1. Match the terms for human grafts with the appropriate description:

<table>
<thead>
<tr>
<th>Terms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Autograft</td>
<td>A. Human</td>
</tr>
<tr>
<td>2. Syngraft</td>
<td>B. Nonhuman</td>
</tr>
<tr>
<td>3. Allograft</td>
<td>C. Self</td>
</tr>
<tr>
<td>4. Xenograft</td>
<td>D. Identical twin</td>
</tr>
</tbody>
</table>

---

### Genetic Transmission of HLA Antigens

<table>
<thead>
<tr>
<th>Family Member</th>
<th>A antigen alleles</th>
<th>B antigen alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 9 10 11 25</td>
<td>5 7 8 12 13 14</td>
</tr>
<tr>
<td>Mother</td>
<td>- + - + - - -</td>
<td>- - + + - - -</td>
</tr>
<tr>
<td>Father</td>
<td>+ - + - - - -</td>
<td>+ + - - - - -</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>+ - - + - - -</td>
<td>+ - - + - - -</td>
</tr>
<tr>
<td>Second</td>
<td>- - + + - - -</td>
<td>- - + - - - -</td>
</tr>
<tr>
<td>Third</td>
<td>+ + - - - - -</td>
<td>+ - + - - - -</td>
</tr>
<tr>
<td>Fourth</td>
<td>- + + - - - -</td>
<td>- + + - - - -</td>
</tr>
<tr>
<td>Fifth</td>
<td>+ - - - + - -</td>
<td>+ - - - + - -</td>
</tr>
</tbody>
</table>

Note: Only HLA-A and HLA-B antigens are shown for simplicity.

In the table above, which relates to questions 2 and 3, the pluses (+) indicate that the antigen was present as determined by HLA typing and the minuses (-) indicate that it was not. An individual's HLA phenotype is determined by HLA typing; an individual's HLA genotype (sum of all alleles) and haplotype (the specific arrangement of alleles on a chromosome) are determined by HLA typing of family members (parents and siblings).

2. The **phenotype** of the mother is:
   A. A2, 9, B8, 12
   B. A2, 9, B5, 7
   C. A2, B8 / A9, B12
   D. A2, B12 / A9, B13
   E. Can't tell

3. The **haplotype** of the father is:
   A. A1, 3 / B5, 7
   B. A1, 3 / B8, 12
   C. A1, B7 / A3, B5
   D. A1, B5 / A3, B7
   E. Can't tell

4. The ideal donor of a hematopoietic stem cell transplant is:
5. Hematopoietic stem cells are given to the recipient by:
   A. Infusing the cells into the patient's blood.
   B. Infusing the cells into the patient's bone marrow cavities.
   C. Infusing the cells into the patient's heart.
   D. Infusing the cells into the patient's peritoneal cavity.
   E. All of the above.

6. Patients who have cancer affecting the bone marrow stem cells or leukocytes are often prepared for a hematopoietic stem cell transplant by:
   A. Chemotherapy and radiotherapy.
   B. Laparoscopic surgery.
   C. Radiotherapy alone.
   D. Splenectomy.
   E. Thymectomy.

7. The cells infiltrating target tissues in chronic graft-versus-host disease are best described as:
   A. Mixtures of mononuclear cells and granulocytes of donor origin.
   B. Mixtures of mononuclear cells of donor and recipient origin.
   C. Mixtures of mononuclear cells of donor origin and granulocytes of host origin.
   D. Mononuclear cells of donor origin.
   E. Mononuclear cells of host origin.

8. Survival of a hematopoietic stem cell transplant and its establishment in the bone marrow is most easily assessed by:
   A. Bone marrow biopsy.
   B. Complete blood cell counts.
   C. Liver biopsy.
   D. Lymph node biopsy.
   E. Measuring creatinine clearance.

9. Which of the following is the LEAST LIKELY complication of hematopoietic stem cell transplantation?
   A. Cancer
   B. Failure of the graft to survive
   C. Graft-versus-Host Disease (GvHD)
   D. Infection
   E. Malignant transformation of the transplanted stem cells.

10. Each of the following has been used as a source of hematopoietic stem cells, EXCEPT:
    A. Blood.
    B. Bone marrow.
    C. Cord blood.
    D. Liver.
Pretest Correct Answers

You have the answers to some of the pretest questions, and other members of your group have the rest. This arrangement is designed to encourage all members of your group to actively participate in an exchange of ideas and concepts. First, study the answers in your booklet and then EXPLAIN them to your group. Please don't just read them to your classmates, and don't let your classmates read their answers to you. In explaining something to another person, most people gain a better understanding of it and often transmit a better understanding. The pretest discussion and patient-oriented problem-solving parts of this activity are "open book." Be sure to refer to textbooks, notes and other written resources whenever questions arise within your group, or discuss questions with your instructor.

You will probably want to make notes on your pretest about questions you missed to help you review. Avoid "collecting papers" for later study and understanding. Learn the concepts now so that later you need only review them.

You should complete questions 1-10 before moving onto the clinical presentation (starting with the green packet).

1. **Correct answers:** (1C), (2D), (3A), (4B).
   **Ask** Why do we bother differentiating between transplants from the patient's own tissues and those from an identical twin?
   **Answer:** Genetically and antigenically they should have been the same at birth. But somatic mutations could cause genetic differences. In addition, a graft from an identical twin could contain an infectious agent (i.e., human immunodeficiency virus [HIV] or cytomegalovirus [CMV]). These are not concerns in an allogeneic-graft situation.

6. **(A) is correct**. An important part of the preparation for a hematopoietic stem cell transplant is to kill off many, if not all, of the abnormal cancerous or bone marrow cells in the patient and to suppress the immune system of the host to avoid rejection of the transplant. This is usually accomplished by a series of treatments with chemotherapeutic drugs and total body irradiation, which eliminate most of the malignant cells (their rapid rate of proliferation makes them more susceptible to catatonic agents and irradiation). The same interventions ablate the patient's immune system. Surgical procedures such as removal of the spleen or thymus or other invasive procedures are not part of the preparation for bone marrow transplantation.

9. **(E) is correct**. Clearly, patients who have undergone the required combination of chemotherapy and radiotherapy prior to receiving the stem cell transplant are at increased risk for infection and sometimes must be hospitalized in special clean rooms to minimize their exposure to infectious agents. In addition, the hematopoietic stem cell transplant often contains immunologically competent T lymphocytes, which can attack the recipient's cells causing GvHD. It is known that there is an increased risk of cancer in recipients of hematopoietic stem cell transplants. This risk is thought to be associated with the chemotherapy and radiotherapy used to prior to the transplants, and the malignancies originate in host tissues, not usually from grafted cells. Finally, not every hematopoietic stem cell transplant is successful; some of these transplants fail to thrive and localize graft in the bone marrow. Others are rejected by residual components of the host's immune system.

START HERE - INSTRUCTIONS

When the designated pretest answers have been discussed, you are ready to begin the clinical problem. Each student has a different part of this patient's story. **Begin with the green booklet (part A).** Discuss this part of the patient data and answer the questions. When Booklet A has been completed, proceed to the yellow booklet (part B), etc. Working together in this way, decide on the best course of action for this patient.

START WITH STUDENT A
Have the students read the Case History from their data pack. The team can view pictures on page one (1) of the supplementary folder. Once the team is done with this, you should start by reading this section and watch this case unfold.

**READ CASE**

Jeff's age suggests two different treatment options. The classic treatment is hematopoietic stem cell transplantation (HSCT); however, the current treatment of choice is a new drug, Imatinib mesylate (Gleevec). Imatinib occupies the ATP-binding site of the BCR-ABL oncogene and prevents phosphorylation of substrates that are involved in regulating the cell cycle (see the second page of pictures #1 and 2).

Jeff opts for Imatinib treatment. In the weeks following the initiation of treatment, his WBC decreased to near the normal range (12,500 cells/mm$^3$). A bone marrow biopsy revealed normal cellular morphology. No Philadelphia chromosome positive cells were detected via cytogenetics or FISH; however, RT-PCR was still able to detect BCR-ABL transcripts. This data indicates that Jeff has achieved a hematological and cytogenetic remission but not a complete molecular remission (see the second page of pictures #3 and 4).

18-months after the initiation of Imatinib, Jeff’s WBC count begins to rise and Philadelphia chromosome positive cells were identified by both cytogenetics and RT-PCR. While this type Imatinib failure appears to be rare at 18-months (<15%) and little data exist as how to proceed, it is decided that Jeff should undergo a stem cell transplant to increase his chances for long-term survival.

**Ask:** What are three different types of stem cell transplant based on the relationship of the donor?

**Answer:** (1) Syngeneic, from an identical twin; (2) Allogeneic, either from a closely matched relative or from a partially matched unrelated individual; and (3) Autologous, the patient's own.

**Ask:** Which type of bone marrow transplant might be most appropriate for this patient? What considerations are there?

**Answer:** Each of the procedures has its own benefits and limitations. Concerns exist over the increased likelihood of recurrence of CML with an autograft and graft-versus-host disease (GvHD) with an allograft. Few people have a syngeneic match, and there can be problems with finding a suitable donor.

**Ask:** What is one of the first things that you need to ask Jeff about when considering HSCT?

**Answer:** If he has any siblings who could be a suitable donor within his family

The probability of finding two humans who are HLA-A, B, C, DR, DQ, and DP matched by random selection is approximately 1/10,000 to 1/1,000,000! Since we do not choose our mates on the basis of shared HLA genes, we find that most parents have completely unrelated HLA haplotypes. Because of this, family members are evaluated first as potential donors.
**Family history**
Jeff indicates that he is the eldest of three children. His mother and his brother and sister all live in town. His father was killed in a car accident ten years earlier.

**Ask:** What is the likelihood that any two siblings would have a “6-antigen match”?

**Answer:** 1 in 4. The likelihood of finding a match increases with each sibling. FYI only Jeff actually has a 43% chance of finding a match because he has 2 siblings. A “6-antigen match” implies that the donor and recipient match at both HLA-A, B, and DR alleles.

**Ask:** What genetic event can complicate this calculation?

**Answer:** Genetic recombination occurs when homologous chromosomes undergo crossing over during meiosis. The rate of recombination between loci is largely dependent on the distance between the loci. The HLA covers a relatively small portion of the chromosome; therefore, recombination rates are low.

Tell your group mates that genetic recombination within the HLA locus is rare, occurring <5% of the time. Thus, the probability of an HLA-haplotype match among siblings is only slightly less than 25%.

To follow this case and address the underlying basic science issues, complete the following:

1. **Review how to do HLA typing through serology**
   Look at the next two pages in your “data pack” entitled HLA-A, B and C typing. The first page diagrams the technique while the second describes it. Work through this technique as a group.

2. **Review MLR**
   Look at the next two pages in your “data pack” entitled Mixed Lymphocyte Reaction (MLR). The first page diagrams the technique while the second describes it. Work through this technique as a group.

3. **Continue with case**

   **Student B will now begin to lead the discussion regarding which of the three family members would serve as the best donor of a bone marrow graft.** The group will discuss the whole yellow packet at this time.
Pretest Correct Answers

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You should complete questions 1-10 before moving onto the clinical presentation (starting with the green packet).

2. (A) is correct. The HLA phenotype of an individual can be determined by HLA typing. Typing identifies the antigens that are present on the individual's cells (the phenotype), but does not indicate the chromosomal arrangement of the alleles (the haplotype) that code for those antigens. (A) is correct because it is the form that merely lists the A and B antigens expressed on the mother's cells without regard to their allelic arrangement. (D) also has the correct antigens, but it gives the additional information including the arrangement of the alleles. One can know the phenotype without knowing the haplotype but not vice versa. (Note: Questions 2 and 3 involve only HLA-A and B genes and antigens. HLA-C and D/DR genes are inherited in the same way. They were eliminated to simplify the problem.)

3. (D) is correct. The HLA haplotype of an individual consists of both sets of alleles arranged to designate which alleles were inherited from each parent. The father's haplotype is determined by examining the HLA antigens of his children. Children 1, 3 and 5 inherited A1 and B5 from the father, so A1 and B5 must be on the same chromosome. Children 2 and 4 inherited both A3 and B7, so they must be on the same chromosome. Make sure that your team can understand the concept of haplotype and the combined inheritance of linked loci.

7. (B) is correct. When a graft containing immunocompetent cells is placed into the recipient, the transplanted cells can recognize the allotypic differences of the host antigens and become activated. These activated donor T cells proliferate and differentiate into effector cells that attack the host cells and cause GvHD.

It is thought that much of the tissue damage is caused by CD8+ T cells while sensitized Th cells secrete cytokines that promote the reaction. If a population of host cells survived the pretreatment protocol (and some often do), they can be mobilized to the site of the reaction and participate in the response. Because these reactions are largely driven by T cells and cytokines such as IL-2 and IFN-γ, granulocytes are rarely seen in the cellular infiltrates characteristic of GvHD.

START HERE – INSTRUCTIONS

When the designated pretest answers have been discussed, you are ready to begin the clinical problem. Each student has a different part of this patient's story. Begin with the green booklet (part A). Discuss this part of the patient data and answer the questions. When Booklet A has been completed, proceed to the yellow booklet (part B), etc. Working together in this way, decide on the best course of action for this patient.

START WITH STUDENT A
STUDENT B

Data and analysis section

Have your group look at the data from HLA typing of Jeff’s family (top table from the next section of your “Data Pack”).

<table>
<thead>
<tr>
<th></th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeff</td>
<td>2, 3</td>
<td>27, 47</td>
<td>2, 6</td>
<td>7</td>
<td>2, 8</td>
</tr>
<tr>
<td>Mother</td>
<td>1, 3</td>
<td>8, 27</td>
<td>2, 7</td>
<td>7, 17</td>
<td>2, 8</td>
</tr>
<tr>
<td>Sibling 1</td>
<td>1, 3</td>
<td>8, 62</td>
<td>2, 7</td>
<td>17, 11</td>
<td>2, 5</td>
</tr>
<tr>
<td>Sibling 2</td>
<td>1, 2</td>
<td>8, 47</td>
<td>6, 7</td>
<td>7, 17</td>
<td>2</td>
</tr>
</tbody>
</table>

**Ask:** Why is there only a single value for some of the HLA genes? (e.g., Jeff’s HLA-DR)

**Answer:** The individual is homozygous at that locus. For example, Jeff inherited an HLA-DR7 allele from both his father and his mother.

**Ask:** What is the most likely phenotype of Jeff’s father?

**Answer:** His father most likely was HLA-A2, 3; B47, 62; C2, 6; DR7, 11; DQ 2, 5. This was determined by subtracting the mother's phenotype from each of the children's phenotypes as each child needed to inherit one allele at each loci from each parent.

**Ask:** Can you determine the father's haplotype?

**Answer:** His father's haplotype is A2; B47; C6; DR7; DQ2 / A3; B62; C2; DR11; DQ5. The phase can be determined by looking at each of the children. They will usually be inherited as a group on a single chromosome. Each child reveals the haplotype of one on the father's chromosomes minus the maternal contribution.

**NOTE:** Keep in mind that the WHOLE MHC is usually inherited as a group to determine if Jeff’s HLA-DQ 2 or 8 came from his mother or his father. If you have any questions about this problem, please ask a faculty member for the “simple trick” to do these problems.

**Ask:** What could make these determinations invalid?

**Answers:** 1) Recombination (crossing over during meiosis) would also complicate the analysis. It should be remembered that recombination between loci within the HLA is rare because they are on such a small region of the chromosome. 2) Nonpaternity. There isn't evidence of either event.

**Ask:** Are any of Jeff’s family members a good potential candidate donor?
**Answer:** The results of HLA typing indicate that Jeff and sibling 1 inherited completely different HLA containing chromosomes. Sibling 2 is a half match so neither is compatible with Jeff. *No, none of the family members is a good match.* According to the National Marrow Donor Program, a related match is only found 37% of the time.

**Ask:** What is Jeff's best option for finding a donor?

**Answer:** An unrelated donor through the National Marrow Donor Program (NMDP) who is a better match than his siblings.

The patient indicates that he would like to proceed with the transplant immediately. You inform him that it can take up to four months to search for a matched, unrelated donor through the National Marrow Donor Program (NMDB) in Minneapolis, MN. The computer database at the NMDP program contains the MHC types of nearly 5 million potential donors in the United States and connects with registries in 14 different countries. These international search increase the possibility of finding a matched, unrelated donor. Efforts have been made to identify donors from different ethnic backgrounds (see folder for data on success of identifying unrelated matches).

You also explain the risks of receiving an HSCT from an unrelated HLA-matched donor. It is possible that the graft would fail to engraft or occasionally it is rejected by the patient's immune response. In some instances, infections have been passed through transplanted materials. Probably the most common serious side effect is the donor lymphocytes in the HSCT could attack the recipient’s tissues causing a condition called **graft-versus-host disease** (GvHD).

The computer database at NMDP identifies four individuals as potential donors: 3242-5423-5, 4328-7895-7, 8493-0983-2, and 3423-5646-1.

Have your group look at the data from HLA data from unrelated donors from NMDP database (middle table from yellow handout).

<table>
<thead>
<tr>
<th>NMDP Potential Donors for Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NMCP #</strong></td>
</tr>
<tr>
<td>Jeff</td>
</tr>
<tr>
<td>3242-5423-5</td>
</tr>
<tr>
<td>4328-7895-7</td>
</tr>
<tr>
<td>8493-0983-2</td>
</tr>
<tr>
<td>3423-5646-1</td>
</tr>
</tbody>
</table>

*Age/Sex/Race

**Ask:** Which one of the donors would you select for Jeff?

**Answer:** Donor 3242-5423-5 appears to be the best match of the four candidates. She appears to be a “perfect” match and is better match than either sibling.

Have the group move onto material to be presented by student C (blue).
You should complete questions 1-10 of the pretest before moving onto the clinical presentation (starting with the green packet).

4. **(D) is correct.** The major barrier to successful hematopoietic stem cell transplantation is the antigens encoded by the major histocompatibility gene complex. Monozygotic twins (identical twins) have identical major histocompatibility gene complexes and major histocompatibility complex molecules. Individuals such as a parent, first cousin, or unrelated individual are not likely to be perfect matches; therefore, they are not ideal donors for hematopoietic stem cells. While gender matched siblings, dizygotic twins, or an unrelated individual might be a 6-antigen match, it should be noted that typing has only been performed for a relatively small number of genetic markers and there is ample room for differences in those that have not been typed. Usually, siblings and dizygotic twins will be mismatched at minor histocompatibility loci. On the positive side, typing includes those markers that have a stronger influence in the outcome of a graft.

8. **(B) is correct.** One of the simplest and least invasive ways to determine if a hematopoietic stem cell transplant is functioning as a source of blood cells is to monitor the red blood cell and white blood cell numbers in the peripheral blood of the recipient. Not infrequently, however, the patients also undergo bone marrow biopsy. The liver and lymph node biopsy do not offer any concrete information regarding the success of a hematopoietic stem cell transplant. Similarly, measuring the creatinine clearance by the kidney is of no value for determining the success of the transplant.

**START HERE - INSTRUCTIONS**

When the designated pretest answers have been discussed, you are ready to begin the clinical problem. Each student has a different part of this patient's story. **Begin with the green booklet (part A).** Discuss this part of the patient data and answer the questions. When Booklet A has been completed, proceed to the **yellow booklet (part B),** etc. Working together in this way, decide on the best course of action for this patient.

**START WITH STUDENT A**
Have your group look at the data from MLR data using cells from Jeff, selected family members, and unrelated donors (bottom table from yellow handout).

<table>
<thead>
<tr>
<th>Responder Cells</th>
<th>Stimulator Cells</th>
<th>Jeff</th>
<th>Mother</th>
<th>Sib 1</th>
<th>Sib 2</th>
<th>3242-5423-5</th>
<th>4328-7895-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeff</td>
<td></td>
<td>483</td>
<td>5,923</td>
<td>9,767</td>
<td>6,824</td>
<td>4,989</td>
<td>896</td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td>15,322</td>
<td>742</td>
<td>17,345</td>
<td>2,348</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sib 1</td>
<td></td>
<td>65,897</td>
<td>22,822</td>
<td>485</td>
<td>8,927</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sib 2</td>
<td></td>
<td>13,092</td>
<td>5,827</td>
<td>27,243</td>
<td>506</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Donor 3242-5423-5</td>
<td></td>
<td>1,892</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>427</td>
<td>ND</td>
</tr>
<tr>
<td>Donor 4328-7895-7</td>
<td></td>
<td>17,854</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>309</td>
</tr>
</tbody>
</table>

The "x" next to the stimulator cells means they have been irradiated to prevent them from responding. The numbers indicate the amount of radioactive tritiated thymidine (3H-TdR) incorporated by the responder cells.
- A large number = more incorporation (proliferation) = higher response = incompatibility.
- A small number = less incorporation (proliferation) = lower response = compatibility.

**Ask:** What do the numbers indicate?

**Answer:** Radionucleotide (tritiated thymidine) was incorporated into the DNA during synthesis. DNA synthesis was induced by lymphocyte proliferation. Proliferation was largely induced by Class II MHC mismatch. The degree of 3H-TdR incorporation is thought to represent the degree of mismatch.

**Ask:** Based on the MLR, who would be the best donor for HSCT?

**Answer:** As indicated by HLA typing, unrelated donor #3242-5423-5 would be the best donor for HSCT because she responded the least to Jeff’s cells. This was tested in the block where the donor cells were the responder cells while Jeff’s were irradiated stimulator cells.

Because Jeff will receive whole body irradiation and high doses of irradiation prior to HSCT, his immune system will be significantly compromised. The greatest risk is of GvH disease where the graft attacks Jeff's cells while HvG disease is not highly likely.

**Remind your group members of this IMPORTANT concept:**

**Host-versus-Graft Reaction** = the recipient's immune response to the graft

The reaction can lead to graft failure. This is thought to occur more often in solid organ transplants. In a one-way MLR, this can be detected by irradiating the graft cells.

**Graft-versus-Host-Reaction** = the graft's immune response to the host (recipient)

The reaction can lead to systemic disease and even death of the host. This usually occurs when the recipient receives large numbers of immunocompetent lymphocytes in the graft. This is common during HSCT and can occur during some solid organ transplants (e.g., intestinal transplants). In a one-way MLR, this can be detected by irradiating the host cells.
**Ask:** Why didn't 3242-5423-5 react strongly against Jeff's HLA-DR 7 as this donor is HLA-DR4, 7?

**Answer:** This donor is HLA-DR4, 7; therefore, her cells are tolerant to BOTH DR4 and DR7. Her cells would not react to Jeff's cells expressing HLA-DR7.

**Ask:** Why is Jeff's response against 4328-7895-7 lower than donor 3242-5423-5?

**Answer:** Jeff is tolerant of 4328-7895-7's class II MHC (Jeff is DR 7; DQ 2, 8 while this donor is DR 7; DQ2). In contrast, 3242-5423-5 is DR 4, 7; DQ 2, 8. Jeff would not be tolerant of her DR 4 antigen.)

**Ask:** If Jeff were to need a kidney transplant instead of a HSCT, which donor would be the best based entirely on the MLR data?

**Answer:** 4328-7895-7 would be a better donor because Jeff has a weaker response against his lymphocytes. The concern in a kidney transplant is the host rejecting the graft not the graft rejecting the host. Therefore, the cells from the individual donating the graft should be irradiated. Please note that in real practice this is NOT used during kidney transplantation. ABO compatibility and Panel Reactive Antibody tests are routinely performed. Cross matching is also done prior to solid organ transplant.

---

**Preparing the graft and the patient**  Jeff is counseled regarding the possible outcomes of HSCT. An HSCT can fail to engraft; this occurs in ~10% of the cases. When grafts fail, patients may develop an infection and die. You isolate and store frozen some of Jeff’s own bone marrow cells for future use in an autologous graft should the first graft fail. You do this as a precaution because cells for a second HSCT from the same donor are not always available.

GvHD is a major concern when using an unrelated donor HSCT. This risk is thought to be higher than when an HLA-matched sibling is identified. A GvH reaction is an immunologic response where graft cells attack the host. GvHD can be mild to severe; when severe, it can be life threatening. The five-year survival rate in patients with CML is 70% if the HSCT is successful.

In preparation for the HSCT, Jeff is given high doses of chemotherapy and total body irradiation to hopefully eliminate all of the malignant cells. During this process, most of his normal hematopoietic cells are also killed. Reconstitution with donor hematopoietic stem cells is required for Jeff to survive otherwise he is unable to make WBCs, RBCs, and platelets.

When the immunosuppressive treatment is nearly completed, bone marrow or peripheral blood stem cells are harvested from the donor. To harvest bone marrow the donor is placed under anesthesia and marrow is collected from the posterior-superior iliac crests marrow (see pictures page 3). An alternative is to administer GM-CSF to the donor to mobilize stem cells from the bone marrow and harvest peripheral blood, from which a stem cell (CD34+) enriched leukocyte preparation can be obtained.

In this case, stem cells from donor 3243-5423-5 are obtained from the iliac crests and infused intravenously into Jeff.
**Ask:** Your group mates why the donor cells are given intravenously. Why not inject them directly into the recipient's marrow cavities?

**Answer:** Although technically feasible, the injection of donor marrow cells into the recipient's marrow cavities would be traumatic to the recipient. The marrow cells are withdrawn from the donor into a sterile syringe and transported to the laboratory, where a cell count is performed. Erythrocytes, which are not required for transplantation and engraftment, are often removed. The cells are then diluted in sterile balanced sodium chloride solution and infused slowly into a peripheral vein of the recipient. This procedure has proven to be the safest and most effective method of transplanting bone marrow. A proportion of the infused marrow stem cells (CD34+ cells) will home to the recipient's marrow and stay there.

Have the group move onto material to be presented by student D (pink).
Pretest Correct Answers

You have the answers to some of the pretest questions, and other members of your group have the rest. This arrangement is designed to encourage all members of your group to actively participate in an exchange of ideas and concepts. First, study the answers in your booklet and then EXPLAIN them to your group. Please don't just read them to your classmates, and don't let your classmates read their answers to you. In explaining something to another person, most people gain a better understanding of it and often transmit a better understanding. The pretest discussion and patient-oriented problem-solving parts of this activity are "open book." Be sure to refer to textbooks, notes and other written resources whenever questions arise within your group, or discuss questions with your instructor.

You will probably want to make notes on your pretest about questions you missed to help you review. Avoid "collecting papers" for later study and understanding. Learn the concepts now so that later you need only review them.

You should complete questions 1-10 of the pretest before moving onto the clinical presentation (starting with the green packet).

5. (A) is correct. Technically performing a hematopoietic stem cell transplant is 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 sites. No invasive surgery or invasive procedures are involved.

Despite the “ease” of transplantation, the preparation (chemotherapy and radiation) and the consequences of transplantation make this procedure both physically and emotionally very challenging for patients and their families.

10. (D) is correct. The blood, bone marrow, and cord blood connecting a fetus to the maternal body are all sources of hematopoietic stem cells. The liver is only involved in hematopoiesis very early during embryonic life (between weeks 6 and 20 of gestation) and has not been used as a source of hematopoietic stem cells.

START HERE - INSTRUCTIONS

When the designated pretest answers have been discussed, you are ready to begin the clinical problem. Each student has a different part of this patient's story. Begin with the green booklet (part A). Discuss this part of the patient data and answer the questions. When Booklet A has been completed, proceed to the yellow booklet (part B), etc. Working together in this way, decide on the best course of action for this patient.

START WITH STUDENT A
Approximately 10 days after the HSCT, Jeff becomes febrile. Blood cultures are ordered but are negative. He was treated with antibiotics and the fever abated. At 20 days after HSCT, his white blood count began to increase compared to Day 0 of the transplant. During this time, he was given two red blood cell transfusions and regular transfusions of platelets. At one-month post-HSCT, a bone marrow biopsy revealed a relatively vacant marrow (the marrow was hypocellular). Philadelphia chromosome positive cells were not found cytogenetically or by RT-PCR. At approximately 36 days after HSCT, Jeff was discharged from the hospital and given prescriptions for antibiotics, and Cyclosporine A.

**Ask:** Why was Jeff treated with antibiotics on day 10? At discharge?

**Answer:** 10 days after the bone marrow treatment, Jeff was severely compromised. His new bone marrow was unable to make neutrophils and the resulting profound neutropenia put him at significant risk of bacterial and fungal infections. These microbes could come from his normal flora. Jeff was given an antibiotic that would cover enteric gram-negative (from the GI tract) and gram-positive (from the skin) organisms.

At discharge, Jeff was given ciprofloxacin (Cipro), to lower the intestinal bacterial load and decrease the risk of infection. Jeff will need to be particularly careful regarding infections for some time after the transplant as his immune system continues to develop. Another consideration is the immunosuppressive drugs that Jeff is being given to avoid graft-versus-host disease.

**Ask:** Why was Jeff treated with cyclosporin A? How does it work?

**Answer:** Cyclosporine was given to prevent the development of a severe form of GvHD. Cyclosporine is a fungal peptide that selectively inhibits the maturation of T lymphocytes and suppresses T cell function. It does this in part by blocking signaling through the TCR. *Cyclosporin inhibits calcineurin, a phosphatase required for activation of the transcription factor NFAT and ultimately cytokine production e.g., IL-2 and IL-2R α-subunit).*

FK506 (Tacrolimus), another immunosuppressive drug, also blocks the same site in T cell signaling.

**Ask:** Is it significant that WBCs appear in Jeff’s blood at day 20?

**Answer:** Yes, it is the first indicator of engraftment and suggests that the new bone marrow is starting to function; however, it normally takes a year or longer for the immune system to respond "normally" following bone marrow transplantation.
60 days after HSCT, Jeff came to the clinic with diarrhea with bloody, liquid stools. A rash had developed on his back, and several lesions were present on the forehands and palms of the hands. Endoscopic examination of the gastrointestinal tract including the colon revealed inflammation and mucosal changes. Several biopsies of the gut and affected skin were taken and the patient was admitted to the hospital. **See pictures for illustrations (top of page 4 in folder).**

**Ask:** How do you interpret the biopsies?

**Answer:**
Low power microscopic view of a duodenal biopsy showed a decreased villous to crypt ratio, glandular loss and mucosal erosion. Brunners glands with dilation are present beneath the *muscularis mucosa.* A submucosal diffuse mononuclear cell infiltrate that is predominantly lymphocytoid cells apparent.

The skin biopsy shows degeneration of the basal layer of the epidermis and a superficial perivascular mononuclear cell infiltrate at the dermo-epidermal junction with invasion of the epidermis by the infiltrating mononuclear cells. High power examination showed apoptotic squamous cells with adjacent lymphocytes in the epidermis. A diagnosis of acute GvHD was made. In addition to systemic administration of immunosuppressive drugs, topical therapy with corticosteroid cream was prescribed and alleviated the rash.

The skin and gut biopsies were compatible with a diagnosis of systemic GvHD. Jeff was treated with intravenous methylprednisolone (a systemic corticosteriod) and improved rapidly over the next three days. Approximately 10 days later, he was discharged from the hospital with a prescription for prednisone, which was to be tapered according to the usual protocol. One hundred days after HSCT the patient reported that he felt well. On one clinic visit at 210 days after HSCT, further treatment with oral prednisolone for a skin rash was prescribed. One year after the transplant, Jeff was able to return to work. He remained Philadelphia chromosome negative via both cytogenetics and RT-PCR.

**Ask:** How can a graft-versus-host reaction develop when the donor and recipient were HLA matched?

**Answer:** The graft-versus-host reaction can be caused by minor changes in the HLA alleles that were not detected by serological testing. Remember, serological testing which is often used for class I MHC typing relies on B cell epitopes that are most likely only a few amino acid residues long. Other variations unique to that individual might not be detected. Sequencing has identified that many of the serologically based designations have multiple subtypes. These minor variations may play a role in the development of graft-versus-host disease. As one would expect, this is more common when the donor and recipient are unrelated.

In addition, rejection can be mediated by genes outside of the major histocompatibility complex. These are sometime referred to as minor histocompatibility antigens. More than 40 genes have been identified to date that affect transplant rejection.

Please note that the additional pictures were included to help you visualize the GvH reaction and understand the process.

You should now lead your group to moving through the final problems 1-4 in the Data Pack and closing comments.
Jeff Thomas, a 43-year-old man, goes to his primary care physician for a general physical. He was in apparent good health with no specific complaints outside of being a bit more tired lately. Blood tests revealed that his white blood cell (WBC) count was elevated to 36,000 cells/mm$^3$ (N 4,500-11,000). All other tests completed fell within normal limits.

The general practitioner requests further blood cell analysis and refers him to your Hematology/Oncology group. These tests revealed that Jeff’s WBC count was reproducibly elevated and the differential cell analysis revealed 85% neutrophils (N 54-62%), 12% lymphocytes (N 25-33%) and 3% basophils (N <1%). Examination of a blood smear by a hematopathologist revealed that the majority of WBCs appeared to be immature neutrophils.

After consulting with the members of your group practice (the group at your table), you perform a bone marrow biopsy and submit it to a pathologist for analysis. The marrow biopsy was reported to be hypercellular (more cells than normal), and chromosomal analysis revealed that the Philadelphia chromosome was present in many cells. Jeff was diagnosed with Chronic Myelogenous Leukemia (CML).

The Philadelphia chromosome results from a reciprocal translocation or movement of part of the long arm of chromosome 22 to the long arm of chromosome number 9. The molecular consequence of this translocation is the generation of the fusion protein BCR-ABL, a constitutively activated tyrosine kinase, which is present in virtually all patients with CML. The Philadelphia chromosome can be identified through cytogenetics and FISH analysis. A test relying on a reverse-transcriptase polymerase chain reaction (RT-PCR) is the most sensitive method for detecting low numbers of BCR-ABL transcripts.

Leukemia can be thought of as malignant neoplasia of white blood cell precursors or stem cells. The normal bone marrow of these patients is replaced with proliferating leukemic cells. Abnormal, immature cells appear in the blood and infiltrate the liver, spleen, lymph nodes, and other sites in the body. The elevated white blood cell count in these patients is not fatal. Rather, CML patients suffer from anemia, thrombocytopenia, and loss of normally functioning leukocytes due to the heavy infiltration of the bone marrow by proliferating leukemic cells. The normal elements are few in number and are unable to maintain an adequate supply of normal cells. In addition, the infiltration by leukemic cells of many tissues and organs leads to widespread organ dysfunction and ultimately death if the leukemia is not aggressively treated (see the first page of pictures in the supplementary handout) to enrich your understanding of CML).
HLA-A, B and C Typing

Lymphocyte Suspension + Antibody of known specificity

Incubate 1 hour at 22°C
Add complement

Incubate 1-2 hours
Add stain

15 minutes later

Examine under phase microscope

Stained lymphocytes
- The cells expressed the MHC allele recognized by the antibody.
- Complement lysis allowed the stain to enter cell.

Unstained lymphocytes
- The cells didn't express the MHC allele recognized by the antibody.
- Little complement lysis so stain doesn't enter the cell.
When instructed to do so by student A, the group will discuss how the HLA - A, B and C typing assay is performed.

The first step in the HLA typing is usually serological screening of HLA-A and HLA-B on lymphocytes. If there appears to be a potential match, class II MHC is typed (and possibly class I MHC is subtyped) using PCR.

Method:
1. The lymphocytes are obtained from 10 to 20 mL of peripheral blood. The lymphocytes are plated into microtiter plates.
2. Aliquots of the lymphocytes are mixed with human serum containing antibodies specific for individual HLA antigens. These sera are obtained from multiparous women or individuals who have received blood transfusions and have made antibodies to HLA antigens to cells of the fetus or in the transfused blood. Antibodies from anti-HLA (each with different reactivities) panels are added to different wells. To simplify the process, the antibodies added to well 1 could react to HLA-A1, well 2 could react to HLA-A31, etc. The samples are incubated to allow binding of any anti-HLA antibodies to the cells.
3. Serum complement is added and the mixture is incubated for an additional 1 to 2 hours.
4. A stain or dye is added and the mixture incubated for a short period.
5. The cell suspension is microscopically examined. If the membranes have been damaged by the reaction of antibodies and complement, the cells take up the stain, while the undamaged lymphocytes will not.
Mixed Lymphocyte Reaction (MLR)

1. Irradiated stimulated lymphocytes
2. Responder lymphocytes
3. Irradiated responder lymphocytes

- Incubate for 5 days at 37°C
- Add radioactive thymidine
- Transfer to filter paper
  - Radioactive DNA
  - Nonradioactive DNA (control)

Scintillation counter
The primary concept in this lesson is that differences in the HLA class II antigens between the cell populations used in the assay will stimulate T lymphocytes to synthesize DNA and divide. The MLR is designed to quantitate the amount of cell division as measured by newly synthesized DNA in responder lymphocytes when exposed to irradiated (non-dividing) stimulator lymphocytes.

The MLR requires peripheral blood lymphocytes from two individuals. As in the diagram, responder and irradiated stimulator lymphocytes are mixed together in MLR. The stimulator lymphocytes are irradiated to prevent them from acting simultaneously as stimulator and responder cells. Irradiation damages the cells’ DNA and prevents them dividing; however, they are still express their class II MHC and induce proliferation in the responder cells.

A "control" MLR consisting of a mixture of responder lymphocytes and irradiated responder lymphocytes is conducted simultaneously. The cultures are incubated for a period of 5 days at 37°C, at which time a radioactive precursor of DNA, tritiated thymidine (³H-TdR), is added to each of the cultures. After 18 hours of additional incubation, the cellular DNA containing the incorporated ³H-TdR is assayed for radioactivity. As is shown in the example in this diagram, the responder lymphocytes recognize the irradiated stimulator lymphocytes and incorporate a significant amount of ³H-TdR. In contrast, the responder lymphocytes do not proliferate when mixed with irradiated responder lymphocytes and do not incorporate radioactive thymidine. This typing assay measures the ability of responder lymphocytes to recognize non-self-HLA-D antigens on stimulator cells and is a measure of the class II MHC antigenic difference between the donors of the responder and the stimulator lymphocytes.

POINT TO NOTE: MLRs predominantly detect class II MHC mismatches.
HLA typing of Jeff's family

Family Study for the Patient and His Family Members

<table>
<thead>
<tr>
<th></th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
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<td>27, 47</td>
<td>2, 6</td>
<td>7</td>
<td>2, 8</td>
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<td>Mother</td>
<td>1, 3</td>
<td>8, 27</td>
<td>2, 7</td>
<td>7, 17</td>
<td>2, 8</td>
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<td>8, 62</td>
<td>2, 7</td>
<td>17, 11</td>
<td>2, 5</td>
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<td>8, 47</td>
<td>6, 7</td>
<td>7, 17</td>
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NOTE: Keep in mind that the WHOLE MHC is usually inherited as a group to determine if Jeff's HLA-DQ 2 or 8 came from his mother or his father.

HLA data from unrelated donors from NMDP database

<table>
<thead>
<tr>
<th>NMDP #</th>
<th>A/S/R*</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
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<tr>
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<td>7</td>
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<tr>
<td>4328-7895-7</td>
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<td>2</td>
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*Age/Sex/Race

MLR data using cells from Jeff, selected family members, and unrelated donors

<table>
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<tr>
<th>Responder Cells</th>
<th>Stimulator Cells</th>
<th>Jeff</th>
<th>Mother</th>
<th>Sib 1</th>
<th>Sib 2</th>
<th>3242-5423-5</th>
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<tr>
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<td>Jeff</td>
<td>483</td>
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<td>Sib 2</td>
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<td>ND</td>
<td>309</td>
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The "x" next to the stimulator cells means they have been irradiated to prevent them from responding. The numbers indicate the amount of radioactive tritiated thymidine (3H-TdR) incorporated by the responder cells.

A large number = more incorporation (proliferation) = higher response = incompatibility.
A small number = less incorporation (proliferation) = lower response = compatibility.