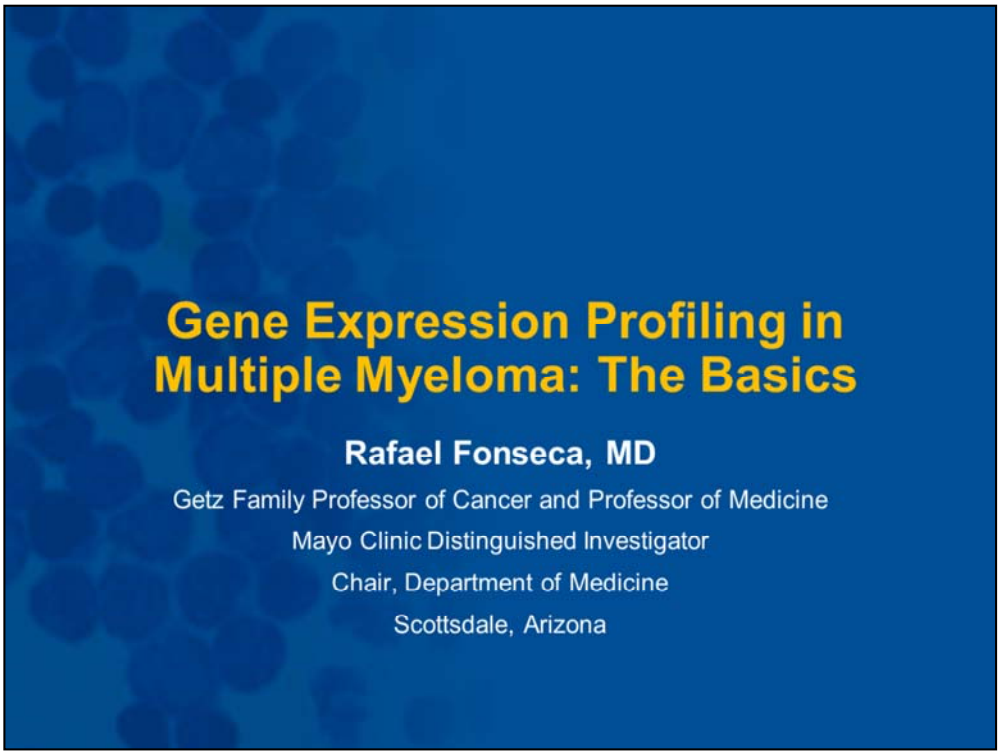
Gene Expression Profiling in Multiple Myeloma: The Basics



Welcome to *Managing Myeloma*. My name is Rafael Fonseca and I am the Chair of the Department of Medicine at Mayo Clinic in Arizona. Today, I will be discussing with you gene expression profiling in myeloma: the basics. This is the first of a series of two products where we are going to talk about the basic genetics of myeloma and the application of gene expression profiling, both to understand the disease as well as for prognostication purposes.

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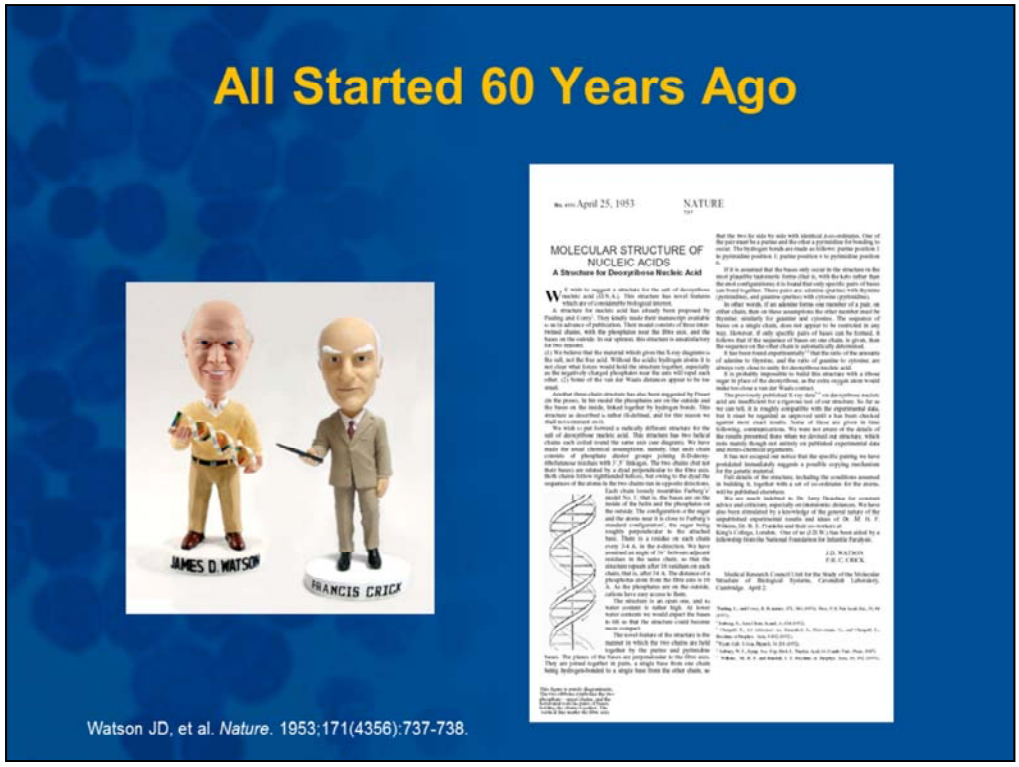

As you all know, it all started 60 years ago with the discovery of DNA, and there is no doubt we have seen a tremendous revolution in our understanding of cancer. Throughout the years, a lot of attention has been paid to the knowledge of DNA-based defects, primarily, of course through the original discoveries of chromosomal abnormalities as hallmarks for the various cancers that affect humans.

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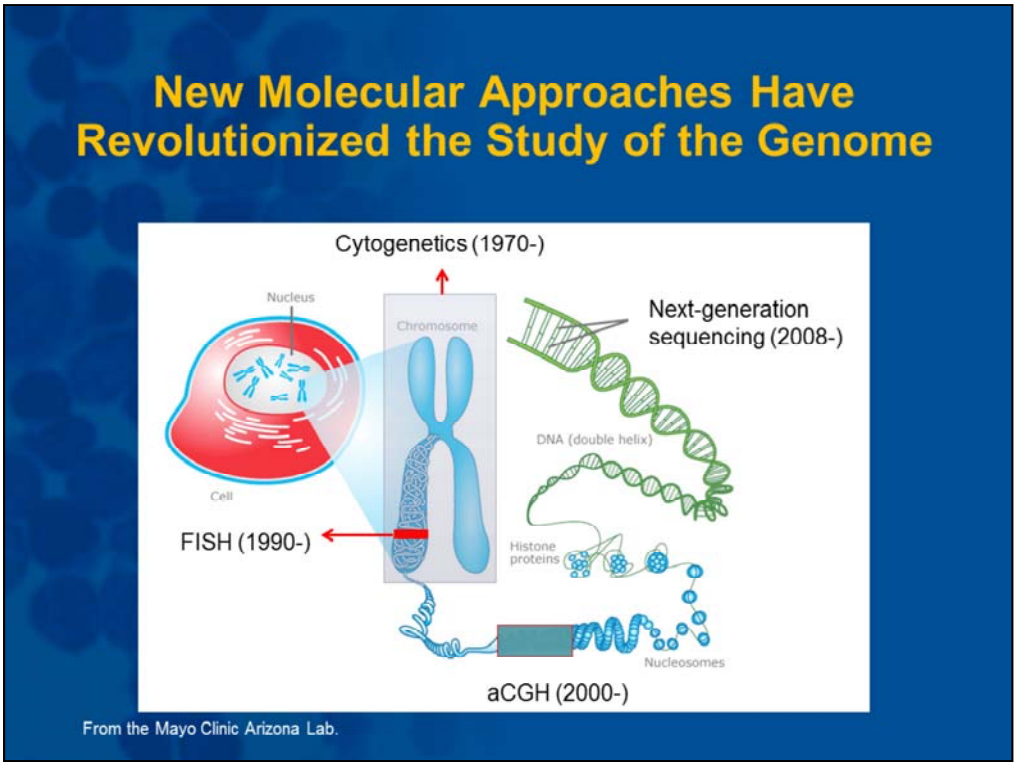

But obviously, that changed quite rapidly, we started by doing chromosome analysis. This was done for the detection of the Philadelphia chromosome, and subsequent to that molecular genetic approaches that could detect mutations or other translocations like in the follicular lymphoma. In the 2000s, we had the advent of array-based aCGH, where we could compare DNA content for tumor types and compare and contrast that of course to the normal genome, and ultimately that led to FISH. But in parallel during this last decade, we had the development of tools that would allow for the rapid, precise, and comprehensive understanding of the expression of genes, so for the study of the RNA.

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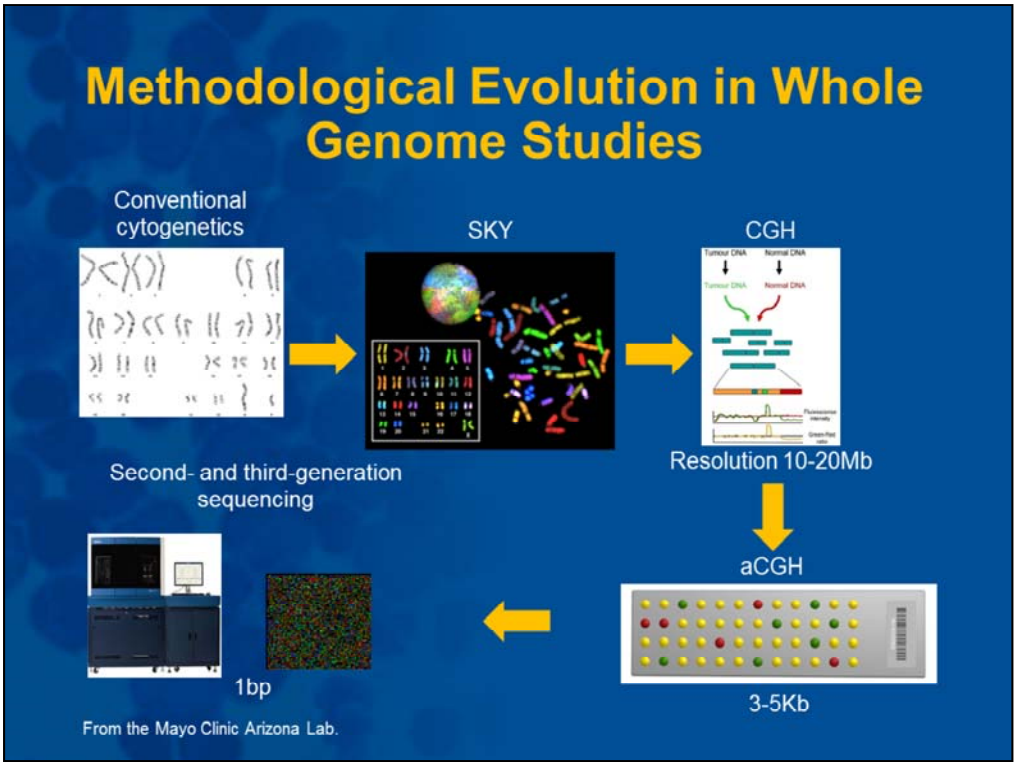

Now some of this, as I mentioned, has been applicable to the clinic. Any hematologist and oncologist is accustomed to the old reports of conventional cytogenetics. That led to SKY, which really did not take off as much as a clinical test. CGH did take off, particularly for some inborn genetic disorders, but perhaps the one that has taken off the most is gene expression profiling, particularly because of its applicability to prognostication in cancer.

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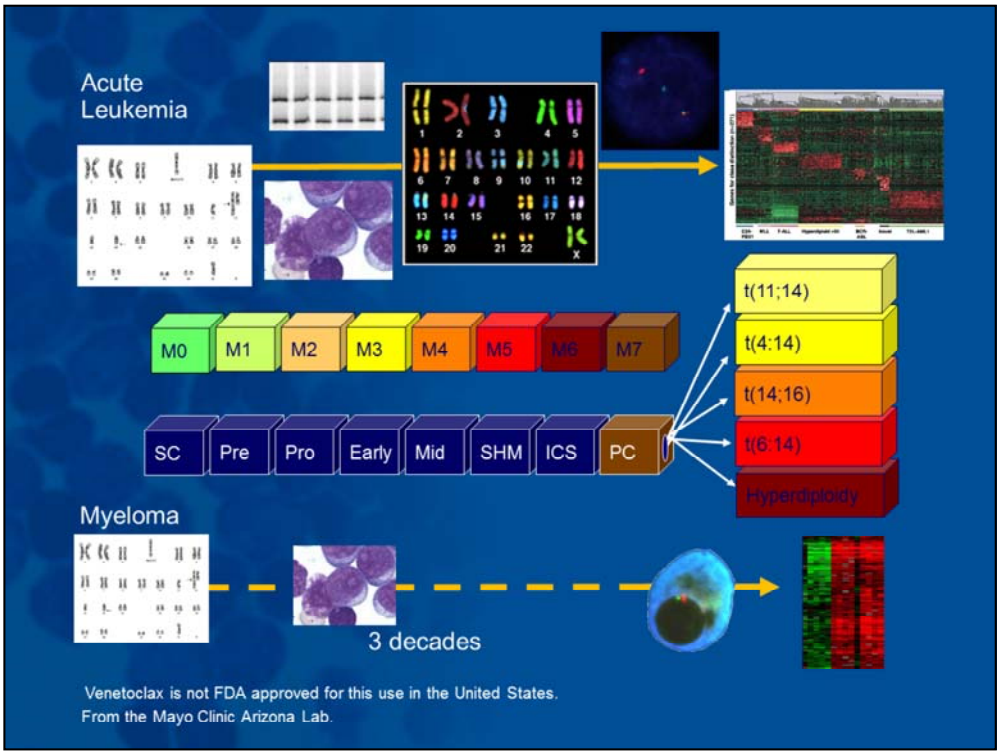

Today, I am going to talk to you about myeloma. This is a classic slide of mine that I have shown at multiple meetings where I compare myeloma to acute leukemia, whereas in acute leukemia the knowledge of the genetics allowed for the precise classification of the disease, and in fact some of the subsets are quite characterized by the specific genetic markers, but we were about three decades behind. The primary reason for this is that acute leukemia cells divide rapidly. So, those cells can give you chromosomes and those chromosomes are then converted into karyotype information, whereas in myeloma that was not possible. So, it is not really until the advent of FISH and subsequently gene expression profiling where we can actually correlate the clonal cells of myeloma for the specific genetic markers. In leukemia, I mentioned, for instance, the chromosome abnormalities could define something as clear as M3 leukemia. That not only has prognostic implications but also therapeutic implications with regard to the selection of therapy. Now in myeloma, the one difference is that we are just looking at the very last set of T-cell maturation, of course the plasma cells, but even within that plasma cell stage which, if you may, would be comparable to the end-stage of a myeloid differentiated cell, we still have genetic subtypes and we will cover this in much greater detail as we talk about the biology of the disease. Now, this is quite beyond just academic interest or knowledge, and we have a number of treatments that are being designed very specifically for some of these genetic markers. This is beyond the scope of this particular presentation, but I will tell you for instance the t(11;14) seems particularly susceptible to medications that affect the BCL-2 signaling pathway, something like venetoclax . So, more and more our ability to understand the disease and more importantly to test it at the bedside is transforming how we approach patients with multiple myeloma.

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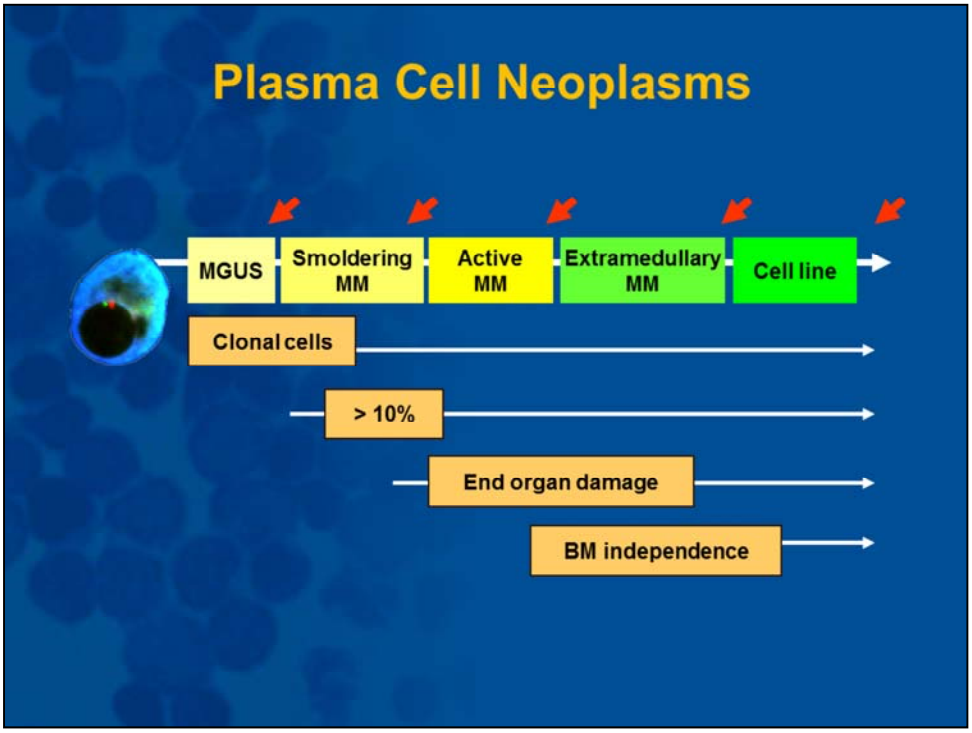

Now before I go into the details of the gene expression, some basic concepts regarding the disease. As you know, myeloma forms part of a spectrum of what we call the plasma cell neoplasms, and they start from the far left with monoclonal gammopathy progressed through smoldering and then active multiple myeloma. Now, with the advent of effective therapeutics, we have seen life span of patients being greatly prolonged. So, this next step of extramedullary myeloma is actually quite common, and as patients progress through these various stages, we have learned that they acquired certain genetic features that were present before. So for instance, we will see the primary genetic markers right from the get-go in MGUS, but we will not see the progression events until we start seeing patients with multiple myeloma, and certainly they will be highly enriched as we reach extramedullary disease. So, one example of this for instance could be p53 abnormalities which are quite common in extramedullary disease, or the definition of very high-risk profiles through gene expression profiling.

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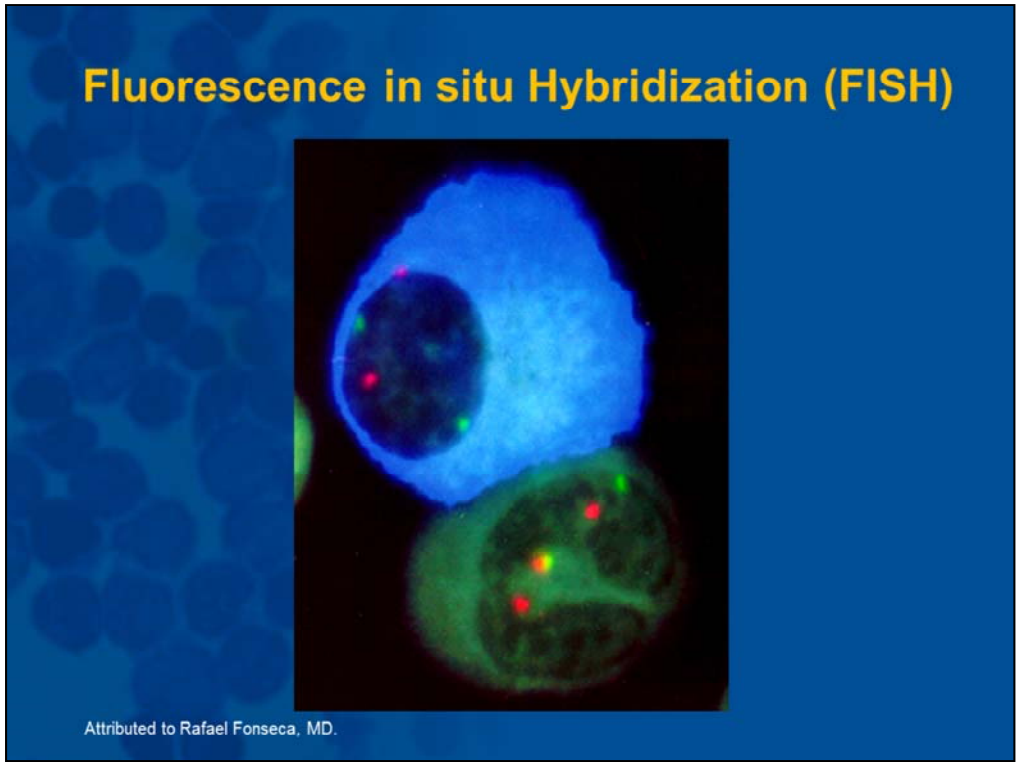

FISH is still one of the important tests that is done in the clinic. This is an example of FISH, and I should mention if one was to do FISH, and this will be relevant of course to gene expression profiling, we must concentrate on the clonal cells. What do I mean by this? Well, when we do FISH, sometimes we will get reports from laboratories that will do it without selecting the plasma cells, and then you do not know what you are scoring. You do not know if you are scoring myeloid cells or if you are scoring plasma cells. Here, I show you an image of a plasma cell that is depicted with a blue cytoplasm and that is because it was stained for the specific cytoplasmic immunoglobulin. Well, the same applies for gene expression profiling. Fortunately, the processes are in place such that when bone marrow samples are received at the testing laboratory they have to be enriched for the plasma cells. So, you get a gene expression profiling exclusively on the plasma cells.

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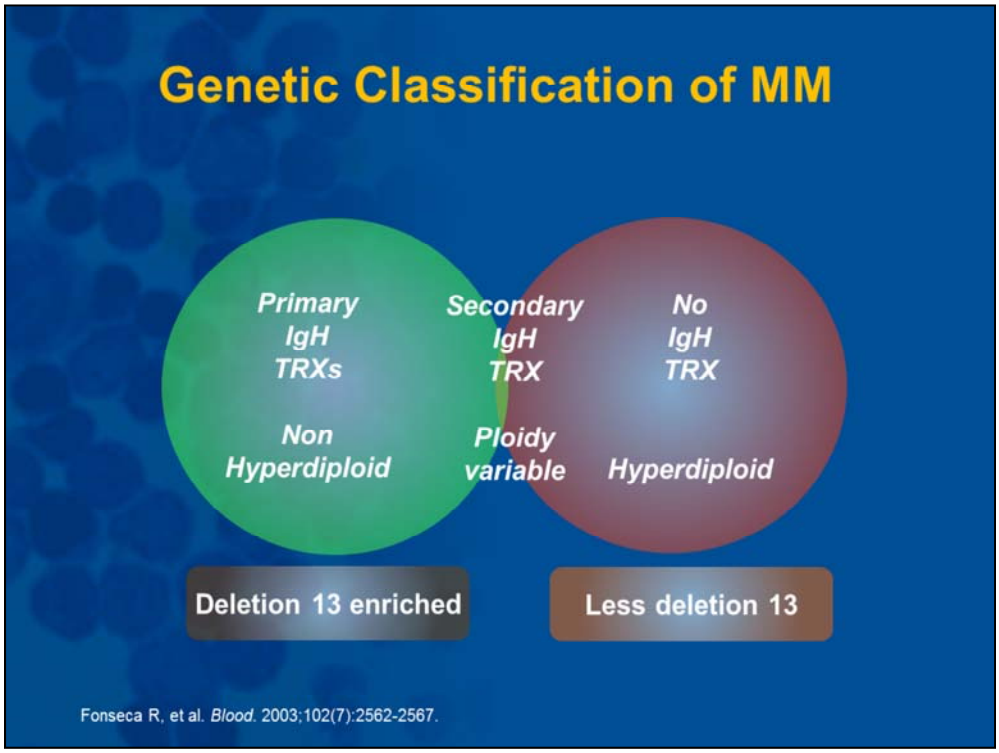

Some general comments about the genetics of myeloma as we dig into the basics with gene expression profiling. Now when you take myeloma from the very top level, there are essentially two major groups. There is what we call the hyperdiploid and the non-hyperdiploid. In the non-hyperdiploid, which is about roughly half and half, you will see patients who have the primary translocations, and those are primarily the 11;14, the 4;14, and the 14;16, and we will talk more about this in a subsequent slide. These translocations are in general associated with more aggressive forms of multiple myeloma, more rapid progression, enriched also for light chain only myeloma and non-secretory myeloma. This is in contrast with the hyperdiploid variant of myeloma that you see on the right. These are patients who have multiple trisomies and who in general have a better prognosis. This is sort of the textbook type of myeloma where you have an elderly male who has an IgG kappa myeloma, who has extensive bone disease but a bit of more of an indolent disease and of course there is a bit of overlap between both groups.

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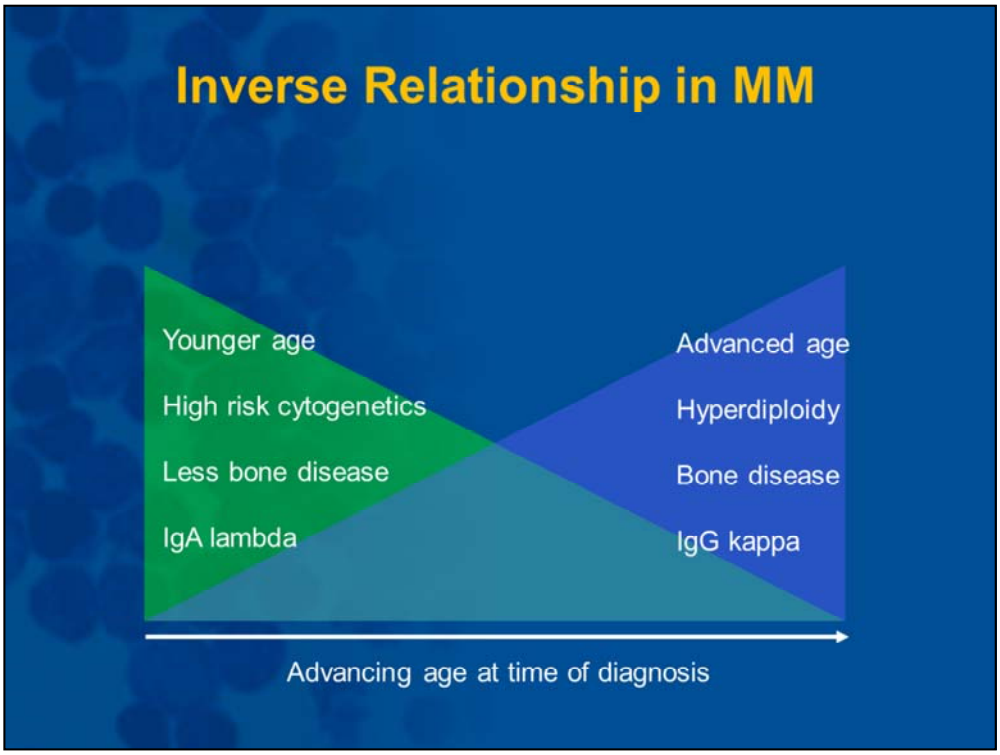

Now, this is important because this information can also be derived from gene expression profiling and what we know is when you look at patients with high-risk myeloma they tend to be younger. I mentioned they have enrichment for high-risk markers and certainly like 1q gains, deletion of 17, and they are also ironically less affected in the bone, so the prevalence of bone disease is lower in the younger patients with high-risk myeloma, whereas advanced age I mentioned previously is a classic sort of textbook type of myeloma.

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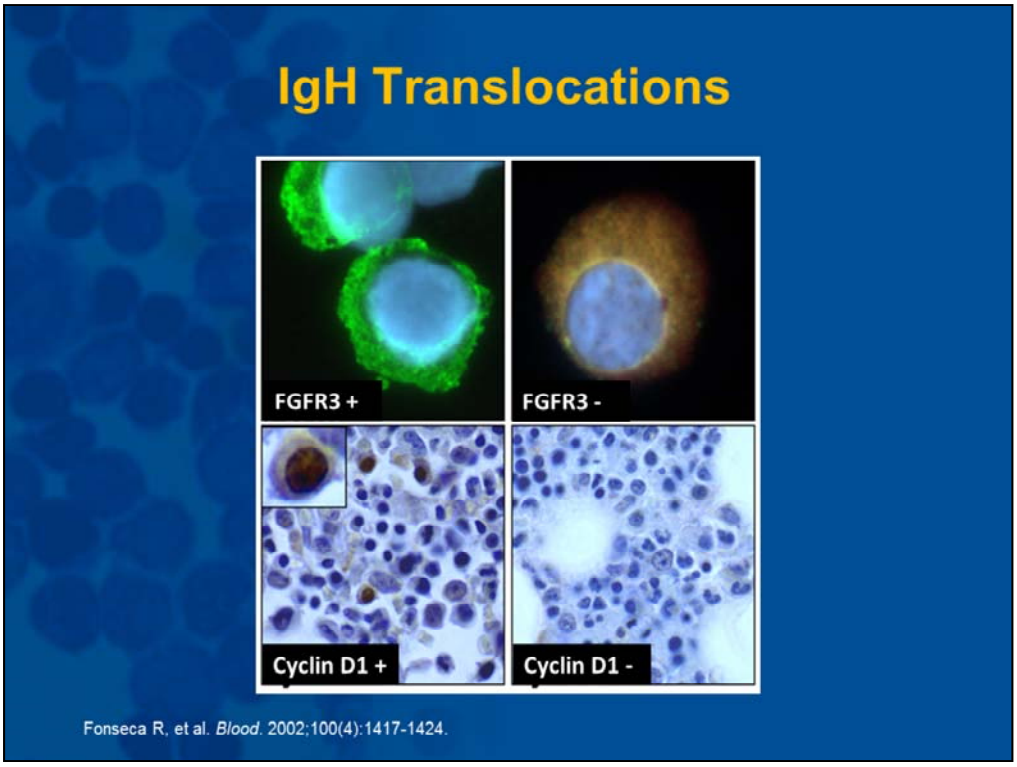

Now, I did mention these translocations are present from the get-go. This is a very important concept. So, a myeloma patient who has an 11;14 was, before, an MGUS with an 11;14. Parenthetically, I wanted to mention that we know that every single time a person is diagnosed with myeloma, we now know that they have the proceeding stage, they have the MGUS. How do we know this? Well, there is an interesting study with Army recruits where thousands of people had their serum collected in a yearly basis. Of this large group, 60 of them developed myeloma. They could go back and test and they all have the monoclonal protein. I show you here examples of bone marrow samples from patients with minimal plasmacytosis. For instance, the patient in the bottom panel that has 11;14 that expresses cyclin D1 and yet this patient can remain stable for a long period of time. It is important to remember that these translocations are persistent and they are present forever for the duration of the disease. So as much as we talk about tumor heterogeneity and intraclonal heterogeneity, the basic genetic subgroup stands, so if you have a myeloma with a 4;14, that means that the patient will always have a 4;14 and essentially all the cells will have a 4;14.

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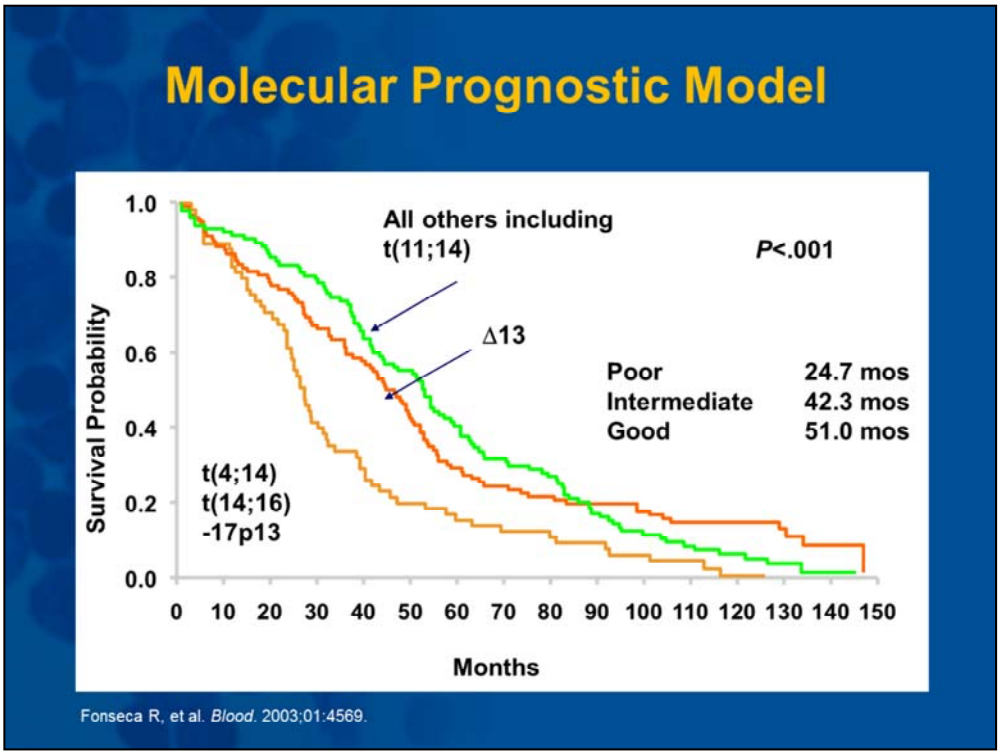

Now, these are older models that we have used before for prognostication, and I will contrast this of course with the power of gene expression profiling, but we and others have shown for instance that these three markers of 4;14, 14;16, and -17 persist as important markers for more adverse forms of multiple myeloma. Now the curve I am showing you was in the time when we were treating patients with melphalan-based combination, and of course, the treatments are so much better nowadays, and in fact we know that some of the subgroups derive benefit from some specific interventions. So, the 4;14 for instance does derive significant benefit from the addition of proteasome inhibitors, which has now of course translated into us understanding high-risk genetic markers by gene expression profiling should also be treated in similar ways even as data continues to emerge.

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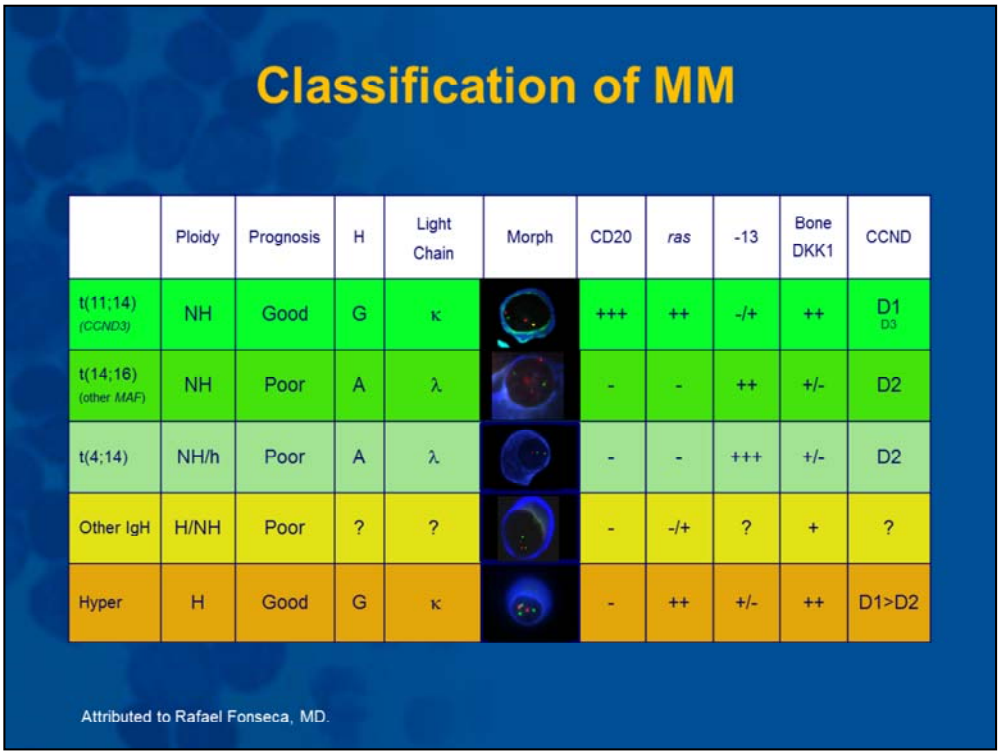

	Ploidy	Prognosis	H	Light Chain	Morph	CD20	*ras*	-13	Bone DKK1	CCND
t(11;14) *(CCND3)*	NH	Good	G	κ		+++	++	-/+	++	D1 D3
t(14;16) (other *MAF*)	NH	Poor	A	λ		-	-	++	+/-	D2
t(4;14)	NH/h	Poor	A	λ		-	-	+++	+/-	D2
Other IgH	H/NH	Poor	?	?		-	-/+	?	+	?
Hyper	H	Good	G	κ		-	++	+/-	++	D1>D2

I will not spend too much time on this, but just want to say again that these are kind of the major genetic subgroups and in particular we are going to pay attention to some of the adverse translocations of 4;14, 14;16, and I have lumped them now of course with high-risk genetic markers through gene expression profiling which traditionally we have tested with FISH, but we can enrich that information out through the advent of the microarrays.

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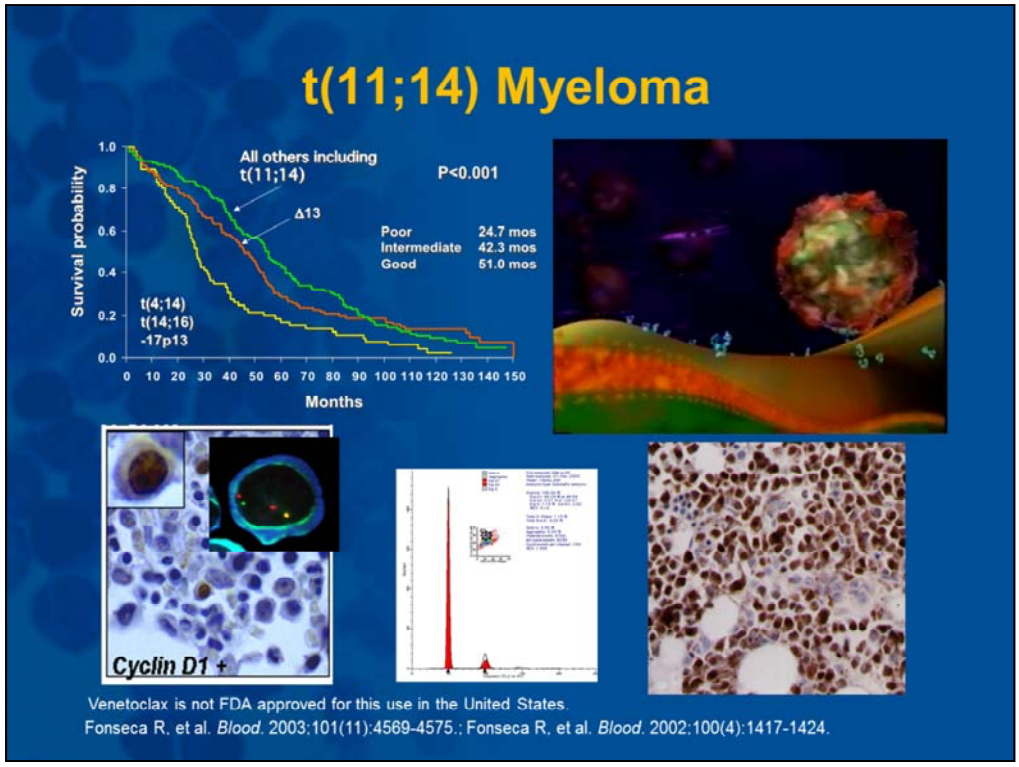

I did mention, for instance, 11;14 is kind of interesting back then when we started with prognosis. The 11;14 actually was considered a neutral to better outcome. It turns out the new treatments are actually not changing as much the prognosis for this patient. So, these prognostic factors are completely linked to the therapy that we provide. These patients expressed cyclin D1 but do not seem to respond as well to proteasome inhibitors or IMiDs, and therefore, new strategies are needed. And I previously mentioned, for instance, the possibility of doing that with something like venetoclax.

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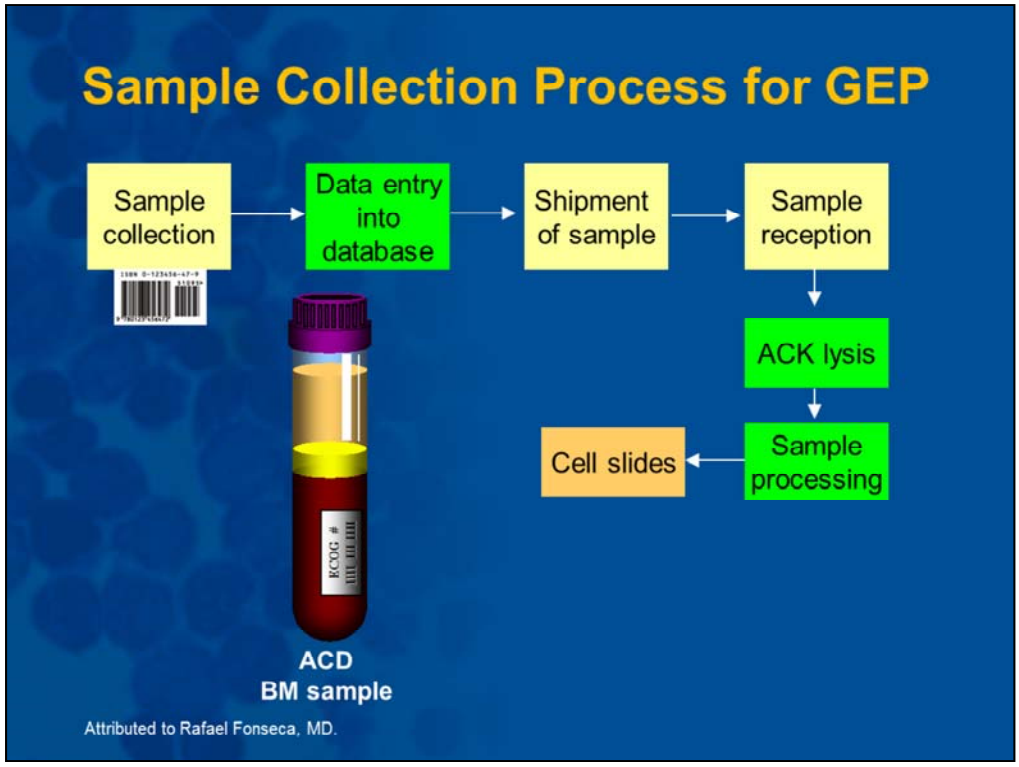

Now, when you do gene expression profiling, and we have done this in multiple realms both as clinical test as well as a research test, you get samples, you kind of enter the samples, and they are received in central laboratories, and it is very, very important that the samples are managed properly. Now fortunately, this has been all sorted out during the second part of the session. I will show you that in fact we have tested that the gene expression profiling can be adequately done with samples that are shipped overnight, but it is important that you go through all the steps of separating the proper cells so that again the gene expression profiling is done in highly enriched plasma cells.

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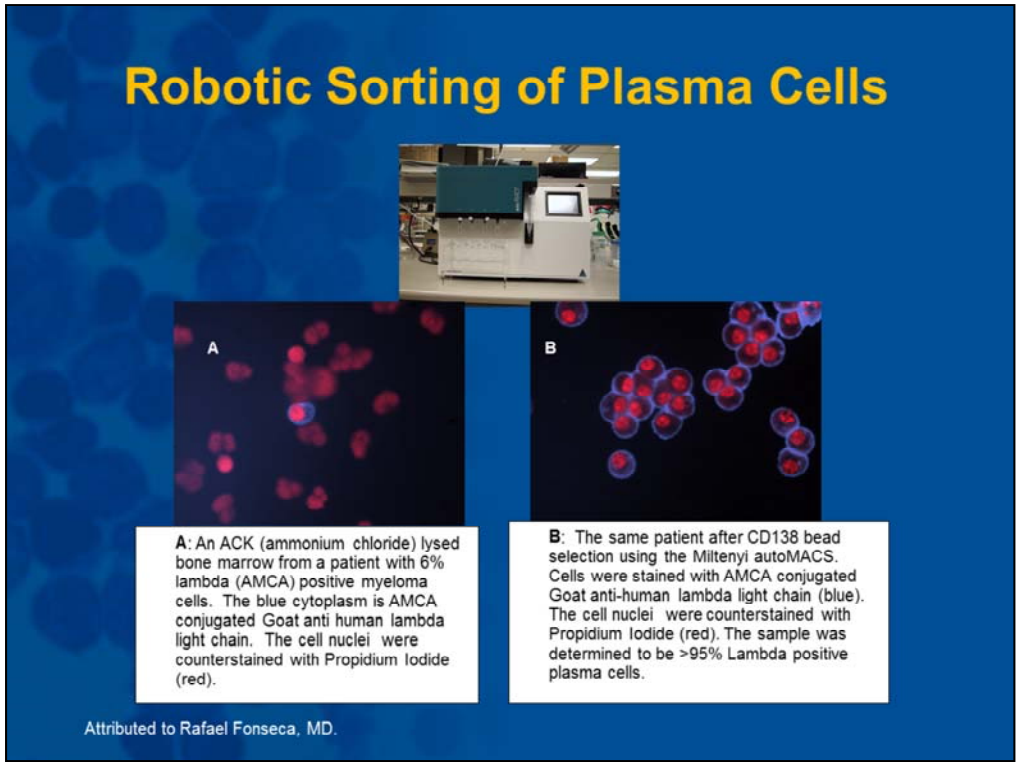

Now one way that one can test for this, and it is routinely done in laboratories that will do clinical testing like this, is that you can measure the percent of plasma cells before and after. This is an example of robotic sorting of plasma cells. On the left side, for instance, is a sample where you see one single lonely plasma cell with a blue cytoplasm, and now mind you, this is not unusual when we get a clinical sample and let's say the patient has 50% plasmacytosis, but you are getting actually second or third pull from the pathology team, there could be significant hemodilution. So, even in a patient with 30% plasma cells you can get a sample as you see on the left of panel A. One of the ways of doing this most efficiently is through use of sorting, and this could be robotic sorting using magnetic beads, and then you can have a sample that is as enriched as shown on the right side. And for all these genetic studies, you want to enrich as much as possible, close to 100%, to get accurate results.

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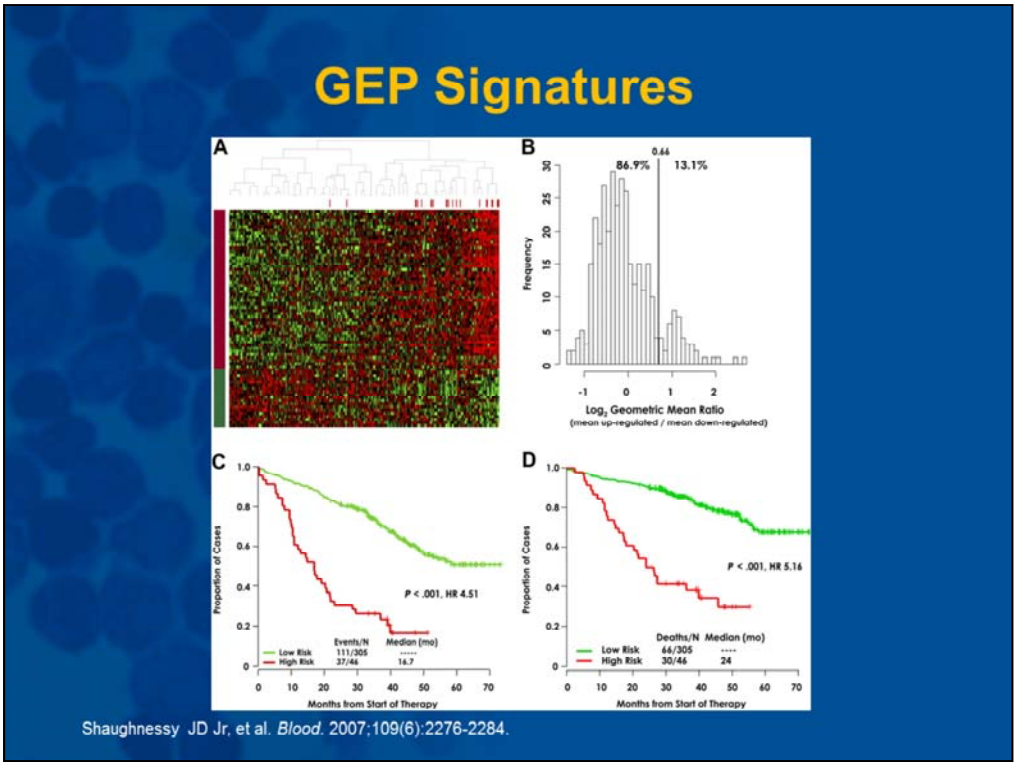

Now during the second part of the series, we will talk about prognosis, but we cannot help to mention that the seminal work done for understanding gene expression profiling signatures in myeloma came out of the University of Arkansas from the work of Dr. John Shaughnessy and colleagues. They were able to identify a subgroup of patients that would have an adverse outcome, even in the context of aggressive therapy such as could be the total therapy regimen provided at the University of Arkansas. Depending on how you establish those cutoffs, they identified approximately 15-20% of patients who would have a shorter survival, as is shown in the slides in the bottom, and again, we will delve into more detail as we talk about the prognostic implications. Now, if you are not familiar with this, let me just take a second to explain what is on the top left that we call the heat maps. So, the heat maps will show in each one of those little squares, each one of those pixels, the level of expression of different genes, and you can have levels that go from very low, that are shown in green, to levels that are highly expressed which come out in red, and that is why this is called a heat map. So, usually what you do is create a matrix where you align your patients. In this case for instance, each one of the columns is a patient and each one of the rows is a gene, and as you do that, you will find out that through computer algorithms you can start doing clustering analysis, which is really the primary tool that has been used for the analysis of gene expression profiling. So, you take all your RNA that you have extracted from the cells that run against the specific chip platforms that are available. You get a computer read-out that tells whether a gene is highly expressed or is low expressed, and that is from where you derive this heat map. So, you do that, you generate your clustering algorithms and you can start seeing subgroups, and in this particular case, one of those subgroups is represented by the red line associated with inferior outcome.

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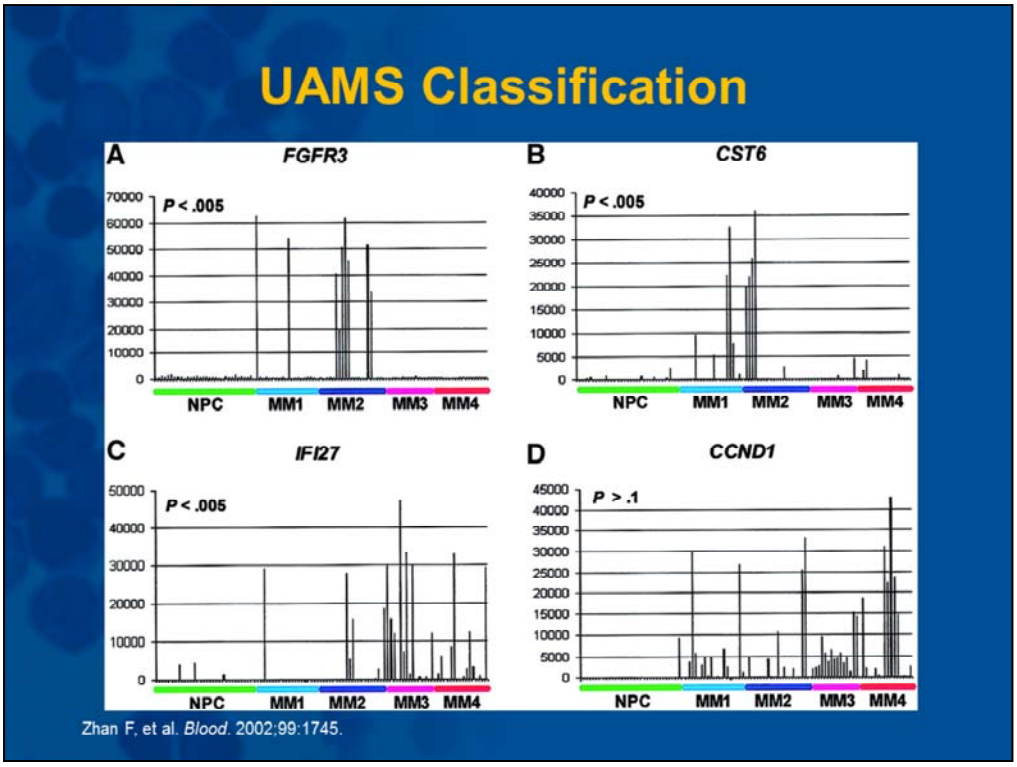

Now , one of the things that was noted out of this data set for instance was that there were some certain genes that were bright red, if you may. So, the genes that were expressed at a very, very high level. Let's take the top left one FGFR3. FGFR3 is a gene that is upregulated in myeloma cases with a t(4;14). So we knew that translocation was present. This is the translocation that has been previously discovered by Dr. Martha Casey, but now, gene expression profiling could very clearly identify that there was a high level of expression, a spike if you may, of the FGFR3 consistent with expression with this genetic deregulation. Similar findings are shown for instance in the bottom right with a high-level expression of cyclin D1 with t(11;14).

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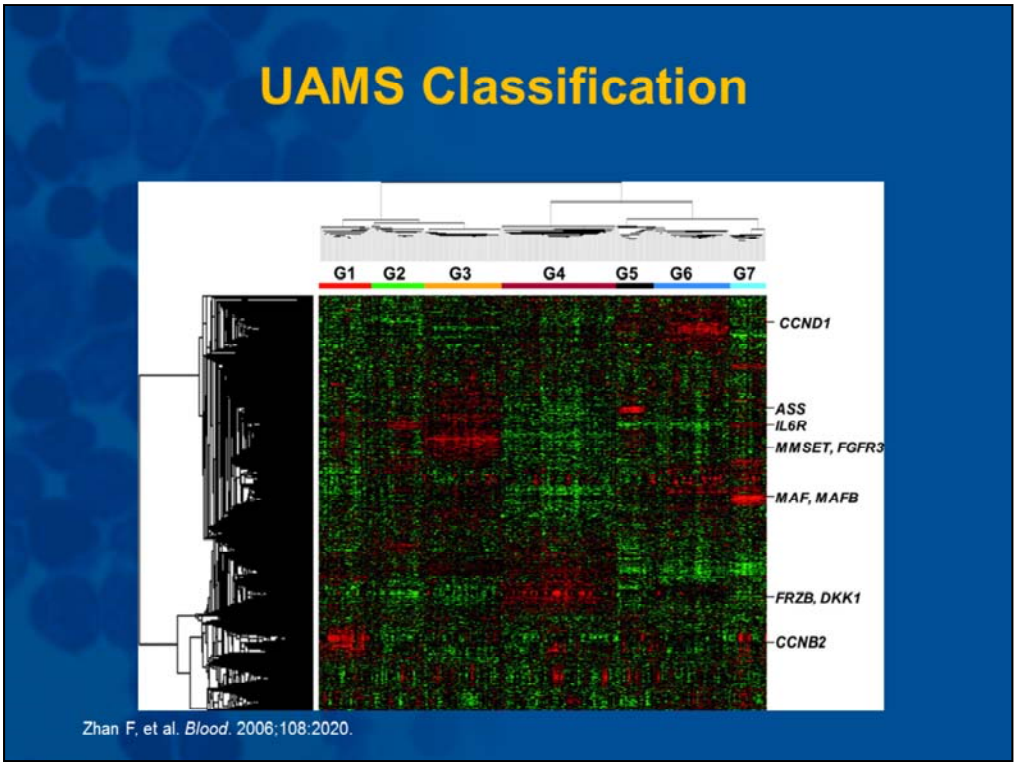

Now if you actually sort of just let things be and you start doing unsupervised or unbiased clustering analysis, you start seeing that some of these clusters are actually reminiscent of what have been seen previously with cytogenetics, and you start seeing subgrouping of patients based on the expression of some of those genes. Sometimes, because they are spiked at very high levels, sometimes because these genes dictate the series of downstream genes that are upregulated or downregulated associated with the specific genetic classification. Take for example the far right . Around the middle of the screen, you will see that there is a designation for MAF and MAFB. In this particular subset of patients, the last columns of this particular heat map talk to you about a patient that has a MAF translocation with very high level of expression of the MAF genes, again consistent with a knowledge that in myeloma, about 5% of patients will have a translocation t(14;16) which results in the overexpression of MAF genes. So again, everything is quite consistent in revealing the underlying biology of the disease.

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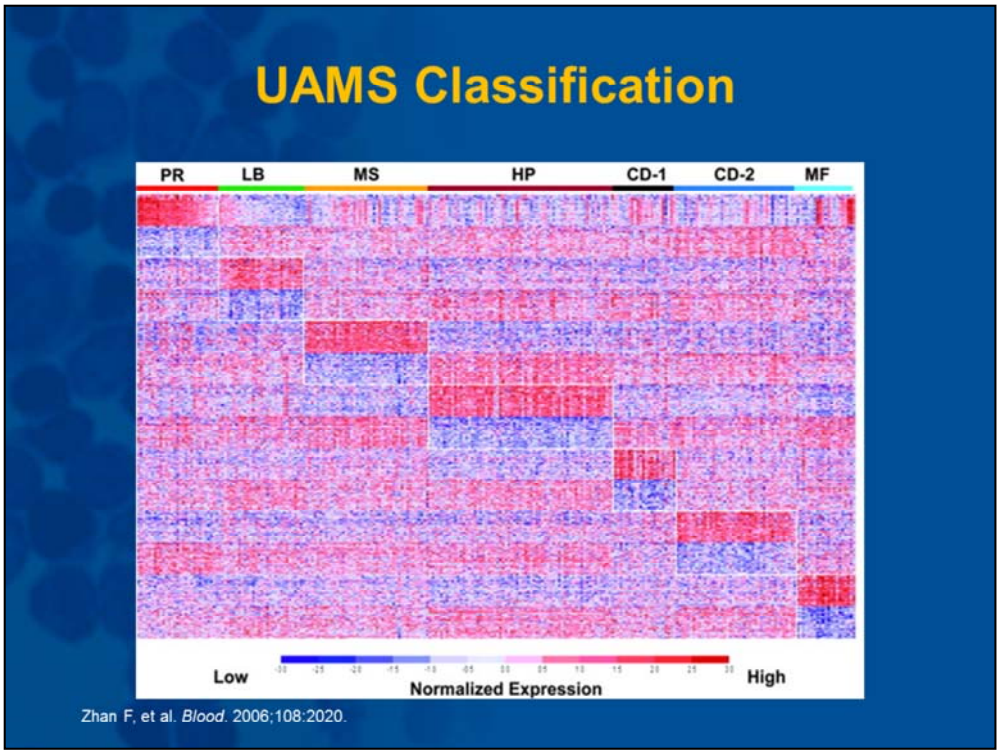

Now based on this information, and with additional clustering analysis, Dr. Zhan and colleagues from the group from the University of Arkansas was able to propose a classification of myeloma into the subset of patients. Patients on the far left, which are called proliferation, would be more aggressive form of multiple myeloma, a second group called low-bone disease, a third group associated with a t(4;14) translocation, a larger group of patients which is in the middle of the graph which is associated with hyperdiploidy, then the cyclin D1 overexpressed genes associated in particular with the translocation(11;14) and on the far right the MAF subgroup of the disease. So, in many ways, this was greatly gratifying because through novel tools through one test, they were able to identify all of the genetic markers that previously could only be described in great detail through FISH analysis.

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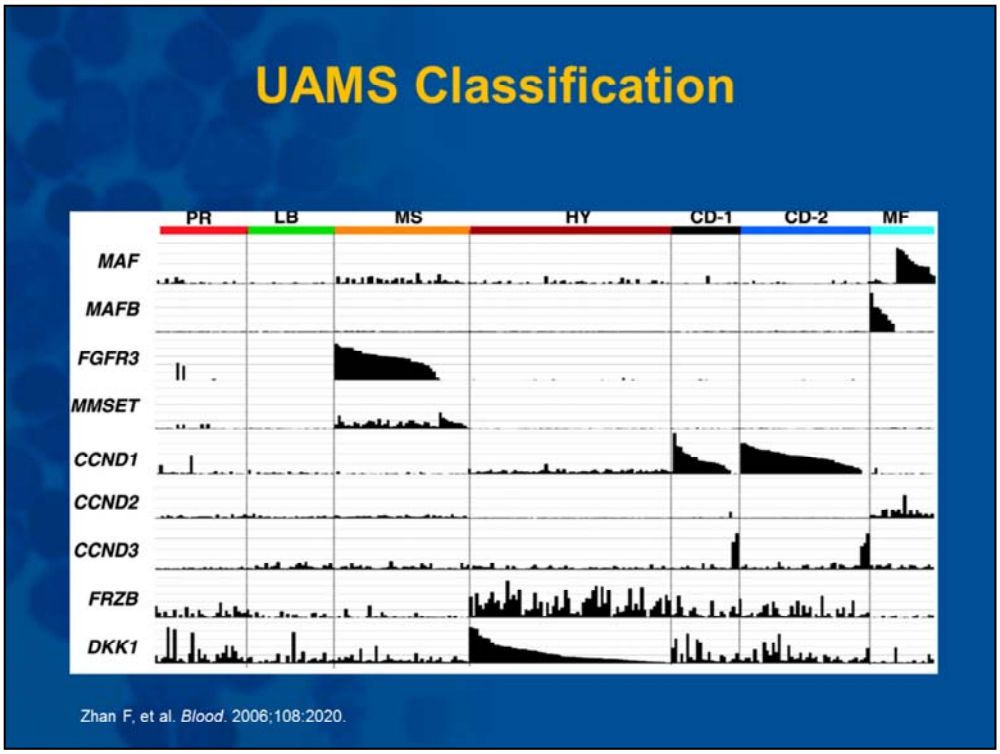

If you apply the same classification, then start looking at the spike genes, for instance, look at MS, MS I told you is a group that is highly enriched for the t(4;14). Most of them have an FGFR3 upregulation. So, you see right there in the middle of the screen an upregulation of the gene FGFR3. You will notice that not all patients have this upregulation or overexpression which is also consistent with what we knew from classic genetic studies. About 25% of patients with a t(4;14) will lose the allele that expresses FGFR3. You will notice that not all patients have this upregulation or overexpression which is also consistent with what we knew from classic genetic studies. About 25% of patients with the t(4;14) will lose the allele that expresses FGFR3, and therefore, those patients will only express MMSET as is shown in the graph below. Interestingly, you will see that cyclin D1 which is for instance in the middle of the screen toward the far right, is expressed in these groups that I call the CD1 and CD2 and they are expressed at different levels, but right in the middle, you will see to the left of it in the group called HY, which stands for hyperdiploid, a low level but increased expression of cyclin D1, which appears to be quite characteristic for this subset of the disease as well.

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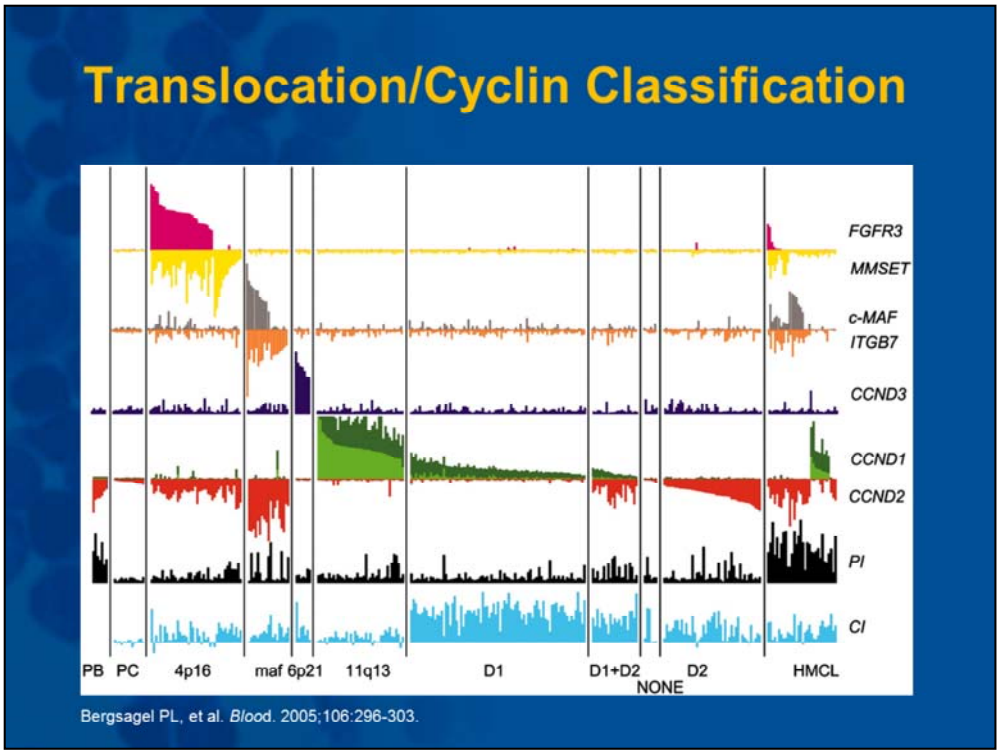
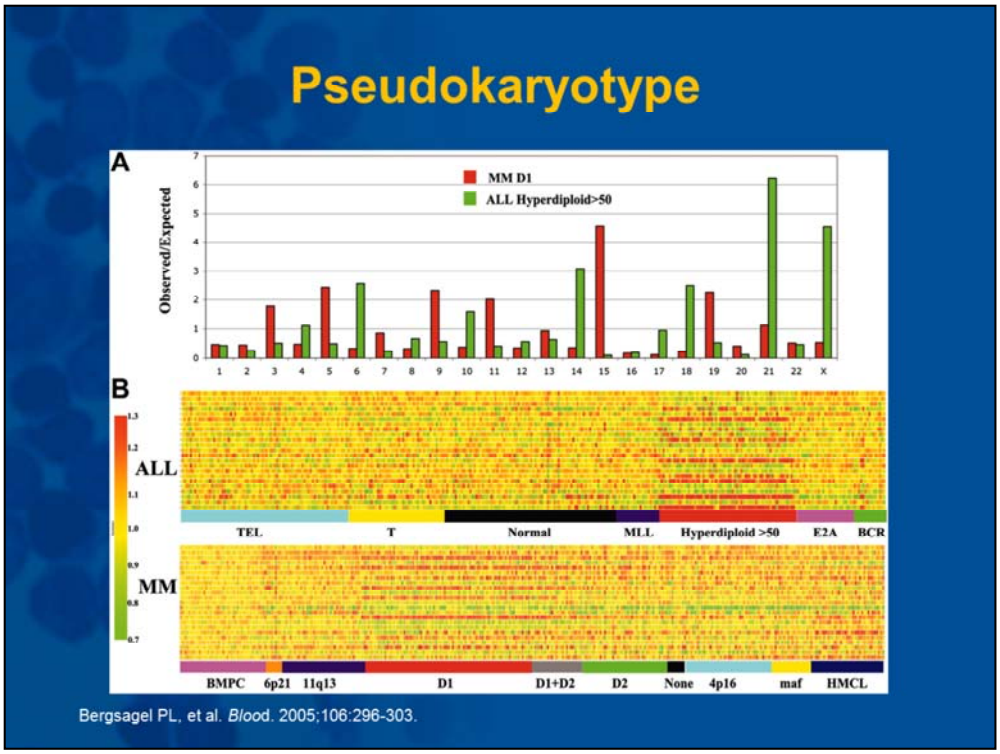


Now based on some of these data sets and given the previous knowledge that Dr. Bergsagel, Dr. Chesi, and Dr. Kuehl had generated towards the understanding of the basic biology of myeloma, they proposed an alternative classification that is called the TC classification. By now, you are familiar with a graph like this, and the translocation stands for T and C for cyclin, and what they proposed is that every subtype of multiple myeloma is characterized by the presence of either a translocation or upregulation of cyclin D1. Now if one starts looking at the cyclin D1 and D2 genes which are shown both in green and in red toward the bottom half of the slide and we can walk through the left. So, patients with t(4;14) have this increased spike of cyclin D2, and it is expressed going downwards, but that means gene is expressed. Next, you see MAF, which is also associated with a high level of cyclin D2. You obviously see the level 14 patients that have high levels of cyclin D1. I previously had mentioned that the hyperdiploid group also has slightly elevated D1. This is the group that they called D1, and this is a group which is a little bit ill defined that is called D1+D2 that expressed the cyclin D1 and D2, and then also this group that also expressed cyclin D2 alone. Now, there is a group right in the middle, a very small group that is marked as none, and these are patients that do not have D1 or D2 expressed and that was of course a puzzle. It turns out that some of these cases have biallelic deletion of the gene RB. So, we know now that all myelomas in one way or another are driven through signaling through the cyclin D pathway and RB dysregulation with that known group being mostly composed of patients with RB biallelic deletion.

Gene Expression Profiling in Multiple Myeloma: The Basics



Now, one of the interesting things is that you can do or you can get from the reports from gene expression profiling is the pseudokaryotype, and this is how this works. Let's say you take all the genes that are contained in a given chromosome, and then you see those levels are high or low, and you will see that if you take for instance genes that are located in a chromosome that is going to be trisomic, the levels are going to be slightly higher. So, indeed having an extra copy of a chromosome leads to extra levels of expression of genes. As an example, you see on the bottom part of the slide I compared ALL to myeloma. As you know, ALL one of the subtypes is characterized by hyperdiploidy. You see a number of streaks there of red towards the right side of the heat map that showed the presence of trisomy chromosomes. Now ironically, these are mostly the even numbered chromosomes. Now if you go down to myeloma, you will see a similar phenomenon, under the group named D1, shown with the red bar at the very bottom of the slide, you will see that there are these lanes that are bright red, and those are the trisomy chromosomes in myeloma which I said ironically before and it is because they are the odd numbered chromosomes. So almost all odd numbered chromosomes can be expressed in myeloma, the most common one being chromosome 15, which is shown by that almost solid red line. Now, if you are paying attention to this, you will see that on top of 15 there appears to be a little green streak, that is chromosome 13. So, chromosome 13 is almost never trisomic in myeloma, less than 1% of cases, and that probably just speaks to this notion that you do not want to have an extra copy of chromosome 13 if in fact you are a myeloma clone.

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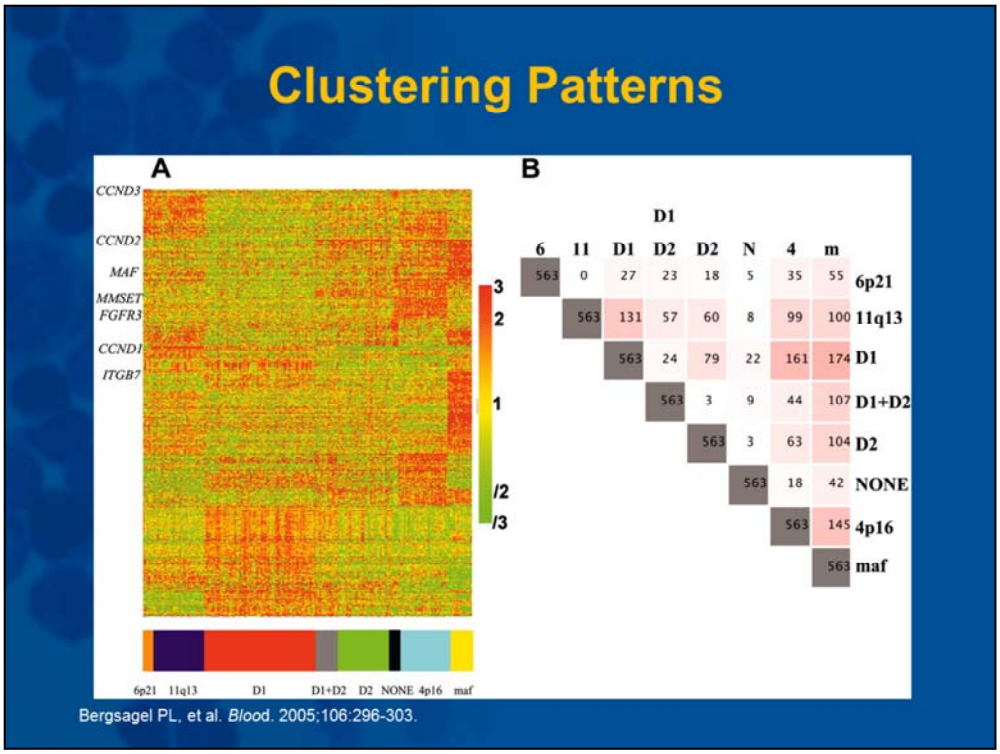

Now, Dr. Bergsagel took this further and there are some clustering patterns, and of course, you can see that you can start contrasting, and at the end of the day this classification, whether it is a TC or the UMS, actually correlate quite well with each other.

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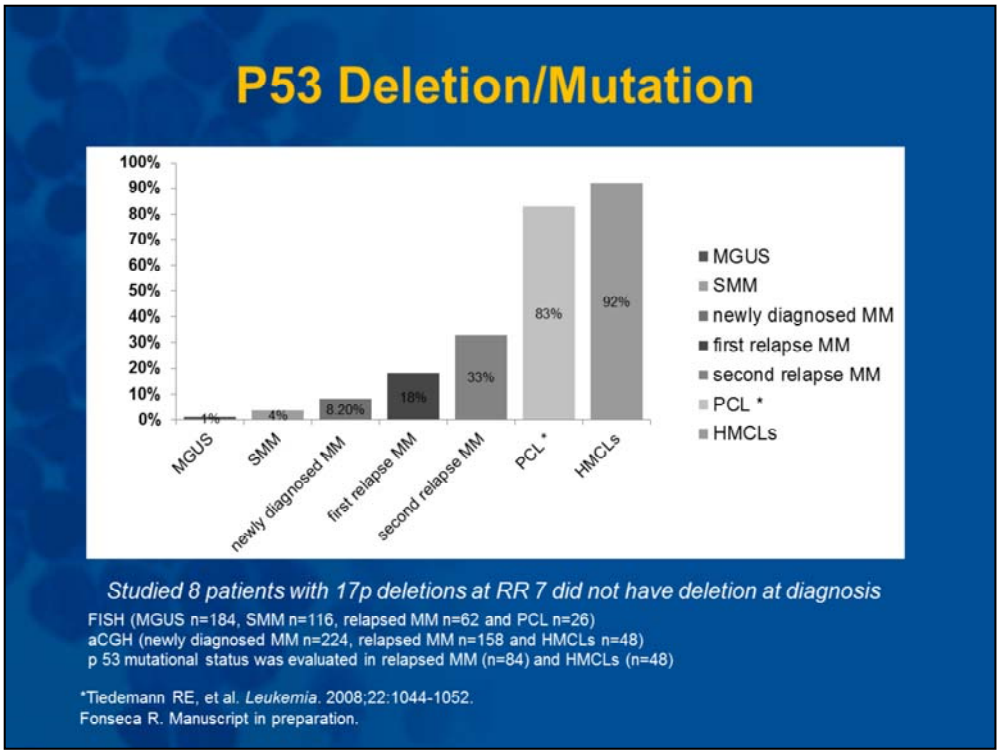

One of the things that is still being explored is incompletely resolved is that in myeloma like is true for many other B cell malignancies, p53 deletions are important, and they dictate more aggressive biology. So for instance, this is data we have compiled over the years in our laboratory and we will see that p53 presence increases over time. Now, p53 is still considered an important marker in the clinic. Arguably, it can be supplemented or replaced by gene expression profiling; however, this is one particular genetic abnormality that we cannot fully predict just based on gene expression alone.

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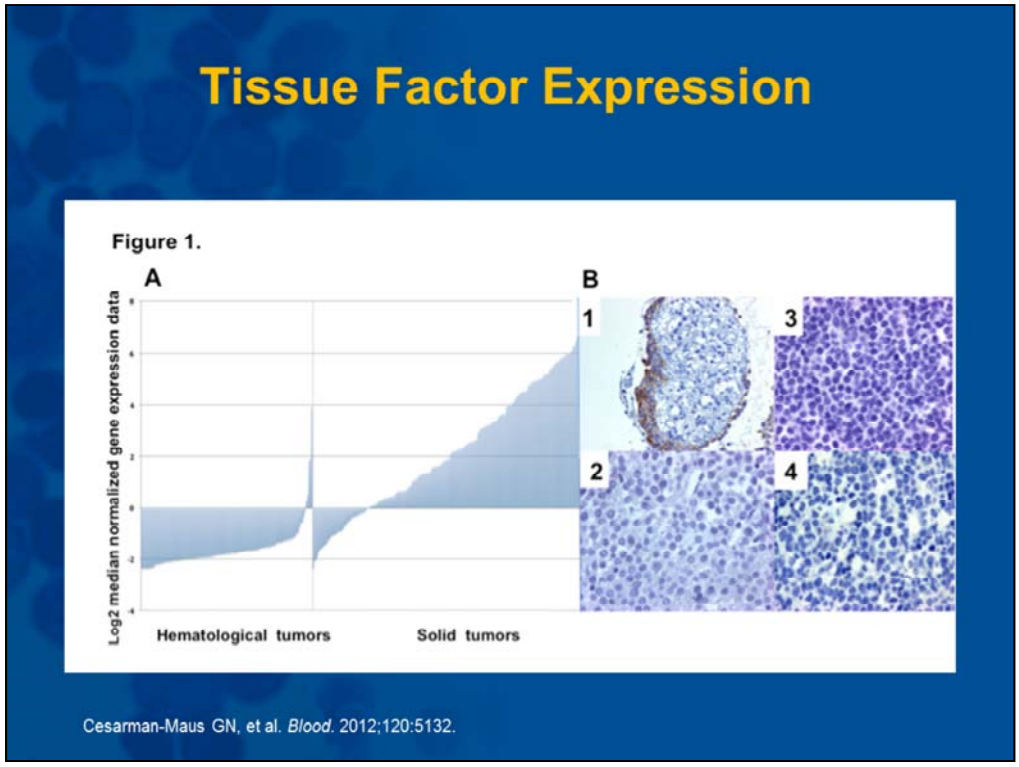

Now, let me give you another example of how you can use gene expression profiling and this is more in the research realm, but you can look for specific genes going back to these large datasets that exist for the disease. So, this is one study that I participated on where we looked at the expression of tissue factor in myeloma. It turns out that tissue factor is associated with cancer and thrombosis, and we were curious to see if heme malignancies express tissue factors as much as solid tumors. That is why solid tumors are associated with such propensity for thrombosis, and we went back to gene expression profiling, and within 5 minutes we can see a graph like this. Solid tumors highly express tissue factors, heme malignancies do not. Of course, a full explanation for this is beyond the scope of this talk, but it just shows you the power that exists with the databases and the ability to create gene expression for myeloma and certainly for any other tumor.

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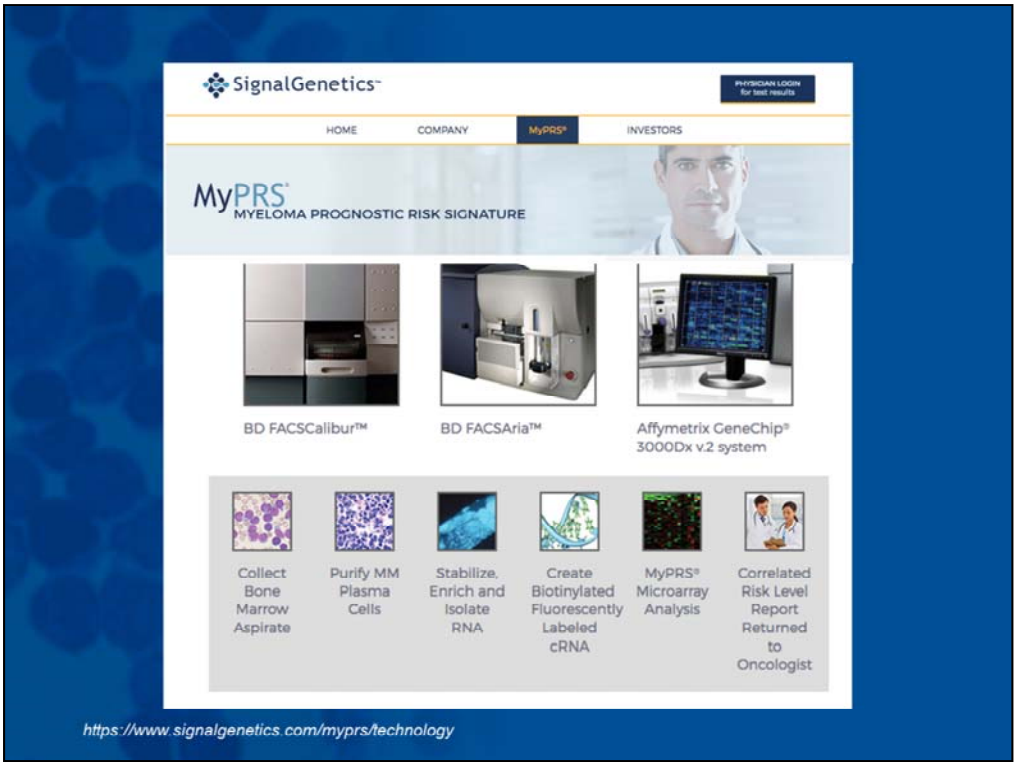

Now, we actually have a commercial test that is available for testing gene expression profiling for myeloma. I will show you a sample report during the second part of my talk. This company is called Signal Genetics. So, you actually send your samples to a central laboratory and you will get back not only the prognostic index but you will get the classification of the disease and the subtype of the, and that allows us again to better understand the biology of the specific patient being cared for.

So I would like to thank you for viewing this activity and invite you to join us for the second part of this talk where we talk about prognosis, and for additional resources, please view the other educational activities on *ManagingMyeloma.com*. Thank you very much.