BBGuy Essentials 063CE
Anti-CD47 Testing Interference with Connie Westhoff
Released February 6, 2019

Connie: This is Connie Westhoff, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hi, everyone! Welcome to Blood Bank Guy Essentials, the podcast designed to help you learn the essentials of Transfusion Medicine. This is Episode 063CE and I am, as always, your host, Joe Chaffin.

I am really, really happy that you're listening! There's two reasons for that. First, my friend, Dr. Connie Westhoff from the New York Blood Center is back on the podcast. I'm so happy to have her! She has a really enlightening interview about the really dramatic impact that a new cancer therapy called "anti-CD47" has on our testing in the blood bank, so really important stuff.

Second, I'm also excited because this episode is being released the same day that Connie's article is actually available in the February edition of the journal Transfusion. Hopefully this is the first of many collaborations with Dr. Rick Kaufman, who's the editor-in-chief of Transfusion, Dr. Aaron Tobian, who's editor-in-chief of Transfusion News, and Wiley Publishing.

First, since this is a continuing education episode, I have to give you a little bit of info, so hang with me for a second. The free continuing education credit is provided by TransfusionNews.com. Transfusion News is brought to you by Bio-Rad, which has no editorial input into the podcast. This podcast offers a continuing education activity where you can earn several different types of credit, including one AMA PRA Category 1 Credit™, one contact hour of ASCLS P.A.C.E ® program credit, or one American Board of Pathology Self-Assessment Module for Continuing Certification (that's CC, which used to be called “MOC”). To receive credit for this activity, to review the accreditation information and related disclosures, please visit www.wileyhealthlearning.com/transfusionnews.

All right, with that aside, about two, maybe three years or so ago, people in the blood bank world were really freaked out about a new treatment for multiple myeloma that had a funny name (and it's memorable). The drug was called "Daratumumab," and it pretty quickly became abbreviated as just "DARA," D-A-R-A, oftentimes all in capitals. DARA is a monoclonal antibody against the CD38 protein on those malignant plasma cells in patients with multiple myeloma.

But...red cells also have CD38, so DARA interfered with our basic antibody detection tests. It was a pain for a while, until we figured it out, and we've really come to a place now where blood bank reference technologists and routine blood bank technologists as well are pretty good
at "smelling that out." We can see when DARA might be an issue when we get histories of patient being on the drug for multiple myeloma. The short version of that is we just treat the test red cells with Dithiothreitol, or "DTT," and the interference just goes away. It's very cool. There's more detail than that, so please check out my interview with Dr. Rick Kaufman on the DARA effect at BBGuy.org/010.

Well, in the last few years, we've been hearing about a new treatment for both hematologic malignancies and solid tumors. Really we've heard about this almost since DARA came out. That new drug is called "anti-CD47," and it's also a monoclonal antibody, shockingly, this time against the CD47 antigen. We've known for a long time, even before this drug came out, that there's a ton of CD47 on red cells and really ALL cells. On red cells it's a really important part of the structure of the red cell, really closely associated with the Rh proteins and Connie will talk about that a little bit.

Because of that, we've been really worried that anti-CD47 could cause a BIG problem for us in blood bank testing, and Connie is here to tell us all about the results that she and her group saw when they tested four patients on anti-CD47 as part of a study.

If you don't know Dr. Connie Westhoff, you should! She directs the laboratory for Immunology and Genomics at the New York Blood Center. She's an adjunct Assistant Professor in the Department of Transfusion Medicine at the University of Pennsylvania. Connie lectures everywhere, nationally, internationally. She's published more than 80 scientific papers [NOTE: It's actually more than 100 now!], numerous book chapters. She's an associate editor for the Immunohematology and Genomics section of the AABB journal "Transfusion." Finally, Connie is actually an original inductee into the National Blood Foundation Hall of Fame.

With that, here is my interview with Dr. Connie Westhoff on anti-CD47 and its effect on routine blood bank testing.

***************************************************************************************************

Joe: Well, hey, Connie. Welcome back to the Blood Bank Guy Essentials Podcast!

Connie: Well, Joe, great to be here! Always fun to talk to you.

Joe: I am truly just ... I mean, I think you know how I feel about you and your skills. You are truly just one of the giants in our field. I'm just so incredibly honored that you're willing to do this, so thanks for being back. Today we're going to talk about something that, man, it's super important, and it's weighing on people's minds. Really, for the last couple of years I have been hearing, "Well, okay, blood banks, you got through the anti-CD38
thing. Just wait till anti-CD47 comes!" I think people are a little scared, Connie, so let’s talk through this.

I think before we get into the details, we just need to set the stage. Can you give us just a little bit of a background on ... Well, first, very generally, what happened with anti-CD38, how that set the stage for anti-CD47? Is this something that we should be worried about going forward?

**Connie:** Well, certainly, Joe always fun to talk about blood bank problems with another blood banker at heart, and especially with your audience.

Certainly, with the DARA and the anti-CD38, we realized that some of these monoclonal therapies that were becoming so popular could cross-react with our red cells and interfere in pretransfusion testing. As you alluded to, there's been a rumor that something interferes even more than anti-CD38 might be coming down the pike, and it was back in I think, 2016 that the first small trials started with anti-CD47.

They haven't moved quite as fast as anti-CD38, CD38 certainly burst on the scene because of its efficacy. I think over one third of the patients responded positively. [CD47] is still pretty much in development and but it's becoming much more common.

**Joe:** Connie, I guess the big question that comes is: What the heck is CD47 anyway?

**Connie:** CD47 actually is a molecule on the red cell that's part of the Rh complex. Now, CD47 is expressed on all all tissues. The reason it's being targeted for tumor therapy is because some tumors, it was recognized, highly express CD47, over-express CD47. The idea was to target CD47, but unlike CD38, it's expressed much higher on red cells, and so that's why it's more of a problem for us. CD47 has a history though of being the "Do not eat me!" signal. Now, that sounds very, very much like a dinner bell, doesn't it? [laughs]

**Joe:** Yes, it does.

**Connie:** The idea is that CD47 tells your macrophages that they're a healthy cell. On the red cell, if CD47 is normally expressed, the cell is very happy and healthy and as it ages that CD47 is thought to somewhat change conformation and be the key that says, "Okay, it's time to remove this old fellow."

The idea is, the same happens on the other cells of your body as they age. Macrophages will recognize CD47 isn't there anymore, or it's time to remove this cell. That's the idea of removing a tumor cell, is telling the macrophage, it's silencing the "do not eat me" signal. It says, "It's time to remove this cell."
Joe: I see. Do we have information that on cancer cells, for example, that you have too much CD47?

Connie: Some tumors have too much CD47. The initial trials in 2016 started with just situations in which the tumor was shown to overexpress CD47, but now it's in much broader trials.

Joe: I see. Okay, so just to be clear, when CD47 is there, it's telling the body, the macrophages as you said, "Leave me alone, don't eat me," as you put it, which I love, but if CD47 declines or is blocked, then potentially the cells would be removed faster? Am I summarizing that correctly?

Connie: Exactly, absolutely. The idea of this monoclonal therapy is to silence the "do not eat me!" signal and so it becomes an "Eat me!" signal. If you cover up the CD47 with monoclonal antibody, then the macrophages supposedly go to town.

Joe: Got you.

Connie: The dinner bell.

Joe: That dinner bell, and that makes total sense. I mean, we're certainly seeing many treatments for different diseases nowadays that are composed of antibodies. The treatment for this obviously is an antibody as well, and so, I guess, Connie, the question is, when you have these antibodies floating around, why is that such a big potential issue for us in blood banking? What do we know, for example, about CD47 on different cells that we deal with in blood banking?

Connie: Well, certainly the problem is CD47 is very highly expressed on red cells. Unlike CD38, which is very low expression, and once you put a patient on the DARA drug, they actually "shed" the CD38 from their red cells within six hours, it's been shown. So, that wasn't so much a problem for us, but CD47 stays on the red cell, the therapy consists of giving high doses of monoclonal antibody, so you have a lot of coating on the red cells, and also you're covering up the "do not eat me" signal on red cells and platelets. The problem with this drug is the side effect of the medication is anemia, and can be thrombocytopenia. Perfect for our blood bank world, right? [Laughs]

Joe: Great, that's lovely. This sounds wonderful already! [Laughs]

Connie: Right, a side effect of the treatment is a mild anemia or frank anemia, depending upon the dosages and the way it's given, and thrombocytopenia, which puts these patients right into the blood bank world and realm.

Joe: Okay, so and we'll get to those effects in just a minute. I hadn't caught before you just said that, that CD47 is also not only highly expressed on
red cells, but it's also expressed, is it "highly" on platelets as well, or do we know?

Connie: There's not a lot of information on the levels on platelets, but actually in the trials that have start, the thrombocytopenia is sometimes more problematic than the anemia for these patients.

Joe: Connie, again, before we get to how this crossed into your attention, and what you guys were reporting, can you tell me a little bit, do we know some background...it sounds like we know more about CD47 on red cells than we do on platelets. Do we have any information on how CD47 may vary depending on I don't know, ABO type, Rh type, any information of that sort?

Connie: Yeah, that's a great question. It was shown already a number of years ago that it has to do with your Rh type how much CD47 you actually have epitope-wise on your red cells. If you're Rh negative, CD47 has higher expression levels on Rh negative cells, so that when we see these incompatibilities, the Rh negative panel cells are usually a little bit stronger than the Rh positive cells, but everybody's positive.

Joe: Okay, and anything about some of the weird Rh types, the Rhnull and the like?

Connie: That's right, so we had known for years ago that D-- [NOTE: Spoken "D-dash-dash"] cells have low level of CD47. It's because CD47 traffics to the membrane with RhCE. If RhCE isn't there, there's less CD47, and so Rhnull cells will have less CD47. So if you test the patient's plasma against D-- cells or Rhnull cells, rare cells, they'll be weaker reactive. Of course, our patients are not usually D-- or Rhnull. They're often Rh negative or Rh positive, and there we see the 3+ to 4+ reactivity.

Joe: Connie, you don't see D-- everyday in your lab, come on?

Connie: No, we don't. I wish we did. We would snap them up as donors, wouldn't we?

Joe: I am sure, I am sure. Now, folks that are out there wondering about D--, that's, we don't have time to go into the details of that today. Connie and I would love to, but yeah, we'd probably have to move on.

Connie: Those D-- lack CE protein, and CD47 traffics with it. But the other thing we've shown years ago is that CD47 is actually linked with Rh to the underlying cytoskeleton. That's why it doesn't "shed" off the cells when the antibody binds, that's what makes it different than CD38: It's not shed from the red cells.

Joe: Got it. Well, Connie, I already mentioned at the top of this podcast that you and your group from New York Blood Center published...in fact, the day
that this podcast is being released is the day that this article that you guys have written about "Monoclonal Anti-CD47 Interference in Red Cell and Platelet Testing," that it's being released this very day that this podcast is being released. So, why don't you, if you would, just take me through how did this come to your attention? You report on several patients that you guys saw. Did this just pop up out of the blue, or were you guys part of a study, or what happened?

Connie: Well, I'm happy that this is very timely, publication at the same time as your podcast, so congratulations on arranging that.

We have been working a little bit with a couple of the companies. This is a very popular area. There are several drugs in the pipeline. We have been working with a couple of them that were developing these monoclonal antibodies, but then two local hospitals were involved in the initial clinical trial, and that's when we saw the samples.

The interesting thing is that it was anticipated that these patients would have multiple anemias or thrombocytopenia, so the dosing started right out as "tiered dosing," where they'd start with just a little bit of the drug, build up, let the patient's reticulocytopenia kick in, and hopefully ... So all of these drugs that are on the market now and in clinical trials are being dosed in small doses first, etc.

When we saw the first patients, it was because we knew they were going to be involved in the clinical trial and we wanted to actually look at patients sera to see how much it was going to interfere in the blood bank. Of course, our first thing was to try and remove it from the red cells like it works so well for DARA! DTT, enzymes... So we threw the kitchen sink at CD47 and nothing "stuck," so to speak. DTT doesn't destroy the epitope, enzymes don't destroy the epitope, papain, chymotrypsin, ficin; none of those things. W.A.R.M. reagent; nothing will denature the CD47 on the test red cells so that we can show compatibility or not.

Joe: Well, you're just cutting to the chase, aren't you? That's fantastic. {laughs] Well, yeah. I mean, that's a scary, scary thing. Before we get to what you did to try and remove that interference, can you just give us a picture of what was messed up when you guys started to do the basic testing?

Connie: Absolutely, because that's what we start with, don't we? An ABO and antibody screen, and they were all positive. The most concerning being the reverse type. There's so much drug circulating that your back type or your reverse type will be positive in initial spin testing. Everything's positive; initial spin, 37 degrees, and antiglobulin testing, 3-4+ incompatibility and an invalid back type.

In fact, after the patients are on the drug a while, sometimes you can even see spontaneous agglutination of the red cells in a degree of +/- or 1+ and
warm washing doesn't help that, so it's really important to have the ABO type done before the patient is put on this drug and everything's incompatible.

Joe: With ABO, let's talk about that for a second, Connie. With ABO, so if I'm hearing you right, you're saying that on the serum grouping, the back type, you're seeing in patients that aren't group O, you're seeing strong reactions with both A1 cells and B cells, but you're also seeing spontaneous agglutination on the front type that this drug is in such strong quantity.

Connie: That's right. Not always interference in the front type, but always interference in the back type. The reverse will not be valid in non-ABO patient. It's because even though this is an IgG antibody, there's so much CD47 on the red cells that it behaves like an IgM antibody. It's a direct agglutinin, it's initial spin reactive.

Joe: That was a point I was going to make, because I saw this in your paper, Connie, that when we talk about antibody reactions, and you had mentioned that everything on your antibody detection tests, antibody ID, etc., everything is strongly positive in all phases in tubes, including immediate spin. Immediate spin positivity is not something we normally (other than ABO) associate with IgG, but that's, I guess that makes sense. You're saying it's in such strong quantity that it's a "pseudo IgM," it's acting like an IgM?

Connie: Exactly. You explain that very well, Joe. Congratulations. [laughs]

Joe: That's because I had to take a while to get it through my slow brain, Connie. That's what it was. Okay, so everything incompatible, everything messed up in terms of your testing. I'm curious, what about the DAT? Did these patients show up with positive DAT's?

Connie: Well that was a little bit of a mystery at first, because the DAT was NOT positive, but then when you did an eluate, it was 3-4+, so that tells you there's a false negative DAT. The cells are SO coated, it's called "blocking" or "prozone," that you don't see the positive reactivity, but the eluate being 3-4+, that much antibody on the cells told us, "Yeah, it's bound to the cells. The DAT was falsely negative."

Joe: That's really interesting.

Connie: A false negative DAT.

Joe: A false negative DAT, and it's because it's so heavily coated with antibody that the test...is it that the anti-IgG can't get in there, is that the deal?

Connie: That's right, it can't. There's no room for it and it's called prozone or blocking.
Joe: Sure, well that makes sense. Everybody listening is thinking back to the whole prozone phenomenon going, "Oh, man. I knew that at one point." [laughs]

Connie: [Laughs] From the Dark Ages, yes. You don't see it very often, but antibodies can prozone and give you a false reaction because there's so much of it there. It actually looks negative, but it's not negative.

Joe: I see. Again, I want to just make this crystal clear for those listening, Connie, that are sitting there wondering about, you mentioned "eluate," and obviously most of us that have been around blood banking for a while are very clear on that. If you wouldn't mind, just give us a little more clarity on that a weakly positive DAT, why does that seem weird when you have a really strongly positive eluate?

Connie: The two should be parallel. If you have a negative DAT, you usually wouldn't have anything coming off in the eluate, because the DAT said, "No, no antibodies bound here." The two usually are parallel. If you have a weakly positive DAT you can get antibody off of them. Eluate is a concentrating method, actually, taking the antibody off the cells.

In total, what you'll see in these patients is a problem in the back type, 3-4+ reactivity, initial spin, gel, solid phase, whatever you're using will be positive. But the real key here was to realize was that this drug, unlike the other drugs that are coming, is IgG4. IgG4 is usually not a problem in the blood bank testing, and so we have a monoclonal anti-human globulin, the monoclonal one made by Gammaclone, sold by Immucor, doesn't detect IgG4.

That was a good way to think about how to get rid of this interference in the antiglobulin test. At least use the reagent that doesn't detect IgG4. Now, an anti-Kell, an anti-Kidd, an anti-Duffy will be detected, but this anti-CD47 that was circulating in the plasma and is now bound to your panel cells or your selectogens, it won't be detected in the antiglobulin phase if you use that reagent.

Joe: Just to be clear, Connie, that is just one specific reagent? Do the other anti IgG's on the market include IgG4 detection?

Connie: That's right, all the others on the market are an anti-total IgG, so this one alone.

What happens then is you can get a negative anti-human globulin test, your initial spin would still be positive, your 37 degree positive, but it should be negative in the anti-human globulin test. Unless there's a little bit of "carryover," and that can happen too, that the antibody's so strong that you if you put it under the microscope (there might be some people still reading under the microscope), but usually in gel card testing, etc., it's negative.
Joe: Okay, so that's really interesting. That's one way potentially around it with using that particular version of anti-IgG. Connie, again before we get to a few more of the ways that you were able to and attempted to get around this anti-CD47 interference. I want to step back for just a second, and again draw us back to the anti-CD38 DARA scenario.

I think most reference labs now, and in fact, a lot of hospital transfusion services are pretty aware of a pattern that they see that makes them say, "This smells like DARA." We've seen so much of it over the last few years, certainly in my reference lab, and I know yours to an even greater extent. It's kind of that general pattern of weak reactivity across the board in your antibody identification panel, etc. Of course, the wonderful multiple myeloma history that does jump out at you.

I'm wondering if you, and you mentioned this a few moments ago, but granted, this is just coming out, and this is still in trials and all that. From your perspective, based on this experience that you guys had with these patients, I guess, are there clues, or is it just screamingly obvious when you see all this? Something's really wrong?

Connie: Well, I think you could be fooled if you weren't given notice that this patient is on anti-CD47. What has happened since the DARA experience is that the FDA is requiring these manufacturers of anti-CD47 to consider the compatibility issues and to actually, in the clinical trials, certainly, let blood banks know that there's a clinical trial going on in your facility.

That's helpful, but this will appear to be, if you saw a patient with everything agglutinating 3-4+ you USUALLY think, "Oh, cold autoantibody, warm autoantibody, autoimmune hemolytic anemia," so it will present like that and the patient will have some anemia. So, autoimmune hemolytic anemia is what it might be confused with.

Joe: Yeah, that makes total sense. That's what I was wondering is what's the big differential diagnosis and a strong autoantibody, I can definitely see people going down that trail. Connie you mentioned earlier, and I don't want to skip this because I think it's important, you had mentioned the anti CD-47 on platelets and you talked about the basic antibody testing that you saw that was messed up pretty extensively. Do you have information on platelet antibody testing? Like platelet crossmatching, or platelet antibody screens, things like that?

Connie: That was one of the concerns is, how's it going to interfere also if these patients need platelet transfusions? Would it interfere in platelet crossmatching? And it does. The Pak Plus, which is a method that actually uses fragments and not whole platelets, actually uses isolated GP proteins, it does not seem to interfere with the Pak Plus type of methodology. But the Capture-P's, etc., and platelet crossmatching give false positive just like our red cell testing. Yes, platelet crossmatching is an
issue. Of course, most patients don't need a platelet crossmatch to respond, hopefully? [laughs]

Joe: Tell that to some of the hospitals I work with. [laughs]

Connie: Yes, that's right. [laughs]

Joe: I can't help myself.

Connie: It will interfere in your platelet crossmatch also.

Joe: Yes. Okay, fair enough. All right, so we've talked a little bit about the background, and we've teased a little bit some of the ... Well, we've more than teased, we've talked about some of the results that you're seeing with your routine red cell testing, and plasma testing. I guess we should cruise on and talk about some of the ways that you tried to get rid of this.

Again, you've mentioned some of them, so why don't you just take us through ... I mean, some of the classic methods that we have used, some of the things in our toolbox in blood bank world to try and get rid of antigens and/or antibodies on the surface of red cells, or those interactions with plasma have included things like enzymes, let's just start there. What did you guys throw it these things in terms of enzymes, and what did it do?

Connie: I'll just remind everyone that what we're trying to do with the enzymes is not do anything to the antibody. We're trying to change the epitope on our test red cells, so to remove that specific antigen so that our test red cells are still valid for looking for specific antibodies.

One of the things you do is see if CD47 can be removed from the test red cells with things like papain, ficin, trypsin; these things all cleave proteins. We've tried alpha-chymotrypsin, we tried DTT, which cleaves the DARA CD38 but doesn't cleave CD47. We tried the W.A.R.M. reagent, and nothing removes CD47 from your test red cells so that you can use those for your antibody screen.

So, you have to think about the other side. Try and get rid of the antibody, the anti-CD47 that's in the patient's plasma, get that absorbed out and removed so that then you could test the patient's plasma. And that's what we do when patients have warm autoantibodies, is we absorb, because we can't remove the warm autoantibody targets from our tests of red cells either. We could only remove the warm autoantibody from the plasma, so we think of CD47 just like a warm autoantibody, try and remove it.

Joe: As we get into that discussion on adsorption, and I know that some people are sitting here breaking out in hives thinking about adsorption, because adsorption is actually interesting to me. It's one of my favorite things to talk
about to people and to teach to learners, but honestly, people have conceptual trouble with it sometimes.

Since we've got a little bit of time here, Connie talk us through conceptually what you guys did? I mean with a warm autoantibody, for example, we always talk about doing autoadsorptions where you take the patient's own red cells and try and pull that autoantibody out of the sample. What did you guys do with these samples that you had? Did you do autoadsorption or did you have to do adsorption with someone else's red cells?

Connie: That's a very good point. It would really be nice if you could use the patient's own cells, even though there somewhat anemic, etc., but their cells are so coated...Remember, I told you about the DAT being false negative, because there's so much antibody there? So, we tried removing the antibody with W.A.R.M. reagent, we tried remove the antibody with AET [NOTE: Another sulfa reagent like DTT], we tried removing the antibody with DTT, but we could never get enough antibody off the patient's own cells to even change the DAT to anything but a 4+.

If the cells are already so coated, autologous will won't work efficiently, certainly efficiently. So we use someone else's cells. We, Reference Lab use a panel of our R1R1, R2R2, and rr cells to do an alloadsorption. We also thought about, "Well, it's on platelets, so maybe platelets would work to absorb," and would be an attractive alternative.

Joe: I have to ask you about that, Connie, because I have to admit, and believe me I certainly claim no level of expertise anywhere in the universe of yours, but I have to admit, when I saw that you guys had used or were thinking about using pooled platelets to do adsorptions to get rid of antibodies floating around in the plasma, I have to admit, that's not something that was super-familiar to me. Is this novel, or is this something that people have been doing for decades that I've just missed?

Connie: Well, that's a trick that's in an old blood bankers bag probably, the blood bank bag of tricks from probably the 70s and 80s, when people were trying to remove HLA-type antibodies from patients sera, they started using platelets. I know one lab that still has frozen platelets, outdated platelets, they freeze their platelets, and they actually have a protocol for adsorption onto platelets when they're trying to get rid of HTLA or HLA-type antibodies.

Joe: Interesting, that's really cool. So, you've tried absorbing with platelets and was that just... Did you just take a bunch of different people's platelets or is there actually a reagent that does that for you?

Connie: We tried both things. There is a reagent out there that's manufactured that is made from platelet membranes. We weren't very successful with that.
fact, we found it very difficult to work with, because the sample would actually "gel" and become very viscous. It may be because the patients, the four or five patients we tried that on, were on very high levels of antibody. There was so much antibody there that it wasn't effective. We were able to use pools of outdated single donor platelets but it took four to six adsorptions but it also took four to six adsorptions on our R1R1, R2R2, rr uncoated cells.

Joe: I see.

Connie: There's such a high titer of antibody, in the thousands, present that it takes multiple serial adsorptions to remove the antibody.

Joe: Okay, so just so everyone's clear on this, Connie I don't think I gave you the opportunity to really describe the results when you did your alloadsorption. You're telling me that alloadsorptions actually work, red cell alloadsorptions work?

Connie: If you do multiple, they work, yes. Yes, you can get rid of excess but you have to do, in our hands at least four serial; take the same plasma on the cells, incubate, etc., and then on fresh cells, again fresh cells, again fresh cells again, and the same with platelets.

Platelets were not more efficient than allogeneic red cells in our hands at removing, but the platelets were attractive option because that's one pool you could use of cells, you don't have to have three aliquots of adsorption.

Joe: That makes sense. Reference lab people being the very compulsive people we are, I think when a lot of Reference Lab folks start getting to three plus adsorptions, people start getting worried about the validity of what's left behind. What's your take on that, Connie, as someone who knows more about Reference labs than the rest of us ever will? How do you feel about that with multiple, multiple, multiple adsorptions?

Connie: That's right. You have to be very careful, and it has to be done in a way that keeps in mind that you are entering into some dilution of weak antibodies. But, if you put this all together in a picture, it is a way to get rid of the antibody, not a very attractive [way], and it's certainly labor-intensive, but you can get rid of the antibody.

Joe: Connie, I have seen a little bit of the...not all of it, but I've been told some of what the manufacturer has stated regarding some of the ways that they have suggested to get rid of some of this interference, and I think you addressed this, in fact, I know you addressed this in your article, some discussion about using adsorption with polyethylene glycol or polyethylene glycol crossmatches. What did you find with that in your hands?

Connie: I'm glad you mentioned that, because I think it was somewhat misleading. What happens with a PEG adsorption or a PEG crossmatch is when
there's a lot of antibody there it actually precipitates, and so it looks like you have a negative test, but it's because it's all precipitated out and it's not any longer available to bind to red cells.

Joe: Oh, boy!

Connie: In our hands, we certainly tried the PEG, but the tests were all invalid when you do the proper controls, because the antibodies, both the anti-CD47 (which you wanted to get rid of) was precipitating out, but so were Kells, Kidd, and Duffy. We showed that they also precipitate out. And, we've been warned before that PEG adsorptions do have that drawback, that you must remember that clinically significant antibodies could be precipitated out and not detected. That was why it was working in initial hands that to get rid of reactivities with the PEG, we feel.

Joe: I see. That makes sense and it's a little scary.

Connie: It is. You don't really want to use PEG, yes...PEG adsorption

Joe: Got it. Okay, Connie, before we finish down the homestretch here and bring this all together and talk a little bit about the comparison with anti-CD38, just bottom line this for us. Based on everything that you guys were able to do with these patients that you saw, is it achievable with anti CD-47 interference, is it achievable to get compatible crossmatches, compatible or negative antibody detection tests, or "accurate" antibody detection tests? I guess would be a better way to put it. Is that something that laboratories can reasonably achieve in your view?

Connie: I think it needs some thought and a process and some discussion with your medical director on how to address these kinds of situations. Remember, you can get a negative anti-human globulin test. So, you can get a negative crossmatch and a negative antibody screen only in the anti-human globulin phase, but that's a pretty important phase, one that we pretty much put a lot of significance on. That still leaves you with a back type issue on the patient. That really focuses you on the fact that you need a pretreatment ABO and Rh and then a discussion about, "Okay, I know what's interfering with the back type. On the next visit, I have a valid ABO/Rh, and I'm confident in the patient's ABO/Rh. So, it's important to have that pretreatment ABO type, and most of these clinical trials are focusing on pretreatment.

Also, typing out the patient. We also showed that in antigen typing, the cells are so coated, you get a false negative on antigen typing. So this makes genotyping something that's a little more feasible, certainly, and having that antigen profile on that patient then allows you to think about maybe getting a better antigen-matched unit to decrease the number of workups you might need to do, especially since these patients, we
anticipate, will need more blood product or transfusion than certainly our patients with multiple myeloma and DARA maybe needed.

Joe: Well, first I know that being able to mention "genotyping" just warms your heart, so I'm glad you have the opportunity to say that. I mean, it's super important, isn't it? I mean when you know that the testing is going to be very difficult to have that information that you have, and as you've told me before, you have that information now and forever and that's so incredibly helpful.

Connie: Exactly. You do it once and then you use it. You use it to actually rule out antibodies. If someone is Kell-positive, you don't have to, when you're doing your antibody screen, you can rule out Kell. The patient's Kell positive, they're not going to make it anti-Kell! I mean, that's kind of a bold clear example, but it's helpful. It really is very helpful, and then you can make some decisions.

Certainly, with warm autoantibodies (like I said, this is presenting as if it's a warm autoantibody), we have a number of facilities that give an antigen-matched unit so that they can decrease the number of times that they feel they need to repeat the workup, so they'll give an extended matched unit. I challenge blood centers to make those affordable and feasible for folks, and that we work together to make it better patient care and also economically realistic. That's my soapbox.

Joe: I'm right there with you. I think that's the future. I think not recognizing that is a huge mistake by people. It is where we are going, in my opinion.

Connie: Exactly, and I know this isn't our topic of discussion today, the use of historically labeled units, which the FDA has just allowed. If you're doing preventative using historically typed unit is very economical.

Joe: Yes, agreed. Look at you, you managed to sneak that one in too?

Connie: A conversation for another day, Joe? [laughs]

Joe: [Laughs] Yes, it's true, but Connie I think as we close this out, I mentioned at the beginning, this has all been discussed under the ... I don't want to say "the cloud of DARA," because that's a little too strong. As I said before, people have gotten familiar with dealing with the Daratumumab anti-CD38 interference.

Folks, just so you'll know, if you are sitting here at this point in the podcast going, "Daratumumab, oh my God..." Let me just mention, I did an extensive discussion on the Daratumumab effect with Dr. Rick Kaufman on my podcast previously. You can find that at BBGuy.org/010. You'll get all you want to know about DARA for that, but Connie, I wonder if you would just take us through, and in your article, you guys did a wonderful table, I believe it's table 3, directly compares and contrasts the effects of
anti-CD38 and anti-CD47. We certainly don't have to go through every line of the table, by any stretch, but can you just kind of compare and contrast the two for our people that are out there that again are a little more familiar with CD38 than CD47?

Connie: Certainly. We'll start with the red cells: CD38 is low expression on the patient and on your test red cells, whereas CD47 is very highly expressed on the patient cells and on the test cells.

In the patient, CD38 will "shed" from the red cell membrane as they're on therapy. It's not going to happen with CD47, they are not going to shed from the red cells.

When you test the patient's plasma, anti-CD38 is an IgG1 antibody and because the CD38 on your test red cells is low, you'll never see more than usually a 1+ reactivity. 1+ and only in the IAT. It's an IgG1 antibody, there's not a lot of CD38 in your test red cells, DARA is 1+ in the AHG phase. In contrast, CD47 is all over your test red cells and the antibody in the patients serum is going to give a positive in all phases, 3-4+, and it's going to interfere in your back type because it is positive at initial spin, whereas DARA never interfered in your back type.

With DARA you're going to be able to use DTT treated cells (or trypsin-treated cells), because you can get those test red cells to get rid of their CD38 so that you can see if there's true antibodies there. But with CD47, there's nothing you can do to treat your test red cells to get rid of their reactivity.

You have to use, in the antiglobulin test, the Gamma Immucor monoclonal anti-IgG for a negative IAT, but if you want to get rid of your back type and if you want to show that there's no immediate spin antibodies there, or 37 degree antibodies, you're going to have to absorb three to four times to get rid of the antibody. Most people if they give an antigen matched unit, it's a medical decision on how much and how far you go as far as adsorption.

It'll interfere in your platelet crossmatching, whereas CD38 doesn't interfere usually. It depends, in about 50% of the patients we see some interference with DARA in the platelet crossmatch too.

The CD47 that we're talking about, I do want to focus on the fact that we're talking about the Hu5F9-G4 antibody, the first one that's come into clinical trials. Now, there are multiple, some of them have been redesigned to be fusion proteins. I mentioned in the article there's a "TT1-621," and we've heard that that has very minimal red cell binding and doesn't interfere like this as much as this Hu5F9-G4 does. There's another I just learned about early this week, a trial on something called "ALX148," and that is interfering in blood bank testing. I know of several others in the
pipeline that do interfere. Most do interfere, but if you're on a clinical trial with the dual fusion protein TT1-621, you may not see interference. Unlike the DARA-based or the CD38, isotuximab, and there's more, MOR204, I believe, it is they all interfere with DARA. DARA kind is DARA. DARA types are DARA. CD47's are going to be somewhat variable.

Joe: That's really interesting. Okay, well as we finish this, Connie, I wonder if..., in the end, this is all about getting our patients taken care of. I know that's where your heart is, as well as mine. Tell us, you guys had these four patients that you were working on. Did you see in them, some of those, the anemia, the thrombocytopenia that was described as possibilities, and were you able to successfully transfuse these folks?

Connie: Actually, only one needed transfusion. The dosing on these clinical trials, the dosing is such that the anemia drops 2-3g and then resolves itself. These patients are very resilient characters.

This dosing effect does work. We're not aware of anyone who needed more than an occasional transfusion. Again, it's early in the trials and they're being pretty careful, so it'll be interesting to see how much transfusion these folks do end up needing.

Joe: It sounds like, Connie, we can maybe try and summarize this with anti-CD47 is going to require more work than patients who are on anti-CD38, but you can get to where you need to go, but just like with anti-CD38, getting that pre-transfusion or the pre-treatment sample and figuring out exactly what's going on with those patients is going to be super important. Is that a fair way to put it?

Connie: Exactly, super important. Also, to know what drug, exactly which CD47 drug the patient is on, because they're not all IgG4, so you're not going to be able to get around all of them with the Gamma monoclonal so you do have to be part of the discussion on what the clinical trial is and what the patient is getting. Because we know there are several that are coming that are IgG1 and you're not going to be able to use the Gammaclonel, you're going to have to use adsorption, et cetera.

As a sidelight, we're trying to convince those manufacturers that they need to use, they need to provide a neutralizing reagent, a neutralizing antibody, and they're working real hard on that! So we hope the other CD47's will have a reagent supply that will help with the blood bank testing. We'll see.

Joe: Connie, I mentioned that I had talked to Rick Kaufman about DARA. This was back in 2016. One of the last questions I asked him in that interview was to break out his crystal ball and tell me what he thought he saw coming with stuff like this, medications like this in the future. He actually, if I remember right, I actually think he may have said something about anti-
CD47 at that time. Let me ask you the same thing, with your magical
crystal ball, what do you see as the future? Are we going to have more
and more and more and more of these things coming out that cause us
problems like this?

Connie: I'm afraid so [laughs]. I think this is just, we're on the tip of the iceberg.
When you think about it, the reason is because blood group antigens
aren't just for red cells. I mean, these proteins are on ... It's only RhD that
it's exclusively only expressed in red cells, but the Duffy, the Kidd proteins,
Kell proteins, they're everywhere in tissues. Anything that's targeted on a
tumor cell may also be on the red cell, and so that's our challenge. I'm with
Rick and predict that this is probably just one of our first headaches. This
is our second headache and there's more to come.

Actually we became aware of few weeks ago of another, it's actually a
drug that's being given for MS patients and Crohn's patients. Now, we
don't often have to transfuse them or work them up, and this was a workup
that was accidentally ordered on a Crohn's patient, and everything was
positive. And it targets alpha-4 Integrin, and LW on our red cells also bind
to alpha-4 Integrin. The interference actually is cross-reacting with LW on
red cells. So, we already have another example, so like I said, this is just
the beginning of ... Immunotherapy is a very hot topic and it's working, but
again because these molecules on tumor cells may also be expressed on
red cells in varying levels, we are at the tip of the iceberg. There's an
editorial that accompanies our paper by Don Branch that expounds a little
bit on what our future might be. He calls it "Immunotherapy: The Good,
The Bad and The Ugly," the real ugly for blood bankers.

Joe: Yes. The paper that Connie has described and the editorial, Connie has
described is available as of the day you can be hearing this podcast in the
February edition of the journal "Transfusion." I want to thank Dr. Rick
Kaufman actually for helping me coordinate this with the release of that
edition of The Transfusion and I hope everyone goes and checks it out.

Connie, before I say goodbye I want to make sure is there anything we
missed? Is there anything I need to hit better?

Connie: I don't think so, Joe, but I want to thank you for the opportunity and for the
great job you do educating us all and sharing our Transfusion Medicine
challenges. I really appreciate it.

Joe: It is my pleasure and it is always my honor to talk to you, my friend. Thank
you so much, Connie.

Connie: Thank you, Joe.
Joe: Okay you guys, I have to let you in on a little secret about Connie Westhoff. You listen to her on a podcast like this and she sounds like such a nice person and all, and then you actually meet her...honestly she's an even BETTER person. She's awesome! I am just really super honored to be her friend.

Again, a real quick summary of what we've talked about. When you have a patient who is on anti-CD47, EVERYTHING is going to be messed up. You can get around that to an extent by using one particular specific anti-IgG that avoids detecting IgG4 and that can help you avoid that interference at IAT phase of testing.

There are some other things that you can do. It may be beyond the capabilities of smaller transfusion services, such as multiple alloadsorptions of the patients plasma using enzyme-treated red cells, and finally adsorbing the plasma with pooled human platelets. Again that might be beyond the capabilities of smaller facilities, but those are some of the options that are out there.

Remember, you can go to www.wileyhealthlearning.com/transfusionnews and you can get your hour of totally free continuing education credit and that's for both docs and laboratorians. Also, you can go to the show page, which I hope you do, and that show page is at BBGuy.org/063. There you'll find the transcript for this episode as well as the link to the article in Transfusion that we were describing.

I have lots of great stuff coming your way real soon, including two interviews that I'm just bouncing off the walls about. One is an interview with Dr. Ron Strauss, who will be talking about granulocyte transfusions, and another is an interview with Dr. Jerry Sandler from Georgetown. Jerry's going try and help us decide if there really is an entity called "anaphylactic transfusion reaction due to IGA deficiency." Jerry has some strong opinions on that, and I can't wait for you to hear. That's coming real soon.

Please go to Apple Podcasts or Google Play or Spotify or Stitcher Radio, or heck, wherever you get your podcasts, and subscribe. Subscribing will make sure that you don't miss any episodes that are coming up. While you're there, again, if you would take the time to give this podcast a review, it really helps me get it in front of more people.

Thank you for being here, and until we meet again, as always, my friends, I hope that you smile, and have fun, and above all, hey, never, EVER stop learning! Thank you so much. We'll catch you next time on the Blood Bank Guy Essentials Podcast.