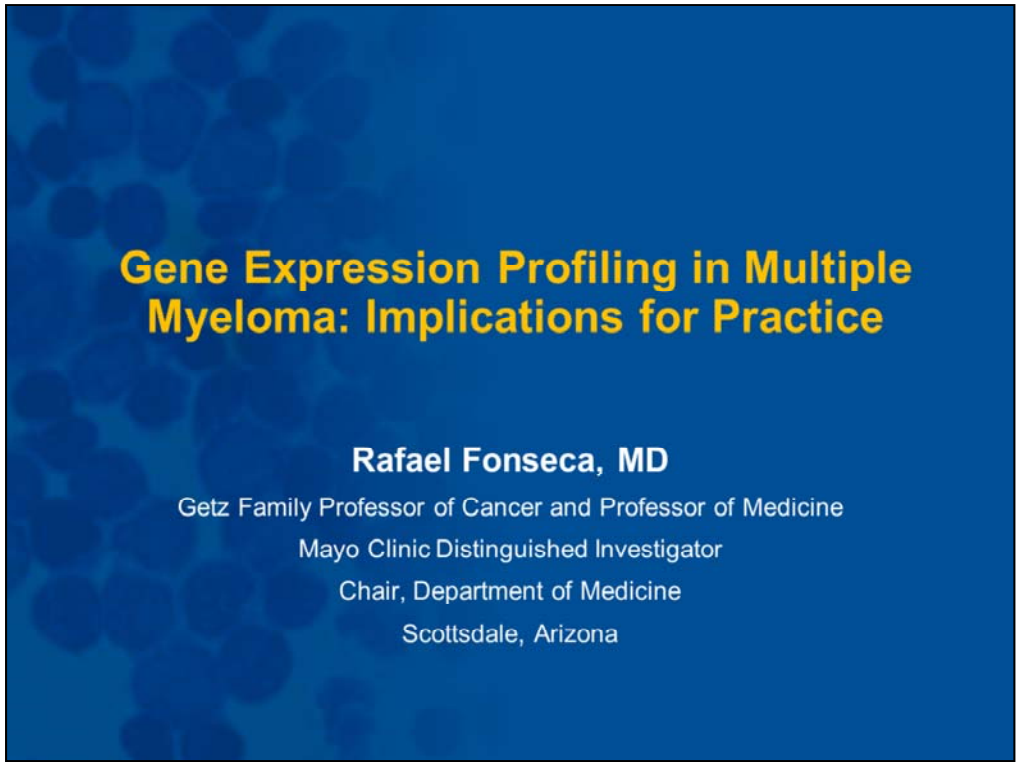
Gene Expression Profiling in Multiple Myeloma: Implications for Practice



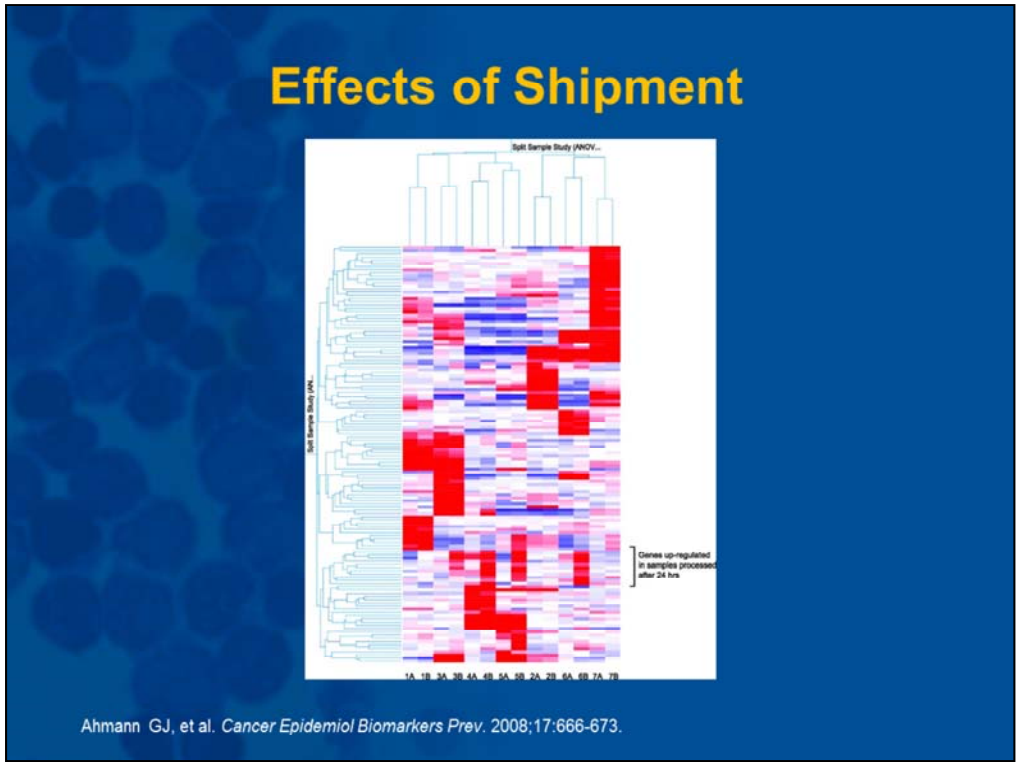
Welcome to *Managing Myeloma*. My name is Rafael Fonseca, and I am the Chair of the Department of Medicine at the Mayo Clinic in Arizona. This is part 2 of the series where we are discussing gene expression profiling for multiple myeloma. In this particular session, we will talk about the implications for the practice and prognosis.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice

Gene expression profiling has worked its way to be considered part of the standard workup for myeloma patients. In fact, the International Myeloma Working Group has recognized that for the risk stratification of the disease, one could employ a gene expression profiling as one of the ways to ascertain the prognosis for patients. This is a paper by Dr. Chng from 2014 that really encompasses the opinion of international experts in multiple myeloma.

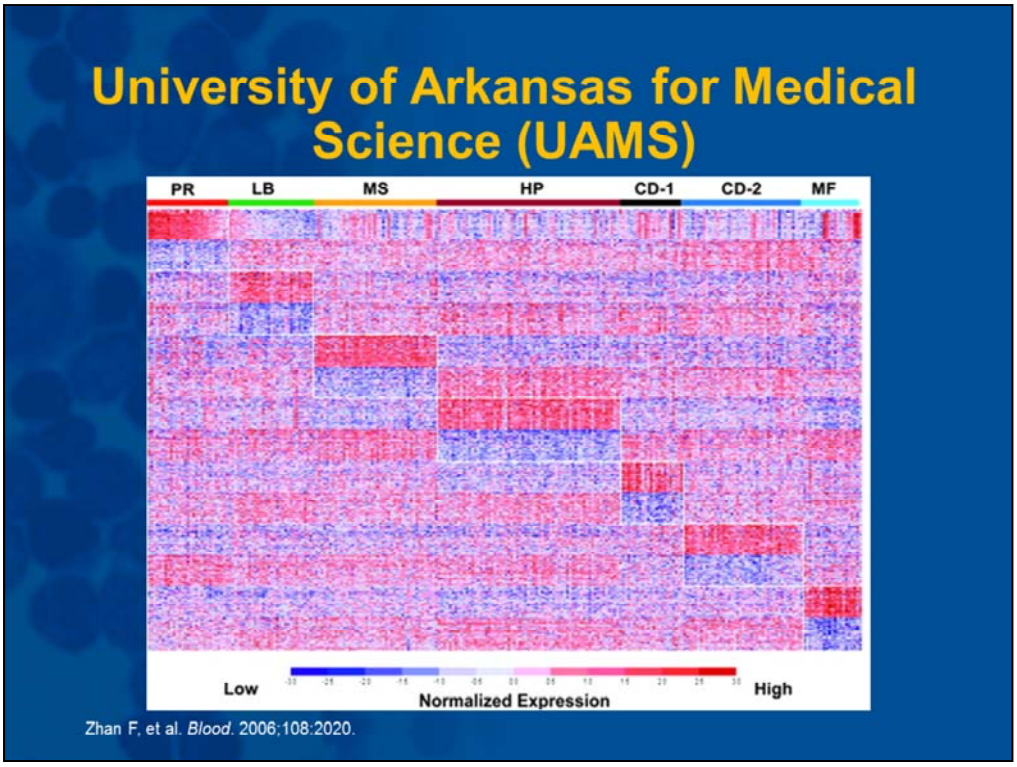
Gene Expression Profiling in Multiple Myeloma: Implications for Practice

Not only that, but actually the same group takes this further by looking at treatment recommendations based on the risk factors for patients. And even though this particular title talks about risk cytogenetics, there is really the full integration now that gene expression profiling is one of the ways to best understand the biology of the unique patient.



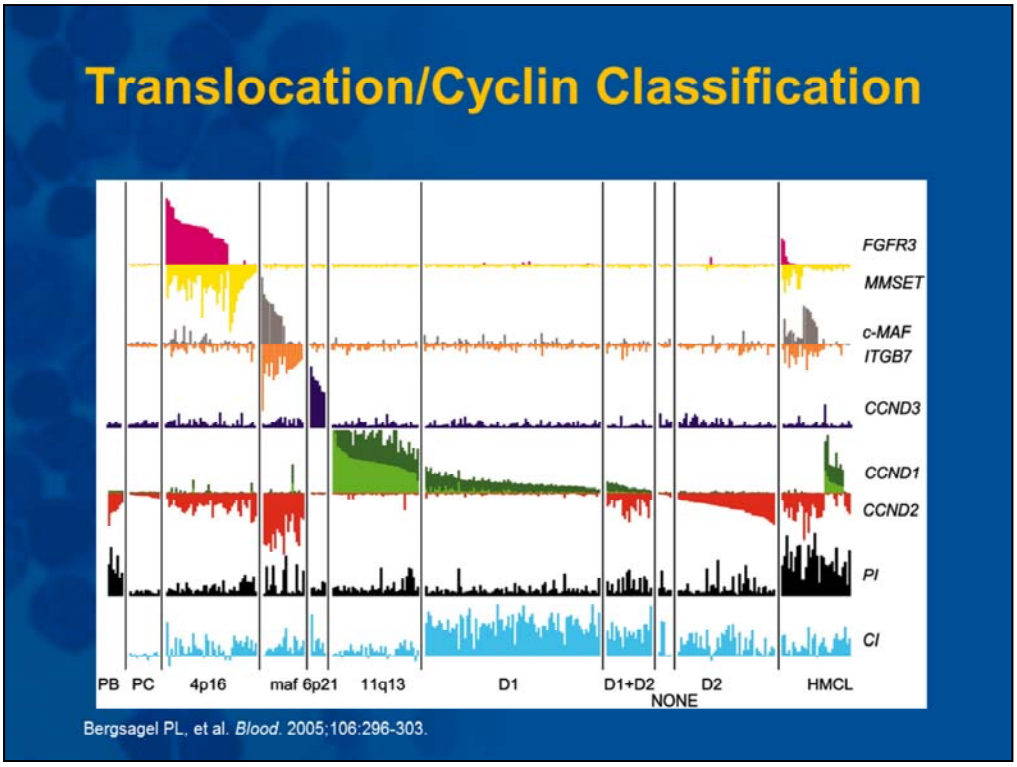
Before we go on to the prognostic implications, I would like to address something that is a common question. Since you have the ability now to send samples for gene expression profiling testing, in the past people were concerned how does that affect the level of expression of your genes and will you get a signature that is truly representative of your patients? This is a study we published now years back where we actually shipped samples to ourselves. So, in collaboration with our colleagues at Mayo Clinic in Rochester, we had samples extracted and tested right away, and then we had samples shipped to Arizona, and then we tested them. What I am showing you here through the dendrogram on the top is that the samples that were shipped and were tested immediately always clustered with each other. So, at the very top, you see those lines that connect samples and each one of those rectangles represents a sample tested locally and shipped, and as you can see not only they cluster with each other but the results are actually identical. So, this gave us reassurance and was published through a peer-reviewed publication, that in fact, the shipment could be done and you could still get accurate results for patients who are undergoing gene expression profiling.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice

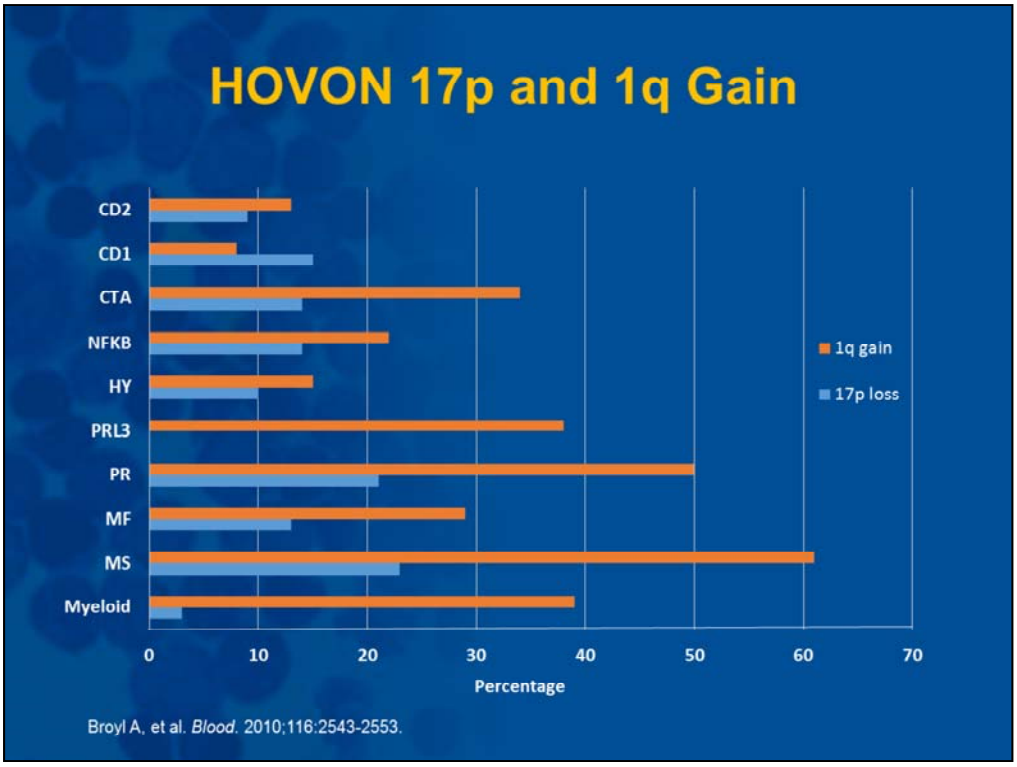


During session 1, I talked about some of the classifications, but I would like to briefly remind the audience that there are several ways in which through clustering algorithms we can start looking at the classification of myeloma. This is the University of Arkansas classification which is work that was led by the group of Dr. Shaughnessy, Dr. Barlogie, and Dr. Zhan where several subgroups of the disease are identified. In particular, for this part of the session, I would like to call your attention to the far left to the proliferative group. This is a group where genes that are associated with cell proliferation and mitosis are upregulated or overexpressed and will be enriched as we talk about high-risk genetic subtypes of multiple myeloma.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice

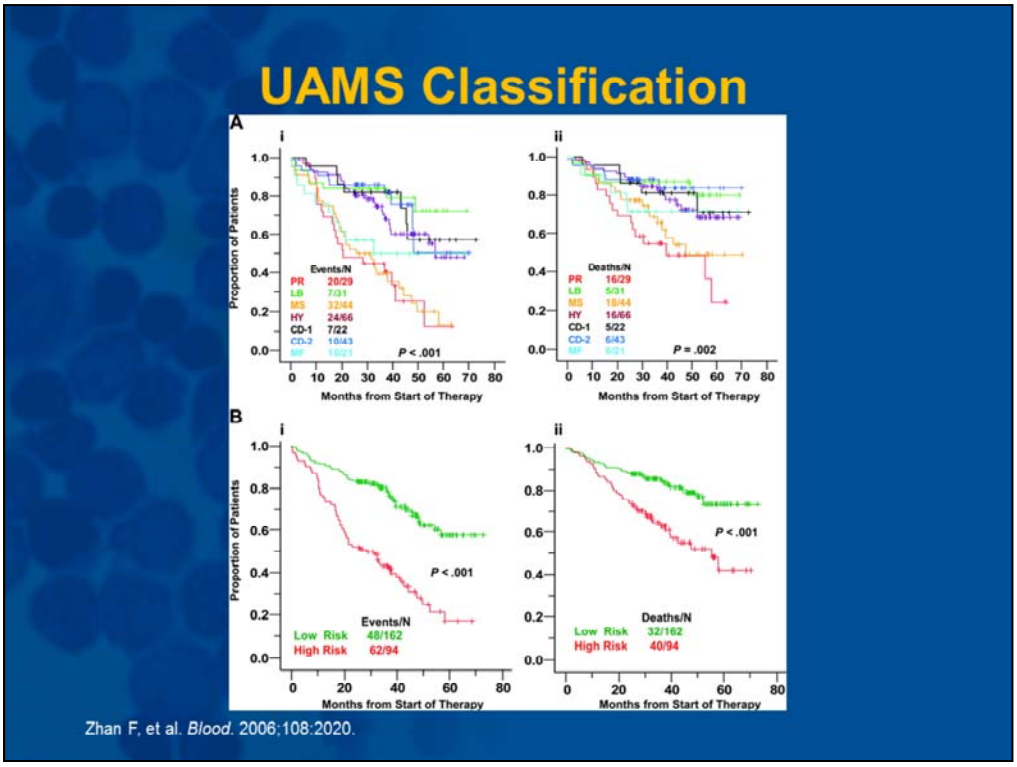


There is another classification that is translocation/cyclin (TC) classification that was proposed by Dr. Bergsagel and Dr. Kuehl. This classification looks at level of expression of certain genes, genes that are “spiked” in association with these translocations that also identified patients with high-risk disease. Now, these two classifications are a bit more biologic, meaning they describe what we know happens in the clinic. Sure enough, we know that patients with a 4;14 for instance tend to have more aggressive myeloma. But the current use of prognosis tries to be agnostic to this biology and rather tries to look for the features – whether you are 4;14, 14;16, 11;14, or hyperdiploidy – that would identify myeloma cases that would show more aggressiveness.



Now, there are several groups that have looked at classifications like this and tried to correlate how they correlate with some of the known prognostic factors. So, this is data from Dr. Broyl from the Dutch group where she looks at the subtypes of the disease, cyclin D1 and D2 from the 11;14, NF-kappa B expression, and hyperdiploid, and what you can start seeing here is that those subgroups that have more aggressive disease tend to be enriched for chromosome 17 deletion and chromosome 1q gains, markers that are associated with more aggressive myeloma through traditional genetic testing. However, it should be noted that the power to discern a very aggressive myeloma is increased through the testing of gene expression profiling of these clonal plasma cells.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice



Now, some of the earlier studies from the University of Arkansas started to look at classic classifiers, so that is those by translocations, and then contrast that with a selection of genetic signatures that will look specifically at patterns that would be associated with an adverse outcome. So, you see in the top part of this graph of course the classification of the disease recapitulates some of what we knew through FISH analysis and through standard genetic analysis, but then, on the bottom part, you can see a much clearer separation with subgroups of patients defined by their profiling as having high-risk disease, and we will go into this into greater detail

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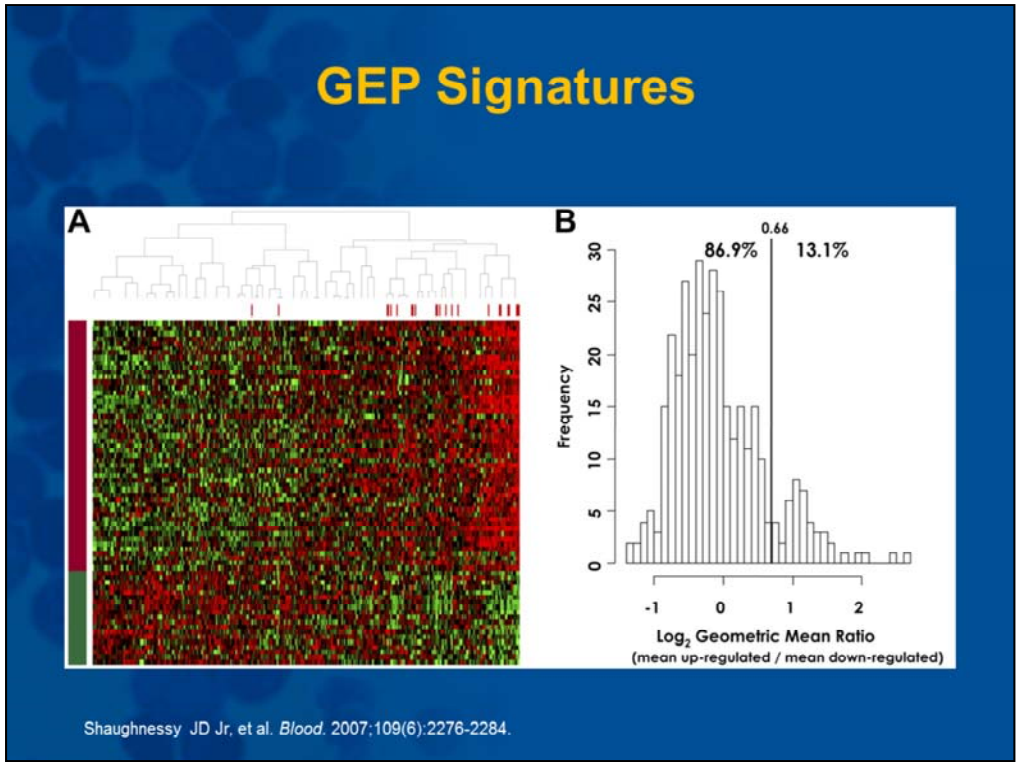

Now during session 1, I mentioned the importance of the clustering analysis. This is the classic picture of clustering analysis for identification of high-risk multiple myeloma. As a brief reminder, if you did not see session 1, we are looking at a heat map on the left and the heat map identifies genes that are upregulated or downregulated. Those that are upregulated are shown in red and the bottom part of the graph that is marked by this green bar on the left identifies patients who have high-risk disease, and those are patients who have genes that are associated with a more aggressive form of the disease. And it is because of this clustering that you can stratify patients and you can query whether this is associated with a more aggressive form of the disease.

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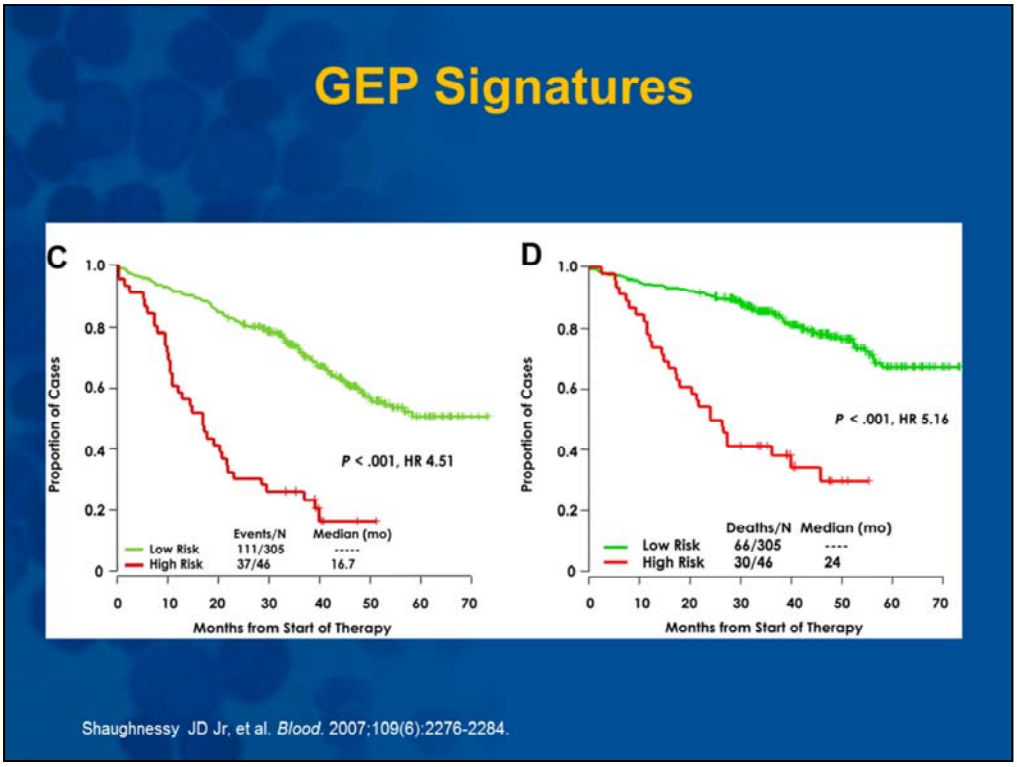

And this is what the group from Arkansas showed that in fact those patients who have a high-risk genetic profile will have a shorter survival. This is done both in a test set and evaluation set as shown on the left and the right side of this curve. Now, oftentimes, we are asked the question, “How does this help you in the clinic?” And this is a very important part for this particular session. There are two things to consider that are absolutely critical. When we think about how does this help in the clinic, the classic response one gets from the physician could be, “Well how is this going to change my management?” Well, there are two things. Number one is patient counseling. If you have a patient that would fall into one of these red curve categories, that is not a patient that we will be seeing myeloma as “chronic disease.” That is the patient that we have to provide more emphasis and perhaps more intensity in the treatment. That is the patient that will need a different approach to the therapy. Whereas perhaps for the patient on the green curve we might be more inclined to talk about that great longevity that myeloma patients are experiencing now and the possibility that this patient could be alive many years from now. So, number one is the counseling of patients is quite difficult. Well, number two is that patients with high-risk disease need more intensive therapy, and through multiple studies, many of which have used some of the traditional genetic markers, we now know that standard approaches are simply insufficient. Perhaps, the clearest recommendation we have from this is that a patient who has high-risk multiple myeloma will need additional consolidation and/or maintenance with a more intensive regimen and one that contains a proteasome inhibitor. Simply doing an IMiD alone would be insufficient. So, both for counseling and patient management, this information becomes critical.

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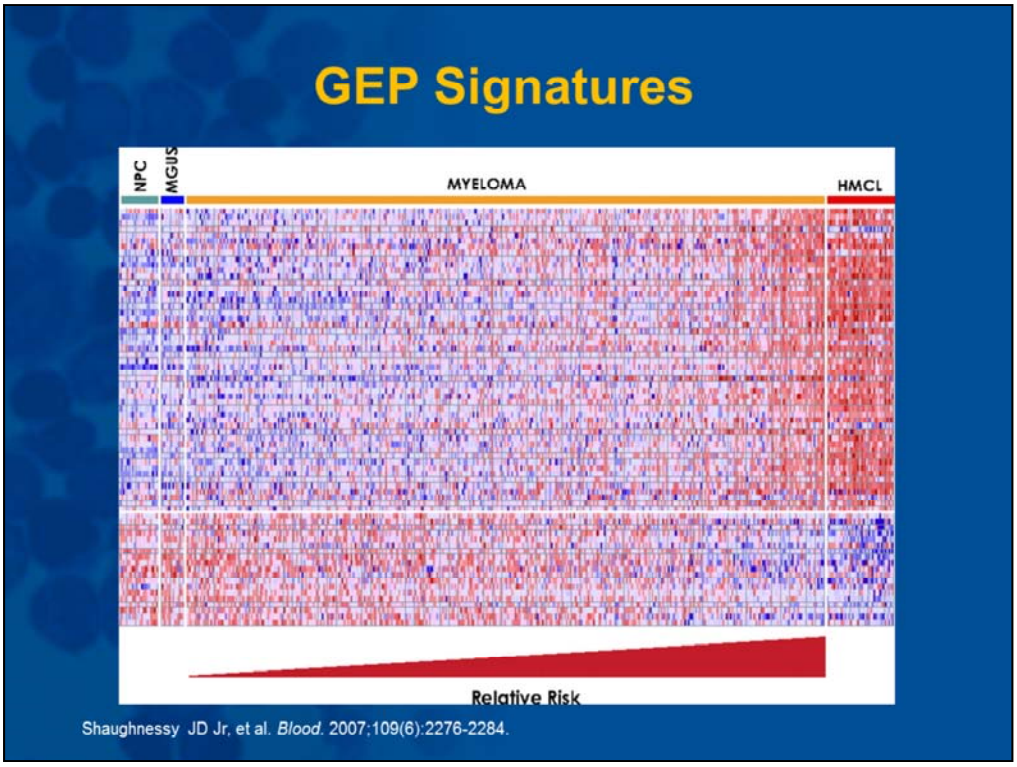

Now, this is another depiction from the early work from the group from Arkansas. This is work from Dr. Shaughnessy where you see the classification of patients with myeloma and the different subtypes, and you actually contrast that to the far right what we see with the human myeloma cell lines. You see that the myeloma cell lines essentially represent very high-risk myeloma and they are highly enriched for genes that are associated with disease proliferation, all of that red part of gene expression, whereas in myeloma there is a full spectrum and to the far left you see myeloma that is actually is more resembling of what we see in MGUS cases and even in normal plasma cells.

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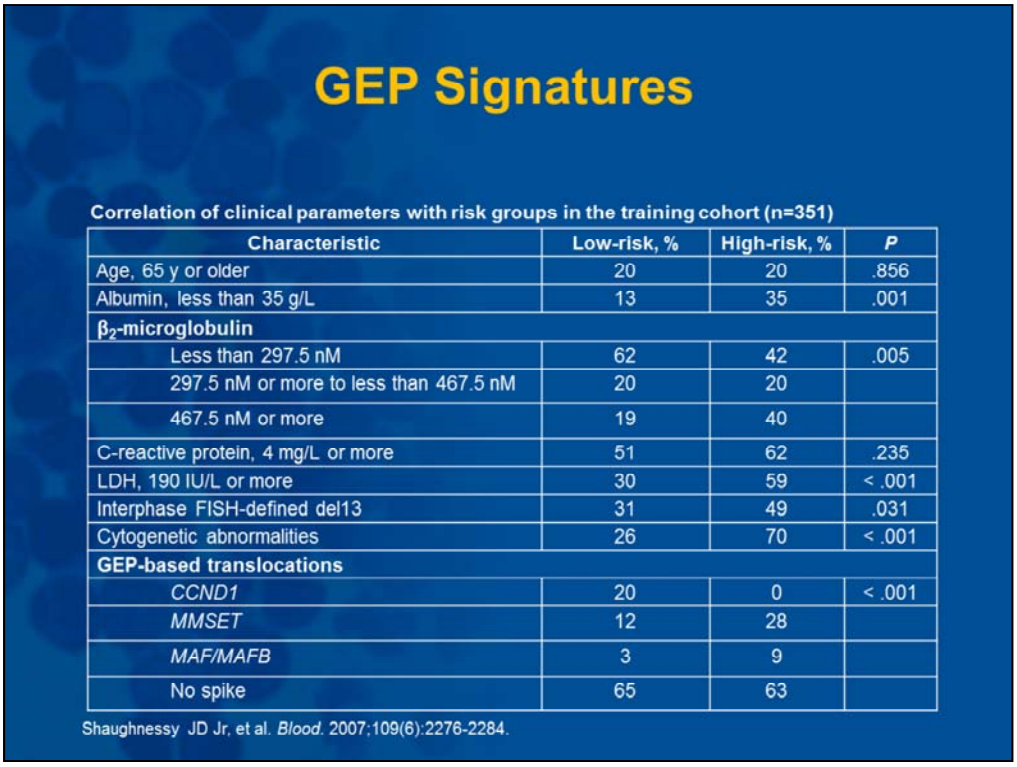

GEP Signatures

Correlation of clinical parameters with risk groups in the training cohort (n=351)

Characteristic	Low-risk, %	High-risk, %	*P*
Age, 65 y or older	20	20	.856
Albumin, less than 35 g/L	13	35	.001
β_2-microglobulin			
Less than 297.5 nM	62	42	.005
297.5 nM or more to less than 467.5 nM	20	20	
467.5 nM or more	19	40	
C-reactive protein, 4 mg/L or more	51	62	.235
LDH, 190 IU/L or more	30	59	< .001
Interphase FISH-defined del13	31	49	.031
Cytogenetic abnormalities	26	70	< .001
GEP-based translocations			
CCND1	20	0	< .001
MMSET	12	28	
MAF/MAFB	3	9	
No spike	65	63	

Shaughnessy JD Jr, et al. *Blood*. 2007;109(6):2276-2284.

When we look at standard clinical characteristics of patients who have high-risk gene expression profiling, you will start seeing, as is shown here, that they will be enriched, in general, for some of the classic markers of more aggressive disease. Now, this is not 100% and just merely represents an enrichment. I will give an example, for instance the level of expression of LDH is much greater in myeloma that has high-risk signatures, but the corollary to this is that you cannot predict a patient being at high risk just by looking at these clinical variables. So, this takes you above and beyond because there are patients that by traditional markers may be considered to be standard risk and yet have high-risk genetic profiling.

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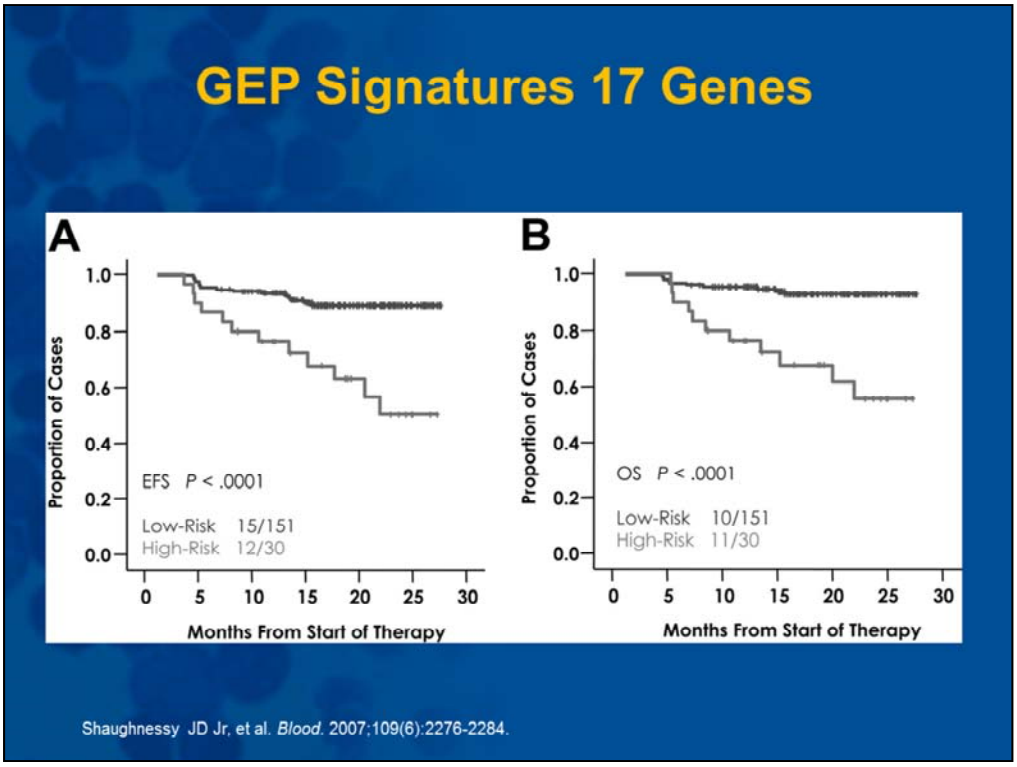

Now, perhaps more to understand the biology of the disease, people have looked at models that could simplify this. So, there is one that goes down to 17 genes, also from the same data set from Dr. Shaughnessy. And you can see that there are separations both for event-free survival and overall survival, now using 17 genes alone. Now, you might ask yourself, “Getting the thousands of genes that I get with microarrays, why I would go down to 17?” Well it is truly more of a research question. It may allow you to pin down what are the genes that are driving disease proliferation, perhaps what are the targetable genes, and we will talk a little bit more about this next.

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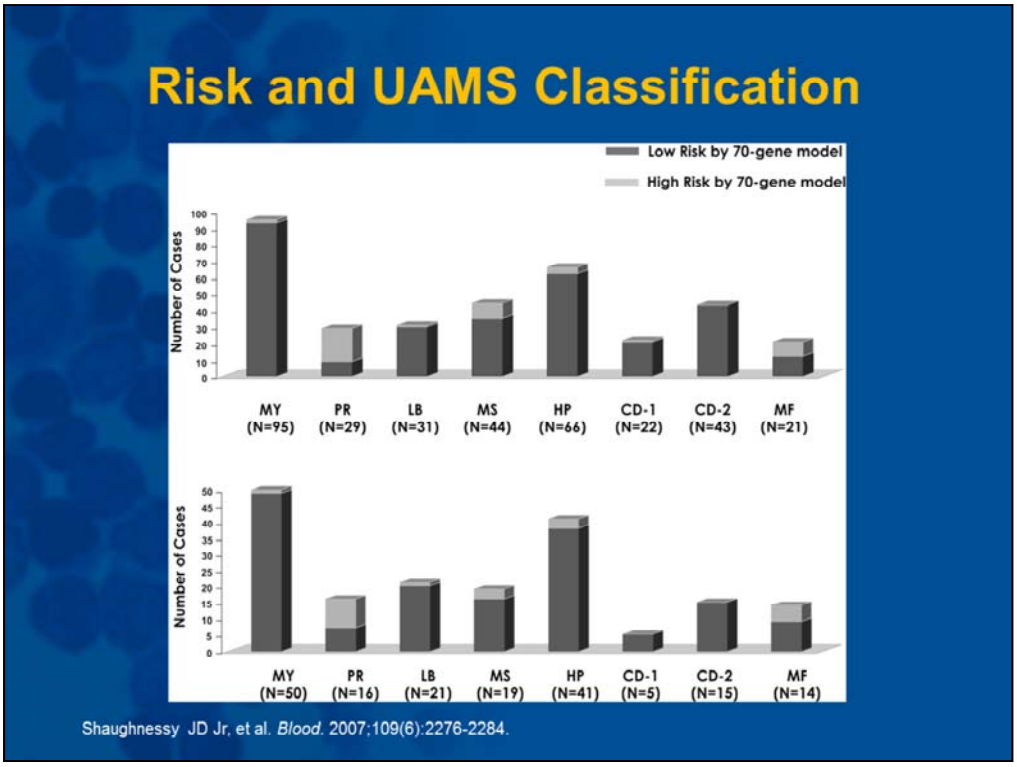
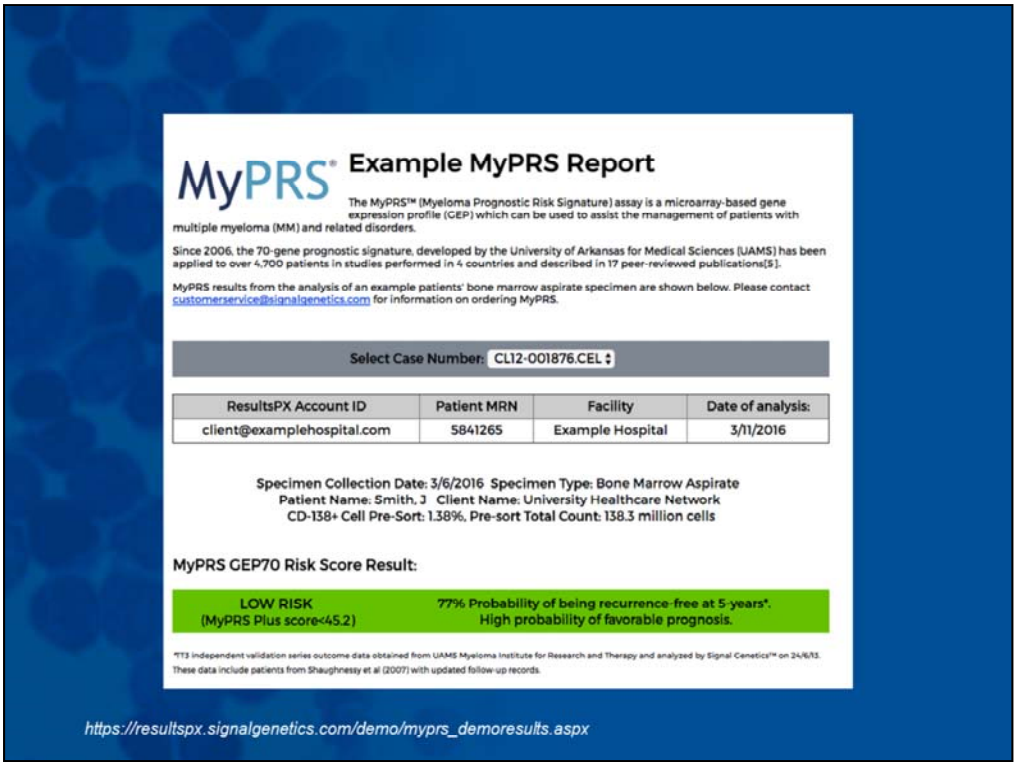


Now, this is very interesting. So, they went back and then they overlaid the risk classification on top of that disease classification that I showed you at the beginning of the session. Now what you would see is in the lighter color are patients who have the high risk by this 70 gene model and then the darker color you see of course the patients that have low risk. What you start seeing is that certain subgroups are enriched for high-risk signature. So, MAF signature enriched for high-risk signature, but in particular, I mentioned before proliferative signature is highly enriched for high-risk genetic signature.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice

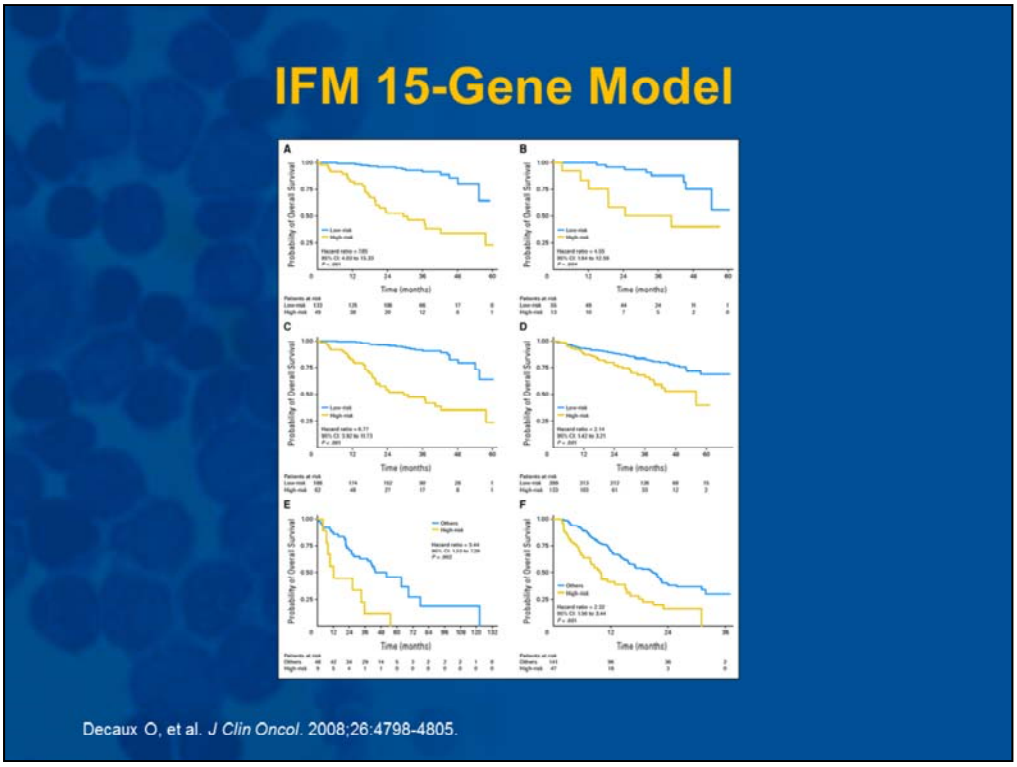
Now, this is one way in which a report from testing as is done by MyPRS® which comes out of the company Signal Genetics that can do gene expression profiling from a reference laboratory would provide you with a number of these features. So, you can get the information based on spike genes and the clustering classification, how the GEP predicts cytogenetic abnormality. So, you can clearly tell from what I have shown you that if you have gene expression profiling you can tell which patient has each one of the translocations or which patient may have a trisomy. You can do things like doing that pseudo-karyotype that actually talks about the extra copies of chromosome. Then you take that and then you actually can look at the presence of low risk and high risk. You will see at the very center of the slide there is this low-risk borderline and high-risk borderline, and this is true for anything we do in biology. So, it is just a continuum of progression for more indolent cells toward more aggressive cells, more resistant cells to treatment, and that is why that definition holds it. But you will see the numbers really hovered between 40 and 50 pretty much. You get a score and that score tells you if you are under 40 you belong to low risk and if you are over 50 you belong to high risk. Obviously, this all has to be coupled with clinical expertise and judgment, but then you start looking at the probability of 5-year survival which actually has been validated now in thousands of patients and needs to continue to be tested, particularly as new therapies are added so that we can refine these numbers in the context of therapy provided.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice



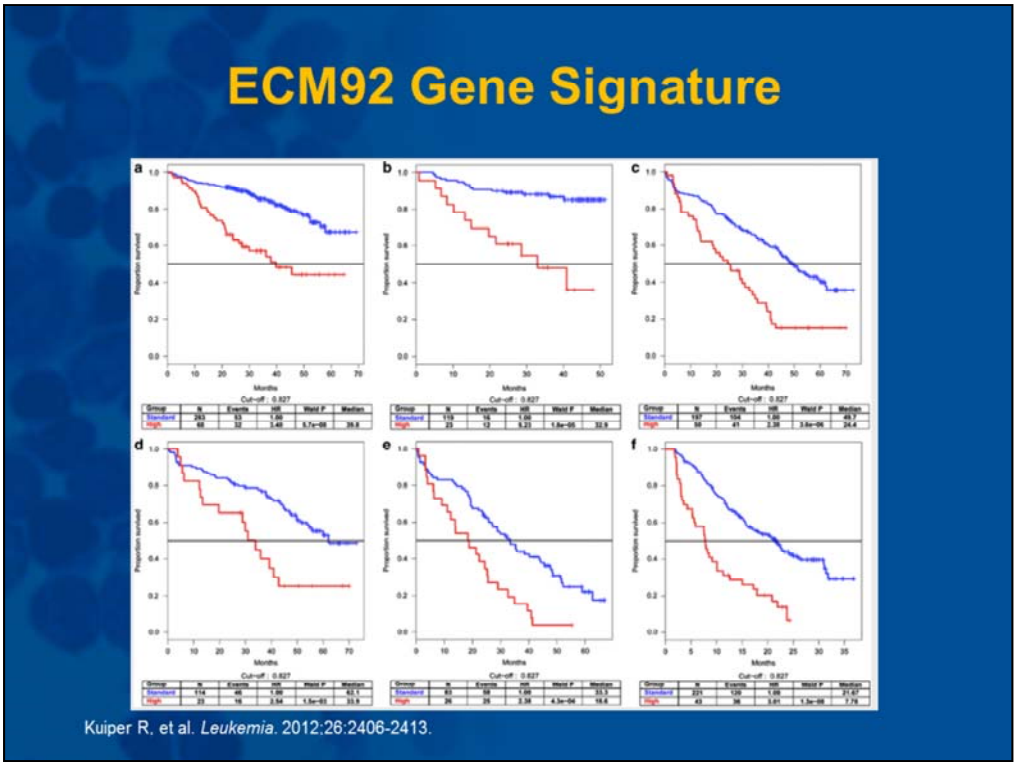
This is a sample of a report you might get from Signal Genetics where you send the sample for MyPRS® testing which will tell you your patient has this risk, in this case low risk, with such probability of recurrence at 5 years.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice



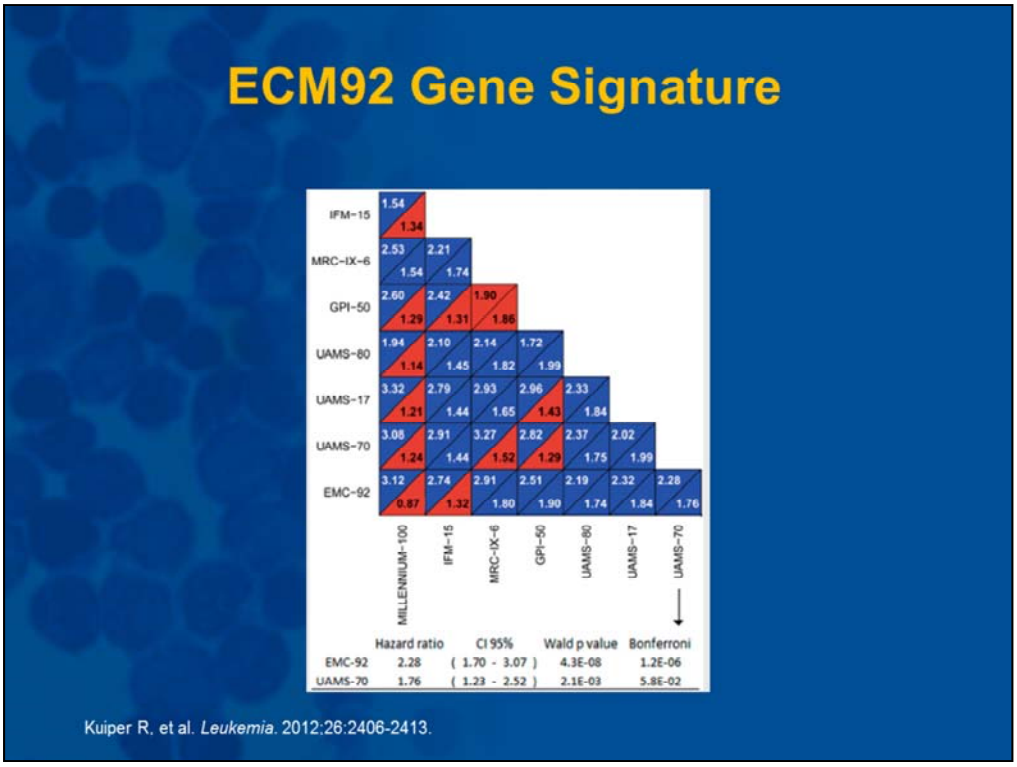
Now, this is a model that has been derived from the French group, the IFM, the French group for myeloma, that looks at 15 genes and also makes association with prognosis. Now, sometimes, these genes do not correspond 100% with different models, and this does not matter because what it means is that there are just different ways in which you can be looking at the genetic features of the disease that will still be prognostic. So, one analogy I use is that you might say, “Well, you have a way that you can discern who is going to run the 100-meter dash.” Maybe, it is who gets the first meter in 5 seconds but also who has such girth for the thigh, but there are different ways in which you can predict the 100-meter dash winner, which is really why sometimes these different signatures do not necessarily have 100% overlap.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice



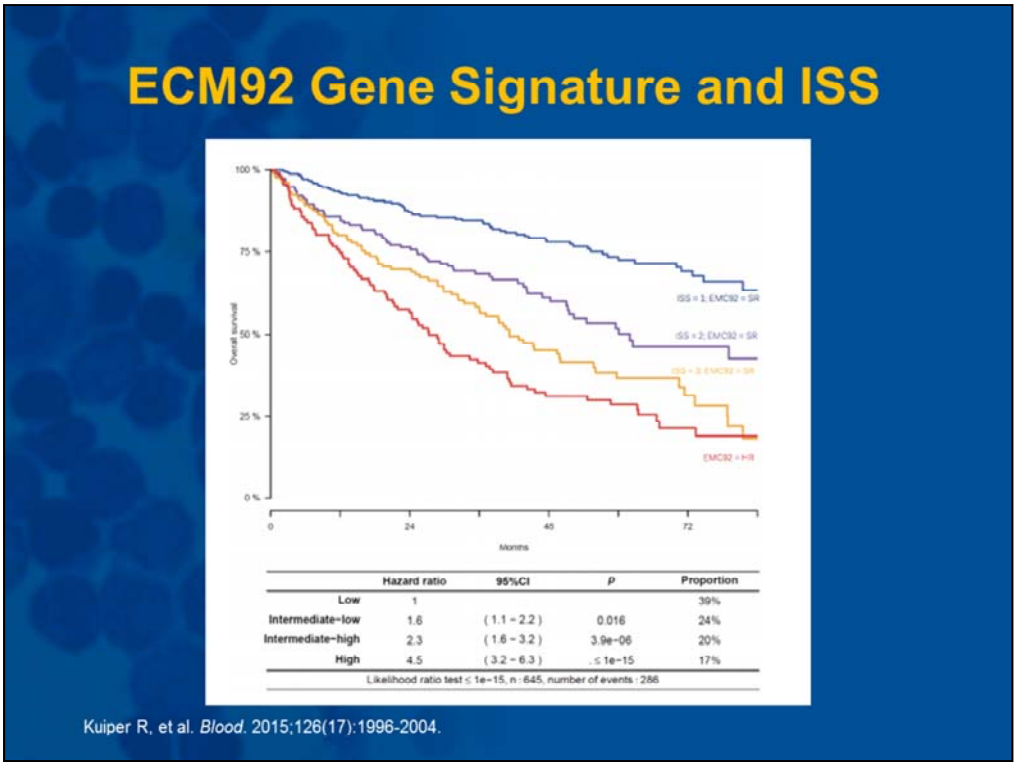
Another study was done by the Dutch group, the so-called ECM-92 gene signature, which is also very powerful in predicting outcomes for patients, and again, I use the same explanation. These signatures do not necessarily all correspond with each other. They do not necessarily have to be overlapping.

Gene Expression Profiling in Multiple Myeloma: Implications for Practice



So, in this particular slide, I am going to show you the comparison of the various signatures. We are looking at the hazard ratios of the signatures as predictors of outcome. Now, there are different platforms and there are different ways in which one can do the analysis for outcomes for this patient, but as you can see, there is work that has been derived from the University of Arkansas. Several European groups including the MRC group, the IFM, and also the Dutch group represented by this ECM-92 signature, all of them with different ability to discern prognosis in different subsets of patients. This was published from 2012.

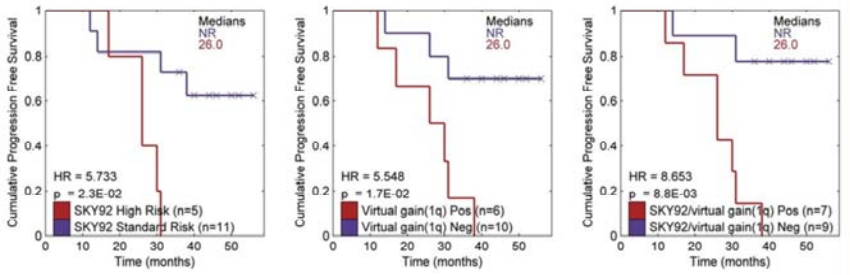
Gene Expression Profiling in Multiple Myeloma: Implications for Practice



Now, one that has been validated perhaps more extensively than the other European one is the ECM-92 that I mentioned before. This is one such analysis of how we can integrate this for instance for prognostication, just published from last year by Dr. Kuiper where you look at the International Staging System, and now enrich that with high-risk genetic profiling. Now, in this particular case, you can see that the standard criteria actually can predict for the lower-risk patients; those are all separated by that. Just in the red curve, you see patients who have high-risk genetic signature. No matter what the beta-2 microglobulin might be, the albumin might be, these are patients of course that have a significantly adverse outcome and therefore need to be managed in a different way.

KRd and Prognosis by GEP

Figure 1. PFS Kaplan Meier curves for 16 KRd pts



(Left) SKY92 signature; (middle) virtual gain (1q); (right) SKY92/virtual gain(1q) . HR, hazard ratio; Neg, negative; NR, not reached; Pos, positive.

K=carfilzomib; R=lenalidomide; d=dexamethasone

van Vliet M, et al. *Blood*. 2014;124:2141.

As we have understood this in the context of very large phase 3 clinical trials of course that now has to be adapted for novel therapeutics. Now, the curves I will show just as a brief example will be by nature quite different because these are smaller studies and these are studies for which there is shorter duration of followup, but even here, you can start seeing that gene expression profiling will and can be used as well for the assessment of prognosis with novel therapeutics.

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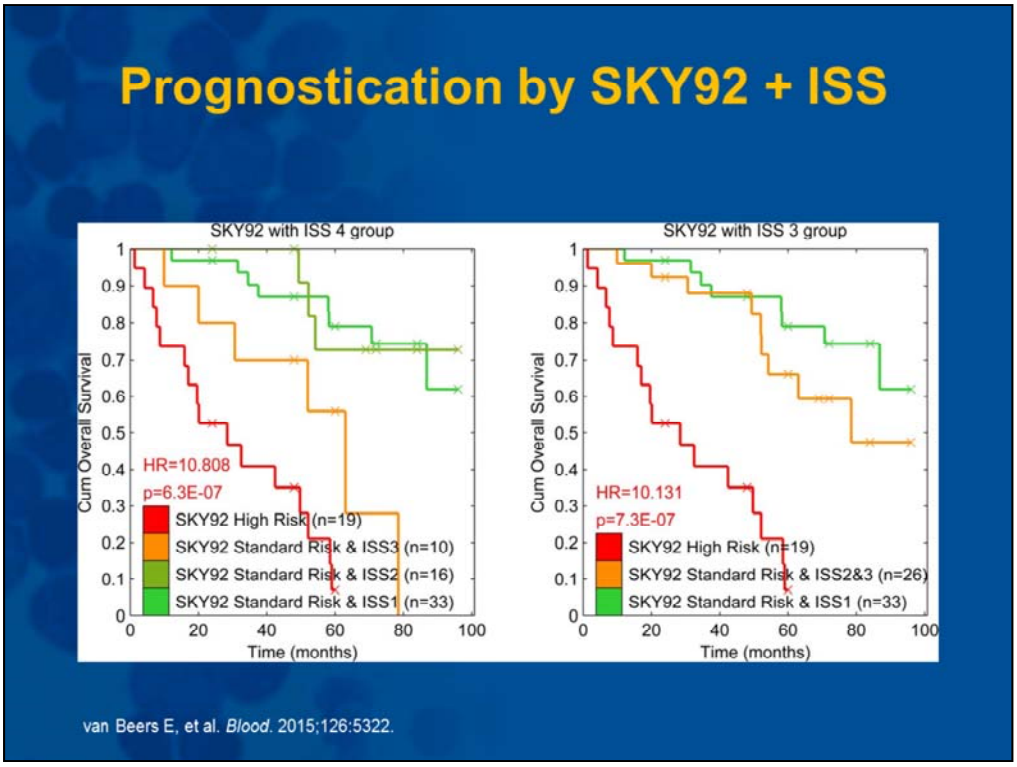

In another example, again Dr. van Beers from the Dutch group last year published that a signature like this could be combined with standard criteria to refine the prognostication of patients. Now, mind you, this is critically important in the clinic and I mentioned this before for two reasons why prognostication is important, but also as we think about the long-term outcomes and the long-term strategy of patients, one of the questions that is at the forefront of how we decide to manage patients is, can these things change over time? So that is, you may have a patient as shown here in this curve that perhaps starts belonging to the green curve, that patient receives optimal therapy and that the patient receives a stem cell transplant. What happens when the patient relapses? We know from previous studies that these patients actually tend to progress toward high-risk disease, which obviously represents a natural evolution into the other disease categories by gene expression profiling, and then the question would be, “How do we integrate gene expression profiling in a longitudinal way for the assessment of myeloma patients from treatment?”

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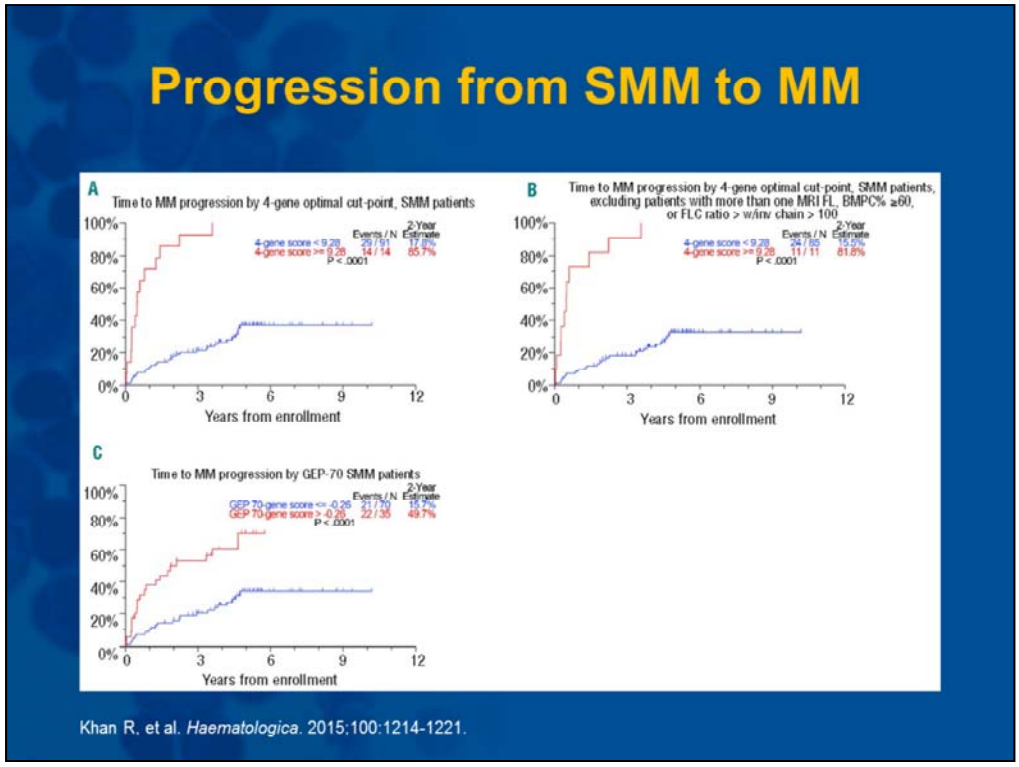

Now, there are other interesting approaches that are being tested and even if they are not 100% accepted or validated at this point are very, very provocative. So, this is data from Dr. Kahn working with Dr. Dhodapkar and others from the SWOG group where they actually looked at gene expression profiling as a predictor of progression from smoldering multiple myeloma to active myeloma. In this particular dataset, they used four genes. As we get more and more information from this, one can only imagine a future where we now see a smoldering myeloma patient, and beyond the standard clinical criteria we might say, “You know you have such gene expression profiling signature, perhaps we may need to treat you, perhaps we need to follow you every 2 months instead of every 6 months, or think of participation in other clinical trials.” Arguably, that is a very, very useful information to someone who has a problem such as smoldering multiple myeloma.

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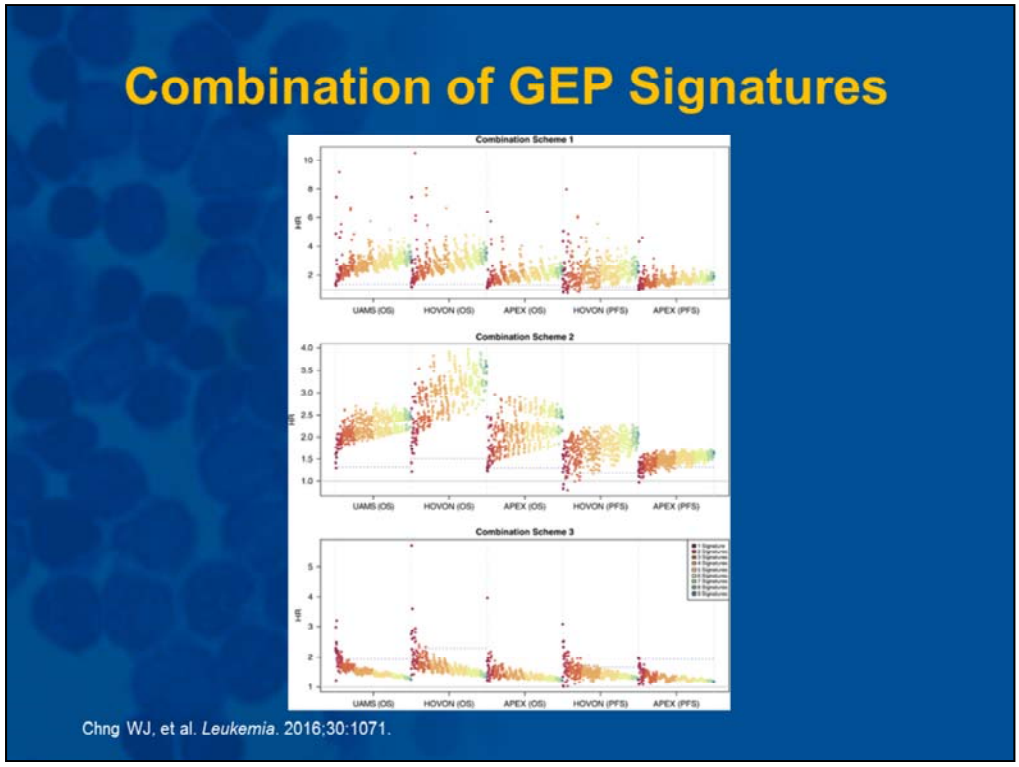
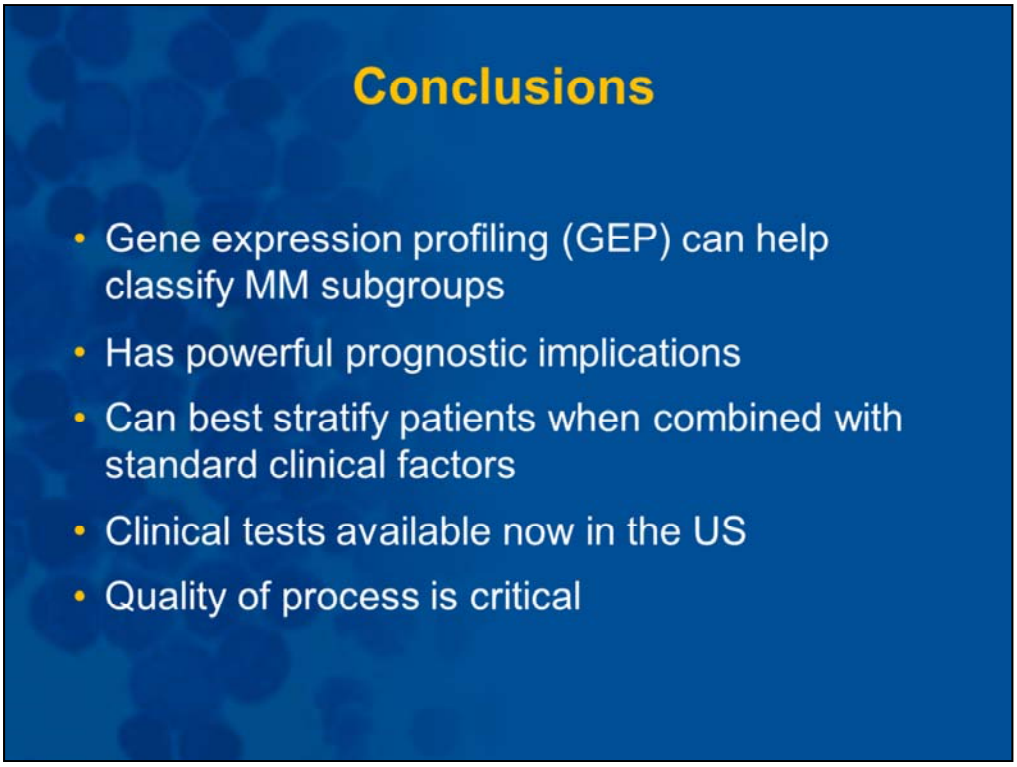


Lastly, and this is recent data from Dr. Chng who has been a leader in gene expression profiling from Singapore. You can see that we will start combining some of the signatures and some of the data which is part of the beauty of doing this analysis, that you have the wealth of information that comes from the microarray-based platform such that we potentially could refine further the sub-classification of the disease.



So, in conclusion, we have seen that gene expression profiling can help classify myeloma subgroups and that it has powerful prognostic implications. In 2016, gene expression profiling has the greatest power to discern outcomes for myeloma patients, and fortunately, we have a clinical test that is available now in the United States. I did stress importance of the quality process, but fortunately, this is something that has been worked out. I did mention that if you combine gene expression profiling with standard clinical parameters you can further refine your understanding of outcomes for patients.

So, with that, I would like to thank you for viewing this activity, and again for additional resources, please view other educational activities on *ManagingMyeloma.com*.