Transcript for Hematopoietic Stem Cell Transplant- Hematopoiesis

KMISHAW@mdanderson.org:
Hello everyone, my name is Kathy Mishaw. I am one of the nursing educators. I am over in the cancer network. Our topic today is to discuss hematopoiesis and immunology.

This is a foundational lecture that will help you understand stem cell transplantation in the Stem cell transplantation and Cellular therapy class, which you will be attending in the future.

The objectives are to describe the structure and function of the bone marrow and then to recognize what is a stem cell and what are their characteristics.

Explain the process of hematopoiesis and identify the different cell lineages that are derived from the hematopoietic stem cell and what their functions are.

And then discuss a couple of the immunologic principles of stem cell transplantation. Hematopoietic stem cell transplantation is the most widely used cellular immunotherapy.

It is a multi-step process that involves the administration of what we call a preparative or conditioning Regimen.

And that usually consists of chemotherapy plus or minus radiation in the form of total body radiation. There may be a targeted agent/ a targeted therapy that's also part of that conditioning regimen.

Once that preparative conditioning regimen is completed, we will follow that with the infusion of hematopoietic stem cells to reconstitute or replace the patient's hematopoietic and immune system.

The doses of chemotherapy are high enough usually that we myeloablate or kind of wipe out the bone marrow.

When we infuse the stem cells, it “rescues” the bone marrow and provide new stem cells, so we regain our blood cell and our immune system function.

This is a potentially curative treatment for certain cancers. Definitely the leukemias, the lymphomas and the multiple myeloma respond as well as some select solid tumors such as some germ cell tumors like testicular cancer.
Now, if someone has a non-cancerous situation, a benign condition, where they need a new immune system, such as aplastic anemia or sickle cell disease as an example, then certainly stem cell transplant may also be a curative regimen for them.

Now to the concept of a stem cell. Stem cells are not found in all organ systems.

But the stem cells we do have in certain organ systems, have the characteristics of, upon a certain command or certain needs/certain signaling, they will self-renew.

And these cells can also differentiate out into mature cells, usually in that same organ system. So they have this lifelong ability to self-replicate and again, differentiate into mature cells. A couple of examples of the adult stem cells that we have, one is what we call a mesenchymal stem cell.

You can see that this cell can replicate or regenerate bone and cartilage.

There are neural stem cells that can regenerate nervous system tissue. And then of course we're interested in the hematopoietic stem cell because we want to be able to regenerate and recover bone marrow function and be able to make different blood cells. In the case of a hematopoietic stem cells, they can differentiate into any mature blood cell, such as a red cell, such as the myriad different types of white cells, we have or mature into a platelet.

And so, as we look at this picture or this cartoon, you can see at the top, is that what we call a multi potential hematopoietic stem cell.

And the reason we call it a multi potential cell is, because depending on the signaling or messaging it gets, it can become a myeloid or lymphoid cell.

Furthermore, if it becomes a myeloid cell, then it can differentiate into a platelet a red blood cell, or here we start with our White cells. The mast cell or any other types of white cell coming from a myeloblast.

And vice versa if it decides to become a lymphoid progenitor. If that was the signaling message, we need more lymphocytes.

It can become a T lymphocyte, a B lymphocyte, or natural killer cell.
And that hematopoietic stem cell you see at the top, you see a CD34 number by it.
That is a nomenclature that we use. All cells of the immune system including the hematopoietic stem cell.

All cells of the immune system, including the hematopoietic stem cell, express different clusters of proteins on their surface which we call these cluster differentiation proteins, CD for short.

It's a nomenclature system. These CD numbers serve as a marker that tell us the type of immune cell that we're dealing with, i.e what cell lineage did it come from,
was it a myeloid or lymphoid cell, how mature or how differentiated is this cell and also tells us about the cell function.

The commonly recognized marker of the hematopoietic stem cell is CD34. Now you might say, that if this hematopoietic stem cell is the mother of all blood cells, how come it's not CD1? Actually, because it was the 34^{th} cell identified so that is why it has a CD34 number.

Now other CD numbers that you will commonly recognize are, for example, CD3 that we see on all T cells. Also you see CD4 on helper T cells. You see CD8 on killer T cells and on regulatory T cells.

On B lymphocytes you see CD19, CD20 and CD21. So these are some examples of the CD numbers that we use commonly.

And the capacity for this stem cell to differentiate diminishes as it moves from this pleuripotential cell up here being able to be plural types of cells.

But as it becomes committed to the myeloid line and even further committed to being a megakaryocyte or platelet, it's now committed...its unipotential it's committed to only a single cell line. It can't go back up and decided it wants to become a red cell.

So the capacity for differentiation diminishes as it goes from this multi potential to a unipotential cell that's committed to that one cell line.

Now we're going to talk about the hematopoietic stem cell. It is influenced by the signaling that occurs with circulating growth factors.

It's also under the influence of the microenvironment of the bone marrow. So we're going to use an analogy of a garden to “seed in the soil” analogy. We're going to say that the CD34 cells are like the seeds. We're going to say the growth factors are like fertilizers and we're going to say the bone marrow micro-environment is like the soil.

Of course we need good seed, good fertilizers and good soil for us to be able to generate blood cells.

These multi potential stem cells, are held in reserve until they are messaged or activated by cell signaling after we've used up some cells in the peripheral blood or maybe the bone marrow has been injured by chemotherapy or radiation.

And then, a message is sent back to the Multipotential stem cell, the CD34 cell, saying that we need more red cells or need more platelet or we need more white cells.

These a hematopoietic stem cells are found primarily in the bone marrow but there'll be a small percentage of these stem cells circulating out in peripheral blood. You will hear us call those peripheral blood stem cells.

Also stem cells are found in very concentrated numbers in umbilical cord blood. So cc per cc, there are a lot of stem cells in the umbilical cord blood, but there's just not much blood in the umbilical cord.

So right there are 3 sources of stem cells, we want to harvest them for stem cell transplant.

We can pull them from the bone marrow, harvest them from the bone marrow or harvest them from the peripheral blood or harvest them from what we call cord blood.

Now the bone marrow itself is very large organized tissue. Skin is probably the only other largest organ. As you remember bone marrow is that spongy bone in the cavities of hard bone. So when you look at a hard bone, 85% of the hard bone consists of that spongy bone marrow. 15% of bone is that hard medullary bone that we're familiar with.

And so, inside the hollow areas of the bone, you will see two compartments of the bone marrow. What we call the extravascular site and the intravascular site.

Extravascular sounds like what it means… it's outside the blood flow of the bone marrow.
But this is where all the hematopoiesis occurs.

These cells grow in discrete little colony forming units. So there is colony forming unit for the red cells (erythrocytes). There is a colony forming unit for the granulocytes. There's a colony forming unit for the lymphocytes.

All of these colony forming units respond to growth factors, such as granulocyte colony stimulating factor, which we call G CSF. And you know these cytokines or growth factors have been formulated into drugs as well.
These cells, as they mature go from kindergarten to 12th grade in these colony forming units. Then when they are mature they're going to get dumped on the peripheral blood.
And that's the intravascular compartment. We have thin walled venous blood vessels, which we call sinuses.

In the colony forming units, these cells lie in between these venous sinuses. When they are mature, they are going to penetrate through the sinusoidal walls and flow out into the blood. That's how we get our blood cells out into the peripheral blood.
Now, the primary function of bone marrow is hematopoiesis… is to make these blood cells.

And that production will occur in the part of the bone marrow called “red marrow”. It actively looks like it is making blood cells.

Then, believe it or not, as we age and get older, our bone marrow gets fattier. Over time, fat cells infiltrate the bone marrow spaces.
And so there will be some space in that spongy bone marrow where fat cells are primarily located. That area is called “yellow marrow”.

And that's important to know just because, if we were doing bone marrow aspirate and biopsy, you want to make sure you are in the red marrow areas of the bone marrow and not the yellow marrow areas.

And so, remember for example, on your long bones when you have a fracture you have to worry about a fat emboli. That's an example of a complication that can occur because of that yellow marrow being in the bone marrow of the long bone.

The two divisions of the bone marrow are the myeloid lineage and the lymphoid lineage. And, of course, you recognize that as how we name our leukemias. We will say they have a myelocytic leukemia or a lymphocytic leukemia. Coming off the myeloid line are our red cells, also known as a erythrocytes.

The big megakaryocyte, when it reaches 12th grade, it breaks open it makes about 2000 to 4000 platelets. Looks like a big plate was broken and we have platelets. Off the myeloid line also comes the granulocytes, which are the basophils, the eosinophils and the neutrophils.

And then also off the myeloid line, come the monocytes. These monocytes can go out into the tissues, swell in size and become other cells known as the macrophages or dendritic cells.
When you look at the lymphoid line there are T lymphocytes, B lymphocytes and natural killer cells. Be aware that the whole lymphoid line are all white cells.
Be aware that granulocytes and monocytes in the myeloid line are white cells.

I want you to know that the cells from both divisions all come from the hematopoietic stem cell. And all of these cells are made exclusively only in the bone marrow **EXCEPT** the lymphocytes. The lymphocytes are produced in the bone marrow but many of them will go to the lymphoid tissue (kind of like their chat room..the lymph nodes).
And they hang there, and if they get stimulated by specific antigen then at that point, they can replicate and divide in the lymphoid tissue and that's a normal process.

So, of all the blood cells that we have, the only cells that are allowed to replicate outside the bone marrow in a normal fashion, are the lymphocytes.

And here's that picture again. You can see the myeloid line and the lymphoid line. We discussed the different cell lines that were coming off each of these division.

Now lets look at the CBC, the complete blood count. When we talk about the differential, that refers to the “different” types of white blood cells. So we'll talk about the CBC with the differential. I am going to talk about the platelets.

I would like to talk about the red cells, and the H/H. Now do you see these values, the MCV, MCH, MCHC and RDW. These are what we call the red blood cell indices and I’m not going to go through their normal values. But these indices tell you everything you want to know about red blood cell.. what's its average size, how much hemoglobin is there in a single red blood cells, etc.

These are used to determine the different type of anemia that you might have i.e. like a megaloblastic or microcytic anemia or anemia from folic acid or iron deficiency.

So they will use these indices to tell us the type of anemia we have. We are going to say we are anemic based on hemoglobin and crit. But the type of anemia will based on the red cell indices.

Now cancer patients pretty much have an anemia of chronic disease. So we're not going to spend a lot of time discussing MCV, MCH, MCHC and RDW. We are going to talk about H/H and the reticulocyte.

We know that the RBCs job is to transport oxygen via the hemoglobin molecule. I don't know if you know this, but the hemoglobin molecule also takes on off hydrogen ions which help us keep our blood pH balanced.So if you are low on your hemoglobin, not only are you having trouble with hypoxia and being oxygenated, but you also may have trouble buffering blood.

Red cells are not a true cell because they got rid of their nucleus about the third or fourth grade. They got rid of the nucleus, so they could pack more hemoglobin into the red cell. What happens when you get rid of your nucleus, all those tissue typing antigens (HLA) on the surface of the cell came off the surface or are “down regulated”. Then up on the surface or upregulated are the A, B and RH antigens which we know is our red cell antigens.

So HLA antigens (called human leukocyte antigen) are the tissue typing antigens. Any cell in your body that has a nucleus has HLA proteins or antigens on the cell surface.

If the cell does not have a nucleus, then you do not have these HLA antigens. So that's a good thing in terms of red cells, because we can transfuse red cells between you and I, without being the same tissue type. All we have to do is match the A, B, and Rh antigens.

And you'll hear this later in the allogeneic transplant setting. It's possible that we have the same tissue type i.e. same HLA type as you donor from chromosome #6, but we inherit our blood type on a different chromosome. So it's possible that you are tissue type to your donor, but you have a different blood type.
So what will eventually occur over several months time is that patient will convert to his donor’s blood type.

The lifespan of a red blood cells about 120 days in the bloodstream. If you donate blood the shelf life of RBCs about 42 days or so.

The growth factor that tells the mother hematopoietic stem cell to make more red cells is erythropoietin. That cytokine comes primarily from the kidney. It's been manufactured into drug formulations. Erythropoietin was our first generation red cell growth factor. It had a short half-life and had to be dosed every week or several times a week.
Darbepoetin was the second generation, and it was formulated to be a longer lasting drug. It was dosed usually every two weeks. Then of course now as these drugs have lost their patents, biosimilars are available and have the same efficacy.
The interesting thing is that the bone marrow does not respond quickly to the red cell growth factors like it does to some of the white cell growth factors.

It takes several weeks, three or four weeks for the bone marrow to respond. Therefore, if you are anemic today, you probably need to transfuse red cells, because you may not be able to wait four weeks to get that hemoglobin for better oxygenation.

And so you can see here, the multipotential hematopoietic stem cells CD34 got the message via erythropoietin that we needed more red cells. The first version is the pro erythoblast then it matured into an erythroblast. There are several versions..the basophilic erythoblast, polychromatophilic erythoblast and then the orthochromotic erythoblast. Here is where hemoglobin synthesis is occurring within that cell. It does eject its nucleus so by the time it's a reticulocyte, an immature red blood cell.

This is a cell that does go out in the peripheral blood, but in small number like .5% to 1.5%. A reticulocyte is a slightly immature red, so it doesn't take on and off oxygen as fast as easily but within 24-48 hrs of getting out in the peripheral blood it matures on into a fully mature erythrocyte and then it has 120 day lifespan.

So only about .5 to 1% reticulocytes are to be out in the peripheral blood. But if the patient starts to bleed or if a patient is breaking down their red blood cells faster than 120 days, then the bone marrow gets a message to make more red cells. As the bone marrow gets that message, then it will start sending some of these reticulocytes. Remember they are like a 11th grader or so.

If we're using up all the mature red cells, the bone marrow may send out the reticulocytes out a little sooner, so you might see that reticulocyte count jump up to 10%.

If you see that retic count in the peripheral blood jump up that much, that tells you that we are hemolyzing blood cells or maybe they're actively bleeding.

When we evaluate oxygenation we look at the hemoglobin. There's the normal values for female and male. Most patients can tolerate the hemoglobin drop until around 7 or 7.5 before they usually get symptomatic. So that's usually, when we start transfusing patients with a hemoglobin between 7-7.5 g/dL.

It depends on your institution, but this is our number here. If a patient has coronary artery disease or has other some type of comorbidity they may get symptomatic well before this so maybe it 8-8.5g/dL and they're showing symptoms like sustained tachycardia, having a little trouble breathing, getting dyspneic on exertion.
Whenever they are symptomatic, we need to transfer or when they're getting 7-7.5 g/dL. we need to transfuse.
Hematocrit is the number of red cells per volume of plasma so it's a percentage. There is usually a 1:3 ratio between the hemoglobin and hematocrit.
So that means that if the hemoglobin was seven, the hematocrit is somewhere around 21%.
Also, when we give a unit of blood and if you are not actively bleeding, it should give your hemoglobin a bump up of about one gram and the hematocrit a bump up of about 3%.

Let's move on to talk about the platelets.

Platelets function by clotting blood or blood hemostasis. They do this by being very sticky and aggregating. Platelets are produced in the bone marrow but platelets again are not a true cells, rather they are fragments of a true cell called a megakaryocyte. So you can see that thrombopoietin is the platelet growth factor. That signal went back to the myeloid stem cell and started making megakaryoblasts which matured on down to 12th grade to a megakaryocyte. In 12th grade, the big megakaryocyte broke open to make about 2000 to 4000 platelets. Now the big megakaryocyte did have a nucleus, and it did have HLA antigens on its surface. When it broke open and made those 2000 4000 platelets, those platelets do have fragments of HLA on their surface.

As we mentioned the production is via the cytokine thrombopoietin. Also Interleukin 11 is a platelet growth factor. We currently don't have an approved platelet growth factor in the US for treatment of a low platelet count due to the cancer disease process or its treatment.

Now there is a drug, Romiplastin (Nplate), that the FDA has given a label for a low platelet count induced by ITP. But we actually do not have one, with an FDA label for thrombocytopenia caused by cancer or it's treatment. Sometimes we use this drug as an "off label".

The other thing you need to know about platelets is about 2/3 of them are circulating in the blood freely so when we do your platelet count in the morning, that's about 66% of the platelets you have. are circulating that's what's in your platelet count.

About a third of the platelets actually hang out in the spleen and are localized in the spleen. Platelets go back and forth between the blood and the spleen. If the spleen is involved with the disease process (the spleen is considered a lymphoid organ) and if you do have some type of lymphoid cancer, the spleen may be involved in disease and then may chew up or sequester the platelets. Then you have a lower than usual platelet count because the spleen is chewing them up/ destroying them.

The lifespan of platelets in the blood somewhere between 8-10 days, (9-12 days) something like. So it's considerably shorter than a red cell life span. The shelf life if someone donates platelets or we pull them off of a unit of whole blood, is about 5 days at room temperature. And the platelets have to stay in a lot of plasma (or a lot of liquid) so they are constantly in solution and need to be agitated so they don't create a platelet plug.

We have a tendency to keep our platelets for about 24-48 hrs. We think they're more viable and we get a better platelet a bump up. If necessary, they could be kept for five days at room temperature and the fact that they're kept at room temperature makes them, probably the most common blood cell that is being transfused that might transmit an infection because of that room temperature. Because it's kept at room temperature so that we're always on the alert watching for that.

Normal count is about 150 to 400,000 h/L. And because they have a short half-life (live only a few days in the body), what they look like today is what the bone marrow is doing today. So sometimes the platelet count is used as an estimate if the bone marrow has recovered from the previous course of chemotherapy and if the bone marrow is ready to take the next course of chemotherapy. So a frequent statement on your order sets might be that if the platelet count has not recovered back up to 100,000, do not start the next course of chemo. The bone marrow may still need a little bit more time to recover.

Now who does that parameter not apply to? Well it is the leukemics of course. They don't have a platelet count of 100,000, rather they sit at 10,000 to 15,000 platelet count due to their disease.

So, if you think you are not going to treat a leukemia pt until their platelet count gets up to 100,000 you'll never get them chemo. Since their disease process in is in the bone marrow, we can't use that as a parameter say the bone marrow is recovered.

If the platelet count drops below 50,000 we do not want to do invasive procedures without either transfusing them up, pre the procedure and/or transfusing them post procedure.

That includes IM injections. We don't want to give Intramuscular injections when the platelet count starts to drop below 50,000. You can give sub Q injections at any platelet count, but IM, you would need to avoid this, otherwise they could bleed into the muscle.

We know that with a 10 to 15,000 platelet count, that patients can be sitting, talking to you and have a spontaneous bleed. We worry about GI/ GU bleeds and bleeding into the skin, but the bleeds that are life threatening are the head bleeds and the lung bleeds. We are very concerned about both of those.

Additionally, sometimes a patient's platelet count looks good but they're on a drug that's anti platelet drug like aspirin or NSAIDS. Alcohol affects patient platelet function, sulfa drugs like bactrim, select antibiotics. And of course, there's a whole category of antiplatelet drugs that we use with cardiac patients who have a comorbidity in terms of the cardiac situation with increased risk for clotting. Patients at risk for strokes and MIs may be on an antiplatelet drugs such as clopidogrel and the other drugs in the same category. So the oncologist need to look at the patients medication profile and scrutinize it closely. When we know the platelet counts are low we may not want an antiplatelet drug on them at the same time that their platelet count is low.
So that was the platelet conversation.

Next we are going into the white cells and have a conversation about the white cells. So you can see on the myeloid line all the derivatives of the myeloblast are white cells. All the derivatives of the myeloblast which are the neutrophils, eosinophils, basophils and the monocytes. The mast cell is also a white blood cell. You can see that the monocyte can go out to the tissues and now become a dendritic cell or macrophage.

So we're going to have a conversation about those cells. The whole lymphoid cell line are all white cells so we'll discuss natural killer cells and then two types of lymphocytes.

When you look at the CBC, the complete blood count, they will list the differential which will tell you that different types of white cells that we have in percentiles. Normal white count is somewhere between 4000 to 11,000 per deciliter of blood (some labs have it at 5,000 to 10,000). Out of that about 2% or so are eosinophils, and somewhere up to half percent are basophils. Now the neutrophils, the segmented neutrophils are somewhere between 40 to 80 OR 50 to 90% of white cells are neutrophils.
There are two forms of the neutrophils sitting out in the peripheral blood. There's the mature form that are fully segmented neutrophils. The band form, which is about a 10th or 11th grader, that's where the nucleus has become slightly banded and then it matures into a mature neutrophils and becomes fully segmented. When you add these both together (bands and segs) we have somewhere between 40% to 80% (or 50-90%) of the WBCs are neutrophils.

Monocytes are by far a smaller number 6-8% and lymphocytes are somewhere between 10 and 14% of the white cells in your bloodstream. Now, remember with lymphocytes, their favorite place to hang out is in lymph fluid and lymph nodes. So really the number of lymphocytes you have in your blood, are not the only ones that you have in your body but all we can quantitate is what is in the bloodstream.

Let's talk about the way you might classify the leukocytes and their different functions.

There are nine major types but notice that only five are in the differential. We have the neutrophils, eosinophils, and basophils, what we call the granulocytes because we see these granules in their cytoplasm.

These 3 cells are in the bloodstream. There is a tissue form of the basophil called a mast cell. This is a white blood cell that is in the tissue. It is not on your CBC because it is not measured in the bloodstream, rather out in the tissue. But the basophils and the mast cells both have the same chemicals in their granules.
The monocytes are in the bloodstream but if they go out in the tissues, they are called macrophages or they can mature on out to a dendritic cell as well.
And then the lymphocytes are the T cells, B cells and the natural killer cells.

Notice that three of these major white cells have granules in the cytoplasm so they're called the granulocytes. These other white cells, monocytes and lymphocytes, do not have those granules.

Most common though, instead of dividing them by granulocytes or agranulocytes, we usually call them myeloid or lymphoid line.

Discussing them individually, the neutrophils we said were by far the most common white cell that you have in the peripheral blood. We call them polymorphonuclear cells, because they have many shapes to their nucleus 2-3 lobes to their nucleus.
They are the primary cell of the natural or innate immune system. They are the first line of defense against bacteria. They phagocytize bacteria and fungus. And of course, bacteria is what you have mostly as normal resident flora on your skin, in your GI tract. The flora all over your body is mostly bacteria and the neutrophils would manage those bacteria if they got into the bloodstream.

If you're neutrophil count falls below what we call 1,000 or 500 u/dL, your risk for bacterial sepsis is very high. We say when someone's neutropenic, they are usually below 1000. We think that the bacteria on their body is going to be their biggest risk for infection, as well as bacteria in their environment.

So eosinophils are only a small percentage of the white cells. What in their granules is how they kill parasites. They also clean up after allergies.

Basophils are only 0.2 to .5% and what's in their cytoplasmic granules are the chemicals that help with inflammation. They release bradykinin, histamine, and serotonin. This causes the blood vessels to dilate and become a little leaky so the white cells can rush to a tissue of origin where the infection is, squeeze out of the blood stream and get to that tissue to help take care of the infection.
If you degranulate too many basophils, you will get a hypersensitivity reaction. If you degranulate a whole bunch of basophils or mast cells, you end up with full blown anaphylaxis.

Monocytes are responsible for phagocytizing bacteria and fungus very similar to the neutrophils, but they are a bigger cell. Sometimes they migrate out into the tissues and when they do, they become much bigger in size and they're what we call the mononuclear (only 1 lobe to their nucleus) phagocyte system (also previously known as the reticuloendothelial system).

And since these cells are out in the tissues, they are patrolling the tissues and serve as the frontline of defense out the trenches.

They are strategically placed organisms are most likely come into tissues.
And these macrophages may have different names, based on what tissue site they are assigned. Macrophages sitting in skin and subcutaneous tissue called histiocytes.
Macrophage in the lymph nodes and lymph fluid, we just call macrophages. The bone marrow has a good number of macrophages in it. In the lung, the alveolar surfaces are just full of macrophages trying to catch organisms coming into the respiratory tract. Those are called lung alveolar macrophages. Macrophages that in the liver, are called Kupffer cells. Macrophages located in a portion of the intestine where most of the absorption occurs and are attempting catching whatever you ingested. Those macrophages are called Peyer's patch. Macrophages in the CNS trying to catch organisms, as they come through the blood brain barrier, are called micro glial cells. The spleen as we mentioned before, is a lymphoid organ, and it has per gram of tissue, more macrophages than any other organ system. And that's because the spleen is responsible for filtering blood for foreign organisms (just like the kidney is responsible for filtering blood for waste product), Macrophages kind of bridge between the natural/innate immune system. They are ready to go at time of birth and they will help activate the adaptive specific immune system because they present antigens that they picked up out in the tissues present antigen to the resting T lymphocytes. This antigen presentation will activate the specific or adaptive immune system.

Dendritic cells are even more potent in terms of patrolling the skin and mucosal surfaces. They pick it up antigens to traffic to the lymph nodes and present to the resting or quiescent T cells.
So dendritic cells are frontline for presenting what we call antigens and activating the T cells. Their role as a "professional antigen" cell is extremely important.

Now again, macrophage and dendritic cells, you can't measure them because they're not in the bloodstream so we can't quantitative them and tell you how much you have. But if you do not have enough dendritic cells or macrophages, you will have a lot of soft tissue infections.

Lymphocytes are 10-40% of your total white count. But remember, I told you there's an awful lot of lymphocytes in lymph fluid which we can't measure and there's a lot of them in lymph nodes. Lymphocytes come from the lymphoid line, the three major types are T cells, B cells, and natural killer cells. The T cells and the B cells are the effector cells or the "worker bees: of this specific or adaptive immune system.

Now they have what we call specificity and memory. So, the T cells are produced in the bone marrow and then migrate to the thymus where they learn how special they are.
They acquire the cell surface markers so they recognize self and don't attack self. So the T cells have receptors for those HLA (or tissue typing) proteins and they know what is self and not self. So, they won't attack self hopefully. The B cells do the same thing. They are produced in the bone marrow, but they actually stay in the bone marrow and acquire those surface markers for self in the bone marrow. The B cells also acquire surface markers that would recognize self or HLA proteins.
Now B cells also have specificity, because the other type surface marker they receive is for a specific organism. So if T cells, learn and acquire the specificity for attacking let's say CMV, they are specific to CMV and if RSV (respiratory syncytial virus) goes by, these T cells don't get activated but if CMV goes by, the T cells are activated and start multiplying and dividing.

So lymphocytes are very specific, unlike the other types of white cells, which are not specific. The other WBCs just generically go after organisms. They recognize patterns primarily of bacteria or fungus. The other types of white cells, such as the neutrophils, and monocytes don't recognize C difficile from bacillus, another type of bacteria.
So the T cells are very specific as are the B cells. These cells have what we call specificity. Every time T cells and B cells are activated and take care of an infection, there always remain behind a small pool of "memory cells". That memory is important, so the next time they see that organism, these cells can respond twice as fast.

Lymphocytes are found in the peripheral blood, in lymph fluid, lymph nodes and in tissues. We can't quantitate how long some of them live. In the blood stream, sometimes we can follow them for 100 up to 300 days and in tissues, that might be years. These might be those memory cells.

The way T cells work, they are not phagocytes. They don't phagocytize fungus and bacteria like neutrophils do and the eosinophils do and like the monocytes do.
Rather, they give us cell mediated immunity because they start producing cytokines that will be used to kill the organism, and they do it via their T cells subsets: the helper T, the killer T and the suppressor/ regulatory T. That is called cell mediated immunity. Also the word cell mediated is used because T cells are pretty much the only type of white cell that can figure out when something is inside a cell. So bacteria, fungus parasites, they remain pretty much outside of the cell. But viruses always go inside a cell and so nobody knows the viruses in the neighborhood because it's already gone into the neighbor's house (the cell) and shut the door.
So the only cell that knows that inside that house is a virus, is the T cells. They can figure that there's viral antigens and they can recognize those on the surface of the infected cell.
So they are frontline for intracellular organisms like virus. T cells are also frontline for recognizes proteins that sit on the surface of cancer cells. So notice that cancer cells will have tissue typing antigens on their surface that say they are "self".
But they also have other proteins that look abnormal to these T lymphocytes. Besides the T lymphocytes cells seeing that self molecule, but they also see these other abnormal tumor associated proteins.

The T lymphocytes recognize when some normal body cells have become rogue and become involved with the cancer. So T cells recognize tumor associated antigens and again they will attack the cancer.

Now, when we talk about this feature of these T lymphocytes being able to attack cancer.
In the stem cell transplant process we refer that as ***graph versus malignancy***. The new graft or the new stem cells we are putting in, are going to produce these T cells which are able to go after the malignancy. These new graft may be be more potent than the original immune system that patient had. These T lymphocytes are able to recognize those HLA antigens as self and non self and because of that, they also may recognize some subtle tissue differences that we didn't completely match between the donor and the patient. So if these T cells notice those subtle differences, they may attack the patient and that is called the new "graft versus host disease", which we will have a big conversation with in our class lectures.

What are the T subsets called? The helper T cells are called CD4. The T helper 1 (Th1) helps the CD8 killer T cell to be effective with cell kill. The T helper 2 (Th2), assists the B cells become activated mature into a plasma cell and start producing antibodies (also known as immunoglobulins).

And then the CD8 are the cytotoxic killer T cells and another CD8 group of cells are the regulatory or suppressor T cells. These are the T cells that when the action/assault or the insult infection was taken care of, these cells down regulate (or shut down) the immune system response.

And then, as I mentioned, they always keep a small pool of memory cells, so that next time we're exposed to that same antigen, these cells can multiply and divide have a much quicker response.

B lymphocytes give us what is called humoral immunity. That is because they make antibodies which we know is immunoglobulin.

So notice T lymphocytes are about 50% of our lymphocytes. B cells are about 30% of our lymphocytes and natural killer cells (NK) will make up the other 26%.
Now, when B cells are stimulated by their specific antigen, they mature into a plasma cell and they produce an antibody, also known as an immunoglobulin that goes after that (recognizes) that antigen. So you can see that in our depiction here.

So there's the T cell, with all its cell subsets and there's the B cell so which matures into a plasma cell, which then makes our antibodies. The antibodies are pictured as a "Y" structure.

Now, what about those natural killer cells. Well, they kill non specifically. They act more like a granulocyte or a monocyte. They don't recognize specific antigens. But they do recognize patterns and can, under the direction of the T

cell, go after primarily tumor cells and viruses. These NK cells don't have memory, (don't keep memory cells) and kill tumor cells and viruses, pretty much under the direction of the helper T the CD4.
Now unlike the other lymphocytes the NK only circulate in blood, spleen and liver. NK cells are not in lymph fluid nor in lymph nodes like the T cells and B cells are.

So we have these two groups of immunity. On group is call innate immunity.
Which these cells are ready to go at time of birth. There is a very rapid response and they don't remember (no memory). They just have to keep responding every time they see the bacteria or fungus, but again very rapid response.
The adaptive immunity which are provided by the T cells and the B cells. Yes it's a little slower response, but it's a very specific response and remember they have memory.
So, even though it takes a little bit longer to get the specific, adaptive immune system up and running, it is extremely effective. It is more potent than the innate immunity. The reason we have some overlap here is because the T cells and natural killer cells do some work in both types of immunity. Remember that the macrophages and the dendritic cells are these are very potent "antigen presenting cells" that go to the lymph nodes and wake up the resting/naive T cells, and get them activated.

So this process of immunology and hematopoiesis is very important in terms of stem cell transplantation. Both of these play a significant and complimentary role. We know that hematopoiesis assures adequate levels of all these blood cells, so that we have enough circulating blood cells in the blood and the tissues.
And we know that specifically the white cells of the immune system provide protection against infection.

We've already told you, these white cells of the immune system, specifically the T cells are the cells that pick up & recognize when a cell has become abnormal..that it has become a cancer cell. And remember we also said we get better tumor cell kill when we have a very potent graft. And that is called "graft versus malignancy" or "graft versus tumor" effect. It is especially pronounced in allogeneic transplant.

So when a cancer patient gets a new immune system from a donor, then that immune system is really very effective or recognize the cancer cells and going after it.
In fact, that graft versus malignancy effect, may have more tumor cell kill than does the high dose chemotherapy.

Now the downside is, of course, that because these cells can recognize nonself, (just like they recognize viruses and they recognize when a normal cells become rogue), they can pick up those subtle tissue differences that we weren't able to completely match, (some of them mismatching at the major/minor HLA antigens) and then they can actually attack the patient. This new graft can actually attack the patient or the host so we call it GRAFT versus Host disease. This has serious side effects.

41:46
We will discuss our blood components next.
If someone donates a unit of whole blood, it is spun down into the following components.
The three cellular components layer out at the bottom. Red cells first, then white cells, and then platelets. By the way in this white cell component are CD34 cells. This white cell layer looks buff color so we sometimes call this the buffy coat.
Now after the platelets then we come to the liquid portion of blood. In the liquid portion of blood that's where all proteins are circulating: albumin, the different types of immune globulin, the clotting proteins such as fresh frozen plasma and cryoprecipitate.

And don't forget with the IVIG component, if there's antibodies against "A blood " or "B blood" that would be an IVIG component. So the cellular components are: pack red blood cells, platelets and white blood cells.
The protein components would be FFP (fresh frozen plasma), cryoprecipitate, serum immunoglobulin such as IVIG and plasma expanders such as albumin and plasmanate.

Now be aware that there's are 3 types of platelet products. Random donors are where we collected six to eight units of platelets from different random donors and put those in one bag. Then we have a single donor where an individual got on the pheresis machine and gave us all their extra platelets. That single donor equals about six to eight units of random

donor platelets. And then the very special “cadillac like” product where we have a single donor who gets on a pheresis machine, but that individual is HLA matched to the patient, so those are considered HLA matched single donor platelets.

The different white cell products we don't give very often. The granulocytes and specifically it's neutrophils we are transfusing here. This are given if someone has a very severe infection that is not responding to our latest generation of antimicrobials.

Donor lymphocytes, however, are specific to the allogeneic transplant patient. This is when we go back to the donor and obtain more lymphocytes and we give them to the patient because we're either wanting to increase / or boost the graph. Or possibly the disease looks like it's coming back, and we would like to use the donor lymphocytes for the “graft versus malignancy effect”. Remember it is the T lymphocytes that are very good at recognizing cancer and attacking.

Now there are two types of processes that we need to do to blood products for stem cell patients. One is leukocyte filter and the other is irradiation.

Now remember pack red blood cells and platelets are on either side of the white cell components. So white cells contaminate both products, even though we spin it down as best we can.
Remember that when we use a leukocyte filter, it will remove about 99% of the white cells that are contaminating but leaves a few white cells are still in there.

Now, the reason it minimizes transfusion of CMV infection is because almost everybody in the country is CMV positive. That doesn't mean they have active CMV infection, they are just CMV positive. So I am CMV positive and if you give a unit of my blood to someone there may be some CMV in my white cells traveling in my bloodstream in my white cells. And if you give my blood to an individual has no immune system, that very little CMV may now become active and causes significant infection in the patient who receives my blood, because they do not have a good immune system. So if we remove 99% of the white cells, we greatly reducing the CMV that might cause an infection.

Remember that white cells are true cells, which is why CMV likes to travel with them because CMV does need a true cell with a nucleus (to replicate).

The other problem is white cells have HLA on them. HLA is very antigenic and patients who receive a lot of blood products develop HLA antibodies. That is what we call alloimmunization. So the more platelets I get the more fragments of HLA my immune system sees and the more anti platelet antibodies I am making. That is called alloimmunization.

So if we can remove white cells and that HLA exposure, that will slow or delay the alloimmunization
And then for that same reason, those HLA antigens on those few white cells cause a fever-chill reaction. When we transfuse that product into the patient, the patient's white cells see those HLA antigens and cause a little bit of a febrile chill reaction as they potentially attack those white cells.

Who (which blood products) do we have to leukocyte filter? Red cells, platelets and we actually will use that as well on FFP.
Obviously, would not use a leukocyte or white cell filter on any white cell product. So the granulocytes do not use a leukocyte filter, that’s what they are white cells or leukocytes. And the same is true for donor lymphocytes, they are white cells.

Now irradiation is another process that needs to occur. If we have removed 99% of the white cells that leaves us with about 1% remain, and some of those might be lymphocytes. If you remember, lymphocytes are the only cell that can replicate outside the bone marrow. Remember, they can replicate out in lymphoid tissue.
Also recall that lymphocytes are the cells responsible for recognizing non self. When you put lymphocytes into a patient, even if it's a few, they recognize the tissue differences, they are very excited and stimulated and they rush to the lymph nodes and replicate themselves. Then they attack the patient. This is called graft versus host disease.
And now, had it been an immune competent person who had received that unit of blood, those white cells would have taken care of those few lymphocytes from the donor. But these patients are immunocompromised. They are not able to

necessarily destroy those few lymphocytes and those five lymphocytes can become an army in about a week and then they can attack the patient in large numbers.

We will irradiate red cells, platelets and granulocytes. FFP and cryoprecipitate do not need to be irradiated.
And one last comment if we have platelets stored in a lot of plasma to help them keep that nice full, three, four or five days of shelf life. If that is an ABO incompatible patient, then they would want to reduce the amount of plasma in that platelet products. So they would rinse or wash or plasma reduces as we say. That would minimize a transfusion reaction.
Let's briefly mention one more time when do we transfuse pack red blood cells? Usually, when the hemoglobin is around 7-7.5 g/dL. Most people start to have some symptoms at that point. But other patients may have symptoms. when the hemoglobin is 8 or 8.5 g/dL. If it is a cardiac patient, they may not tolerate that 7 to 7.5g/dL.

So what are the symptoms that might tell you that they need to be transfused? Shortness of breath, they are dizzy or faint, chest pain, sustained tachycardia or blood pressure trending down.

Pack red blood cells need to follow the ABO type specific indications. We mentioned RBCs need leukocyte filtration and they have to be irradiated.

Pre-meds such as diphenhydramine, hydrocortisone or acetaminophen might be used.
If they have a febrile chill reaction that might require Demerol (meperidine). That opioid is very good for chill reactions/rigors. (not for pain control). Packed RBCs cells are usually infused over two to four hours. If they hang past four hours, they will come to room temperature and then RBCs hemolyze and bacteria start to grow. Both of those are problematic. That is why RBCs are not to hang past four hours.
We do need to infuse RBCs slowly for the first 15 minutes to make sure there's no severe reaction to them.
That helps us, just in case someone has made an error with the type or cross match with the paperwork etc. We would be able catch a severe reaction before too much blood went …if a mistake had been made.

We go very slowly, for the first 15 minutes. We should have baseline vital signs and the RN should be the one who goes into do the first 15 minutes vital signs to make sure that the patient looks okay do a quick assessment and then resume the regular infusion rate. Check vital signs every hour thereafter. Once the blood is finished, I need one more set of vital signs at least 30 minutes afterwards.

Platelets are usually indicated when the platelets count gets around 10,000 or so. That's low enough that the patients can spontaneously bleed. So, they do need to be transferred somewhere in that 10,000 range. Some people use 10,000 to 20,000 range. We use the 10,000 cutoff. Now is there any time when the platelet count is above that range and we would still want to transfuse? Yes, if they have a reasonable count between 10 and 50, but we think those players are dysfunctional or they're actively bleeding, then we will transfuse. If there is a planned invasive procedure, we may want to keep those platelet count above 50,000. So we may transfuse prior to that procedure and/or maybe we are going to transfuse them every six to eight hours after that invasive procedure for a period of time to assure they don't bleed.
Now we should have post platelet transfusions check periodically to make sure that they are responding well to the platelet transfusions. So, what we'll do is, about 10 to 60 minutes after a transfusion, we will check a platelet count. If it fails to go up by at least 5000, then they are considered allo-immunized. That means they've got enough anti platelet antibodies that they are now eating up/ chewing up the platelets. The other word we use to describe this is “platelet refractory”. When that occurs, we need to consult transfusion medicine before we give additional platelets. They can work on getting us a special platelet products such as single donor or HLA match single donors.

Another complication that may occur with regard to blood products and blood transfusions, are the difference between a patient and their donor when they maybe the same tissue type, bu a different blood type. This will only happen on allogenic patient i.e. when someone else is the donor, not the patient for himself, rather someone else is the tissue matched donor.
We will match your tissue typing or what we call your human leukocyte antigen. These are inherited on chromosome 6. We will map those out and tissue match those as best we can with your donor. You inherit your blood type proteins on chromosome 9, which we did not necessarily try to match. So it's possible to be the same tissue type as your donor, but

a different blood type. That happens about a third 30 to 40% of the allogeneic patients. Now, if the patient is not the same blood type, and is a ABO compatible with their donor, there are two points in time which this becomes problematic. It becomes problematic on Day Zero only if we are giving fresh cells back on day zero. Because when we have fresh cells, then we possibly have some red blood cells as well as plasma in the stem cell product. And so our options are to just give the stem cell (AKA buffy coat) only. Or we could give the Buffy coat back with the red blood cells and plasma. But if they are ABO incompatible, we have to process that product based on the ABO and incompatibility issues. Why on day zero transplant day, is it not a problem if they're going to get peripheral blood stem cells or umbilical cord blood? Those two products have been frozen. When you freeze a stem cell product you remove all the red cells and the plasma, so that the product takes up as least storage space as possible., So removing RBCs or plasma is not an issue for frozen cells (they have already been removed). Problematic only on the fresh product which of course is only when using bone marrow stem cells. (PBSC and Umbilical cord stem cells are frozen).

And what would we do with those bone marrow stem cells on day zero?
If the **patient has antibodies against the donors red cells**, then we'll just simply deplete the red cells or wash those off. That's called a major mismatch.

However, if the **donor has antibodies in their plasma, to the patient's red cells**, we will plasma deplete those cells. This is called a minor mismatch.

Well, what if the **patient has antibodies to the donors red cell AND the donor has antibodies in their plasma to the patient's red cells,** that's considered both the major and a minor mismatch (sometimes we call that bi directional mismatch….goes both ways). Then we will need to take off the red cells and the plasma (red cell and plasma depletion). In this case the patient is only going to get the Buffy coat. So it will be very small concentration in the syringe to transfuse.

The second time the ABO incompatibility becomes problematic is as the patient changes over (seroconverts) to the donor's blood type. It takes about three to four months, maybe up to six months if we have a mismatched allogenic donor, or if we have a matched unrelated donor.

And so the patient may do a slow hemodialysis over that period of time. We will closely monitor the patient to make sure that the hemolysis does not get out of control.

So what they do is a chimerism test (or what we call those DNA test), on the patient's blood on day 30, day 60, day 90, and maybe beyond, to see how much blood is the patient's original blood type and how much of the blood is the new donors. That was a call a chimerism test. And that way we know what degree of seroconversion has occurred and when did they finally become their donor's blood type. As you can imagine, accurate blood typing is critical during this process so we can minimize or watch and monitor for that hemodialysis. Transfusion medicine is always consulted on these types of patients so they can always tell us what type of red cells, platelets and plasma products, we're going to give on this patient until they convert over to the new blood type. Of course you could always use the universal donor for red cells which is "O" type. We could always use the universal donor for plasma products, which is "AB" blood type. So if we had to give FFP or cryoprecipitate, you would use "AB" FFP and cryo.
Now, if we're unable to get that donor for the platelet transfusions, if the platelets are in large amounts of plasma, then we are going to simply do a plasma reduced platelet product.

And lastly, we provided a table for you if you do not remember what antigens are on a red blood cells, what antibody that patient has in their plasma. Also on the table we have included the blood types they can receive and the plasma types, they can receive. So please use this as a reference.

Universal donor for red blood cells is "O" blood, and the universal donor for plasma is "AB" plasma.
Any questions, there's my email, please email me if you have questions and we'll respond. Thank you very much.