. There are some problems that can occur with this type of transplant. The mature T-lymphocytes contained in

the bone marrow of the haploidentical parent would be able to recognize the HLA alleles that are unique to the patient, and would thus cause GVHD.

In order to prevent this complication, it is essential to remove the mature T-lymphocytes (called T-cell depletion) from the bone marrow before infusing the stem cells into the patient. This is done with a preparative regimen before the transplant. After the mature T-cells are removed from the HSC, the risk of GVHD is markedly reduced.

T-lymphocytes of donor origin that develop from the transplanted HSC and reconstitute the patient’s T-lymphocyte immunity will remain haploidentical to the rest of the cells of the patient. However, the risk of GVHD from these T-lymphocytes is low because these cells develop inside the new host from immature precursor cells in the grafted marrow. Like a person’s own T-cells, they are “educated” during their maturation to ignore or “tolerate” the cells and tissues of the host.

It may take as long as six to eight months for the stem cells to reconstitute T-lymphocytes and for these newly generated T-cells to mature and learn to work with other cells in the host. Therefore, restoration of immune function after T-cell depleted HSCT takes longer than after fully matched HSCT (where mature T-lymphocytes contained in the graft may immediately provide some immune function).

Sometimes, complete immunologic reconstitution may not occur after HSCT. In some cases after haploidentical T-cell depleted HSCT, more than one transplant has to be performed to achieve T-cell reconstitution. Full immune reconstitution (including antibody production) is achieved less often than after fully matched transplantation.

Some centers use T-cell depleted HSCT for treatment of babies with SCID who do not have a matched family donor, while other centers believe that the search for a

### (Selecting a Donor continued)

matched unrelated donor is the best first choice option.

The best choice depends on many factors including:

- The type of SCID or primary immunodeficiency disease
- How much immune function remains
- The degree of matching of potential donors
- The types of HSCT available (cord blood vs. bone marrow)
- The age of the patient
- How sick they are and what types of complications they have had

## Procedures

HSC are “harvested” from the donor by removing bone marrow from the pelvic bones. Bone marrow is removed by drawing the marrow up through a needle that is about 1/8 of an inch in diameter. Only two teaspoons are taken from each puncture site because, if more is taken, the sample is diluted with the blood that flows through the bone marrow space. Bringing blood with the bone marrow increases the risk of the sample carrying the mature T-cells that have the potential to cause GVHD.

Usually, two teaspoons are taken for each two pounds of the recipient’s body weight. The average donor might have only a few punctures performed to get enough stem cells for a baby, but more than 100 punctures may be required to get enough stem cells for a teen or full sized adult. The procedure may be performed under general anesthesia or under spinal anesthesia. The discomfort after the procedure varies from donor to donor.

Almost all donors will require some type of pain control medication for two to three days after the procedure, but most donors are not required to stay in the hospital overnight and are able to return to full activity shortly afterwards. The donor’s immune system is not compromised because HSC and marrow quickly regenerate.

Once it has been harvested, the bone marrow is passed through a fine sieve to remove any small particles of bone and processed further, if necessary, to remove incompatible red blood cells, or to remove T-cells. It is then placed into a sterile plastic bag and infused into the host intravenously just like a blood transfusion.

As an alternative to bone marrow harvesting, HSC can be obtained from peripheral blood and then purified via a process known as apheresis. The donor’s blood is collected from an arm vein, using a needle that is connected with a machine that removes the white blood cells. After white blood cells are removed from the blood, the remaining red blood cells are then returned to the donor via a vein in the opposite arm. The HSC are then purified from the other white blood cells. Typically, in order to enrich the amount of HSC in peripheral blood, the donor receives subcutaneous injections of granulocyte-colony stimulating factor (G-CSF) or of plerixafor in the days that precede the blood collection. Both G-CSF and plerixafor mobilize the HSC from the bone marrow, transferring them into peripheral blood, so that a large number of HSC are present in the peripheral blood before the apheresis procedure.

## Results of HSCT

HSCT between HLA matched siblings has been successfully employed in the treatment of primary immunodeficiency diseases since 1968. The first child to receive a transplant (a patient with X-SCID) is still alive, healthy and has a family of his own. This case suggests that, as best as can be determined, the graft is very long lasting and appears to be permanent.

In the case of infants with SCID, HSCT involving a matched marrow has minimal graft versus host disease risk and is associated with an overall success rate of as high as 90%. Results of HSCT from unrelated donors from a haploidentical parent are not as good, yet approximately 60-80% of the infants survive and demonstrate robust T-cell reconstitution.

The chance of survival depends on the health of the patient at the time of the transplant. If the patient is in relatively good health, free from infection at the time of the transplantation and does not have lung damage from previous infections, the outlook is very good. Because of this, survival is very good (>90%) in infants with SCID who receive HSCT within 3-4 months of age, even when the donor is not a family match. This emphasizes the importance of early recognition of SCID, and the benefit of newborn screening for this disease, that is BEFORE the patient has a serious infection.

While reconstitution of the number and function of T-lymphocytes is the rule after HSCT for SCID, normalization of antibody production occurs in some, but not all, patients. Reconstitution of antibody production after HSCT for SCID depends on the specific form of SCID, on the type of donor (matched vs. haploidentical) and on the use of chemotherapy as part of the preparative regimen before the HSCT. If antibody production is not reconstituted after HSCT, patients will require Ig replacement therapy indefinitely to help protect them from infection. Even if replacement therapy is required, these patients usually enjoy a good quality of life after transplant.

HSCT is also an effective form of treatment for other forms of primary immunodeficiency diseases, including WAS, IPEX, HLH), XLP, X-linked hyper-IgM (also known as CD40 ligand deficiency), CGD and other primary immunodeficiency diseases.

In most of these conditions, conditioning with chemotherapy is required before the transplant to allow engraftment of donor-derived stem cells, even when the donor is a matched sibling. The success rate after HSCT from an unrelated donor in these cases is nearly as good (70-80% survival) as using a matched sibling for the donor. Here again, the initial health of the patient is extremely important and the best survival rates are in children who are transplanted under the age of 5, who are relatively free of infections and who do not have pre-existing lung or liver damage.

Mixed chimerism (that is persistence of the patient's immune cells along with donor-derived white blood cells) after HSCT is sufficient to cure the disease in many of these disorders (IPEX, HLH, XLP, X-linked hyper-IgM, CGD), and this may allow doctors to use less intense chemotherapy, thus also reducing the risk of related toxicity. In boys with WAS, mixed chimerism is associated with a higher risk of complications (autoimmunity, persistence of low platelets) and more intense chemotherapy regimens are typically used for this disease.

HSCT is not always indicated in patients with CD40 ligand deficiency and CGD, as many of these patients do well on medical management. The risks and benefits of the procedure must always be carefully weighed.

It must be noted that HSCT from a haploidentical parent is not as successful in primary immunodeficiency diseases other than SCID and is typically reserved to very severe cases that cannot be safely managed otherwise. Again the risks and benefits must be carefully addressed.

## Gene Therapy

Most primary immunodeficiency diseases are caused by errors (mutations) in specific genes. It has long been the hope that one day it would be possible to cure these diseases by fixing the mutation that causes the disease and thus affect a cure. As a result of the human genome project and similar efforts to map all of the genes present in human beings, we now know the identities of the specific genes involved in many diseases, including the vast majority of primary immunodeficiency diseases. More genes are being identified nearly every week. We have finally reached the stage where that long held hope is becoming a reality.

Not every genetic disorder, including some primary immunodeficiency diseases, will eventually be correctable by gene therapy. However primary immunodeficiency diseases, as a general rule, may be better suited for this therapy than almost any other class of genetic disease. Transplantation of HSC taken from a normal donor has been successful in curing many of these disorders, so it should theoretically also be possible to take the patient's own HSC and correct the genetic defect in those cells by adding a normal copy of the gene that is causing the disease.

To introduce the gene, we take advantage of the ability of some viruses (retroviruses) to penetrate into cells and to insert their genome into the patient's own DNA. For the purpose of gene therapy, viruses have been modified so that their own genes have been largely removed and replaced with the normal copy of the defective human gene that is causing the primary immunodeficiency diseases.

To perform gene therapy, the patient's HSCs are first isolated from the bone marrow or from peripheral blood, and they are then cultured in the laboratory with the virus containing the gene of interest. Various growth factors are added to the culture to make HSC proliferate and to facilitate infection with the virus. After two to four days, the cultured cells are washed to remove any free

virus, and then they are transfused into the patient. The cells that have incorporated the gene of interest into their chromosomes will pass it to all cells that will be generated when these cells divide. Because the gene has been inserted into HSC, the normal copy of the gene will be passed to all blood cell types, but not to other cells of the body. Because primary immunodeficiency diseases are caused by gene defects that affect blood cells, this can be sufficient to cure the disease.

Gene therapy represents a life-saving alternative for those patients with severe forms of primary immunodeficiency diseases, who do not have a matched sibling donor. In these cases, performing an HSCT from a haploidentical parent or even from a MUD would carry some significant risks of GVHD. In contrast, GVHD is not a problem after gene therapy, because in this case the normal copy of the gene is inserted into the patient's own HSC, negating the need for a HSC donor.

Until now, gene therapy has been used to treat patients with SCID secondary to adenosine deaminase (ADA) deficiency, X-linked SCID, CGD and WAS. The first clinical trial of gene therapy was at the National Institutes of Health in 1990 and treated a 4-year-old girl with ADA deficiency. The design of this first trial did not attempt to correct the defective HSC, only the T-cells. This girl is now clinically well and still has about 25% of her circulating T-cells carrying the corrected ADA gene more than 20 years after her treatment. After this initial clinical trial demonstrated that gene therapy could be carried out safely and that gene-corrected T-cells could survive for years and function normally, follow up trials were initiated attempting to cure children with ADA-SCID by targeting HSC for gene correction. The results have been spectacular with most of the more than two dozen ADA-SCID patients attaining a significant long lasting increase of the T- and B-lymphocyte count

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and a remarkable improvement of immune function. Importantly, no episodes of serious adverse reactions or cases of leukemia have occurred in the patients with ADA deficiency treated by gene therapy.

The next primary immunodeficiency disease to be treated by gene therapy was X-linked SCID. This trial also targeted the HSC using a retrovirus to deliver the gene. Beginning with a groundbreaking study in Paris followed by a similar experience in London, there have been 20 X-SCID babies around the world that have been treated with gene therapy. In these infants, gene therapy was performed without any need for chemotherapy prior to the transfusion of HSC that had been cultured with the virus. Eighteen of these patients are currently alive, and in 17 of these 18 children gene therapy alone was sufficient to restore development of T-lymphocytes and immune function and no other treatment was needed.

Unfortunately, while the SCID was cured, five of these patients developed leukemia. Four of the children's leukemia was cured, but one child died.

Gene therapy trials are ongoing with patients with other primary immunodeficiency diseases. Overall, the experience with gene therapy in primary immunodeficiency diseases has demonstrated that it is possible to cure the disease by inserting a normal copy of the gene into the patient's HSC. However, there are some risks that need to be overcome and safer vectors need to be developed. Various laboratories around the world are working at modifications of the viral vectors in order to improve their safety. Nevertheless, gene therapy must still be regarded as an experimental therapy. It is likely that the inherent problems will be worked out in the coming years and that a larger number of primary immunodeficiency diseases will be cured by gene therapy.