



**Joe Chaffin:** Alright, everyone! Welcome to the Blood Bank Guy Essentials Podcast! This is episode 010, and I am delighted to have as my guest today Dr. Rick Kaufman from Brigham and Women's Hospital in Boston, and Harvard Medical School. Rick, welcome to the podcast!

**Rick Kaufman:** Thanks a lot Joe! Very happy to be here.

**Joe:** Well, it's really an honor to have you, Rick! I would like to take just a minute to introduce you to everyone, for those who don't know you. Dr. Rick Kaufman is a native of Pittsburgh who did his undergraduate work at Duke and got his MD from Washington University in St. Louis. He trained in Clinical Pathology and Transfusion Medicine at Washington University before he relocated to Boston. He is currently the medical director of the Brigham and Women's Hospital Adult Transfusion service, and he is also an associate professor of Pathology at Harvard. You have a lot going on, Rick!

**Rick:** I've been a little busy this year for sure!

**Joe:** It sounds like it! In addition, in your spare time, you are serving as a member of the AABB Board of Directors, and as an associate editor of "Transfusion." Dr. Kaufman, everyone, has done a lot of work in a lot of different areas, but over the last couple of years, he has focused really extensively on strategies to mitigate the daratumumab interference with blood compatibility testing, and that's actually what we are going to spend some time talking about today.

Rick, with all that being said, I usually start my podcasts the same way, because there are a lot of people that are listening to this podcast that are trying to decide what they are going to do with their professional lives, and some of them, hopefully, are thinking about Transfusion Medicine as an area of specialty. I'm just wondering, for you, what was it that kind of got you started along the pathway to Transfusion Medicine? What was it that keeps you going there now?

**Rick:** Well, like most people in the field, I didn't grow up thinking that I would be a Transfusion Medicine specialist. I actually never heard of it until I was finished with medical school. I started in Internal Medicine, and during my first year, I realized that it wasn't exactly what I wanted. I had an idea all along that it would be fun to be involved in some sort of research, that is, I was kind of eyeing an academic job, and it seemed like to be a really good Internal Medicine clinician was going to require so much time, and it would be difficult to combine with a research career. And so, I left that program and I went to work in a laboratory, and it happened to be the lab of Dr. Doug Lublin, who was the blood bank medical director at Wash U. He's now retired. But anyway, I kind of got interested in blood banking from talking with him, and so I later started in the CP residency program at Wash U and continued on. I think if I had stayed in Internal Medicine, I probably would have ended up in Hematology, but who knows?

**Joe:** Right, and now that, obviously, you are very well-established and well-published and thought of, etc. in Transfusion Medicine, what is it about the day-to-day work in Transfusion Medicine now that keeps you interested?

**Rick:** I like the opportunities to both teach and also do research in what some people really think is kind of a mature field, but the indications for blood products, the products themselves, and the problems that the field faces really continue to evolve. We are now dealing with Zika virus, and a lot of new technologies, and I think transfusion continues to be an incredibly important therapy in support for a lot of different areas of medicine. So, I just find it fascinating.

**Joe:** Let's go to the topic at hand. As I mentioned earlier, today we are going to be discussing how to resolve the anti-[CD38](#), or the "daratumumab" interference with blood compatibility testing. This is really something that blood bankers everywhere need to completely understand. There have been webinars done on this (some of which you've actually done, Rick), as well as AABB bulletins put out on this topic, and yet, I personally still find that as a reference lab director, there's just not *quite* enough understanding out there in hospital world about this! So, I really wanted to have you on as an expert to give us some practical tips for how to deal with this.

So, Rick, why don't we start from the beginning? Can you tell us a little bit about the background of why this is even an issue? What's the disease that anti-CD38 treats, and why is this particular drug coming onto our radar screen right now?

**Rick:** So, daratumumab or it's actually much easier to say, "DARA," which is how we usually refer to it, is actually one of what may turn out to be a group of anti-CD38 medications. These are IgG monoclonal antibodies that are directed against the CD38 antigen and that's a protein that's highly expressed on myeloma cells. Multiple myeloma is a hematologic cancer, a disease in which a plasma cell clone expands—proliferates within the bone marrow. Typically patients with multiple myeloma will also have high levels of a protein called "M protein," which is basically these cancerous myeloma cells cranking out IgG or light chains, some part of antibody or a complete antibody, but it's basically all being made by their tumor. And this M protein as it's called, is usually detectable in the serum or in the urine. Patients with multiple myeloma have a variety of different problems, they often have renal involvement of their disease, they always have marrow involvement, of course that can lead to things like anemia and other problems with producing cells from the bone marrow. The main issue is that it's currently not curable, that is, everyone eventually dies from multiple myeloma. Having said that, the treatments have gotten quite a bit better over the past several years. People are now living years longer than they used to, after getting treatments like autologous stem cell transplants or proteasome inhibitors or immunomodulatory drugs. But no matter what treatments people have gotten, the disease always comes back, and it eventually relapses and the patients die. So, there's been a lot of interest in trying to develop better therapies for it and given the success of things like rituximab for other hematologic malignancies, and rituximab being anti-CD20 antibody, for a long time there's been this idea that it would be great if you could create a therapeutic antibody to treat multiple myeloma and that's what daratumumab represents. I think as I alluded to earlier, I think

that's a first and what's likely to be a whole bunch of antibodies for multiple myeloma, and I think we're going to be seeing more and more therapeutic antibodies, in general, some of which will undoubtedly affect testing in the blood bank and I think this is, kind of "tip of the iceberg."

**Joe:** So, before we go any further, you mentioned rituximab, obviously we're talking today about daratumumab, and I think sometimes, one of the things that people are learning about this get a little bit befuddled by—as obviously, the names are complicated, weird, and strange. Obviously, I don't want to go into all the details about what means what, but there is some convention to these and at least, the last part of it —the ending in "-umab" means something, right?

**Rick:** That's right. So if it ends in "-mab," it really means monoclonal antibody. It's an antibody that's directed against the very specific protein that's a target that's expressed on the outside of a cell that you want to kill. In the case of DARA, that protein is CD38. These anti-CD38 monoclonals are produced at very high levels and they're infused to patients at very, very high levels, with the idea that they'll bind to the target tumor cells, and through a variety of different mechanisms, produce tumor cell killing.

**Joe:** Okay. As of now, I know that there are a lot of studies going on, but as of now, is there a particular group of myeloma patients that might be eligible for this drug, in terms of what's approved at this point?

**Rick:** So right now, the myeloma patients that are approved to receive daratumumab are those that really have refractory disease. It kind of makes sense, it's something that's often seen as new therapies are introduced. They're first tried on very sickest patients with a particular condition. So, the patients that were in the trials that resulted in daratumumab getting licensed last year were those that really had refractory disease—meaning that they'd gotten various lines of treatment and still relapsed.

**Joe:** So primarily at this point, for relapse, but I've heard you say before—and you alluded to earlier—that there is certainly interest in moving the patient group beyond that group of patients, correct?

**Rick:** That's right. So, the use of daratumumab and drugs like it, will definitely be studied in patients in different stages of multiple myeloma. It's also being studied in malignancies other than myeloma. So, CD38, the target antigen for DARA, is expressed by lots and lots of cells, and so diseases like lymphoma are potentially treatable with daratumumab, though we will have to see what the studies show.

**Joe:** Right. That's obviously important, though, and we'll talk shortly about what effect it has on us in the transfusion service, but if the medication is going to be growing in use, at least potentially, rather than shrinking in use, it has the potential to affect us a lot more as we go down the line. But I did want to ask you before we get to that effect: What are the studies showing? Is this medication working? Is it having any effect?

**Rick:** In the published studies of daratumumab where it was used in refractory patients, about a third actually showed a response when they received DARA as a single agent only. And so, this was really quite an exciting finding! These patients had gotten proteasome inhibitors, they had gotten linalidamide and other drugs, and their disease had still come back. And yet, a third of them, approximately, had a response to daratumumab alone. Those responses are graded as complete response, partial response, or something in the middle, very good partial response, essentially based on how much M-protein is left circulating and if there are plasma cells still detectable in their marrow. Within these very, very sick patients, there were actually some patients that had a complete response, that is, their marrow is entirely cleared of myeloma cells, and so that was obviously quite exciting. Now why some patients respond better than others is really unclear. But this approach definitely seems to be helpful for quite a few myeloma patients.

**Joe:** Rick, you mentioned that these patients take fairly high doses of this drug. Would you mind just kind of outlining for us: What is a course of DARA? How long are people on this medication?

**Rick:** They can be on for really several months. Basically, the current dosing is 16 mg/Kg, the patients get it weekly for 8 weeks, then every two weeks from weeks 9-24, and then they would get it monthly from week 25 onwards, and they would basically keep taking it until disease progression, at which point they would go off the drug.

**Joe:** So, obviously, potentially *long* periods of time that people are taking this medication.

**Rick:** That's right. And even when they go off it, we know that the drug continues to circulate for up to six months afterward.

**Joe:** So this is not your little medication that somebody goes on and they are on it for a week, and hooray, the effect is done and it goes away! This is something, once somebody goes on it, they are *on it* for awhile.

**Rick:** That's right. It's a chronic infusion therapy.

**Joe:** And so with all that being said, I think that you have definitely helped us understand that this is a medication that seems to have a lot of promise in these patients that are notoriously tough to treat, and that it's something that they are going to be taking for awhile, and that there does seem to be some great effect, so I guess we come to this: So why do we care, Rick, in the transfusion service? We are transfusion medicine docs, you know, let the hematologists do their whole deal with the new drug... yay, hooray, happy for them...why should a blood banker care that a patient's on DARA?

**Rick:** Great question! The reason why a blood banker should care that a patient's on DARA or one of the other anti-CD38 antibodies which are being studied now, is that they will very consistently and reliably interfere with a lot of tests in the blood bank.

Basically, any indirect antiglobulin test or any test that involves AHG detection. And the reason why this happens is that it turns out that CD38 is expressed not only on myeloma cells, but it's also expressed, at least at low levels, on red cells. What we found was that in patients on DARA, in doing [antibody screens](#), that those **antibody screens will invariably be positive, usually weakly positive, and it's because the DARA that's in their plasma binds to CD38 on reagent red cells, the screening cells.**

**Joe:** So, obviously with those antibody screens, you are going to see positives, and is that like 10% of people, is that 100% of people that you guys have studied? What's the proportion?

**Rick:** It's approximately 100%.

**Joe:** (laughs) Close enough, right, to 100% to be 100%!

**Rick:** Close to 100%, and the other thing that makes really in some ways perfectly designed to drive blood bank techs crazy, is that both two or three antibody screening cells will be positive, and the next step is to do an antibody identification panel, and all of *those* cells will be positive. So, **it typically looks like an autoimmune hemolytic anemia, perhaps, maybe someone with a [panreactive autoantibody](#).** The DAT is variable, it's actually usually negative. And then the part that makes it particularly difficult for the blood bank is that, at least at reference labs or academic medical centers, it's routine to do [adsorptions](#), to try to remove what looks like panreactive autoantibody. And the **adsorptions invariably fail**, when it comes to DARA. It turns out there's just not enough CD38 that's on the adsorbing cells, and so you can adsorb for days and days, and you still can't get rid of the interference.

The problem with that, of course, is that now you've got a patient that has all these positive reactions, and if you go to crossmatch blood for that patient, you will also find doing an [AHG or "Coombs" crossmatch](#), that every single unit in the blood bank will also be crossmatch-incompatible! And so, patients often have the potential to go for a long time without being able to get red cells. We've seen this happen where labs just didn't know that a patient was on daratumumab. The patient comes in maybe from out of town for an outpatient transfusion, and they just can't get any blood because the blood bank can't figure out exactly what's going on. That seems truly to be the biggest problem with this drug; that is, it's certainly possible that it could mask an underlying alloantibody that might cause a hemolytic transfusion reaction in someone that was on the drug. But the biggest problem in practical terms is that it has great potential to cause delays in patients being able to get blood.

**Joe:** Rick, I think that you've said some really important stuff there. I think it's really important for people that are listening to this podcast to understand if this isn't your day-to-day work, people in transfusion services and reference labs, if they don't know that this medication is causing this issue, they will spend tons of time working up a panagglutinin, basically, working up for the presence of a warm autoantibody, trying to identify underlying alloantibodies with, as you said, using absorptions to try and pull the

presumed autoantibody away and if it doesn't work, that gets really frustrating for people. So, I guess there must be some techniques that we can use, when we know that someone has DARA. I think we'll get to that in a second. But actually, you know what? Before we get to that, Rick—forgive me, I'm going to digress just for a second—you mentioned the antibody screen and the antibody ID being positive. There's obviously, one other set of tests that we do routinely in the blood bank. What about ABO and Rh testing? Does DARA affect that at all?

**Rick:** Oh, so that's a great question. ABO and Rh testing is not affected at all by DARA.

**Joe:** Okay. Why is that do you think? I guess I could figure it out logically, but what's your rationale for that?

**Rick:** Oh, so, the tests that get affected are really those where you're using red cells and antibody that IgG binds, and then to detect that binding, you add in anti-human globulin or Coombs reagent. Basically, antibody against Fc that will cause cross-reaction and cross-linking and agglutination of antibody-coated red cells. In the case of ABO and RhD testing, the reagents that are used are IgM, so they're sort of directly agglutinating and so the DARA doesn't affect them at all.

**Joe:** Got it. Okay. So forgive me for that little digression, that's obviously important to understand that those tests are not affected, but pretty much everything else is! So Rick, you and your group—you were the senior author on a paper that was kind of a definitive paper that has looked at this so far, published in "Transfusion" in June of 2015. Would you mind just kind of taking us what you and your group did? Because you guys did some really cool experiments! Can you just take us a little bit on a high level summary of how you investigated this in terms of looking at how CD38 played into this and how the CD38 expression on the red cells played into this?

**Rick:** Sure. So, basically my blood bank at the Brigham provides testing and blood products, not only for the Brigham, but also for the Dana Farber Cancer Institute. There's a prominent multiple myeloma group here that's done a lot of nice clinical and translational research studies on that particular disease, and so, when studies began in the United States of daratumumab, they were, I believe the first group to start using it. And then other sites around the country began using it in trials, and so, we just happened to get samples from the very first patients, in the U.S. anyway, that were treated. We had some sense that there would be a problem. In the protocol, it said something like, "the antibody screens may be positive." We kind of knew that ahead of time but we didn't quite know what we would be dealing with until we saw the first samples start to come in. And then, when these samples arrived in the lab, the situation was kind of like I described before—so we would get positive antibody screen, positive identification panel, and then the techs started doing absorptions and nothing would go away. And so they started coming in my office, kind of asking me what to do. So, after about the third time that it happened I decided, "OK, well this sounds like a real problem. I should start to look into it."

So, I just did some reading of old studies about CD38, the antigen itself, because I didn't really have a great idea for getting rid of the antibody. It was possible then and it's possible today to buy **CD38 purified recombinant protein** online, but it's really expensive. So, I decided to focus on methods of maybe getting rid of the CD38 antigen, thinking that maybe the problem was that it was just drug in the patient that was binding to CD38 on red cells. And in fact, many groups in the past going back to the 90's had shown that there's at least a little bit of CD38 on red cells and one group had shown that if you prepared CD38 from red cell membrane, and then treated it with beta-mercaptoethanol reducing agent, that they would lose the ability to detect that protein. That is, it would seem like that agent was destroying the CD38. So we don't use beta-mercaptoethanol in my lab, but we do use **DTT**, "dithiothreitol" or DTT, which is a reducing agent, and it basically breaks disulfide bonds. So we thought, "Okay, well that really might be a good way to try to get rid of this antigen on red cells."

The approach that we took was basically, there were two main approaches: 1) To do tests using, kind of standard blood bank methods, using red cells that were treated or not treated with things like DTT and looking for agglutination, as our read-out. The second, kind of independent line of experiments that we did was to try to use flow cytometry to directly visualize binding of DARA and then, looking at the effects using flow of different ways of getting rid of that binding. We started out trying to use red cells in flow cytometry, but like others, before I found that there's such a low signal, that is, there's so few CD38 molecules on a typical red cell that flow cytometry wasn't going to work. And so, we ended up using a cell line that expresses lots and lots of CD38 and that gave us really a nice way to visually, in a sense, see binding of DARA, and then we tried different ways to get rid of it. And the methods that we tried were essentially to use DTT, we also tried using **trypsin**. There was some old data suggesting that maybe, instead of denaturing the protein you can clip it off the surface. That seemed to work, as well. Certainly, in the serologic model, that is using red cell agglutination as our read-out, it worked quite well. We thought that DTT would be a little bit easier for other labs to use. In our flow cytometry model it was a little more robust, so that was kind of the method that we focused on. And then we also tried neutralizing the antibody in solution. So we bought some of the expensive CD38 protein and did get that to work. You have to add, something like five-fold excess of that protein, in order to mop up the anti-CD38.

**Joe:** I see. You're basically trying to overwhelm the antibody with a bunch of free-floating CD38 so that it doesn't bind to the red cells?

**Rick:** That's right. And actually, there was a group in The Netherlands that had been working on this problem before us. We were the second group to start dealing with this. The very first group was in the lab of Dr. Karen De Vooght, and their lab really focused on these neutralization methods, so what was nice is that we were kind of doing complementary work and ended up publishing back-to-back in "Transfusion" initially. Karen's lab really focuses, as I said, on the neutralization methods, and they showed that recombinant CD38 worked. And the other thing that they made was a really neat reagent, an **anti-idiotypic antibody**. This is an antibody that binds to daratumumab specifically, and where it binds is to the Fab portion, that is the combining part of the molecule that would normally be used by DARA to bind to CD38...

**Joe:** The “business end” of it, basically...

**Rick:** The business end of DARA, the binding end, it actually could be thought of as an antigen itself, and they raised an antibody against that. And so, they basically have an antibody that will stick to DARA, itself an antibody, and that also works beautifully. It's not currently available, and its future availability is a question. One advantage of using the recombinant CD38 vs. the anti-idiotypic antibody is that there are going to be other brands of anti-CD38 coming on the market. A company called Sonofi has one, DARA made by Janssen is already out, and there is a company called MorphoSys that has one at least in studies. If you used recombinant CD38, basically that approach would work for any brand or any manufacturer's anti-CD38, but if you were to try to use an anti-idiotypic approach, you'd have to make a different one for every brand.

**Joe:** I see, that makes sense.

**Rick:** The DTT method, getting rid of the antigen, well the good news is that works for any brand of anti-CD38. And, by the way, these other manufacturer's antibodies, to the best of our knowledge, they react essentially the same as DARA does in the blood bank. So it really is considered to be a “class effect.”

**Joe:** OK, so you guys kind of came to the point where for your lab, and correct me if I'm wrong with that impression, but for your lab, you decided that the best choice was to use the DTT to strip the CD38 from your reagent cells, the cells that you're doing the testing on, is that correct?

**Rick:** Yes, that's exactly right. And then of course, that has its own disadvantages...

**Joe:** Ah, tell us about that! You mean, wait, Rick, other stuff gets affected by DTT? How can that be? (laughs)

**Rick:** DTT is a pretty “blunt instrument!” But we were lucky in that there are many different blood group systems, and there are only a subset that are sensitive to DTT. Within that subset, there are some clinically important antigen systems: Kell, Dombrock, and others, some more rare ones. The antigen that is most important, though, is the “Big K” antigen in the Kell system, also known as “Kell 1.” Anti-K is seen all the time in blood banks. It will cause both hemolytic transfusion reactions and hemolytic disease of the newborn, and apparently the K antigen is the second most immunogenic antigen after D, which we are already prospectively matching for. So blood banks like ours see anti-K *all the time*. The good news is that 90% of people or more are K-negative, or at least among the blood donor population. Of the units on the shelf, 90 or 91% are expected to be negative for the K antigen. So, it's relatively easy to issue units to people if you are worried about them making an anti-K antibody that would not be detectable if you used our method.

**Joe:** So, just to make that clear to the students that might be listening to this: So when you treat your reagent cells with DTT, and then you react your patient serum against those cells, obviously if there is an anti-K, for example, or anything else that DTT strips



from the surface of those reagent cells, you lose your ability to detect it, is that a fair way to put it?

**Rick:** That's right. And I guess another way to say that is, **we absolutely know that if you use DTT-treated cells for an antibody screen, that you will absolutely lose the ability to detect antibodies against a number of red cell antigens.** All the antigens in the Kell group, of which K is just one, all of them in Dombrock, and Scianna, Indian, and there were some others. But of the ones that you lose, you *just don't see* patients form antibodies against them all that often. The main exception is the anti-K, and so if you deal with that, essentially, the vast majority of the time, you really in practical terms won't be missing anything, although the potential is there. People need to be aware of that, if using DTT.

**Joe:** So Rick let's take this home. Let's imagine, you and I, for just a moment, that we are living in a "perfect world." If we are sitting in that perfect world, and there was a patient with myeloma who is going to go on DARA, how would you describe for us the perfect world scenario for how communication should go, for how the blood bank should handle the testing, and just take us through really practically what the strategy should be from start to finish on how to get as safe a transfusion product to this patient as possible.

**Rick:** Okay, so that's a great question! The first thing that should happen, is that the **blood bank should be alerted that a patient is about to start DARA, before they ever get the drug.** What we recommend that the blood bank does, is to get that baseline sample, do a **type and screen**, and additionally, if it's feasible, we would recommend doing **genotype or phenotype** for clinically important red cell antigens. If you have that information, for example, it helps reduce some of the risk of missing antibodies if later on the patient goes on DARA and you're using DTT. So honestly, that communication is the very biggest deal. We don't have a perfect way of achieving this yet. Right now, at my hospital, I would say about half the time we're informed that a patient is going on DARA, and about half the time we're not. We've tried to educate our clinical colleagues to let us know, but it's definitely a challenge. What some hospitals have done, which I think is really clever and we're trying, is when an electronic order for DARA goes into the computer system, that the blood bank is automatically notified, by email or something like that and we've put in a request for something like that. Otherwise, you do run the risk of patients going on this drug without knowing it. I will say that our techs are getting better at picking it up. They can kind of recognize now what this typically weak panreactivity looks like, if they know the patient's an oncology patient, a myeloma patient, and so on, they can usually make a phone call and find out. But getting a good drug history is absolutely pivotal. So then the patient's sample comes in and what we recommend is to **do a screen using DTT-treated red cells.**

**Joe:** And this is after the patient goes on the DARA, right?

**Rick:** This is after they go on the DARA. That's right.

**Joe:** Got it. Okay.

**Rick:** Now we use a machine called the Tango. We know that if a patient is on DARA or if he's even got a single dose, it's possible for us to get carry-over, or what we would call "DARA-over," where there's so much antibody in the patient's sample that however the rinse step goes in the machine, typically the next sample in the rack will also show the same panreactivity.

**Joe:** Wow! That's news to me! I did not know that!

**Rick:** So, we know that this happens on the Tango. And I've heard other labs that use that particular instrument have this issue. I've not heard of it coming up with other instruments, but it may be a problem. I just don't know.

**Joe:** Sure, understand.

**Rick:** But anyway, in our lab we do everything on the bench, at that point. So, after they would started DARA, we would do an antibody screen using DTT-treated cells. If that screen is negative, which it typically is, then we would **issue blood by electronic crossmatch**. If the screen is positive, using DTT treated cells, then **we would use DTT treated red cells and an antibody identification panel and identify what antibody the patient's made**. There again, having that phenotype or genotype can be quite helpful if you know, for example, a patient's negative for the Rh E antigen or if they're negative for some other antigen, then it can really help you nail down what else they've made and can help you figure out what blood will be safe to provide them with.

**Joe:** So, a couple of questions about that, Rick, in terms of that process. First, you mentioned if the antibody screen is negative, with the DTT-treated cells, you're issuing things by electronic crossmatch, which obviously not every facility does. I'm assuming that immediate spin crossmatch would be acceptable as well?

**Rick:** You could also do immediate spin crossmatch. I think that would be equally acceptable in the case if someone that has no detectable antibody in their plasma and also no history of alloantibody. The patients that are trickier, are those that have made antibodies. We've been lucky and haven't seen that many. When they do, you're kind of stuck either doing a crossmatch with anti-human globulin, that you know will be incompatible or you can DTT treat a little bit of the red cells from the unit that you want to transfuse and use that as a crossmatch.

**Joe:** That is what I was going to get to. That's a little trick, because obviously, we know that if the patient's got DARA on board, and we do an AHG crossmatch with units off the shelf, they're gonna be positive. I mean you've already shown that and discussed that. You'll actually do that? You'll treat the sample from the cells to be transfused with DTT to do the crossmatch?

**Rick:** We will do that sometimes. It depends on the variety of different logistic factors within the blood bank. Sort of tech availability and turn-around time, that sort of thing. In some cases, what we'll do if we have a genotype is do more of a "dry crossmatch," that is, we will provide blood that's matched against for things that the patient has a potential

to make an antibody, but may or may not have actually made that antibody, simply based on the genotype, if that makes sense.

**Joe:** It does, it does. That's specifically in the cases of when people have made it? Have a positive antibody screen with the DTT-treated cells?

**Rick:** That's right. Although, I will say there is a variety of different approaches taken. Some of the national level reference labs with a large inventory of genotyped red cells may go to that approach, sort of earlier than we would.

**Joe:** Right, understand. So you've mentioned what you are doing with those screens, once the patient is on the DARA. One thing I cut you off and I know I didn't let you finish, in terms of your selection of cells off the shelf, even if the screen is negative, you're not just pulling any random cell off the shelf—you've already mentioned the Kell issue. Could you talk about that for just a moment?

**Rick:** So many of the patients we would be transfusing would be expected to be negative for the K antigen. If we know that that's the case, and we're using a DTT-treated red cell screen, then we **provide red cells that are negative for K**, just because we know that is one we don't want to miss and could easily be missed with the method that we're using with DTT. If the patient's known to be K positive, then we don't worry about it.

**Joe:** Got it. And that's important to recognize and obviously, if the person has antibodies, clearly, it goes without saying that you would give cells that are negative for the targets of those antibodies, as well. Have you had issues, Rick, when you are sending out stuff that is crossmatch incompatible, I mean we've all dealt with that with warm autoantibodies when we're sending out things that are crossmatch incompatible. Any resistance from your clinical staff or are they savvy to all that?

**Rick:** Basically, no. Our oncologists are now pretty savvy about the issues related to transfusion patients that are on DARA, so they are reasonably accepting of units that may look incompatible in-vitro.

**Joe:** That's good that that's true in your facility. I think that obviously does vary from place to place. We've seen a little bit of resistance, on occasion, from my reference lab with some of the smaller hospitals that we serve with patients on DARA, with the clinicians, not all that excited about getting units that are crossmatched and compatible! (laughs)

So, that's a real practical approach. Obviously, as you said, alerting the blood bank, prior to the transfusion or prior to the DARA starting is *so key*. And then, managing things that way, as you recommended with the DTT treatment, I think that is certainly a practical strategy. Before I let you go, Rick, I've seen some other things that people have mentioned, and you mentioned one of them earlier. I wanted to circle back to this, you mentioned trypsin vs. DTT. DTT, in my experience, has a little bit of a "negative rep"

as being a little bit hard to work with and I haven't personally worked with trypsin. You had felt that DTT was easier, was that the case? DTT is easier than an enzyme?

**Rick:** DTT smells terrible! It's actually is the main ingredient in whatever it is that skunks spray!

**Joe:** (laughs) Oh nice!!

**Rick:** In fact, I learned recently, that that's how they found it initially! That is, someone use to study what was in skunk spray and DTT was isolated from there. So it does smell terrible. But having said that, it's really not bad to work with. You can make up a big batch and then freeze aliquots. Some labs I know are exploring, actually DTT-treating red cells and then droplet-freezing them, so they have little batches of treated cells ready to go. Trypsin, while not nearly as malodorous as DTT, has some problems of its own. It can be kind of tough to dissolve, and it has to be made up fresh all the time, and it's perfectly fine and used all the time in reference level labs. It's just used less commonly and maybe a little more difficult to be used at academic hospital-level labs. Then there are community hospital blood banks and they have to think about how they want to deal with this problem, too. I think DTT is relatively easy to use for—I don't know, a 500 or 1,000 bed academic hospital—for smaller community hospitals, I think that may be beyond what they are comfortable with. Just as many labs in the community will often, basically beyond an antibody ID, really won't do any kind of more extensive testing and prefer to send out to a reference lab. There are some other methods, it's been recorded that you can use **cord blood cells**. Cord cells appear to express little or no CD38 and we've confirmed that in our lab. It's necessary to phenotype cord cells in order to make them into useful, sort of reagents. That one, while possible, hasn't really caught on all that many places. And then, the second issue with the cord cells is that, you can use them, for example, for an antibody screen, but then if you got a positive, it's not clear that it would be practical to then try to make an antibody identification panel out of cord cells. So that's one.

**Joe:** Yep, I gotcha. And the cord cells is what I was going to go to next, so thanks for addressing that. I know there was a...I can't remember if it was a letter to the editor or something that came out a few months after your paper came out, that described the use of cord cells in a way that I hadn't seen before.

So, one other question, Rick. Obviously, it's possible for someone to transition from having a negative antibody screen to a positive antibody screen while they are on DARA, I assume. But I'm wondering in your experience, have you seen that? Have you seen someone go from negative, where you're just using K negative (most likely) units off the shelf with the electronic crossmatch, and then they make an antibody and you have to move to giving them a tighter-matched product, is that been something you've experienced?

**Rick:** We've only had, I think one patient where that's happened out of group of maybe, 25 or 30. Actually, our approach has worked fine. I know that there are other individuals who have seen a lot more patients on the drug. Melissa Cushing, at Cornell, for

example, I think has seen 100 patients. She has many patients that have formed alloantibodies or autoantibodies. In general, she's worked out a nice algorithm to deal with these cases. What's been a real challenge is cost. In patients that have formed, let's say, a few different alloantibodies, it's necessary to get antigen-negative units, and certainly, the cost for those can go up pretty quickly.

**Joe:** You bet. As a medical director of a blood supplier, I can certainly agree with that! And further, you get the challenge of when someone has a type that's not your standard, every day, easy phenotype, that can be a struggle just in the supply area to actually to be able to find units that are matched closely.

Rick, I think you've answered every question I can think of. Before I let you go, I do want to ask you; I think that we've learned a lot of lessons from DARA, and I think we're continuing to learn those, but I think it's important for people to understand, this probably isn't the last time we're going to have to deal with issues like this, is it?

**Rick:** No, this is going to be the rest of our lives! For anti-CD38, specifically, it's very clear that, as with rituximab, it's not going to be just restricted to a single disease. It absolutely will be tried for other things, lymphoma in particular, but also other conditions, probably things people haven't even thought of yet. So blood banks will be seeing more of this. There is an anti-CD47 antibody that's now being studied, and it also binds to red cells and will cause serologic interference. That may be the next one that people see. But there is unquestionably going to be a lot of these, and it's something that we as a transfusion medicine community are going to have to work together on to come up with solutions. I think the one constant is that we have to know the patient's on the thing, and so I think getting drug histories is going to become more and more important for blood banks, and communicating that likewise is going to be important for the clinicians.

**Joe:** I think that's such a great point. Depending on the blood banker, it's fairly common when they see an antibody for them to pull up a drug history, but I think we are going to have to really get used to looking more critically at that drug history, and at diagnosis. I had mentioned off-mic to you when we had a previous discussion; we had one in my reference lab where it had the same pattern that you were describing. We asked the hospital about four times whether the patient was on DARA... "nope, nope, nope, nope" and then finally we got from them the drug list, and there it was! I'm sure you've experienced that as well.

**Rick:** Many times, unfortunately!

**Joe:** (laughs) Many times! Well, Rick, again I can't thank you enough for taking the time out of your busy day to do this podcast with me! I think you've really helped us look through this and understand some practical strategies moving forward. Hopefully, everyone listening will have a better idea of what daratumumab and drug therapies like it are all about, and how we can deal with it. So again, thank you so much for being here!

**Rick:** My pleasure! Thanks for inviting me.