 In particular, we have a class quiz up there. Take a moment if you haven't. You've heard a lot of these terms before. One you haven't. I have a particularly wonderful item to make up for the tough exam. It is a flashing, isn't that so cool? It's a flashing jellyfish ball. OK, so let's see what we can do. Yes, I know, this is a post-test item. OK, settle down. Please, we have a lot to cover. You've heard most of these terms before. This lecture is all about these terms phrased in a different way. So I want to make sure we are together before we start. [Student speaks] Yes, ma'am? OK, so the final function of a cell, OK. Potency in the pink top. I saw your hand first, yeah? Yeah, OK, potency. This is a very big one in stem cells, the number of possible fates that a cell can assume, commitment, decision to make a cell type, differentiation, someone on the side of the room must have some thoughts. Yeah?

OK, I'll give that to you, to the final transition here.

Do you want one that's orange rather than pink? God knows I don't want to be genderist here, but there we go. The differentiation, the process by which a cell assumes its final fate. And what we are going to discuss today is the kind of gradations of commitments in differentiation, commitment particularly, some of the steps of commitment.

The last one we have not discussed in class, but if anyone wants to have a go, I will, yeah? Lineage, the term lineage in biology.

Ish, ish. Let's try another one. Good, OK, a group of cells or a group of cell types that descends from a common precursor, so, this is a funny term, lineage, because it's used a lot in stem cell biology. Let's think about it for a moment. The definition I have is the set of cell types that normally arise from a precursor cell. Now, of course, if you think hard, all cells arise from a fertilized egg from a zygote. So, really, all cell types are of a common lineage.

And this gets to the question of semantics, and where you put your definition boxes. OK, in stem cell biology, and a lot of biology, the term lineage refers to a subset of cells arising from a common precursor that is somewhat arbitrarily defined. All right, so this diagram which you've seen over several lectures is really important. That's just what we've been talking about, the progression from uncommitted cells through committed cells through differentiated cells. And so, we are in the how-to two module, talking about stem cells. Stem cells are in the news all the time, and I want to, today, take you beyond the newspapers, beyond the magazines, and tell you what I think stem cells are all about. So, if you look at our game board of life, we are, I would say, halfway plus through the semester, and we've gone through all of these things, sticking out here into stem cells. So, where are we? OK, so what's a stem cell?

Let me go back for one moment, and let's talk about stem cells.

For those of you who don't have a handout, you really should get it.

It's two pages up front here. So, let's do some board stuff, stem cell, which I will heretofore abbreviate SC is a cell type that I am going to refer to as somewhat committed. So that's deliberately vague. This is not a committed cell

type, but it's somewhat committed.

It knows more or less what it's going to become.

It can be either unipotent or pluripotent or totipotent. And that stem cell, by definition, will go on and make an asymmetric cell division. So, it will give rise to itself, another, not itself, but another stem cell.

And it will also give rise to a committed, and here's jargon you should know, progenitor. And so, this is an asymmetric with one S, asymmetric cell division. So, you'll end up with two daughters that are different from one another, a committed cell, and a stem cell.

And so, the stem cell in a sense is self renewing, or is self renewing. And then, this is one of the big deals about stem cells. That committed progenitor will go on to divide, and it eventually gives rise to one or more differentiated cell types.

OK, in those differentiated cells will come from a specific lineage.

So, the stem cell is undifferentiated. The committed progenitor is undifferentiated, and eventually you'll end up with a differentiated cell. You can look at your first handout. Here it is; I've drawn it for you out. You need to know this, so I've done it in two places. There is the asymmetric division stem cell plus progenitor, and the progenitor goes on to give these differentiated cell types. There is no magic about this. This is the same process we've been talking about all along except for the fact of the self renewing thing. So, in the normal embryo, cells go on down the pathway of commitment towards differentiation.

But they generally do not renew themselves.

So, a zygote, for example, although it's totipotent is not considered a stem cell because it doesn't self renew. And that's true of most embryonic cells. They do not self renew and so they are not considered stem cells. So, what's the deal with stem cells, and why, when I Googled stem cells in Google News last night did I come up with several thousand entries for the previous week?

This is the deal. One of the great goals of biologists is to repair organs, to repair damaged tissues. And this has been very difficult to do. We touched on this a few lectures ago., stem cells have some kind of promise a some kind of universal repair kit.

And the idea is this, that one could isolate some kind of magic stem cell, or some kind of stem cell, and it would be autologous, which means it would be matching your own cells. OK, so you isolate some kind of autologous, self matching stem cell. That stem cell under ideal conditions is pluripotent, or that set of stem cells, and can be

coaxed to form many different cell types depending on how you treat it. So, you can imagine adding some kind of factor. And if you like, you can call that an inducer as we have been talking about secreted factors in developmental biology. And that inducer would turn those stem cells into progenitors. And those progenitors would have a specific -- -- future fate by virtue of which factor you treated the stem cells with. You would then take those progenitors, inject them into someone whose body needed repair -- And the notion is that these progenitors would go on to differentiate and repair whatever needed repairing.

OK, so this is the dream. And this is why you can find headlines like this everywhere. These are some of the ones I pulled out last night: Stem Cells May Help Repair Stroke Damage. Stem Cells May Repair Broken Bones. Note the use of the term may. OK, there's a lot of hype about stem cells and not much that's been proven. There's a lot of money involved in stem cells, trying to isolate stem cells to repair people, and there is also, there are also advertisements where you pay people to take your stem cells, and to store them. So, poured blood refers to the blood of the umbilical cord of a newborn, which is believed to be, it was known to be full of stem cells of a certain kinds. And you can pay someone a couple of thousand dollars to freeze that group of cells and keep it in case the child needs some kind of stem cell therapy in the future. So, what's the deal? Do stem cells actually exist?

Well, yes, OK.

Do stem cells exist or are they just hype? They do exist, and the idea is that sometime in embryogenesis, as cells are normally going on and making the different cell types, some cells are put aside, that the body is going to use later on for repairing itself. And there are some examples, and I will tell you a couple which are really fantastic illustrations of this. So, do stem cells exist?

Yes. Where? Likely in the older embryo, and the adult, and the particular organs that contains stem cells is not clear.

There are several very good examples, but it's not clear whether or not some, yes, all, not clear. It's not clear whether all organs contain stem cells. So, let's look at this a bit more, and let's go back to talking about how stem cells were discovered. Why are we having this conversation?

And I'm going to be referring to number two on your handout. So, let's talk about the discovery of stem cells. The discovery of stem cells was an accident, and it came about when people started looking to see how long cells lived in a particular organ.

And the way you do this is by using a protocol called a pulse chase experiment.

And a pulse chase experiment gets to the question of turnover rate, or if you want, half-life of cells in an organ.

OK, this is the way it goes. You've got this as number two on your handouts. You take a cell population, or you just take the whole organism if you like, you feed it something called bromodeoxyuridine. It doesn't have to be this, but this is a good one.

Bromodeoxyuridine is a nucleotide. Deoxyuridine, so, you know uracil is normally an RNA, but if you make the deoxy form, it gets incorporated into DNA. The bromo part allows it, later on, to be detected by various colorimetric assays, and you can add BRDU to an organism for a short time. This is called a pulse. The BRDU is incorporated into DNA of those cells that are undergoing DNA synthesis, and then by various means you wash out the BRDU. And what you get is the labeled cell population that had undergone DNA replication during this pulse period. And then, if you follow this group of cells over a period of hours or days or weeks or months or years, you can look and see what happens to those labeled cells.

So, in my example, I started off with for labeled cells over some period of time, the number of labeled cells per total unit number of other cells decreases by half. And that gives you the half-life of the population. And if you do this for many organs, you find that adult organs do not just sit there with a cohort of cells that doesn't divide. There is an enormous amount of cell division in adult organs. And we talked briefly about this previously. So, for example, red blood cells have a half-life of about 120 days. And that actually means that there are more than ten to the 7th new cells produced per day.

The intestine is something we touched on previously. Cells in the intestine, some of the cells in the intestine had a half-life of three to five days, and you're producing about ten to the tenth new cells per day. Obviously, the number that you're producing per day depends on the total size of the population.

Skin has a half-life of about 14 days, hair on your head, a half-life of about four years, and your eyebrows and eyelashes, half life of about 30 days. One of the mysterious half-lives are the neural cells, your nerve cells. It was believed for a long time that nerve cells never divided, and once you've got all your nerve cells by about age two, you never made any more. In fact, that doesn't seem to be true. And there are certainly populations of neurons that divide. But we don't really know the half-lives for those cells. So, this was very interesting data, and it said that there had to be some way that the organism was using to replenish these cells that were dying, and to repopulate the organs so that things functioned properly. And in fact, you can go further than this because you can not only count the labeled cells, you can ask what those labeled cells become. So, you can look at your labeled cell population and assay the fate of those labeled cells.

And if the cells want to differentiate from an initially undifferentiated population, you know that these differentiated cells must have been derived from stem cells or from progenitors by definition. OK, so what organs do we know have got stem cells? Let's talk a bit about this. The test is a great example, and the spermatogonia, the diploid

precursors of the spermatozoa are dividing cells that divide throughout life, and go on to give rise to themselves so they can replenish themselves. And they go on to give to these primary spermatocytes, which are the first step in the cascade or in the lineage of cells that are going to differentiate as a spermatozoa. OK, and you know you can do these. You can look very clearly, and mathematically look and see the number of cells, do this pulse chase analysis, and know that the spermatigonea must be stem cells. This is a very important stem cell lineage. It's the hematopoietic lineage.

In the bone marrow, there is some kind of pluripotential hematopoietic cell that gives rise to all of myeloid progenitors, so all the red blood cells, and the various other cells in the blood stream as well as to all the immune cells. And those come from a single progenitor, a single pluripotent cell. This is called the hematopoietic lineage. And we will talk more about that in a moment. This is an example that I mentioned to you many lectures ago. Newt limbs, if they are amputated, will regrow. The reason that they regrow is that there seem to be a population of cells in the lab which are called blastema cells, and those are the cells which can go on and to form the entire limb again. And that's something obviously we can't do, but people are very interested in. That's been a tough system to look at. This might be a better system to try and understand the details of regeneration. So, this is a planarian. This is a flatworm. These are little guys. They can grow up to a couple of centimeters, or a few centimeters. They are very pervasive animals in the animal kingdom. And they have this extraordinary property, as many simple animals do, that you can cut them into pieces, and they will regenerate the whole animal. So, you can take out the head. And over time, it will regenerate the tail. You can take off the middle; it will regenerate a whole animal, and so on. And this bottom picture is a planarian that's stained for BRDU incorporation, or for another marker as well.

And each of these dots is a cell called a neoblast.

The neoblasts are the stem cells of planaria that can regenerate the whole animal. And if you look right up front here, it's not very distinct, but you will see a region where there are no neoblasts. And that is the one region of the animal that cannot regenerate. So, if you cut off the very tip of the sort of nose equivalent region. It will not regenerate a new animal because there are no neoblasts. And the system is being studied here at MIT by Professor Reddien of the Whitehead Institute, who is a new faculty member, and who some of you might went to Europe with sometime. OK, so let's talk about isolating stem cells, and how you do this. If one is going to use stem cells for repair, you've got to be able to isolate these things.

And the challenge of isolating stem cells is that they are rare. For example, in the bone marrow, the hematopoietic stem cells, which I'm abbreviating HSC, comprise about 0.01% of the bone marrow.

And I would say it's fair to say that no one has really seen a cell and said, oh, this is a stem cell.

It's hard to pinpoint exactly what a stem cell really looks like. It's just a cell. But it's got particular properties, and

you need the appropriate way to look at the cells and see these properties. One way that's been used to isolate stem cells is something called FACS, or fluorescence activated cell sorting. I'll talk about it in a moment. And there are two ways that FACS is being used to isolate stem cells. One is by getting a group of cells called SP cells where the SP stands for side population.

We'll talk about that more in a moment. And the other is through the use of cell surface proteins that are characteristic, or enriched in stem cells. And this has been many decades of work by many people to come up with a set of criteria by which one can enrich for stem cells in a particular population.

So, here's the way. Actually, before I go through that, let me go through the assays, and then we will go through all the slides together. How do you assay for stem cells?

Well, one of the ways you know you have a stem cell is if you have something that can act as a stem cell. And there are two assays that you should be aware of. One of them is a repopulation assay usually done by transplant, and very often you remove some endogenous group of cells, and then try to replace that group of cells by using stem cells. So, you remove some set of differentiated cells.

And then, you try to replace with transplanted stem cells. And the other way you can do this is in some kind of in vitro culture assay which I will talk about later on. All right, so with this in mind, let's go through some of the slides. You've all heard of bone marrow transplants, which people who have leukemia and other associated disorders undergo to repair themselves to get rid of the leukemia cells to get rid of the cancer.

This is how it works in a mouse. And this is a repopulation assay.

I'm going to use bone marrow transplants as an example. You take your mouse, or your person if you are undergoing bone marrow transplants. And you irradiate to destroy the bone marrow. The reason you do that is to make space for new cells to come in to expand and grow. If you just put your new cells into an animal with an intact bone marrow, they kind of disappear amidst the masses. So, you have to give the cells your assaying a chance. And then, that irradiated mouse would die.

That irradiated person would die. But you get them, now, an injection of normal bone marrow. And if things go well, the mouse or the person lives. And there was a Nobel Prize given out some years ago for developing bone marrow transplantation. One of the gold standards in the stem cell field is asking whether or not this rescued mouse has regenerated stem cells because you can imagine that what you're doing in this case is giving cells that are the progenitors. They are one step down from the stem cells. Or they might even be partly differentiated. So, you can imagine that you are restoring this mouse is life by giving cells that are not self-renewing. Excuse me, one of the gold standards in the stem cell field is to take this rescued mouse, isolate more stem cells or more putative

stem cells, and take those and then tried to rescue another mouse. And in the hematopoietic system, you can do this over and over again.

So, how many stem cells do you need to repopulate a mouse?

Actually, you need one, and I will tell you how this is done. So, the idea of the first thing when you are trying to do assays to figure out how many stem cells you need for rescue. You take your bone marrow; you stain it for some stem cell marker. You have this in front of you. OK, this is number nine of the first page of your handout. You stain somehow for a stem cell marker.

I'll tell you in a moment how you sought the stained cells through this fluorescence activated cell sorter.

And you isolate from it a pure-ish population of stem cells, an enriched population of stem cells. And then, you do a dilution assay where you inject different numbers of cells into a recipient irradiated mouse. Now, you don't actually injects one, ten, and 100 cells. You inject one cell, and millions of carrier cells to help those cells along, OK? The one cell would just disappear in your syringe. So, you've got to give it some companions. But the bottom line is you really only need one stem cell to rescue the life of that mouse. It can go and repopulate the entire hematopoietic system, all the blood cells, all the immune cells. What is this fluorescence activated cell sorter?

Fantastic machine. It looks like this. Again, you have this in front of you, so look up here. The idea is that you take a reservoir of cells that you have labeled with particular antibodies. And these are living cells. OK, you label them in certain ways, and they can be labeled with fluorescent antibodies or fluorescent dyes. I'll tell you a dye example in a moment. And you put them in a reservoir, and you trip them out so that one drop of liquid contains one cell on average, or zero cells. And you let those cells drip through a laser and a fluorescence detector. The laser activates the cells. They fluoresce, and you set the detector to activate a charging collar at a specific wavelength of fluorescence. And when the charging collar is activated, it will activate some deflecting plates which will give charge to cells of particular colors, and move them into particular tubes so that they are sorted on the basis of their color. OK, this is done one cell at a time but it's really quick. And you can purify millions of cells to do these kinds of assays.

This kind of thing is not done for human bone marrow transplants.

There, you take a much bigger population of cells from the bone marrow, and you generally don't purify them very much. But if you want to do specific stem cell assays, and many others, the fax machine is really fantastic. So, what do you get out of this?

Well, you can plot what the cells look like. So, here in this example, I've got cells that are labeled in red and green. And you can label them according to this plot as to whether they have no label, red label, green label, or both red

and green. This is a real example of an experiment that was done here at MIT a decade ago in Richard Mulligan's lab. And this is what a real FACS plot looks like. OK, it's a mess. Every little is a cell. But for reasons known only to the Mulligan lab, Peggy Goodell who is now a professor in her own laboratory, they assayed this little region of cells in the bottom left-hand corner for their stem cell properties.

They called these SP cells or side population cells, and they found to their surprise that these SP cells were highly enriched for hematopoietic stem cells 1,000 fold or more. And in fact, if you take the very bottom left-hand corner where there are almost no cells, you get a 10,000 fold enrichment.

So, the way they sorted these was with a dye called Hest 3342.

This is a vital dye. It stains the DNA but it doesn't tell the cells.

And somehow, these SP exclude the dye, or efflux it, remove it from the cell. And it's really not clear what that has to do with stem cellness, but this is still one of the very best ways to isolate stem cells from almost every organ. These SP cells, these things that don't stain with these DNA dyes seem to be the ones that are stem cell-like. OK, so let's move on to the question to several questions that I want to discuss with you that fall under the umbrella of regulation and control of stem cell fate. And there's several questions I like to pose to you. Firstly, what makes a stem cell self renewing? What's the molecular basis for that?

What makes a stem cell decide whether it's going to make progenitors or not?

And the big one for the stem cell field, what controls the potency of a stem cell? Well, this is where we step back into the developmental biology that we've been talking about because it all controls at some level by gene expression.

And in particular, there are both intrinsic and extrinsic factors that seem to control certainly the first two points on my list, the self renewing and the progenitor aspect, and perhaps also the potency also.

Intrinsic factors, cell autonomous factors, determinants -- -- and extrinsic factors, non autonomous factors, secreted ligands, inducers, and these and extrinsic factors have been given a special name in the stem cell field just for argument's sake. They are called the niche where the niche contains all the cells that influence stem cell activity, cells that influence stem cell activity. OK, this is just a term. You should know it because if you read it you will know it. So, here's how it works. The surrounding cells in the niche are cells that seem to maintain stem cells usually in acquiescent state.

So, it's believed that in most organs, stem cells are sitting there quietly. They're not dividing very much, but they can be stimulated to divide, and this is on the second page of your handout. They can be stimulated to divide by

some kind of environmental input, and this changes the surrounding cells. And the environmental input could also be a secreted ligand for example. And the surrounding cells then induce the stem cells to be activated. And they go on to make progenitors, and of course also to self renew. This is a fancy way of saying that cell fate is controlled by induction. OK, so you should be nodding. The should not be anything new for you at this point. It's phrased a little differently, but that's all. This is a very interesting example of control of cell fate by surrounding cells. This is from my colleague, Professor Fuchs at Rockefeller who studies the hair follicle, and who has shown that this region called the bulge is the source of stem cells. So, this brown thing is the hair that sits in a shaft of cells that got a bunch of interesting cells around it. In particular, there's a rather inconspicuous group of cells on one side called the bulge. And some years ago, there is another group of cells. I'm also going to refer to two at the bottom, which is called the dermal propeller.

But some years ago, Professor Fuchs took the cells of the bulge and she transplanted them into a mouse that didn't have any hair. It's called a nude mouse. It has lots of problems including no hair. But when she did that, here's the control and here's a mouse into which these stem cells have been transplanted. And you can see all of a sudden this poor little nude mouse has got tufts of hair. OK, and in fact, these hairs actually glow-in-the-dark. They've been labeled with green. It's really cool, OK? The cells that were transplanted, this is something you know, as well. They were lineage labeled, and so, you could prove that these hairs came from the transplanted cells. All right, so let's look. So, during the hair cycle, so you're hairs grow cyclically.

And there are a whole bunch of processes including growth, regression, induction of growth, and new growth. And during that period of time, the bulge and the dermal papillae are in relatively different positions.

So, during a process of growth, they are far away from each other, and then as the hair and growth regresses, they come closer until they're actually touching each other during the process of induction of new growth. And it's at that time that new growth in the hair is stimulated. And it's clear that the dermal papillae is signaling to the bulge cells. And here's what it's using to signal. It's using something called the wind pathway, and in particular, a molecule called beta-catenin. If you think back several lectures, we talked about beta-catenin is one of the things that told the embryo to make its back rather than its belly. So, here's a different use of the same molecule in stimulating hairs to grow. And so this is a particularly cool example of cells coming together at particular points to stimulate stem cells to go on and to make progenitors. All right, let's move on to something important called embryonic stem cells And all right -- -- also called ES sells. So, although I told you that most adult organs are likely to have stem cells, and this has been very clearly shown for many, there are many organs where it's not clear whether they have stem cells, or it's very difficult to isolate them. Stem cells are rare in all organs, and things like neural stem cells in the nervous system seemed to be a exceedingly rare and difficult to isolate. So, the push has been to try to find a source of stem cells that would be more plentiful and more useful for repairing lots of different

organs.

And that's where the embryo comes in this particular kind of stem cell called an embryonic stem cell. And the idea is if one takes an embryo or part of it, puts it into culture, after many steps you get out cells that are called ES cells. And these ES cells are pluripotent. They are not totipotent. But they are very, if I can be forgiven, they are very pluripotent bouquet, and they are pluripotent, and you can control their cell fate.

So, let me going to the slides, and we will talk more about this through the slides. So, in mammalian development, and we're going to talk about mammals here specifically, mammalian development, the blastula forms. And at a certain point in development, a group of cells called the inner cell mass segregates from the rest of the cells which form a shell around the embryo.

We mentioned this previously. This yellow stuff is fluid, and this little group of cells called the ICM, or inner cell mass, is the thing that's going to give rise to the embryo proper. The cells surrounding are going to give rise to the placenta and the other extra embryonic components. So, the idea in trying to get these ES cells is to take the inner cell mass of an early embryo, take it out of the embryo, and put it in a Petri dish that's got nutrients and various factors to disperse the cells such that you've got single cells dispersed in the plate, and give them nutrients. And over time, those cells will grow, and they will form clumps, each of which is derived from a single embryonic cell. OK, now, normal embryonic cells do not do this. OK, they will normally go on and differentiate, and stop dividing, but there's something that happens during this culture process that is abnormal.

And it turns the cells into groups of cells that can self renew. OK, so you've turned these cells into self renewing cells. And each of these groups of cells or colonies that you get may be able to grow into a stem cell line. And I'll talk about cell lines in a moment. I'll talk about cell lines now. So, you might not be familiar with the term cell line. What is the cell line?

A cell line is a cell population, a homogeneous cell population that could grow continuously in culture. So, it's self renewing. OK, so all cell lines are self renewing. A stem cell line is a cell line that has the capacity to go on and differentiate into specific normal cell types. So, there are many cell lines that will grow continuously in culture, but they will never go on and differentiate as anything. They are very abnormal cells.

They are useful for many studies, but they are not stem cells. The stem cells not only can grow continuously, but they can go on and differentiate. But you are dealing with an abnormal cells here. This is not a normal embryonic cell. OK, how do you test the potency of these ES cells done in the following way? The ES cells from a mouse, a black mouse, are taken, and they are injected into an early embryo of an embryo derived from white parents.

And if you do that, the ES cells incorporate into the embryo. You then take the embryos, and put them into a

surrogate mother. And when the mice are born, when the babies are born, you can see that they often are not just pure white. They often got black stripes, and you can look at the various organs and show that these ES cells have incorporated into various organs. So, these ES cells are highly potent. And the idea is that you can take these ES cells and add to them various factors. So, you can add a particular red factor that will turn an ES line into heart muscle cells or pancreas cells or cartilage cells. And you can use those cells in your stem cell repair assays. OK, and this is true. You can take ES cells, and you can do exactly this to them. And then, they will become these different kinds of differentiated derivatives.

So, this is really cool. And the push has been to try to get this to work not for mice but for humans. And this is where the huge controversy in the stem cell field comes from. So, the controversy comes from the fact that you need embryos to get these stem cells. You get the embryos from in vitro fertilization that we touched on a few lectures ago. Eggs are isolated by ovarian stimulation.

They are fertilized in vitro, and they are allowed to grow in vitro for a week or so. And at that point, there are harvested or killed to make the ES line. And this is the very controversial point, whether or not it is ethically OK to harvest these embryos, and turn them in to stem cell lines or not. Presently, there is no federal funding that is allowed to be used to make human ES cell lines. There are some that exist, and President Bush has told scientists that they need to use the ones that exist. However, they are not very good cell lines, and so scientists have used private funding to make new human ES lines that hopefully will be more useful. I don't think there's any right or wrong answer whether this is OK or not. My opinion is that this is an OK thing to do, with all due respect to the embryo.

I think one can save people's lives, well, and the embryo at this point is not a differentiated entity. But it is an embryo. And so, this is an ethical issue. There's opinion here, and it's good for you to think about what your opinion about this type of research is. OK, so let's move on. And I'm going to, in the last minute, just touch on something called stem cell plasticity.

One of the things that has come out of the human ES work or all the ES work, and that has come out of the quest not to use embryos for stem cells is to ask whether or not one can turn one stem cell line into another. So, it's easy, or relatively easy, to get hematopoietic stem cells. And wouldn't it be wonderful if you take those hematopoietic stem cells and turn them into brain stem cells, and fix people who have Parkinson's or Alzheimer's? OK, wouldn't it be wonderful to fix people who have muscular dystrophy by turning hematopoietic stem cells that you can get lots of into muscle cells? So, the idea is maybe you could turn something from one lineage, a hematopoietic stem cell into a stem cell from another lineage and get it to do something else? And the hypothesis, then, is that if you do appropriate experiments, you may be able to figure out where the stem cells from one lineage can contribute to another. This is the second to last slide on your handout. So, this is the way the

experiments were done. It's the last thing I'm going to tell you. Bear with me. You can take a mouse that's been dyed green, and its hematopoietic stem cells are expressing GFP, which is a green fluorescent protein. You use that mouse as a daughter for bone marrow transplants.

You put in the great cells. Not only do you rescue the bone marrow, you also ask whether other organs have got green cells in them, indicating that the hematopoietic cells can contribute to other lineages. And when you do that, initially people got very excited because you saw results like this.

And this is data from my colleague, Dr. Camargo over at the Whitehead Institute. When this experiment was done, he could show that the liver of such an animal had lots of green cells, suggesting that the hematopoietic stem cells could also be liver stem cells.

But when he looked more closely, this is a complete artifact. And what had happened was that the hematopoietic stem cells had actually fused with the liver cells, making the liver cells green. And in fact, presently, there is no data to suggest that you can interconvert stem cell lineages. And I'll stop there. Thank you.