Advances in Immunotherapies and Tolerance

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[00:00:04] **Dr. Michael Yeaman:** Hello, everyone. Hope the meeting is going great. I'm sorry I'm not able to attend in person, but really delighted to have a chance to chat with you today. In the interest of time, I'm going to go pretty quickly to review Advances in Immunotherapy & Tolerization. It's a very exciting field and one that we've been working on for quite a while in the background, and now it's really beginning to emerge in the foreground.

[00:00:35] So let me just dive right in. The presentation is going to be, as I say, very much of an overview but happy to talk with anybody who'd like to learn more about this. Here are my disclosures. It has been a pleasure for me to work with the Guthy-Jackson Charitable Foundation for more than 15 years toward understanding these diseases, in particular NMO, and trying to really advance some of the solutions that we'll talk about today.

[00:01:07] So very briefly, what I'd like to just touch on are four points. First, the revolution in NMO research and therapeutic landscape that has been the focus of the work that many of us have been doing, but it's a model for what is to come. And I'll touch on that a little bit. There has been a lot of great progress in therapeutics, but there are still unmet needs and they have to do with burdens and risks. And I'll talk a little bit about that.

[00:01:38] We really can't talk about immune tolerance and tolerization without a crash course in these topics, and so hopefully we'll do that in a way that's very relatable and you'll be able to tell everybody that you know all about these topics. And lastly, just a quick update on how the field is accelerating toward antigen-specific tolerization cures. So let's just go right in here.

[00:02:03] First, a quick review of the NMO revolution. We like to talk about what's happened in this field because it's a reminder of I think what Shakespeare said, which is, "Past is prologue." And I think we are entering a new era that will reflect the kinds of advances that you see here. This is just in the last 15 or 16 years where you can see a lot of effort and resources went into the field in such a way that strongly promoted publications, collaborations, and international diversity focused on NMO and NMOSD.

[00:02:42] And there were several milestones that includedsome of the work that Guthy-Jackson Charitable Foundation began. Its CIRCLES study was launched, clinical trials began, the IPND criteria emerged, clinical

trials continued, three approved therapies in 2019, 2020, with a fourth in 2024. Over that time, 9,300 papers, I think there were about 100 back 15 years ago. So a lot of great activity and success as measured by these variables.

[00:03:14] If you look at it in terms of what has this done to improve clinical care, I think you can see here that, back in 2008, the mean global prevalence was considered to be about one in a million. In 2015, maybe more like 1 in a 100, 000. Today, it's up to 7 in a 100, 000. So there's much greater recognition of NMOSD around the world.

[00:03:40] Are we happy that there are more patients diagnosed? No. But if they are having this disease, we want them diagnosed quickly and accurately. And you can see the rate of misdiagnosis has dropped considerably. As has the annual relapse rate, largely in part due to advances in diagnosis and clinical awareness and clinical care. But, of course, the new highly effective therapies are beginning to really shape these numbers, and you can see there's been a drop by about tenfold in annual relapse rate over the last 15 years.

[00:04:17] The clinical trial landscape, I think Michael just reviewed this but, went from zero over a 100 years of no clinical trials and no approved therapies to a number of clinical trials that has led us to four approved therapies today. So, incredibly amazing progress, but there's more to do. And I'm just going to summarize the science that has occurred over the last 15 years or so that has been the backdrop to a lot of this work. This is a very high level summary, so pardon if I'm not going to go into a lot of details. But for NMO, at least as a model, astrocytes express aquaporin-4 on their surface.

[00:05:03] And for reasons that we don't fully understand, but I'll come to in just a moment, certain types of cells like dendritic cells or antigen-presenting cells process these kinds of antigens and display pieces on their surface. This is called antigen presentation. And depending on how the antigen is presented and in what grammar, cytokine molecular grammar is used to do the presentation. T cells can become either stimulated to be inflammatory. They can be stimulated to be anti-inflammatory. In the case of NMO disease and other like diseases, there's inflammatory stimulation that leads to active T cells.

[00:05:46] And I just want to continue this line of thinking to the next slide because here's that active T cell, which now does a couple of different interactions. We call these molecular handshakes with B cells that then become activated to make antibodies. The antibodies penetrate the blood brain barrier, interact with astrocytes that are expressing aquaporin-4. The interaction between the antibody and the target aquaporin-4 causes complement activation and one piece of the complement protein called C5a activates neutrophils and other granulocytes to enter the CNS and cause trouble. So that is a one-minute overview of about 15 years of science. But it's important to really understand these steps because it's essential to deal with these steps if we're going to retolerize.

[00:06:46] A couple of other highlights regarding recent advances in NMOSD research, again as a model for other autoimmune diseases. We now understand that FDA-approved therapies for NMO reduce relapse risks by at least 90% over time. We also know that full immunization to mitigate infection risk is very important. There's a lot of work going on with non-cell based assays to detect antibodies, both anti-aquaporin-4 and anti MOG and others perhaps. And if we can identify these antibodies without high-tech cell based assays, that means more of the world can use these types of assays.

[00:07:29] There are also new insights into genes that might be associated with risks for either onset or severity or therapeutic benefit in NMOSD. These include the HLA antigens you probably heard about earlier. And more recently, we now have a multi-protein signature that in preliminary studies can forecast relapses up to 180 days in advance to give time for interventions to prevent or mitigate risks of those relapses. And

we know that we need to do some work in terms of the instruments that are used to measure disability, including EDSS, and there's a lot of work going on there.

[00:08:12] So let's talk about the unmet needs that remain despite these amazing advances. Well, one aspect is how we deal with relapses if they occur. We like to think about corticosteroids and plasma exchange and IVIG as interventions that can rescue patients during a relapse, and they can be very helpful for sure. But they are 50 years old, there's new technology, and we should be able to come up with approaches that can help us move forward with better and safer and faster protective activity.

[00:08:49] We also know that even the best therapies can have risks. We know that each of the approved therapies does have some infectious disease risks and other risks. These are summarized here. They can be urinary tract infections that are due to multi drug resistant pathogens or even extensively drug resistant pathogens. Respiratory tract infections, also due to resistant organisms and capsule-positive organisms. CNS infections due to Neisseria like meningococcal disease. These can be multidrug resistant and some are vaccine-evasive so we have to be careful there.

[00:09:31] For some of the therapies, there are latent viral viroid or prion associated infections that can lead to things like PML, and we really have to be careful with that. And of course, whenever you tamp down the immune system, cancers can emerge that are due to lack of immune surveillance. So, even the best therapies, which absolutely have saved and improved lives, can have some risks. And so there's still a little bit of work to do there.

[00:10:01] The other thing, just to comment, is there is what I call this vicious cycle of conventional therapeutics in immunologic disease and, in particular, in CNS autoimmune disease. So, for example, if we start with autoimmune disease here, let's say there's a patient who's diagnosed with autoimmune disease, they will typically be treated with therapies at least conventionally that suppress the immune system. One of the effects, as I just mentioned, is a greater risk of infection, and there are a lot of infections that occur everywhere in the world, just these numbers from the United States.

[00:10:36] There's about 3,000,000 multidrug resistant infections per year in the United States that lead to about 70,000 deaths. But beyond that risk, 1 in 5 cancers is due to a preventable infection. So when we alter the immune system by treating for autoimmune disease, we open up other doors of risk. And one of those is infection and one of those is cancer. And unfortunately, even some of the best new cancer therapies proliferate this problem because they can cause autoimmune disease. And so the cycle continues. And we want to break this cycle and that's where we're looking toward better immunotherapeutics and tolerizing cures. And I'll come to that in just a second.

[00:11:24] So crash course in immune tolerance. Most of you know all of this, I hope, but if not, it'll be hopefully a refresher course that will help us think about the technologies that I'll talk about in just a second. First, what is immune tolerance? Well, big picture, it's how your immune system protects you against threats, internal and external, such as cancer or infection, but without harming any healthy cells, tissues that make up your body. It's a system of checks and balances that prevents the immune system from being too aggressive or too passive.

[00:12:04] Of course, we want the immune system to allow or permit things like healthy microbes, healthy cells, healthy tissues, normal wound healing, normal growth, all of that good stuff. We also want the immune system to defend us against some bad things, pathogenic microbes, precancerous cells that lead to cancers or tumors, abnormal wound healing or growth. The immune system has to be able to tell the difference between normal self and abnormal and non-self. And that is really in essence what immune tolerance comes down to.

[00:12:42] The cells that largely control immune tolerance are T cells. B cells, of course, have a huge role, but I want to focus on T cells for just a moment. And I like to think about the thymus as the T cell university. So you all, I'm sure, remember that most blood cells, including our immune cells, are made in our long bones, in particular, the femur, the iliac crest. So that's where these cells are made and they proliferate there.

[00:13:13] In the case of T cells, they are recruited through a very interesting and miraculous way to the thymus. The thymus is a gland that at maximum size when you're about 25 years old wraps around your esophagus. It's about as big as your fist. T cells undergo three exams in that gland that I'll tell you about in just a second. But basically, the T cells that are made in the bone marrow move to the thymus and they undergo these immunologic examinations.

[00:13:47] The first of the three exams that T cells must pass in the thymus is, can they recognize an antigen-presenting cell? If the answer is yes, they move on. If the answer is no, they are deleted. That's called negative selection. So cells that pass the first test then undergo a different test in a slightly different part of the thymus. And that is, can they do the molecular handshake with the antigen-presenting cell? Can they exchange signals that allow the T cell to know that there's something that the antigen-presenting cell thinks is abnormal? If the answer is yes, they move on. If the answer is no, they're deleted.

[00:14:30] Finally, the big question that relates to tolerance is, can the T cells distinguish between self and non-self? That's a really complex set of molecules and it involves how tightly the T cells bind antigens and in what part of the pocket they are bound and that sort of thing. Bottom line is if the T cells can tell the difference between self and non-self, they move on. If they cannot, they are deleted. Only about 1% of T cells successfully pass all of these exams.

[00:15:07] The graduates, the T cells that pass these exams move from the thymus to the lymphatics. Again, I'm overviewing this. These are a lot of nuances that I'm not going to be able to talk about today. But essentially, T cells that graduate these exams go to the lymph nodes, where they wait. They wait for antigen-presenting cells that monitor and surveil the periphery and other tissues will bring antigens to them in the lymph node to allow the T cells to decide whether it's a threat or not a threat. And that in essence is immune tolerance.

[00:15:47] If the T cells and the antigen-presenting cells signal to one another that there is a threat, the T cells will become activated and leave the lymph node toward the sites at which the antigen-presenting cells are telling them the threat exists. So it's a fascinating area, that is a massive overview, but I hope that helps because we're going to talk about these points in just a second.

[00:16:14] How is immune tolerance then lost? What goes wrong? Basically, it's a case of mistaken identity. At each of these signals, either at the self antigen, perhaps it's abnormally structured. It could be the antigen-presenting cell or its interaction with T cells. It could be the T cell response and its interaction with B cells.

[00:16:39] At any one of these steps, if there's a mistaken identity, tolerance can be lost. The kinds of ways that tolerance can be lost are summarized here. There can be abnormal self-antigen structure or ectopic expression. For example, if an antigen is expressed in the wrong place or at the wrong time. That's usually a DNA and RNA kind of a problem. There can be dysfunctional antigen presentation. So that can be the genes that encode antigen presentation. Those are called the HLA genes. And that also involves T and B cell receptors, which are encoded by different genes.

[00:17:20] And these can be abnormal. They are often altered in patients who have immune tolerance loss, and they involve the RAG gene system or what's called the recombinase gene system. Of course, infectious disease or environmental antigens through antigenic memory and other mechanisms can lead to loss of

immune tolerance where an antigen, a protein from a microbe or some environmental agent looks enough like a human protein that the immune system just mistakes it for a human molecule and then begins to react to the human molecule.

[00:18:06] And finally, there can be, what are called neoantigens from pregnancy, cancer, or transplantation that can lead to loss of immune tolerance. So a lot of reasons for possible loss of immune tolerance, but the question is, can immune tolerance be restored? And that's what we've been focusing on a lot.

[00:18:28] The latest meeting, the Guthy-Jackson Charitable Foundation held its third summit for NMO tolerization in January of 2023. You can see the usual suspects. Here's Dr. Greenberg, here's Dr. Levy, here's Dr. Wingerchuk, et cetera. And folks got together to really brainstorm and understand the technologies and what we can do to apply it to central nervous system autoimmune diseases.

[00:18:56] So ways that are now really priorities for restoring immune tolerance are shown here as strategies. And then I'll show you a few technologies right after this. So first would be to correct any gene mutations or dysfunctions. You've all heard of CRISPR technology now that can do very precise gene editing. That's a possibility for certain types of mutations. We may be able to correct the production or the folding or the structure of proteins that act as autoantigens. And that involves changing RNA expression or the way that the antigens are produced and folded.

[00:19:39] We can reprioritize how antigen-presenting cells present antigens to T cells and/or B cells, and that can be done through something called immune deviation. Of course, we can get rid of the bad actor T or B cells or antigen-presenting cells. Those kinds of therapies are already in place for some diseases, but they really involve censoring or deletion of autoreactive T cells, B cells, or antigen-presenting cells. And finally, the best case scenario is to reeducate the immune system and its antigen-presenting cells, T cells, and B cells through the process of tolerization. And that's where we want to go for the remainder of the few minutes here.

[00:20:26] There are a number of technologies that are emerging to focus on tolerization. Again, I've just used NMO as a model here. You can see a bunch of different abbreviations. I'm not going to go through all of these, but some of them involve dendritic cell vaccines. This is an antigen-presenting cell, and it's a way to actually use it to tame the immune system rather than promote inflammatory autoimmunity. There are a number of companies that are making antigen-specific tolerogens, those are decoy antigens that tell the immune system to turn off the response to those proteins.

[00:21:04] Likewise, antigen-specific tolerizing nanoparticles. I'll mention one or two of those in just a moment. There is a lot of buzz right now about what are called CAR-T or double A CAART cell therapies. These are chimeric antigen receptors, and I'll talk a little bit about that. Antigen-specific checkpoint inhibition, certain ways to use normal healthy tolerogenesis as might occur in the case of pregnancy called HLA-G tolerogenesis. A lot of excitement about inverse vaccines that turn down the immune system to specific antigens. Of course, going all the way back to marrow, rebooting, including stem cell transplant, et cetera, is a possibility. And there's some other ways to maybe leverage endogenous tolerogenesis or even tell the cells that are causing trouble to simply die on their own. That's called programmed cell death.

[00:22:04] So very quickly, how does some of these things work? If you think about CAR-T or double A CAAR-T cell therapy, basically, you take T cells from a patient, then you engineer them with DNA to become cells that express these chimeric antigen receptors that now tell these cells to go look for specific target cells and ideally kill them. These would be, for example, autoreactive T cells or autoreactive B cells that are in the patient. So these engineered cells are returned to the patient, where ideally they kill just the target cells that are causing trouble.

[00:22:48] That's been used in cancer in many ways to a lot of benefit, but we still have some work to do because when you use CAR-T or double A CAAR-T cell therapy, you have to condition the immune system by creating a bit of a window where your immune system is really significantly suppressed. And that can cause a few problems but, again, newer methods are being focused on that.

[00:23:13] Another approach would be tolerogenic nanoparticles. These, I'm sure you've remembered nanoparticles from the COVID vaccines. The particle composition can actually do a lot to change the molecular handshake between antigen-presenting cells, T cells, and B cells. So, in this case, tolerogenic nanoparticles that might contain little pieces of the autoantigen, for example, pieces of aquaporin-4 or pieces of MOG protein could be used in a way with the formulation of the nanoparticle such that the dendritic cell or the antigen-presenting cell becomes a cell that tells the T cells and B cells not to react to that antigen. So now they are tolerogenic T and B cells in the patient.

[00:24:04] Similarly, dendritic cell vaccines have been used where dendritic cells are removed from patients. They're shown the autoantigen that would normally cause disease, but they're shown this autoantigen under conditions that make the dendritic or antigen-presenting cell tolerogenic. So the tolerogenic dendritic cells then return to the patient where it calms down the T and B cells that might otherwise react to that antigen.

[00:24:30] And then lastly, I think we have the example of inverse RNA or DNA vaccines. Very interesting, very exciting work going on here, where basically the whole concept of a vaccine that might promote the immune system in the case that would defend against an infection is turned upside down. Now we load the inverse vaccine antigens with the autoantigen in such a way that the DNA or RNA within that particle tells the cells that process this vaccine to turn itself down to tolerize to the antigen to which it was presented. And again, we would end up with tolerogenic T cells in these patients.

[00:25:19] So, if we think about the work that's been going on, and again, this is just a brief summary, but I wanted to leave you with the idea that the pace of science and clinical trials in the advanced immunotherapeutics and tolerization space is accelerating. So, very briefly, several years ago, Richard Burt and others tried hematopoietic non-ablative stem cell transplant in NMOSD and there were at the time some good results, not perfect. And we're seeing that there were some imperfections that have led to non-durable kinds of responses, but nonetheless, a lot of work in that area.

[00:26:06] More recently, there was an attempt to use dendritic cell vaccination in NMOSD, and this was a phase 1b trial where mainly the results were focused on safety and tolerogenic response, and that looked pretty good. More recently, there was an experimental demonstration in a mouse model of inverse vaccination in the EAE model. And this was against MOG, which demonstrated a very promising response, again in an experimental model, but just illustrated the potential for this approach.

[00:26:44] And then from an even more recent example, the use of nanoparticles loaded with gliadin that strongly suppressed autoreactivity in celiac disease. I mentioned these together as examples of antigen specific or advanced immunotherapeutic approaches that turn the immune system down. The alternate is listed up here. And again, these are just some examples, but these approaches turn the immune system up, which sounds counterintuitive, but it turns the immune system up to go after and kill bad T cells and B cells. So it's a little bit of a counterintuitive concept there.

[00:27:31] But there was a lot of excitement in 2022 of a CAR-T cell approach that focused on CD19, targeting that was used in lupus patients that were refractory to many other types of treatments with dramatically positive results. Similarly, an anti B-cell maturation antigen CAR-T cell therapy was used in patients with very

severe and refractory NMOSD. There were 12 patients, 11 of them completed the treatments. After about 12 months, one patient had a mild optic neuritis event. All the other patients have had no relapses.

[00:28:15] Those studies are being followed up. And now the company making that CAR-T cell therapy has just received FDA approval to do a clinical trial in the United States. Furthermore, there are companies now that are using what are called bispecific CAR-T cell therapies. For example, the anti-CD19, anti-CD20 technology now being used by the ImmPACT Bio company that was just approved for fast track clinical trials by the FDA about a month ago.

[00:28:51] And even more recently, a double approach using the CD19 and a PD-1 knockout CAR-T where by eliminating PD-1, you prevent T cell exhaustion. So these CAR-T cells should be able to do more work for a longer period of time to track down and kill autoreactive B cells and T cells. And of course, all of this stuff is just an example of how there is now a vastly accelerating and ever increasing interest in and resources provided for the ultimate goal, which is antigen-specific tolerance to cure autoimmune disease. So that's where things are right now.

[00:29:42] I just want to leave you with a couple of other concepts. So, conventional immunotherapies and therapeutics in autoimmune disease often turn the immune system down. They ask the immune system to wait on the sidelines and that can lead to risks. Tolerization asks the immune system to actively participate, but in a different process, that is one of immune peace or tolerance. And I think this illustration really represents how what we're all trying to do now in the field of tolerization is reeducate the immune system so that it ends the autoreactive process once and for all.

[00:30:29] And curing diseases is a big and bold audacious goal. You probably heard Dr. Greenberg talk about it's not enough to simply stop the disease, you have to repair the damage it caused. And that is true.

[00:30:45] But I'll just leave you with this idea. And this was Christine Peterson, a NASA strategist, one of the earliest women in the NASA program and a true leader. And she said, "If you're looking into the future and an idea today seems like science fiction — it may be wrong... but if it does not look like science fiction — it will definitely be wrong."

[00:31:08] So I hope that helps summarize where we are with advances in immunotherapies and tolerization. It's a big goal and there's a lot to do still, but there's a huge amount of momentum. I'll leave you with this. In case you'd like to learn more, you can go to the Guthy-Jackson Charitable Foundation website, go to pioneering curative therapies. It's really cool. It's a fascinating topic. We're all going to be talking about the immune system and tolerance in the coming years. And I think I'll stop there. It looks like, Ben, you're all set up there. So I hope that's helpful. Thanks a lot.

[00:31:52] **Dr. Benjamin Greenberg:** Michael, we really appreciate it. Great talk. And as people have questions, we'll ask them to reach out to you. I know we have your contact information, the foundation's. We appreciate you taking time out of your Sunday in beautiful California to Zoom in with us. So, appreciate it. Thanks, Michael.

[00:32:08] **Dr. Michael Yeaman:** Thank you, Ben.