



Speakers:

Elizabeth A. Griffiths, MD

Sheila Tighe, NP

MDS Foundation: ... Please feel free to stay for that event as well. We have two speakers today. We have Dr. Elizabeth Griffiths that will speak first and then we'll have some question and answer. We'll have lunch and after that we have nurse practitioner Sheila Tighe who will speak and, again, with a lot of open discussion. Unfortunately, my colleague and I will have to leave a little bit early. So, at lunchtime we'll be sending around an evaluation form that I'll then collect from you before we leave. We have Cindy Anthony from the Aplastic Anemia and MDS Foundation in Canada and she's actually going to be helping out this afternoon with the microphones. Lastly, I think we mentioned parking validation. If you still need your parking ticket validated, back on that table where the waters are there's a machine. If you need help with that machine just let us know and that's it and we'll get started. Thank you so much.

Elizabeth A. Griffiths, MD: Welcome, everyone. Thank you so much for showing up. I'm free but this mic is also live and this thing will cause feedback. I don't know if there's a way to turn this off. Can you hear me alright with this microphone on the lavalier right now? Okay. Just switch this slide deck.

Thank you all for coming today. I understand that this is an usual and remarkable turnout for one of these events and so I'm extraordinarily grateful to all of you who I know and those who I don't yet know for showing up for this. I hope that we can provide some additional information and that we can provide some background on MDS and please feel free to interrupt me at any time during the presentation if you have additional questions. If you raise your hand I will interrupt the presentation and we can talk some more about specific topics. Many of you sent in questions prior to today's event and so I've tried to address many of those topics in the presentation that I'll be providing today. If you have additional questions as the day goes by please feel free. We have some microphones and we are being recorded. So, this is anonymous in the sense that if you don't say your name your name will not be recorded, but your voice will be recorded and that way people can watch this online and benefit from whatever questions you have.

So, for an outline for today's presentation I'm going to talk about what is MDS and where does it come from, a little bit of background; what are the subtypes of MDS, what treatment options are available as standard of care and what are new things that are coming down the pike for MDS.

So, many of you are already familiar with this. Can everybody hear me in the back? A little bit louder. This only has an on and off button. It doesn't have volume controls that I'm in charge of, but I think our helper is in the back room back there. So, maybe he can adjust the mic volume. So, many people when they present with MDS are asymptomatic, but those who are symptomatic the chief complaint is really fatigue and this is data from David Steensma whose at Harvard now, previously was at the Mayo Clinic and he sent out a survey to all of his patients some years ago now where he asked 359 patients to report on their fatigue symptoms, patients with MDS, and the thing they complained of the most frequently was fatigue followed by bruising or bleeding and night sweats. I assume that this reflects people's experience. Yes? Okay. I think the other thing that people might

observe from looking around is that this is, in fact, a disease that is a function of age. So, a majority of patients with MDS will be older and we'll touch a little bit on the epidemiology of the disease and the acquisition of the disease and the mechanism that we think is underlying the development of MDS. It's a phenomenon on of aging. We see this predominately in older patients. Median age at diagnosis for patients with MDS is in the late 60s and early 70s.

In the United States between 10,000 and 20,000 people every year are diagnosed with MDS and the spectrum of this disease ranges from low risk MDS to higher risk MDS and we'll talk about the features of the disease that make us describe the disease that way. Based upon statistical data that means that there are between 60,000 and 170,000 people in the United States living with the diagnosis of MDS and as I said before the median age at the time of diagnosis is 74.

In order to make a diagnosis of MDS you have to have several criteria. Some of you may have found that your doctor was suspicious about low blood counts and maybe did some testing early on during your diagnosis and that they weren't sure about making that diagnosis. Maybe they said you had a pre-MDS or that they said you had low blood counts for a period of time and this is because in order to make this diagnosis you have to have sustained low blood counts for at least three months that are low but not dramatically low and that's because we want to exclude patients who have rapidly progressive disease that's AML or leukemia. So, low blood counts. That is to say hemoglobin less than 11 grams, a neutrophil count less than 1,500 and platelets less than 100,000. In addition to the exclusion of other causes of MDS. So, your doctor may have done a ton of blood work. Those of you who are my patients often come to me and say the first time you see me you have a ton of blood work done and that's because we want to exclude other diagnoses that can cause low blood counts but that are not MDS. In order to do this we check your B12 levels. Vitamin B12 is an essential nutrient and your folate levels. We check for HIV or other viral infections that people endorse a history of having had blood transfusions or potential occupational or personal exposures. We check for the presence of copper deficiency. We don't talk a lot about copper and I don't think many of you take a copper supplement, but it turns out that copper deficiency can also mimic MDS and look for all the world like an MDS diagnosis and there are two tests that we do to look for copper deficiency. We always ask about alcohol consumption. Heavy alcohol consumption can actually cause low blood counts that look exactly like MDS and sometimes bone marrow findings that are suspicious as well. We ask about medication history. So, we take a comprehensive medication history and ask about whether or not you're taking any medications that might cause low blood counts. We often in younger patients talk about congenital risk for MDS things like fanconi anemia which are germ line or mutations that occur in every cell in your body that are associated with a diagnosis of MDS. This is historically not been thought of as being common in patients with MDS, but actually turns out to be a little bit more common than we thought and I'll touch on that briefly and that's an area of ongoing research right now and we also want to exclude other hematologic malignancies. So, you have to have exclusion of all of those things in addition to the persistent low blood counts and then you have to have also a decisive characteristic of MDS and that means that when we look inside the bone marrow you have to have evidence of dysplasia and I'll show you some pictures of what that looks like. I think a picture is worth 1,000 words and at least 10 percent of the cells of at least one or more lineages. You have to have an increase in blast... So then you have to have MDS diagnostic criteria as well and that means dysplasia that we see in the bone marrow when we look. So, when we talk about it often I tell you we're looking inside the bone marrow to see if we can see findings that

are associated with MDS. Some of those findings are dysplasia as I touched on, an increase in blast percentage. Now, blasts are young looking cells when we look in the bone marrow. They are cells that look younger than the majority of other cells. That means they're bigger, the nucleus is more soft looking, more velvety we call it, the chromatin is open. Blasts of 15 to 19 percent are associated with a diagnosis of MDS as well as a typical karyotype abnormality or evidence of clonality. Now, this is a little bit complicated or can seem a little bit complicated, but when you think about it I want you to think about the fact that in our bodies all of us are born with an instruction manual and that instruction manual is our DNA. DNA in humans is packaged in chromosomes. We all have 23 pairs of chromosomes, 46 chromosomes in total. In women XX and men XY and so if we see an abnormality that's present only in the bone marrow they're only in the bone marrow we say that's... some of them are recurrent and we say that those are MDS associated abnormalities and there are certain abnormalities which are very characteristic of MDS and there are others which are not so characteristic and we'll touch on that again.

So, what causes MDS? I mentioned briefly that less than a small percentage of patients probably have a familial or congenital form of MDS, a germ line, a gene that's present in every DNA copy in their body that's present in those... in every cell in the bone marrow is associated with blood counts and the development of bone marrow failure in MDS. Classically, we think of these abnormalities of things like fanconi anemia and other gene mutations but we're increasingly recognizing that some patients have mutations in the germ line that are associated with development of MDS and this is an area of active research. I actually have a collaboration with Lucy Godley at the University of Chicago in which we're doing germ line testing on some patients. The second most common cause is exposure to chemotherapy or radiation in the past. So, some of my patients have previously received chemotherapy or radiation for other cancer treatments, treatments for which they are now cured things like lymphoma or breast cancer, for example, and those exposures to chemotherapy and radiation we know that those are dangerous and we know that there's a risk of developing MDS. It's actually a relatively low risk. Less than half a percent of patients for example with breast cancer who get chemotherapy will then go on to develop to develop MDS, but when we think about the burden of disease in terms of breast cancer therapeutics and our increasing use of chemotherapies this can be a relatively large number of patients. Certainly very extensive chemotherapy and radiation, things like autologous bone marrow transplant for a lymphoma can substantially increase the risk of MDS, but a majority of patients actually have what we call de novo MDS and this means that the MDS showed up out of nothing. So, they had no history, they have no other findings and a majority of these occur in patients with normal aging and we'll talk about that. Aging, median age in the mid-70s.

So, in order to make this diagnosis we do a bone marrow biopsy. I think many either have had personally or have observed one of these procedures. Not the most fun procedure, but necessary. We stick a needle inside of the bone and we take a sample of the blood from inside the bone. What you can see in this picture here below is what the normal bone marrow should look like. So, what you can see here just from low power even if you're not a pathologist is that there are a variety of different types of cells in this picture. You can see big cells, you can see some smaller cells. You can see these things that look like little squiggle with dots in it. You can see these guys. These guys are red blood cell precursors. These guys are neutrophils and neutrophil precursors and there are a variety of different sizes and shapes of cells and they look all a little bit different and that's characteristic in normal bone marrow and when we look at the bone marrow we want to see whether we see

abnormal... or abnormalities or dysplastic cells, funky looking cells or if they all look normal. So, under normal circumstances we are all born with a certain number of stem cells. We have the maximum number of stem cells in our body when we're born and then these stem cells they self-renew to a certain capacity. So, when they divide one cell stays sort of a seed in the bone marrow and the other one grows up to become adult cells. The first split in terms of differentiation is between myeloid cells and lymphoid cells. Myeloid cells go on to produce all the stuff we're used to seeing, red cells, platelets and neutrophils. These are the infection fighting foot soldiers of the body. These cells have sort of a clumpy nucleus like this and they have a pinkish cytoplasm and they usually have little dots inside of this cytoplasm. Those little dots contain important weapons that the neutrophils use to kill bacteria and viral infections. Those little dots, granules, contain hydrogen peroxide just like you use to kill bacteria in your... they actually contain hydrogen peroxide. They contain something called super oxide and they contain a whole bunch of other enzymes that are designed to kill things in a nonspecific way. In MDS the problem is largely in this group of cells, these myeloid cells and lymphoid cells we think are probably okay. So, myelo, bone marrow. Dysplastic, funny looking. Group... and syndrome group of symptoms. So, that's very simple. It's derived from Greek and Latin.

So, I want to show you some pictures. This is what we're looking at when we look inside your bone marrow. On the left here in the left two images we see what looks like normal cells. So, this is, remember, a neutrophil. I told you about that before. Clumpy nucleus with these little dots in the cytoplasm. This is a neutrophil on the right here you can see with my pointer I'm pointing at. This is a neutrophil from somebody with MDS. What you can see about this cytoplasm here or the liquidity part around the nucleus is that this cytoplasm is missing those granules and when that happens patients with MDS can become immunodeficient as a result of that because those granulocytes, those foot soldiers are missing their primary weapon and that can result in immune deficiency. They can also... the neutrophils don't develop normally. They look a little funky under the microscope. So, instead of having this nice clumpy nucleus they have just a bilobed nucleus and you can have an increase number of blasts. This is a megakaryocyte or a platelet parent. Normally, platelet parents have multiple nuclei and this one has single one. On the left here is a finding called a ring sideroblast. Ring sideroblasts many of you may have already heard about ring sideroblasts. There's a lot of information about that. We'll talk about that a little bit more because we see a genetic abnormality that's associated with ring sideroblasts and associated with an undulant clinical course for MDS. That is to say a slow clinical course. No progression, no leukemia. This is normal. Normally, you do not have that many ring sideroblast in your bone marrow. Ring sideroblasts are red cell parents that are growing up to be adult red cells. You can see that this one on the left has very few. This is an iron stain. On the right you can see that most of the red cell parents are surrounded... the nucleus is surrounded by these purple or bluish dots. Those purple bluish dots represent abnormal handling of iron by the red cell parents and as result these red cells are deficient in their capacity to grow up and do their job correctly and the result is anemia or low blood counts, low hemoglobin in the peripheral blood and so people who have abnormal handling of iron in the bone marrow have often anemia as the primary presentation of their symptoms. This is a megakaryocyte. Megakaryocytes are platelet parents. They produce adult platelets in the peripheral blood which are little tiny cells. In the bone marrow. They're actually the biggest cell. Normal platelets have an even number of nuclei and sort of a dark cytoplasm like this. In MDS, sometimes the nucleus doesn't grow up properly. Again, dysplastic, abnormal looking and the cytoplasm also can be pale and so the platelets often are not

working as well and so you can see people who have platelets as well as low red cells and low neutrophils. So, MDS, a group of symptoms. They stem from this abnormal population of cells in the bone marrow and in general these cells are all derived from the same abnormal stem cell population which is growing up and taking over the space in the bone marrow and pushing out normal production of cells. The result is low blood counts which can cause infection, bleeding and fatigue. Dysplasia that we can see on the bone marrow aspiration and a variable likelihood of disease progression to the more deadly form of leukemia called AML.

So, what drives this? Increasingly, we recognize that what's driving MDS is the developing in one precursor cell of an abnormal genetic complement. So, you look inside these cells and we actually look... when we do a bone marrow biopsy we take the cells and we stick them in a dish and we grow them just like in a garden and we look inside the cells and the first thing the most primitive thing we can look at is we can actually look at their chromosomal complement. So, you take that same picture and you blow it up to be big and they are all kind of gnished across like this and so you have a person who actually goes through and cuts out... it used to be done by hand, but now it's done on the computer each of these chromosomes, each of these things look like a... each of these are chromosomes and then they get lined up into a karyogram and this is a normal looking karyogram on the right. It's a karyotype or cytogenetic profile. Most patients, about 50 percent of people with MDS will actually have a normal looking karyotype. Again, we have 23 pairs of chromosomes, two chromosomes each... of each type look the same. We get one from our mom and one from our dad and if you're a man you have an X and Y and if you're a woman you have two Xs. Now, patients with MDS, a subtype of patients, will have an abnormality in which chromosome 5 you can see here. This is a copy of chromosome 5. If you're not a cytogeneticist you might not appreciate that all of these chromosomes are looking the same. I think I would have a hard time if I was asked to pick out the chromosomes here from this on the left. I don't know about you. This is why it's very important to have a really expert cytogeneticist who does this job all the time. So, one of the things that we have here as Roswell that's very special is our cytogenetic group is extraordinarily gifted and they do a great job with this and they can get me the results of this within a very short period of time. So, this chromosome 5 can be abnormal and that produces the syndrome called deletion 5Q syndrome where people have an isolated deletion of... the only problem they have is a problem with chromosome 5 where they lose the short arm of chromosome... the long arm of chromosome 5 here and then some people with MDS can actually have a complex karyotype and what that means is we see three or more abnormalities and if you look at this chromosomal karyotype above versus below this patient actually has an abnormality in chromosome 2. They have an abnormality in chromosome 5 where they're missing actually the long arm of chromosome 5. They have extra material or sometimes abnormality here on chromosome 7. They have extra material here on chromosome 8. They're missing entirely one copy of chromosome 12. They're missing one copy of chromosome 20. They have extra material here on chromosome 21 and they've got three copies of chromosome 22. So, this is what we call a complex karyotype and you can imagine that under normal circumstances your cells are really set up to deal with this normal chromosomal karyotype and if this is allowed to happen that means that there's something fundamentally wrong with the ability of those cells to identify mistakes because under normal circumstances if your cells have this abnormality they trigger apoptosis or cell death and they kill themselves off. So, if your cells can continue to survive with this abnormal karyogram that implies something fundamental about the underlying disease that says that there is an abnormality in the process of sensing mistakes.

So, it turns out that among people with normal karyotype, in fact, a majority of patients will have mutations that are not visible when we look just at the chromosomal complement, but if we sequence the DNA, if we look deeper remember in my previous slide we have chromosomes, more sensitively we have genes and then we have the DNA itself and if we look at the gene level we can actually identify mistakes in genes in a majority of patients with MDS and this is true whether you have chromosomal abnormalities depending on irrespective of the type of MDS that you might have. These are the subtypes of MDS and you can see a majority of these abnormalities are in genes like TET2, SF3B1, ASXL1, SRSF2. I know many of you were asking me about... in your questions were asking about genetics and about molecular profiling in MDS and this what we're talking about. We're talking about looking specifically for mistakes in specific genes that are recurrently mistaken in patients with MDS.

So, this identification has actually resulted in an ability of doctors to start looking at patients who have those low blood counts where we don't actually know for sure that they have an MDS diagnosis because they don't have those features that are definitive. That is to say they don't have an increased blast percentage. They don't have dysplasia or abnormal funny looking cells in the bone marrow, but they do have low blood counts that are persistent and they don't have any abnormalities in terms of loss of building blocks for genes like a B12 or folate deficiency and that helps us to define those patients into people who have what we call non-clonal low blood counts, your idiopathic cytopenias of undetermined significance, a terrible mouthful. I apologize. Historically, we used to call these people idiopathic cytopenias. So, unknown cause, low blood counts of undetermined significance. So, we didn't know what was going to happen to those people if something was going to go bad or not. So, we can divide those people now into what we call non-clonal idiopathic cytopenias of undetermined significance and a majority of people where we cannot find an abnormality those people are likely to not have a problem from their low blood counts. Those low blood counts are probably from something else. You're likely not have any problems and then we described patients who have what we call CHIP, clonal hematopoiesis of indeterminant prognosis and these people often do not have necessarily substantially low blood counts, but they may have slightly low blood counts and then a little bit further on we have this entity called clonal cytopenias of undetermined significance and these are people where we have evidence of clonality. So, we can identify a mutation when we look for it. They have mild cytopenias or they have some cytopenias, but we don't know because we haven't got long term data about following these patients over time what's going to happen to those people and part of what's going on the field right now is that we're starting to mutationally profile patients at the time of original diagnosis in order to be able to follow them long term to figure out what does happen. Should we be worried? At what point should we be worried? We're starting to get some insights about that because we're starting to look at patients and follow them retrospectively. So, from people where we know what has happened we can look backward and some things we found are that people who have very high percentage of cells that are contained in those mutations. So, more than 20 or 30 or 40 percent and people who have more mutations. So, if you have two or three or four mutations that's much more likely to cause problems in the future. They're just people who might have only one or two and that helps us to distinguish patients and then now we have patients with low risk and high risk MDS and we can help distinguish what's going to happen in the future based on looking at the presence of those mutations.

So, I think it is also important, however, to highlight that a mutation alone is not enough to make a diagnosis of MDS because it turns out that if we take normal people in their 50s, 60s, 70s and 80s. These are people who do not have blood disorders. These are people who have normal blood counts who are following up in things like the nurse's health study and the long term health study people who get blood work done for other reasons because they're part of a registry study. Many thousands of people in fact these are two different studies which were published in 2014 which looked at more than 10,000 people in one and more than 20,000 people in another and what you can see is that if you look in their blood in their arm in up to 15 or 20 percent of people as we get into our 70s and 80s we can actually see mutations and the mutational spectrum is identical to what we see in patients with MDS. We see mutations in the gene DNMP3A, ASXL1, TET2, JAK2, SF3B1, TP53. These are genes that we recognize as being common in patients with MDS that we're seeing also in health older adults and so at the present time although these patients where we identify mutations do have a slightly increased risk of developing blood disorders and hematologic malignancy, it's certainly not the overwhelming majority of patients. It's less than one or two percent of those patients who will go on to develop a blood disorder. So, I think it's important to recognize that the presence of a mutation does not equal an MDS diagnosis and some patients have mutational events done and they become very concerned about that, but it's important to recognize that it's not just the mutation itself, but it's also the amount of the mutation or the variant allelic frequency and the number of mutations.

So, historically we have divided MDS largely in terms of molecular division based on cytogenetics. That's the more gross way of looking at things and in 2012 we revised the International Prognostic Scoring System based on a much more careful evaluation of cytogenic risk groups in more than 2,000 patients with MDS who received no disease modifying therapy. So, these are patients who did not get a transplant, who did not get hypomethylating agents, Azacitidine or Decitabine, who didn't get Lenalidomide where there was data in the registry from outcome and they were able to divide patients based on these cytogenetic risk categories into five different risk groups and you can see them listed here. For the sake of time I'm not going to go through this in detail, but you can see that divides patients into patients who have very good risk disease. That is to say people whose disease is likely not to progress. That is to say a single abnormality involving deletion 11Q seems to do very well. People live a long time. Loss of chromosome Y is an abnormality that's associated with a very good prognosis. Again, numbers are very small in the very good risk group. In the IPSS we said good risk cytogenetics include normal cytogenetics, the deletion 5Q and the deletion 20Q. We've now added deletion 12P and any double abnormalities including deletion 5Q as long as you don't have a chromosome 7 abnormality. We have this intermediate group which comprises a large number of abnormalities and then we have a group of patients who have what we call poor or very poor risk cytogenetics and that includes complex abnormalities, abnormalities involving chromosome 7 or chromosome 3 and double abnormalities include deletion 7 or deletion 7Q and then those patients with very complex karyotype which tend to do very badly. I mentioned before that those abnormalities reflect the fact that those cells in the bone marrow are able to live with a genetic catastrophe and this confers generally a poor prognosis and using the cytogenetic list groups in association with the percentage of bone marrow blasts the degree of low blood counts in terms of your hemoglobin, your neutrophil count and your platelet count we divide patients into... we stratify patients in terms of the likelihood of being alive at various time points in the future with a range of some scores between zero and 10 and just like golf, less is more. Right? We all want a lower number here. So, that's how we divide patients, but it turns out that in addition to the cytogenetic

abnormalities molecular profiling can actually add to that information. So, if we add the molecular profile and this a paper published in 2014, so several years ago now using just a five gene profile. It turns out that if you look at any... if you have any one of these five genes mutated in association with each of those risk categories that I described for the revised IPSS that you get prompted to the next highest risk category and so that means that the cytogenetic and the molecular characterization are additive in terms of their ability to prognosticate for patients. So, if you had low risk disease, intermediate risk disease this is a core of the patients from Harvard. If you then take patients with this Intermediate 1 risk disease and you add any of those mutations that pushes you to the next highest risk category. So, that's been incredibly valuable for us in the field to start thinking about patients and how we're going to approach patients because if you have low risk disease you might not receive any therapy, but if you have Intermediate 1 or Intermediate 2 risk disease we might be inclined to treat you and we might incline to think differently. We might be inclined to think about more aggressive therapies including allogeneic transplant.

So, it turns out that certain mutations actually predict a very indolent or slow growing clinical picture and the most notable of these is the gene called SF3B1. So, SF3B1 is actually associated with those ring sideroblasts that I mentioned to you before, the presence of this picture on the microscope where you see these iron dots around the nucleus of the red cell precursors and this the presence of an SF3B1 mutation in patients with low risk disease actually predicts a very prolonged survival and so we're increasingly recognizing this is a distinct clinical entity. In the old days by a pathologic evaluation we used to call these patients refractory anemia with ring sideroblasts. So, when you think about pathologic...

Yes?

Q1: I have the ring sideroblasts, but I don't have that mutation. How does that (inaudible 29:12)?

Elizabeth A. Griffiths, MD: So, we don't have a full characterization of the mutational spectrum in ring sideroblasts, but in many patients it turns out to be still quite indolent. So, ring sideroblasts are still associated with a relatively indolent prognosis. I can't speak to that personally, but I can talk about some of the responses to treatments in the future that are associated with that in just a moment.

So, I think fundamentally the take home message about all of this molecular data which is quite a bit of alphabet soup even for many of us in the community is that gene mutations and cytogenetic abnormalities are frequent events in patients with MDS. Most people with MDS will have either a mutation or a cytogenetic abnormality or both. In patients with MDS there are usually between two and three mutations per patient. The range here is between one and 12 and in patients with true MDS the allelic burden is usually at least 20 percent. So, if we look at the total percentage of cells in the peripheral blood and the bone marrow that contain that mutation compared to the normal at least 20 percent of the total cells in the bone marrow are associated with the abnormality. We see more than 40 genes recurrently mutated in patients with MDS and this has provided a little bit of insight in the pathogenesis of the disease.

So, now I'm going to transition briefly to talking overview about therapy. I think many of you are already very familiar with this data. We think about MDS treatment intensity as being for patients with isolated cytopenias. We think about the erythropoietin stimulating agents for lower risk disease. Sometimes we add GCSF to the erythropoietin stimulating agents to enhance response and then more recently we started to use Thrombopoietin in the medics. That is to say copies of the growth hormone to make platelets as well as red cells, but these approaches are usually confined to patients with lower risk disease and so we usually tend to keep these approaches for lower risk disease. For those with more substantial low blood counts and those with higher risk disease by the IPSS scoring system we think about hypomethylating agents and the goals of therapy for these agents is to improve blood counts and to delay transformation to acute leukemia and to prolong survival. These drugs, hypomethylating agents, include the drugs Azacitidine and Decitabine and there are several novel compounds that are being developed that are in this family and we'll talk about those briefly in a moment and for patients who are relatively younger and by this I mean people less than the age of 75 we think seriously about allogeneic bone marrow transplantation which comes with a fair number of real potential toxicities but does offer the potential for longer term survival in patients with higher risk disease who otherwise have a pretty poor median survival.

So, in terms of approach to therapy, I think the first thing we need to talk about is best supportive care. Best supportive care includes transfusion support, blood stimulating growth hormone support including growth factors like Erythropoietin and Thrombopoietin potentially, but it also involves thinking about iron chelation. I think there's an increasing body of evidence to suggest that especially in patients with lower risk disease or those who are eligible for allogeneic bone marrow transplantation chelation of iron or removal of excessive iron can improve long term outcome. There's certainly in vitro data, data in mice and other animal models to suggest that if you have iron overload that that can actually impair the ability of the bone marrow to produce cells and so there is some data to suggest that by chelating or removing iron from the body we can improve hematopoiesis in patients with MDS and using registry data largely by the group in Canada, but in other places as well we can show that in a prospective cohort of patients with true low grade MDS that is to say people who are very unlikely to die of their MDS transformation to acute leukemia but are likely to die of cytopenias or low blood counts that by chelating the iron we can actually improve survival. So, I think in my practice I'm pretty aggressive about removing iron in patients if I possibly can get away with it and that's based on this emerging data to suggest that it can actually improve survival. One can assess the burden of iron by checking the blood levels of a hormone called ferritin which is actually a protein that binds to iron in the circulation or by doing an MRI of the liver for iron content. In patients who are on treatment with iron chelation one should check the iron levels monthly using ferritin monitoring. The insurance companies will also require hearing and visual testing once a year and many of you who are on iron chelation this is a real pain to go and have it done, but the reason we do that is because when we chelate iron and we mobilize iron from its storage spots in the body it can actually increase the risk of developing cataracts and can give you high frequency hearing loss. So, we want to make sure we document the hearing and visual testing before we begin and

document any cataracts that might be there and then repeat that evaluation once a year to make sure we're not precipitating worsening of those things.

The main options for treatment with chelation for patients with MDS are the oral drug Deferasirox which has various brand name formulations include a dispersible tablet and a pill as well as now sprinkles which can be used on food. The chief toxicities of this is as many of you are familiar is diarrhea and a change in social quotient to quote one of my friends as well as rashes and kidney problems. As I said before, hearing and cataracts can occur as a result of treatment and the old drug, Deferoxamine which can be given either intravenously or subcutaneously, again, has a similar spectrum of complications related to it. This is a pain in the butt, butt is acceptable to use in patients who have low platelets and people with low platelets there's a black box warning for the drug Deferasirox because of increased risk of GI bleeding. So, we try to avoid if your platelets are less than 50 we try to avoid this drug.

The other therapy we use are drugs like ESAs erythropoietin stimulating agents or erythrocyte stimulating agents. The chief drugs we use here are Darbepoetin and erythropoietin. They can include hemoglobin levels although not in everybody. The response to these therapies is more likely if your blood EPO level, the EPO is a hormone that's made in your kidneys. It's designed to sense that amount of oxygen that's being delivered to the kidney and that's an important thing. Your kidney gets about 20 percent of your total blood volume and so it's a very careful sensor of you're not getting enough blood or enough oxygen delivery and so in response to the low oxygen delivery when people are anemic the kidney starts to make a hormone called EPO. EPO is the same hormone that they use for blood doping. Right? If you're a performance cyclist and you take EPO supplementation you can actually drive your normal bone marrow to make your hemoglobin 15 or 16 or 17 grams which can be a problem. We don't do that, but in patients with MDS if the EPO level, the endogenous production of EPO is lower or EPO levels of 500 are pretty high, but if it's lower than 500 you can actually see an improvement in hemoglobin response to supplementation. Patients with that abnormality of ring sideroblasts tend to be less responsive to EPO in the upfront setting and if we add GCSF to the EPO sometimes that helps. We're not exactly sure why that is, but it's certainly been documented that you can take about a 25 percent of people who failed to respond to EPO as a single agent and add GCSF and you can improve responses in a subset of patients.

Lenalidomide is another drug that's frequently used. The brand name for this drug is Revlimid. This drug has significant activity particularly in patients with deletion 5Q disease. So, if you have isolated deletion 5Q this drug can improve it. It turns out that this drug actually alters the level of proteins inside your cells and if you have a deletion of chromosome 5Q the results is a haplo insufficiency or a partial loss of a protein called casein kinase N1 which is a complicated name which is an unimportant except that by giving Lenalidomide it actually changes the level of this protein in your blood in your cells and decreases it such as those MDS associate cells die off and so you get growth of more normal cells and that's the direct mechanism that is very beautiful work that was done by the group up at Harvard and I was lucky enough to participate in that. The Lenalidomide also has some activity in non-deletion 5Q MDS and there was a recent

phase three study in low risk patients. Again, patients with transfusion dependent anemia but who did not have this deletion 5Q abnormality and they were able to take a third of patients and improve their hemoglobin with the drug Lenalidomide. Again, this is not a slam dunk for our patients. Still a third of patients and the toxicities are real because many patients who get Lenalidomide will have very low blood counts especially in the first three or four months after treatment. Those with low platelets in addition to low hemoglobin are generally not likely to respond to Lenalidomide. This drug, again, improves hemoglobin, but it does not alter survival as far we can tell.

The drug Azacitidine is standard of care for patients with higher risk MDS. This drug has been shown in a prospective fashion and these were patients with high or a very high risk MDS in the study who were randomized by their doctor to receive either Azacitidine or what they called conventional care regimens and so if you were the doctor treating a patient you had the choice of giving people induction chemotherapy. So, that napalm like chemotherapy like we give for AML or you could give patients low dose ARC, subcutaneous ARC which was the old treatment that we used to use for MDS or you could just transfusion support. So, as the doctor you made that designation and then patients were randomized to either receive what you suggested or the Azacitidine. At this point we didn't know that Azacitidine was better than anything else. In the phase three trial we were able to show that Azacitidine treatment actually improved survival in patients who got the Azacitidine compared with conventional care. Again, a small number of patients go induction chemotherapy, but in those patients if you look by subset analysis Azacitidine was at least as good as induction chemotherapy and so I think this is the basis upon which Azacitidine has really become the standard of care for our patients and any patient who showed an improvement in blood counts of any time either improved platelets, improved hemoglobin or improved neutrophils showed a survival benefit. These patients what's distinct about this study is these patients were treated for a long time. The intention was for patients to receive at least six cycles and there was no end point on treatment. So, in general when people come to ask me look, I've had a great response to my Azacitidine. Can I come off? The answer is no because, in fact, when you stop drug early patients progress and we know that when we stop those drugs that there's a substantial risk of progressive disease.

Decitabine is the other analog of Cytidine which is used for patients with MDS. It was approved in 2006 for all MDS subtypes based on phase three data which compared Decitabine to best supportive care alone. In this study 30 percent of patients had a response, but this was using an old schedule of Decitabine, a much more dose intensive schedule which caused a lot more cytopenias. The modern schedule of Decitabine of dosing is five days in a row every... IV every 28 days. Response rates between 40 and 70 percent were observed in some studies although I think this is a little bit of an overstatement. Current practice guidelines really use Decitabine interchangeably with Azacitidine, but there has never been a prospective study that showed Decitabine improved and so in general I would argue that many of us in practice would favor the use of Azacitidine over Decitabine as our first line therapy based on the survival benefit.

A word about allo transplant. There was a question about allogeneic transplant. This is strongly recommended for all patients with a suitable donor who are less than 60 years old. I think that the field is changing a little bit these days, but I still think this is probably a very reasonable recommendation. For younger, lower risk patients we want to wait to transplant them until they show disease progression because there's some data to show that if you transplanted early you had the risks of transplant without the potential benefits for very low risk disease. For those who have more aggressive disease we would think about treating them with a hypomethylating agent before transplantation. Patients who achieve a CR or a PR to an HMA appear to have a better outcome following allogeneic transplant and there are ongoing clinical trials in Medicare beneficiaries, in older patient populations to ask the question of whether allo transplant either matched unrelated or related allogeneic transplant or haplo transplantation or half match transplants might provide survival benefit for our older patients with MDS and this is an ongoing question.

So, it turns out that mutational profiling might actually help us in terms of figuring out which patients are likely to respond to the treatment. Within the patients with deletion 5Q the co-presence of a P53 mutation predicts a group of patients who are less likely to do well. I told you before we think about deletion 5Q as being a very good risk prognosis, but within those patients with deletion 5Q if you identify a mutation in P53 outcome is less likely to be good and so we want to identify those patients relatively early and consider the addition of other therapies or even allogeneic transplants because these patients tend to do less well. You can see here time from randomization in years these patients do less well than those with normal P53.

So, we also wanted to ask the question was there a mutational profile that predicted response to the hypomethylating agents and this has been a long time goal of the field. There is certainly incredible enthusiasm once epigenetic modifying mutations were identified. So, it turns out that TET2, DNMP3A and IDH mutations are actually mutations that alter the epigenome. I don't know if you guys have heard about epigenome. You heard about... has anybody heard about epigenome? Is this too much? No. So, these enzymes are important in a pathway that Azacitidine effects is the bottom line, Aza and Decitabine, and so there was a lot of thinking that maybe the fact that MDS responds to Azacitidine and Decitabine and that genes within the pathway that Aza and Decitabine effects might be important to modify response and so again Rafael Bejar did this beautiful study with a variety of different co-investigators where they looked at 213 patients with MDS who were treated either Azacitidine and Decitabine. These patients were predominately male. They are mostly older, so they do reflect a real population of patients with MDS. More than 90 percent of these patients had a mutation. You can see these are the common actors that we talked about before and they ran the spectrum from low risk, intermediate risk, intermediate risk and high, but again intermediate and high are predominately overrepresented and what they found with the study is that if you had a TET2 mutation you were much more likely to respond to Azacitidine or Decitabine and that was even more true if you had a TET2 mutation but you didn't have an ASXL1 mutation and the likelihood of response was almost twice as much compared to each other mutational profiles and so I think this is not definitive, but certainly suggestive that by mutational profiling we might be able to identify better therapies for

some patients and because Azacitidine and Decitabine response is observed in only about half of patients who get them if we can do mutational profiling and we can figure out which patients are more likely to respond we can offer the patients who are less likely to respond some other therapy if we found something better and I think that's an important modifier for us in the field. It turns out that mutational profiling can also help to inform outcome after allogeneic bone marrow transplant. So, we talked before about how allo transplant is really the most intensive therapy but also the therapy that offers the best potential for long term survival in selected patients. Again in order to get an allo transplant you have to be young and fit. We prefer marathon runners really, but in general these patients represent the most fit and youngest patients with MDS, but you can see here that if you have a P53 mutation you're likelihood of doing well after transplant even among a very selected group of patients is actually not very good and so maybe we should start thinking about changing our practice. Right now what this is meant is for patients with P53 mutations we tend to do more aggressive conditioning regimens. So, we alter the way that we condition patients before the transplant and we treat them with drugs that seem to clear out the P53 mutation. There was a recent publication that suggested that those with P53 mutations respond extraordinarily well to the drug Decitabine and so if you give patients Decitabine first you may be able to really push down the percentage of cells carrying that P53 mutation and then follow on with an allogeneic transplant. There are recently studies looking at the addition of hypomethylating agents in the post-transplant setting to maintain those responses and maybe some immunotherapy as well and I think that's an emerging field.

Other mutational events like TET2 and DNMP3A are associated with less good response in this small study, but in larger studies these have not been validated to be associated with poor response to transplant. Also the presence of a P53 mutation can actually replace complex karyotype as a prognostic factor. So, if you have a patient who has a complex karyotype if one has a complex karyotype, but you don't have a P53 mutation actually transplant outcomes are pretty good and so I think that's an important modifier of our prognostication for patients and this is very important.

So, those are the standard therapies for MDS. I'm going to talk now a little bit about emerging therapies and I think the point here even though there's a lot of alphabet soup, again, I apologize is to tell you that we do have new therapies that are coming down the pike and I think there is something to be excited about in this disease. Many of you are probably are aware that within the last three months after having no new approvals in the field of acute leukemia for the last 30 years. We actually have had four drugs approved by the FDA. One of those drugs is a drug that targets one of the genes we have been talking about a gene called IDH2 and there are IDH1 inhibitors as well which are seen we've currently mutated in patients with MDS that are also coming down the pike for which we have targeted therapeutics. There are a whole other host of drugs now that we are understanding a little bit the molecular mechanism of the disease that we're hoping to develop for patients specifically based on their molecular profile.

So, Luspatercept is essentially a hormone trap. This drug is given once every 21 days and this depletes inhibitory factors that block the terminal differentiation of red blood cells. So, in

patients with SF3B1 mutations particularly but in patients with ring sideroblasts this drug was tried. There was a recent phase two study which actually was done in 58 anemic low risk MDS patients and this drug was given, again, every 21 days followed by an extension phase which allowed patients to remain on treatment for up to five years. Responses were seen in a large percentage of the patients who got the higher doses of therapy. They were seen in people who previously had ESAs and failed and in those who hadn't and in these patients the presence of an SF3B1 mutation predicted an extraordinarily good response. So, if you had an SF3B1 mutation 77 percent of those patients responded in terms of their transfusion dependence and among patients who did not have an SF3B1 mutation who responded a majority of those patients actually had ring sideroblasts in the marrow. So, think that speaks to something about the biology of the disease and the mechanism of this drug. I'm going to do this again. I'm going to show you these pictures. So, the first of these pictures is the development of transfusion independence. So, you can see that these patients who got treated. This is the first set of patients who were followed for a period of up to 14 months getting the higher dose of this Luspatercept. Once they start a drug their changing baseline hemoglobin went up by between 1 ½ and 2 ½ grams and for those of you have hemoglobin's around seven or eight, 2 ½ grams might make a substantial improvement in quality of life and transfusion dependence and you can see here these patients did pretty well and it was sustained and then furthermore if you looked at the group as a whole those who had low and high transfusion burdens prior to entry into the study most of those patients actually became transfusion independent. So, what this is a waterfall plot. If you go up that means you were requiring more transfusions. If you go down that means you're requiring less transfusions. Each of these bars represents an individual patient. So, in this patient population here you can see the change in red cell transfusion burden as a percentage. So, 100 percent change in the negative, 100 change in the positive. Let's just say the person became transfusion independent and you can see a majority of the patients in this study showed a substantial improvement in their transfusion burden.

So, based on these data from the phase two study was actually published just this past week in the *Lancet Oncology*. Based on these data there's a phase three study in development and underway. We're hoping for some preliminary information about this at ASH and this study is a double blind placebo controlled randomized study which is randomizing patients two to one to receive the drug versus best supportive care. They're enrolling 210 patients and the primary end point is the rate at which people become independent of red blood cell transfusion after eight weeks for more than eight weeks in the first 24 weeks of treatment. Patients who are eligible have low risk MDS. They have to be transfusion dependent and they have to have previously failed in the SA therapy and they're going to be randomized to receive either Luspatercept or placebo and hopefully we're going to have some updated information about this study at ASH.

At Roswell Park, we have a robust portfolio of clinical trials for patients with MDS. We've actually participated in the development of several novel hypomethylating drugs. I'm just going to highlight them briefly here. SGI-110 is a form of Decitabine that's complex to another nucleotide called Guanosine and the bottom line is that while Decitabine and Azacitidine hang around in your body after we give them for a period of 15 to 30 minutes, SGI-110 hangs around

for up to 50 to 75 minutes or even longer in some cases and so it exposes the MDS cells in your body to a slightly longer period of the drug and based on that we see substantial activity in patients with AML and we actually have a study here at Roswell Park for patients with MDS who have previously failed a hypomethylating agent offering them it's a randomized study because we saw preliminary results in phase one and two testing that showed activity. So, it's randomized between supportive care, chemotherapy or subcutaneous Cytarabine and SGI-110 and we have that study here open.

We also have a study looking for newly diagnosed patients with oral Decitabine. So, instead of getting your shots intravenously you can potentially get pills. We participated in the phase one and two program for the development of this compound and we're now about to open the phase three study at the end of 2017 and this is... we'll talk about this a little bit more detail later on. There's a huge amount of data and a huge amount of stuff in the news and I'm sure all of you have heard about using the immune system to target cancer. I think in MDS this is actually a very exciting concept and I'm very excited about it. There are multiple studies many of them being done at MD Anderson and other places. Here at Roswell, we have a particular study that's work that I done based on work that I've done in the lab using Nivolumab which is an PD1 antibody in combination with Decitabine and a vaccine that's active going on now. I think a very exciting combination that we don't have open here, but is that is an active development for MDS is a combination of the drug called Venetoclax which is a BCL2 inhibitor with Azacitidine. This is potentially and particularly interesting in those patients with P53 mutations. We have new drugs targeting splicing factor mutations. So, SF3B1 as well as several other mutations that I listed there before are in genes called splicing factors called splicing factor machinery. The splicing factor machinery is an important way that your cells interpret the information that's read from your DNA. So, your cells and your body translate from the DNA message to an mRNA message and that mRNA message gets spliced and cut up and moved around in different ways and then gets produced as a protein. It turns out that in MDS frequently we see mistakes in the ability to process the MRNAs and that in MDS there's a selective potential we have targeting that mutation by killing the one functional copy of the splicing mutation that you have and in doing so you have a specific selective killing capacity for MDS cells versus normal cells. So, it's very exciting. We also have specific inhibitors that I touched on briefly before. IDH inhibitors we have currently studies of IDH1 and 2 inhibits going on at Roswell Park. These are likely to be open in the next month. One of them is open now and we have an ongoing study of a drug called an LSD1 inhibitor which is targeting another pathway that's abnormal in MDS.

So, briefly about oral hypomethylating agents. I think all of you would be much happier if you didn't have to come in every day for five or seven days in a row to get shots or to get IV therapies. We've been actively working on this process and this problem. The problem with oral hypomethylating agents is that there are a huge number of enzymes that are in our blood and also in our GI tracts that are designed to protect us from invaders, protect us from things like bacterial DNA and other people's DNA and these actually break down Azacitidine and Decitabine very quickly in the GI tract, but you can inhibit those enzymes at least briefly and by doing so you can use a drug called E7727 which actually inhibits this enzyme called cytosine deaminase and by

doing so you can actually enhance the exposure to Decitabine for a brief period and so we have developed a drug I collaboration with Aztex Pharmaceuticals that's doing this and so we have that Decitabine drug and in fact if you camper this is data from an Aztex I participated in using this drug called ASTX727 which is, again, the combination of Decitabine with the cytosine deaminase inhibitor drug and the idea here is by giving a pill that we can exactly copy the same exposure that you get by giving IV Decitabine. So, the way this study was done is that patients received either IV Decitabine or the pills in the first cycle of treatment and then we did blood levels to check to see what the blood levels of those different... of the Decitabine was and in the blue here you can actually see the blood levels in concentration in the blood from one patient and from the co-arm... from sort of all te patients together kind of put together who got IV Decitabine and you can see they peaked right here and then it fell off and it was quick. It was gone... all the way gone by two hours, but mostly gone by one hour and then we gave oral Decitabine and we compared it and this is the drug levels on day two and this is drug levels of day five and you can see that by day five with pills we can actually exactly copy when we get to the higher doses of oral Decitabine or we can exactly copy the IV exposure window with the pills. So, this is very, very exciting and based on this there's actually a phase three study looking for newly diagnosed patients with MDS to look to see if we can prove that this true in a larger cohort of patients because, again, this is only 43 patients. We want to do a larger cohort. We're going to put about 300 patients on this study and the idea is really to look to see if we can exactly copy the exposure to IV Decitabine with oral pills and so patients will serve at their own controls. In the first cycle they get either IV or pills and second they get the opposite and then they go on to get the pills and so that's the idea here is to recapitulate so to make it easier for people to take the drug.

The next point I'm going to focus on is the development of checkpoint inhibitors or immunotherapies for patients with MDS and there's a huge number of these studies going on all across the country particularly with a group at MD Anderson. We think, in fact, that these drugs, HMAs, might change the ability of the immune system to see the cancer, to see the MDS in the patient. It turns out that you give HMAs to the whole person and we talked before about we're mostly focused on the myeloid cells, red cells, white cells and platelets, but we give these drugs to the whole person. So, you get them in all over your body. They also can affect your T and your B cells and particularly in the T cells they can actually de-methylate or re-express the gene PD1 and that might block the ability of the immune system to respond to cancer and we think that it's possible that the HMAs one of the things they do by re-expressing a whole bunch of genes is they might actually make your cancer more visible to your immune system and we might be shooting ourselves on the foot a little bit because the Decitabine also hits the T cells and so if we combine a T cell unblocker with the HMA we think that maybe we can enhance the ability of patients' own immune system to see their cancer and we believe that transplant is a paradigm for this because in a transplant we give a new immune system to a patient and we're hoping that the new immune system will recognize the cancer and kill it off. Well, we're hoping to unmask the MDS cancers to your own immune system and to get that immune system to kill off the tumor and that's the hypothesis behind this. That's the thinking behind this. So, we have a study that's developed based on work from my own laboratory which is combining a vaccine

against a gene that gets turned on in response to the exposure to Decitabine or Azacitidine. It's a gene called NY-ESO-1. We know about this gene because it's expressed in a whole bunch of solid tumors things like lung cancer and breast cancer and in that context people have developed vaccines for patients and in fact, we have T cell therapies that are targeting NY-ESO-1 that are being developed.

So, it turns out that if you look at patients who are getting Decitabine or Azacitidine if you look in their blood after they get the drug, this gene is also turned on and expressed and I can take cells from a patient who has leukemia getting Decitabine and I can mix those cells after exposure to Decitabine with T cells that recognize that gene and I can get those T cells to kill it and I've done that work in vitro. So, based on that we developed a phase one study where we combined Decitabine, standard of care Decitabine, with a vaccine against NY-ESO-1 and we were able to show that in a small number of patients we could get a response, but it wasn't as robust as what you see in solid tumor patients. So, now what we want to do is we want to do exactly the same thing, combine the vaccine with Decitabine, but now we want to add that checkpoint inhibitor called Nivolumab because we want to unmask the T cell's ability to respond against that antigen and so the plan is to do a study in patients, a small number of patients first, about six and then to expand it to about 12 patients to combine the Nivolumab with our Decitabine and vaccine and that means that the... and that we'll give the vaccine first and then the Decitabine and the Nivolumab together and then every two weeks people will get an additional vaccine and then people will go on to get maintenance therapy with Decitabine and then vaccinations only every four cycles and that's very exciting and that study should be opening at the end of September. This is going to be confined to patients who are not eligible for bone marrow transplant since we don't know if Nivolumab is safe to give to patients who are going to transplant right now. You can imagine that if I have an antibody hanging around that activates the immune system and then I transplant that patient there might be an increased risk that the new transplant might cause graft versus host disease and might be very dangerous. So, we're holding off on that right now.

I think another study that's very exciting that's being done in Boston and many other places around the country is a combination of Azacitidine with a drug Venetoclax which was just approved for CLL, but which also has substantial activity in AML and probably also in MDS. This study is comparing Venetoclax with Azacitidine at a lower dose with Venetoclax with Azacitidine at a higher dose, with Azacitidine as a single agent. This is for patients who previously never seen a hypomethylating agent and will be followed up. This preliminary data, hopefully, will be seen at ASH this year and we can move on from that. This is based on really exciting 70 percent response in patients with AML who got this combination and, again, we talked a little bit about using mutations to target MDS cells. I said RNA splicing is a very frequent problem in patients with MDS. So, among those gene mutations I talked about before among those more than 40 genes that we see recurrently mutated in MDS a set of them are associated with this phenomenon of RNA splicing that I talked about and it turns out that you could potentially target this splicing using drugs. There's a drug which is being developed by a company called H3 Biosciences which is actually a splicing inhibitor. It's an oral drug and it modulates this SF3B complex. It seems to be really efficacious in splices of cell mutant CMML

venal graph. So, that means if I take a patient's blood or a bone marrow who has CMML and I inject into a mouse that's been engineered to accept human cells and you can imagine that's a little bit mind-blowing, but it turns out that we have these mice that actually secrete human cytokines and support the development of human cells inside of a mouse and if we get these mice to grow these abnormal MDS cells if we then treat them with the splicing inhibitor they work really extraordinarily well and there's a doctor called Omar Abdelwahed who's done some very beautiful work showing that in these mice. It's very exciting. We have this phase one study has been open for about the last 10 months and they've enrolled several patients in this. It seems like the drug is well tolerated. They're doing this thing called dose escalation of the drug. So, we started at very low dose and we slowly go up and we are planning to expand each of the dosing cohorts when we see disease activity and indeed at a second dose cohort level there has been some activity and so they've developed an expansion cohort. So, we are participating in this study and after extensive discussion with the company over the contracting it's finally going to open hopefully in late September. So, we're very excited about that study for patients who have mutations in this splicing factor genes.

One other study that's developed out of work, again, from our laboratory is a supportive care study looking and I'm going to plug my own study here. This is about to open. This is a study looking at response to influenza vaccine. So, you would think that we would understand everything about the response to influenza vaccine in every patient. Wouldn't you? You would think we already would know that. Yeah, well, we don't. So, it turns out that we give influenza vaccine to everyone particularly patients who we deem immunocompromised and ask that their family members also get vaccinated because we actually don't know how well patients who are getting chemotherapy are responding to influenza vaccination. There was a recent study published by a collaborator of mine and from the NIH that showed that in patients getting induction chemotherapy seven plus three, for example, the response to influenza vaccine as one would expect really not that great, but we actually don't if patients who are getting Azacitidine and Decitabine have a similar defect in response to the influenza vaccine and we don't know if patients who don't receive therapy, patients who have MDS who I told you before their immune system might not be completely normal. We actually don't know if people who are getting supportive care are responding to influenza vaccination even though we give it to everyone. Now, there's no risk to giving the influenza vaccine. We give killed vaccine. We give the dead vaccine. We tell people not to get the nose one. Just get the shot and we do that because that's not likely to put people at risk for side effects and it's not likely to be dangerous, but we actually don't know what the response is like. So, here at Roswell we've developed a study where we're actually going to look using high dose influenza vaccine in everybody because there's data to show that high dose influenza vaccine might be better for people who are getting chemotherapy. We're going to look to see if patients with MDS who are not getting any disease modifying therapy who are just getting supportive care, erythropoietin stimulating agents, etc., if those people respond normally to influenza vaccine. We're going to look at patients who've received Decitabine or Azacitidine but who have received a small number of cycles and then patients who've received very many cycles. So, late in the disease process where we think that the MDS is actually very well suppressed and the blood counts are actually pretty good and the immune

system should be pretty good. We want to see if the Azacitidine is actually having an effect on the ability to respond to vaccine and there's actually arguments on either side. There are some people who think that the Azacitidine might blunt the immunologic response and there are others, in fact, work from our lab suggest that maybe the Azacitidine might improve the immune response. So, it depends a little bit on the context and then we're going to enroll a cohort of healthy controlled who are similar in age. So, family members if you're eager to get vaccinated. We can provide the vaccine for you and if you're willing to give us your arm we can do blood work on you and we'll check to see what the response to vaccine is among age matched cohorts and so this is our question. Do MDS patients respond normally? So, I'm very excited about this study. It's been a long time in the making and I think it's an important question to answer.

So, I'm a little bit over in time. Oh, I'm so bad. Okay. So, I want to thank everybody, my patients particularly and their families and all of you who came here today to hear me speak. I hope you've learned something and it hasn't been too complicated. I want to thank my lab group and the group here who works with us and research (inaudible 1:07:23) protection as well as the people who funded my work. Thank you.

(Applause)

We have a microphone. We have a question here at the front of the room.

MDS Foundation: (inaudible 1:07:38)

Elizabeth A. Griffiths, MD: Can I give the lavalier to some... because I have this thing here.

Q2: How often do you suggest the bone marrow biopsies be done once you're diagnosed?

Elizabeth A. Griffiths, MD: So, for patients who are just getting supportive care and being followed, I generally do a bone marrow biopsy at baseline and then I do a bone marrow every one to two years sometimes less frequently if the counts are very stable or at any time if the blood counts change. So, if somebody suddenly becomes more transfusion dependent or if I'm thinking about changing therapy I would definitely do a bone marrow biopsy.

Q2: So, I'll be coming up to the four years that I should have another one.

Elizabeth A. Griffiths, MD: Probably.

Q2: Okay. Second question is...

Elizabeth A. Griffiths, MD: And you might want to have a mutation profile at that time when you have your bone marrow biopsy just because that might help inform the long... I mean, if you have a very good risk mutation maybe you don't need it so often.

Q2: Okay. I'll tell Dr. Tabson when I see him in two weeks.

Elizabeth A. Griffiths, MD: He'll do that.

Q2: The influenza study when...

Elizabeth A. Griffiths, MD: When is it going to open?

Q2: Yeah.

Elizabeth A. Griffiths, MD: So, hopefully soon. It's going to go to the IRB on the 14th and so hopefully after the 14th it'll be open and I think the influenza vaccine is not yet available to us at least yet. So, I'm hoping that it'll correlate with availability of the vaccine because I think everybody will get their vaccine immediately and I want to be able to check to what the responses are and so I need to draw blood before and then we're going to draw blood about a month later and then about three months later to see if the response is sustained.

Yes?

Q3: For these studies do you have to be a Roswell patient or can you be a patient, say, you are at All Cancer Center.

Elizabeth A. Griffiths, MD: So, for most clinical studies you have to come to the institution where the study is open. Now, many studies have multiple sites open to make it easier for people. So, depending on which studies. The University of Rochester has a portfolio of studies that are open and we try to not overlap too much. We try to be able to offer complementary studies. Sometimes there are studies that are more broadly available that are available at both institutions. We collaborate very closely with a group at Rochester and we actually have a conference call to discuss different clinical trials that we're bringing every month to six weeks to discuss that. In terms of these studies, most of them require many of the studies that we're talking about here are earlier phase clinical trials. That are later phase clinical trials require less frequent monitoring, but for most of these clinical studies you want to have a single place that collects the data. So, when you're doing a study if you have a drug that's not proven to be better than the standard of care and you're trying to prove that. You have to check the response to the treatment and that means checking the blood counts and usually that requires checking them at a single location at a recognized location so that you can collect all that data. One of the biggest things that we run into with clinical trials is that people don't get the testing done that's necessary for the study and then you can't say whether the response has been good or not. So, in terms of participation in clinical trials generally you have to go to the place where the study is open and you have to get the treatment there. For some studies particularly ones where they're doing frequent PKs. I showed you that picture of the Decitabine levels in the blood and you can imagine that's an eight hour day. So, you come in the morning, you get a blood work done to show the level at the beginning. We do them multiple times through the day and that allows us to really track what

happens in the blood in terms of the exposure to the drug, but that requires really the generous donation of time from the person getting the drug and then we have to follow up to see what the response are to that treatment. Now, some studies are more... require more frequent blood count checks than others and more frequent (inaudible 1:11:33). So, for example my vaccine study we're really looking very carefully at what happens in terms of the immune system response and we're doing that initially twice a week and then every two weeks as well and then we're doing more frequent bone marrow biopsies and that's really to be able to answer questions about what's happening because unfortunately many... even though we've had many of these drugs for a long time we sometimes don't fully understand. I mean, it was really only three years ago that we understood the mechanism of Lenalidomide. That drug has been around for a long time, but it was only very recently we understood the mechanism. In fact, one of the reasons we were able to understand that mechanism is because at Roswell Park we saved samples on a lot of patients and many of you have agreed to let us save your samples and I was able to send samples to Benny Burt at Harvard from patients with deletion 5Q MDS and based on that he was able to prove that his hypothesis of what we saw in the laboratory was actually true in our patients and that's a unique resource that Roswell has provided that's part of the reason I came here actually to do work is because of the ability to use patient samples and look back in time to see if there are questions that we can answer using them and then look forward with acquisition of samples to ask questions about response to treatment.

Q4: Do you have an opinion on using Procrit compared to the side effects of high blood pressure and heart problems and...?

Elizabeth A. Griffiths, MD: So, for some patients who have bad high blood pressure and have cardiovascular disease the risks outweigh the benefits. I think for every individual person you have to sort of take the disease and the patient and the clinical context into consideration and these are not simple... this is not a simple thing. It's important to have a conversation with your doctor about what are the potential benefits and what are the potential risks and if you start a drug and you start to have side effects related to that drug then I think the answer is to stop it. The benefit of Procrit or erythropoietin stimulating agents in general is to improve the hemoglobin. It doesn't happen in everybody who gets those drugs. In some people you see an improvement in the hemoglobin. If you see an improvement in the hemoglobin and people feel better than I would continue that. If you do six to eight weeks and you haven't responded and you have side effects then the answer is to stop it. For people who respond, but have side effects then you have to ask the question what are the risks of those side effects, how serious are they and is it worth it to take a pill for blood pressure to manage those if you feel substantially better or not and, again, I think that's a conversation you have to have with your doctor and I guess one of the things that I try to get my patients to participate in is that from my perspective this is not an autocracy. I'm not telling people what to do. I want to be a partner with my patient to make the right decision for them and that's going to be different for every single person and some things that are right for one person are not going to be right for another person and that's not a... I'm not going to tell people what to do and I never want to do that. I want to be a partner in

discussing that and I think that everybody's doctor should be able to have that conversation with them and be very straightforward about that.

Q5: Is everybody molecular tested when they first have a bone marrow transplant?

Elizabeth A. Griffiths, MD: So, these days when they have a biopsy we do molecular testing on all of our newly diagnosed MDS patients. Now, many of you may have seen or seen gotten things from your insurance company about rejections for payment for this genetic testing and that's what we're doing. We're sending actually the sample to a company called FoundationOne Hematology which provides very broad molecular profiling and which... one of the reasons we chose them is because that they don't balance bill people. So, we have an agreement with them that they will support our patients. So, if the insurance company doesn't cover that testing because the testing itself costs the list price is about \$7,000. So, it's very expensive to do this molecular profiling. As time passes this sequencing testing is become much less expensive. So, I foresee a time I think in real time that cost is going to come down to more like \$1,000 and hopefully in the near future it'll come down to even less and as we get more and more technology. What we do is we take the cells from you just so you know about what we're doing this testing and what that means when we say sequencing. It means we take the cells, we extract the DNA and we also extract the RNA. So, the DNA is the original message. The RNA is the message that gets transcribed from that initial message to make as an intermediary to make proteins. Does that make sense? Do people kind of get that? So, you start with DNA. That's the instruction manual. You interpret the instruction manual to make the little messages. So, memos. Right? Get sent out and then based on the memos people, the cell, produces something. So, we actually look at the memos and we look at the DNA message. So, the instruction manual and the memos and we actually look at the DNA signature by doing something called sequencing where you actually read A, T... your DNA the message is A, T, G, C and you read out those As, Ts, Cs and Gs and you compare them to a reference sequence. So, we have sequences from people who don't have disease. We have what we call that germ line sequence and you compare the sequence in the patient with a known sequence and that's important because there are actually variations in our DNA that are different depending on your genetic background, depending on where you're from. That's why we can do 23andMe testing and say my background is from Europe or my background is from somewhere else so that we compare the reference genome to the mutation... to the MDS genome and then we read out those differences and there are some differences that occur routinely that we see again and again. That's what we're describing when we're describing these mutations. The Foundation test, the FoundationOne Heme test tests more than 500 different mutations. Many of them have not been well characterized, but we're trying to do sort of a broad test or a broad net because actually we don't know what we don't know. There may actually be things that we haven't looked for. Again, you're only sequencing specific genes currently 500 or more. We don't do whole genome sequencing. There's a distinction between sequencing the many thousands of base pairs, many hundreds of thousands of base pairs in the genetic code many of which is we don't think functional versus sequencing specific genes, specific messages. Is that too complicated or do people follow?

Yes?

Q6: I do have a question. If you're considered low risk and yet you have a high allo frequency of mutations how does that fit in terms of figuring overall assessing that risk?

Elizabeth A. Griffiths, MD: So, the presence of a higher allelic frequency in the absence of dysplasia and the absence of low blood counts just tells us that that person should be watched. It doesn't say that we should alter therapy and, in fact, we don't have... As I said before we don't have prospective information about allelic frequency and gene mutations going forward in people who don't have disease. I told you before that if you look at these large cohorts of normal older people and you sequence you actually find mutations in many of them up to 15 to 20 percent of people as we age and those people only develop malignancy or problems a very small percentage of the time, .5 to one percent of the time and so that's actually very important information which tells us we shouldn't overreact to the diagnosis or the presence of a mutational event. We should just watch it because right now we don't have anything that can get rid of them first of all, any treatment that I have has real potential side effects. If I do a bone marrow transplant, bone marrow transplant comes with an upfront mortality of 10 percent or more. That means if I take 100 healthy people to transplant 10 of them will die in the first 100 days after I give them the transplant. That's real mortality and in the longer term more than a third of them will die related to complications from graft versus host disease and from complications from the transplant. So, when you think about the therapies that we have available unless I have something that's really not going to hurt people, a Pap smear is a good example. I can do Pap smears. It's not a big deal. Everybody gets that done. Mammograms a little less clear. You do the mammogram that hurts a lot. Nobody likes that. PSA testing. Again, another question. Should we do PSA testing or not? There remains a huge amount of disparity in the field about that because if we identify early cancers that you're going to die with rather than if I do a surgery on you today to remove your prostate based on a PSA test and I make you incontinent and impotent the rest, 20 years of your life you're not going to be able to pee properly and you're not going to be able to have sex and I've just done something to you that might never have effected your survival and that's a real problem in medicine because we don't know if we identify this and we do something about it if it's going to hurt people or help them and so I think one of the things that as a community we have to be careful about is proving the things that we're doing are benefitting people and making sure the advice that we offer to patients is realistic and honest and provides as much information as we can without putting people at risk. When I took the Hippocratic Oath the very first line in that oath is first do no harm and I think in medicine now we think that this is all just easy and quick that we can do one thing and another and we see these television programs and that's just not true. We are all... each of us represents a careful homeostasis, a careful possibly and health represents balancing on the tip of an iceberg and maintaining that is incredibly important and hard. All of us spend a huge amount of energy and we don't know it, but our bodies are spending that energy to keep us in homeostasis to keep us exactly where we need to be and if we mess with that we run the risk of putting people at risk and having people die and it's very easy to have problems and so I think it's important for us to be realistic and also honest about what we

can and can't do and what we should and should not do. Anyway, that was a long winded answer. Sorry.

Q7: My question is about remaining transfusion independent. At various points in your remarks you kind of talk about things that I believe are new about how you can remain transfusion independent longer. Is that right or if you are transfusion dependent that you can then get off that and...

Elizabeth A. Griffiths, MD: Well, novel therapies and all therapies using erythropoietin stimulating agents is the oldest one we have to try and improve transfusion dependence. Some people respond, but not everybody does and for people who don't respond who are anemic transfusions are a huge boon because they make people feel better. We know for sure that people who are transfusion dependent who have low grade disease or say for example a non-malignancy things like thalassemias where people are born with a genetic defect which blocks their ability to make red cells. We know that transfusion support in those people or people with sickle cell disease, for example, transfusion support can allow people to live relatively normal lives at a relatively long time and we know that iron overload is the problem that gets those people. So, we know that by chelating iron we can improve their ability to tolerate long term transfusion support. So, although nobody wants to be transfused because transfusions are a pain. Two hours of sitting around, an hour of waiting for the blood work to come back and nobody wants that. I would love to be able to fix that for all my patients, but if the difference is transfusion dependent and quality of life on the times when you're not getting the transfusions or no transfusions and dying of anemia or feeling rotten all the time, I would choose transfusions every time. I am hoping that we're going to develop new drugs and that these new drugs that are being developed will help people to become transfusion independent, but it's my suspicion that not everybody will benefit. Certainly patients with those mutations that predict good response that's going to be a great ability for us to identify people up front who are going to benefit from the treatment that we give. Many times in cancer therapies we actually don't know who's going to benefit. So, we give a lot of people who have a disease that looks under the microscope like the same thing, the same treatment and we see that some of them get better and some of them don't. I think as we begin to understand what's underlying in each of these malignancies we sort of divide them into groups so that it's not all one big thing. MDS is very variable. We know that there are some people who do very well for a long time. We know that there are others who progress very rapidly and die of the disease. How do we identify more effectively those patients and identify therapies for those patients that are going to be best for them, provide them the best quality of life, the longest life expectancy and the least toxicity and I think that's really the point of all this work is to try and better understand what we're doing and offer better therapies that are less toxic.

Q8: Getting back to the concept of homeostasis how in the world do you determine dosage?

Elizabeth A. Griffiths, MD: Hmm. So, when you say dosage you mean for chemotherapy drugs?

Q8: Well, yeah. You got new drugs coming on. You have obviously changes in outcomes and then buys maybe different levels of dosage. So, there has to be protocols that you use.

Elizabeth A. Griffiths, MD: Sure. So, during drug developing when we do clinical trials we do... I'm sure you've all seen phase one, phase two, phase three clinical trial. So, phase one clinical trials are new drugs that we think we know how to use in mice and in the dish and in monkeys where we see toxicity. So the way that new drug trials work is you start by giving a new drug to animals and to cells in a dish. You believe you understand the mechanism of how the drug works. You give it in a dish. You do a whole bunch of studies to figure that out and then you have this new drug you think wow, this is great. It works great in a dish. So, then you give it to mice and you show that mice that carry human tumors respond not all drugs can work this way because mice are not exactly the same as people but that's sort of the idea beginning. After that we start giving the drug usually to the closest human species... the closest to human species that will predict toxicity and usually what they do they use cynomolgus, I guess, monkeys. I don't if I'm saying this... cynomolgus monkeys. I always mispronounce it. It's the C monkey. Not the sea monkeys, S-E-A, but there's a cynomolgus monkey. It's a monkey species that's relatively less expensive, smaller and doesn't live so long. So, you don't feel so bad treating them and you give these monkeys the drug and you look to see do you see toxicity in the monkeys and you look for toxicity in various organ systems after you give the drug and usually you give the drug at very high dose to the monkey. So, you start by giving the drug at a lower dose and you titrate it up. You give higher and higher doses to the monkeys and once you see a level of some toxicity from the drug then when you go back to start giving it to people for the first time you actually take that drug and you give it at a dose that's one-tenth of the drug that produces toxicity in monkeys and that may be below the level at which the drug is active in people, but hopefully will not be toxic and so you start by giving the drug at this low dose to people and you look by... and we do it using something called a cohort. So, you start by enrolling a person, giving the lowest dose that you think is not going to be toxic and you give that to three people and if no toxicity is seen you give it to another three people and if you see still no toxicity in those people and you believe that that's true then you dose escalate. You increase the dose and the first step you dose escalate in a phase one study you double the dose. So, you go from 10 percent of the dose in monkeys to 20 percent. So, you double that dose and then you do another six patients and then you do again a dose escalation. Again, only a third higher this time. Instead of 100 percent higher you do 50 percent higher and then 30 percent and then lower and then you titrate up until you start to see a response. So, the initial studies of drugs often are called the phase one studies are titration studies where you're trying to see if you can get a dose that is not toxic, but at the same time has some efficacy. Now, many times in the modern era when we believe the drugs are targeting a particular molecular features of the disease we... and when we have more targeted drugs we actually will select patients in the phase one setting who have the mutation. So, that gene, the splicing mutation study that I told you about you're only eligible to get on that study if you have the mutation and so we suspect that the drug will be more active in that patient population because in vitro and in mice if you give the drug to mice that are carrying tumors that have that mutation they're much more likely to respond than mice that are bearing tumors that

don't have the mutation and so you hopefully select for a group of patients who are more likely to respond because if all you show is toxicity data in the phase one and you don't have any efficacy data the drug company and the group... our group as investors as a whole are unlikely to be excited about funding a drug that has no activity because it costs many hundreds of thousands of dollars to do studies like that. So, it's not just the cost of the drug. It's the cost of the testing, it's the cost of the analysis, it's the cost of holding the database and hiring somebody to do that work and so it turns out that even early phase clinical... even small early phase clinical trials cost \$100,000 - \$200,000 to do because of that. Did that answer your question?

Q9: What is the difference between MDS and leukemia and also does MDS patients develop into leukemia and if they do like what percentage are we looking at?

Elizabeth A. Griffiths, MD: So, leukemia, white blood, low blood counts is defined by the presence of more than 20 percent blast cells in the bone marrow or the peripheral blood. Leukemia can be AML, acute myeloid leukemia, or ALL. When we're talking about MDS, we're mostly talking about the development of acute myeloid leukemia or we are entirely talking about the development of acute myeloid leukemia. About a third of patients, 25 percent of patients, 25 to 30 percent of patients with MDS will go onto develop more than 20 percent blasts in the bone marrow and that is an arbitrary cutoff that says that at this point when you have more than 20 percent blasts in the bone marrow we call you instead of calling you MDS, we call you AML. Now, from a molecular perspective the disease has not changed. It's just the accumulation of more of the young cells in the bone marrow and so historically we treated all patients with AML differently than we treated patients with MDS. I think now we're thinking that these people who have MDS associated AML or AML that is associated with molecular features more close to MDS tend to respond less well to conventional treatments and so we're trying divide patients early on between MDS and AML and the patients who have AML that arose from MDS because we want to treat them a little bit differently because there's things that work really well in people who have real AML. So, real AML you're fine, your blood counts are completely fine today. One month from now I have a repeat blood work done, all of a sudden I have no blood counts, I have all blasts, all blasts in my bone marrow. Mostly people comes from nothing like that. AML that arises from MDS is more like a cockroachy disease like today I have 20 percent blasts... today I have 10 percent blasts in my bone marrow and I've been watched for a long time and my blood counts have been low for a long time. In a year I have 15 percent blasts in my bone marrow and now I have 20 or 30 percent and that's a much slower disease. The cadence of the disease is a little bit different. Sometimes people with MDS can develop an extra mutation. So, the MDS cells they have those mutations we talked about. They can add extra ones because the housekeeping machinery of the DNA is faulty and so you can start to add extra mutations and some of those mutations can actually cause the leukemia in the MDS cells to grow really, really quickly which is different from the way they normally grow and in that case you can see outgrowth of AML, what looks like real de novo AML in those patients and sometimes can chemotherapy can be effective. Sometimes people with MDS can develop a mutation in a gene called FLT3 which is a gas gene. It actually turns on signaling and it's a growth factor gene in myeloid cells and it tells them to grow as quickly as possible. Under normally physiologic

circumstances that gene is designed to if you get nuclear holocaust and your bone marrow was wipe out to grow back you cells as quickly as it can, but if it gets coopted by the MDS cells or by the leukemia cells then the cells can grow really quickly. That's a long way of answering question. What was the other question you had? What percentage of people go on to... Yeah. So, about a third, but certain mutations and certain cytogenetic abnormalities are more associated with that than others and certainly when we look at patients particularly those who have sort of the better risk prognostic features of mutational profile they seem to develop leukemia at a much lower rate or much less frequently than others. So, we're starting to try and divide people based on molecular profile to predict outcome. The presence of P53 mutations in general tends to be associated with a more higher likelihood of developing problems.

Q10: So, because of a question I'm wondering am I then looking at the right site for CMML? I'm stuck between two worlds. So, where do I go?

Elizabeth A. Griffiths, MD: So, CMML is increasingly recognized as a distinct clinical entity. We use to lump it. We used to kind of lump it underneath AML.

Q11: What's CMML?

Elizabeth A. Griffiths, MD: Oh, chronic myelo monocytic leukemia. The mutational spectrum in CMML is a little bit different than the mutational spectrum in classical MDS. The MDS and Aplastic Anemia Foundation and the MDS Foundation they still cover CMML. So, you're in the right place. Therapies are largely similar. Some patients with... there's good data now from retrospective studies looking at survival in patients in the hypomethylating agent era compared with essentially matched cohort controls from the past. So, patients with a particular diagnosis of CMML, for example, who have similar clinical features based on looking at their medical records and then looking back to try and match them with somebody from the past who had the same features who did or did not get a hypomethylating agent and it certainly looks like hypomethylating agents are prolonging survival in CMML patients based on that retrospective... I don't have prospective data. I don't have a large study in patients just with CML that says that they respond, but certainly from those data it certainly looks like it. So, it's a similar related but somewhat distinct molecularly disease. The more we understand it the more potentially we'll be able to be intervene with specific things. Does that answer your question?

I've really gone very long now. So, we're good? I'm okay? I was afraid I was... Any other questions? At the back of the room?

Q12: How do we find out if we're appropriate or eligible for any of these research studies?

Elizabeth A. Griffiths, MD: So, depending on what study you're interested in at Roswell Park we have several research coordinators and if you're interested in participating. We can have you call and speak with the research coordinators over the phone who can essentially take your history over the phone and say it looks like you might be or you might not be. If you have a

particular interest in one we have a Roswell Park website that lists all the clinical trials that we have that are active. Some of these studies are not going to be on there because they've not been released yet, but those that are coming, I think... if you're interested... if you call and you say I have an interest in this. I want to be screened for one of them you can call and speak to one of our research coordinators and they can help you.

Q13: If you have MDS is your life the rest of your life when you have MDS, are you much more susceptible to colds, flu, everything else than the average person?

Elizabeth A. Griffiths, MD: The answer is we think yeah. So, first of all if you have MDS and you don't get an allogeneic transplant MDS is not in general a disease that is going to go away. So, in the absence of allogeneic transplant MDS is not a curable diagnosis. We think that patients with MDS are at increased risk of infection. Certainly we know that people with MDS who are neutropenic whose neutrophil count is less than 500 or less than 800 are at increased risk susceptibility for infections. With respect to viral infections we actually don't know that detail and that's one of the things we're interested in asking with this vaccine study that I told you about. Because we think that it's likely that MDS patients are at increased risk for viral infections, but there's not good prospective data to say that. Certainly patients with MDS tend to get infections and infection is one of the three main causes of death in patients with MDS. So, we always worry about that in our patients and we are always checking. Many of my patients whose neutrophil counts are low I put them on preventive antibiotics and I'm always giving them a hard time and they don't like to take those antibiotics, but I'm always telling people to take those antibiotics. That's why is to decrease the risk of infections. Most of the infections that people get when they're neutropenic those infections are largely not infections that are viral or that you get from other people like flu. Those are more likely to be related to the fact that we live in a soup of bacteria and as we learn more and more about the microbiome, I think many of you probably heard about that in the news. The bacteria that live with us on our skin and inside of our mouths and in our GI tract in many cases are actually really important. They seem to modify weight. So, certain bacterial colonializations are associated with certain weights. They seem to modify the risk of developing diabetes. They certainly protect us from (inaudible 1:38:57) infection, but if you have a low neutrophil count then those bacteria can sometimes cause us a problem and can escape across the barriers that we have into the blood stream and can cause sickness. So, sort of a double edged sword of the bacterial soup in which we all live. It's a normal part of our biology that in MDS is maybe a little bit more risky, but that's the source of most infections.

Yes?

Q14: Can you just speak briefly about the thalassemia connection with that? It's always been the low blood count, low blood count forever and now the MDS on top of that. How does that work after a year of Procrit?

Elizabeth A. Griffiths, MD: So, I'm not exactly sure what your question you're asking, but I'm going to divide it into two things. So, thalassemia is a genetic disorder in which the instruction

manual for how to make hemoglobin chains has an error in it and there are various different thalassemia that are described based on which of the chains are involved. It turns out that to make hemoglobin you need four chains of protein. There's the product I was telling you about and if you have a mistake in one of the genes for each of those chains, the alpha or beta chain, that your red cells get messed up because they get an unequal production in each of those types of chains and so people with thalassemia don't make normal numbers of red cells. If you have a thalassemia gene, but it's only one copy of the thalassemia gene, people can have a little bit of mild anemia from that. So, hemoglobin that's a little bit on the low side, but not requiring blood transfusions and it turns out the presence of thalassemia gene actually is protective from malaria just like the presence of one copy of sickle cell gene is protective from malaria. So, within people's whose family history and family backgrounds arise around the Mediterranean where there was a huge amount of infection with malaria there's a selection for people. So, people survive better if you had one copy of the thalassemia gene, but that thalassemia, the carriage of the gene, can make you have a little bit of a low hemoglobin and a little bit of a low MCV, the size of the red cells a little small. People who have thalassemia trait or who have thalassemia can also develop MDS. It's not thought to be related. I certainly don't see an overrepresentation of people with thalassemia who get secondary MDS as a result of the mutation like other germ line mutations can result in an increased risk of MDS in genes whose job it is to husband the production of normal DNA, the copying of DNA. In our bodies remember we all started when we're in utero when we're made if you take a sperm and an egg and you make that's one cell. All of us started as one cell. So, in a sense all of us are clonal from that one cell that started us. So, that one cell divides and divides and divides and gives rise to differentiated or grown up cells that professionalize in production of skin, liver, heart, brain, blood cells. There are some genes that are present in that one cell that we started with that can cause damage to production of particular cells because the parts of the instruction manual that are important for that are involved in a muted. Thalassemia is a good example. There are other genes that are important for husbanding the ability of the genome to copy itself. DNA repair genes. P53 is one of them. RB is another one of them. BRCA is another one of these genes. These germ line mutations result in a failure of the genetic machinery ability to check its own fidelity. So, it makes mistakes when it's copying itself and so tissues that undergo frequent copying that are using that husbanding gene are at increased risk for developing cancers because of the ability to acquire mistakes that are not caught. Does that make sense? That was a long winded way of answering your question.

Q15: Quick question. So, for your patients that you typically treat with something like Vidaza. I apologize. I can't remember the generic name and they don't really respond well to that. What other therapies or options do you consider?

Elizabeth A. Griffiths, MD: So, I think first of all what is good response or poor response and I think it's very important and I think one of the things that I see frequently is that people are declared to not have responded too early. So, in general for Azacitidine it takes at least four to six cycles to see a good response. Some people have responded after as many as nine cycles or more. Decitabine seems to happen a little earlier, but it's important to make sure that people are getting the drug on time and on schedule every four weeks for at least four to six months before

we declare failure. Now, if the disease progresses if you see an increase in blast percentage in the bone marrow that goes up very, very high and the person is not improved in terms of their hemoglobin or in terms of other parameters then sometimes people are said to have failed earlier than that, but in the absence of really overt progression of the leukemia cells in the bone marrow I usually want to make sure that people get a really good exposure and a prolonged exposure and a really good test because the truth is that we do not have other drugs that work well for MDS right now. Azacitidine and Decitabine are really the mainstay of our first line approach. Now, there are some other drugs that are coming down the pike. In those patients who truly fail a hypomethylating agent depending on what you started out with in terms of your disease status, depending on the IPSS score, survival is actually quite limited and so if you truly have failed a hypomethylating agent outcomes are best if you get a clinical trial and/or an allogeneic bone marrow transplant and so for younger patients who have failed a hypomethylating agent that's usually our first approach is to put people in a clinical trial. Now if now clinical trials are available or if people are not eligible sometimes I'm the kind of person who tries lots of things. Those of you who know me know that I'm a back of the pocket kind of a person. I always like to have a backup plan and so for patients who are not transplant eligible or for who don't respond then sometimes we look together at literature and we try drugs that have been shown to be active in the phase two setting and sometimes we work on getting drugs like that for patients, but I think ideally a clinical trial in which novel therapies are offered is the best choice followed by allogeneic transplant if people are eligible.

Q15: Thank you.

Q16: What makes a person non-transplantable?

Elizabeth A. Griffiths, MD: So, the number one reason is age. Historically, we cut off... we say we will not transplant people who were older than 60 and then it was 65. More recently it's really 75 is the upper cutoff although people who are in very good shape who are older I don't rule it out as a possibility. So, I have some people in their 80s who look and feel and behave much, much younger and so I don't exclude it as a possibility. I don't use it as my first go to because, obviously, toxicity is a really substantial problem. To address that were looking at novel immunotherapies, but allogeneic transplant is usually reserved for younger healthier people.

Does that...?

Q16: Yes, thanks very much.

Elizabeth A. Griffiths, MD: I think we use really performance status and by that we mean your ability to get up from a chair and run across the room more than anything else as a better predictor of outcome with transplant than we've ever used... than we're using age. So, that's really more of the line.

Yes?

Q17: Do you have any sense of the causes (inaudible 1:47:24)?

Elizabeth A. Griffiths, MD: Of idiopathic cytopenias. Yeah. So, remember that your hematopoietic system is one of the most frequently copying systems in the body. So, remember we talked about those stem cells that you're born with that they... so, we start out as a single cell. We grow up. We make all the different parts. One of the parts we make are stem cells. Those arise actually in the kidney first and then they migrate from the kidney to the bone marrow. They set up shop in the bone marrow and then they set up there and when you're born you have the maximum number of stem cells that you're ever going to have in your life and each year as we age that self-renewal of stem cells is actually not entirely perfect. So, we lose we think about one percent of your total stem cells per year. So, when you look in the bone marrow at the time you're born the cellularity in the bone marrow if you think about the bone marrow as a garden where you have some fat in some cells. When you're born it's 100 percent cellular and all those are differentiating cells. As we age some of that space gets replaced by fat, about one percent per year so that the total cellularity in the bone marrow which is, again, reflective of the total stem cell pool decreases substantially. So, by the time you're 50 you should only about a 50 percent cellular. One of the features that's characteristic of patients with MDS is a hypercellular marrow. So, more cells than we would expect for the age of the person. So, if you're 50 having a bone marrow cellularity of 80 percent suggests that there's something wrong that the bone marrow is trying to work a little harder and... lost my train of thought here. So, every single day all of us are replacing parts of our blood. So, you replace your whole red cell population under normal circumstances all of us replace that every 120 days about. If you have a fever, your red cells break down and get used up more quickly and so you have to replace them more quickly. If you have an infection likewise. You replace all the platelets in your body. You make new ones every eight to 10 days. That's why they tell you you have to stop taking aspirin seven days before you have a surgery because aspirin poisons platelets and you make new ones at the rate of one-seventh of your total platelet population every day. So, if you... you have to replace those platelets all the time and neutrophils have a life expectancy about nine days. So, your bone marrow is constantly working. I told you about it before that physiologic preface. We're constantly making new red cells, new white cells and new platelets and so MDS is a disease in which the ability of that bone marrow space to create all of those things and to replace them is limited. So, there's a defect in production of cells, production of new cells and so people become anemic, thrombocytopenic and neutropenic. During that process those cells in your bone marrow are being turned over. So, the stem cells are being turned over and over the course of your life you can imagine that we all see a whole variety of different potential toxins. So, I don't know about you guys, but I like to do a little bit of home improvement. So, I like to paint and I've done a lot of verathaning over the years. So, we know that verathane and paints those contain... some of them particularly the ones that are oil based contain benzene. If you take a snootful of benzene or a deep whiff off the back of your car exhaust or you get exposed to something, who knows what. You get radiation or when you were a kid you went to Buster Browns all the time and you x-rayed your feet your stem cells got exposed to those insults and as we age the pool of stem cells that we have left over decreases a little bit every year. So, if you have a stem cell that's

been hiding in your bone marrow and was damaged at some point in the past as we age that stem cell has a higher likelihood of being called up to cause us trouble and so when that stem cell starts to divide if it's damaged in some way that gives it incorrect signals to die off or to grow up properly that stem cell can become a dominant population in the bone marrow. That stem cell can start to predominate because at some point in your life that stem cell got a mutation, got damage from some exposure. Does that make sense? And so all of us over the course of our lives may be exposed to something that damages stem cells, but if that stem cell doesn't divide it's never going to cause a problem, but if it does get called up to divide then it's missing the message to turn itself off and it became the dominant thing. I said that twice, but does that make sense? Does everybody follow me? And so likely the reason that we see clonal cytopenias in the peripheral blood is because as we age those mistakes that come something that's more common. They get called up into circulation and they show up in the peripheral blood and that's not a problem unless you get a second hit probably. It's not a problem if you only have one. In mice if we insert the genes that I talked about, DNMT3A particularly and TET2 so if I take a mouse bone marrow and I make a mistake in those genes on purpose. It's a mean thing that we do to mice. We do a lot of mean things to mice. I'm sorry. And if you take those stem cells from the bone marrow of those mice and you insert the gene and you take normal bone marrow stem cells from a mouse and you compete it with the mouse that has the mutation in TET2 is this too complicated? And you would do a transplant where I take those cells, mutant and normal and I inject them into mice and then you grow them up and passage them in mice. So, you do that and then you kill the mouse and you take that bone marrow and you do it again. Over time the TET2 mutant mice and the DNMT3A mutant cells win in terms of growing. So, they have a survival advantage in the bone marrow, but if all you have is TET2 or DNMT3A in the bone marrow of the mice those mice actually don't get cancer. They don't get leukemia certainly not frequently. But they do rarely get it and if you do a second hit, if you add a second then they're more likely to get a cancer. Does that make sense to everybody?

Thank you all.

MDS Foundation: Thanks Dr. Griffiths for providing some amazing information.

(Applause)

MDS Foundation: Thank you. So, we'll take a break now for lunch and if you did have a specific dietary restriction that you mentioned to us when you registered you can see Janice and she can help you in the back. And let's just line... Thank you.