Title: MMRF Patient Webinar: Biomarkers in Multiple Myeloma

Date: February 19, 2024

Mary DeRome: Hello and welcome to the MMRF Patient Webinar Series brought to you by the Multiple Myeloma Research Foundation. I'm Mary DeRome, senior director of medical communications and education at the MMRF.

Today we have with us two myeloma experts who will be discussing the role of biomarkers in the management of multiple myeloma. Dr. Benjamin Diamond specializes in the treatment of plasma cell disorders, including monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), and multiple myeloma. His research interests include maintenance therapy, the genomics of multiple myeloma, and minimal residual disease (MRD) in clinical decision-making. Dr. Diamond serves as assistant professor of medicine in the Division of Hematology at the University of Miami's Sylvester Comprehensive Cancer Center. Dr. Francesco Mora has conducted genomic investigations on cancer genome evolution and the chronological reconstruction of early and late driver events in hematological cancers. His recent research has focused on modeling and integrating clinical and genomic data to better characterize the pathogenesis and subclonal evolution of hematological malignancies such as multiple myeloma, lymphoma, and therapy-related myeloid neoplasms. Dr. Maura is an assistant professor and co-principal investigator of the Myeloma Computational and Translational Laboratory and Associate Director of the Myeloma Research Institute at the University of Miami Sylvester Comprehensive Cancer Center.

Let's get started with our first speaker, Dr. Ben Diamond.

Dr. Benjamin T. Diamond: I'm going to be talking about some of the biomarkers that you're going to be seeing on a daily basis and how you can interpret them, and I'll try to demystify a lot of these common lab results that you're going to be seeing all the time. We'll segue into some of the less common things and maybe more complicated biomarkers and how they're becoming more important in clinical practice and how you're going to be seeing a lot more of them. And this really includes genomics, which is a primary focus of a lot of the research that's being done in multiple myeloma these days.

So, first, "biomarker" is a very nebulous and kind of big term, but what does it mean? It's actually quite simple. It's basically a characteristic that we can use to objectively measure and evaluate a biologic process. In this case, our biologic process is multiple myeloma, and any of these characteristics are going to be lab tests or imaging studies that we can use to tell us something about the disease and how it's acting. There are multiple kinds of biomarkers. There are diagnostic, prognostic, and predictive biomarkers.

An example of a diagnostic biomarker would be the monoclonal protein. This is something in the blood helps us diagnose whether multiple myeloma is present or absent.

A prognostic biomarker would be something like MRD, where the measurement of this marker can tell us something about how we can expect the disease to perform over time; it measures outcome.

Then there are predictive biomarkers, which are more rare in multiple myeloma. They basically tell us what we can expect will happen when a given treatment is applied to that specific disease. One example is the 11;14 translocation, which in multiple myeloma helps us predict whether a patient may or may not respond to a drug like venetoclax, which targets BCL2 and the biology of the translocation 11;14.

There are a ton of biomarkers, and there‘s no way we‘re going to be able to talk about all of them. So I decided that we would choose some that you‘re going to be seeing most commonly. These are going to be things that are drawn from both the blood and the bone marrow—so sequencing tests, flow cytometry, and also our bread-and-butter paraproteins like the M spike and the free light chains.

These are things that you‘re going to be seeing from day one. Some blood tests that you might see at the very beginning of the journey are going to be beta 2 microglobulin, lactate dehydrogenase (LDH), and albumin. Combined with fluorescence in situ hybridization (FISH), which is a basic genetic test, these things are going to be able to help us stage the disease. You may see these at one of your first office visits, and they‘ll help us come up with a Revised International Staging System (R-ISS) score. These scores are becoming more outmoded by some more sensitive biomarkers, but they still hold true; even in a lot of the newer models, R-ISS is still important.

The detractors will say that these measurements are nonspecific, that they basically tell us first how active the disease is and also how sick a person is. Like the albumin, for example, tells you how sick you are. But they don‘t really tell us a whole lot about the disease biology. But that‘s a question for another day.

Some of the other tests you‘re going to be seeing are the light chains, the serum protein electrophoresis, and the immunofixation electrophoresis. These all together bundled up are the paraproteins. These are the tests that measure how much myeloma there is in the blood. They’re surrogate markers for what‘s going on inside the bone marrow.

For example, in serum protein electrophoresis, a sample from a patient is put on a gel and electricity is applied to it. Because all protein has mass and it has charge, as you apply electricity, proteins in the sample will migrate down the length of the gel, separating according to how big and how electrically charged they are. If there‘s a very big peak in the gamma region, which corresponds to an

abnormal protein, this is described as an M spike. If it‘s in the gamma region, it would be a gamma globulin.

What we‘re going to do then is measure the height of the peak to give you the value that you‘re seeing on the lab test. We can also use an antibody known as the immunofixation to figure out exactly what kind of protein it is. Is it an immunogloubulin (Ig) E, is it an IgG, or is it an IgM? That‘s what immunofixation will tell us.

Now, what is monoclonal protein? You‘re going to hear this all the time. Monoclonal protein not only tells us what the protein is, it tells us about how we can measure MRD. The basic idea is that, in every person, we have normal healthy B cells, and these B cells, as they mature, undergo a process in which their IgH locus, which is the antibody locus of the cell, becomes mutated.

We want these mutations to happen, because for a person to have a nice diverse immune system, you want to have a lot of different cells with a lot of different sequences that ultimately identify a lot of different pathogens—viruses, bacteria, you name it. You want to have a very big polyclonal population of cells that‘s going to be able to identify a lot of different targets, and that‘s healthy. But in a person that has multiple myeloma, what happens is that you get hypermutation, and you develop a specific VDJ sequence in that IgH region. That myeloma cell, now that it has this unique sequence, is going to turn into a clone, it‘s going to become an army, and it’s going to multiply, and these cells are all going to be producing protein. But because they all have the same sequence, they are all making the exact same protein. We call that monoclonal protein, because it comes from a monoclonal cell population.

This concept that each myeloma disease basically is made up of the cells that are expanded with the same sequence is going to be very important for us in the future. But for now, what we see is that if, let‘s say, at the beginning of the journey, we see that there are 5 grams of this monoclonal protein in the blood, and we benchmark that—we do a bone marrow biopsy—and we say that there are 50% plasma cells or abnormal cells in the bone marrow, but we give some treatment. Then, over time, we see that now there‘s only 1 gram of protein there. I don‘t really want to do a bone marrow after every single cycle. You don‘t want to receive a bone marrow after every single cycle. So what we can do then is see that there‘s a fivefold decrease in the amount of protein in the blood, and we see that that corresponds to about 10% bone marrow plasma cells. This is a back-of-the-envelope calculation, but we can benchmark the protein to what we originally had in the bone marrow to give us an idea of how the response is going over time. This helps us get past the need for invasive sampling. This is basically a surrogate marker that we can use.

The next thing to talk about are the light chains. Now, these are tricky, and I only really want to make the point that they're tricky, because you may see a lot of common misinterpretations of these tests. What these are, basically, are a very small component of the antibody that can fall off of the antibody. They can float around in the blood and be measured freely. They're tricky, because they're very sensitive to kidney function, and on top of that, it's hard to confirm that they are clonal, because they're so small and the sequences can overlap. What you basically have is a decent biomarker, but it's not as specific. certainly, It's very sensitive in people that have fluctuating kidney function. The point that I can bring up here—the perfect example, I think—is one of these patients who is being followed for SMM.

What we know is that part of the diagnostic criteria to give somebody a diagnosis of multiple myeloma is to have a ratio of the involved to the uninvolved light chains of over 100. If a person comes to us and that ratio is 117 or 118, that in and of itself, plus having myeloma cells in the bone marrow, should give a diagnosis of multiple myeloma. But if later, at the 7-month time point, the kappa has risen a couple of points and the lambda has risen just 0.1, just one decimal point, that's enough to bring the ratio down under 100.

The reason I bring this up is because it's a disservice for anybody to be diagnosed with multiple myeloma on just this one test at one specific point in time, because it's so sensitive to all of these things—to kidney function, to immune response—that it's tricky to be able to use as a one and done. This is something that needs to be watched over time, because, depending on when you look at it, the person either has multiple myeloma or they don't. Either way, we can still use this to track disease over time.

The reason we measure all of these things together is that we use them for diagnostics to define states of MGUS, SMM, and multiple myeloma. We also use them for risk ratification. Depending on the numbers, what's going on in the bone marrow, we can figure out whether somebody has high risk for each of these conditions or low risk.

Finally, we can use them to measure response. We always want to know how people do once we give them treatment. Measuring these proteins can help us understand how this is going in a longitudinal way. What I want you to see is that, for the traditional response criteria that have been used for many years, a complete remission is defined as having less than 5% of these plasma cells in the bone marrow.

That's not specific, and we can do better. That's where MRD comes in. This is a topic that is extremely hot in multiple myeloma; if you haven't heard it yet, you will soon, because we're very interested in measuring MRD. The reason is that it can get us much more sensitive than just looking under the microscope and counting

the number of plasma cells. So this is a test. There are a couple of ways to measure it. One is next-generation sequencing (NGS). There‘s really just one platform that‘s very commonly used in multiple myeloma these days, that‘s the clonoSEQ test, which can get us down to basically one in a million cells, which is far more sensitive than anyone can do just looking into the microscope.

Every myeloma cell is unique in that it has a shared IgH sequence. We can leverage that. If we measure that sequence at the very beginning of treatment, we can track it over time. That‘s exactly what clonoSEQ does. So even if there‘s very little disease left in the bone marrow, you look under the microscope, there‘s nothing there. You can use this test to measure myeloma cells at a level of one in a million, because it can detect at very small levels the presence or absence of any residual sequence that might still be left over.

Now at face value, when the number of myeloma cells is shown with this test falls to zero, everyone says, “Great! MRD negative!” But the things that you need to be able to take away and always know are that there are some caveats here. For an MRD test to be accurate, you want to see between 2 and 3 million cells evaluated. These tests are so sensitive that the more we give them, the more accurate they‘re going to be. With smaller samples, you can‘t rule out that, if we had given a bigger sample, there might be something there.

The next test to look at is flow cytometry. This is a common test. It‘s one that many different laboratories and many institutions can perform. But because there‘s a lot of difference between some of the different labs in the antibodies that are used, and certainly there‘s a level of technical expertise that‘s required, there‘s sometimes a disagreement between labs and difficulty interpreting labs from different institutions. But the basic way this works is that you take a sample of cells, you apply a bunch of antibodies to them, and each of those antibodies has a color. You run the cells through a column, and you can actually count them one by one by measuring the colors that you see within those cells. This is a test that also can get down to similar levels of sensitivity as the NGS test, as the adaptive test, generally down to the one-in-a-million level, depending on who performs it. The Spanish are particularly good at this, and you‘ll see that in all of the trials they report, they get down to very deep levels measuring MRD there.

MRD tests have to say what the detection limit for the assay is, whether it‘s 0.001% or 10^{-5}, so you know exactly how sensitive it is. They also tell you how many white blood cells—leukocytes—were tested. You want to supply between 2 and 3 million cells, so you have a 10^{-5}, or one in a hundred thousand cell–level detection. When you‘re looking at a result, make sure that when your physician is explaining these things to you, that they go over all of these things, making sure that the number of cells and the limited detection are precisely annotated in the test.

Good MRD tests are really the most important results that you can get a response in multiple myeloma. In a really nice study done by the Spanish, patients getting Velcade + Revlimid + dexamethasone (VRd) plus transplant who were MRD negative were the ones that did quite well, both in progression-free survival (PFS)—in other words, time until disease recurrence—and in overall survival (OS).

Other traditional response markers—complete remission, partial response— all did the same. Showing us that MRD is really the utmost important prognostic marker that we're looking for in response.

There were a few meta-analyses. These were studies where basically multiple different clinical trials are pushed all together and statistically evaluated together, so that we can increase the power to detect a lot of different patterns. Studies done by Dr. Langren and Dr. Munshi showed that patients that were MRD positive at the end of treatment did worse than patients that were MRD negative, again, both for PFS and OS.

Finally, MRD is more complicated, because response over time matters. When we looked at a few patients that were on maintenance, measuring MRD every year, we saw a couple of interesting patterns. The first is that people that are MRD negative on maintenance for 2 years basically did not progress for as long as we followed them. On the other hand, patients that had a conversion or a resurgence of their MRD even after 2 years were patients that did the worst. It really matters that we watch this over time and not hang our hats on a single value.

Some final notes about MRD before we transition to genomics. What we know right now is that MRD is very prognostic at the individual patient level. But there's more difficulty in adopting it as a regulatory end point. We've seen a lot of the clinical trials these days use MRD as a primary end point. The nice thing about that is, first of all, it tells us an answer to the trial very quickly after starting it. We don't have to wait many years for survival. But because of the variations and measurements, and across institutions, it's not quite yet adopted as a full regulatory end point. But again, there is quite a move towards that. Quite a number of trials have used MRD as their primary end point.

How are we going to use MRD in the future? There are a lot of things that we're interested in doing. What we want to know is that not all multiple myeloma is the same, and yet we treat it the same. But MRD after we give therapy can help us determine whether we should intensify or give more treatment or de-escalate. Maybe if a patient got a good MRD result, we can actually slow down on the therapy and not go too hard. It can also tell us maybe whether we should give combined maintenance regimens over time or maybe how long we should give them. For people that have been on maintenance for quite a long time and are

doing really well, maybe simultaneous MRD-negative tests over time can tell us that it‘s time to de-escalate or slow down on therapy, and that can save us on financial and also medical toxicity.

To transition to genomics, I want to make a point about SMM and where we‘re going. Over time, we‘ve seen that SMM, which is an asymptomatic condition, may progress to multiple myeloma. If we give single-agent lenalidomide, we can delay the time until the disease progresses. But what we really want to know is which patients are at the highest risk for progression, because we don‘t want to treat everybody with SMM. It‘s very common, and not everybody needs to be treated.

A lot of different models have been used to determine who is at the highest risk, from the Mayo 2008 to PANGEA. We know from single-arm studies that if we give treatment to people with this high-risk disease, they do pretty well over time. We don‘t know if that‘s because these people are very fit, they‘re asymptomatic, they have no signs of disease, or if it‘s because the criteria that we‘re using to define high risk simply include a mixture of people that are actually doing quite well, along with the people that are actually destined to progress to multiple myeloma.

We did a genomic analysis of a couple of different trials looking at high-risk SMM using whole-genome sequencing and whole-exome sequencing, which are more in-depth NGS tests that look at the DNA of each of these diseases. We also compared this to newly diagnosed or full-fledged multiple myeloma from the CoMMpass study.

As a quick aside, no matter which risk criteria you were using, there was complete disagreement among those risk criteria. In other words, you can try and use a risk criteria from Mayo 2008. You could use a risk criteria from the Mayo 2020 or Athena. There was no agreement between them; whether a patient had one of these or two of them couldn‘t tell you whether they were going to progress once they were given treatment for their high-risk SMM.

But when we use genomics, what we can see is that there are certain combinations of mutations, certain mutational signatures or patterns of mutations, and also complex structural variants that are much better able to pick apart the patients that actually have real high-risk disease and who are going to have the strongest need for therapy.

Dr. Maura will comment on how we can use genomics to get that better granularity into understanding which patients are truly high risk past some of the biomarkers that we‘ve been used in the past.

With that, I‘m going to turn it over to him.

Dr. Francesco Maura: As Ben summarized at the end of his talk, we are moving from blood test measurement of proteins to more accurate measurement of which kind of myeloma patients have, in particular looking at the DNA.

So this is the overall outcome for multiple myeloma; it's not updated. We just overlay the CoMMpass on the historical clinical data from the Mayo Clinic, and what people every time say that myeloma is having a great time. So many drugs, so much research, and it's true. Unfortunately, we still have around 10%, 20% of patients that experience a complete refractoriness to most of our treatment. We still don't know how to approach disease that is more aggressive than others; we don't even know how to recognize it.

On the other hand, there are old studies where patients were treated with what we consider today a suboptimal therapy. Despite the suboptimal therapy, 20% or 30% of patients are still in remission—not just alive but in remission—for more than 10 years. This clearly shows that we don't need to treat every patient with five drugs, CAR T, transplant, and maintenance until disease progression. This patient will not maintain. This patient gets just four cycles of thalidomide, dexamethasone, transplant, and that's it. So there is definitely a need to better recognize this clinical pattern to do something better for these patients and maybe something less for the ones that have good outcome.

So as my colleague Ben Diamond mentioned, we have some prognostic scores in myeloma, the International Scoring System (ISS) plus integration with some of these FISH reports. So we have this translocation 4;14 or deletion 17p that you probably have seen in some of your reports that are usually done at the diagnostic stage. But these, just to make it clear, are relative risk. So when a patient is said to be ISS-2, it means that his values are similar or identical to the one patient in ISS-2. if you put together all these patients in a survival curve, they have this clinical outcome. So this is what we call relative risk; it's not the individual risk of your patient.

If you want to develop a strategy that says this patient has this alteration and this risk, and I'm going to treat with transplant, and this patient had this risk, so I don't give him transplant, these scores are not very useful. Furthermore, in the last 10 years, we have learned so much more about genomics, in particular why multiple myeloma happened, and which patients had genetic alterations that underline multiple myeloma evolution that this course looks quite inadequate to really characterize the disease of each individual patient.

I want to introduce the concept of genomics and why genomics is important. Cancer is a disease of the DNA. The cell become crazy and acquires alteration, genomic damage mutation, and according to the different types of alteration you have, you have different clinical outcomes: patients with one single mutation

have maybe an intermediate outcome, whereas a patient with a lot of alterations in his DNA has very aggressive disease.

If we know the specific alteration for a patient, you can identify new strategies and what‘s called target therapy. So all of a sudden, the patient with the same mutation that was high risk is now intermediate risk, which is very good, because we have an effective therapy. That‘s why we‘re studying DNA and biomarkers.

If you think about how Google or chatGPT works, what we want is to ask these tools is, what‘s the best restaurant in town? We provide information—what the tool knows about us, our Gmail, our information, what we looked at in the past, or where we live. The same can be done for multiple myeloma, and that‘s our overall aim. That‘s where we should go. Every patient that comes to our hospital should be profiled, his information matched, like a classic artificial intelligence approach, to a large database where we have thousands of patients with this information with treatment history and clinical data. Based on this information, we can identify which outcome. This artificial intelligence–based tool or knowledge bank dataset is the future of medicine, and we need to work this also in multiple myeloma. To integrate all the information that matters, we need to identify which are the real drivers, what really is important.

APOBAC is a very complicated topic for genomics. In all multiple myeloma, there is a protein that is basically crazy. Instead of repairing the DNA, it causes mutations. What we found is that more or less all multiple myeloma patients have APOBEC, these mutations. We can detect these mutations using whole-genome sequencing. We know that a group of patients has a very high amount of these mutations. In these patients, this protein APOBEC has gone ballistic and introduced thousands of mutations.

Why does this matter? Because patients that have this very high number of mutations caused by this crazy protein, what we call hyper APOBEC, have a very short survival compared to all the others. We are talking about less than 3 years median OS. These are really a population of very aggressive disease that we need to do better with.

Other alterations that we know can only be detected using DNA and not by FISH or any other tool is, for example, chromothripsis. For example, for chromosome 16, everyone has one copy from his mom and one copy from his dad. In a myeloma patient, this chromosome is completely different, with numerous alterations—pieces of DNA that are broken. This suggests that the multiple myeloma is very complex. This DNA is very unstable. In patients whose DNA is more unstable, is more damaged, survival is much worse.

It‘s not just about the crazy protein or how much your DNA is altered. There are also distinct mutational alterations. Most of the patients in the U.S. that are living with myeloma should or are getting treatment with anti-CD38 antibodies. Patients

with this alteration tend to be refractory to dara-KRd, so anti-CD38 antibodies, or without anti-CD38 antibodies. Because this protein, this gene, is critical for the efficacy of lenalidomide. If you lose this gene, lenalidomide will not work. That‘s why these patients have such an aggressive disease. So it‘s not just marker of tumor instability, but also a specific mutation that we can use to adapt our treatment for patients with alteration; you don‘t want to give lenalidomide, because you will just give toxicity without any efficacy probably.

The second point is how we can improve our approach to immunotherapy. Immunotherapy is what you hear about in every conference on myeloma, because it‘s the hot topic. Immunotherapy works in a way in which we have these monoclonal antibodies, CAR T, and bispecifics that basically move our immune system against the tumor and clear the tumor. To make it work, they need an antigen; they need the protein. If you lose BCMA, the myeloma will be refractory to CAR T against BCMA. They need to interact with the immune escape. If the tumor loses all the interactions with the protein for the immunity, you will not be able to interact. The different T-cell NK cells will not be able to kill your tumor cells.

It‘s very important to understand, because unfortunately multiple myeloma immunotherapy is not curative as, for example, in lymphoma. The immune environment is important, because if you don‘t have a good immune environment before treatment, immunotherapy will not work. So we need to consider these other additional layers. But we also noticed that patients with very aggressive genomic and unstable genomes are usually the ones with an innate immune environment.

Why are DNA alterations important for immunotherapy? One therapy that is currently leading the field of immunotherapy is antibodies and CAR T against anti-GPRC5D, a protein that is expressed in normal multiple myeloma. Again, this is not curative. Most patients relapse after these therapies. Why? Because the tumor is sneaky and basically knocks down, or reduces, or completely loses this alteration, this protein, so the protein is not expressed any more on the surface, and the tumor cannot be killed. That‘s why it progresses. That‘s happened in all patients, more or less, that we know.

We also have another marker that is more famous, probably, to most patients and doctors. That is anti-BCMA CAR T and bispecific antibodies. We have a lot of products right now, but again, this is not a curative therapy. Patients who were previously treated, heavily refractory, have great responses, but they don‘t last, except for a few exceptions. In a study was done by our group in collaboration with Calgary University, we found that BCMA, which is the target of these therapies, is lost in a very small fraction. Myeloma cannot really afford to lose BCMA; BCMA is much more important than GPRC5D. You can think of GPRC5D

as like a piece of hair, and BCMA is like one of your kidneys. BCMA is definitely more important; it‘s very hard to live without BCMA for myeloma.

What we found is that—because it‘s so hard to lose BCMA and myeloma is very sneaky—myeloma acquired mutation on the BCMA that doesn‘t alter the BCMA function but blocks the interaction of the CAR T or bispecific. So the CAR T bispecific proteins interact, and they need to bind this the BCMA, but if the binding site is mutated, then they cannot work. In fact, these patients relapse after acquiring these mutations. Understanding comprehensively the genomics of our tumor at the DNA level is important to understanding why the tumor progress and how to select the best treatment. If you lose BCMA completely, you cannot get another BCMA. But if you get one of these mutations that, for example, confers resistance to teclistamab, you can still be treated with another anti-BCMA if the binding site is different. This is what we call precision medicine and individualized treatment, and that‘s where we should all go.

We want to build a knowledge dataset. To do that, we recently in a collaborative effort between multiple institutions collected all the clinical demographic, ethnic, treatment, and genomic data—basically, everything you need to know about these patients. We did a deep-learning neural network model to understand how we can use this large dataset to predict individual risk—like the chatGPT of myeloma.

What you have in this model is that around 40% of patients progress during treatment with bortezomib or bortezomib and cyclophosphamide. These are the refractory patients. The real aggressive patients. Their outcome was extremely poor, unfortunately. Thanks to this model and how it is built, we can identify these patients better than was done before.

We validated our model, then we took another dataset—HD6 from Heidelberg University, who kindly offered this dataset as a validation. What we found is that the model could predict exactly the outcome of this completely independent population. So we use our 2,000 patients to predict the outcome of each patient in the German dataset.

To come back to genomics, the features that are important to define high-risk myeloma include chromothripsis and APOBEC. We cannot ignore that genetic markers are very important to predict clinical outcome and to define the risk.

The IRMMa risk model for myeloma is able not just to predict the outcome of a single patient, but also to predict 36 possible outcomes according to 36 possible drug combinations. For example, we simulate for each patient in our model what the outcome would be if he was treated with bortezomib, Revlimid, dexamethasone plus transplant, maintenance, or both, or just one of the two, or none. We identified a group of patients, determined their risk, and assessed the outcomes of, say, transplant and no transplant. We can predict that this patient

will have an excellent outcome without transplant. Or for a patient that has a lot of genetic alterations that are bad in myeloma, for instance ISS 3 and deletion 17p, we can see that the patient needs the transplant. These are just examples of how the model could work.

For research purposes, we developed a website where people can have a taste of what could be the future. The output is not easy to interpret; you need to follow the guide that has a lot of technicalities. It‘s not great for people to use the information for their own lives and their own clinical decisions, but it provides an idea that it is possible to develop a chatGPT for multiple myeloma. That‘s why we are working with our collaborators to generate more and more data. The more data we have, the more information we can provide to our patients, with better accuracy. And finally, precision therapy—identifying for each patient which is his or her best treatment that provides the best efficacy with less toxicity.

All this work was a very extensive collaboration with multiple institutions. As I mentioned before, Memorial Sloan Kettering, Heidelberg University, and Moffitt Cancer Center were the best partners we had, but many, many others that I cannot list for the sake of time.

Thank you all for your attention. Happy to answer all the questions.

Mary DeRome: Thank you both for those great presentations. I‘ll ask some of the questions that came in.

There were a couple of people who asked about non-secretory multiple myeloma. Dr. Diamond, can you talk about that?

Dr. Benjamin T. Diamond: Yes, I‘d be happy to. It obviously is a very vexing condition, and a lot of us are trying to understand it better. But basically, when we‘re talking about that monoclonal protein coming from that specific IgH sequence, there are some patients that just frankly don‘t produce any protein. We call this non-secretory disease. The diagnosis of myeloma in these patients can be very difficult, and tracking the disease over time can be very difficult as you can imagine, because we are out of a blood test that we normally use for everybody else. This happens relatively infrequently, but we see it. If you‘re a myeloma specialist, you‘re going to see a decent amount of this.

It‘s not so much that the disease acts any worse than somebody that has secretory disease, it‘s just more challenging to treat because, frankly, it‘s harder to measure. A lot of the time what you‘re stuck with is having to perform repeat imaging and repeat bone marrow biopsies, because you have to go straight to the source to measure how the disease is doing.

We don‘t fully understand just yet why some patients have this versus not. There‘s likely something that turns off the protein secretion for these patients, but we don‘t quite know what it is yet. So just make sure that if you do end up having

it, that it‘s being monitored with some other needs. That‘s not a blood test, for example.

Mary DeRome: There are couple of questions about bone marrow biopsies that were inadequate for the test that was being used. There‘s one patient who was asking about having a defective, original bone marrow biopsy for MRD, and that‘s it‘s helpful to have an original sample to be able to do MRD via the clonoSEQ test. If the patient‘s original bone marrow biopsy was defective, would they still be able to use clonoSEQ, or would like a mass spec test be the next choice?

Dr. Benjamin T. Diamond: I wish I had mentioned this during the talk, but I‘m glad you brought it up.

This is the advantage of having multiple different ways to measure MRD. For the clonoSEQ test, you need to have that baseline sample to establish a sequence. The nice thing is that that sequence can be obtained from multiple different sources. So even if the aspirate, which is the actual blood that gets extracted from that bone marrow is not adequate for the test, sometimes there‘s an archive slide, the actual core that can actually be sent to Adaptive for them to be able to try and find a sequence there.

If all of those things fail and it‘s been a number of years and there‘s just no sample left over, this is where the flow cytometry can be really handy. This is because you don‘t need a baseline sample for this. On any bone marrow you can send flow cytometry, because you‘re not looking for a sequence, you‘re just physically counting the abnormal cells with these antibody probes. If you don‘t have that baseline sequence, flow cytometry of the MRD flow can be a good way to get around.

Mary DeRome: A patient asked about having a bone marrow biopsy that didn‘t have enough information for a FISH test. It‘s pretty well known that genomics from a FISH test is what many people get, but it‘s certainly not the most accurate of genomic tests.

Dr. Maura, can you comment on that?

Dr. Francesco Maura: I want to add one more thing on the question before. For a patient that is in remission, every time the patient progresses, you can still repeat the test and identify the VDJ and the clonoSEQ even if that was not performed in frontline. So not having done the baseline in early samples doesn‘t preclude the patient from monitoring for MRD in the subsequent line.

For this question, you should see FISH like a steam engine. If you want to go from Miami to New York, you can use a steam engine, but you probably want to take an airplane. Genomics is the airplane. The fact that no one does genomics basically means that the information that you can get from FISH is extremely limited. It‘s important to identify 11;14 for the patients that may relapse because,

as Dr. Diamond mentioned, you can access a specific drug that seems to work in 40% of patients with 11;14. That is venetoclax. Also, it‘s important to identify patients with, for example, markers of very high risk, like two extra copies or 1q gain or deletion 17p.

Now, after saying that, if the patient is monitored and has sustained MRD—so an MRD that is negative over time with the current technology that we have—that probably is the best indicator of your risk. So even if you miss the patient in one time point, that will not affect your clinical treatment and your decisions because, as I mentioned, these are relative risk scores. They are not designed to change the treatment of patients. Not yet.

Mary DeRome: Dr. Diamond, we have somebody who‘s writing in on behalf of their aunt who is a myeloma patient seeing a community oncologist. They‘re interested in having an MRD test. How would a person who‘s seeing a community oncologist advocate for having MRD test?

Dr. Benjamin T. Diamond: It can be a little bit of a challenge if, in the community, there‘s not access to the flow cytometry test, which is the easiest way to just send it right at the point of care. If you don‘t have that, then what you need to ask for is the clonoSEQ test. If you haven‘t had the baseline sample sent, and you don‘t have evidence of disease or if there‘s very little amount of disease, you‘ll ask the oncologist to see if they can locate the original sample, which is usually at some lab on archive. Adaptive can actually help a lot of the time in trying to locate that sample and certainly try and acquire that sample, so that they‘re able to do it.

What it takes is having a conversation with the oncologist and seeing if they‘re willing to help track down that sample and help send it to Adaptive. If they need help, they can reach out to Adaptive‘s customer service.

The other way to do it, of course, would be to go somewhere close by at some institution that‘s able to do the flow cytometry test, because there, again, you don‘t need that baseline sample. You can just do it right then and there.

Mary DeRome: Dr. Maura, we‘ve got another patient who asked, is there currently a way to determine MRD status without having a bone marrow biopsy? The answer to that currently is no, unless you‘re in a clinical trial.

Dr. Francesco Maura: Correct. There is no assay that is currently approved to do MRD on the peripheral blood, although there is advanced research suggesting that ultrasensitive technology like mass spectrometry or circulating DNA—can be used to predict MRD. We don‘t yet know the concordance, in terms of accuracy—probably the accuracy will be okay—not as accurate as the bone marrow but with the advantage of using peripheral blood. That‘s why so many groups, including ours, is investigating. Myeloma is a patchy disease. It‘s not only the bone marrow. The myeloma can be in other bones, in the sternum,

in the shoulders, in the femur. Sometimes, the more you treat the disease, the more the disease is not present in the bone marrow and can grow in extra sites.

In these settings, relying only on bone marrow would actually create blindness of the systemic disease. You can be negative in the bone marrow, but you can have the disease growing in your sternum, and this disease will only be captured on the peripheral blood, for example CT DNA technologies. It‘s very important not to think about one single MRD assay, but measurable residual disease—combining multiple technologies, including imaging, to really assess whether the disease is still there or if it‘s coming back.

Mary DeRome: We had another patient, Dr. Diamond, who asked to clarify what is a primary refractory patient.

Dr. Benjamin T. Diamond: These days, thankfully, we‘re not seeing as much primary refractory disease, which is a very feared complication. We‘re trying to come up with better ways to assess risk other than what we have. Because, currently, we‘re assessing risk based on some of these biomarkers that are measured in the blood, plus FISH. Even people with the highest-risk disease tend to respond at least relatively well to the frontline therapies that we offer. A lot of clinical trials report that people have 99% to a 100% response rates.

Now, primary refractory disease will be disease that is not responsive to that initial therapy or that relapses within the confines of that initial few cycles of therapy. Obviously, you don‘t need a FISH test or a blood test to tell you that this is a bad situation. Because this is the kind of myeloma that is not responding to our most powerful induction therapies. That‘s primary refractory disease. it segues into things that are like functional high-risk disease, which are diseases that relapse very quickly after initial treatment. They‘re very similar entities, and this is the area of the highest risk that we really need to be addressing. Our treatments are really good, but they‘re not really good for these patients that have clear resistance to our best therapies, and we need to find some alternate strategies to be able to best serve them.

Mary DeRome: Dr. Maura, here‘s a question for you about the IRMMa model. What are the confidence interval levels for the current sample space used in the IRMMa model, and what is needed in the sample space to further improve that confidence?

Dr. Francesco Maura: That‘s a great question. The confidence interval usually ranges around an accuracy according to different analysis, because there are several analysis in the paper is very dense. But overall, I would say that it is around 0.1 in c-index, which is a lot. That‘s why it‘s still a research tool. Also, the accuracy is around 0.7, so it‘s not like 0.9. It‘s still very high, if you consider that ISS is 0.6. But it‘s not yet there. What we need to make it better, so we need more patients treated with the current drugs like daratumumab in particular and

isatuximab, which are both approved anti-CD38 antibodies. We have very few patients. The more patients you have, the more the model can learn and can predict with accuracy the outcome.

So that‘s first. The second is the model is already designed to include MRD. That‘s something we developed, and the model is a learning model. It is based on machine learning, so the more information you provide in the future, the more the model will learn and increase the accuracy. I expect that over the next few years, thanks to the collaboration we are trying to build, we will able to include more and more cases, including also maybe MRD. That will allow us to probably reach more than 0.8, 0.9. At that point, the model can be tested in clinical trials for development for clinical practice.

Mary DeRome: This is going to be the last question. So this gentleman said, “My mother had myeloma, I have myeloma. What blood test markers should my son monitor prior to having a bone marrow biopsy?”

Dr. Diamond, you can go first.

Dr. Benjamin T. Diamond: This is a difficult situation. Depending on who you ask, you‘re going to get different answers. The traditional teaching is that myeloma can run in families, and when we say run in families, we mean that there‘s a higher risk if a relative has multiple myeloma for an immediate family member to also get multiple myeloma. But that relative risk, although they say it doubles, it only translates to an absolute risk increase of maybe like a fraction of a percent. In truth, there is not a mechanism in place or a recommendation in place for us to screen family members of patients that have multiple myeloma. That being said, some studies have come out where they‘ve looked at CoMMpass data and have seen that there are certain genetic predispositions that may run in families that could help predispose somebody to developing multiple myeloma.

As Dr. Maura alluded to earlier, the development of myeloma is very complicated. It‘s not the product of one specific genetic alteration, it‘s a combination of environmental factors, inflammation, et cetera. So having one single predisposition gene is not going to be enough. That‘s part of the reason why we don‘t see this complete penetrance. But, that being said, if a person is very anxious, you know, sometimes what will end up happening is that the usual blood test can be sent off for that patient, which includes the paraprotein test that normal myeloma patients are going to get. Again, I can‘t recommend for or against that. It‘s a very individualized decision, but those same tests can be sent for family members.

Mary DeRome: Dr. Maura, anything to add?

Dr. Francesco Maura: I agree with Dr. Diamond. There are cases of families where you have a lot of cancers in general, like many, many cancers. We know

that there are certain genes that predispose these patients and these families to have more cancers. One is BRCA, for example, the most famous one, the Angelina Jolie one. In myeloma, we don‘t have the Angelina Jolie gene. We don‘t have BRCA and the family that we have screened, and we participate in some of those studies, they all have a different alteration or genetic alteration. So it‘s very difficult to identify the cause. After saying that, I would say I agree with Dr. Diamond that as a reasonable approach, if two generations have myeloma, and if I was in the third generation, I would probably check my serum protein electrophoresis here and there after age 35, 40, just to make sure and monitor. But that‘s a personal decision. It‘s not based on evidence; it is more like gut feeling like what I would do.

Mary DeRome: I also want to add that there is a study where you can get a free blood test done, which is located at the Dana-Farber Cancer Institute. It is called The Promise Study. So you can go to their website, they will send you a blood kit for free, and you can bring it to a nearby lab near your house, have some blood samples taken and send it off. They will test your blood. Really if you have a first-degree relative who has multiple myeloma, they will test your blood and send back the results of that. There‘s no out-of-pocket cost for that. That might be a good option for people who are interested in something like that.

That is all the time that we have today for questions. I‘d like to thank our audience for their attention and for these great questions that were submitted. I‘d also like to thank our speakers, Dr. Ben Diamond and Dr. Francesco Maura, for their time and their contributions to our program today.