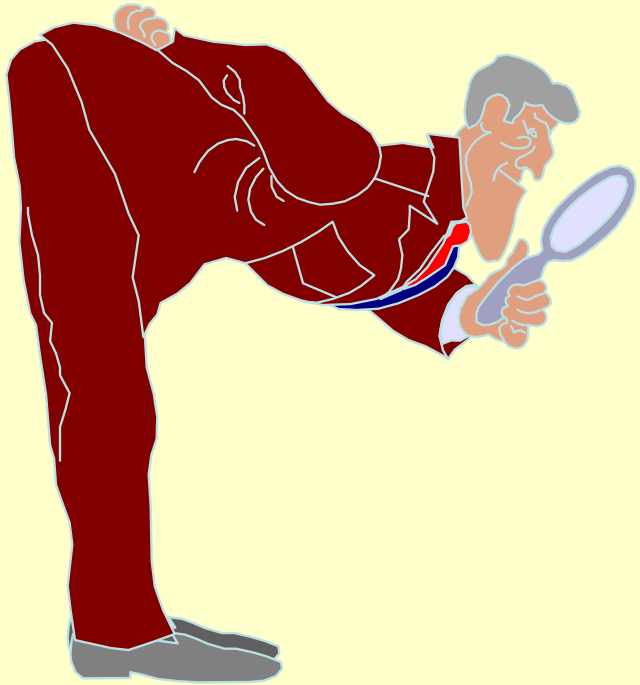
BIOSTATISTICS FOR TRANSLATIONAL & CLINICAL RESEARCH



Blood-&-Marrow Transplants & CANCERS

Stem Cells

Stem cells are immature body cells that act like "starter dough" because they can make identical copies of themselves. This keeps a constant supply of **"starter" cells ready to mature within several distinct tissue layers** --internal, middle, or external--in response to the body's needs. Stem cells mature within these layers, replacing aging or damaged cells in their respective body tissues. Once they mature, stem cells lose the ability to duplicate themselves.

What is “Bone Marrow”?

Bone marrow is spongy tissue found inside bones. The bone marrow in the breast bone, skull, hips, ribs, and spine contains stem cells.

In a “transplant”, marrow and blood stem cells are harvested and administered together; **blood stem cell transplants** are also called **bone marrow transplants (BMT).** However, the focus are stem cells, not bone marrow; there are other sources of stem cells.

Blood Stem Cells

Blood stem cells, known as hematopoietic stem cells. These "starter" cells **re-supply three types of blood cells**: erythrocytes, commonly known as **red blood cells**; **platelets**, also called the blood-clotting cells; and leukocytes, the **white blood cells** of the immune system.

From Bone Marrow to the Bloodstream

Immune cells--also called **white blood cells or lymphocytes-**-leave the bone marrow while still immature, and they migrate through the bloodstream on their way to the thymus and other lymphoid organs. During their journey, they mature into specialized T or B cells of the immune system. Other blood components, like red blood cells, completely mature in the bone marrow before being released into the blood.

Blood Stem Cell Transplants: When?

In patients with leukemia, aplastic anemia, and some immune deficiency diseases, the stem cells in the bone marrow malfunction, producing an excessive number of defective or immature blood cells (in the case of leukemia) or low blood cell counts (in the case of aplastic anemia). The immature or defective blood cells interfere with the production of normal blood cells, accumulate in the blood stream and may invade other tissues.

Blood Stem Cell Transplants: Why?

Hematopoietic or **blood stem cell transplants** are also called **bone marrow transplants** or peripheral blood stem cell transplants, depending upon the location of stem cell collection.

A blood stem cell transplant can restore normal hematopoiesis. It enables physicians to treat these diseases with aggressive chemotherapy and/or radiation by allowing replacement of the defective, diseased, or damaged stem cells and/or bone marrow after the chemotherapy and/or radiation treatment.

Stem Cells from Self to the Rescue

To minimize any damage to blood stem cells from cancer treatment, these stem cells from the bone marrow are removed and preserved before patients receive chemotherapy. The stem cells are then re-infused into the patient after chemotherapy where they migrate to the bone marrow and begin producing new blood cells. Patients with multiple myeloma as well as non-Hodgkin's and Hodgkin's lymphoma may be offered this approach as part of their treatment.

Stem Cells from Donor to the Rescue

Once collected, stem cells are usually infused immediately into the patient, where they migrate to the bone marrow and "settle in" or **engraft**. Once there, stem cells can repopulate the bloodstream with normal red blood cells and immune cells that "rescue" the patient. For some cancer types, this feat is curative.

Alternatively, stem cells are harvested from a donor source and "cryopreserved," which means they are frozen in liquid nitrogen. At some later time, they are thawed and infused into a patient.

Engraftment: Crucial Event

The two to four weeks immediately following transplant are the most critical. The high-dose chemotherapy and/or radiation given to the patient during conditioning will have destroyed the patient's bone marrow, crippling the body's immune or defense system. As the patient waits for the transplanted bone marrow to migrate into the cavities of the large bones, set up housekeeping or "engraft" and begin producing normal blood cells, he or she will be very susceptible to infection and excessive bleeding.

Not Just Any Blood Stem Cells Will Do

The success of a blood stem cell transplant relies upon the interactions of markers on the surface of all body cells, including the immune cells of both the patient and the donor. Normally, all cells within the patient's body coexist peacefully in a state known as self-tolerance because all bear the same "self" marker proteins. These proteins are also called **antigens** because, should they be introduced into a new environment, they are capable of stimulating a powerful immune reaction. This is what occurs when a blood stem cell preparation with "non-self" antigens is transplanted into a patient whose "self" antigens are very different.

Host vs. Graft/Graft vs. Host

Donated blood stem cell transplants bring with them their own distinctive "self" antigens. The immune system of the patient, who is called a host, senses that these antigens are "unmatched" or "non-self." This prompts the patient's immune cells to attack the donated (transplanted) cells, which are called a graft, and this assault can lead to the patient's rejection of the transplant ("host vs. graft"). More often, though, some mature donor immune cells that are mixed in among the transplanted blood stem cells recognize the antigens on the patient's body cells (host) as "non-self." This causes the transplant to attack the patient's tissues and organs. The result is "graft vs. host" disease, which can be very serious.

When patients donate their own stem cells (**autologous**), "self" antigens meet "self" antigens and there is enough similarity to lessen the risk of immune cell warfare. The same advantages are associated with **syngeneic** (from identical twin) transplants, although few patients have identical twins.

In most cases, the transplant is **allogeneic**, meaning the blood-forming stem cells come from a "non-self" donor. These transplants will contain antigens that are somewhat like but not identical to those of the patient.

Many Names for the "Self" Antigens

The scientific name for "self" antigens is major histo-compatibility complex (MHC) proteins, and each person bears a unique set. To make matters even more confusing, when these MHC proteins mark the surface of immune cells called leukocytes, the antigens are dubbed **human leukocyte antigens** (HLAs). Since leukocytes are the cells used for tissue typing, HLAs are what clinicians use to find reasonable matches for their patients. Whether called MHC proteins or HLAs, all are the same thing--"self" antigens. Technicians remove human leukocytes from the blood of potential donors and determine what HLA markers are present.

Haplotypes:
Passing on Genes for "Self" Antigens

Genes for "self" antigens are passed on from one generation to the next in a clump called a haplotype. A **haplotype is a combination of genes** --HLA alleles, for example--that are located closely together on the same chromosome and tend to be inherited together as a package deal. Alleles are genes that come in many varieties, all of which function reasonably well at making the related protein.

6 Major Genes: 10,000 Antigens

Six important genes for human leukocyte antigens are contributed by each parent. So each person inherits a *double set* of **six major genes that produce six major corresponding antigens, HLA-A, -B, -C, -DP, -DQ, and -DR**. Since as many as 20 varieties exist for each of these HLA-producing genes in different individuals, the total possible human leukocyte antigens that can mark "self" and trigger an immune response number about 10,000!

Three Most Important Antigens

Some donor "self" antigens are more antigenic than others, which means they are better at triggering an unwanted immune reaction in the transplant patient. By studying successful cases of blood stem cell transplantations, doctors have identified the three most important antigens to match when choosing "non-self" donors for a transplant. Matching these helps to minimize the chances of graft vs. host or host vs. graft attacks. The antigens are **HLA-A, HLA-B, and HLA-DR**.

A "Clinical Match"

An ideal 6:6 antigen match, known as a "clinical match," means that both sets of the three "most important" inherited human leukocyte antigens in the donor match perfectly with those on the body (and immune) cells in the patient. Below, only Child E would serve as a 6:6 clinically matched donor for Child A; one sibling is a total mismatch; the other siblings and both parents would be haplo-identical because only one of their two inherited haplotypes matches Child A.

Some Haplotypes Occur More Often

Because certain haplotypes are inherited among siblings more frequently than expected merely by chance, there is a 25-30 percent probability of finding a 6:6 match within a family. If one antigen is mismatched, it is a 5:6 match, which clinicians will accept because it increases the odds of finding a donor. When doctors accept a 4:6 match, the likelihood of finding a donor increases even more. As the number of HLA antigen matches decreases among donors and recipients, the chances of finding a donor increase.

Sometimes a 3-Antigen Match Is Necessary

More than 90 percent of patients can find a haplo-identical donor if they are willing to accept a 3-antigen match. Sometimes this is medically necessary, as when severely ill patients cannot wait the several months it may take to find a 4-, 5-, or 6-antigen matched donor. Patients accepting a 3-antigen haplo-identical match also accept a serious risk of suffering an immune cell attack on their tissues by the transplant (graft vs. host disease), or of having their body reject the transplant (host vs. graft).

Delicate Balance:
Graft vs. Tumor/Graft vs. Host

Haplo-identical transplants may provide a possible benefit. They may enable an attack on cancer to occur. A graft vs. tumor or graft vs. leukemia effect occurs when a donor's mature immune cells come along with the stem cells in the transplant, and these immune cells recognize and attack as "foreign" the cancer cells found in the patient's body. This phenomenon helps prevent relapse and, in some cancers, can even be curative. To boost this graft vs. tumor effect, patients are sometimes given an infusion of a donor's haplo-identical immune cells (white blood cells) along with the stem cell transplant.

A risk with haplo-identical transplants is that of enabling an attack on the patient's tissues. This can happen when a donor's mature immune cells, called T cells, contaminate the stem cell graft. To minimize this risk, researchers sometimes deplete all T cells from the transplant, leaving the graft with stem cells only. While this can minimize the chance of graft vs. host disease, it also reduces the transplant's ability to attack any loitering cancer cells. An alternative option is for the doctor to prescribe steroid medications for the patient pre- and post-transplant. This helps to prevent graft vs. host disease, yet leaves T cells available for graft vs. tumor action.

Success in Matching Varies With Population

An alternative to a haplo-identical transplant between related individuals is a matched transplant between unrelated individuals. For Japanese patients, because their island geography increases the likelihood of marriage within a limited gene pool, the chances of finding a 6:6 clinical match are better than for patients in larger gene pools. Of course, much depends upon getting enough donors. Like North American Caucasians, African American and Asian patients are part of a large gene pool, but they are under-represented in the donor pool and only have a 50 percent probability of finding a 6:6 clinical match.

General Preparation:

A successful transplant requires the patient be healthy enough the undergo the rigors of the procedure. Age, general health condition, diagnosis and stage of the disease are all considered in determining whether the patient should undergo the transplant. A battery of tests (heart, lung, kidney, and other vital organ functions) are carried to form “baseline data” against which post-transplant tests can be compared to determine if any body functions have been impaired.

Preparing Patients for Myeloablative Allogeneic Transplants

Before receiving allogeneic transplants--the most common type--patients with blood cancers must undergo a conditioning regimen of high-dose chemotherapy or radiation to kill any resident cancer cells, suppress the patient's immune system, and leave a disease-free environment into which healthy new blood stem cells can be infused. Known as myeloablation, the regimen prepares the patient to accept a "non-self" graft without triggering a severe attack on the patient by the transplant (graft vs. host disease).

Preparing Patients for Reduced-Intensity Allogeneic Transplants

Because many patients are unable to withstand myeloablation, clinical researchers are studying the feasibility of reduced-intensity nonmyeloablative regimens. Before infusing donated blood-forming stem cells, the patient receives low-dose or standard-dose chemotherapy and/or radiation therapy. Afterward, the patient is given immunosuppressant steroid drugs to help prevent the body from rejecting the transplant. Sometimes the patient also receives an infusion of donated immune cells (white blood cells from the same donor who supplied stem cells) to enhance the graft's attack on the patient's cancer (graft vs. tumor benefit).

This approach is new, so clinical researchers do not have extensive data on its effectiveness. Graft versus host disease sometimes occurs. So far, rates of graft vs. host disease using this approach are similar to those after high-dose conditioning regimens, but the onset is often delayed by weeks and months. Insurance companies and Medicare administrators, who view reduced-intensity transplants as experimental, often do not cover them.

Preparing Donors for Allogeneic Transplants

Blood stem cells are extracted most often from the peripheral blood of donors and occasionally from their bone marrow. Because there are significantly fewer stem cells in peripheral blood than in bone marrow, doctors prepare peripheral blood donors by injecting them with a series of growth factors to move blood stem cells from their marrow into their bloodstream. This increases the blood stem cells concentration 10- to 100-fold in the blood.

If stem cells are harvested from bone marrow, the donor is placed under general anesthesia. In a procedure that takes about an hour, marrow is removed through a large needle inserted into the donor's pelvic bones, and it is processed to remove blood and bone fragments. After the collection is completed, the donor may be given a transfusion of his or her own red blood cells from units of blood that were self-donated a week earlier.

The bone marrow (a thick and red liquid) is extracted with a needle and syringe. Several skin punctures on each hip and multiple bone punctures are usually required to extract enough amount of bone marrow. There are no surgical incisions or stitches involved – only skin punctures where the needle was inserted. The amount harvested depends on the size of the patient and the concentration of the bone marrow cells in the donor's blood; usually about 2 quarts of marrow and blood are harvested – which the body replaces in about 4 weeks.

Apheresis:
Harvesting Stem Cells From Peripheral Blood

Nurses harvest blood stem cells from a donor's peripheral blood using a process called apheresis. This process involves removal of whole blood from the donor. As blood is drawn from the donor, circulating stem cells (and sometimes mature immune cells) are extracted. A centrifuge-type machine then separates the components. The blood itself, minus the stem cells, is returned to the donor. Apheresis requires no anesthesia but can take several hours.

Preparing Patients for Autologous/Syngeneic Transplants

Like allogeneic donors, patients who donate their own peripheral blood for an autologous stem cell transplant, as well as identical twins making a syngeneic donation, receive injections of growth factor prior to collection by apheresis. This amplifies the number of blood stem cells in the bloodstream. Almost all autologous/syngeneic transplants are now taken from peripheral blood.

The patient will also receive high-dose chemotherapy before his self-donated stem cells are reinfused. Some clinical trials even repeat the procedure twice for these autologous transplants, so high-dose chemotherapy is followed by a self-donated transplant two times. This back-to-back clinical protocol is called a tandem transplant.

Cord Blood as a Source of Stem Cells

The human body's most primitive stem cells form in the fetal yolk sac and move to the fetal liver before entering the baby's bone marrow during the third trimester of pregnancy. When a baby is born, the placenta and umbilical cord still contain a rich supply of immature blood stem cells in the very process of migrating. These stem cells are capable of rebuilding all three types of blood cells in the body (red blood cells, immune cells, and platelets). First used in 1989 to help children with leukemia, cord-blood transplants have since proven themselves effective for adults too.

Placental and Cord-Blood Stem Cell Transplants

Cord and placental blood are retrieved in a single procedure right after a child is born. Nurses remove the majority of the red blood cells and the plasma (which aren't needed for transplant) and concentrate the immune stem cells. Then they add an agent to protect these cells from damage during storage at extremely low temperatures. This helps to assure that enough blood-forming stem cells are cryopreserved successfully for later infusion into a patient.

Using More Than One Cord-Blood Donor

Clinical trials using cord-blood units from two different donors to increase the number of stem cells infused in a single transplant show that cells from one unit may dominate the other, although both can attack the host's immune system. This phenomenon, which seems to facilitate grafting and boost the rate of hematopoiesis, is the subject of ongoing research.

Placental and Cord-Blood Transplants: Pros

For many patients waiting to be matched to a suitable donor, or for those unable to find one, umbilical cord blood is now a readily available, easy-to-store alternative. Even with a 1- or 2-antigen mismatch, cord-blood transplants succeed in a greater percent of cases than occurs with equally mismatched bone marrow transplants. This discovery greatly increases the transplant options for minorities who are under-represented in the donor pool. And cord blood is unlikely to harbor a virus called the cytomegalovirus that can cause life-threatening infections and contaminates about 10 percent of marrow donations.

Placental and Cord-Blood Transplants: Cons

There is a slight risk that maternal cells or genetic disease in the child could contaminate the cord-blood donation. And cord transplants contain only about 1/10 of the number of cells that can be harvested from a bone marrow transplant. Another concern is that cord-blood transplants require more time to "take" in the recipient's bone marrow, leaving the patient vulnerable to infection longer.

When a Blood Stem Cell Transplant Works

The newly transplanted cells home to bone marrow, engraft, then begin to produce healthy new blood cells. Peripheral blood stem cells generally restore the bone marrow within about two weeks, but it can take up to five weeks if the stem cells come from the marrow itself. Restoring complete immune function can take several months in autologous transplants, and one to two years in allogeneic transplants. During the patient's recovery, doctors can determine whether the cancer has returned by taking blood samples or aspirating small amounts of bone marrow through a needle for biopsy.

STATISTICAL ISSUES

- Major statistical issues are:

(1) Monitoring adverse effects: Non-engraftment and GVHD; GVHD is also an “outcome variable”

(2) Competing risks and competing risks with covariates

References:

a) Jeffrey et al. (JASA 88: 400-409, 1993)

b) Gooley et al. (Stat Med 18: 695-706, 1999).

c) Gray RJ. (Annals of Stat 16:1141-1154, 1988).

d) Fine JP & Gray RJ. (JASA 94: 496-509, 1999)