[MUSIC PLAYING]

MAXIMILIAN MERZ: Knowing and Finding the Enemy, I mean, right now, in cancer, there's so many different developments going on with molecular diagnostics. And I just saw also some of the speakers are advising some of these companies. But I want to make a strong arguing point at the beginning that basically the FISH analysis is still the only established and validated analysis for myeloma to make decisions for treatment also for prognostication. And I will show you why that's actually-- why I think that's the point. But like Ian said, I mean, if you have questions or completely disagree with something, I would be happy to have, like, an entire discussion about that.

So almost half a century ago when this young man-- I'm not sure if somebody knows him. Maybe one of the esteemed faculty knows this guy-- established the criteria of diagnostics for multiple myeloma, the work was pretty simple. You had basically three things that you have to look out for-- M-protein, plasma cells, and bone disease. And back in the day, that meant basically serum protein electrophoresis, just a regular bone marrow [INAUDIBLE] and a smear and an H&E staining. And bone disease was still just a conventional X-ray.

So nowadays, in 2019, we have, even for simple things like M-protein, several different things that we can assess. The plasma cells, we moved from morphological findings to next generation sequencing. And nowadays, on a single cell, there are basis and imaging bone disease. Of course, I won't talk about that because we have the guest expert in the room today with Jens.

And I want to start with spectrometry because I think the assessment of M-protein seems to be a bit boring. But actually, last year, at the [INAUDIBLE] in Stockholm, these three also esteemed myeloma researchers discussed this topic in an extension. And actually, I think that's a very promising thing. Mass spectrometry is just another way to highly-- with a high sensitivity assess M-protein-- monoclonal protein-- serum, [INAUDIBLE], or urine.

Nowadays, they moved back again from urine to serum because the background noise might be better urine. But in serum, it's more reliable because of the renal function in myeloma. But basically, if you move through these slides, it's an interesting thing. If you look at the patient here at the beginning, it moves clockwise. You see the monoclonal protein and the serum electrophoresis and also the immunofixation. And then the patient gets treated with daratumumab.

And what you see is that basically, the serum [INAUDIBLE] freezes. Does not change at all. So if you see the M spike, it's basically the same and stays the same during the treatment. But if you look at spectrometry, in spectrometry, you really can assess the molecular weight of the protein. You can see that basically, the patient goes into remission. And truly, M spike goes down. And you can measure daratumumab as a monoclonal antibody is going up.

So basically, using serum electrophoresis, it would be bad to say this patient doesn't achieve any remission because, basically, what you see in the electrophoresis or what you measure in the serum would be daratumumab. And you would miss that kind of omission.

And another interesting thing is, of course, coming back to Robert Kyle. So the mystery is relieved. He nowadays looks like this, still very fresh for his over 90 years. I'm not sure how old his is. But he is-- I guess he's over 90. And what he did back in the day-- and that's pretty amazing, especially when you're a young resident or fellow and start with myeloma. And you have like a full clinic. And they're like, let's say, 5Q MGUS patients.

You always are very relieved because MGUS, you know, I just have to tell you a story. It's 1% per year risk of progression into symptomatic disease. But basically, it's a pretty easy talk that you have. And usually, you probably won't see that patient again because 1% per year, even if it's a younger patient, he won't progress.

So what he did back in the day, he's-- almost half a century ago, he just collected serum probes-- serum samples from MGUS patients. And now he's able to go back with this spectrometry and basically can see 10 years after he started this that back in the day when he saw it now in an active myeloma patient, the positive serum electrophoresis immunofixation, that when you apply the spectrometry, you can diagnose MGUS very early.

So that's maybe becoming a new diagnostic criteria because you are just more sensitive to detect monoclonal protein. So I think that's another application beyond the measurement of disease with treatment with a monoclonal antibody but also to check for MGUS in otherwise healthy-- healthy individuals.

So I'm moving back to the genetic and to the myeloma cell itself and now to the surrogate markers of tumor load. Basically, there are different steps that cause myeloma. And with every different step, you can perform another test.

So from the way-- from DNA damage and mutations to the functional protein and basically the phenotype of the cell, there's so many different steps that can be assessed. And I want to start with DNA. And, of course, the most important thing, like I said, is cytogenetics in myeloma. Might sound a bit boring. But actually, the chromosomal aberrations are still the most important prognostic effect on myeloma.

So I'm-- back in the day, like three years ago, Salomon Manier published this very nice paper on the clone heterogeneity of myeloma because myeloma is a very heterogeneous disease. And I will go into detail on that later on.

But based on the cytogenetics, you all might know that you can classify myeloma into different categories. One is hyperdiploid myeloma caused by the gain of autonomic chromosomes. Here on the right side, the autonomic chromosomes, if you just have two or three of them in the cell, then you can be considered hyperdiploid which is associated with a better risk.

And the nonhyperdiploids are myelomas basically characterized by IgH translocations. That means translocations involving the heavy chain locus and chromosome 14. And there is a big heterogeneity regarding the prognostic value of these transactions. While (14;16), (4;14) are non-high-risk translocations. Some of them, for example t(11;14) are considered standard risk. But basically, if you look at subclonal analysis in patients with translocation (11;14), you might also have a high risk even if you have-- your main clone is a translocation at (11;14) clone.

So back in Heidelberg, we perform this analysis, including t(11;14), (4;14) and (14;16). Check for the deletions-- the most important deletion, of course, is MVP with the locus of TP53-- and the gains to check for the hyperdiploid karyotype. But here, again, for example, chromosome 1q is associated also with adverse prognosis in myeloma. But it also depends on the load. How many chromosomes? If it's just a gain or an amplification. So if you have a cell consisting of five or six copies of chromosome 1q, it's worse than if it's just three or four copies.

And like I said, this has been established like 10 years ago or 20 years ago. And this paper is seven years old. But basically, nothing has changed since then because the biggest analysis by the IFM back in the day included more than 500 patients with a very long follow-up. And they found basically that what I just said for 14 deletion delusion 17 and gain of 1q were significantly associated with adverse progression for [INAUDIBLE] overall survival in these patients treated with high-risk disease and also novel agents for induction therapy.

And again, you can argue whether cutoffs are important in cytogenetics. So back in the day, they used 30% of positive cells for 1q as positive, and for deletion 17, over 60%. That again, is not completely without any discussions because in Heidelberg, we always do it like this, that we perform plasma cell purification.

So you get a sample with 90% of plasma cells. Then you apply your different probes. And we had a cutoff back in the day of 10%. In our collective of patients, it's always shows up that if you have positivity in 10% of cells, it is associated with higher risk. But basically, there's no consensus out there how many cells need to be positive.

That is another thing that needs to be argued. But in my opinion, nowadays, everything is moving so much forward to imitation and gene expression, single cell analysis, that these basics of myeloma and also the genetics nobody cares about them anymore. But actually, I think it would be worth looking into this in a meta-analysis combining different study groups. But I think that won't happen in the near future.

So another thing is that, of course, the chromosomal aberration are the broadest change that you can have on your DNA. But of course, everybody carries mutations in myeloma. And basically, the largest study that analyzed the prognostic value of mutations in myeloma was performed by the UK senior author with Garris Hmong.

And basically what they found is that known mutations that are known to several types of solid tumors as well, like NRAS, KRAS, BRAF V600E very frequently mutated in myeloma. And of course, there are some trackable mutations in myeloma. But again, although these mutations correlate to adverse outcome if you have-- basically you can say, if you have more mutations, the outcome is worse.

But it's still very costly. There's no validated system how you should perform whole genome sequencing and whole exome sequencing. It's not clearly established what should be a source material for that. So it's still very interesting to decipher the biology of the disease. But for prognostication, it's still too early to say that's the new gold standard.

Back in the day, they calculated also mutation-based ISS score. They combined the ISS score with the mutation and load. And they were able to group these nice three different kinds of patients. But again, this has been the only study investigating mutations, spectrum, and pyroclastic value in transplanted patients. So it's still too early, in my opinion, to say we can move away from cytogenetics to mutational analysis. Nevertheless, it's very costly.

When you see your mutations in the DNA, it's always important what was your sequencing. Was it whole genome sequencing or whole exome sequencing? And then, of course, it's the question whether the mutation leads to a different differential gene expression. So almost 20 years ago, first analysis were performed on gene expression profiling in myeloma. And what you saw was-- what everybody saw is this works pretty well.

So several groups, starting in Arkansas that was the first publication by Shaugnessy almost 15 years ago that analyzed a panel of 70 genes that was associated with high risk and low risk disease. Later, the IFM, the French group followed, followed by the European endeavors to check gene expression profiling and transplanted patients.

And then we performed the same analysis back in the day, in Heidelberg, on over 600 patients. And what we saw is that all these different gene expression profiling patterns basically produced the same [INAUDIBLE] curve. So you see the [INAUDIBLE] is pretty stable. PFS and OS looks pretty much the same across these different gene expression profiles.

But what was very interesting, and also I think it's-- nobody has an answer to that-- is that when you compare these different gene expression profiles, you'll see something very, very similar, I guess. So if you look at the most established profiling systems-- that's the Arkansas group, the Dutch group, and the IFM-- you see basically no overlap between the genes that were checked in these different gene expression panels.

So basically, they use 70 genes. The European endeavor was with 90 genes. But there was no overlap between these genes whatsoever. So I think, for example, if you look at something very easily to be checked, like hemoglobin level, and somebody came up with a test, and somebody else came up with a test, but there was no overlap in the results, I think it would be very worrisome to say, OK, let's do gene expression profiling in every patient to get an idea of the prognostication.

So from the very same publication that came to the same conclusion, it was entirely ready for the rework. My answer to that is a strong no because there's no-- it can be reproduced. It's prognostic in several populations. But there is no overlap and I think also no consensus on which genes are the very most important in these profiles.

So of course, there are other steps, for example, epigenetic modifications, like histone acetylation and DNA methylation. But this is assessed right now also in bigger populations but still very costly and not applicable for the regular clinic. And also this, you see after the DNA gets transcribed into RNA, you'll see that after that there's a whole different so-called RNR world that can be explored in myeloma. And it's very complex. And it's still very early to say if that has any prognostic significance or maybe can be targeted also with different medications.

And of course, the evolving field of proteomics and phosphoproteomic needs to be assessed in the future because myeloma is a disease of a very, let's say, not only [INAUDIBLE] disease but also when it comes to protein expression. So I think this will be another endeavor that needs to be picked up in the future.

So now I will show you one example that was very interesting. Back in the day when I was a resident, we had a patient that was 58 years old working-- still working full time. No signs of disease whatsoever. But during routine assessment, we found a monoclonal protein. And afterwards, we saw osteolysis NCT. And then we started the workup.

And then we performed our ISS staging and find out, OK, ISS 1. Good sign. With transplant, this kind of patient can live 10, 15 years. Then the official results came back. And we saw translocation (4;14) and so on. That's a good, high risk. But what do you do with ISS 1 translocation t(4;14) present? That's, on the one hand, a good sign. On the other hand, biology tells you that the patient won't do well without transplant and even with novel agents.

So we performed gene expression profiling. [INAUDIBLE] established and all the score. The Arkansas group showed us high risk disease. OK. That's very confirming with the t(4;14). But since we had the RNA expression, we said, OK, let's test the yellow scores as well. And we did the IFM score and found out, OK, according to the IFM score, [INAUDIBLE], this patient's considered to be low risk.

Again, what should you tell that patient? Luckily, there's a third score that we can assess-- our own score. And then we saw it's medium risk. So basically, with all these tests that we had performed back in the day, we had no answer at all. It was just too confusing. If you tell that kind of reason-- that kind of result with patient, I guess you will also lose some kind of confidence that what you are doing is pretty much working.

So I guess we have to run even more tests. And we have nowadays-- and that's what we are working right now on [INAUDIBLE] in the lab that there are some reasons why these-- there's so much heterogeneity. Even if you apply the same analysis-- which was, in this case, for example, gene expression profiling-- there might be some explanation for that coming from the clonal evolution of the disease itself.

So this has been described for several solid tumors that basically you have this stem cell concept that every tumor starts maybe with one cell that gains certain mutations or chromosomal aberrations over time. And then you create these different ecosystems. And every time you apply therapy, that's a bottleneck for the ecosystem. And then arising from a subclone [INAUDIBLE] present subclone, you have multiple clones arising. And that causes basically the heterogeneity of the disease.

And that was very similar when you looked at this. That was very similar to the evolution of species that was initially described by Charles Darwin. So when you stay within this tree model, you can see, for example, in hairy cell leukemia, you have one mutation that's pretty common-- BRAF V600E. But for example, in metastatic colorectal cancer, it's more like a mangrove tree. You have so many different roots. And some of the colorectal cancer patients carry the BRAF mutation. And that can be tracked.

But in the hairy cell leukemia, it works pretty well. But metastatic colorectal cancer, it doesn't work at all if you just apply, for example, vemurafenib. And that's the same as in myeloma. Myeloma is very heterogeneous. Some patients, about 5% to 10%, carry the BRAF mutation. But it's not really worthwhile to try only the BRAF inhibitor to treat myeloma.

And what we are doing right now is basically to assess from the asymptomatic disease, starting with MGUS, over smoldering and symptomatic relapse disease to assess the genetic heterogeneity over time. Because in newly-diagnosed patients, you see the number of mutations-- kind of mutations is higher than at the MGUS level. But that's only descriptive because we-- longitudinal analysis are very complicated, especially in MGUS patients because you never know which MGUS patient develops multiple myeloma.

So another layer of complexity is added by the fact that when you look at, for example, cytogenetics, like I said, you can just apply a threshold of 60% positivity for cells. But that's not only-- that's not only always true. So for example, if you look at the deletion 17-- and that's what we did in our HD4 trial that compared thalidomide maintenance therapy to bortezomib maintenance after transplant that basically if you have no further subclonal cytogenetic aberrations, your outcome is remarkable good even if you look at high risk operation.

So the top curve here represents basically only patients that have a major clone operation and no further subclone operation. That includes also patients with deletion 17. So we had a couple of patients that had 60% of plasma cells positive for deletion 17 and no subclones at all. So no deletion 8, no deletion 13, no translocation (4;14). And when you apply bortezomib to these patients after transplantation, you see that the outcome was pretty-- really significant.

And the other groups were patients that had, for example, 100% of plasma cells positive for, let's say, deletion 8, which is not considered a high-risk aberration but had further subclones, for example, a deletion 13. And once these subclones are present-- and this can be considered because it's a single cell analysis, iFISH analysis-- this can be considered a surrogate for heterogeneity. The outcome was worse, even with bortezomib.

So it gets even more complicated if you're biopsy different regions. And that's something we are very interested right now because two years ago, also, from the very productive Arkansas group, we had this amazing study performed on retrospective biopsies because the old chief, Bart Barlogie, started this 20, 30 years ago. If you had, for example, a PET positive lesion, he did not only biopsy the area crest but also biopsied the PET positive lesion.

And what they did in this specific application is that they checked, of course, the cytogenetics, the mutation load, and also the gene expression profiling. And very remarkably, you can see, for example, in this patient, from the area crest, you have a hyperdiploid karyotype, standard risk. Then you have a BRAF mutation. And you have a gene expression profiling-- a score that considers this patient as low risk.

But then if you look at the heart lesion here in the fourth lumbar vertebra, you see the gene expression profiling is high. No hyperdiploid. And you have deletion 1p, which is considered to be a high-risk disease associated and also biallelic activation of TP53, which is basically the worst thing that you can have in myeloma.

So that's even more confusing right now because we-- right now, everybody's performing biopsies from the iliac crest. We know that 80% of patients have focal lesions or [INAUDIBLE] or PET positive lesions at primary diagnosis. So it's really the question if we are looking at the right spot to check for the disease.

And because of that, we started a clinical trial in April this year where we basically offer the patient who is newly diagnosed or with-- so newly diagnosed relapse disease before the initiation of retreatment that we perform biopsies from the iliac crest and also from the focal lesion. Here it's a focal lesion on MRI. But we perform MRI on PET CT. And then we perform next generation sequencing-- whole exome, whole genome sequencing from both locations and [INAUDIBLE] analysis to check if the disease from the iliac crest-- the regular bone marrow-- matches the disease from the focal lesion.

And so we really hope that also the classification of patients will be different when we look at the focal lesion because sometimes, for example, if you look at solid tumors-- I think it's always good to look at other disease-- what other doctors do with that kind of disease.

And if you look at, for example, in metastatic colorectal cancer, any kind of other metastatic solid tumor, the endeavor to check the disease-- the extent of the disease also from the metastatic side is pretty common. So if you have an easily reachable region that you can biopsy, I think it's really worth for the patient to try that.

We had a couple of patients that the diagnosis was not so clear from the bone marrow. But then from the focal lesion, it was full of abnormal plasma cells, full of mutations that potentially trackable, of course. So that might even change not only the course in our assessment of prognostics but also the course of our treatment.

So totally confusing, because, I mean, we have two different layers of complexity right now. We have the heterogeneity. So basically, plasma cells are completely different in every patient. Then you have to look at the location. Some locations are completely different. And nowadays, we have this method that I am completely obsessed with right now-- single cell sequencing.

So back in the day, everybody did the same, of course. We had the tumor represented by these different kind of cells. Then you do your DNA extraction, put it in a vial. Then you do, for example, the gene expression profiling. And you get an average of the gene expression for all cells. But you don't know which type of cells contributes to the gene expression.

So nowadays, that's pretty easy in myeloma because you can do the CD138 purification. You can get the cells into a single-cell suspension, of course, and then barcode every of these cells and perform, for example, a single-cell RNA sequencing. And you can see for every population delineated by different colors that the gene expression is completely different based on the cell that you are analyzing.

So now we can basically say, OK. The cells are itself completely different. The location is completely different. And the population of the cells you are assessing is also completely different. So it's really hard to say if these, for example, biogene expression profiling patterns are really telling you to the full extent what kind of disease you are dealing with.

And that this is applicable was shown for the first time last year by an Israeli group also supported by Dr. Landgren who is was speaking today and published in *Nature Medicine.* And you see here, basically, it's quite easy to understand because these plots look very familiar like, for example, flow cytometry plots. You have these t, s, and e dimension redacted plots which basically gather the cells based on their gene expression profiling.

So here, they have different patients. MM stands, of course, for multiple myeloma, AL for amyloidosis because there are also different patients with amyloidosis. And if the cells are based on the gene expression profiling very close together, they just build this cloud. And then you have this big brown cloud here. And these are just background noise, nonplasma cells, et cetera, also in the bone marrow [INAUDIBLE].

And if you look, for example, at this patient here, the smoldering myeloma patient number 2, in the patient you see that he harbored three different clones based on the gene expression profiling. And each of these clones consists of 10 of [INAUDIBLE] cells. So it's really hard to say if by sequencing approaches are really showing us the entire picture. And if you imagine how you would perform single cell sequencing, which is commercially available now right now for $3,000 per patient and also for a sample.

So if you really want to imply this in your routine clinical practice, it's pretty challenging also from the financial toxicity otherwise. So I guess we should stay with cytogenetics for now because it's quite cheap, easy to understand, and the only thing that tells you really about the prognostication of patient.

But also very important is that we have these several layers of complexity of in myeloma. And what was also described in this review by Salomon Manier is that with every further step of disease-- and especially if you have patients that have like six, seven, eight lines of prior therapy-- the disease heterogeneity increases. That's something that's also pretty obvious in clinic.

For example, a patient with plasma cell leukemia-- secondary plasma cell leukemia or a secondary [INAUDIBLE] disease. They have thousands of lesions sometimes in the skeleton. And to my understanding, right now, is that these lesions basically are a surrogate for heterogeneity because nobody wants to stick 1,000 needles into a patient to assess these cells. But I guess if you would do so, you would get 1,000 different clones per lesion.

And the thing is when-- the more heterogeneity over the cause of disease you acquire, the sensitivity to your drugs, of course, decreases. That's also a problem. If you look at targeted therapy, like the example I showed to you with colorectal cancer, it's-- [INAUDIBLE] always says, it's called dirty drug. So you can be very specific and very targeted with your treatment. But for example, if you just target your risk mutation, which will be available in a couple of years I guess, what about these other clones?

So you need also dirty drugs like methylone or high-dose therapy that covers basically every cell and not only the mutation of a single cell. And that this concept somehow of decreasing sensitivity with increasing heterogeneity works was also shown amongst others by Dr. Landgren who treated together with, of course, some colleagues, high risks as smouldering patients with KRD.

And what you see is here these number of cycles with KRD that were applied. And you see that the depth of responses is quite high. So after eight cycles of KRD, you basically have every patient in a near complete [INAUDIBLE] app.

And that's compared to a, for example, KRD treatment relapse disease. The number of patients in [INAUDIBLE], that's unprecedented. So basically, that's some kind of indirect proof that if you treat early, you might get the chance to, of course, achieve MRD negativity and cure. And I'm sure Dr. Landgren will elaborate on that a little bit more extensive in a couple of hours.

I guess, always a good end to mention the cure of myeloma. And since it's always complicated to say something about war as a German, I guess war is over now if you're a diff MRD negativity. And love is all you need. So thank you.

[MUSIC PLAYING]