[MUSIC PLAYING]

AMR H. SAWALHA: Thank you for attending this talk about lupus, where I'll be providing some updates regarding the pathogenesis of lupus, the etiology of lupus as we currently understand it, as well as review the clinical aspects of lupus and some of the emerging treatments and newly approved medications that have been shown to work for lupus patients.

So lupus is a systemic autoimmune disease that was first recognized by skin involvement. In fact, the first image of what we now know as the malar rash in Lupus, appeared in the textbook of dermatology by von Hebra in 1856. And von Hebra was actually the first physician who invoked the term butterfly rash, which is another term we use to describe the malar distribution of the malar rash that happens in about 2/3 of Lupus patients.

Now, Kaposi was another dermatologist. He was the first to recognize that lupus is not necessarily only confined to the skin, but can also involve organs within the body to become known as a systemic disease. In fact, the disease is so systemic that it can affect multiple organ systems. And in fact, any organ system potentially can be involved in lupus. Patients with lupus do have the mucocutaneous involvement.

As you can see here, we listed the ACR classification criterias for lupus that recognizes the malar rash and the discoid rash. And I'll show you some pictures of that later on. Photosensitivity, oral ulcers. And patients with lupus can also get arthritis, although the joint involvement of lupus tends to be deforming, nonerosive type of arthritis. Unlike what we see in rheumatoid arthritis, for example.

Patients can get serositis. Serositis primarily pleurisy or pericarditis and pericardial effusions. Renal involvement is a major complication in lupus patients. And that's one of the most important complications to screen for in patients and make sure that we detect it early. If it happens, to try to prevent kidney damage and chronic renal insufficiency.

Patients with lupus also can present with a variety of neurologic manifestations that can range from cognitive dysfunction to different types of headaches to even mental fogginess or depression and psychosis or even seizures. And there's a lot of other neurologic manifestations that are becoming more recognized in lupus. Lupus is an autoimmune disease, so it's not surprising that there is an immunity component to it.

And almost all lupus patients, if not all lupus patients have antibodies to the two antigens that are in the nucleus, which we call antinuclear antibodies. And some of the most common ones. There are hundreds of them. There are hundreds of antinuclear antibody specificities in lupus. And some of the more common ones that we test for in the clinical laboratory are shown in this box over here.

So I mentioned classification criteria for lupus. And this is really important because classification criteria is not diagnostic criteria. We do not use the ACR or SLICC or the ACR, UNR, the new 2019 criteria to diagnose lupus. The diagnosis is clinical. Only an experienced clinician or rheumatologist can make a diagnosis. And the classification criteria help to classify patients for research purposes.

In fact, this is the reason why these classification criteria were put together to uniform our approach to enrolling patients in research, whether it be clinical research or transertional or even basic research. We use these criteria for research purposes only. Classification criteria.

Now, there are three main sets of classification criteria for lupus. There are more than the three main ones that you've probably heard about, or the ACR of criteria, which were modified in 1997. So we know them as the modified 1997 ACR classification criteria for systemic lupus erythematosus. The SLICC criteria put together in 2012 and then the EULAR/ACR criteria in 2019.

And you can see here the differences in terms of sensitivity and specificity between the different criterias in both the derivation cohorts, as well as the validation cohorts in the data that were used to derive and validate the 2019 classification criteria. Lupus is very heterogeneous. That's why it's important to classify it for research purposes.

Lupus rashes that I mentioned before include a malar rash that you can see here. , First, the involvement of the malar eminence sparing, of the nasal labial folds. It's typical for malar involvement in lupus patients with malar rash. And it's important to know that this is a photosensitive rash, so it gets worse on sun exposure. It's very important to prevent sun exposure as much as we can in lupus patients.

Because not only does it worsen the skin involvement, but it can also be associated with a systemic flare up of the disease, including even renal flare up in the disease. Patients can have photosensitivity, as we can see in this picture in the upper left side. And then, subacute cutaneous lupus erythematosus, as seen.

There are two different forms. There's a psoriasis form here. And then there is a annular form that can happen in patients with subacute cutaneous lupus. Subacute cutaneous lupus is associated with the presence of antiviral antibodies, for example. So there is some importance of knowing the antibody specificities in lupus patients because they can-- some of them at least-- predict or inform about the possible clinical involvement that we see in this disease.

This is an example of discoid rash involving the scalp on the far upper right here. This rash, discoid involvement is deeper. It's more intense in terms of inflammation. And because it's deep inflammation in the skin, it will lead to scarring, permanent hair loss as it involves the scalp, and discoloration.

Patients can get nephritis, glomerulonephritis. And I'll talk a little more about that later on. Patients can have arthritis. Again, this is nondeforming arthritis, nonerosive arthritis. And like we see in other inflammatory arthritides like rheumatoid arthritis. And then, oral ulcers. You can see an example in the middle right panel here.

And then, despite the variability in the clinical presentation of the disease, again, almost all-- if not all lupus patients-- have antinuclear antibodies as shown in the lower panels here, that we test for the HEp-2 cell lines. And we can see evidence of antigen antibody complex position in organs involved with lupus, such as the glomerulonephritis immunofluorescence picture shown in the lower left panel here.

And we do different types of tests to identify the specific antibodies that are present in each lupus patient. Because as I mentioned earlier, the various antibodies can predict-- to some extent-- how complicated lupus might become, how much organs can be involved, and so on and so forth. This is an example of an ouchterlony plate, which is a traditional way to look at the immunoprecipitation lines in patients using patient serum. And of course, no other methods have been used to detect these antigen and antibody specificities in lupus patients.

So at UPMC, within the Lupus Center of Excellence, we have a lupus cohort, which now exceeds 1,000 patients. Recently, we reported upon over 700 patients who meet the ACR classification criteria for the disease. And we used clinical clustering analysis to try to come up with clinical sub-clusters of the disease. They mentioned it's a heterogeneous disease, and therefore patients can present with a variety of combinations of different manifestations.

And it turned out that our patients, at least here in Western Pennsylvania, in our cohort, cluster in one of three possible sub-clusters. And we have a cluster here we called cluster 1. That's characterized by discoid rash and renal involvement, hematologic disease. And, in a sense, it's a more severe form of lupus. And so not surprisingly, this cluster's enriched in male patients who tend to have a more severe disease than females when affected.

Even though lupus is much less common in men. It tends to be more severe when they're affected. And then African-American patients are also enriched in this cluster. And as we know, African-American individuals tend to have a more severe disease in lupus.

The second cluster is characterized by malar rash and photosensitivity. And third cluster by oral ulcers. And both clusters 2 and cluster 3 also tend to have more arthritis than we see in cluster 1, for example. And so it turns out that if we look at correlation between the various manifestations of lupus, and this is correlation coefficient here. Blue means positive correlation and the more red, it's negative correlation. There is actually a protective effect for having photosensitivity or oral ulcers against the development of lupus nephritis.

So patients and cluster 2 and cluster 3 are protected, in a sense, from lupus nephritis compared to cluster 1. And it seems like the presence of oral ulcers and photosensitivity in our cohort predicts a less severe disease and deaths here in Western Pennsylvania. This nephritis, as I mentioned, is one of the complications for lupus that we are most interested in preventing, detecting early and treating aggressively to prevent interstitial disease.

And lupus nephritis comes in a variety of flavors. We have different classification system for lupus nephritis that depends on the type and the degree of inflammation within the glomerulus. The most severe forms involve what we call proliferative lupus nephritis, where you have increased proliferation and similarities within the glomerulus and by proliferative.

Yet really, what we mean, is proliferation that leads to obliteration of the capillaries within the glomerulus, which then will render these glomeruli ineffective in performing their functions. And histologically, we see evidence of benign nephritis. But we also, to confirm that the nephritis we see on biopsies is actually likely from lupus, we always do immunofluorescence looking for antigen or antibodies complexes. And in lupus, what we see is a pattern that we call a full house pattern, where you can see immunofluorescence for almost everything you look for in terms of different types of immunoglobulins, complements, and so on and so forth.

Now, one thing we don't necessarily always talk about when we talk about the classification of glomerulonephitis in lupus nephritis is how much tubular interstitial inflammation there, which is not part of the classification system we use for glomerulonephitis or for the lupus nephritis. But it turned out that actually the degree of inflammation in the interstitian, so around the renal tubules, is a major predictor of the outcome in lupus nephritis patients.

And in fact, recent data using single celled RNA sequencing approaches where a kidney biopsy was taken from lupus patients can be looked at at the single cell level by doing RD sequencing at a single cell level. We can actually distinguish based on the transcriptional profiles, based on the MRNAs presence in each cell, what type of cells you're looking at. But what type of anatomic [INAUDIBLE] do they belong to?

Proximal, tubular cells, for example, are clustering or differentiated or we were able to see them separate from loop of Henle cells and from [INAUDIBLE], for example. Or distal tubular cells in red here. And it turns out that the tubular cells-- and I'm not talking about inflammatory cells that are present in glomerulonephritis and lupus nephritis. Just talking about the actual tubular cells.

It turns out that they actually turned up the inflammatory transcriptional profiles in lupus nephritis, including overexpression of interferon at regulated genes, which is something that know of happens in the peripheral blood and other immune cells in lupus patients. And it turns out that in patients who have higher transcriptional up regulations interferon regulated genes, there is evidence that they tend to be less responsive to treatment of lupus nephritis. And patients who have lower interferon score or transcriptional profile in the kidney tubular cells are more likely to be responsive to our treatments for lupus nephritis. So again, the tubular interstitial involvement is critical and we have to look at it in the context of lupus nephritis in addition to also looking at [INAUDIBLE] life.

So if we think about genealogy and the pathogenesis of lupus, the current paradigm suggests that this starts with genetic predisposition. Which makes sense. We're born with our genes. Were born with the genetic variants that we have. And there are hundreds of genetic susceptibility [INAUDIBLE] that I'll talk about in more detail that have been described in lupus.

So we are born with a genetic predisposition and then we think that on top of that, we might need an environmental trigger. That environmental trigger might be an infection. There is evidence for involvement of Epstein-Barr virus, for example, in lupus. Or it might be a chemical. Or it might be an environmental agent that we still have to invest to actually recognize in certain individuals who then attempt to develop lupus.

And then after that, after having the genetic predisposition the environmental triggers that are necessary to develop lupus, then there's evidence for immunity. Serologic immunity. Or the presence of autoimmunity, and by itself, before the development of pathologic injury that results from the position of the antigen activity complexes in

addition to other immune disregulatory pathways that are involved in lupus. And then after that, we get clinical disease. So autoimmunity first, and then we have clinical disease. And then patients will present with a variety of manifestations that we described earlier.

In fact, there is strong evidence that systemic autoimmunity and lupus starts with a serologic involvement. Starts with the presence of antibodies that can happen years before the clinical disease presents. This is a classic paper from [INAUDIBLE] in Oklahoma, Oklahoma Medical Research Foundation, led by Judith James and John Harly with Arbuckle as the first author, showing the development of [INAUDIBLE] antibodies years before the appearance of the first clinical manifestation of lupus and before patients fulfill the classification criteria of the disease.

In terms of epidemiology, lupus is a disease that tends to be more common in women and tends to also be more common, and more severe, in minority populations. Namely African-Americans as well as Hispanics. And so if you look at epidemiologic studies, lupus-- this is a landmark study from the Michigan Lupus Epidemiology and Surveillance program, MILES that was published relatively recently-- looking at incidents on the left hand side and prevalence on the right hand side of lupus across the different populations. Black, white, male, and female.

And what we see is that the highest incidence for lupus, and this is per 100,000, highest incidence is in African-American women followed by European-American women. But we also see is that the disease onset in African-American women tends to be a decade earlier compared to European-American women. The peak onset as you can see here. And that's very important, because lupus, when it starts earlier, we know it's more severe. So the earlier the disease starts, the more severe the course of this is. And so lupus is probably more severe in African-Americans because, in a sense, it starts early. And I'll come to explain a little bit of why we think lupus tends to start early, or is more severe therefor in African-Americans in the subsequent slides.

You can also see that the disease. This is a prevalence and the mid-western United States, which is probably going to reflect the incidence and prevalence of the disease in our region here in western Pennsylvania. So one in about 500 African-American woman, one in about 1,000 in white or European-American woman. And then the prevalence in male patients is about nine to 10 times less compared to women.

So I talked about genetics and the genetic etiology of lupus a couple of times. And so let me walk you through how we got to the knowledge of the lupus genetics that we have currently. So before 2008, we were doing a lot of [INAUDIBLE] gene studies. And then the GWAS, first Genome wide Association Study in lupus, started in 2008. And then you can see the number of confirmed loci, genetic severity of loci for lupus, really increased significantly. And we have well over 100 loci now.

In fact, the first indication that lupus might have a genetic component came from familial aggregation reports. Lupus tends to be aggregated in families. You have one family member affected with lupus, you're more likely to have lupus than the general population. And that was in the 1950s. And then came the twin concordant studies, which also suggested a higher concordance rate in monozygotic twins compared to dizygotic twins and lupus. Then the linkage studies, which was before we were doing candidate gene studies and snip based studies on GWAS that I described earlier. And all of that resulted in pretty much identifying a large number of genes and therefore, pathways involved and pathogenesis of the disease that will help us to understand the etiology of lupus.

This is an example of a recent genetic study in lupus that involves over 27,000 individuals, including European, and African-Americans, and Hispanic Americans. And what you see here is what we call the Manhattan plot where you see the number of chromosomes here on the x-axis. With the variants represented by dots. Each dot on this figure represents a genetic variant across these different chromosomes. And then the y-axis is the minus log 10 p values. So the higher the number here, the more significant the genetic association. And we call this the Manhattan plot because it looks like buildings in the skyline of Manhattan.

And so in a sense, you can see that everything here this is five times 10 to the minus eight, which is our threshold for GWAS level of significance. You can see that everything above this grey line actually is a susceptibility locus for lupus that we consider to be established for the disease.

So if we take the over 100, 110, 120 now, genetic susceptibility loci discovered for the disease and try to cluster them into what we call meta groups, functional analysis, to try to come up with what pathways and what functional categories they may reflect to help us understand the disease better. And I've done that in over about 110 of these loci. You can see that the interference signature we talked about is represented also at the genetic level by a number of genetic susceptibility loci for the disease.

Involvement of T cell responses and dysfunction, therefore, of T cell immune responses is involved in the genes that we found to be associated with lupus. Cell adhesion, JAK-STAT signaling it's also involved. Phagocytosis-related genes, and allograft rejection cytokine activity related genes. Cell response genes. TLR signaling. And again, Type-I interferon. So in a sense, you get that the genetic susceptibility loci for lupus tend to show us the type and the pathways within the immune response that might be associated with the development of the disease.

And the genetic risk for lupus is really not linear. Meaning it's not that the more genetic risk loci for lupus, the more likely you're going to get the disease. But this relationship is not linear. You can see here in the example. Odds ratios developing lupus on the y-axis. The genetic risk, in a sense, that we derive from this genetic susceptibility loci is weighted here based on the effect size of each locus on the x-axis.

And so the higher the weighted sum variants, the higher the odds of developing lupus. But then all of a sudden, there's an exponential very, very steep exponential phase of this graph or this relationship, worthy even a small increase in the number of genetic risk score here is associated with a significantly higher odds of developing lupus. So in a sense, there is a threshold that once you pass from a genetic standpoint, you increase your risk for lupus. And that threshold can be lowered significantly if you have a high genetic risk for the disease in certain situations.

And so why do African-American individuals have more prevalence of incidence of lupus? So we thought maybe it's the genetics. What we did here in the study, we looked at a relatively small number of patients. But you can see that even with a relatively small number of patients, African-Americans in blue dots and European-Americans in red squares here, genetic risk plotted on the y-axis. These are the patients.

You can see that even with a small number of patients, we can see a significantly higher genetic risk in African-Americans compared to European-Americans for lupus, which might, in part, explain why the disease is more common in African-Americans and it's more severe. And I mentioned earlier that the disease tends to be more severe in younger individuals when it starts early. So the earlier the onset of the disease, the more severe the disease. And childhood onset disease, therefore is even more severe than adult onset disease symptoms.

And so what we did here we looked at a number of individuals affected by lupus. Children, as well as adults. And plotted the relationship between the age of onset of lupus and the number of risk alleles, the genetic risk score, in a sense. The genetic risk present in each individual. And you can see that the higher genetic risk, the lower is the age of disease. Onset, or the earlier, the disease onset. And so in individuals with high genetic risk, that is the disease starts early, and as the genetic risk goes down, the disease tends to happen late in life.

And it actually makes sense. Because if you have high enough genetic risk, then you don't need much environmental triggers to develop the disease. So you develop it early. But if you have low genetic risk, then you need to be exposed to environmental triggers, maybe for a long period of time, to be able to develop that risk that puts you above that threshold to develop the disease. At least that's our working hypothesis, based on our data.

And I mentioned that childhood onset disease is more severe. And you can see this is, again, our data here looking at individuals. Over 100 childhood onset lupus. And childhood onset defined by this study is less than 18. And over 1,200 adult onset lupus, again, with an age of onset more than 18. And you can see the proteinuria, selective lupus nephritis again, signifying a significant, or more severe disease, significantly more common in the earlier and the later onset disease.

And even in adult onset disease, those who have onset between 18 and 49, have a more severe disease, as you can see here, compared to patients who develop the disease at a later time point in life.

So we talked about male lupus being more severe. And the question is why? Why is male lupus more severe? Is it the hormones? Or is it something else? Or is it genetics? So this is a really nice study from again the group in Oklahoma, which did a lot of work on the genetics of lupus. And what they did here is they wanted to answer this very question. And they looked at a group of male lupus patients that come from families who also have female lupus patients within the same family. Compare them to female lupus patients who do not have a male lupus patients in the same family.

And so look at nephritis here. So this is proteinuria, cellular casts, [INAUDIBLE] classification criteria for [INAUDIBLE] involved with lupus. And you can see that in both males and females, the frequency of proteinuria and cellular casts is actually similar between males and females within the same family.

However this is significantly higher, compared to females, who don't have a male relative with lupus. Suggesting that the reason why males have a more severe disease is not because they're males, but because they come from a family that have males affected. Meaning that it's genetics. Because the females from the same families, in this case, have the same, if not even a little higher, frequency of the higher severity of the disease.

So what we did to test this hypothesis a few years ago, we looked at the group of patients, 344 affected individuals, and over 1,100 controls. Again, males. And then we had a group of almost 3,600 female lupus patients and 2,300 controls and looked at the genetic risk. Again, we calculate the genetic risk score that we weigh based on the effect size.

And you can see very clearly how the curve is shifted for men to the right compared to women, meaning that there is a significantly higher genetic risk, on average, in men compared to women with lupus. And that, again, suggests that the reason why lupus might be more severe in men is because we have a higher genetic risk. And the reason, also, why lupus is less common in men is probably also because they have to have a higher genetic risk to develop the disease. So there is a higher threshold to cross. But once you cross it, then you get a more severe disease. At least that's our hypothesis in that sense.

And then what we want to learn from genetics is whether or not it can help us at the clinic. And so what we did here we studied thousands of patients across ethnicities, as you can see in the lower left panel here. And we decided to look at the different genetic susceptibility loci that have been discovered for lupus and determine whether or not there are some of these [INAUDIBLE] polymorphisms more common in different subgroups of patients.

What we found is, for example, the presence of polymorphisms, lupus associated polymorphism TNFS4 and ITGAM was associated with more renal disease in lupus patients. The presence of STAT4, more oral ulcers. ITGAM [INAUDIBLE] discoid rash.

So there is a potential, in a sense, to use some of the genetic information that is being generated, or has been generated, to help us predict which manifestations lupus patients might develop. And that's something that potentially can be very helpful in the clinic.

And rarely, we see what we call monogenic lupus. So what I mentioned so far about the genetics of lupus is this polygenic lupus, where we're talking about a hundred plus genetic polymorphisms. And a number of combination of which probably are likely needed to develop lupus in individuals.

But there are certain mutations. So rare polymorphisms mutations, less than 1%, that's how we define a mutation, that can by, themselves, lead to the development of lupus. So they're in critical genes. They're critical mutations lead to protein dysfunction in these affected genes that may result in the development of lupus, even in the absence of anything else from the environment on our genetic influences. These are rare, but I've summarized some of this data here in this figure and also in this table with the inheritance model of these various first mutations.

We talked a little bit about the translational impact of uncovering the genetics of lupus, or complex autoimmune diseases in general. And what we hope to do from the genetic information is to understand the mechanisms, as I alluded to earlier in the talk. What pathways, what type of immune disregulation is involved.

But in addition to that, we want to understand the heterogeneity of the disease. Why some lupus patients develop nephritis and others don't. Why some lupus patients have arthritis and others don't. And so on and so forth. If used and successfully use the genetic information to understand heterogeneity of the disease, then we can monitor the patients in a way that is tailored to their genetic risk or to their predicted organ involvement.

We want to also potentially be able to use the genetics diagnostically. We're not there yet, but that's the hope at some point. We want to use the genetic information that will show us the specific pathways and molecules and functional groups that are involved to target those specific pathways and molecules, and so ON for treatment.

And we also hope that some of these polymorphisms might predict which specific treatment patients might respond to. And there's a lot of heterogeneity in the disease. So if we have a tool to tell us what type of treatment will work best for which patient, then that will help us tremendously in reducing non-responsiveness in lupus treatments and be more efficient in preventing disease complications.

The disease, as I mentioned, is heterogeneous. And it's not only heterogeneous and the clinical level, but it's also heterogeneous at the transcriptional, or molecular, level. This is a landmark study that shows the transcriptional signatures, or transcriptional changes that's happened, in lupus patients' blood cells as a disease flares. So the study included a number of patients. They were able to have multiple time points across the disease course. And then at each time point, they did RNA evaluation of the transcriptional profile of the RNA profile in these individuals.

And see which transcripts correlate with the disease flare. Or the disease becoming more severe over time. And it turns out that these transcripts are not the same in every lupus patients. And they're actually different. And they can classify lupus into seven different subgroups based on what transcriptional signature they will have as the disease flares.

For example, you'll have a subset of lupus patients with an interferon signature. So meaning that the interferon regulated genes tend to go up as a disease flares. But then there are other groups of lupus patients who do not have an interferon signature flare. For example, patients who have less plasma blast signatures. So they have more plasma blast-related genes that go up as a disease flare. Or a lymphoid lineage signature. And so on, and so forth.

So in a sense, lupus is heterogeneous at a molecular level. And if we know up front what type of molecular subgroup lupus patients belong to, then we can tailor treatments accordingly. And for example, it doesn't make too much sense to use a treatment that targets the pathway that is not necessarily involved in the flare in that individual lupus patient that you're treating. So that's something to keep in mind, and hopefully, we can apply this clinically in the future.

So in terms of treatments that we have available for lupus. Obviously, there are many treatments that we use in lupus patients. But to mention some and go over some of the key recent clinical data available, I will list here a summary of the categories of treatments we have. Antimalarials, specifically hydroxychloroquine has been used for many, many years in treating lupus. And it's something that I will mention a little more about. But in a sense, lacrimal is so good, or hydroxychloroquine is so good as a treatment in lupus patients that I think every single lupus patient has to be on hydroxychloroquine unless contraindicated. And I'll show you why I'm saying this.

Steroids. If we use steroids, we usually use them if a disease is flaring up because it's really the fastest thing that works to suppress the immune system. It's fastest to suppress an active lupus nephritis as immunosuppressants take effect, for example. But when we use steroids, we use them sparingly in lupus. We tend to want to use the lowest needed dose for the shortest duration of time possible because steroids are such a significant side effects, as you know.

We use a variety of immunosuppressants in lupus. Azathiprine, for example. Mycophenlolate mofetil we use for lupus nephritis And cyclophosphamide. We're using less of that now because of mycophenlolate effectiveness against lupus nephritis in a good proportion of lupus patients. Methotrexate and other immunosuppressants are also used. And then we have B-cell targeting biologics. And I'll mention some of the newer ones as we speak.

So back to hydroxychloroquine. Why do I say that every single lupus patient should be on hydroxychloroquine unless contraindicated? Because it has all these beneficial effects that are summarized on the slide. Hydroxychloroquine has been shown to reduce lupus flares, reduce organ complications, including lupus nephritis, recurrence of kidney involvement in lupus, reducing even lipids and thrombosis. this becomes especially important in patients with anti phospholipid syndrome, for example. But also because lupus patients are more likely, much more likely, to have cardiovascular disease than the general population.

So it's important to have a treatment that mostly helps in that regard. Improves blood sugar control. Even treatment failure in lupus nephritis. Whether patients received cyclophosphamide or mycophenolate. Regardless of what treatment arm they were on in the [INAUDIBLE] trial, for example treatment responsiveness was better in patients who had Plaquenil or hydroxychloroquine as part of their treatment regimen. And overall, it improved survival in lupus patients. So hydroxychloroquine, hydroxychloroquine, hydroxychloroquine. Every single lupus patients should be on it unless contraindicated.

So some of the newer treatments target B-cells as I mentioned earlier. One of the molecules that we target, and you've heard about more actually that even more recently, is this molecule called the lymphocyte stimulator, or BAFF/BLyS, list or BAFF.

This is, as the name indicates, a B-cell stimulator. It works to a number of different receptors to result in B-cell activation in a sense. Increase B-cell maturation and survival, class switching, and so on.

And so the data from involvement of BLyS in lupus come initially from my studies. It turns out that if you overexpress BLyS in mice, mice developed a lupus-like disease. They get glomerulonephitis, they get the antibodies. And it turns out that if you look at some of the commonly studied lupus mouse models, namely the [INAUDIBLE] PR mouse and the NZBW mouse, both of these mice tend to have high levels of BLyS in the circulation. And if you block it, the disease gets better.

And so based on this data, the initial studies done on BLyS, looked at this expression-- or BLyS presence in the serum. Concentration of BLyS in the serum. Comparing lupus patients, this is one cohort and then a second cohort. Comparing them to a normal control. And it turns out that BLyS was actually also more present in higher concentrations in the serum of lupus patients compared to controls.

So then, based on the data, and the mouse that I mentioned earlier, it makes sense to develop a way to target BLyS. And this is called belimumab, which is a fully humanized anti-BLyS monoclonal antibody and which actually inhibits soluble BLyS. And there were two landmark phase three trials, BLyS 52 and BLyS 76, that were done. And both met their primary endpoint of reducing glucoses activity based on a complex measure we call SRI. So they actually developed for the purposes of these trials. And SRI stands for SLE Responder Index. And you can see at the bottom here that's defined.

And so not only that this trial number of secondary endpoints were also met, that are also listed here. And based on that, belimumab was the first FDA-approved treatment for lupus in 60 years. About 60 years, which is a major, major landmark in the treatment of lupus.

And more recently, even though the study in children was not powered to detect statistically significant difference, because it's harder to do studies on children. There's less clinical trials than in children with lupus. But nonetheless, there was a difference between belimumab and placebo. And based off that, even though the difference was not statistically significant, the treatment with belimumab was approved by the FDA as the first FDA approved treatment for lupus in children.

Anifrolumab, which is another newly approved or newly shown treatment to be effective in lupus, this is a molecule that targets the interferon receptor. That talked a lot about type-I interferon, interferon signature in lupus. And so the interferon receptor that is shown here is a common receptor to a number of type one interferons. So instead of blocking each one of these interferons separately and trying to guess which one is more important, anifrolumab was designed to target the receptor.

So in a sense, you can block the effect of all of these type one interferons. And so this was tested in two phase three trials, TULIP one and TULIP two. And even though TULIP one did not meet its primary endpoint, TULIP two did. And so we were able to show that patients who were treated with anifrolumab had a higher response rate compared to patients treated with placebo. And this is, again, another composite measure for disease and activity in lupus called BICLA. Which is based on the BILAG. And again, it's a composite measure defined here at the lower portion of the slide. But this was a landmark study, again showing the effect of anifrolumab to treat this patient.

And it's not only that anifrolumab was also shown to be effective in improving skin involvement in lupus. And even though I mentioned once that TULIP one failed to meet its primary endpoint, it actually did meet the endpoint of improving skin involvement. So we have both TULIP one and TULIP two show positive effect for anifrolumab against lupus this can [INAUDIBLE].

More recently, even more recently, there was a study adding on belimumab, which we discussed earlier, but added to treatment in lupus nephritis to see effectiveness, or response, at the renal level. And so you can see here. This was recently published in the New England Journal of Medicine. Standard of care for lupus nephritis, which is either mycophenolate or cyclophosphamide, plus belimumab, versus standard of care plus placebo. And you can see that there was a significant effect in terms of renal response in patients on belimuab. And so based on that data, the FDA approved belimumab as an add on therapy in lupus nephritis.

Now there is a caveat here. There is a difference, there is an improvement, in outcome. However, minority populations were underrepresented in this trial. So Hispanic and African-American patients were underrepresented and they tend to have a more severe lupus nephritis as we know. And so it's important to take this into consideration when applying data from clinical trials into practice. But at the end of the day, adding belimumab did improve the response in patients with lupus nephritis to a significant extent.

The other successful phase three trial in lupus nephritis is voclosporin. Voclosporin is a calcineurin inhibitor. It's very similar to cyclosporin in terms of structure with a really minor change. But that change results in a more potent effect and at the same time more stability at the pharmacodynamic level and cyclosporin. And so this was, again, an add on design. So obviously, you have to treat lupus nephritis this with what we know works for lupus nephritis. So standard of care plus placebo or standard of care plus voclosporin. And here, we see a very significant difference really. 40.8% compared to 22.5% in the placebo arm in terms of renal response.

And this trial resulted in FDA approval for voclosporin and lupus nephritis as an add on therapy. And this trial did actually include about a third of patients were Hispanic, which is great. Now if you look at the phase two data of the voclosporin, there is actually increased mortality in the voclosporin arm. Which, fortunately, we did not see in the phase three trial. So there was no change in mortality-- the difference in mortality between placebo and voclosporin in phase three. But you have to keep in mind that the phase two triall did shrink mortality. So these are some of the details that one has to consider when trying to decide which treatment to use in treating individual lupus patients.

So the challenges in lupus clinical trials stem from multiple factors. As I mentioned, the disease is heterogeneous or heterogeneous clinically. Heterogeneous in terms of disease severity. Also at the molecular level. And we potentially can do better in terms of reaching better trials outcome if we take the heterogeneity of the disease, including heterogeneity into the design of the clinical trials. Background polypharmacy that our lupus patients are on can complicate the interpretation of some of these clinical trials, adding to the challenges in trials in lupus.

And then we don't have a perfect activity measure in lupus. And so the reliability of the primary endpoint for clinical trials and what endpoint to select remains a challenge. I've shown you data on-- I told you to if one failed to use a different response-- primary endpoint response than TULIP two that succeeded. So SRI failed in TULIP one. But BICLA, which is the other composite response measure was used, and was successful in TULIP two in achieving a successful result. So there is work to be done to find the perfect endpoint for clinical trials in lupus.

And then, there's really a limited number of patients in trained centers, like ours for example the lupus center, the UPMC Lupus Center of Excellence. We are a training center, can do clinical trials. There's really a limited number of centers like this to be able to enroll patients to reach enough sample size for a clinical trial to be successful.

There are a number of other molecules that are being targeted in different clinical trials as we speak. Some of them are summarized here. Many are focused on targeting cytokines and others are focused on the T-cell B-cell reaction. And also B-cell response in lupus. And we shall see if we have even more molecular targets that are successful in future clinical trials in lupus.

And so we at UPMC would like to have the opportunity to take care of your lupus patients. We provide integrated state of the art clinical care for lupus patients, so feel free to refer your patients to us. Can see our address, our phone numbers, fax, and also the email of the administrative coordinator. You can email me directly as the director of the Lupus Center of Excellence at UPMC and we'll be happy to take care of your lupus patients. So thank you very much.