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SPEAKER 1: We're very excited today to introduce Dr. Thomas Marron, for his grant rounds on cancer immunotherapy. Dr. Marron as our very own, completed his medical school here, as well as his PhD at Mount Sinai. His doctoral research focused on human immunology and primary immunodeficiencies. He then went on to receive his internal medicine, as well as his hematology oncology training here at Mount Sinai, as well. He did a post-doctoral fellowship in the lab of Dr. Brody, studying immunotherapy in cancer.

Dr. Marron currently serves as Assistant Professor of Medicine, and is the Assistant Director of Early Phase and Immunotherapy Clinical Trials here at Mount Sinai. So it's going to be a very exciting talk. Please join me in giving a warm welcome to Dr. Thomas Marron.

[APPLAUSE]

THOMAS MARRON: Thanks, everyone, for having me. I think that this is definitely the most interesting part of Internal Medicine, so I'm happy you're all here--

AUDIENCE: Yes, it is.

THOMAS MARRON: --to hear about it. It's most interesting because it's the first time that, as oncologists, we're really interacting with all of the specialties because of the toxicities that we actually cause with some of these therapies. Although there is, I believe, some significant benefit. In my title, which I didn't include here, I got a lot of weird emails from people wondering what that c word was. But it's the cure word that we oftentimes are not murmuring to our patients who have stage 4 disease. Before, that was a no-no to say that word to folks and give false hope.

I don't have any personal financial disclosures, but I do have research grants from BMS, Merck, and Regeneron. I'm going to be talking about some off-label use of their agents that we're doing and some novel investigator-initiated trials. And just about everything I talk about, because I'm a Phase 1 doc, is off-label. So don't try this on 10 Center. Let's have some supervision of some sort. So I'm just going to talk about three different things.

First, I'm always going to sort of start with a little bit of Immunology 101 as a refresher. I'm going to tell you about the current state of cancer immunotherapy-- here in the States, at least. And then our approaches to how to move this field forward, specifically here at Mount Sinai, that I've been working on for about four or five years at this point. So my father is actually a scientist, and he still reads all my grants for me. And he always makes fun of immunologists for speaking a weird anti language. And he says that we're not very good at communicating with the rest of the community.

So I always like to take a step back and remind everyone about some basics of immunology and a relatively complex part of immunology, which is the priming of an immune response by dendritic cells. So as everyone knows, there's a very delicate balance between activation of the immune system to recognize foreign cells or tumor cells, which appear foreign, and then tolerogenic mechanisms to keep the immune system from recognizing normal tissue and attacking normal tissue causing autoimmunity. And the process of carcinogenesis, whether this be through smoking or radon exposure or just sunlight exposure in Australia, basically makes your normal cells look more and more foreign, and so recognizable to the immune system.

And the way in which this happens is that over the course of our life, we're constantly accumulating mutations in all of our cells. Cancer cells are growing very rapidly, in general. And they accumulate even more mutations than normal cells. So most of the cancers that we look at have dozens, if not hundreds or thousands, of mutations. And many of these mutations kind of result in transcriptional and translational changes that result in a mutant protein. So this mutant protein is a foreign antigen, in that it's something that you were not born with, something that you were not tolerized to in your thymus. And so you have the ability to elicit a T-cell response to recognize that foreign antigen.

And the way in which we teach those T-cells what to be on the lookout for is through presentation of antigen by a professional antigen presenting cells to CD4 and CD8 cells, among some other cells as well. So all throughout our body and our skin, in our bowel, everywhere, we have different subsets of dendritic cells. And these dendritic cells are constantly sampling the micro environment and taking up antigen from dying cells that are releasing their contents, whether they're just normal cells sloughing off, dying in a normal fashion, or if they're cells that are dying because they have become cancer or if they're infected with viruses.

And so, as the dendritic cell takes up these antigens in the environment and samples the environment, it's also looking for dangerous signals or conversely tolerogenic signals, saying this is infected tissue and there might be bacterial DNA, RNA that can bind to receptors in the dendritic cells, or the dendritic cell has receptors for ligands and antigens that are being released from normal tissue. And so, not necessarily something to be worried about. So dendritic cells then, directly present antigen on MHC Class II or they cross present the antigen onto MHC Class I. And this allows for interaction with CD4 and CD8 cells.

So the MHC that is loaded with antigen is interfacing with the T-cell receptor. But alongside that interaction, you have what we call Signal 2. Oftentimes, there's many different Signal 2s, but these are the co-stimulatory signals that are coming from the dendritic cell to the T-cell, telling the T-cell either to become activated and go on the lookout for this antigen that it's being presented or to senesce, and it's basically exerting a tolerogenic signal. Because we don't want to activate these T-cells against self antigen.

So within Signal 2, I showed you Signal 2, which is an interaction between the antigen presenting cell and your T-cell, but you also have a Signal 2 of sorts between the tumor cell and the T-cell. So if we are, in fact, able to prime a T-cell to recognize this tumor antigen, once it finds that tumor cell along with interacting with MHC on the tumor cell presenting this new antigen, tumor cells are able to up-regulate senescent or sort of silencing co-stimulatory signals, such as PD-L1. So PD-L1 is the one that many of you have probably heard about. It's the one that we-- that interaction with PD-1 on the T-cell is really what we're targeting with most of the therapies that are currently FDA-approved.

But along with putting up lots of these stop signs on the cell surface, the tumor has found a whole slew-- dozens and dozens-- of different ways to really turn off the immune system. One way, that I'm showing here, is that it produces a soluble factors, such as chemokines and cytokines, that recruit immunosuppressive cells to the tumor micro environments. These include T-regulatory cells, as well as an umbrella term of MDSCs, which are really just a variety of different myelin lineages which are able to really turn off the immune response within the tumor, so that the tumor can protect itself from T-cell assault. So as I mentioned, PD-1, PD-L1 is really where a lot of the research has been to date and where all of the FDA-approved drugs are to date.

And a lot of you have heard about this. Not only because your great aunt Millie is calling you, asking about this immune therapy things she heard about. It's because we've had some really notable successes. So Jimmy Carter had metastatic melanoma to the brain, which previously was a near universally fatal diagnosis. And along with radiation and surgery, he received Keytruda, which is a PD-1 blocking antibody-- pembrolizumab. And he's now been in remission for more than five years, so we consider him cured of his melanoma.

Melanoma-- Stage IV melanoma, particularly, when it's metastatic to the brain-- was previously completely incurable. As it is just about every Stage IV cancer maybe, outside of head and neck cancer. But most of you have also heard about these because they're advertised all over television-- Monday Night Football, Food TV. I mean, any type of program that you're watching, they're going to advertise immune therapy.

In some cases, they might be overselling it a little bit, so it's always good for us to know some about these agents, whether you're an oncologist or not, just to temper the excitement that some of these patients come in with. But they are very exciting drugs. These seven drugs that we're talking about the target two different pathways, they block checkpoints. Checkpoints are basically a-- it's like an umbrella term for an on/off switch. And there's dozens of on/off switches for the immune system within the tumor. So your tumor cells are chocked full of different immune cells.

As I said, on the left here, you have some of the suppressive immune cells that are being recruited to the tumor. So you have the t-regulatory cells, tumor-associated macrophages, and these myeloid subsets, which really are able to turn off the T-cell response by secreting a variety of different soluble factors. And on the right side, we see the interface between the antigen presenting cells, such as the dendritic cell, and the T-cell, where the priming of the T-cell response is really occurring.

And if you take a look on the right hand here, you see that there's a whole slew of different on switches and off switches. So these receptor ligand interactions between the T-cell and the antigen-presenting cell are either turning on the T-cell and at the same time also activating the antigen-presenting cell. But there's lots of inhibitory signals on the bottom, such as PD-1, PD-L1, where when you have receptor ligand interaction, you're really turning off those T-cells and blocking the immune response to whatever antigen is being presented by the antigen-presenting cell to the T-cell.

And so, you can sort of summarize these as on the top, we're looking at a bunch of different ligand receptor interactions that result in a kill the foreigners signal, really activating those T-cells to go out and be on the lookout for what the antigen is that's being presented. While on the bottom, we have these stop, don't hurt yourself signals. And these signals are extremely important. We're disrupting them in cancer, but these signals have been around forever. And it's really the way in which our body keeps our immune system in check, so that we don't develop autoimmunity.

And currently at Sinai, we have over 30 trials that are really looking at modulating all different parts of this sort of immune micro environment within the cancer. Both at the interface between the T-cell and the antigen-presenting cell. We have different cytokine factors that we are injecting either into tumors or into patients to hopefully rev up T-cells and turn off all those off signals. And we also have a program of *in situ* vaccines that look at a tumor-associated antigens or damage-associated molecular patterns, which are basically TLR ligands, like synthetic viral RNA. And we're injecting those into tumors in what we call *in situ* vaccines-- and I'll get into that a bit as well.

So taking a look at this interface a little bit closer, the way in which we're modulating these in the clinic currently-- and most of this is in clinical trials-- is on the top side here. So these are the on interactions between T-cells and APC. We can create agnostic antibodies, and these really mimic binding of the receptor to the ligand to activate either the T-cell or the antigen presenting cell. So as an example, right now we have an anti CD-40 antibody in the clinic that we are giving folks that binds to CD-40. It mimics the binding of CD-40 ligand on the T-cell to CD-40. So it really revs up the activity and helps mature that antigen presenting cell. So the antigen presenting cell can go out and activate further T-cells.

And then we have these antagonizing antibodies. And these are the ones that we have that are already FDA-approved. And the concept here-- with the agonistic antibodies, we're kind of pushing on the gas and revving up the immune system. Here, we're really just taking our foot off the brake and allowing for the immune system to do what it wants to do, without inhibiting it with interaction such as PD-1 and PD-L1. And as far as agents that are already FDA-approved, we have six agents that are approved that are targeting the PD-1, PD-L1 axis. We have one antibody, ipilimumab, which binds to CTLA-4.

But most of our success has been on the bottom. And so we're really look different ways in which we can modulate the activating pathways as well as the inhibitory pathways, as we think that maybe a combination between those two sort of pushing on the gas and taking off the brake at the same time might be the best combination. And right now, we're looking at a whole slew of different targets. We have antibodies and small molecules that are really inhibiting all of these different interactions. Trying to find the perfect combination where we'll get the best clinical benefit with the least amount of toxicity.

So the reason that we're super excited about checkpoint blocking antibodies and why it's on the cover of *The New York Times* regularly and why everyone is not an oncologist probably gets bored reading *The New England Journal* is that we have had a lot of success. There has been 35 FDA approvals in the last four years of checkpoint blocking antibodies in over a dozen different histologies, because of really amazing responses that are things that we've never seen with chemotherapy. Chemotherapy, we were always kind of inching forward and adding three to four weeks of life to the overall survival.

Here, this is a curve from a study looking at patients who had metastatic melanoma. So as I said, universally fatal, not a lot of good treatment options. Dacarbazine is the straw horse here. That is the blue line. It's a very unpleasant chemotherapy. It's not particularly successful. And you can see that when these patients were treated with decarbazine, the overall survival was a mere nine months and the vast majority of the patients were dead within a year and a half. But if you take a look at the gold curve, you can see that the nivolumab significantly-- and early on-- breaks off from the dacarbazine curve.

And you really see significant benefit in these patients who are receiving nivolumab. And if you look at a larger cohort looking at nivolumab, which is a PD-1 blocking antibody, or that in combination with ipilimumab, which is a CTLA-4 blocking antibody, this is a progression-free survival-- so not even overall survival-- curve. And you see that at three years, over 30% of the patients have yet to progress. So a real significant improvement on the curve that you're seeing here with decarbazine. And the most important thing about these Kaplan-Meier curves is that we appear to be seeing plateaus in these curves.

And we usually didn't see plateaus in stage four cancer trials. And the main reason that we're excited about this is plateaus really suggest the concept of cure. These are durable responses that we're seeing in many of our patients. And most of these patients have gone off therapy after two years typically. And so-- we're always nervous to use the C word-- but I think that when you look at data like this, we are getting a little bit more comfortable. Once we have patients who've been on therapy for one or two years, considering taking them off therapy and just monitoring them as if they were a patient with a durable remission.

And we've seen similar curves. Not quite as impressive as that, because melanoma is extremely immunogenicity. But we've seen similar impressive responses in over a dozen different types of cancer. This is from a *New England Journal* review. On the y-axis, you see the response rate. On the x-axis, you see actually the number of neoantigens or the number of mutations. And so there appears to be a direct correlation between the response rate that we're seeing and the number of antigens within these tumors.

However, what I think is most important about this is actually fewer than 20% of these patients are actually attaining an objective response-- And that's either a partial response or a complete response-- radiographically. And this includes patients with lung cancer and bladder cancer, where we're using immunotherapy in the first line setting, because it's that much better than chemotherapy. So we're patting ourselves on the back for some pretty amazing leaps forward in the last five years. But we have a long way to go if 80% of patients are not actually achieving an objective response.

And not only that, we also have to think a little bit about the toxicity. So immunotherapy is much better tolerated than chemotherapy. Chemotherapy is pretty brutal for a lot of patients. It's much better now because we have good agents to manage symptoms. But in general, I would say well over half of patients experience some sort of significant toxicity from chemotherapy. While with immune therapy, about 80% of patients actually-- who are getting monotherapy-- do not experience toxicity.

But those that do, they can be significant. And they can also be difficult to diagnose, which is one of the reasons why we incorporate the help of many people in this room-- because we need our endocrinologists and our rheumatologists on speed dial-- so that we can understand exactly what's happening when people have relatively odd presentations. And the reason for this is that checkpoint antibodies, they bind not only to the check points within the tumor and that are being over-expressed by the tumor.

But they're also binding to the checkpoints that are all throughout the rest of your body. These checkpoints are evolutionarily conserved, because they really are a mechanism to keep your body from attacking itself, to really inhibit autoimmunity. So when take off the brakes and particularly we start pushing on the gas, we're developing really significant autoimmunities and inflammatory disorders-- that we've never seen before in many times. And these can attack just about any part of your body.

Actually, they attack every part of the body. There's case reports of just about every type of autoimmunity and inflammation you could think of. And they're all relatively rare. So dermatitis is probably the most common. Maybe about 10% of patients overall across studies are experiencing dermatitis. Somewhat less common, but still something that you're seeing in the hospital are pneumonitis, colitis, myocarditis, nephritis, hepatitis. But then also, there's this odd tendency to develop these autoimmune endocrinopathies. So autoimmune thyroiditis is relatively common.

Obviously, that's something that's a little easier to treat. But we also are having patients present with new onset Type 1 diabetes, from autoimmune pancreatitis. A year ago, I admitted a patient to the hospital who, all of a sudden, had a new onset adrenal insufficiency-- and she had developed adrenalitis. And I didn't even know adrenalitis was a word until I diagnosed her with adrenalitis and I looked it up. And there was maybe two case reports of it. And so we really need everyone in the room's help whittling down the diagnoses with these patients.

Because the way in which we treat these toxicities is we give high dose steroids, typically. And those steroids, they tend to turn off a lot of the benefits that we're getting from immune therapies. They don't totally negate the benefit. But they really are taking an entire therapeutic approach off the table when we determine that they have a very severe autoimmune manifestation from these toxicities. So we really want to know what we were talking about before we treat them.

And another interesting thing and an important thing to note about these toxicities is that unlike chemotherapy-- if I give chemo on a Monday, I know that by Friday, Saturday, Sunday that's usually when the toxicities are going to pop up, that's when people are going to call me and they're fatigued or maybe they're having some nausea and vomiting that's not being controlled by their PRNs. These toxicities don't tend to show up in the first week, two weeks, three weeks.

They typically show up after about two to three cycles of immune therapy. So six to seven weeks, you might start seeing some toxicity. But some of these patients actually won't develop an immune-related adverse event for months, if not even a few years after starting these therapies. There's been case reports of three years after starting therapy is when somebody finally develops a toxicity. And it's important to know that because these antibodies stick around in your system.

But they're also priming a T-cell response that remains even once the antibody is out of your system. So let's say you're in the emergency room and somebody comes in and they say I had nivolumab in February. But now it's December and they're presenting with Type 1 diabetes, new onset. That's probably autoimmune pancreatitis caused by the nivolumab, just sort of a delayed response. So it's something to always have in the back of your mind if a patient has received one of these immune therapies.

And so, as I said, only 20% of patients really seem to be responding and there's real toxicities. And so there's a lot of questions that remain. The number one question, which usually surprises people that I'm asking this, is how these agents work *in vivo.* We don't really understand how these agents work*in vivo.* We have a lot of mouse data that gives us suggestions of how it works. But we don't really know how PD-1 blockade is disrupting the normal tumor immune micro environment and the MLu, which is suppressing the immune system within the tumor.

And not only do we not know that, we don't really know how to predict who's going to benefit and who's not going to benefit. So we really need bio-markers to determine who we should be giving these to because they'll have a clinical benefit versus who we shouldn't give them to because they just are going to run the risk of toxicity without any benefit. And most of these drugs cost anywhere from $150,000 to $500,000 a year, which is completely not tenable for the large population.

So we can't give every lung cancer patient immune therapy. And so the goal is really to identify who's going to benefit most from this therapy and who we should be putting on clinical trials or who we should be trying standard chemotherapy on. And finally, what a lot of our research is focused on is what additional targets should we look at other than this PD-L1, PD-1 axis. I showed you there's literally dozens of different ways in which the tumor suppresses the immune system. And then there's actually hundreds of different ways in which we can prime an immune response through vaccines, using CAR-T-cells, TIL therapy. So

There's all these different agents that we could incorporate. We just don't know exactly what the best therapies are and how we shouldn't mix them together. And this is some data from the Cancer Research Institute, from earlier this year. And there's currently more than 3,400 agents that are in development within the umbrella field of cancer immunotherapy. And this includes cellular therapies, vaccines, but particularly antibodies targeting these checkpoint or ligand receptor interactions. And they're looking at 417 different targets.

And so I mean, the amazing news, we had maybe a handful of different chemotherapies that we used to mix in different ways for the last few decades and try to find the best combination. So now, we actually have 3,400 agents. That's the good news. The bad news is that we have 3,400 agents and we have no idea exactly how to combine them. And most importantly, *The New York Times* had this article about six months ago that pointed out that we have too many drug trials and we actually don't have enough oncology patients to fill those trials.

So unfortunately, a lot of the times, to see a significant clinical benefit-- if we're just looking at a clinical outcome-- we need a relatively large cohort to really statistically power that trial. And so if we have all these large cohort, large trials going on, a lot of these trials are either negative trials and they're not showing any benefit over giving pembrolizumab alone or those trials don't accrue. And at that point, we've wasted our own time and, most importantly, we wasted our patients time and not getting a definitive answer from that trial.

So this is a big problem. I would argue that this basically amounts to just us throwing spaghetti at the wall. Because we're not making these combinations in the most intelligent way possible. We might have some preclinical data and a mouse saying if we mix PD-1 blockade plus X, it seems to work in this inbred Miriam model of melanoma. And then we immediately are jumping to humans. I think that we need to better understand how these agents are working in humans, to make smarter decisions about how to combine these therapies.

So this is a problem that we are trying to tackle here at Mount Sinai. And we're going to-- hope to do that using deep tumor immunophenotyping to really aid in target selection. And what a lot of you actually don't out-- I found out that most people aren't quite as aware of what's going on over in the Cancer Institute and up in the Icahn building-- is we really have an unparalleled immune monitoring collection of platforms, as well as expertise here. There's very few institutions-- probably one or two in the world-- that really have all of the capabilities that we have here at Sinai to look in-depth at the immune micro environment within tumor, within blood, and understand the interactions there.

And these are just a few pretty picture examples of some of the interesting things we can do. One thing that we have really optimized are multiplex immunohistochemistry platforms. So we to take a small biopsy or a chunk of tissue that's taken out-- and normally with immunohistochemistry, you just take one slice on one slide and you stain it for one thing, so stain it for CD8, stain it for cytokeratin. With these multiplex platforms, we're actually able to stain it with at least 10 things.

The first platform that was developed by Sacha Gnjatic and his colleagues here, which is called MICS, was able to look at 10 different markers on the same piece of tissue. And that really allowed us to understand the interface between certain dendritic cells, the stroma, and the tumor itself. We now just acquired something called a MIBI, which is a new multiplex immunohistochemistry platform that came in the last few months, that were still in the process of optimizing. But this platform can actually look at 50 different markers within a single slice of tissue.

By looking at 50 different markers, not only are we looking at phenotypic differences-- so we can see different t-cell subsets, exhausted T-cells versus activated T-cells versus central memory T-cells-- we can also look at their activation status at the transcription factor level, at the soluble or the cytokine levels-- see what cytokines they're producing-- and really understand the importance of their location within the tumor, the importance of the stroma within the tumor at excluding these immune cells, and within the interaction directly with the tumor itself. We also have many different platforms.

CyTOF is a mass cytometry platform. It's basically flow cytometry, but instead of 8 or 10 colors in flow cytometry, we can look at 50. So we can get lots of information about the T-cells. And not just the T-cells, but the dozens of different T-cells subsets that we can sub classify T-cells into. We have a single-cell transcriptomic platforms that really allow us to understand the activation status and different pathways within the infiltrating immune cells. And by mining all of this data, we can really find different ways to exploit what's happening in these cells and target these cells. So that we can hopefully activate the immune cells that are already within the tumor or within the patient themselves to attack the cancer more effectively.

So we're going to use these platforms in some trials that are going to open this February. The first trial, which is a neoadjuvant window of opportunity trial. Blue trials is what we call them. And we think that we can use this to really elucidate the intertumoral effects of these immuno-oncology agents. And the goal of these window of opportunity trials is basically we take a patient who's going to go to the operating room anyway-- and most of these patients have a very high risk of a recurrence. And so even if we cut it out and we give them some adjuvant chemo, most of them have over a 50% chance of that tumor coming back.

So if we could prime an immune response against their cancer to kill off micro metastatic disease and keep the cancer at bay, that's obviously a big win for us and the patient. So these trials, what we're doing as we take a biopsy of the tumor. We give them four to six weeks of an intervention, whether that be chemotherapy, radiation, and/or one of these new immune therapies. And then we take the tumor out. So by comparing the pre-treatment tumor biopsy and the tumor itself as well as blood and stool from these patients, we can really assess the dynamic changes that have happened over the course of just four to six weeks.

By looking at those dynamic changes, we can understand what exactly is happening in the tumor in response to that therapy. And that will allow us to really make some smart moves going forward. And for the patient, the neoadjuvant setting I think is extremely beneficial. Much more so than in the metastatic setting. So these patients they have greater immune fitness. These patients have not seen chemotherapy. We haven't destroyed all of their T-cells and many of their myeloid cells with chemo. Their bone marrow has not been taxed, as most of our patients have after chemotherapy.

And then we also know from the metastatic setting, those who have less tumor burden-- which these are people who start with a small tumor. We're then cutting it out, so their remaining tumor burden is just their micro metastatic disease. Those patients who have smaller tumors are more likely to attain a durable complete remission. And those who have less tumor heterogeneity-- so less differences in the antigens that are being expressed by the tumor here versus here, if you have metastatic disease. Less heterogeneity means more potent response to an immune therapy.

And then for the physician scientist, the neoadjuvant setting is extremely exciting in that it really allows us to create these dynamic atlases to determine what each of these therapies is doing by themselves and how they might be synergize. So we're already making treatment-naive tumors. Yonit Lavin is one of the interns currently in internal medicine. She did some amazing work during her PhD with Miriam Merad. There's a cell paper-- you should definitely check it out-- looking at treatment naive lung cancer tumors.

We're going to be looking at the dynamic changes following standard adjuvant therapy, following these novel immune therapies, PD 1 blocking antibodies and other immune therapies, and then also following combination immune therapies. So in lung cancer, when I have a patient who comes to me-- not for a trial, but just as a new diagnosis lung cancer metastatic lung cancer-- all of them now-- small cell, squamous, adeno-- they're all getting a combination of immune therapy and chemotherapy.

And immune therapy is full of steroids, so you'd think that would negate the benefit of the chemotherapy. But there's actually a really impressive synergy that's seen when you add immune therapy to chemotherapy. And we have a lot of theories about why that is, but we haven't really ever shown it *in vivo.* And so these atlases-- well, if we have data on both therapies alone and then the combination therapy, theoretically we can identify some mechanisms of synergy. We can also compare responders and non responders. That will allow us to identify bio-markers of who we really should be giving these meds to and who we should not be giving them to.

And then most importantly, we want to propose rational combinatorial approaches. And this isn't a concept that I thought of-- or anyone at Sinai thought of, for that matter. This is something that has been underway in the last year or two. This is a paper that came out from our colleague Dr. Drew Pardoll down at Hopkins and colleagues at Memorial as well. Here, they took 20 patients with resectable lung cancer. They gave them only four weeks of nivolumab. And they saw that-- they weren't really expecting much of an pathologic response.

They really just thought they were going to be priming a bit of an immune response-- so once they take the tumor out, you're kind of vaccinated. But they actually saw after four weeks, there were many patients who had a really significant pathologic response. And here in the waterfall plot, you actually see that 45% of the patients achieved a major pathologic response, which means more than 90% of the tumor-- in that short period of four weeks-- had been killed off. And there really wasn't any toxicity in this short period because, as I mentioned, toxicities from these drugs really pop up around week seven, week eight, after the surgery. So after they're already recovered.

And here at Sinai, we have some experience in the neoadjuvant setting as well. This is a patient of Myron Schwartz, who came in who had a large hepatocellular carcinoma that was abutting the portal vein, was deemed borderline resectable. The patient received two cycles of nivolumab. Not only did the tumor shrink significantly, if you look on the right, there was significant intertumoral hemorrhage. There was lots of fibrosis that developed. There was this really robust immune infiltrate.

When we compared the immune infiltrate before and after and looking at the quote, unquote normal liver tissue around the tumor versus the area inside the tumor and the peritumoral stroma. We saw very significant dynamic changes in the types of cells that we were finding and also the activation status of those cells. So you know very exciting data from a relatively short intervention in these patients. That's very well tolerated. That we think, eventually, it will translate to an overall survival benefit, because we're priming an immune response.

That's obviously a little bit more TBD because we'll need larger data sets there. But based on this success, we've developed this clinical trial with Regeneron, our partners up in Tarrytown. And this is a neoadjuvant trial where we're going to be giving neoadjuvant cemiplimab, which is a PD-1 blocking antibody, to patients who have head and neck cancer, hepatocellular carcinoma, or lung cancer. And within lung cancer in particular-- lung cancer is kind of one of my biggest interests. Here, we're actually going to be giving patients either a PD-1 blocking antibody, the combination chemoimmunotherapy, or just chemotherapy. And after surgery, everyone gets immune therapy.

But this initial three cohorts is really going to allow us to better understand what the synergy is that we're seeing in all of the metastatic patients that we're treating with chemoimmunotherapy. Most importantly, because we don't really understand-- they made a trial, they designed it, they executed it. They didn't look at different dosing regimens, different dosing schedules. And so, hopefully, this will allow us to possibly plan a more significant response in these patients.

And the most exciting thing about this is we're going to get a lot of bio-specimens. We're going to get blood, tissue, and stool from these patients before and after their neoadjuvant therapy as well as after their adjuvant therapy as well, to better understand the dynamic changes. And really understanding the effect of PD-1 blocking antibodies. Chemotherapy, we're also looking at radiation in liver cancer patients. Hopefully, this will allow us to plan more rational approaches.

So sort of taking a little turn to the left. I'd like to discuss some of the research that we're doing here at Mount Sinai. Most of this I am doing in conjunction with Josh Brody. And our work here-- the work I just described to you is really focused on finding new targets and finding how we might combined those new targets with PD-1 blocking antibodies. Here, though, we really wanted to focus on finding ways to get patients who are not good responders to checkpoint blocking antibodies that are already FDA-approved, that have lots of safety data out there. How can we increase the response rate?

So you can see down in the lower end here, we have lung cancer and bladder cancer, as I mentioned. We're giving all those patients PD-1 blocking antibodies. Only 20% them are responding. But three of the most common cancers that there, which are breast cancer, prostate cancer, and lymphoma, they have virtually no response to these therapies. It's all in the single digits. And so these patients really have a significant need for some sort of benefit from immune therapy. Because they typically will run out of options after chemotherapy.

So we'd like to find a way where we could use what we've already got, what's already FDA approved, and just kind of rev up the response to those therapies. And to do that, I think that we really need to rev up the T-cells, along with taking off this roadblock. If you say our t-cell is this green car-- you can see I'm quite an artist-- if we take off-- so your T-cell is parked out front of Mount Sinai on Madison Avenue, and you have this big roadblock in front of it, which is PD-L1. If we remove PD-L1, that parked car is not going anywhere.

You have to turn on that t-cell and you have to rev up and gas up that t-cell, in order to get it on its way. So what types of gas, what types of stimuli can we give to this t-cell in order to get it to drive down Madison Avenue? And so there's two different approaches that we've really been looking at, which are to further prime t-cell responses. So increase the education of T-cells as to what tumor antigens are and what they should be attacking.

And the second approach that we're using is to promote the expansion of these T-cells. So we want to teach more T-cells what's to be on the lookout for. And then we want those T-cells to grow and expand so that they're at significant numbers to kill the cancer. So first taking a look at priming of the T-cell response. All of us have been priming t-cell responses throughout our career-- and that's through vaccines. We got primed early on in childhood to many different antigens. And I should preface this by saying all the micro people in the room have to close your ears. Because I'm in a bastardized the explanation of how a vaccine works.

[LAUGHTER]

But basically a vaccine has two different signals. So the first signal is the antigen. So in the pneumonia vaccine, our antigen is the pneumococcal antigen. And the second signal is really the adjuvant. So that's the thing that recruits your immune system to the injection site and activates your immune cells. So in the pneumococcal vaccine, you inject a pneumococcal vaccine into your arm and then the conjugate, which is CRM 197, which is a modified diphtheria toxin. That's what recruits your immune system to your arm and activates your cells that have taken up that antigen so that you stimulate a whole systemic response.

So similarly, in cancer are tumors that are constantly growing and dying. So they're growing kind of aberrantly and dumping their antigens into the tumor micro environment. So our tumor is already chocked full of Signal 1. So an *in situ* a vaccine is a vaccine that we're doing in the site of the tumor. So let's say I have a big tumor here and I'm going to inject *in situ* into the tumor, Signal 2. Signal 1 is already there because there's a lot of dead antigen. Signal 2 is basically going to recruit my immune system to my tumor.

It will take up all those neoantigens. And, hopefully, it will primal response that will be a systemic response. Because I'm not trying just to kill this tumor. I'm really trying to prime a whole systemic response, where not only will it kill any micro metastatic disease, but will also develop an immune memory against those new antigens that'll be with us throughout our life-- just as we have measles-informed T-cells from our childhood. So Signal 2, we're injecting into the tumors.

But we know that neoantigens themselves are not enough. So not only do we need the neoantigens there in the inflammation, we really need these antigen presenting cells there to take up those neoantigens and present them to T-cells. And we know this because within-- there's not a lot of great bio-markers for response to immune therapy. But one thing we know is that hot tumors, which are tumors that have lots of T-cells in them, are the ones that respond best to PD-1 blocking antibodies.

And that makes sense, because you have this tumor that's full of T-cells that most likely some of them are recognizing the cancer, but they've been turned off by PD-1, PD-L1 expression by the tumor. So they're just kind of sitting there in limbo, not really knowing what to do with themselves. And so we know from some TCJA analysis that was done by Tom Gillespie's group at the University of Chicago that if you look at the t-cell signature, which basically just means lots of T-cells or not a lot of T-cells, there is no correlation at all with the number of neoantigens with mutations.

So no matter if you have never smoked or you smoked a lot, it really doesn't dictate whether or not you're going to have a lot of T-cells within your tumor. But the one thing they found that does correlate with t-cell infiltration-- specifically, CD8 cytotoxic t-cell infiltration-- is the expression of something called Batf-3. And Batf-3 is a transcription factor that's extremely important for the production of a rare subset of dendritic cells called DC1s. And in mice, these are called CD103 dendritic cells. And in Brad Pitt, they're called CD141 dendritic cells.

And these dendritic cells we know are the most potent cross presenters of antigens, the best professor cells to teach the CD8 cells what to be on the lookout for. And this is a paper that really demonstrated this, also in 2016. And there was multiple papers that copy this paper, but this is from Sinai, so obviously the best of the papers. This is from Miriam Merad's group that showed-- the curve here is a B-16 melanoma model. So basically they've implanted this tumor into the mouse and the dark curve, that you see going up, is how the tumor grows unabated, if there is no therapy.

When you give this mouse a PD-1 blocking antibody, you significantly shift the tumor growth to the right. So you're having a significant quote, unquote clinical benefit from the PD-1 blockade. However if you deplete these key DC1s, these Batf-3 expressing dendritic cells, you lose all benefit from PD-1 blockade. So we really need these cross-presenting dendritic cells to teach our T-cells what to be on the lookout for. So that those T-cells can then exert an anti-cancer effect, particularly when we give them a PD-1 blocking antibody.

So it begs the question, how do we get more of these Batf-3 dendritic cells, if they're such great teachers of the immune system? And one way is that we can use Flt3 ligands. So a Flt3 ligand is kind of like the Neupogen for dendritic cells. So we give Neupogen to patients to bring up their neutrophils. Flt3 ligands really is a growth factor for dendritic cells. And in particular, we see a significant expansion of the dendritic cells we're most interested in, which are the DC1s.

So we've done this. This is some work by Linda Hamrick is one of the post-docs in Josh Brody's lab. She took a tumor-bearing mouse and she injected that tumor-- [CLEARS THROAT] excuse me-- everyday with Flt3 ligand. And as you see-- you look before and after-- the red is the dendritic cells and the green is TLR3, toll-like receptor 3. And those cells that are expressing both red and green, those are the DC1s that we're most interested in. So compared to before, you see that there's a whole slew of these dendritic cells in the tumor after we've injected the tumor with Flt3 ligand.

We then give a little baby dose of radiation to kill off some of the cells and release tumor antigen. And then we activate those dendritic cells with the TLR3 ligand poly(I:C). So what we're doing is we're recruiting dendritic cells to the tumor. We're loading those dendritic cells. And then we're activating those dendritic cells, so that they can teach T-cells what to be on the lookout for. And when you give this triplet combination, you significantly decrease the growth of these tumors. And you also prolong survival in these mice as well.

So we see a really robust immune response to this triplet therapy. So we actually put this into humans a few years ago. We started vaccinating patients with indolent Non-Hodgkin's Lymphoma. And this is a patient that I treated in March of this year. She came in and she had a diagnosis of follicular lymphoma, which is a slow-growing lymphoma. She had very significant bulky axillary lymphadenopathy, lymphadenopathy in her cervical region and throughout her groin.

So what I did was I injected her right inguinal region with the Flt3 ligand every morning for nine days in a row. On that ninth day, we then gave her-- I'll show you first. If you look at the tumor before and after the Flt3 ligand, we see a significant increase in the CD141 dendritic cells. So this is a CyTOF analysis. Basically, just fancy flow cytometry showing pre, post dendritic cells. The red correlates with a 1log or 10-fold increase in the number of those cells. So we're seeing a 10-fold increase in these very potent cross presenters of antigen.

So we then gave a very tiny dose of radiation, 2 grey times 2, which we call it boom-boom in the lymphoma world. Because it's a palliative dose that's regularly given. So that releases some tumor antigen. And then we activate those dendritic cells that are gobbling up that antigen using poly(I:C)-LC, . the TLR3 ligand. And you can see at six months after this treatment, she has a near resolution of all of her lymphoma. She really only had some shoddy axillary lymphadenopathy leftover.

And we actually took out one of those lymph nodes and compared it to a lymph node that we had excised before the treatment started. And not only did we see a really robust CD8 infiltrate into that remaining lymph node-- and there's some fibrosis in there as well-- interestingly, when we did flow cytometry analysis of those lymphoma cells, really comparing the normal B cells to the malignant B cells, we saw that all of the remaining B cells had actually down-regulated to MHC Class I. So this is an escape mechanism by which they become basically invisible to your T-cells.

So it was a very exciting response. The problem is in this trial we've had four significant responses-- one of them is not on this curve-- at the abscopal site, so the injected site we're irradiating. So we expect it to shrink down somewhat. But the abscopal site is the distal site-- outside of the radiation field, where we're really trying to see if there's an immune response-- and it's killing off the other sites as well. So only a subset of patients have actually benefited. And so, we weren't sure exactly why this is, so we went back to our preclinical model.

And here, Linda noticed that if she took out a tumor after the vaccine, the intertumoral CD8 cells had become activated, but they also expressed a high level of PD-1. And PD-1 is a marker of activation, but also a marker of functional exhaustion at the same time. And at the same time, she looked at the tumor. And the tumor, as well as the dendritic cells infiltrating within the tumor, expressed high levels of PD-L1. So you have high levels of the off signal on the tumor and high levels of the off signal on the t-cell.

So it's not surprising that we're not having continued tumor killing. So we took a look at our humans and, actually, we saw similar data. So on the top row, this is CD8 cells from a patient who had achieved a complete response. On the bottom row, it's CD8 cells from a patient who had a progressive disease during the vaccine protocol. And we noticed that in the peripheral blood-- this is at week four, so after the majority of the therapy has been received-- the patients with progressive disease had a very large population of these exhausted T-cells.

We don't know the specificity of these T-cells, because we didn't have tetramers in this analysis. But we saw this with a few of the patients with progressive disease and a few patients with complete response. And the exhausted phenotype that we were relying on is just if you have high levels of PD-1 Lag3, and TIGIT, as well as a few other markers. That's a pretty reliable marker of these T-cells being exhausted. So when your T-cells are exhausted, it basically means your T-cells are tired and they're not working well.

We have to reinvigorate them. So the way that we reinvigorate these T-cells and wake them up so they recognize the cancer is we give a PD-1 blocking antibody. So we did that in mice, and we saw that we saw an even better regression of the tumors. Nearly all the tumors melted away. And we saw a significant improvement of survival over the previous triplet therapy. So now we have this quadruplet therapy or Flt3 ligand, radiation, poly(I:C), and PD-1 blocking antibody.

So based on the success that we saw in mice, I wrote this trial awhile back and got some grant funding to support it. And it's going to be opening in the next few weeks. And this is going to be doing our normal *in situ* vaccine protocol, but combining it with pembrolizumab, which is a PD-1 blocking antibody. This is going to be opening in patients who have metastatic cutaneously or superficially accessible Non-Hodgkin's Lymphoma, metastatic breast cancer, as well as head and neck cancer.

So along with priming these T-cell responses, we want to look at different ways where we can further promote T-cell expansion and the growth of these T-cells. So we know from the transplant world and from the cellular therapy world, such as CAR-T-cells-- which you guys might have heard a lot about-- as that lymph depletion-- if you give a patient who has a bunch of T-cells in their body lymphodepleting radiation, chemotherapy, and you wipe out their T-cells-- we notice that there is an increase in the homeostatic cytokines such as ILs 7 and 15.

So these homeostatic cytokines are basically T-cell food. So all of a sudden, a little bit of T-cell food turns into a T-cell buffet. And nobody's at the buffet, so you've got a lot of food there. So all of a sudden, when you then transfer in T-cells to these patients, they grow like gangbusters and they become hyper-activated. And so this is one of the thoughts why autologous stem cell transplant is very beneficial for patients. Not only do we give them high-dose chemotherapy, but when we give them their T-cells back-- because we harvest their stem cells peripherally, so it's a bunch of stem cells and a bunch of T-cells all mixed togethetr.

We know that those T-cells grow very rapidly, rapidly expand, and they also rapidly become activated. And, hopefully, some of those T-cells are actually recognizing tumor antigen. So we took a look at some of our lymphoma patients that were undergoing bone marrow transplant. We looked at their T-cells before transplant and 10 days after transplant. And, interestingly, we saw that they expanded rapidly and they attained an activated phenotype. But by 10 days, counter-intuitively, they actually had a very exhausted phenotype. So they had lots of PD-1, lots of CTLA-4. So these markers that basically say, these cells are probably not working too well.

And we also saw that they were functionally exhausted, not just phenotypically exhausted. And we saw this not only in our human patients, but also in our mouse patients as well. And so Antonia, who was one of the post-docs that used to be in Josh Brody's lab, she took this model of bone marrow transplant, which I won't go through. But it's basically similar to the transplant that we were doing in our humans in many ways. And she just started giving PD-1 and CTLA-4 blocking antibodies before and after the transplant.

So we know from the lymphoma studies that are done that PD-1 and CTLA-4 blocking antibodies don't really do much for lymphoma. So if you look at this curve, in black here you see-- this black curve right here is basically just how the tumor is growing without any treatment. So this is a lymphoma tumor in a mouse and it's growing pretty unabated. If you give CTLA-4 and PD-1 blocking antibodies alone, you see a little bit of delayed growth, but not much of a benefit. Similar to what we're seeing in humans. And then in the red here, you're seeing the benefit from bone marrow transplant.

So you get even more slowing of the tumor growth initially, but then eventually the tumor is still taking off despite the immune cells. Probably because you're having exhaustion of those transferred T-cells. However, when you combine bone marrow transplant and checkpoint blocking antibodies, you see this very impressive synergy. And we don't just see this synergy in this B-cell lymphoma model. Because B cells are an antigen presenting cell. People could argue. This is kind of a unique thing.

We also see it in a model of T-cell lymphoma, in a model of melanoma, and in a model of lung cancer. And the most important thing about these, not only is the synergy that we're once again seeing. It's that if you look at melanoma and lung cancer, the black curve is untreated. The blue curve is getting these checkpoint blocking antibodies. This is a standard therapy for lung cancer and melanoma in humans. And it's not having any benefit in these mouse models. These mouse models are very tough to treat. Much tougher than your average human being.

But you're seeing this amazing synergy, which you see in the purple here, which is an immunotransplant. That's what we call it, because we're transferring the revved up immune system from one mouse to another. And so based on this data, I wrote a trial during a fellowship that I opened in April of this year, in which we're giving two cycles of checkpoint blocking antibody nivolumab and ipilimumab, so PD-1 and CTLA-4 just like in the mice. We then harvest their peripheral blood mononuclear cells. So we're not taking any stem cells out.

We're just taking their T-cells and some monocytes and some B-cells. We're throwing them in the freezer. We give them fludarabine and cyclophosphamide which lymphodepletes their body. This is the standard chemotherapy we're giving the CAR-T-cell patients, which is FDA-approved. We then a few days after the FluCy is out of their system, we give them back their T-cells. Those T-cells expand like gangbusters, as we would expect, and become activated. And then once they hit neutrophil recovery, we give them two additional cycles of Nivo/IPI, the combination immunotherapy. And then they go onto maintenance nivolumab therapy after that.

This was the first patient I treated on this trial. She's a delightful 69-year-old lady. She'd been dealing with lymphoma for almost 10 years. She'd blown through many lines of chemotherapy. She'd had a transplant-- that didn't work either. And when she came to me, she had this very large, painful mass in the root of her mesentery. She was not able to eat. She had intractable nausea and vomiting. And she was on morphine four to five times a day. She was really an unhappy camper. And we gave her two lines of nivo/IPI. We then admitted her and gave her this modified transplant.

And before she was even discharged, I knew something was working. Because she was eating normally and she was off the morphine. We gave her two additional cycles of Nivo/IPI. And she came back and she had a PET scan that showed that we had a near resolution of the hyper-metabolism in her abdomen. And at this point, we don't even know if this cancer or if this is just inflammation related to the treatment that we're giving her that's showing up on the CAT scan. So she then went on to nivolumab maintenance. I actually saw her yesterday and she's working out more than I am, which is at the low bar.

[LAUGHTER]

But she is eating really well. She is pissed off at me because she's gaining weight because she's eating so well. And her most recent PET scan has shown a further improvement in the metabolism of her tumor. So it's a very exciting result. And most importantly, we're giving this lady two different therapies. Both of which will rev up your T-cells and activate your T-cells. So we were concerned that we might see some of these immune-related adverse events even worse than we would in a patient receiving just nivo/IPI, which a significant percentage of those patients do as well.

Neither the first nor the second patient, who's also had a pretty impressive response, have had an immune-related AE. They're doing extremely well so far. Knock on wood. So we currently are in a safety expansion, where we can only enroll patients every 42 days. We're enrolling our third patient this week or next week, I believe. And then, assuming that we get through this safety cohort, we're planning on expanding to additional tumors. Most likely patients who have metastatic lung, breast, and liver cancer next.

SPEAKER 1: Five minutes.

THOMAS MARRON: So with that, I most importantly want to thank all of the patients and families that really invest a lot of faith and spend a ton of time with me here. And they spend even more time with our nurses. Our nurses in the research unit are absolutely some of the most amazing, awe-inspiring people I've ever met. Josh Brody, who's been a great mentor, and his lab. Particularly Linda and Antonia, who did a lot of the preclinical work I showed you. The HIMC takes all of our samples and processes them.

The neoadjuvant trial that I mentioned really takes a gigantic village of medical oncologists, surgical oncologist, radiation oncologist, radiologists, pathologists. Basically everyone in the hospital has to be participating in these. And it's been a real pleasure developing those. And I always like to thank all of the mentors, who let me putter around in their lab over the last 25 years. Particularly Charlotte and Lloyd, in the middle here, who really transformed me into the nerdy immunologists that I am. And then, Josh and Nina and Miriam, who've helped me develop into a cancer immunologist in this very exciting field.

So with that, I'll take questions. This is my contact info. This is my personal cell phone, if the house staff want to write it down. If you ever have any questions about these weird drugs we're putting into people and any of the side effects that they might be causing. Thank you.

[APPLAUSE]

AUDIENCE: So I have a question. So what if the-- we've looked at the security [INAUDIBLE] sort of that we're all connected to each other, sort of.

THOMAS MARRON: Yes.

AUDIENCE: So with CTLA-4 and PD-1, there's a lot of polymorphisms within those genes themselves. In fact, we've published [INAUDIBLE] showing there's different immune responses if you use blocking agents, by blocking antibodies with CTLA-4, you have totally different response depending on the genotype and PD-1 is very [INAUDIBLE].

THOMAS MARRON: Yes.

AUDIENCE: So is anybody starting to look at that individual response to the check point inhibitors based on the mutations in those genes themselves?

THOMAS MARRON: We definitely-- larger groups are. I mean, they're within pharma, where they have larger sample populations, they are looking at it. There hasn't been any published data or even an abstract form that's shown that those might be a bio-marker of who would gain the most response from those therapies or who might really gain a benefit from the combination of those therapies. But, yeah, it's an interesting line of thinking definitely.

AUDIENCE: And a very interesting one also.

THOMAS MARRON: Question?

AUDIENCE: So it's a very impressive talk. To put to the first part, with the toxicity in with the second part, do you worry that in the long run it may take longer for those T-cells that are exhausted against normal tissue-- so you get the autoimmune toxicities-- it may take them longer to recover. So that you may later see a lot of toxicity?

THOMAS MARRON: Yes. That's a great question. I'm actually writing a trial right now, really dedicated to seeing how long we have to treat these patients for. Because lots of these patients would get the optimal response within three to six months, but the trials have either been written to give therapy for two years or to give therapy indefinitely. And a lot of the times when we're doing that, patients might develop a partial response and then it just stays put at that partial response level continuously. But then a year later, they're developing a toxicity.

So why run the risk of giving them a year of therapy, if maybe four cycles, six cycles is more than enough to get them to the best benefit that they'll get from that therapy? So that's an extremely important question. It's a tough question to answer for many different reasons. Funding is one of those reasons. But we're working on it. I'm actually working with a group, with the Society for Immunotherapy of Cancer, to develop a trial specifically to address that in melanoma.

AUDIENCE: We thank you very much, Thomas.

THOMAS MARRON: Sure.

[APPLAUSE]

AUDIENCE: We're looking forward to hearing about--