

Speakers:

John F. DiPersio, MD, PhD

Denise McAllister, MS, ARNP, AOCNP

Denise McAllister: And the food is to the left of our room.

Dr. John DiPersio is Chief of the Division of Oncology and Deputy Director of the Alvin J. Siteman Cancer Center at Barnes-Jewish Hospital in the University School of Medicine here in St. Louis. He has published over 100 articles contributing to the science and the wellbeing of those with hemalignancies. He was recently honored with the American Association for Cancer Research Award for his outstanding achievements in clinical cancer research. He's consistently recognized as a top physician and a best physician in America and he's also internationally recognized for his work in T cell function, stem cell research and hemalignancies such as myelodysplasia and acute leukemia as well as gene therapy. So, it is my pleasure to introduce to him today as a contributor to folks living with myelodysplasia as well as to the science of the disease. Thank you.

(Applause)

Dr. John F. DiPersio: Okay. Thank you, Denise, for that kind introduction and welcome everybody. I guess some of you have come from a long way. Is that right?

Q1: Yes.

Dr. John F. DiPersio: Where are you from?

Q1: Flagstaff, Arizona.

Dr. John F. DiPersio: So, you're used to the heat.

Q1: Yes.

Dr. John F. DiPersio: We add a lot of water molecules into the heat here.

So actually, I wanted to just go over some of the basic issues. Denise has reviewed some of them. I want to be relatively quick because I want to save some time at the end, so you can ask me any questions you might have. I'm sure you've been thinking about this disease. Many of you may have it or you know people that do have it and so I want to just provide you with some time so that I can answer some of your questions. I also wanted to spend a little time at the end. The last half of this brief presentation will focus on what we know about what causes it and what our recent understanding of the disease is. I think you'll find that kind of interesting as well.

So, Myelodysplastic Syndrome and acute myelogenous leukemia are almost Siamese twins linked at the waist. They're very similar in many ways and share many of the same features. In fact, the distinction between advanced Myelodysplastic Syndrome and treatment related or secondary AML is very limited because they're probably the same disease. I'm going to show you what I mean later on when I talk about the genetics of this disease, but generally what happens is that bone marrow cells develop a series of events, catastrophic events that results in their inability to mature normally to the normal blood cells that you form. Now, the nice thing about your hematopoietic or blood forming system is that these cells that you make are for the most part all destined to die. So, that's one of the interesting things about you produce blood cells and if they're normal they mature and then they die. Sort of like us really and unfortunately or fortunately none of us can live forever, but some of these cells, actually, develop a number catastrophic events which allow them to survive and not mature normally and so their normal pathway of maturation then senescence and death is interrupted and that results in not only an expansion of this early compartment of cells in this region these early cells, but also they become disordered. They become dysfunctional and disordered. So, they don't mature normally and so they don't develop those normal mature functions like being able to fight infections like these cells over here. So, they're stuck in the middle and they can't fight infections normally. So, people develop decreasing numbers of blood counts. These guys are stuck in the bone marrow. These guys leave the bone marrow, but if you're stuck in this range here, you get low blood counts and you also have a defect in being able to fight infections and so this is just a picture of some of the crazy kinds of forms of cells that you can see when you have Myelodysplastic Syndrome. So, these... look at the number of nuclei in here. There're a crazy number of nuclei. So, this should be a very ordered hematopoiesis and these are very disordered. These giant bilobed nuclei and nuclear blebbing and sort of very bizarre forms which suggest that the normal differentiation process is interrupted and if it's bad enough and if it's severe enough it can result in a complete block of differentiation and we call this acute leukemia and in the case of Myelodysplastic Syndrome, it's just a matter of quantitation. Right? It's always happening. It's always happening, but when it gets to a certain level, we just name it acute leukemia and so in patients with Myelodysplastic Syndrome, especially older patients with Myelodysplastic Syndrome, the functional distinction between acute leukemia and Myelodysplastic Syndrome is very limited. They're very similar and that's why sometimes when your doctor walks in the room and says, "Oh, you had Myelodysplastic Syndrome, but today you have AML," and you say, "Well, how could I have AML? I have the same blood counts. Everything looks exactly the same," and the doctor says, "Well, now it's a serious problem and your blast count has increased from 18 percent to 24 percent and so now you have acute leukemia and you need emergent treatment." Well, that's absolutely false. There's no difference between someone with 24 percent blasts and 18 percent blasts that have Myelodysplastic Syndrome. It's just a little bit worsening of the underlying process which is the same and that is this block in differentiation in the piling up of these cells early on. There is a catastrophic event that can occur when enough of these cells builds up and they start to proliferate in very rapid order, and that's called a proliferative acute leukemia and those kinds of patients do need emergent intervention.

So, just to review for you Myelodysplastic Syndrome is a clonal disorder. That means we think that there is one or multiple abnormal cells that develop a number of these catastrophic events that allow them to progress and survive long term and the dominant feature of this disease is bone marrow failure, the decreasing blood counts and ineffective hematopoiesis. That means disordered blood cell production. There is over many, many years a high mortality rate from this, but fortunately many patients that get this disease are older and so for some patients it doesn't really alter the natural history of their own lives and supportive care has been the standard care for treatment and I'll show you that we had many treatments for Myelodysplastic Syndrome, but we need to do better. You'll see what I mean in a minute.

The epidemiology of Myelodysplastic Syndrome is that about 20,- to 30,000 patients a year develop this in the United States. It's more common than AML, about two to three times more common and it's predominately a disease of the elderly meaning we define elderly in hematology oncology as anyone over 60. So, it's not physiologically being elderly. It's just chronologically being... That's how we define the disease. So, don't get... don't take this personally. As I get older and older, I always set the elderly limit older than I am. That's how I define it and I think that you'll agree me that's rational and the survival can be very... can vary tremendously between patients from just a few months to many, many years. Now remember, median survival, people think about this. They ask their doctor, "Well, how long could I live with this?" and the doctor says, "Well, the median survival is nine months," and of course you're in the room with the doctor and you're life just kind of crumbles in front of the physician. You're thinking, oh, my God. My life is over. Remember when we use these terms, these are what's called Poisson distributions. These are distributions. So, that means the median. That means where half of the patients will succumb. So, patients can actually live even though they have a very poor prognostic disease, because there's a bell shaped curve, some patients can live an incredibly long period of time with disease that you would normally say you could only live a few months with. So, patients can do well even though they have being a typically or objectively poor risk disease. So this is the age distribution and you can see that the vast majority of these patients occur in patients over the age of 55 and 60.

Risk factors. What caused this disease? What happened to me that caused this disease and I'm going to tell you later what caused this disease, but there's a minor contribution of things that we know about. The major contribution is simply age. That's the sad part of this. We were not built to live forever and we have a very fine mechanism of replicating cells and each time we replicate a cell in our bone marrow... a bone marrow is really one of the few organs in our body where cells are turning over all the time and dividing. Most other organs in our body know that your kidney, there's not a division... no cells are dividing in your kidney ever. Your GI tract has a little bit of dividing cells, but for the most part your blood forming system is the most rapidly dividing organ in your whole body and so with every division, problems can occur. There is approximately 1 billion base pairs, nucleotides, in your genome and every time you make a new 1 billion base pairs, every one of those has to match up perfectly and we don't do that. In fact, we now know, I'll show you later that as we get older over our entire life this process is

inherently slightly inefficient and for most of us, we are very lucky and we don't develop kinds of repair abnormalities that result in disease. So, we're all accumulating these diseases over time and some people just get unlucky and they accumulate either an important abnormality or a gene defect or a combination of them that causes the disease, but in addition to the usual stuff that's related to age and the number of divisions your bone marrow cells undergo, there are certain things that when you're exposed to these things can cause Myelodysplastic Syndrome and we know these are things like radiation therapy if you've had radiation in the past, exposure to certain solvents like Benzene, cigarette smoking and there are some congenital disorders which are very rare like Li-Fraumeni which actually is a DNA repair abnormality and for some reason male gender is associated with a higher frequency of MDS. We haven't quite figured that out yet, but there's a reason for it. We just haven't figured it out yet and there are families that develop MDS and now we know that there are certain genes involved in the familial MDS syndrome.

Symptoms I'm not going to go through because Denise went through everything beautifully and the symptoms really relate to low blood counts for the most part, but some patients also develop constitutional symptoms. By that I mean, fatigue or sometimes bone pain or sometimes fevers or sometimes just weight loss and lack of appetite and so what is that caused from? It's caused from the production of cytokines or proteins from these abnormal blood cells in the bone marrow and they die prematurely in the bone marrow and when they die they release stuff and that stuff makes you feel ill sort of like when you have the flu and if it's bad enough, it's an indication for intervention with therapy or if you're fatigued enough, it's an indication for therapy or if your transfusion requirements are increasing to a point that's an indication for treatment although we can't cure this disease with the exception of stem cell transplantation. For the most part, we like to leave this disease alone until you actually are symptomatic from it.

So, these are the survival curves when we classify... This is the old classification. So, this is very simple. If you've never seen these curves. These curves... this is years and this is survival. This is survival and this is the time to AML transformation and one of the... the only thing that's important here is that if you have low risk disease, low risk disease means few blasts in the bone marrow and other features that are low risk disease based on this old FAB classification. You live longer as you might expect. If you have a high risk disease, you live less long and you progress to AML more frequently. So, low risk disease you can live a long time and not develop AML and high risk disease you live your predicted survival is less long and you have a higher rate of developing acute leukemia, which is usually can be problematic and now we have a new classification, you know, this is a bunch of guys that sat in a bar in England and they really are not scientists. They're clinicians and they're very famous people though, but none of them actually do anything except pontificate. So, they came up with these new classifications for MDS not knowing anything about the biology and so they came up with this new classification. You'll see that this new classification dramatically changes things. So, it looks exactly the same. So, it's just using slightly different criteria to look at risk and it does, in my view, add very little if anything relating to survival and so, again, the low risk patients have a shorter survival and they have a less frequent progression to AML. So, this fancy new International MDS Risk

Classification if you're going to ask your doctor has not really improved our understanding of the disease from many decades ago.

This is the NCCN. You probably heard of the NCCN. These are the 22 largest NCI Conference of Cancer Centers get together, actually the NCCN's an interesting organization. To be an NCCN member you not only have to be a comprehensive cancer center, but you have to pay the NCCN a lot of money to be part of this group. So, it's a little whacky doodle, but, of course, the hospitals use this as bragging rights. So, they're happy to pay for it because they think there's going to be an economic end here, but in fairness to the NCCN, the experts in the field do convene, the clinical experts and try to develop pathways for treatment and so these are the pathways for low risk disease and as you can see patients with low risk disease sometimes just observation is or supportive care is the approach and then there are certain triggers that actually start the therapeutic modalities moving forward, progressive anemia, constitutional symptoms and these supportive cares or the issues are oftentimes just transfusional support or the treatment with the Erythropoietin and sometimes therapy with things like antithymocyte globulin and hypomethylating agents like Decitabine and Azacitidine. I don't really want to get into a big discussion about the treatment because I'll show you that we need to do better with the treatment. This is the approach for high risk patients. These patients... the high risk patients have a much, as I just showed you no matter which classification criteria you use, they have a much shorter predicted overall survival. So if you're very old and you have high risk disease, we still consider sometimes just supportive care. Sometimes no interventions whatsoever. If we think you may benefit from the treatment then some kind of treatment is initiated, but if you're young and you have high risk disease. So, those patients are going to have a predicted survival, which is very short and so we have to think about changing the natural history of the disease because they haven't expected... if they had a normal lifespan they would live for 10 or 20 or 30 more years and so we feel compelled to intervene in those patients where it's life threatening, but curative therapy and that's called stem cell transplant. So, this is the only group, I think, for the most part where we're really from the very get go we're thinking about those kinds of interventions and as Denise said older people are older physiologically not chronologically and we know now from retrospective studies that if you are over 60, over 65 and even over 70 and you are in great shape meaning your performance status, you're active, you're running around every day, you're working, you have normal organ function then it looks like the outcome of transplant in a patient at 70 is no different than a patient at 60 or 73 than at 60. So because you're 70 and you're incredibly active and you have a really bad disease like advanced MDS, most big centers now are not eliminating you as a candidate for curative therapy because your predicted survival is so limited. Does that make sense? So it's always a tough conversation with older patients and the more... the reason to go to a large center, I think, is that they have the experience to be sensible and not just impulsive with their recommendations. Small centers like to be impulsive and recommend treatments that they're not doing that much, so they can say to other physicians that they're doing them, but it's your life that's in the balance. Right? And so you want to go to a place that has enough balance and experience to say, "You know, I've done this 1,000 times. I think we should wait and not do this," as opposed to saying, "Oh, you're going to live just a few

months. We have to rush into a transplant,” and you’re 73 years old. So, that’s my plea there to go to a big place that does a lot of these and probably before you make a decision regarding anything like that get another opinion.

Treatment. There’re all sorts of treatments. Hypomethylating agents. This is what hypomethylating agents simply do. They take this methyl group off cytosines and that effect results in the production of... here are these methyl groups that are present on many genes in the human chromosome and when you use these hypomethylating agents, you demethylate these genes so that they get produced. So, many genes that are silenced are methylated and if you over... if you get rid of these methyl groups by these hypomethylating agents like Azacitidine and Decitabine, you convert... you take these methyl groups off and now these genes are expressed. They were normally silenced and so those are the genes that... We’re very simple minded about this. This probably has nothing to do with the way these drugs work, by the way, but our simple minded view is that if we take the methyl groups off and the genes are expressed that those genes help those cells that are stuck in limbo differentiate into the normal mature cells, but we have no evidence of that. There’s no doubt that these drugs work in some patients and there’s no doubt that the drugs do this, but there’s no doubt we haven’t the faintest idea how they really work to benefit the patients and how they really improve their clinical situations. So, there’ve been some trials looking hypomethylating agents and this is why I think this is the trial that got... This is the only trial done by a cooperative group to my knowledge that actually was adequate to get FDA approval for a drug. Most other trials are done as sponsored studies. So, this was done through the old CALGB and patients with Myelodysplastic Syndrome were randomized to either best supportive care or Azacitidine and this is what got the drug approved. So, you can see from these modestly good results that the benefit to patients is limited and the outcome is not changed. So, that’s why we consider transplant in patients that are younger with this disease because the long term outcome doesn’t change and all we’re doing is shifting the curve to the right modestly by giving therapy. There’s no doubt that some patients can get therapy and do well for a long period of time. However for the most part, the group as a whole is benefited modestly by our most effective therapies right now. So, that’s Azacitidine. There was a confirmatory phase three Azacitidine study that was done that showed about the same thing. Survival was enhanced. Decitabine, I heard someone ask about Decitabine. The same trials were done with Decitabine and this trial randomized patients to get supportive care versus Decitabine, same kind of design. The only difference here was that Decitabine was continued only for four treatments whereas in the Azacitidine trials it could be continued indefinitely. The indefinite continuation of therapy actually resulted in the prolongation of overall survival. So in this trial even though there was a modest response, the overall survival did not change and so that was the problem with Decitabine and that’s why I think Azacitidine, the company that makes Azacitidine, aggressively and inappropriately marketed this drug as a better drug than Decitabine. So in my view, it was only better because the trials were designed differently and one can’t imagine how they could be that much different because they’re essentially almost identical drugs.

And this is the time to AML or death for the Intermediate 2 or high risk patients. So in the high risk patients, there seems to be even a more beneficial effect and this is in the high risk patient. So, it's the same for Azacitidine and Decitabine especially Decitabine that the real benefit occurs in patients with advanced disease. Okay. So if you're going to treat a patient and you're going to predict whether the patient will have a response, it's ironically a better chance if the patient has advanced disease.

Now, the last couple minutes I want to talk about what the heck this is all about and what causes this disease. Okay? So to get to this point, I want to tell you about a story that we've embarked...we embarked on here about 12 years ago and we decided that we knew nothing about cancer and we were going to actually sequence the cancer genome and so the first cancer genome sequence in the world was done here and published in 2008 in the journal *Nature* and this was a patient with acute myelogenous leukemia here and subsequently we've been leaders in the world in this area in the area of cancer genomics. We've sequenced probably 1,000 patients with MDS and AML and we've gained some limited insights, which I'll share with you about what may contributing to this disease, but we've taken... this required and I'll show you how hard this was to do and how expensive it is and remember it is looking at... It's figuring out how to sequence the entire human genome and then looking for mistakes and if there's 1 billion base pairs, 1 billion words, let's say, and you're looking for one letter that's misspelled in 1 billion words, you can imagine that would take a pretty good proofreader. Right? That would be like filling this building with dictionaries and looking for 10 or 15 mistakes in those dictionaries. So, we need some pretty fast computers and so we have a thing here at Washington University called a Genome Institute and it's along with the Broad and MIT in Boston, the two largest genome centers in the world, and so we've had... we have an advantage and we had an advantage over other investigators trying to do this. So, it's provided some insights into the biology of this disease, but historically we looked at MDS using these very gross approaches called cytogenetics. That's if you have deletion of chromosome 5 or a 5Q. That's what cytogenetics is and then we started to look at maybe certain genes and sequencing just certain genes, but remember there's 27,000 genes and probably 200,000 gene variance and 1 billion base pairs. So if you're sequencing five genes, it's not really going to give you very much information and then whole genome sequencing is what we decided to do because had no way of knowing what was going on and instead of looking for something that we thought was wrong, that's looking under the lamppost, if you will, we had no idea what we were looking for so we took a less biased approach and looked at the whole thing. To do that was impossible when we started and we figured out ways to do it. So, this is sort of little cartoon saying what you can sort of get from cytogenetics and from candidate gene sequencing and really get all the information from whole genome sequencing.

So, what are the mutations that are present at diagnosis? This was one of our questions. We want to catalog these mutations. Did the mutations that we find affect prognosis? And so we want to correlate mutations with outcomes and do the mutations affect treatment recommendations? Were there mutations that we could find that would really tell us which treatment to use and then

in order for this to occur, we have to have the effective treatments. Right? You don't have very effective treatments right now sadly.

So, this is sort of a cartoon of what we know so far. So, these are examples of clonal populations. So, these green little balls are normal cells and the rest of the cells in this, let's say, tumor, right, are all tumor cells. They all derive from a single cell and they're all blue. Right. These are oligoclonal. This means that the disease process starts with not one abnormal cell, but several different abnormal cells and this is called oligoclonal. You can see that there's normal cells here, but there's a clone of blue cells and a clone of white cells and the founding clone is this blue cell. So, this blue cell is the founding clone and then this clone actually derived from the blue clone and then there are in MDS, we now know that there are multiple unique clones. We never knew that before. So, the disease is not a clonal disease, but an oligoclonal disease and there are anywhere between one and six or seven different clones when the disease starts. So, the question is which is the driving clone? Which are the mutations in the original clone that drive disease forward because you got to target that original founder clone if you want to get rid of the disease. All the little subclones... if you're the mama and you're creating all the... if you're the mama spider and you're making all the baby spiders and you kill all the baby spiders and then the next thing you know there are more baby spiders and there's more baby spiders and so you have to go to the founder clone to get rid of the disease. So, you can only do this by doing what's called digital sequencing and so these data suggest that knowing the gene mutations and the clone that they occur in may be clinically important especially for targeted therapy. So, here's an example of what we did. This is sequencing 3 billion base pairs. That's going back and forth. Actually, the number of genes... the number of base pairs is around 100 to 150 million, but we do it 50 times over and over again backwards and forwards, so we're sure about the sequence and you can see in this particular patient there is one founder clone and then at the time of the MDS develops, there's two clones. There's one founder clone and then there's one subclone and when the patient develops secondary AML, this founder clone develops another mutation and you still have some of the original founder clone with this additional mutation here and then this subclone actually starts to disappear a little bit and this is the clone that actually causes the acute leukemia and you might say that this just a carton. How could we figure this out? But actually we figure out exactly quantitatively what mutations occur in what clones and we can follow these over time in patients. So, this is going to provide the first insight into what happens to this disease.

Yes? I thought you were asking a question.

So, this is the subclone that actually causes acute leukemia and we now know the genes that are present in these clones. This is an example where the gene in the original... the driving gene in this original population, founder clone, is STAG2 and so you can see that each patient is different. These are called fish bowl plots of all these different patients. You can see how complicated each patient is, but we can now in real time within a few weeks generate all of the sequence data and we know exactly who has oligoclonal disease, what mutations are present, which clones disappear with treatments, which clones stay, why are they staying, why are the

persisting and there are going to be some rules that we'll figure out. We're going to figure out the rules of the game and when we figure out the rules of the game, we're going to identify what founder mutations, what founder mutations really drive the disease forward and those are the genes that we want to target for getting rid of the disease.

One other thing. This was a surprise, too. So, you have MDS. Denise doesn't have MDS probably. I have MDS. I don't have it either hopefully and she has 19 percent blasts in her bone marrow and I have one. In fact, there's no blasts in my bone marrow and my blood counts are reasonably well preserved. Her blood counts are terrible. So when we look at her bone marrow by using these clonal studies here, we find that all of her cells can be defined by three or four different clones of MDS, which makes some sense because she's got a lot of blasts in her bone marrow, but me, here I am down here with very few blasts in my bone marrow. What we find out is I also, all of my cells are a clonal or oligoclonal. So by the time you develop even low risk MDS and you have almost well preserved blood counts, you already have a clonal disease. Clonal diseases are called malignancies, but they don't manifest themselves as bad blood counts or progressive disease. So even low risk disease, all the events have happened to develop clonal disease, so and people are fixated on the number of blasts in their bone marrow, but here's prima facie evidence that the disease is already evolved to a clonal malignancy even though you only have one percent blast in your bone marrow. So, all of that stuff that happened is happening years previously. I'm going to show you how that happens. I'm going to just skip this.

So, here is what we did. This is an interesting experiment that is kind of depressing, but it gives you some insight into why I said that MDS is a disease that occurs as you get older. So, what we did is we used these sequencing techniques to look at bone marrow cells and when we took bone marrow from young adults that were normal and we sequenced their entire genome and we compared the sequence of the genome compared to the DNA that they were born with. So, how did we figure out what DNA they were born with? Well, we took the bone marrow out of their bone marrow cavity and we made DNA from that and we then took skin DNA as the DNA you were born with and compared the two. We found no mutations and if we went up to a 70 year old person that had normal blood counts and had no evidence of MDS, we found no mutations and went up to a 90 year old person and we sequenced the bone marrow and the skin. We found no mutations in anybody. As long as they were normal and then we started saying, "Well, wait a minute. What if these mutations occurred differently in each stem cell?" So then, we took bone marrow from cord blood. That's even before you're born or people in their 20s, 30s, 40s, 50s and 70s and we grew those bone marrow cells in a little medium so that this one cell grew up to thousands of cells. So, the one cell is growing up to thousands of cells and so we didn't mix all the cells together. We took each stem cell and let it grow up on its own and then did the sequencing and what we found is that as you get older in every single clone, you accumulate all of these mutations that are some of the mutations are the same mutations that we see as present in MDS and AML. So, the point is that mutations occur as we get older in every one of our stem cells and these mutations accumulate in every single stem cell and those stem cells give rise to normal blood cells and they accumulate over years and decades and something happens at some

point. The incidence is high in those cells that have accumulated the most events and so one cell that's accumulated six to 10 mutations has one additional event and that cell gets an advantage and then you have a disease called MDS or AML and so this is the same thing showing you the frequency for AML.

So, this is what it looks like. You have a one stem cell and every stem cell in your body is the same and you start to accumulate mutations. Nothing happens until this fourth mutation occurs and that that cell starts the oligoclonal expansion and then there are additional mutations that occur as subclones develop and you develop AML. So, this is, I think, this is now the model that everybody in the world is using and this is something that we published recently in *Cell*.

So, the questions are what mutations are present at diagnosis? Did the mutations affect prognosis and did the mutations affect treatment recommendations? These are works in progress. So, clinical implications. Can we target the MDS founding clone? Well, we're starting to look. We can now figure out what mutations are in the founding clone. So, we want to ignore all the mutations in all these subclones because they're all derived from the founding clone and the founding clone drives everything else. We got to go at that clone. So now, we have the machinery to identify which mutations are maybe essential for the initiating event and that's what we have to target if we're going to get rid of the disease.

So one of the things that's come from our efforts and has really identified the major abnormality in MDS is a series of mutations. So, a two groups that reported this at the same time, our group and a group from Tokyo reported at the same time that mutations in genes called splicing genes were very frequent in MDS. We never seen it before and the only way we could of done this would be by whole genome on bias sequencing and so now we know that the most frequent mutation in MDS is splicing gene mutations. So, what are they? They are genes that actually allow you to make normal proteins. So, you have DNA. DNA makes RNA and RNA makes protein and to make the proper RNA, you have to splice these coding regions to other coding regions and that requires about 200 different proteins. Mostly they're managed by about 10 or 20 different proteins that allow the RNA to bleb up like this and to cut off the stuff that's not useful between these coding regions of DNA and put everything together and we find that in MDS the biggest and most dominant recurrent mutation in the founding clone are mutations in splicing genes. So, this is a beacon. This is it. This is what's going on and so the question is how does that really make the disease? So, we're working on that, the biology. How does splicing abnormalities result in this ineffective blood cell production and the progression to AML? The second is if it's a splicing defect, how other drugs that we can actually use to actually repair the splicing abnormalities. So, we're working on that as well as well as other groups. To show you that this important, here are the mutations that are present in these various kinds of MDS and you can see that these are splicing genes and you can see that they really define which kind of MDS you have. So if you have refractory anemia with ring sideroblasts, you for the most part just have this splicing gene mutation. If you have CMML, you mostly have this splicing gene mutation. If you have CMML or AML, you mostly have these. So, we're now starting to put... piece this

together. That's a big deal. These definitely contributing to the disease how and how to actually inhibit their... how they actually cause the disease will be the next step. Sixty-five percent of patients have these mutations. That's pretty much probably... and if we look harder it's probably going to be much higher than that. Probably 80 percent. So, that is one of the key driving factors of the disease. So now, we got to spend the next 1,000 years figuring out the biology and how do we intervene and fix this.

So in summary, MDS is highly clonal and with the founding clone that persists and progresses in patients with secondary AML. Targeting mutations in the founding clone may have therapeutic advantages and interaction of gene mutations in iron overload, which I didn't talk about. You know that all patients with MDS get iron overloaded, too. Anybody in the room know that? Even without transfusions. So the iron overload, it turns out that the mutation in this gene also results in iron overload syndrome in mice. So, probably the iron overload associated with MDS is not just transfusional iron overload, but there's an inherent pathway for iron overload due to the mutation in this gene.

So this is why all this stuff takes so long and is so hard. So, you know the human genome, the first... the human genome was sequenced. It took actually most of this human genome sequence was done here at Washington University and it took about 12 years to complete sequencing of the first human genome. This was a project funded by the NIH. It cost \$1.7 billion and one human genome was sequenced. The first cancer genome was sequenced here in 2008 and it cost us \$1.6 million. The second cancer genome sequenced here six months later cost about \$500,000 and took us six months and for AML genomes done here, numbers three through 50, it cost about \$50,000 a case and took two months. Now, we do a single genome every two to four weeks at a cost of \$12,000 and probably next year it'll be around \$8,000. Even though it's cheap, it still takes an army of people to do this and here is some of the people that just focused just on the MDS and AML project. It's a very small snapshot of all the people that were involved in this. So, it takes an army of people to do this, but I think for the first time, we're gaining some real insights into the disease. I think that this is important if we want to improve the treatment of MDS from what we have now and in my view it's inferior and it's got to be improved and I also think that... We didn't talk about transplant, but I think that transplant even for older patients is a real viable option now and we're doing incredibly better with older patients and transplant, as well, and so I think that the future looks good and I think that our insights into the biology are going to make all this happen and I want to thank you for your attention and I'm ready to answer any questions you might have.

Yes?

Q2: I appreciate you sped up the research. That was really (inaudible 45:20). If you... like you're early diagnosed .Why do you wait? Wouldn't that put (inaudible 45:29) bone marrow transplant just waiting (inaudible 45:33) much worse? Why is... There's nothing going on, no treatment or anything until you really need it. It didn't make sense to me.

Dr. John F. DiPersio: Yeah. Because we haven't been able to do bone marrow transplants safely enough in older patients until very recently and I'm not saying we've fixed that. So, the reason we don't send patients for transplant is that remember I told you go to a place that does a lot of transplants. They'll give you the fairest balanced impression about whether you should undergo that. The transplant is as soon as those cells enter your bloodstream, it's over. Your die is cast. It could be great or it could be a life of debilitation and agony or it could be death.

Q2: So, you just have that transplant (inaudible 46:27) going for it early on instead of late.

Dr. John F. DiPersio: Yes. So, that's a very good point. So, I think we're getting closer to exactly what you want to do. That is that instead of waiting, we're... if we could offer transplant with complete safety and with not making your life worse. Now if your life... if you're 65 years old and you're going to live another 10 years and I'm going to transfuse you, give you therapy and your life is pretty well preserved, but if I transplant you and you live another 10 – 12 years, but it's agony, I haven't really done anything good. So, if we can actually fix the transplant so that the toxicities of the transplant are eliminated, believe me, we got a ways to go, but we're making progress there. Then we could offer this treatment to everybody as soon as we make the diagnosis.

Q2: And that would (inaudible 47:23) all this horrible stuff that could happen later on.

Dr. John F. DiPersio: Now remember... You are confused, but remember the pictures I showed you about all the mutations in these clones. As you get older, there are many more mutations that develop and these clones are hard to get rid of and so one of the things we've noticed about transplanting patients with MDS is it's hard to get rid of the disease. It's even harder than acute leukemia because acute leukemia when it develops out of the blue in a younger patient is just associated with 10 mutations. MDS in an older patient may be associated with 40 or 50 mutations and so it's a lot more complex genetically and it's harder to get rid of. So, if we make transplants so it's completely easy, are we going to end up with just everybody relapsing in which case it would futile effort as well. So, the goal is to do exactly what you say. That is to fix transplantation so we can offer this curative therapy early, but also be sure that the therapy, the transplant therapy, is going to help people.

Q3: I'm low risk, intermediate (inaudible 48:36) and I'm on Procrit and I have been for about six months. My white count is critical (inaudible 48:44) and they put me on Levaquin and it gives me (inaudible 48:50). I just feel terrible when I'm taking it, 500 milligrams. So, I'm down to 250 milligrams, but I just started having fevers below 100 the last two weeks.

Dr. John F. DiPersio: Your neutrophil count is below 100?

Q3: My what?

Dr. John F. DiPersio: Neutrophil count is below 100 or...?

Q3: Yes.

Dr. John F. DiPersio: Yeah. So, you know, if your neutrophil count is 100, I don't put people on prophylactic antibiotics. I never do unless they've had recurrent infections and I'll tell you why. Because if you have a neutrophil count of 100, you have 100 billion neutrophils in your body. Now, they may function abnormally because people can have MDS. People with MDS can have neutrophil counts of 3,000 to 4,000, but those cells do not function normally. So, they can't kill organisms well and so they have recurrent infections and it's something that's very hard to deal with the exception of a transplant or some medical therapy.

Q3: How critical are the infections? They said if my temperature went up like over 101, I should go emergency (inaudible 50:07).

Dr. John F. DiPersio: That's right. That's right. Again, but I actually don't like to put people on antibiotics unless they've had recurrent infections.

Q3: He put me on that almost from the start. Then another doctor at KE said you shouldn't be on it.

Dr. John F. DiPersio: I agree with the KE doctor.

Q3: But then when I got the infection, then I started to take...

Dr. John F. DiPersio: Well, if you're getting infections, I mean, again, getting infections you treat the infection and my sense... my approach and most people's approach is if you have a neutrophil count of 100 and you've had an infection, you treat the infection and then you observe the patient even though their counts are low. If they have recurrent infections and they're getting infected every week, that's different. You need maybe some kind of medical intervention then. So, that's a reason to actually intervene with some kind of therapy for your disease if you have recurrent infections like Decitabine or Azacitidine. Okay.

Q3: Thank you.

Dr. John F. DiPersio: Sure. Yes?

Q4: Do you have anything to say about the possibility of MDS being in remission as a result of being on the Revlimid for several years?

Dr. John F. DiPersio: In remission. You mean genetically in remission or if you've been on Revlimid for a few years...

Q4: No.

Dr. John F. DiPersio: Yeah and your... Do you have any chromosomal abnormality like a 5Q-? Yeah. So, that's a really good question. That's a question that only we can answer. Only we could tell you, but the reality is people with 5Q do respond nicely to Revlimid and they can get normalization of their blood counts and transfusion independence and their 5Q- goes away. So, that's part of the clone. Right? The question is are all the other subclones or is that founder clone which may be very small. Is that still there and so that's a good question. Nobody's looked. We've looked at patients who have AML and our AML patients that have been treated with... older AML patients... So, they had MDS. As I told you right in the beginning, elder AML and MDS are very similar diseases and so we've had patients that go into complete remission, everything goes away and we do their sequencing and we find out that those clones are still present. So, they don't completely go away, but if the clone that causes the problem has gone away. So, there's one of these subclones that is suppressed, but the backward... the back clones, the founder clones are still there. So, that's why the disease sort of always comes back eventually, but it doesn't change what I would recommend. You should continue on with your current approach, right, if things are going well.

Q5: I have a question. I guess, I don't understand not necessarily how you get it, but why does one person get it and the other person doesn't? Is it something...? It's not hereditary is it?

Dr. John F. DiPersio: There are, as I said, remember we've been studying... there are families that develop MDS. So, we know with the mutations that cause about 30 percent of those familial forms of MDS. So, that's new. We haven't published it, but I can tell you that one of them is called a gene called GATA1... GATA2 and this is a gene that's mutated and another gene is called RUNX1 So, RUNX1 and GATA2, those two genes can... those mutations in those two genes, the genes you were born with. So, that's inherited, passed through the germ line, can cause familial forms. So, that's very rare. That's one to five percent, one to three percent of all MDS syndromes. The vast majority of people have this develop spontaneously over time and so there probably are... there's a reason for everything. There probably are reasons why some people develop it and others don't. There may be mitigating features or factors that cause it - environmental exposures we don't know about, but we think that the vast majority of these things occur what's called stochastically or randomly meaning that you accumulate everyone, me, you everyone, we all accumulate about almost exactly the same number of mutations every decade as we get older and each one of our stem cells develops... each one of them within each of us develops a different array of mutations and so it's just one of those cells being hit at the right time with the right mutation in the background of all these other mutations causes this to progress. Those cells somehow live longer, don't divide and dominate. They must gain a competitive advantage. Right? Because if they didn't gain a competitive advantage your normal

blood system would just always take over. Right? So, they must have some competitive advantage and so what if there's anything that enhances someone's chances of developing this, I'm sure there are issues and I'm sure there are things, we just don't know what they are yet and maybe even if there are things that means that there may be ways to reduce that risk. Right?

Yes?

Q6: I've been on Procrit for 16 (inaudible 55:58) and my red count or hemoglobin has been right eight. It goes down a little bit and it stays a little bit about eight. Is that (inaudible 56:09) would that be the best thing for me to be taking? I'm not getting anything for my white count at all.

Dr. John F. DiPersio: I think that if you're stable and you're getting... are you getting transfusions or no?

Q6: No.

Dr. John F. DiPersio: Then I think that that's perfectly reasonable. I think the reason to treat you with something like Azacitidine or Decitabine or put you on a clinical trial... I didn't talk anything about clinical trials, did I? What do you all think about clinical...? You don't like clinical trails.

Q6: I think that would be more of a (inaudible 56:43)

Dr. John F. DiPersio: Okay. Remember this. Just something to think... The solution to the problem is clinical trials and unfortunately for better or for worse if no one participated in clinical trials, we would not have Azacitidine. We would not have Revlimid. We would not have Decitabine. We would not have transplant. So, it's a tough pill to swallow when someone says try this something new and you're going I don't want to trying anything new and obviously, you know, placebo control trials are very infrequent. Most of the trials are you get the agent no matter what and I think just a proponent just when your doctor if your doctor ever says think about this, you should seriously think about it because in today's world at a good place if you're being seen by a guy who knows or a gal who knows what they're doing in academic center, not necessarily in private practice, but in an academic center. All of these trials are well conceived and they give you the almost always the basic treatment, which everybody can get plus something and there well thought out and I think there's always reason to believe they can help people and that's how we got Decitabine. That's how we got all of these drugs.

Q6: My neutrophils are between 7 and (inaudible 58:14). He wanted to put me on Vidaza. Would that be a (inaudible 58:18)? It goes down to .5.

Dr. John F. DiPersio: Neutrophils? Again, I would only consider starting therapy if something changes in your blood counts like you start requiring blood transfusions. If you're stable and you

have an ANC of .7 and you're not getting recurrent infections and you're otherwise just getting good solid supportive care and your bone marrow has not developed advanced disease. So if everything's stable, I would consider it.

Q6: I'm supposed to get a second opinion and bone marrow biopsy at KU next Friday. (inaudible 58:55)

Dr. John F. DiPersio: Right. I don't hear anything...

Q6: The last treatment is better probably?

Dr. John F. DiPersio: I don't know that for sure.

Q6: More or less.

Dr. John F. DiPersio: I don't know that for sure.

Q6: (inaudible 59:13)

Dr. John F. DiPersio: I think... I have to remind you that every treatment, every standard of care treatment, by the way, has risks and people... and so if you have a low white count to start with then one of the obvious things that's going to happen especially with that first cycle is your counts are going to drop even further. So, there's always a risk that someone could develop a serious infection with treatment. That's why unless the disease is changing or the clinical symptoms are changing, driving me to recommend a treatment which is inherently there's some risk to every treatment and there's also a lot of cost. Think about it, but wait until you see what the bills are for Decitabine and Azacitidine.

Q6: Almost \$2,000 (inaudible 59:58)

Dr. John F. DiPersio: That's nothing compared to these other things though.

Q7: You talked about environmental factors. I grew up in Roxana, Illinois. My elementary schools here and junior high, my high school and this right here was Shell Oil refinery surrounded us. The football field it even came down along here and we have a very high incidence of leukemia in Roxana and (inaudible 1:00:32) Shell Oil, Sinclair Oil, Standard Oil, all of them used to be headquarters there. So, is that possibly is what happened to me? Is there a long period of time that benzene... if I had an environmental factor, would that have happened when I was from five to 18 and it didn't manifested itself till I was 63 (inaudible 1:00:59)?

Dr. John F. DiPersio: We know from Hiroshima and Nagasaki what the average time between an event and leukemia is. So for radiation exposure, the average time is about two to three years.

For exposure to chemotherapy, there are two phases depending upon which chemotherapy you get. It's either one to two years or five to seven years, three to seven years. So... and for normal environmental exposures, we have no idea. So, all we know is from those little snippets of patients developing acute exposures to environmental stimuli like radiation, benzene and toxin exposures or chemotherapy and we know what the time frame is. So, I would say that if the insult is not ongoing, if you've lived 10 years after the insult has been completed, it's probably safe to say you've survived the event.

Q7: So, that wasn't the issue. Something else got me. Not living... my high school class ring has that refinery on it.

Dr. John F. DiPersio: So, I'm just going to ask you to do something for me as an example. You're going to go home. I want you to copy your dictionary and then I'll see how many mistakes you made and then I want you to take the same dictionary in ten years and copy it and I'll see how many mistakes you made. What happens is over time as you get older the frequency of these what's called repair abnormality mistakes is slightly increased, but more importantly your ability to repair these abnormalities decreases. So as you age, your DNA repair capabilities drop slightly and your increase rates of mutations increase slightly and so that results in a slight increase rate of DNA repair abnormalities and this is what happens as you get older and you can't repair these abnormalities and mutations start to accumulate and they accumulate at a more rapid rate over the age of 60 and, again, I would say although there may be specific events that contribute to this, this is stochastic. It occurs in every cell and it's just bad luck that the right combination of mutations accumulate at the right frequency at the right time and the right cell and it causes this event.

Q7: Well before I met (inaudible 1:03:49) I was at another doctor in St. Louis and, of course, that was the first comment to him was it was some assault to your... he said... I said how did this happen? Well, it's an assault to your body and of course, I go why did somebody hit me? I fell and broke my leg. What happened to me? So, that sentence, the initial sentence, has kind of stuck with me and I tried to think, but you're saying this might happen because just because and I should take that sentence out of my paradigm.

Dr. John F. DiPersio: Yeah. Exactly.

Yes.

Q8: If I'm listening to you carefully it's not only happens with MDS, but it also happens with other types of illnesses, cancers and that to me whatever you can't replicate the same or there's an assault to your DNA then that's what causes the cancer.

Dr. John F. DiPersio: Yeah. We know that treatment related acute leukemias and treatment related MDS, it's a different disease completely than age related MDS or spontaneous AML. The

mutations are completely different. So, that's one thing. So, if your leukemia or MDS is generated by a previous exposure to chemotherapy, the disease is different than if it just develops based on age. The mutations are different. So, there is... and in patients with other cancers, I should have brought of picture of a cancer genome from a lung cancer patient that had not been a smoker. We sequence the cancer genomes of many lung cancer patients here and they have a limited number of mutations. If you look at a lung cancer genome in a smoker, there are hundreds more mutations. So, we know for sure that external environmental exposures can alter the genomics' background. So, very simple and also much more treatable lung cancers that develop in nonsmokers versus smokers that have terrible lung cancers because the number of mutations caused by the smoking is 100 times higher. Now when you are smoking, by the way, blood is circulating in your lungs and so there's probably maybe even some indirect effect on your hematopoietic cells that circulate if you're smoking a lot and now... and we do know that smoking is also associated with an increased risk of MDS. It's modest, but it's definitely... and AML. So, these kinds of environmental factors can play a role potentially at increasing the risk slightly, but in solid tumors like smoking and also head and neck cancer. Head and neck cancers that are caused by HPV virus is a very uncomplicated simple cancer genome, not many mutations. If you look at a smoker's head and neck cancer, it's exploding with mutations and rearrangements and those things are impossible to treat because there's so many genetic changes that you can't possibly repair them all with any treatment.

Yes?

Q9: So, if you live a more active life, instead of you going to the couch because you don't feel good and you get up and do your normal things and try to live a normal life that can be better for you and possibly help you.

Dr. John F. DiPersio: I think it's just common sense. I mean, if you live a healthy lifestyle, you're going to be able to tolerate anything and everything better. Let's just take transplant as an example because if you're physiologically young even though you're 70, you'll do as well as a 55 year old or a 50 year old.

Q9: Yeah. That's what I wondered if you're...

Dr. John F. DiPersio: So there is incredible benefit of being physiologically in good shape and eating well and exercising and being active. Does that specifically change the natural history of these diseases? I think it's good for you. I think you'll tolerate everything better, but I have no evidence that it changes the natural history of the disease and I wish I could say it did, but I'd be lying or I'd be Tony Robbins or something like that.

Q9: (Inaudible 1:08:33) make you feel better physically and spiritually to...

Dr. John F. DiPersio: I have twins age 3 ½. I suggest you all get a couple twins at home. That'll keep you running around.

Q9: I have grandchildren, great grandchildren.

Dr. John F. DiPersio: That's better. Grandchildren, you can leave them at the end of the day.

Q10: I just have one really quick question on this. (Inaudible 1:08:58) the MDS jumped into AML. Do you know (inaudible 1:09:03) bone marrow the blasts (inaudible 1:09:04).

Dr. John F. DiPersio: Yeah. So, remember I told you in the beginning that distinction is bogus. There's no difference between someone that has 15 percent blasts...

Q10: Yeah. What's the normal blasts?

Dr. John F. DiPersio: Normal blasts are between zero and five percent in the bone marrow. So, if you have MDS and you go from 15 to 22 percent blasts, even though an inexperienced oncologist will say oh, this is terrible. It's the end of the world. It doesn't mean anything. It just means that your disease has moved along its expected course.

Q10: But there's other (inaudible 1:09:40).

Dr. John F. DiPersio: Yeah and so what can really also cause you to know that someone's developed acute leukemia. So, an event can occur, a genetic event can occur, which makes the cells suddenly not just differentiate less well, but also grow faster. So, some patients that have MDS instead of just their counts getting worse and worse, which is by far the most common, some people can actually develop rapid progression of their white count like leukemia and any of these leukemic... So, that's not very common, but that's also a way to tell if someone's developing acute leukemia.

Yes.

Q11: The problem is I'm (inaudible 1:10:22). I'm lucky I didn't (inaudible 1:10:25) as I know that (inaudible 1:10:31) discussion about Agent Orange cause a lot of cancers and things. Does that have any connection with (inaudible 1:10:44)?

Dr. John F. DiPersio: I don't think so. I mean, we have no evidence that Agent Orange contributes to MDS. Agent Orange probably has some... has probably some effect on the development of non-Hodgkin's lymphomas and skin cancers, but other than non-Hodgkin's lymphomas and some skin cancers and that's, obviously, debatable. Right? I mean,

Q11: (inaudible 1:11:10) I have the MDS.

Dr. John F. DiPersio: I don't there's any evidence that... I get this question a lot because many people are Vietnam veterans. They're about at the right age now to develop MDS and so I get that question a lot and I just them I just don't know of any evidence and this has been contested in lots of different VA courts and VA systems and VA complains and it's the way waste the rest of your quality of life battling this stuff in my view.

Q11: Well, I had no complaints (inaudible 1:11:51), but you hear a lot of evidence.

Dr. John F. DiPersio: I don't think there's any evidence that...

Q11: (inaudible 1:11:56) ten years (inaudible 1:11:59) doing good.

Dr. John F. DiPersio: How many people have MDS in the room here if you don't mind me asking?

Q12: He has CMML which is a version of MDS.

Dr. John F. DiPersio: I know him. So, CMML. Yeah. CMML is a form of MDS. Also that's another good disease. The mutation that is... we have a mouse when we delete this gene results in a disease that's CMML like. So, we know if we delete one of the genes that's been commonly seen in CMML. In fact, the most common mutation in CMML is a gene called TET2 that's mutated. So, we discovered that as well and now TET2 is often in these... there's commercial panels to look at various genes that are mutated in hematologic malignancies and TET2, if you delete the gene TET2 in a mouse, the mice develop CMML and then AML over time. So, CMML is... behaves... has its own sort of world of or staging and classificat... but I think it's... it is a... even though it's been pulled out, it is in my view very similar to patients with intermediate risk to advanced risk in MDS patients and patients can live with it for a long, long time and some CMMLs are caused by completely different mutation and those patients just need to be on a pill forever and there's no evidence that intervening with transplant early is the thing to do. Patients that have CMML, we watch them very closely like we watch you very closely and we make difficult decisions about when to treat and when to subject someone to a transplant.

Okay. So, good luck everybody. Wish you all the best of luck and I hope you enjoyed... had fun today and enjoyed some of this. Okay.

(Applause)