4. (A) is correct. Technically, performing a hematopoietic stem cell transplant is one of the simplest transplantation procedures. The hematopoietic stem cells are infused into a patient's blood stream using an intravenous infusion system. The surface adhesion molecules of the transfused stem cells will mediate their migration to the appropriate territory(ies) No invasive surgery or invasive procedures are involved.

7. (B) is correct. When a graft containing immunocompetent cells is placed into an immunocompromised host, the transplanted cells can recognize the host antigens as "non-self". In response to these antigenic differences, the donor T lymphocytes become activated, proliferate and differentiate into helper and effector cells that attack the host cells and cause GVHD. The crucial role played by the donor T cells in GVHD is demonstrated by the fact that their elimination from a bone marrow graft avoids the reactions, although the therapeutic benefit is also significantly reduced. However, as the GVHD evolves, the majority of the cells infiltrating the target tissues affected by the GVH reaction are of host origin and include T and B-lymphocytes, as well as monocytes and macrophages. The proliferation of host cells is probably a result of the release of high concentrations of non-specific mitogenic and differentiation factors by activated donor T lymphocytes. Granulocytes are rarely seen in the cellular infiltrates characteristic of GVHD.

9. (D) is correct. This is common, since the recipients of hematopoietic stem cells have undergone chemotherapy and radiotherapy to eliminate all the cancerous cells and, as a consequence, all of their normal stem cells are also severely affected. As a consequence these patients develop a transient anemia which must be treated by red cell transfusion. Incidentally, some patients do receive tetanus immunization or tetanus booster shots, but this is performed at a later stage, to help determine whether the stem cell transplant has established itself. Interleukin-2 therapy is not known to be of value in assuring the success of a hematopoietic stem cell transplant. Obviously, some patients may need psychotherapy after having gone through such a traumatic procedure, but not all patients require this. Megadoses of folic acid are unlikely to accelerate bone marrow regeneration.
Introduction

Thousands of transplants of organs, tissues, and cells are performed throughout the world annually. Transplantation of bone marrow cells or hematopoietic stem cell transplantation (HSCT) is performed to correct failures in the production of red or white cells, to repair immune deficiencies, and as a means of replacing malignant cells which have taken over the bone marrow. The major barrier to organ, tissue, and cell transplantation in humans is the immune response to the graft or, in the case of HSCT, the immune response of the graft against the recipient.

This POPS leads to a discussion on the indications and problems associated with HSCT or allogeneic bone marrow transplantation (ABMT) in patients with leukemia. Approximately 70% of the patients undergoing ABMT for the treatment of certain types of leukemia live at least five years. A major barrier to the success of HSCT is the fact that the patient's immune system can reject the donor stem cells or that lymphocytes in the HSCT will attack (reject) the recipient. Donor selection is very important in order to prevent or minimize immune reactions of host against graft and graft against host. It is important to find the best possible histocompatibility match between the donor and the patient.

There are at least six genetic loci in the major histocompatibility complex (MHC), located on chromosome 6, which code for HLA antigens. Each of these six genes is polymorphic meaning that there are many different versions of each gene which differ from one another by a different sequence of nucleotides. Each of these gene variants, called alleles, codes for an antigenically different HLA antigen. In the human population there are many, many thousands of different MHC gene combinations or genotypes, as they are called. Faced with this huge variability, it is very difficult, although not impossible, to find two human beings who have identical HLA antigens by searching in the population at random. Since HLA antigens are genetically determined, it is often possible to find individuals who are HLA identical, or matched, within a family.

The HLA antigens that receive the most attention for the purposes of transplantation are HLA-A, HLA-B and HLA-DR. Since each person carries a chromosome six of maternal and paternal origin there are two copies of each HLA gene all of which are expressed (co-dominant expression). Therefore, a “match” typically refers to a “six-antigen match” (2 HLA-A, 2 HLA-B, and 2 HLA-DR). Other antigens are also important, including HLA-C and, to a lesser degree, HLA-DQ. Consequently, an 8/8 match refers to an eight-antigen match (2 HLA-A, 2 HLA-B, 2 HLA-DR, and 2 HLA-C), and a 10/10 match includes 2 HLA-DQ. The HLA genes on a particular chromosome 6 constitute a “haplotype” (derived from “haploid genotype”). Each person has one maternal and one paternal haplotype based on which of each parent’s copies of chromosome 6 was inherited.

The most likely place to find a perfect HLA match between two people is among full-blooded siblings. According to Mendelian inheritance principles, one has a 25% chance of inheriting the same HLA molecules as a sibling, a 25% chance of inheriting none of the same HLA molecules as a sibling, and a 50% chance of inheriting half of the same HLA molecules as a sibling. When HSCT is considered, the first thing to do is a “family study” by which the patient and all available siblings and parents are HLA typed. This allows for identification of potential “six-antigen” matches among the siblings and analysis of the segregation patterns of the HLA molecules. The segregation patterns are obtained from determining which of the parental haplotypes are inherited. Each sibling from a given couple should have one haplotype from each parent.
In the example shown below, five of the seven MHC genes of the two families were determined. A number identifies each HLA antigen coded for by an MHC gene. Since we inherit one set of genes from each of our parents, we all have two versions of each MHC gene and thus there are two numbers given for each gene. Because data from both parents and two siblings was available, it was possible to establish the genotypes for the different family members. Discuss the interpretation of the HLA typing data below within the group before turning this page.

### HLA Genotypes

<table>
<thead>
<tr>
<th>Family Member</th>
<th>HLA Gene</th>
<th>Family 1</th>
<th>Family 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Mother</td>
<td>1</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>Father</td>
<td>3</td>
<td>7</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>48</td>
</tr>
<tr>
<td>Child 1</td>
<td>1</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>48</td>
</tr>
<tr>
<td>Child 2</td>
<td>2</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
<td>47</td>
</tr>
</tbody>
</table>

The family members in Family 1 are different from each other and there is no HLA match in the family. However, in Family 2 it is apparent that Child 1 and Child 2 are HLA identical. These examples are shown to make a point about the inheritance of MHC genes and the possibility of finding HLA identical individuals within families. If a sibling or other relative is thought to be a potential match, further testing is done to ensure that the person is the best match possible. This is called “higher-resolution” testing, and is performed by DNA testing utilizing a polymerase-chain reaction (PCR) based assay system.

If a match cannot be found within a patient’s family, a search for a matched, an unrelated donor search can be performed through the National Marrow Donor Program (NMDP). The NMDP has a computerized list of people who have agreed to be stem cell donors and their HLA types. Currently, the NMDP has over 4 million people in its registry. Although this sounds like a lot, it must be realized that there are hundreds of thousands to hundreds of millions of different combinations of HLA genes, depending on the number of HLA loci considered. To complicate matters even further, certain HLA antigens are found in greater proportions among members of different racial groups. To put it another way, an African-American patient has a greater chance of matching certain HLA antigens with another African-American. Unfortunately, African-Americans are greatly underrepresented in the NMDP registry thereby decreasing the likelihood of a match for that ethnic group. The same could be said for most minority groups in this country.

Information about joining the NMDP registry can be found on their website, www.marrow.org.
Case presentation

A 6-year-old girl presents for her annual follow-up in the pediatric oncology clinic. She was treated as a toddler for Wilms tumor with chemotherapy and radiation. A complete blood count (CBC) shows that she is neutropenic, anemic, and thrombocytopenic. A bone marrow aspirate and biopsy are obtained and 40% abnormal myeloid blasts are present. Cytogenetic analysis reveals the presence of monosomy 7.

She is started on induction chemotherapy with the goal to get her leukemia into remission. Her parents are told that her leukemia has a very poor prognosis.

What do you believe led to her leukemia?

What steps should be taken next for her treatment?

Up to 15% of childhood cancer survivors will be diagnosed with therapy-related acute myeloid leukemia (t-AML). This is typically a result of exposure to alkylating agents (such as cyclophosphamide) or topoisomerase inhibitors (such as etoposide). Therapy-related leukemias from alkylating agents typically have a latency period of 5-10 years and are associated with deletion of 5q and/or 7q. Therapy-related leukemias from topoisomerase inhibitors typically have a shorter latency period of 2-5 years and are associated with an 11q23 chromosomal abnormality. Prognosis is uniformly poor, as therapy-related leukemias are often refractory to chemotherapy, and stem cell transplantation (HSCT) offers the only chance for long-term survival.

HLA-typing should be done on the patient, parents, and siblings at diagnosis before chemotherapy begins. Once chemotherapy is given, the reduction in white blood cell counts can make it difficult to obtain an adequate specimen for HLA-testing.
The patient has no siblings, and a preliminary search of the National Marrow Donor Program (NMDP) registry and the cord blood registry demonstrates that there are several possible 6/6 unrelated donors as well as two 4/6 matched cord blood units. The physician explains to the parents that the graft-versus-leukemia (GVL) effect is particularly important to treat t-AML. Because it is currently not possible to separate the GVL effect from GVHD, it will be important for her to have some degree of GVHD to have the best chance for cure.

*Would you pick an unrelated donor or one of the cord blood units for this patient's transplant?*

*What factors should be considered?*

Compared with unmanipulated bone marrow, T-cell depleted bone marrow (TCD BM) has the advantage of producing less GVHD. For this reason a mismatched transplant with TCD BM may be preferable to a mismatched unmanipulated marrow, but transplants with TCD BM are associated with higher rates of graft failure, infection, and relapse.

Compared with unmanipulated bone marrow, mobilized peripheral blood stem cells (PBSC) have the advantage of faster engraftment, decreased infection, and reduced relapse, but transplants with PBSC are associated with higher rates of debilitating chronic GVHD. Also, a normal donor is exposed to G-CSF although to date increased risks to the donor have not been detected.

Compared with unmanipulated bone marrow, cord blood units are more “immunologically immature” making mismatched transplants more feasible, as there is a decreased or equivalent risk of GVHD for a 4/6 matched cord blood transplant compared with a transplant with a 6/6 matched unrelated bone marrow donor. Conversely, there is significantly slower engraftment with increased risk of infection as well as an increased risk of graft failure. Search time is reduced, as a cord blood unit can typically be available for transplant in about two weeks, compared with the median search time of 51 days for an unrelated donor in the NMDP registry. However, the “donor” for a cord blood transplant would not be available again if a second transplant or donor lymphocyte infusions were needed for graft failure or relapse. Another issue is that outcome after cord blood transplant is clearly related to the total nucleated cell dose of the cord blood unit per kilogram of the recipient. Often, there is not an adequate cell dose in cord blood units for patients weighing more than 20 kilograms.

For this patient, because of the importance of GVL in treating this disease, transplant with unmanipulated bone marrow from a fully matched unrelated donor would be the preferred graft source.
Discuss the following points with the group members:

Children who undergo HSCT have lower rates of GVHD than adults who undergo HSCT. *Why is this the case?*

Unlike recipients of solid organ transplants who require lifetime immunosuppression, recipients of HSCT who are doing well with no signs of GVHD are able to discontinue their immunosuppressive medicines six month to one year after HSCT. *Why don’t HSCT recipients require lifelong immunosuppression?*

As mentioned previously, T lymphocytes are the predominant effector cells of GVHD. A typical bone marrow graft contains 10% lymphocytes, 90% progenitor cells, and 0.01% stem cells. The post-thymic donor T lymphocytes in the graft represent adoptively transferred donor T lymphocytes in the host. The pre-thymic donor progenitor cells in the graft should mature in the recipient’s thymus. Positive selection occurs in the thymus cortical epithelium for T cells that recognize self MHC, and negative selection eliminates clones with excessive affinity for self MHC with self peptides. Therefore, although these cells are donor-derived, they should become host-tolerized after maturing in the thymus. The thymic involution that occurs with aging is possibly the cause for the overall increased rates of GVHD in adults versus pediatric recipients of HSCT.

There are a number of differences between solid organ and bone marrow transplants. In solid organ transplantation, there are few immune cells in the graft and no preparative regimen is given to the recipient. The primary clinical problem is rejection, and life-long immune suppression is required. In bone marrow transplantation, the graft obviously contains a large number of immune cells, and the recipient typically undergoes immune ablation. The primary clinical problem is GVHD, and immune suppression is typically only needed for months until the pre-thymic donor cells in the graft become host-tolerized after maturing in the thymus.
Closing comments

Whenever a patient receives a graft of an organ rich in immunocompetent cells, there is a risk that **graft-versus-host disease (GVHD)** may develop. GVHD is a significant problem in up to 30% of transplant recipients and is the primary cause of death in 15-20% of transplant recipients. The probability of developing GVHD is greatest in the two-month period immediately following transplantation.

When a graft containing immunocompetent cells is placed into an immunoincompetent host, the transplanted T cells can recognize host-derived peptides as non-self. As a consequence the donor T lymphocytes become activated, proliferate and differentiate into helper and effector cells that attack the host cells and tissues, producing the signs and symptoms of GVHD. The crucial role played by the donor T cells in GVH is demonstrated by the fact that their elimination from a bone marrow graft avoids the reactions. However, as the GVH evolves and reaches its highest intensity, the majority of the cells infiltrating the different tissues affected by the GVH reaction are of host origin and include T and B lymphocytes as well as monocytes and macrophages. The proliferation of host cells is probably a result of the release of high concentrations of non-specific mitogenic and differentiation factors by activated donor T lymphocytes.

The initial proliferation of donor T cells takes place in lymphoid tissues, particularly in the liver and spleen (leading to hepatomegaly and splenomegaly). Later, at the peak of the proliferative reaction, the skin, liver, and intestinal walls are heavily infiltrated leading to a skin eruption that may progress to exfoliative dermatitis, hepatic insufficiency, and severe diarrhea or even intestinal perforation. The splenic involvement results in a loss of function not unlike that seen in splenectomized patients. The patients often develop *Streptococcus pneumoniae* bacteremia and antibiotic prophylaxis is necessary.

All immunosuppressive drugs used in the prevention and treatment of rejection, including cyclosporine, tacrolimus, sirolimus, and mycophenolate mofetil, have been used for treatment of the GVH reaction. In addition, cytokine modifiers, such as infliximab, and thalidomide, the tranquilizer drug that achieved notoriety due to its teratogenic effects, have been also used with mixed success for the control of chronic GVH unresponsive to traditional immunosuppressants. One of the more promising agents for the treatment of both acute and chronic GVHD is pentostatin, a drug that initially was developed to treat hairy cell leukemia. Pentostatin inhibits adenosine deaminase and renders patients functionally similar to SCID patients, with the accumulation of dATP leading to lymphocyte cytotoxicity. Patients with GVHD require infectious prophylaxis and close monitoring for infections, because both GVHD itself and the drugs used to treat it lead to profound immune dysregulation.

In leukemic patients receiving allogeneic bone marrow or stem cells as part of the treatment for their disease, GVHD may be prevented by **T cell depletion** of the graft. This can be achieved by pre-treatment of the bone marrow with anti-lymphocyte/thymocyte immunoglobulin, or with monoclonal antibodies specific for T cells (e.g., anti-CD3). However, the rate of cure is also decreased, the risk of graft failure is increased, and immune reconstitution in the recipient is delayed. A low grade, controllable GVHD is associated with better outcomes in leukemic patients, likely as a result of the elimination of leukemic cells by the grafted lymphocytes ("graft-versus-leukemia" effect).