

CHIMERIC ANTIGEN RECEPTOR THERAPY

SANDRA E. KURTIN, RN, MS AOCN, ANP-C Our next session --
I'm very excited about this, friends and colleagues here to speak, and it's entitled Understanding the Mechanism of Action for Chimeric Antigen Receptor, or CAR T Therapy. Please join me in welcoming Ms. Patricia Mangan of Abramson Cancer Center and Dr. Edward Stadtmauer of the University of Pennsylvania.

DR. STADTMAUER It's our pleasure to be here today to talk to you about CAR therapies.

Disclosures -- both Trish and I have worked as consultants for various industry advisory boards. In terms of the learning objectives, we're going to describe the mechanism of action of the CAR T cells and how they differ from other forms of cancer medicine, discuss the logistics of autologous T-cell therapy, including pheresis, processing, replacement infusion. We're going to review the steps needed to address the major treatment risks associated with CAR T cells, especially that cytokine release syndrome, and we're going to show how we monitor patients' response to treatment and management signs and provide long-term support for care who received these therapies.

The rationale for this cellular immunotherapy -- and I'm going to emphasize B-cell cancers, but we have these chimeric antigen receptors, or engineered T-cell therapies, studies going on for brain tumors, for breast cancer, for pancreatic cancer, for mesothelioma. Those are a little less advanced in development, but this really is potentially a therapy for all of oncology.

The rationale really started with the obvious fact that, unfortunately, despite all of the chemotherapy, radiation therapy, surgery that we have for various diseases, that advanced and relapsed and refractory disease still has a poor prognosis and survival is limited.

T and NK cells -- these immune cells from B-cell cancer patients can kill autologous cancer cells in the laboratory. We've always done it, we've done experiments where we activate T cells, and we can see the cancer cells be destroyed. Then, of course, for decades we've been doing allogeneic stem cell transplant, which is, in many ways, just the infusion of donor T cells into a patient. We've obviously seen these responses and cures when we infuse these donor T cells by causing a graft-versus-tumor effect, a graft-versus-leukemia effect, a graft-versus-lymphoma effect. But, of course, there's high morbidity and mortality usually associated with the graft-versus-host disease.

The philosophy is if perhaps we could engineer our own immune cells so they could specifically attack a tumor, we could get a good graft-versus-tumor effect, but since they're your own cells, you wouldn't have the graft-versus-host disease that leads to so much of the problems.

Targeted cellular immunotherapy could overcome the limitations of conventional chemotherapy and immunotherapy. The advantages of autologous T cells that are genetically modified is they can be a super antibody therapy and with the cellular component of an amplified response and lead to memory cells; the cells just stay in the patient's body to keep eating up any potential tumor that comes back.

The first thing you have to do when you make a cellular therapy or an immunotherapy is figure out what target you're going to use. It's really important to determine what is the best target because you want a target that is uniquely on the tumor cell and not on other parts of our body. These cells can sometimes be so active and so aggressive that if by chance the heart has the target on it and the same target as the tumor, then these cells are going to attack the tumor, but also attack the heart. So you have to be very choice in your decision of what to direct these cells against.

CD19 has been the original or the first target that we've directed a lot of these cells to because CD19 marks primarily lymphocytes and lymphocytes at all levels of development. So that's why you'll see that most of the original cancers that have been treated are the cancers that are CD19 positive or B-cell malignancies, and you can see on this chart that the earliest B cells or CD19 positive -- and those are the cells that lead to acute lymphocytic leukemia. Then, as they mature, which leads to the chronic lymphocytic leukemia, and the lymphomas, they store CD19 positive. Once they get to be plasma cells, the most mature B cells, they tend to start losing CD19.

What are the ways that we've tried to overcome immune suppression and immune tolerance to cancers? You all know of the monoclonal antibodies, rituximab being really the first major one, and most recently in myeloma we have two new ones, that elotuzumab and the daratumumab. Chemotherapy and immunomodulatory agents are somewhat immunostimulatory, particularly the lenalidomide and the pomalidomide, etc. Then you're going to hear, as I'm sure

you're hearing every day -- and you'll hear a little bit more from Tricia -- is the checkpoint inhibitors and how they are stimulating the immune system.

What I'm going to emphasize today are the cellular therapies and what we call the "tumor-infiltrating lymphocytes," the CAR T cells, which are the chimeric antigen receptor engineered cells, and the T-cell receptor engineered T cells. There are three major approaches that are shown on this diagram and they all are basically the same thing. The first thing you have to do is you have to collect lymphocytes from the patient and with the CAR cells and the TCR cells, it's mainly from the blood; we do leukapheresis to obtain these cells.

But also you can go to the tumor itself and in the case of a hematologic malignancy, it's the bone marrow, so the guys particularly here in Maryland at Johns Hopkins have done a lot of work with marrow-infiltrating lymphocytes. The lymphocytes that are around the lymphoma cells or the myeloma cells are the cells that are most likely to be directed against the tumor. So if you do a bone marrow aspirate; get those cells. You can then cook them up and activate them and potentially use them. I'm not going to speak much more about that, but the other two is to take the blood T cells and, again, you engineer them to put on their surface a receptor that will basically be a warhead to find the tumor cells and then you, outside the body, expand them and activate them and then give them as a blood transfusion.

You hear a lot about chimeric antigen receptor, and here's what, in a diagrammatic form, they are. A chimeric antigen receptor has three different parts; one part is outside the cell and it's an antigen recognition part, so it's like

an antibody receptor and it's a light chain, it has a heavy chain. And that's what's directed against CD19, for instance, or BCMA or whatever it is you want to direct.

Then there's a part that goes through the cell membrane, and then inside the cell are these what we call stimulatory and co-stimulatory molecules; one of them is called 4-1BB, another one's called CD28, another one's called CD3 zeta. What these are are molecules that are inside the cell that when the receptor gets attached to something sends a signal into the cell for the cell to start proliferating and get activated. It's really cool.

So what they did was they made genes, genetic material; DNA and RNA that code for these receptors. So the first thing that you had to do was create a receptor like this, get the DNA. The next step is you have to insert this DNA into your T cell, and the vehicle that we've used to do that is a lentiviral vector, so in effect we infect these cells with a virus, a lentivirus. And then it does what viruses do, which is to insert genetic material into the T cell. So getting a lentiviral vector, making the DNA, and then finally you have to have an artificial T-cell-stimulating tissue, and we call it an "artificial dendritic cell," which is magnetic bead that has on its surface two antibodies; an anti-CD3 and an anti-CD28 antibody, which makes the T cells when they are incubated with these cells think that there's an infection going on and makes them proliferate and get activated.

It was these three molecular biology techniques creating DNA to code for the chimeric antigen receptor making a viral vector that infects these cells, then having a technique to cook them up that really took 20 years in many ways. It seems like this is just in the last couple of years this happened. It is 20 years of

work to do this. In fact, my son is a senior in college and he's an evolutionary biology major, and when he took molecular biology, the first lecture in his course was about the chimeric antigen receptors to try to motivate them and show them how the stuff you're learning might be useful sometime in the future.

This just in a cartoon, once again, to show. Remember, you take this chimeric antigen receptor and you put it into a virus, you infect the cell with the virus, and then the cell then becomes a machine to put on its surface these chimeric antigen receptors, and then they find the tumor cells and induce apoptosis and cell death.

Every time you hear about these cellular therapies, this is the diagram of how they're used. You take a patient -- it has to be an appropriate patient, a patient with a disease that is not clearly, easily treated, with the current therapies, but is well enough to undergo this procedure -- and the first thing you do is you do a steady state T-cell leukapheresis; you take out the T cells. And then outside the body, those T cells are incubated with the magnetic bead-laden artificial dendritic cells, they're cooked up, they're infected with the cells, and you make a batch of these cells. You can make numerous batches so the product can be infused in whatever batches that you need.

Meanwhile, the patient usually gets some therapy to suppress their immune system to allow these T cells to engraft -- we call it lymphodepleting therapy -- and then it's just a transfusion, a quick transfusion of these cells.

CD19-directed chimeric antigen receptor T cells for B-cell malignancies have now been widely published, ever since -- I think 2011 was the first

publication of three patients with chronic lymphocytic leukemia. And the only reason why it was only three patients that were presented was because the money ran out. It was very hard to get grant funding for this sort of thing because it was so science-fictiony and so much in the future.

But when all three of those patients responded beautifully to therapy -- and I think two of them remained long-term alive, like one of those patients is 6 years out, complete remission, doing well -- that then stimulated the whole system. But it was only since 2011 that the first reports came out, so probably that's old news. Throughout the world there are many of these things going on.

To give you a summary, in chronic lymphocytic leukemia about 50% of the patients -- these are relapsed/refractory patients -- have no other options. About 50% of the patients respond nicely, and about half of them, or 25% of the total group, are long-term doing well. The real miracle is in acute lymphocytic leukemia where somewhere in the neighborhood of about 90% of the patients who have received these cells all relapsed/refractory, no other options, respond and maybe two-thirds of those people are long-term doing well. And though I said greater than 3 years, we now have patients who have durable, complete remissions 5 years, 6 years out.

This shows you the survival curve. This is from the *New England Journal of Medicine* in 2014, where about two-thirds of the patients -- most of these were kids and, in fact, probably pediatric B-cell ALL -- will become the first indication that might be FDA approved, but also adults having very nice responses.

In fact, the famous patient is Emily Whitehead. She was the first pediatric patient that was treated, horribly relapsed, refractory leukemia, got really sick in a way that Trish will discuss, but miraculously got through it and is in continued complete remission now 5 years after therapy.

ALL, CLL -- probably what you don't know as much about, so I'll give a little bit extra on it -- is non-Hodgkin's lymphoma. Obviously, non-Hodgkin's lymphoma is one of the more common diseases in B-cell hematologic malignancies. Since 2014, we've been doing a study in relapsed and refractory diffuse large cell lymphoma, follicular lymphoma, or mantle cell lymphoma, and these patients, just like in the same diagram that I showed you, get their T cells harvested, get some lymphodepleting chemotherapy, and then get infusion of the cells. We wanted to see how well this would work in the CD19-positive disease.

The choice of lymphodepleting chemotherapy is really the physician's choice. We try to direct that to what is logical for the particular disease. For instance, in non-Hodgkin's lymphoma, sometimes that hyper-CVAD type cyclophosphamide is reasonable, for myeloma a slug of cyclophosphamide, for ALL it might be high-dose ara-C, but we give some sort of lymphodepleting therapy and then infuse the cells. So this is a report of the first report given by Steve Schuster, who's the lymphoma specialist at our center who did this study -- 43 patients with a combination of follicular and diffuse large cell. Just looking at the large cell lymphoma -- this is 26 patients and they had an average of about three prior lines of therapy -- about a third of them had had a prior stem cell transplant. You can see that the lymphodepleting therapy that they got was a

whole concoction of different things. Some people got that EPOCH regimen, some of them got cyclophosphamide, some of them got infusion of etoposide.

In the end, about 50% of those patients responded to this infusion of therapy. And what's most important is that after about 6 months, no patient has relapsed from that. The median follow-up is only a year and a half so far, at least from this report, but these are durable. That's the key to this whole thing, is durable responses with these infusions. This is -- a picture is worth a thousand words; you can see the PET scan lighting up in the pelvis and completely going away. Look at that, just literally 1 month, 4 weeks, from the infusion of these cells the PET scan normalizes.

How about follicular lymphoma? Follicular lymphoma is a more chronic and indolent disease; we have a lot of therapies for it, but once it goes bad, it can be really bad. These patients had an average of five prior lines of therapy. Many of them had had transplant, and so this was a heavily pretreated group of patients. Seventy-seven percent of these patients, 80% of these patients, respond just to the infusion of these cells. And more impressively, that in this case, unfortunately, there was one late relapse at about a year, but virtually after 5 months, every patient remains in remission who hadn't relapsed within 5 months; so durable remissions, rivaling the ALL responses that we've seen. And a picture -- you can see that this is a PET scan that was lighting up all over the place, and literally a month after the infusion of these cells, a completely normal PET scan.

What about myeloma? Myeloma, as I suggested to you that CD19 may not be the most logical target, and we know from that daratumumab medicine, CD38, it seems like a really nice target and then elotuzumab has that CS1 (SLAMF7) as a target. But maybe CD19 isn't so insane. Let me show you that -- as I showed you, the plasma cells don't generally have CD19 on their surface. But we had a hypothesis that to make a myeloma cell, you have to first be a lymphocyte, and lymphocytes are CD19 positive, so perhaps the mother cell is CD19 positive even though the progeny aren't -- so, CD19-positive stem cells.

Additionally, there was evidence that there is a back and forth to cells, that plasma cells can de-evolve into CD19 positive, maybe even chemotherapy-resistant clones, and that there's a back and forth. So maybe focusing on CD19 maybe won't kill every myeloma cell, but it might kill the mothers and it might kill the most resistant cells.

We designed a study that would at least give us some suggestion as to whether this hypothesis is correct. What we did was we took patients with multiple myeloma who had very bad prognoses. They had had a stem cell transplant, an autologous stem cell transplant, but had less than a year of progression-free survival, so we knew that transplant was not the answer for them. Then we let them have whatever therapies they were going to have after that and then if there was ever a time we thought it was reasonable to do a salvage transplant, another stem cell transplant, which we commonly do in multiple myeloma, we would then do that, but follow it with an infusion of these anti-CD19 stem cells. Then if you have a remission -- traditionally, the second

remission after stem cell transplant is shorter than the first remission. So if we had remission inversions where the remission lasted longer in this salvage stem cell transplant, then that would suggest that these cells are doing something useful.

It was just a 10-patient pilot study, same as what we did before; we took some T cells out. In this case, though, the patients got a stem cell transplant and then we infused the engineered T cells. And the bottom line was there was no significant toxicity attributed to these cellular therapies; we did have one episode of what Trish is going to talk about: cytokine release syndrome. But in general -- and also we didn't know whether it was feasible to collect T cells from patients with myeloma; they're so heavily pretreated -- can you still get good, healthy T cells? But it was very feasible.

This is our famous patient that we published in the *New England Journal of Medicine* who was the first patient on this clinical trial. She was 48, she had an IgA kappa myeloma, and she only had about 6 months of remission after her first stem cell transplant. If you look at that curve looking at her IgA level, she never went into complete remission after her stem cell transplant and, in fact, never went into complete remission before getting any of this treatment at all. She had had 10 prior lines of therapy over 5 years and was basically dying of multiple myeloma. She had all the treatments that you would expect and, most importantly, her plasma cells did not have CD19 on them, so it's not a direct CD19 effect.

Then as you can see from the next curve, that we kept her in a little bit of a remission by giving her doses of cyclophosphamide. Then we gave her a big slug of high-dose melphalan, and you can see we gave 140 mg/m^2 , which is less than the amount of melphalan that she had the other time, and then we squirt in this cellular therapy, and she went into a complete remission. And that complete remission was MRD negative; we did every test we could to see whether there was any evidence of myeloma still in her. And this is the infusion of the cells with the whole team.

So what you can see -- this is the graph that we published in the *New England Journal of Medicine* -- that she went into complete molecular remission and 15 months later she was still in complete remission, so definitely a remission inversion. Interestingly, the T cells as far as we could tell, all of these engineered T cells did go away by about 3 months and she did recover some of her B-cell function. But this just shows that we did a bone marrow biopsy that was packed with myeloma beforehand; it was completely empty of myeloma after the treatment. This is 6 months after her treatment against medical advice skiing in Vail. I still can't believe she did that. Anyway, really cool.

We treated 10 patients and three of those patients had these remission inversions, but seven of them didn't, had shorter remissions, didn't get a lot of toxicity. So the logical thing is, why don't we get a different target on the myeloma cells themselves? And BCMA, it's called the B-cell maturation antigen, is on all of the plasma cells, but not on the younger myeloma cells, and so we've created a CAR against BCMA. This is the study that we've been doing -- it's not

completed -- we've treated about 12 patients, and the patients, again, get leukapheresis and then they get infusion of the CARs. We gave it in a number of different boluses over 3 days.

This is -- let me show you again, patient number one. We've been very lucky with patient number one; treat the first patient very nicely and they treat you nicely.

This guy was 66 years old, actually a cell biologist believe it or not, and his diagnosis was a decade ago with myeloma. He had 11 prior therapies, was progressing, really packed with myeloma; his myeloma cells did express BCMA. Then he got just an infusion of these cells and just like the other patient, went right into a beautiful remission, within 4 weeks no evidence of disease. He did have some cytokine release syndrome, but otherwise did well. A bone marrow biopsy day 28 was negative after being packed before that. And we're seeing him in 2 weeks for his 1-year follow-up completely in remission, so very cool.

The NIH has been doing this BCMA-directed cellular therapy, and just like us, they see very similar results. It's only about a third of the patients having these wonderful responses, but seems very reproducible.

To conclude my portion of this talk, we're seeing really successful cellular immunotherapy. Immunotherapy for myeloma and lymphoid diseases started with the immunomodulatory agents; lenalidomide, pomalidomide, and, of course, those monoclonal antibodies that synergize with this and checkpoint inhibitors have the potential of stimulating this. But there are many promising targets, many

promising ways of making these cellular therapies. We've proven that you can get functional cells that are active against these lymphoid diseases.

PATRICIA: What I wanted to address after these exciting results -- and clearly, this is very novel and new -- it is not without toxicity and it's important to be aware of the potential toxicities. When the cells are infused, there is really no significant infusion-related toxicity. To give you a sense, for those of you that may have participated in some of the trials, but for those that haven't, the amount of infused cells -- it's very similar to a peripheral blood stem cell, but it is very purified and very small amounts; 10 cc is what's infused. It's frozen after all that processing occurs in the lab, brought to the patient's bedside at the time of the infusion, defrosted, and just 10 cc is administered.

It can be administered over just one infusion, depending on the protocol. As you saw in some of the protocols, it's split up over 3 days where a small amount is given day one, second day a little higher percent of the cells, and by day three the remaining amount of CAR cells are infused. It is a biologic therapy, so it does sometimes have some fever, some chill associated with it and so it's not a 0 -- it's not uncommon to see a little fever with that. Tumor lysis syndrome, just through the description of the patients that we saw, they were very heavily treated, high tumor burden, so it is a potential risk of tumor lysis, so we're always observing for tumor lysis labs, hydration, utilizing uric acid lowering medications, and things like that.

There are toxicities, as Dr. Stadtmauer mentioned. We do utilize lymphodepleting therapy. And why do we do that? Depending on the disease, we

try to clear out some space in the marrow to allow these T cells to grow and multiply, and that's why we give lymphodepleting chemotherapy.

In the myeloma trial that we highlighted with our patient who was skiing on the top of Vail, the mountain in Vail, she underwent a high-dose stem cell transplant, so we are dealing with the toxicities related to high-dose therapy, too, in that setting. There are some issues, some cytopenias, that we're dealing with at the time of the infusion.

B-cell aplasia -- we're targeting B cells and these T cells it's nondiscriminatory, it kills B cells. There is potentially a very profound B-cell aplasia and it's something to be aware of and monitor over time. We evaluate IVIG levels and administer IVIG, usually in a monthly like fashion, and we monitor that to prevent recurrent infections. Up to now, we haven't really seen too many issues with infection related to the aplasia created by this.

Cytokine release syndrome, though, that is the more serious and more common toxicity related to this. So I will now highlight that a little more specifically because it is somewhat specific to CAR therapy, unlike other things that we have seen. It mimics very similar to a septic event where we see very high fevers shooting up, some hypotension, and some hypoxia. These patients -- sometimes these infusions that the T cells are administered as an outpatient. Sometimes we administer them as inpatient. We are very closely monitoring them, and if they are home, they are monitoring their temperature very closely, calling us immediately if they have fever. If we're concerned with a fever, very commonly in that divided dosing of the cells, day one, day two, we'll see a little

mild fever, but it's associated now with a little hypotension. We might admit that person just to observe them for the evaluation of cytokine release syndrome.

To give you a sense when is the peak activity that we see, the cytokine release, it's usually around day 10 after infusion of cells, although it can happen any time after the infusion and even later than day 10. But if you look at all the patients that have been treated, the most common time of the real cytokine release is around day 10.

It is associated -- it's cytokines, and in particular interleukin 6. It is really stimulating this release syndrome. It is similar to that HLH macrophage-activating syndrome, which is usually seen in pediatrics, so we as adult practitioners weren't that familiar with it, but we're learning. It's very similar to that where there's a cytokine release happening and it is associated with a very high ferritin level, so we monitor ferritin. To give you a sense, a normal ferritin is around 300 and these can drive up very quickly to right -- it's listed here greater than 50,000 or higher than that even. Also, C-reactive protein, as we know, is a surrogate marker for interleukin 6 and so that can definitely be elevated, as well, so we do watch that.

C-reactive protein -- the test that we do measure, though, is the noncardiac C-reactive protein because sometimes you'll check one and it looks okay and the other one's elevated. To give you a sense, that's the one that we're looking at.

There is a mental status change that occurs and it's very vague. And it's not a classic thing; sometimes patients -- could it be associated or is it just

because they're having high fever and they're sick? But sometimes they're a little confused. We had some people that had word-finding problems, and some become obtunded. But we had one patient who was from Italy and he spoke English perfectly, but when he became change in mental status, he only spoke in Italian. And then as he got better, guess what? He started speaking in English again. So it could be you start speaking in different languages, but that's not true.

We know what turns this process off -- steroids. What do steroids do? They decrease inflammation and they kill lymphocytes. So that's great, we have that, but we don't want to kill the T cells, right? We want them to proliferate and grow. Miraculously, we have found that the antidote, over time we figured it out and it's tocilizumab, or toci, and it is an anti-interleukin 6 monoclonal antibody. We have pulled people out of a very serious illness with the use of this.

So the interesting thing with this, and our experience, is that the patients who have experienced cytokine release syndrome tend to respond. There have been a few patients that developed cytokine release but ultimately did not respond. We've had some people that responded without cytokine release, but generally cytokine release is commonly seen and it may herald the event or response. So when do we give tocilizumab is we have gotten very good at doing this and over time you'll figure out, it is not very quickly given, it is a very serious discussion usually in the middle of the night with all the PIs and everything, "Should we turn it off now?" And things like that. So these are the things that we are aware of. As you can see in our results, we've given toci as soon as day two and as late as day 11 commonly or even a little later at this point.

Let me give you a sense of this. This is in hours, so we're looking at day 0 is on the far left of your screen and then you see the temperature curve was normal then. The patient developed some fever, but the temperature did go as high as 103, toci was given, and you can see how dramatically that just chilled out that reaction. Temperature resolved very quickly and, again, the ferritin levels -- this is a different patient, but to give you a sense -- normal ferritin and it's always important to get a baseline. It's not really the absolute number of the ferritin, it's within that patient and the change that occurs. So some people are starting with a higher ferritin level than someone else. You can see this was a relatively normal ferritin level, jumps up with this whole cytokine release, and given toci, it dramatically improves. Graphics are impressive when you see this.

We know that CAR therapy does hold great promise for patients with CD19. How best to engineer these cells Dr. Stadtmauer led to. There's just been an evolution of how best to keep these cells persisting in the body; how best to deliver them; how best to manage this toxicity we're still working on; and, certainly, when to time the turning off of the cytokine release if it occurs; and how to maintain this persistence. But when you maintain persistent -- which hopefully in medications keeps people in remission -- we're going to always deal with B-cell aplasia as a component of that, so administering IVIG is always helpful.

Then, just to summarize, we talked about the toxicities of the cytokine release syndrome, the tumor lysis, and neurotoxicity. Again, seen in many of the trials, this mental status vague thing, that can vary. Usually it is self-limiting and does resolve over time. And we talked about the B-cell aplasia.

Now, that is very patient-specific T-cell immunotherapy and it's expensive and it's time consuming and you have to grow these cells up and things like that. So what are out there? Wouldn't it be great if we had T cells that could activate T cells within a patient and we didn't have to take them out first? We could just pull some medicine off a shelf and give it to you? And this is what these bispecific T-cell engagers are, and it's cool where these are very small molecules - and they're called BiTes. If you look at the center of this diagram, there is a piece of the BiTe that attaches to the T cell, CD3 on the surface of a T cell, and engages there and it activates the T cell. The other component of the BiTe is attaching to a cell surface tumor surface protein. In blinatumomab, the target is CD19 and so it latches on to that CD19 and causes a more activated T cell to attach to this and promotes more cell death or apoptosis.

These are really cool molecules. Right now, to give you a sense, blinatumomab is FDA approved for B-cell ALL that is pH negative, and it's been on the market for about a year now. It does have some potential toxicities, and if you look at these, it's very similar to cytokine release. Other symptoms that we've had with our CAR infusions as well: flu-like symptoms, some tremors, some electrical abnormalities, the immunoglobulins do become suppressed. There are mental status changes with this.

Just recently I was walking out of work the other day -- and that's when I check my mailbox because it's where I get on the elevator -- and there was a handout I just received that there is a new warning of pancreatitis associated with this, so that's something too. We're always monitoring electrolytes as well.

Blinatumomab, for those who haven't given it, it is an infusion over a month and it's titrated; people get admitted to the hospital for that first week, and it's slowly titrated up. It's very interesting, if you have to stop the infusion for any of these potential toxicities, it has a very short lifespan, very short half-life, and it very quickly resolves. But unlike CAR therapy where we don't want to kill those activated cells that we cooked up, we can turn this off also with steroids. So this is the antidote for this, although tocilizumab can also be administered, as well. But, generally, just the stopping of the infusion and then after 4 hours if the infusion hasn't restarted, you have to go back to the first stage again and then slowly escalate up again.

To give you a sense, a little bit about these BiTees and maybe there'll be other targets in the future, so it's definitely a cellular therapy. I know there was a big talk on checkpoint inhibitors that we know that T cells have evolved over time; they're a part of our immune system to help us fight infection, and we know that they're very active and very good at fighting infection. But to self-preserve ourselves, T cells have created inhibitors or brakes on their own cells so that we don't attack our own body too much. So these are program death PD-1 and program death antigen on a T cell has cancer cells, also have a PD-1 ligand and when they connect together, it prevents the T cell from actively killing it as protective mechanism.

Let's put a brake on that. Let's inhibit that interaction with a breakpoint inhibitor or checkpoint inhibitor and it allows the T cell to be more anticancer against that particular cancer. We know that the FDA indicated some of these

PD-1 inhibitors tremendously efficacious in melanoma and lung cancer, and we're now looking at it in blood cancers as well. Nivolumab was approved and we know it's approved in Hodgkin's disease -- I guess is the first blood cancer that it's been approved in. But we are looking at it even as a mechanism of action in other blood cancers and we have a trial -- there's been a couple trials and I know in the audience some of my myeloma friends here today that they're combining pembrolizumab along with pomalidomide, and dexamethasone has been shown some really exciting -- so it's more of a cellular stimulating our own T cells to be anticancer with some nice responses.

I know we're running out of time here, so I wanted to just end with that -- the age of immunotherapy for blood cancer really is upon us. As Dr. Stadtmauer said, it's probably been upon us for about 10 years, but we just have been becoming aware of it recently. So the sky's the limit so much, so how best do we utilize this technology? Do we move it up sooner in the disease state, earlier patients being treated with these long response rates? If we can move that up further, that would be tremendous. How best to make these T cells stay in the system longer for that persistence and working on that and making them more potent, making more targets that we can treat many different cancers with this. Clearly, like with every other therapy in cancer, combining it with other agents.

As you can see, hopefully we gave you some of the thought process of this whole thing and going forward maybe some newer cocktails to keep people with blood cancers in particular, but all cancer, doing well for a long time and maybe cured. We couldn't do this without our whole team.

Listed here are just some of our patients and our staff, and our laboratory research guys have worked very well. And then, finally, once questions end, please post questions that you can answer. Hopefully, that was helpful, and I'll now open it up to any questions that you may have and I'll ask Ed to come up and see if he drops his mic.

FEMALE The patient who was skiing, is she still doing well?

DR. STADTMAUER Yeah. In fact, I saw her yesterday and she's great. She's 2.5 years now from the infusion of those cells.

MALE I have a couple of questions. One is, the patients receiving infusion, did you guys do it in an outpatient setting or inpatient setting? And the next question that I have is, the toci -- because you say you guys did not treat the neurotoxicity you guys saw or very few of them -- so, the question is, whether the toci was given as a drip or boluses?

DR. STADTMAUER Yeah. Our style and our plan is to always give these infusions as an outpatient. As of this moment, since every patient we've given an infusion of has been on a clinical trial, we do it in our outpatient clinical research unit and with monitoring. But usually I would say the most common time that they get infusion is around 11 o'clock in the morning and usually by 3 or 4 o'clock in the afternoon they've left. We have patients coming from distant areas and we do ask that they stay in the Philadelphia area for 4 weeks, and at least for the first week, we watch them pretty much daily, but then afterwards we see them at least once a week for 4 weeks.

In terms of the toci, the toci is given as a bolus; we just give a single bolus, we watch them. As Trish was mentioning, the art of this is to -- since we don't know, we haven't done a randomized trial of giving toci versus no toci. We don't think it has a negative effect on the effectiveness of these cells, but since we don't know we try to wait until it's the -- this is the art of this -- we wait until just before they might need to go to the intensive care unit for more monitoring, and we've avoided that circumstance in the vast majority of patients.

FEMALE Thank you very much; it was a great presentation. I have two questions. The first one is, what is the time interval for monitoring the CAR T levels? And then can you talk a little bit about the survival of the CAR T cells and it's correlation with survival?

DR. STADTMAUER Sure. It depends on the disease it seems like right now. In those original studies of the acute lymphocytic leukemia -- and I guess in particular we do monitor every month and then it turns into every 3 months. We monitor levels of the CAR T cells in the patients, both by blood draws, as well as because there's a genetic change we can monitor by like PCR, the genes. So we monitor the levels of that, and for our patients with chronic lymphocyte, our long-term patients, virtually all of them still have some of the detection at very low levels. We do think that the levels of these cells will respond to the disease burden, that if they start relapsing the cells will go up, then hopefully zap the tumor, and then they go back down. But not everyone.

In our myeloma experiments, the patients within a month or certainly within months, their levels go down to nothing and yet, we have long-term, so I

guess it depends on the disease. In some patients, hopefully this zap kills everything and cures the disease; for others, they may need more of a serial thing.

FEMALE Are you taking patients to transplant after they get the CAR T cells or they just the CAR T cells and then you just watch and wait?

DR. STADTMAUER That's a really good question. There are differences, but there are many centers throughout the country that are doing this work and I think that different centers have different philosophies. So a number of the centers have used CARs as a bridge to allogeneic transplant with success. For us, we've been primarily focused on trying to use it as the cure and so we haven't generally done automatically allogeneic transplants. We've had some patients who have relapsed after the CARs who've had transplants or patients with ALL, like adults, who had really difficult times who we've done that, but it's the minority of our patients and not of our plan to do allogeneic transplants.

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