CAR T-CELL THERAPY AND THE PHARMACOLOGY OF MANAGING CYTOKINE RELEASE SYNDROME

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MODERATOR Welcome back, everyone. Our next session is on a topic that is at the top of our mind in this field: CAR T-Cell Therapy and the Pharmacology of Managing Cytokine Release Syndrome. Please welcome our wonderful speakers, Dr. Jae Park and Dr. Amber King, both of Memorial Sloan Kettering Cancer Center.

DR. PARK Thank you for inviting us to give a talk. We're both from Memorial Sloan Kettering Cancer Center in New York. I'll be doing the first half of the talk and talk about a little background and clinical information about the CAR T-cell therapy in ALL and large cell lymphoma, and then Amber will talk a little bit about how to manage some of the side effects that we often see associated with the CAR T cells.

These are the learning objectives. Hopefully, by the end of the sessions we can identify and, hopefully, I can highlight how to identify those patients who meet the criteria as candidates for CAR T-cell therapy, consideration for the efficacy, and then the side effects. While as much as it works really well, it may not be for everybody. And then, the second objective is to devise strategies to mitigate cytokine release syndrome and other side effects associated with the CAR T cells.

So, first to start off, what is CAR? And you guys may all be familiar with it; at least you guys have all heard about it. Now they are an FDA-approved therapy. There are two approved therapies for CAR T-cell therapy for ALL and DLBCL. So, a CAR is the combination of an antibody and then the T-cell receptor.

You can see here the first part of the CAR T-cell therapy. The CAR T-cell construct comes from a binding domain from an antibody. This is what we call single chain variable domain, so you kind of link them together, heavy and light chain into a single chain like this. And then you connect the head of the antibody to a body of a T-cell receptor. So, the body that's embedded in the transmembrane domain here and then the cytosolic domain here, the T-cell signaling domain. Then, these are now combined to the antibody.

So, in a way, what it allows us to do is that it has a specificity of an antibody target recognition, just the same way that any antibody is targeting tumor antigens on anything that's expressing on the cell surface, but it acts like the T cells. There's a combined advantage of both the cellular or the immunotherapy.

So, how do we really move the CAR T-cell therapy into clinics and then make it successful as a cancer therapy? So, this is a simple structure or kind of a very simplified schema of a CAR T cell, as we talked about. Again, the binding domain from an antibody against a specific tumor antigen and then the T-cell receptor body.

Once you create the construct and you have to insert the CAR into—CAR stands for chimeric antigen receptor—into a T cell. And the T cells can come from either the patients or the other allogeneic donors to talk a little bit about them, but the most common is coming from the patients themselves.

So, how we insert the CAR T car into the patient's own T cells is that we use a replicase incompetent, either retro or lentivirus. So, these viruses really acting as a bacteria carrier to carry the genes, so it's a DNA replicase of the patient's own T cells.

Some of the T cells will then take them into the retroviral DNA, so any T-cell DNA that has taken in the retroviral DNA, all the progenies of those subsequent T-cell expansion will carry the CAR that you want them to. So, you then select out those CAR T cells that you want to, then you infuse the T cells back into the patient. And once they're in the patient's body again, these CAR T cells then travel to the side of the tumor. Because now they are equipped with this CAR, it is against the specific tumor antigen, and then start eradicating the tumor cells. So, that's really the basic concept of the CAR T-cell therapy.

So, in reality, how that works is that we take the T cells from the patient. In a cellular manufacturing process there is what's called cell culture or the activation happens, so this whole process can take about 7 to 14 days. It really varies depending on the manufacturing process and how you are modifying them, either retro or the lentivirus. So, once these cells are made, they can either be infused fresh or more commonly cryopreserved. So, they are frozen and then they ship to the site of the clinic where the patients are being treated. And then

once they arrive at the site, they are thawed at either the bedside or the cell therapy center and then infused back into the patient.

This is what we call vein-to-vein. One factor to really consider is that they are not readily available like any other drugs that we have. It's not quite off the shelf, at least in the approved way now. So, this whole process—even though the manufacturing can take about 7 to 14 days—there's a pre-collection and there's a quality control that needs to happen after to make sure these cells are clean and not contaminated, also viable. And this can take about 3 weeks on average, but up to 4 to 5 within some cases, which implies that some patients may not even survive long enough to allow the T-cell infusion. So, that's the first factor to consider. It's just really the right patient who is able to at least survive through the process of a 4-week period of time to receive the T cells.

And during the manufacturing process, the patients are able to receive some chemotherapy or any therapy to stabilize their disease. But some of these patients, if they're really, really refractory to all the lines of therapy, you may not have a lot of options to control the disease and those may not be the best patients to be treated with the CAR T cells.

So, why do we have to create this CAR and go out of the way to create the combination of this from an antibody and the T-cell receptor? What it allows us to do is initially an independent antigen recognition. So, the native T-cell receptor T cells generally have is that they require specific HLA type for the antigen to be processed and then be presented to the T cells and that's how T cells recognize them.

But, the HLA is very specific to the patients themselves or the people themselves. So, the antibody is universal, so you don't really have to consider the HLA. Anything that is expanding to the cell surface, you can target them. So, the universal applicability is really the one benefit of the CAR T-cell therapy.

So, any target in the cell surface can be targeted. And the rapid generation tumor is supposed to be T cells. And when we say rapid, again, it's about 7 to 14 days. Depending on how you look at it, they could be fast and 14 days is longer. It is getting better and better since there are some ways that cell manufacturing may take even less than 7 days.

And these T cells because they're coming from the patient themselves and there is really very little risk of graft-versus-host disease as opposed to allogenic stem cell transplant, which is really the biggest side effects of that, is the graft-versus-host disease. And the biggest potential—the reason that we are excited about the CAR T-cell therapy even though we still need to modify it and have work to do—it's a living drug, it's a potential for the lasting immunity.

As opposed to antibody or antibody-drug conjugate that you need to continuously administer these drugs to really get the most benefit from this therapy, the cellular therapy is just kind of the one-shot deal in a way as we're kind of envisioning is the one single cell infusion. I mean, one infusion of the CAR T cells can generate, once they're in the patient's body, they can replicate, they can expand usually 1,000-fold, 10,000-fold, sometimes even a million-fold expansion, and they last to provide the longstanding immunity.

Now, it's not always successful that way that some of these patients are able to get really the maximum benefit and are alive after 5 years. And these are the ones that we often hear about and generally a lot of excitement and the potential of the cell infusion can be kind of the one-time and the living drug that continues to replicate. As the tumor cells arise, T cells arise at the same time too and kill the tumor cells again and go into a quiet state and they replicate the stage to provide the long-lasting immunity. That's really the holy grail, where the biggest potential of the cellular therapy is and that is what we are working toward.

Where this CAR T-cell therapy is currently being used as a cancer therapy is a CD19; it is really the best target for the B-cell malignancies. So, any B-cell malignancies or ALL—acute lymphoblastic leukemia—or the lymphomas, a lot of B-cell lymphomas, we then have the CD19 universally expressed on all stages of the B-cell maturation, including the BLL. And, again, the B-cell lymphomas, and then as opposed to CD22 and CD20, which are also expressed in some of the BLL patients may not express CD22 and CD20 as opposed to 19.

But, importantly, it's not expressed in hematopoietic stem cells. The one factor to consider is that these CAR T-cell therapies because they are very potent and they can expand 10,000 times kind of once in the patient's body and they're much more potent than the antibody therapy if they were to work really well and survive long-term. If they target really vital cells, then we're in big trouble.

So, it is very important that they are not expressing hematopoietic stem cells so we don't have to worry about eradicating or bleeding the stem cells and

these patients do not need a subsequent bone marrow transplant to rescue them. So, that's kind of the big factor to consider. The selective expression of the target is really the key to make the CAR T-cell therapy a success.

CARs have come in many generations, so this is kind of the first generation they came about is really the very simple – the antibody or the body of a T-cell receptor that didn't really work quite well. And then the second generation, which is kind of what we will be talking about most of the time and these are what's in clinic now, is the same as the first generation, but there's one additional component to it, which is a co-symmetry domain of a CD28 or 4-1BB.

So, this is what's called signal two is that these T-cell receptors or T cells in need of signal one, which is the antigen recognition, that's the very first step the T cells require to be activated, it needs to recognize the antigen. But that's not enough. If that's all they get and they get exhausted, they get anergic very quickly. So, they need a signal two to excite them even more, so these are real tumor antigens, they really need to be activated. Signal two needs to be provided. But a lot of tumor cells get smart and they don't express the signal two ligand for the activity of the T cells.

What the secondary CAR T cells have is that within them – even if the tumor cells do not express a signal two ligand CD80, for example, and for 1BB ligand that express a signal in itself, so they can activate themselves once it's recognized by the signal one of the antigens. So, that's really the biggest potential.

Where we are with the timeline of the clinical development – MSK was actually, and it's probably a well-known fact, is the first site to ever treat oncology patients with a CAR T-cell therapy. So, the first CLL patients were treated with MSK and that was in 2007. So, it's about 10 years ago is when the journey actually began.

After that and then subsequently, the other disease process either would be some malignancies that we're doing about and then ALL patients and in the lymphoma patients and follicular lymphoma patients. And then it took about 7 years until we actually really got the phase 1 clinical trials in ALL and in non-Hodgkin lymphoma to really get the process going.

We'll first talk about the CAR T-cell therapy in ALL. So, this is just to provide that not everybody may be treating adult ALL patents, the leukemia patients, so these are a very, very poor prognostic group of patients. This is really an outcome of relapsed ALL in adults with a chemotherapy alone.

The graph on the left is showing the largest data from the MRC, the UK, and then the US data showing that if your first-line chemotherapy fails, you have less than a 10% chance of long-term survival. And then the second curve on the right is showing another French – the European data showing a very similar outcome. So, the relapsed patients even if it's their very first relapse, they don't do quite well, and there's a huge need for these patients to improve their survival rate.

Where we are with the CAR T-cell therapy in adult patients with ALL and how we're really improving the outcome is that this is a phase 1 clinical trial that

we conducted at MSK using one of the second-generation CAR T cells targeting CD19; that's where the 19 comes from. And then we have a CD28 as a closed-in domain and we talk about the second-generation CAR and one of the signals, and then what we are using at MSK is predominantly CD28. Others have used a 4-1BB, and I'll show that data, so, that is why it is called 1928z is actually the signaling domain, the zeta chain.

The 1928z CAR T cells in relapsed/refractory ALL at MSK, and this is the study outcome that we published earlier this year and our long-term outcome. With chemotherapy in this patient population—and I don't have a table to show you—but it's about 20% CR, which we'll get excited about. The complete response is what's expected for conventional chemotherapy.

But in this data that we are seeing a CR rate of about 85%, which is significantly higher than we have anticipated in seeing with the chemotherapy alone. And this is replicated across all different CAR T-cell trials in ALL. And this data of an 80% CR rate is actually what generated the initial excitement of the CAR T-cell therapy several years ago. That's probably when we all started to hear about, you know, what's really the potential, this has a true promise, and kind of a very encouraging therapeutic option for these patients.

And this is really small, but just to show you there are a variety of the subgroups that we have looked at, the patients with a very large or small disease burden or the patients where there are a lot of prior lines of therapy. And as you can see here, most of the patients had at least three or four prior lines of therapy, meaning these are really super refractory patients to the conventional treatment.

We got less of that in the age groups' all groups really benefited from the CAR T-cell therapy with an 80% complete response rate.

Now, this is a long-term outcome that we saw with those and then it shows you the 80% complete response rate. But when you actually look at the survival curve, there are a couple points to point out. The one is that it is not as good as we hoped it to be. And even though we have a long-term survival, the median follow-up is about 30 months in this patient population and we have certainly room to improve.

Because despite the 80% complete response rate initially, there are early relapses that do happen in these patients and we need to watch out for these patients very carefully. So, it's really too early to celebrate until about 6 months after. If you don't really relapse the first 6 or 8 months, the chance of relapse after that is significantly low.

But what is really exciting in this patient population is, again, these are the patients that we don't expect to survive long-term beyond 1 year, so these are the dismal prognosis patients. But, we can see the patients are surviving beyond 5 years; this is just kind of going above the 60 months altogether. So, this is what is also generating excitement in a way that even though there are early relapses, they are long-lasting responses these patients do experience and what we need to really work on is how can we improve the response rate?

In this very same study, we also looked at what are the factors that determine really the best success of the therapy? And then we looked at a bunch of different factors; there are disease characteristics, there are cytogenic and

molecular status age, prior lines of therapy. But, regardless of what has really come out to be the best predictor of their long-term response is the disease burden, so the lower the disease burden that you have—in ALL patients we define them as less than 5% leukemia cells in their bone marrow—the better the outcome these patients got.

The low disease burden patients, which is the curve in the red box there, is significantly better than the high disease burden patients. Almost all high disease burden patients eventually relapse and then, unfortunately, succumb to the disease. But it's really the low disease burden patients who are really enjoying the long-term benefit.

So why then maybe in the case is one thing that we continue to work at MSK to try and find out what really separates out the good responders and then the not so good responders and there are a lot of factors to consider. T-cell quality for these patients are different. And the one other thing is the low disease burden patients, they have a better control of the disease at the time of our T-cell therapy. And that's the one factor that we try and emphasize at least in adult ALL patient populations to think about what's really the best fit for the CAR T-cell therapy.

When we think about the referral to the centers who really deliver this CAR T-cell therapy are really not the best patients are the ones with rapidly progressive disease. And those are really very difficult to get on to the CAR T-cell therapy trial or even the commercial therapy because it's very hard to pick a time

to collect the T cells and wait for the 3 to 4 weeks of a T-cell manufacturing process when the disease is taking off so quickly.

So, really, the best population with somewhat stable disease, they could have a high burden disease, but stable disease is really the key at the time of the T-cell infusion to get the best benefit. And, of course, the lower the disease burden is better, as well. And we think it is really driven by the better effective target ratio, meaning these patients get the same number of CAR T cells. But, if you have a low disease burden, there's much more T cells relative to the amount of the tumor cells, and the favorably effective target ratio may be what's driving the benefit of these low disease burden patients.

So, we share about the data at MSK that we have done, but what about the other centers? So, this is tisagenlecleucel. It used to be called CTL019, that was originally conducted at Children's Hospital of Philadelphia and then now it was a global study and this is a global study result in children and young adults with the relapsed/refractory B-ALL. So, what I just shared with you is our data with adult patients 18 and older and this was up to the age of 25.

So, again, this is a 25 global study and the 92 patients enrolled and 75 patients were treated and then the T-cell dose and then the design was pretty similar. And this is a survival curve from the data. Then, you can see this is already better than what we have seen with adult patients.

The one thing to keep in mind—and I will show you the data overall at the other centers—the pediatric ALL patients unfortunately tend to do much better than adult patients too. And so, even the less refractory patients when they get it,

the disease is very different. So, not only the age of these patients, tolerability of the therapy, and then ability to go through the side effects, but their disease itself is actually better responding to any therapy they do get. So, that's kind of what is driving the differences in the survival.

But the same pattern emerges here, too, the early relapser. You can see about in the first 10 months or so, there's a continued decline of the curve, meaning there are relapses that happen during the first 10 months or so. But, after that, if you don't relapse during the early part of the time, then your chance of relapse is very significantly lower. Because it is a long-term follow-up, this is holding true for an even longer follow-up now that the responses are durable.

So, based on that result – and the response rate, again, in that study was about 80% as well. So, this is what generated the first ever FDA-approved CAR T-cell product that was done in August 2017 for treatment of the patients, but only up to the age of 25 because that was really the data for the tisagenlecleucel for the relapsed or refractory B-ALL. So, we still do not have an approved CAR T product for adult patients older than 25. So, 26 and older, all CAR T-cell therapy is currently done as clinical trials for ALL patients.

So, this is an overall composite data of the clinical course after CD19 CAR T cells; again, similar patterns, so the pediatric ALL in the top panel there tend to do much better. In an adult ALL study, there are fewer because it's a much rarer disease in adults than in children. But, even though they're better, they will be expecting to have conventional chemotherapy, they tend to do slightly worse than what we expect in the pediatrics.

And this is just composite data just to highlight that regardless of which CAR you use, which centers, which manufacturing process. It is one thing that is encouraging, it is a very consistent result, about 80% complete response rate, and similar relapse rate is about 40% in this patient population.

So, we will move on to the DLBCL patients—diffuse large B-cell lymphoma—we focus about in ALL. They are now an approved product for that, too. The first one is axicabtagene, but this is based on ZUMA-1 trial. This is again showing 1928, so the construct is actually very similar to what we are using at MSK, targeting CD19 with the CD28 coasting domain.

For overall response rate in this, also, again heavily pretreated patient population was 82%. Now, the 54% of this was complete response and about 28% were partial response. And then ALL data, all the responses we're talking about really complete response. And partial response in ALL is really not that meaningful because almost all of those patients will relapse.

And DLBCL is a somewhat truth, as well as the aggressive large B-cell lymphoma is really the complete responders who do much better than the partial response. And this is kind of the curve that shows that the complete response—which is the green line on the left side of the curve there showing the best responses—and then the partial response. If you get more than 50%, but not completely, those patients now tend to not do very well too. So, really, it is really the complete responders who do very well.

But the relapse rate even though they happen, they are less than what we get with ALL. So, what is really quite interesting is that using the same number of

the CAR T cells, that we're getting a different response rate. The complete response is about 50%, so lower than 80% that we see in ALL, but their relapse rate is slightly different; there's only about 30% or so and it's very durable. So, there are actually a lot of differences, so it really makes it also very difficult to interpret the data and go trial by trial too.

Based on that—and I didn't share the data with tisagenlecleucel, which is the compound that we reviewed the data for pediatric ALL—they have results in a very similar data in adult patients with DLBCL. So based on these two different clinical trials, there are now two products: one is axicabtagene that is approved in October of 2017 for relapsed/refractory large B-cell lymphoma after two prior lines of a systemic therapy. And then there are some histologists that are really applicable, which is the DLBCL not otherwise specified, primary large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL that arise from follicular lymphoma.

And the tisagenlecleucel, which was subsequently approved in May of 2018 for very similar indications for this too.

Where we are now is that now we have two approved products; one for both DLBCL and pediatric ALL—that's tisagenlecleucel—and then we have one approved product for large B-cell lymphoma, the axicabtagene. So, this is quite exciting. There's now more prospective clinical trials to understand where the CAR T cells fit in as well.

We already talked about low burden disease is probably the best setting to do. It is really not best to do CAR T-cell therapy, which is also true for the large

B-cell lymphoma where the tumors are rapidly progressing is really not the best setting to try for the CAR T-cell therapy, so a lot of trials are now ongoing trying to move the T-cell therapy to earlier lines of a therapy too, rather than waiting until their fourth line of therapy, trying to move them to a first or second—the relapsed line of therapy.

For sake of the time, I'm going to just talk about the toxicities a little bit because I think it is very important to focus on. We talked a lot about the clinical efficacy, which is what generates side effects. But what we really do see day-to-day from the ER nurses and the advanced practitioners who take care of these patients hour-to-hour, minute-to-minute, is really the toxicity aspect of it and that is kind of where we spend a lot of time educating. And then what our nurses and APPs have been really the greatest for is going to try and establish a standard of practice of how to manage and watch out for these symptoms.

There are two main side effects that we do watch for associated with a CD19 target of CAR T cells. One is cytokine release syndrome and the second is neurotoxicity. Cytokine release syndrome is abbreviated CRS; it is really a result from an activation of the T cells. And the clinical symptoms are really from the fever, hypertension. The fever could be very high to up to 40 degrees Celsius, 41 degrees Celsius, so we are talking about a very high degree of a fever that can last several days. Three to 4 days of a 40 degree Celsius will really exhaust our patients too.

And then just buy acetaminophen and ibuprofen and other things you will do for these patients. So, even though there is a fever, some can be very mild.

And there are very different degrees of a cytokine release syndrome, too, but these are the factors to consider.

Hypertension – and, again, it could be very mild and the blood pressures in 90s or 50s that require one liter of bolus and then it comes right up. But some patients require three pressors and need to go to ICU because it is a very severe cytokine release syndrome. So, these are really just an immune activation state, the body's response from activation of the CAR T cells. It is very similar to a flu-like illness. So, when we just explain to patients the potential side effects they could get, we really just talk about this could be severe flu-like symptoms and some can be very mild and then some can very severe.

Respiratory insufficiency. The other things that we think about is that because of the capillary leak syndrome, these patients can get, especially if they get a lot of fluid boluses for their hypertension, they can also get hypoxic, increased respiratory rate, and some of these patients may also require intubation for this too. So, again, those are very severe side effects and it is very rare for patients to get them, but there is a whole range of side effects that we have to think about.

When we see patients who start to get fevers, then we know that these are warning signs and this is the beginning of something that could get potentially worse. These are the patients we just like to admit and monitor more closely. And then rather than vital checks every 6 hours or so, it is much more frequently. And depending on the nurse-to-patient ratio, some of these patients may also need to be transported to ICU, not necessarily because they need the pressors or

intubation or special medications, but just simply for the amount of care they require and the frequent check-ins sometimes to do often on the floor.

The second is neurotoxicity, which is very unique and it is something that we did not even know to expect when we were first treating patients with the CAR T-cell therapy, so now it is a very well-recognized side effect. But when we were first treating these patients 10 or 8 years ago, we were not even expecting this. The first time we saw it, we were not even sure what those were, whether these are side effects of high fevers or delirium or these were just kind of distinct side effects. Now, we do know that these are very much a distinct side effect these patients do get. And then the symptoms can range from confusion/disorientation, which is really the first symptom they do get. And then they progress usually to aphasia, so difficulty speaking, stuttering, and not able to name objects. So, these are really very sensitive test that we do with a consultation with neurology how to best – that is really the hallmark of this neurotoxicity, to not be able to name the objects and blank stare and stuttering is where they begin.

Now, they can stop there and spontaneously recover without any further intervention or they can progress into the next symptoms, which is seizure or seizure-like activity, or hallucinations. And then the severe case is a global encephalopathy and comatose state, so unresponsiveness and they are just in a deep sleep and not able to arouse. And they can be sleeping for 3 days, 4 days, and not arousable during that time too.

Now it sounds all scary and then I'll pass along to Amber to say what are we really trying to do to minimize the side effects too. But I just spend a good amount of time on the side effects with the patients, too, because it is really important to really set the expectation from the very beginning. A lot of times we will be – and the patients do come in and they are thinking this is going to be the great therapy, 80% response and durable response and of course I want this therapy for my own self and then more disease too.

But the one thing that we have to think about is that it is not so much the patients, too, the caregivers that come with the patient. When they see the patient is not able to speak, stuttering and not able to communicate, it could be very scary for them. So the more that we spend the time with them, these are the things that you can expect to see.

We are hoping that you don't get any of the side effects. You may not get any, but if you do see these, these are well-known side effects we are expecting to see, these are the ways that we can manage them. We found that those strategies have been very helpful to alleviate the anxiety that usually comes with this type of therapy too.

I am going to talk briefly – I know I am kind of running short and I want to give Amber plenty of time to talk about her stuff. What are the rates of these toxicities? We talked about CRS and the neurotoxicity, two common side effects associated with the CAR T-cell therapy.

So, CRS all gray, it says about 80% or so, meaning most patients will get some degree of a CRS. And these are just isolated fevers. It could be 38.5, 39, it could be 40, but a day or two. These are what we call mild or low-grade CRS.

But what we really need to focus on and what we really need to get concerned about is the severe CRS; those are the ones who require ICU level of care for pressors or intubation. So, the rate of those is about 26 or 25% overall for adult patients with ALL. And DLBCL is slightly less too.

And one thing to keep in mind is that the rates of these side effects are different based on the products themselves. So, who is manufacturing them, the structure of the CAR T, how quickly the T cells get activated. The quicker the T-cell activation, intuitively, the more likely you're going to get—and quickly—the severe cytokine release syndrome. Once you know the characteristics of a CAR T-cell expansion and 1928 in that aspect, they are the fast expanders.

So, 1928 is one of the CAR T-cell therapies. We think of it as kind of the racing horse where as soon as the gate opens, they go really quickly and just go all the way out there. Within a couple days, they can get 40 degrees Celsius of fever right away.

1-4BB is kind of more of a marathon runner. They get a slow start, but they eventually get to the same place as the 1928, but they get there slower. So, because of that, the side effect profile may be a little bit somewhat more expected and slower too. And that is an important distinction, too, because when you see a 40 degrees Celsius for one product that you know can get bad very quickly, whereas the other ones that need to stay there and then you may have

more time to intervene. The wave—and Amber's going to talk about some of that—the medications that we may not wish to intervene and why we worry as much about them.

So, it is important to recognize the side effects, the rate, and really the severity depending on not only the CAR T-cell product, but also the disease in itself too.

Adult ALL patients tend to get the most degree of side effects too. They are the ones who get the highest rate of a complete response, but they are also the ones who get the most toxicity. DLBCL is slightly less and pediatric ALL is also slightly less too. But the lymphoma patients, about 20% or so of these patients will get.

And I will go over what are the ways that we can actually treat CSR is the better the T-cell expansion, the more the CRS neurotoxicity, which implies that the side effects are somewhat associated with the CAR T cells. It is not so much the CAR T cells themselves are directly responsible for these side effects. We actually don't know the mechanism quite well for the neurotoxicity, but they correlate with – something about the T-cell activation and an immune expansion is what is leading to the side effects. And this is some of the data to show that too.

And the one key thing to expect, which Amber will talk about, is the CRS, one of the hallmarks of the cytokine release syndrome. As the name implies, cytokine release syndrome – there are several cytokines that get elevated during that time, and interleukin-6, or IL-6, is just one of them. And then we have several

IL-6 receptors and IL-6 inhibitor, which are used and approved for this specific indication to prevent the CRS. These are the things that Amber will talk about and how to manage them.

The key thing in managing these types of side effects is early anticipation. Who are the patients who are likely going to get the severe cytokine release syndrome? Again, adult ALL patients receiving 1928 are probably the most likely going to get them. DLBCL patients with a 90 4-1BB it's less likely. And then the higher the disease burden is also associated with severe side effects too. And then that's kind of intuitive in somewhat of a sense. The more disease you have, it's more antigen and then the more chance for the T cells to activate.

So, even if we actually give a very little dose of T cells—like one million as opposed to 3 or 30 million for the other low-burden disease—even if you get a small disease burden, that antigen is so much, as soon as the T cells get infused into the patient's body, they immediately encounter the tumor cells and they get activated very quickly. So, the more disease you have, the more toxicity you are going to get.

But, also, we talked about more disease are the ones who also get less durable response too. That's another reason that we advocate that really the best patient population is not only for the side effects, but also the efficacy, is the low disease burden patients.

This is just one slide to show that neurotoxicity – we don't know what the mechanism is. We have done a lot of work in MSK and other – the Fred Hutchinson in Seattle has done a lot of other great work there too. This is just to

show what the time frame – the CRS and neurotoxicity, just to close it, is transient reversible side effects. And as much as the terrible things that I just talked about, and I talk with the patients, I usually end the conversation that they are to expect the side effects, but that we know how to manage them. And there are a couple different ways that we can manage them and these are reversible side effects too.

And there have been a few ALL cases and these are rare events, but something for us to consider. And that's really all the more reason to be vigilant for these side effects and intervene at an appropriate time point. But these are really the transient ones. The timing for CSR is usually the first week or so. If you really don't get it for the first 7 or 10 days after a T-cell infusion, you are really not going to get the side effects.

Neurotoxicity comes slightly after, so where the orange is there in this curve, is usually about the 7 days or so. So, the first neurotoxicity really happened – the median time is day 9. The first fever median time is day 2. So, meaning immediately after the T-cell infusion, we expect the fever to happen. Neurotoxicity comes after. And they can last—depending on the degree of the neurotoxicity—for a few days to a few weeks too. Two weeks is probably the long period of time for this to last. When they last that long and they are not able to be mobile, especially in a very encephalopathic state, these patients also require some physical therapy to get better too.

But, again, the important point is that these are transient even though there is a 3- to 4-week period of time and there is nothing lasting. there are no

chronic side effects at least as far as we know now that we need to deal with as opposed to graft-versus-host disease and other transplant-related symptoms too. These are kind of intense side effects that we need to monitor really immediately after the T-cell infusion. But, again, beyond 3 to 4 weeks, if you don't get those, it is unlikely to happen for those patients.

So, with that, I am going to give this to Amber to see how we are going to really manage the cytokine release syndrome and to prevent it from happening.

DR. KING Thanks, Dr. Park. So, as Dr. Park essentially mentioned, cytokine release syndrome is the most prevalent adverse effect following CAR T-cell therapy. CRS must be identified promptly using patient symptoms, such as fever, rigors, or hypotension, and also lab changes and inflammatory markers, such as C-reactive protein or ferritin or other sophisticated inflammation markers.

Following diagnosis, there is a delicate balance between mitigating this immune cascade while salvaging the efficacy of CAR T cells. The early recognition and optimization of supportive care should be consistent among all grades of CRS for your patients. It is important to employ strategies to maximize patient comfort and symptom relief.

The first step is utilizing antipyretics. And the antipyretic of choice at our institution is acetaminophen. Often, we try to avoid things like NSAIDs and ibuprofen just because persistent and high doses of these medications can lead to acute kidney injury, which in a critically ill patient can be ultimately devastating and add insult to injury.

Secondly, these patients are hematologic malignancy patients that are immune suppressed, so a sign of fever is not only indicative of CRS, but could also be an overlapping infection. So, it is essential that practitioners recognize this and cover your patients with broad-spectrum antibiotics. We employ broad-spectrum Gram-negative coverage for things like pseudomonas and Klebsiella, along with radiographic studies and frequent vital sign checks for these patients.

Finally, we conservatively use G-CSF, or colony stimulating growth factors. These inherently are cytokines in themselves and could exacerbate cytokine release syndrome. However, they can be essential and lifesaving for a patient who is neutropenic and bacteremic.

In addition, with fevers, another big step in CRS is hypotension. As you can see, the management is very similar to that of a septic shock patient. So, we usually start with fluid boluses of normal saline and use about a half liter to a liter. After failure of about two to three fluid boluses, our shift is escalation of care. On the right you'll see a table of vasopressors and equivalents. We usually start with low-dose vasopressors, plus or minus anti–IL-6 therapy—which we will get into detail later—and see how a patient responds. Overall, a patient that requires high-dose vasopressors or multiple vasopressors should be strongly considered for anti–IL-6 or CRS-directed care management.

Now we will go into detail about the pharmacotherapy and CRS. By a show of hands, who has had to use tocilizumab for a patient? Okay, so there are a few of you. So, we will talk in detail about the signs behind tocilizumab and what are some steps and just how to decide when to use it for a patient.

So, as Dr. Park extensively mentioned, there is a correlation between the severity of CRS, the peak of the CAR T-cell levels in the blood, and the elevation of IL-6. IL-6 can signal in multiple different ways, but it is thought that the trans-signaling is the prone inflammatory component of IL-6.

Tocilizumab is an IL-6 receptor antagonist. It is available as a subcutaneous or IV infusion. It is essential to note that when used for CRS, it should be used in the intravenous formulation only for reliable peak levels and quick onset of action for patients. It's FDA approved and it is the only FDA-approved agent for cytokine release syndrome for patients 2 years and above. And the CRS has to be deemed severe or life threatening and secondary to CAR T-cell therapy.

Tocilizumab binds to all IL-6 receptors, mitigating this downstream activation of inflammation. The serum IL-6 levels have also been shown to be displaced after tocilizumab utilization. At our institution, we have some early data that suggests that the use of tocilizumab may actually shift IL-6 levels into the CSF and could possibly increase risk of neurotoxicity. Now, this by no means precludes our use of tocilizumab for CRS, but there should be caution used when a patient has isolated neurotoxicity, as tocilizumab may not be that effective and can also add insult to injury to your patient.

As far as administration, the dose is strictly capped at 800 mg and it is weight-based. So, if your patient is above 30 kg, the dose is 80 mg per kilogram. Patients less than 30 kg receive 12 mg per kg. It is a relatively quick infusion and could be given via central or peripheral access.

If no clinical improvement of the patient is established, you have the ability to redose tocilizumab. There has to be a minimum of an 8-hour interval between doses and the recommendation is to cap at four doses of tocilizumab. An extremely important admin component is that if your institution utilizes commercial CAR T-cell products, patients must have two vials of tocilizumab on site.

As far as kinetics, tocilizumab has been demonstrated to be efficacious and has a quick onset of action. In the retrospect of trials—which we will touch upon in a few minutes—the median time to effervescence was approximately 4 hours and hypotension tends to resolve within the next few hours.

You will see a lot of recommendations for renal and hepatic adjustments, but our current practice and the recommendation is to give patients full, unattenuated doses of tocilizumab to give them the best chance at efficacy. Often in CRS, the organ dysfunction was present and may not be a secondary to drug, but actually due to the CRS itself.

Finally, a theoretical risk is drug-drug interactions. During cytokine release, there are thought to be a blockade of CYP enzymes, enzymes important for a metabolism of a lot of drugs during CRS. There is a theoretical risk that you can experience increased drug levels of sensitive substrates, like voriconazole, posaconazole, or a lot of other medications. Fortunately, the function of CYP enzymes is thought to be restored after tocilizumab.

So, our current practice is to not do any empiric dose adjustments, but to consider therapeutic drug monitoring levels for sensitive substrates to make sure your patient is neither under-dosed or overdosed from medications.

As far as the adverse effect profile in tocilizumab, it is important to establish there is two different profiles. There are adverse effect profiles for the non-CRS population—which you will see before you—and on the right, there is adverse effects profiles for the CRS population. And the retrospect of analyses that got tocilizumab the FDA approval, there are no adverse effects that were independently associated with tocilizumab therapy and the lack of differences were seen in both the pediatric and adult patient population. So, it is important to consider when you utilize tocilizumab, the benefit almost certainly always outweighs the risk.

Next, we will dive into the clinical evidence that established tocilizumab as an agent for CRS. There are pooled retrospective studies from the major CAR T-cells trials that Dr. Park spoke about previously. They included 60 patients that were deemed to have severe or life-threatening CRS and they evaluated patients for CRS resolution. Now, CRS resolution was defined as the absence of fever and the absence of vasopressors for at least 24 hours. In the intervention group, you can see it is the FDA-approved doses of tocilizumab at 8 mg/kg or 12 mg/kg based on adult or pediatric dosing.

As far as demographics, you can see the characteristics of the patients. The CTL019 CAR T cells were the younger group with all B-cell ALL, and the KTC-C19 CAR T cells were our adult patients with a few large B-cell lymphomas.

The majority of the patients had severe or life-threatening CRS and a decent amount had CRS that persisted for 4-plus days. Most patients received only one dose of tocilizumab.

As far as efficacy results, you can see the impressive response of tocilizumab. Remember, these patients were severely ill with life-threatening CRS in the intensive care unit. And over 68% of them responded by day 14, most receiving only one dose of tocilizumab.

As far as safety, again, it is important to harp on that no adverse reactions attributable to the tocilizumab were seen. Overall, because of the patient population, there were five deaths seen within each group after the first 30 days of tocilizumab, but this was attributed to disease progression and critical illness.

Finally, the kinetic data in these studies supported the safety of dosing with up to four doses given 8 hours apart, which is now in the FDA-labeled recommendations.

Whenever we talk about abrogating inflammation, there is also a theoretical concern that we are actually harming the efficacy of CAR T cells. Fortunately, tocilizumab has not been demonstrated to blunt efficacy of CAR T-cell therapy, and there are some early studies to try to officially establish this fact. In the Axi-Cel trial, which garnered the FDA approval for adults diffuse large B cell or T cells, there are no differences in overall response rates between toci users and non-toci users.

So, to summarize the evidence for tocilizumab, it is the only FDA-approved agent for CRS management. And it is strongly recommended for

patients with grade 2 or beyond CRS. This is CRS that has progressed beyond vasopressors, fluid boluses, and other supportive care management.

The FDA approval was unconventional and based off of retrospective series, but it is appropriate considering the rarity of these cases and the critically ill nature of this patient population. But, it is always important to note that these studies were not directly powered to assess for efficacy or toxicity of tocilizumab.

Fortunately, tocilizumab has not been demonstrated to diminish the efficacy of CAR T cells based on the currently available data. Overall, more studies in real-world experience are needed to establish the true role and timing of tocilizumab therapy.

Another IL-6 agent that is approved on the market is siltuximab. Siltuximab is an antagonist of interleukin-6. Think back to tocilizumab; it is an antagonist at the receptor of IL-6. Siltuximab is available as an intravenous solution only, but it is extremely important to note that the use is restricted to expert opinion as salvaged therapy for CRS that has progressed beyond tocilizumab, glucocorticoids, and supportive care measures. Siltuximab, again, binds directly to IL-6, thus preventing IL-6 from activating all IL-6 receptors and leads to a net lowering of IL-6.

As far as administration, the optimal dose of CRS, again, is unknown because siltuximab is in the infant stages of being used for CRS. The dosing, however, is based off of weight at 11 mg/kg. And the dosing is extrapolated from the FDA approval of Castleman's disease. It is a quick infusion of about 60 minutes, but it is important to note that it is short, stable. So, if at any point your

institution decides to use this, there is a coordinated effort that must be established between admixing and administration. And, again, since there is a lack of data in CRS, there is no data available regarding the dosing intervals.

So, to summarize siltuximab, the routine first-line use is not generally recommended. There is overall addressed and absence of published data. The mechanism action suggests less risk of an IL-6 flare that can, in theory, can be seen with tocilizumab. Siltuximab should be considered for severe and refractory CRS that has failed after optimization of tocilizumab, glucocorticoids, or there is a concern for overlapping neurotoxicity. Further studies for siltuximab are being established to determine the true role of siltuximab in CRS management.

Next, I'll move on to the final and probably most controversial agent in the management of CRS: glucocorticoids. We are all familiar with the mechanism; steroids tend to just fix everything. They decrease inflammation, they decrease transcription of interleukins, and also increase transcription of interleukin receptor antagonists, overall leading to a net degree of anti-inflammatory effects. Inherently, you can see the theoretical risk of using this medication, as glucocorticoids are directly lymphotoxic and can destroy T cells.

As far as glucocorticoids, there are two major agents that are recommended in guidelines: dexamethasone and methylprednisolone. Now, the choice of these agents depends on the institution you are working at, the clinical symptoms of the patient, and what expert opinion guideline you are looking at. But I'll list below for you the differences between the two agents.

First, is dexamethasone, which is our long-acting glucocorticoid. It has a half-life of about a day and a half to 3 days and is overall five times the potency of methylprednisolone. It has partial metabolism in the CNS, thus giving an adequate CNS penetration. There is a proposed dosing schema below before you, but by no means is this a strict recommendation. You should titrate the dose based on patient clinical scenario and symptoms.

On the right you will see methylprednisolone, which is a shorter or intermediate acting glucocorticoid. The half-life is shorter, which gives you the benefit of quickly tapering off and having the effects wear off. Compared to dexamethasone, however, it has poor CNS penetration. And there is a proposed dosing schema below before you. Overall, when the choice to use glucocorticoids in these patients is established, it is essential to make sure that you use the shortest amount of dose for the shortest amount of time because we are unsure of how long-term steroids can adversely affect these CAR T cells.

We are all familiar with the adverse effect profile of glucocorticoids, but there are some notable adverse effects for use in these patients that should be highlighted. Endocrine metabolic effects, such as hyperglycemia, should be well controlled, especially if a patient is in the intensive care unit. There is a risk for GI hemorrhage and stress ulcers, so ensure your patient is on adequate PPIs or stress ulcer prophylaxis.

And, finally, there is an increased risk for opportunistic infections, especially in this hematologic malignancy patient population. Patients should be

on adequate fungal and PCP prophylaxis, especially when they are receiving therapy with glucocorticoids.

So, going back to our concern with efficacy for steroids, inherently steroids can diminish the expansion of T cells in a healthy patient, so there is a concern of using glucocorticoids with limiting the effectiveness of CAR T cells. So, our practice and the expert opinion practice is to attempt to reserve steroids only for CRS that is refractory to supportive care and tocilizumab.

There have been several attempts to establish the true nature of glucocorticoids. What is the dose and how long is too long to be on these for these patients? In the Axi-Cel trial, the rates of overall response actually did not differ between glucocorticoid users and non-glucocorticoid users.

So, to summarize glucocorticoids—the overall suppressed and inflammatory response—they should be strongly considered for CRS that is refractory to tocilizumab. Dexamethasone and methylprednisolone have emerged as the glucocorticoids of choice. The dosing range in which agents use is based on the grade of CRS and the concern for overlapping neurotoxicity. Some data suggests that corticosteroids might not actually mitigate response to CAR T cells; however, it is important to note that more prospective and controlled trials are needed to be established to elucidate this true effect of glucocorticoids on CAR T cells. So, overall, the concern for decreased T-cell expansion and decreased efficacy, we use caution with routine glucocorticoid use in this patient population.

I only looked at all the agents that we can use for a cytokine release patient; we'll go through some case studies. And the methodology behind this is what we would use in our practice and a lot of the guidelines suggest they should be used in sequence.

So, the first case is AK, who is a 24-year-old female with relapsed/refractory B-cell ALL, who is day 2 of anti-CD19 CAR T cells. The RN pages you with a high fever, T-Max of 101.3, and hypotension. The patient is fortunately A&O x4 after exam. Which of the following is the most appropriate intervention for AK at this current time?

You will see below there is a host of different options, including supportive care, corticosteroids and vasopressors, tocilizumab, and dexamethasone and broad-spectrum antibiotics. But it is important to sequence these and reserve extreme therapies for patients who need it.

The correct answer here is a fluid bolus, antipyretics, broad-spectrum antibiotics, blood cultures, and x-rays. This patient has new onset and grade 1 CRS, or fevers. It is important to control her for infection, control her symptoms of fever, and ensure that she has enough fluid boluses onboard to support any method of hypotension.

You are called to the bedside about an hour after your previous intervention. AK is now persistently febrile with a lower blood pressure. The ICU is consulted and the team decides to start low-dose vasopressors on the floor. The patient remains alert and oriented upon exam, but is visibly diaphoretic and

is still febrile. Fortunately, the blood cultures returned negative. Which agent is the most appropriate to consider at this time?

So, you will see there is a choice of broadening antibiotics further, anti–IL-6 therapy with tocilizumab, glucocorticoids with dexamethasone, and, finally, siltuximab. This patient has CRS that has progressed beyond supportive care measures and vasopressors and so, the next appropriate agent to employ at this time would be anti–IL-6 therapy with tocilizumab.

Finally, AK's symptoms resolved within 3 hours after receipt of tocilizumab. Unfortunately, she experiences a resurgence of febrile episodes and hypotension. She is transferred to the ICU for further management. After three more doses of tocilizumab and high-dose vasopressors, she remains hypotensive, febrile, and is now only minimally responsive. Which agent is the most appropriate to consider at this time?

So, you will notice the patient now has essentially grade 3/grade 4 severe CRS. She has already optimized supportive care, she is on vasopressors for blood support, and she is essentially deemed refractory to anti–IL-6 therapy. This is the point where we consider glucocorticoids as our fail-safe option.

So, as far as future directions, there is a lot to be known about CRS. There is a role discovering the prophylactic use of tocilizumab for at-risk patients, the true role of siltuximab for the prevention and/or management of CRS, other inflammatory therapies. Finally, a formal consensus CRS guideline needs to be reached sequencing agents, when to use them, and how to combine them, if applicable.

DR. PARK Thank you, Amber. the one thing is that tocilizumab, as Amber pointed out, is really the IL-6 indicator and is the standard therapy for the management of cytokine release syndrome. It doesn't really work very well for the neurotoxicity, so I think dexamethasone is the preferred therapy for patients either isolated neurotoxicity, which is not very common, or severe neurotoxicity.

And the last acknowledgment section. This is, as you can imagine, a very intense teamwork. It is from the Leukemia Service from our first CLL and ALL patients, not the lymphoma patients, and our tumor CAR T patients in clinical trials where we have realized it is really good to have a collaborative team from the different department services—a point person—one from the APP side, the nursing staff side, and ICU side, and neurology side, and infectious disease team. Having that really improved the quality of the care and then the communication that we need to have and then, also, setting up some consensus guidelines.

So, what it is, if there is really no absolute kind of perfect way to manage these patients, a lot of it indeed is a guideline and is what Amber just went over the questions and what's also in the REMS, the therapy that each of these commercially approved products because of the side effects, they are required to really complete kind of this training too.

These are some of the members that we are working with at MSK, Center for Cell Engineering, where the cells are actually being manufactured, Cellular Therapeutic Center where we are a part of or where the actual cells are being administered and the clinical care is given. And then the leukemia service,

including the Ambers and the pharmacists, and of course the patients who have dedicated and participated in the clinical trial that allow us to kind of advance this therapy to clinics.

And thank you for your attention too.

MODERATOR All right. Thank you very much, Dr. Park and Dr. King, for the presentation. If anybody has any questions, feel free to come forward and talk to the presenters.

[END]