[MUSIC PLAYING]

MALE SPEAKER: These are my disclosures. So let me start by saying that immunotherapy has transformed the management of cancer. So when I was in medical school which increasingly with every passing year appears to be longer and longer ago, now actually 16 years ago when I graduated, we had nothing that was effective for the treatment of advanced cancer.

In fact, when I was in medical school, melphalan was just being used as part of autologous stem cell transplantation. High dose chemotherapy for breast cancer was a thing, even though it was not thought to be completely effective. And melanoma was a disease of dead and dying people, because we had nothing that worked outside of tilt therapy and cytokine based therapeutics.

And then over the last decade, we've got these new drugs known as immune checkpoint inhibitors. And this drug has really transformed the management of cancer. For anybody who's older than 45 years old, you probably have seen how the migration on the board exams has gone from having to recertify an era in which cancer was not thought to be important to an era in which now cancer has actually got treatments. And not all cancer patients are patients with whom we have hospice conversations.

So these new agents, these immune checkpoint inhibitors, essentially produce durable responses in multiple tumor types. They're precise. They're durable. They're effective. And they're effective across a broad array of cancers. They're used singly and in combination with other immune checkpoint inhibitors. Number of diseases and number of trials that these agents are utilized in, it's just expanding on an annual basis.

So right now, there's thought to be approximately something like 2,000 therapeutic [INAUDIBLE] trials across the world. This is a $22 billion space for anybody who's counting. And it's all because we've now got these new agents that just work across cancer. And the primary reason why they work is because they essentially uncouple an interaction that the cancer uses to hide from the immune system.

So all of us are discovering and developing cancers on a daily basis. And all of us with intact immune systems actually are undergoing cancer killing on a daily basis. The only difference between the people who have cancer and the people who don't is the fact that the people who have cancer, the immune systems go to sleep. The immune system goes to sleep, because the cancer uses an interaction to hide from the immune system. And these drugs, essentially, block that interaction and make the cancer more obvious to the immune system and thereby unleash an effective anticancer immune response.

So just in melanoma, this is what has happened. So when I started fellowship training, which is the first part of this, this is all we had. And over the last couple of years with the advents of targeted therapy, what has happened is the five year progression free survival in melanoma has transformed from 8% ipilimumab to 29% with single agent immunotherapy to 36% with combination immunotherapy. And we don't know what it is with triplets.

But the point is with these new agents, about a third to 40% of melanoma only, but increasingly in other cancers too, but melanoma alone, 36% of advanced cancer patients are rendered disease free. So why is that such a big deal, and why is the intestinal microbiome such a big part of this? Well, the intestinal microbiome is really the collection of all organisms you have inside your gut.

And the reason that this is important is, because firstly, the number of organisms that we have that don't belong to us actually outnumber us. So we have about 10 to 30 trillion human cells in the human body but about 100 trillion microorganisms. We have 30,000 human genes but three million genes that belong to the microbes.

So if anybody is trying to try to figure out how much that is, when you try to think about how to conceptualize what 100 trillion is, I'll give you guys simple figures. All of us pay taxes, and the debt ceiling has gone up under the prior president. Who knows what's going to happen now. But the amount of money in the national debt is $25 trillion. And that's a lot of zeros. But the number of organisms that we have in our gut is 100 trillion. So it's four times the amount of dollars in the national debt.

And we now know that the gut microbiome plays a tremendous role in human health and disease. The gut microbiome affects the onset of diabetes. It's been linked with the efficacy of lipid lowering therapies such as statins. It's been shown to influence the development and the severity of autoimmune diseases. It's been linked with the development of a gut-brain axis that affects the severity of microbial-- affects severity of GI problems in autistic children.

So the point is that alterations in the gut microbiome have tremendous effects in multiple organ systems, in multiple disease states, ranging from fatty liver disease, cardiovascular disease, antibiotic resistance, metabolic syndrome, inflammatory bowel disease, and increasingly cancer. But why is that the case? Well, as it turns out, the response to immunotherapy is dependent on multiple factors. Now, we have traditionally in the context of cancer and cancer biomarker development have focused on what we see on the right, which is the tumor genome and the epigenome as well as the tumor microenvironment.

So all our drugs are essentially targeting elements of this genome, elements of the cancer genetic cycle, alterations in cancer-causing genes, as well as alterations in the tumor microenvironment, looking at agents that block vasculature, for example, such as bevacizumab, looking at agents that block certain cells that are important in the tumor microenvironment such as macrophages, such as CSF1R receptor antagonist, and so on, and so forth.

Or we try and increase the effect of the host immune system. So beyond PD-1 blockade that increases the amount of CD8 immune cells, so the activated killer T cells of the human body, we have a bunch of other agents that target essentially macrophages, NK cells, and so on, and so forth. However, we've completely neglected the human microbiome, which is really the second genome, as we talked about earlier. And the question of whether or not we can do this is the subject of the next 30 to 45 minutes.

So we know that responses to immunotherapy are increasingly dependent on factors such as these. But why is this a problem? Well, as it turns out, even though immunotherapy works in cancer, the best response to immunotherapy across trials is 50%, and not only that. Even when immunotherapy is effective, and even sometimes when it's not effective, immunotherapy causes tremendous side effects.

And many of you guys in the audience who are members of the subspecialists in this hospital have gotten calls from our ever expanding legion of alphabetic soup services, A service, B service, hit service, and so on, and so forth regarding consults on patients who have side effects. And the incidence of these side effects is ever increasing, ranging from 15% to 55% of high grade severe side effects. And given the increasing use of these agents, this is basically an increasing problem.

So therefore, we have-- at least my group has chosen to study these patients. And we have now seen two opportunities basis. The first are patients who feel immunotherapy, best response 50%, so the 50% of patients who don't respond, and also patients who develop severe side effects. So I'll talk about the second, and then I'll talk about the first.

So the question is, what do we know about the intestinal microbiome composition in relation to response to immunotherapy and side effects to immunotherapy? And then second question is, can you alter the microbiome, particularly in patients in whom immunotherapy does not work to effect anticancer immune responses? So let's start with the first question, which is what is the role of the composition of the bugs in relation to response?

The first thing is people have been thinking about this for a very, very long time. Now, you could wonder why this could possibly be the case. But very interestingly, if you go back to about 15 years, people have shown that responses to chemotherapy, particularly immunogenic chemotherapy such as taxanes, are dependent on the constituent bacteria that are found in the gut of mice. And this has been known for a long time.

So when these new drugs came along, people started to study this. Three years ago, seminal papers came out that linked the presence of response to immunotherapy to certain bacterial species. In this case, a group from MD Anderson suggested that firmicutes as well as the increased diversity of the bacteria was associated with response. Another group from Chicago showed that bifidobacterium was associated with response. And yet another group from France looking primarily in non-small-cell lung cancer as well as kidney cancer linked response to immunotherapy to akkermansia and firmicutes. So first two studies were melanoma. Third study was non-small-cell lung cancer and kidney cancer.

But the first thing that you see is across all these three different diseases in three different cohorts that happened in three different geographical locations, the first thing that shows up is that the bugs are the same. So this is a summary slide looking at all of these different cohorts including two of the smaller papers. And the first thing that you probably get struck with is the fact that when you look at all these different data sets, there appears to be a tremendous lack of concordance. And when you think about the number of organisms that we talked about earlier, 100 trillion, it's not surprising that there's actually a lack of concordance. But the question is, why?

So what we set out to do was we firstly set out to develop a new cohort. And this cohort was significantly larger than any of the other cohorts. But we also did a meta-analysis of four prior cohorts. All the melanoma cohorts along with our cohorts was analyzed. And that's the subject of the next set of slides.

So the first thing we said is, one of the things that cancer doctors do is we like to analyze things using metagenomic sequencing, which essentially is DNA sequencing. And when you sequence something, we're essentially trying to see whether the depth of sequencing-- whether it's shallow sequencing such as 16S sequencing or deep sequencing which is the sequencing of the entire depth of the metagenome-- was the depth of sequencing that was different across these different studies-- because as you see some studies utilized 16S sequencing. Some studies used metagenomic sequencing.

So the first question that we had was, is the depth of sequencing one of the reasons why these responses were different, these organisms were different? And so we have responders in blue, the nonresponders in red. And we showed that in this data that's currently been submitted to *Nature Medicine* that the depth of sequencing was not different. It did not defer. It did not significantly account for the differences that were observed across the different cohorts, meaning regardless of the sequencing depth, responders and nonresponders were segregated.

The second question is when you look at responders and nonresponders, you can very clearly identify some very, very, very nice groups of people. So you see here, in nonresponders, there's a big cluster here of these organisms. And then in responders, there are two distinct clusters down here. And these organisms, eubacterium rectale, ruminococcus gnavus, as well as [INAUDIBLE], are organisms that appear to be important as I will show a little more earlier.

The second thing that we tried to do is we also looked at side effects. So the first thing we tried to say was, is the intestinal microbiome implicated in the occurrence or nonoccurrence of immune related side effects? So the key thing that it's saying here is that we're looking at specifically immune related side effects. We do not look at gastro, GI bleeds, cardiac events, strokes. We specifically focused on immune related side effects, such as rashes, diarrhea, thyroid problems, vitiligo, immune mediated hepatitis, immune mediated colitis, and immune mediated lung joint problems as well as neurological side effects.

So the first thing you see is completely unexpectedly. For the first time, we show that the metagenomes of people, the contents of your gut bacteria, predict the kind of side effects that you have, because when you look at people who have side effects and people who don't have side effects, their metagenomes are very, very, very different with a very significant p value.

And second thing is when you break these things down by categories-- so let's say you group people with side effects-- and then you look at the proportions of different bacteria in these different side effect groups, you see some very, very interesting trends. So the first thing is streptococcus is a very interesting organism, because it's primarily an organism that is found in people's oral cavities. It is not native to the gut. And it's actually very interestingly associated with one or two or three particular side effects. So we've separated these side effects out.

And what you can see here is that streptococcus is very interestingly associated primarily with hepatic side effects, with joint side effects, with adrenal side effects, and with pneumonitis. Now, very interestingly, streptococcus is an organism that is not native to the colon, which is where we obtain the vast majority of stool from. So how is it that the strep, which is an oral commensal, makes it into the gut? So our first hypothesis was that maybe some people just have strep that is colonized in the colon. Is that even possible? As it turns out, that's not possible.

And the reason it's not possible is because streptococcus is something that requires a certain pH. And that certain pH is a pH that is slightly above acidic, slightly more alkaline. And that pH, if it's too acidic, streptococcus gets destroyed. But very interestingly, when we tried to think about why this might happen, why streptococcus can actually make it into the gut, we then found that-- we looked for the presence and the association between streptococcus abundance, these side effects, as well as the use of certain medications known to alter the gut pH, alter the pH by increasing the pH. And that is proton pump inhibitors.

And we found that, again, very surprisingly, but retrospectively not that surprisingly, that streptococcus abundance was directly very compellingly linked to proton pump inhibitor use. And why is this important? Well, as it turns out, side effects are both good and bad. Certain side effects are very good to have. But streptococcus is a very, very adverse organism, as we've shown earlier. It's an organism that is strongly associated with adverse outcomes.

So when we looked at this, what we were able to show, again, for the first time, is that the survival differences in people who developed immune related adverse events was largely explained by the abundance of strep. And so when you look at all people with side effects, people with side effects generally do better. But when you separate these side effects by people who have high strep and low strep, the people who have high amounts of strep-- that is that line in the bottom in red-- do terribly.

And the people who have low amounts of strep do well. And very interestingly, this is extremely concordant with reported data from other groups, linking the exact same pattern with the use of proton pump inhibitors. And other groups have shown that proton pump inhibitor use is associated with adverse outcomes in patients with immunotherapy. And I think now we have been able to explain why this might be the case. And again, these are very, very significant p values and hazard ratios.

So the summary of this is that one, for the first time, we've now been able to link the metagenomic composition of somebody's gut bacteria to not only response but also in clarifying why there all these different bacteria are associated with response. But more interestingly, we've been able to show that the development of immune related side effects are distinctly, and compellingly, and clearly associated with the metagenomic composition of the individual and that this is sometimes adversely altered by the use of certain medications such as PPIs, with resulting negative outcomes upon response to immunotherapy.

So as we were developing this, which is our first story, we actually started a second trial in parallel. And that study was to try to figure out if we know that the microbiome is implicated in people who do well to immunotherapy therapy, can we potentially alter the microbiome in patients who don't do well on immunotherapy for potentially therapeutic effect? And so that was a second question.

And the reason why we asked this question was because it has been shown, in some of the papers that I showed earlier, that the administration of either beneficial organisms-- in this case, bifidobacterium longum-- was associated with an anticancer phenomenon. So here, you see when you add bifidobacterium, what you can do is you can make tumors a little smaller.

And so the question was, if you can show this with both single bacterium and you can also show this when you use entire consortium-- in this case, fecal microbiome samples-- So in these experiments that were done by the MD Anderson group that were part of the papers from three years ago, what these investigators showed was that when you took beneficial organisms from patients who did well on immunotherapy, as shown in blue, or adverse organisms obtained from patients who did poorly on immunotherapy, as shown in red, you were able to recapitulate the effects.

So beneficial organisms resulted in a rejection of cancer in the mouse that synergized with the administration and the co-administration of checkpoint inhibitor therapy in the mouse. And the administration of adverse organisms from nonresponders actually resulted in rapid tumor growth. So the question that we had was, instead of treating mice, which we were not very interested in doing, can we do this in humans?

So this was the subject of a first in human study that we put together here about five years ago now. Firstly, this is a huge team effort. So I want to call out some key members of the group that actually at the University of Pittsburgh and Hillman Cancer Center [INAUDIBLE]. And so for example, the group consisted of myself, and Hassane, who's the translational cancer immunologist, a couple of people at the NCI who helped us with computational work on this. But there were two gastroenterologists who were actually on the paper. And one is Howard Dubner, and the second is Marc Schwartz.

So the key thing that I want to emphasize here is that this is uniquely an experiment done at the University of Pittsburgh, one of only two studies worldwide, the second that happened in Israel. And investigators at the University of Pittsburgh were the first people that came up with this idea. So it's, again, cool.

And so what we did was we took patients who were completely refractory to immunotherapy. So these are people who never had a response to immunotherapy at the time at which they went on this study. And we know that in cancer patients, that accounts for about 60% of the patients that we see. Even though immunotherapy kills about 40% of cancer, 60% of cancer does not respond. And a small fraction of those patients have cancer that doesn't respond at all. And those are the people that we utilize.

And the important thing here is to keep in mind that when you take people like this and you give them an intervention, the question is always, what is the likelihood of the intervention working? Well, as it turns out, in this particular patient population, you don't expect immunotherapy to do anything, because they failed it. So anything that produces a response can clearly be linked to the outcome in question. So that's part of the reason why we did this study, which is a very high risk, high reward type setting.

And what we did was we gave them a single donation of fecal transplant, a single fecal transplant, that came from a donor who had done exceedingly well. Now, obviously when we get [INAUDIBLE] between people-- we're talking about literally stool transplants-- you have to be very, very concerned, because stool is not exactly the world's most sterile substance. So what we had to do was we had to really type the donors as well as the recipients for a variety of different infections.

And so this is where our infectious disease colleagues at the University of Pittsburgh also came into play. And they helped us come up with this algorithm that allowed us to define who could and who could not receive stool from patients and from donors. And increasingly, of course, we had to test for a whole host of organisms, all of which are listed here. And people were sero matched on the basis of their infectious serologies to appropriately commensurately sero match donors and then got a donation.

And after they received this sero matching, they received a single transplant on day one, followed by pembrolizumab, a drug that most of them did not respond to prior to coming on the trial. And then they continued that for as long as they appeared to benefit or they developed side effects. And the trial was set to go on for about two years. And they could receive pembrolizumab for up to two years.

So this is a profile of the donors that we had utilized. Again, you can see a whole host of people. We identified about 25 to 30 people that we could use as donors but settled finally on a total of eight donors who have a profile as listed here. And all of these are patients with advanced melanoma, extensive metastatic disease ranging from either skin mets to potentially even brain visceral metastases and brain metastases, all of whom had advanced disease and all of whom were cured as a result of immunotherapy.

And we know that they're cured, because all of these people are currently in surveillance. And it's not been just one year or two years since they've stopped their treatment. Many of them had to be in three or four years up to five years since they had finished treatment, so truly durable responders to immunotherapy. So we took these people. And then we got stool donations from these people, and we administered them to our recipients.

So before I talk a little bit about what the effects were, one question that people have is, we know that patients have a variety of different good responses to cancer therapy. Sometimes, it's a complete eradication of cancer, which is termed a complete response. Sometimes, it's a near complete eradication of cancer, but it's termed a partial response. And oncologists do not feel that there's any major difference between these two groups. So we think that these two groups of people are ideally really the same.

So when I counsel patients who have had a partial response that is a near complete eradication of cancer, I tell them, you know what, don't worry about it. Your likelihood of having a cure is just as good as somebody who is at a complete eradication of tumor. And when we set out to do this experiment, we assumed that the metagenomes of people with complete responses would be identical with people with partial responses. But we were wrong.

When we looked at complete responder donors, even though in terms of every other parameter these people are almost identical, and the fact that when we looked at the outcomes in transplantation by donor response that was also identical, because there's no difference by a chi-square test, when we looked at the metagenomes of the people who were complete responder donors, complete responder donors were a little better than partial responding donors.

So again, going back to the idea that one, we don't truly understand everything that we think we understand about cancer patients, and two, even though something doesn't necessarily have a clinically meaningful difference, whether it's in terms of the donation result in a cancer patient, one, or two, how these responders do long term-- see, our donors do just as well as PR donors-- one very interesting thing is that your metagenome may make the difference between tipping you towards a complete response versus holding you a little back and giving you just a partial response. So we found that these donors had a greater bacterial diversity if they're CRs, or complete response, compared to partial response.

So what about the patients that we treated? So we treated a total of 16 patients, 15 of whom were evaluable. Their characteristics are shown here on this particular slide. And they basically represent a very, very, very extensively pretreated patient population with many adverse characteristics, including the presence of large quantities of visceral disease, elevated LDH which is a barometer of high disease burden, and most of whom had received multiple prior therapies, including a median of two prior therapies in our patients, some of whom, a third of whom, a fifth of whom had received actually more than three or more prior therapies.

And these are results. So the first thing that we were able to find is with a single fecal transplant-- so again, I want to emphasize how crazy this is-- but with a single fecal transplant, what we were able to observe was that 6 out of 15 people had a response. So these are people who were not supposed to respond to anything at all, let alone continuation of the immunotherapy that they had just failed. And a single fecal transplant resulted in an arrest of their cancer growth kinetic.

Now, again, we have to acknowledge that 9 out of 15 times, nothing happened. These people progressed, and they needed subsequent other treatments. But 6 out of 15 people had either a response, a slow response, or long term disease stabilization. And the patterns of response that we observed include the traditional cancer responses that we see, which is a rapid reduction of cancer burden, what is termed a complete response, a slow evolving response that sometimes grows a little before it shrinks when it's time to see the progression, or just complete disease stabilization, a nongrowth of cancer.

And all of these people were treated the same for the purposes of our correlative analysis. And you can see that these responses are durable. One of our responders, unfortunately, passed away because he decided to-- he had actually severe cervical spinal stenosis, underwent surgery for the cervical spinal stenosis. And unfortunately, he had a spinal cord infarct that was a complication of the surgery itself and died as a result of that, had an autopsy, and was found to be NED at the time of autopsy.

But the other five patients who were responders had ongoing response at this time and have not initiated any further treatment. In fact, one of the patients actually stopped treatment and is actually currently under surveillance. And these are what you see in some of these people. So you see in this case a guy with a liver lesion and a tumor in the left armpit that essentially initially increased in size but then rapidly reduced in size. And at least at this point in time, this patient has ongoing shrinkage of all his radiographically evident disease.

Now, what we were able to show-- and these are some slightly technical information, but I'll walk you guys through this-- so the first thing is that we were able to show that the microbiome transplant engrafted in most patients. Basically, if you gave somebody a stool specimen, most of the time the stool specimen engrafted, which means that it colonized the recipient. And colonization here is represented by what is termed Euclidean distance. So that is the degree of similarity between the host and the recipient, meaning the donor.

And so the donors start out in 0, and their degree of approximation to their donor sample is indicated by a positive direction. The amount of time we had engraftment was actually quite significant. 10 out of 15 people had relatively rapid engraftment. However, while engraftment happened in 4 out of the 9 people who did not respond, engraftment happened in every single responder.

So what is really interesting was that engraftment was actually quite common. It happened 10 out of 15 times. But while all the responders engrafted, for some reason, even though engraftment occurred in some of the nonresponders, it was not sufficient to turn the tide. And these engraftment changes are actually rapid and profound, when you look at the rate of compositional change in the responders versus the nonresponders.

Now, what we also know is that when we give people a living drug, which is the microbiome, the administration of a nuclear agent, such as an antibiotic, is sufficient to eradicate established engrafted transplants. So this is a case of a single patient who was one of our responders who was given antibiotics, because she developed cellulitis. And this cellulitis actually resulted in her transplant essentially being lost.

So what you see here is her metagenome is indicated here in the circles in blue. And what happened after she received the transplant was that she completely migrated away from her original state. So this is where she started. This was bad. This is what happened after she got transplanted. After she got antibiotics, she went back to being bad. But then we gave a second transplant, and she went back to being good.

And all of this was associated with commensal changes in her radiographical disease burden. So she started out pretty terribly with lots of disease. When she got transplanted, her disease burden reduced. When she got antibiotics, the disease burden increased. And then when she was retransplanted, her disease burden went back to being a little better.

So the point is-- and that's illustrated here-- when you see the first transplant, you see a bloom of these species in green after the first transplant, which are beneficial, and a reduction of the species in dark brown here, and an expansion of the species in yellow. But after the antibiotics are administered, you see a rapid bloom of the species in green, which are adverse. And all of this is associated with an increase in her disease burden. And then when you give her a second transplant, her cancers start to shrink again. So the point is that this particular patient illustrates the profound and profoundly deleterious effects of antibiotics upon what is essentially a living drug.

Now, we do know that some organisms are good, and some organisms are bad. And how do we know which organisms are good and which organisms are bad? So what we were able to find was that overwhelmingly, the organisms that were increased in people who did well were these organisms, firmicutes and actinobacteria. Alternatively and conversely, organisms that were increased in people who did poorly primarily belong to the bacteroides and proteobacteria phyla.

Now, in order to see what the effect of this was at the level of the host, what we were able to do was essentially what is known as immunoglobulin-seq, which is essentially a technique of looking at the immunological response to the bacteria at the level of the person's blood. So remember, we're giving a fecal transplant. The transplant is literally going via colonoscopy into somebody's stool, into someone's rectum, into their proximal colon.

What is the effect of that? And how do we measure the effect of that in somebody's blood? Well, as it turns out, you can do it. All you need to do essentially is measure anti-commensal immunoglobulin. So this is basically a form of an indirect Coombs test. You're looking for bound immunoglobulin. And so essentially, what you're giving somebody, you're taking serum. You're measuring the presence of immunoglobulin that is bound to a particular bacterium by looking at antihuman IgG.

And you're looking for the combination of the complex, the complex that is the bound immunoglobulin that you give as well as that is bound to in this case particular bacteria. And what you can see is that responders in blue compared to nonresponders and red have a far greater amount of on treatment increase in the amount of bound immunoglobulin using this assay compared to nonresponders. And this difference is statistically significant.

But what happens at the level of the immune system? So we were able to show that the combination of microbiome modulation not only resulted in immunological changes that happened at the level of the blood, as I showed earlier, which are humoral changes, but we were also able to show that the combination resulted in the elaboration of increased amounts of immune cells that were detectable peripherally. And these immune cells fell into the adaptive T cell cascade as well as a memory immune response.

So we are able to see that essentially large amounts of CD8 T cells elaborated activation markers as well as NK-like CD8 T cells that also elaborated activation markers. Now, very interestingly, we were also able to show an increase in the amounts of effector memory cells that recall antigens specific responses and, very interestingly, MAIT cells, or mucosal associated invariant T cells that specifically respond to microbes. And these will also increased in our responders, as shown here in green.

At the level of the tumor, we were able to show that this combination reduced the amounts of myeloid cells. And myeloid cells are well known adverse cells that actually are the immediate nonresponse to anti-PD-1 or anti-CTLA-4 immune checkpoint. And these adverse myeloid cells were increased in nonresponders but were actually decreased significantly in responders. And along with that, we were able to show that responders had reduced amounts of these cytokines that were associated with myeloid cells, such as CXCL8 to IL-8, and high amounts of type 1 cytokines such as IL-21, IL-10, FLT3 ligand.

Along with that, when we visualized the metabolome that is the effect of the microbiome upon circulating metabolites, what we found was that responders had much greater serum bile acids, that the responders had a greater transformation, a more efficient transformation of primary to secondary bile acids, as shown here. And some of these compounds, including [INAUDIBLE] sulfate and hydrocinnamate, have been described as biomarkers of microbiome diversity and correlated with the presence of the taxa that we previously showed as being a response to anti immune checkpoint response.

So in summary, we were able to show that when you gave people stool, that resulted in a bunch of different changes at the level of the microbe in the gut as well as a bunch of changes in the cytokines as well as a bunch of changes in flow cytometry, or circulating immune phenotype. But the key thing that we tried to do here-- and this is computationally intensive work-- is how do you know that all of this is causal as opposed to just a correlation between organisms that are very, very, very numerous and, therefore, the pulsatility of drawing false conclusions increases because of the sheer number of organisms?

So if you have 10 to the trillion organisms, so 100 trillion organisms, you might have a p value that is significant just because you have 100 trillion organisms. So what we did here is we did a computationally intensive neural network analysis to identify causal relationships between the host, the microbes that were independent of a particular group or a particular patient by creating the statistical model of interactions between these different players-- the microbiome, the metabolite, the cytokine, and flow, so at the level of the microbiome and at the level of the host to try to see whether or not these relationships held true.

Using this transkingdom network, we identified a couple of different key nodes, which were all these different things highlighted here in either hexagons, squares, triangles, or circles. And we were able to show that the vast number of interomic edges belong to the microbiome metabolome data set, which had the greatest number of positive indicated in blue or negative indicated in red connections with the metabolome data set. And these microbial data sets were more densely connected to each other and to the other data sets than any other network.

So you see that the connections that emanated from the microbiome were greatest compared to any of the other data sets, indicating that the microbiome governed the interomic changes following checkpoint inhibitor therapy and microbiome resensitization. And along with that, we were able to show that when you look at one specific network, we were able to link the adverse cytokine that we had shown before, IL-8, with the presence of certain beneficial adverse bacteria and the myeloid cells that I highlighted earlier.

So in conclusion, why is this a big deal? Beyond being a cool science experiment, what is the big deal about all of this stuff? Well, the first to understand is that what we have shown so far is that in multiple cohorts of anti-PD-1 treated patients, for the first time, deep computationally intensive sequencing and computational analysis of deep data sets undercovered distinct microbial signatures not just associating responders and nonresponders but also for the first time indicating and linking metagenomic changes with side effects, immune related side effects, which happen to between 15% to 55% of patients, result in a large number of admissions to the hospital, cost the health system a large amount of money, and don't have a solution other than nonspecific administration of steroids.

So to try to further evaluate this, what we have done at the level of the cancer center is partner with a couple of people who are actually on the call in this to answer the following questions. The first thing is, can microbiome signatures predict immune related adverse events? And then can a microbiome specific intervention reverse immune related adverse events? And so to do this, we've developed an immune related consult service.

So some of you guys will be seeing this over the next couple of months. It's basically a large group of people. My interest in this, obviously, is from the research side. But this will be held by Jamie, and Robie, and myself. We've got Matt Hensley from [INAUDIBLE], Josh Levinson, Hunter Champion-- I'm sorry-- [INAUDIBLE], as well Tim Patton and a couple of other colleagues from rheumatology.

And we welcome many other people who might be interested in this and sitting in the audience. And if you are interested in something like this, please don't hesitate to email me and advertise your interest, because we're developing this both firstly to fix the problem, which is the fact that side effects exist and side effects need better management, but also to develop a microbiome focused biorepository to try to answer whether or not side effects and microbiome focused efforts can potentially be used to ameliorate side effects.

And actually, we're developing side effect focused trials. So this is an example of a trial that we've already developed that is pending funding, with a partner of ours, a pharmaceutical partner. And this is going to be a trial specifically of a microbiome intervention in people with tremendously serious side effects that do not respond to steroids. And the idea behind this is we are hoping to try to overcome the next wave of problems, which is not that we've cured cancer. I thought at the time in these people with cancer that is reproducibly now exterminated, we've now caused a second problem, which are side effects. And we want to reduce the burden of side effects in our cancer patients.

Now, the second question is, in checkpoint inhibitor refractory melanoma, we and two other groups have shown in these exciting science papers that microbiome modulation using fecal microbiome extracts can produce responses. So right now, this is just a cool experiment. This is an experiment. This is our data. This is the data from Israel. They were both published in the same edition of *Science.* And most likely, these happened to people who had the ability to respond to immunotherapy but whose ability to respond was thwarted by an unfavorable microbiome.

And all that we did was we merely restored the balance. We put the yin back in the yang. And we put the species back where they belong to allow that to help the immunotherapy to work. And the question is, can this now be done beyond melanoma? How can we extend our work? And so to do this, what we're doing is we've now developed new trials to treat other cancers, including non-small-cell lung cancer, a subsequent trial on melanoma, as well as a further trial in kidney cancer. And hopefully other cancers will be the focus of such an intervention in the near future.

Next, we know that these organisms appear to be good. But the overlap between the organisms that immediate beneficial effects, as I showed in the first part of the talk, as well as organisms that cause the antitumor side effects based on the microbiome transplant, what actually-- the overlap is very limited. And the overlap between these organisms and actually consortia that are currently in development to be used in cancer therapeutics is actually even further limited.

And what that suggests is we are merely at the tip. We've only understood the very, very most-- we've just started to plumb the depths of our understanding of the microbiome and its role in cancer therapeutics. And any such consortia is going to require further refinement before it's near to be used in cancer patients.

And so for example, this is an example of a consortia that is being developed by a company known as Vedanta Biosciences. And what you will see here is that there's none of the organisms that I highlighted earlier, whether it's in the groups of bugs that was shown to be important in our large data set or in the groups of organisms that were shown to be important in cancer patients treated with microbiome modulation. We don't see a lot of overlap between these 11 bugs and those bugs. And that suggests that these consortia need to be further refined.

So what I see as being the future for this line of work, which is actually a huge focus of the cancer center, where there is actually a microbiome shared resource-- of which I happen to be the clinical director, along with Hassane who's the scientific director, and Jesse [INAUDIBLE], who's the manager of the facility-- is the intestinal microbiome composition, is this group of bugs, or what we call now our microbiotype, our enterotype, a biomarker that cannot only predict response but to potentially predict side effects to immune checkpoint blockade. And so this is an area of work that we'll be doing in the future.

As I mentioned, microbiome modulation has been shown to be feasible in melanoma. But can we look at this in other cancers where resistance to immunotherapy is common and potentially linked to alterations in the microbiome? And so to answer this question, we have developed trials in kidney cancer and lung cancer that will shortly be open soon. And finally, very interestingly, how does the microbiome differ amongst patients of different ethnicities, particularly in non-Caucasian populations, mainly because the vast majority of the data that I've shown you has come from about 139 different white people?

So what is the microbiome in non-Caucasian populations? Particularly because, as it turns out, while melanoma is a disease primarily of the Caucasian race, other cancers besides melanoma such as lung cancer and kidney cancer actually do happen in other races too. So what is the microbiome? How is it distributed in other races where their diets are different, where their clinical characteristics are different? And along with that, we're hoping to identify consortia of commensal bacteria that can favor clinic response to checkpoint inhibitor therapy and ally that with hos specific perturbations.

So besides PPIs, which we've now shown to be adverse, besides antibiotics, which clearly harm the effect of anti-cancer immunotherapy, there will shortly be data showing that probiotics are adverse. So we need to identify and integrate this knowledge along with our knowledge of hos specific perturbations that affect outcomes with checkpoint inhibitor therapy and control for this, with the overall goal of essentially extending our efforts to cure more patients with cancer and also eliminate side effects. And with that, these are the people that I'd like to thank, a very big group here at the University of Pittsburgh. These are our funding sources. And I'm happy to take any questions.