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Advancing Noninvasive CRC Screening: Exploring an Aptamer-Based Assay

Mr. Quigley:
Welcome to *Project Oncology* on ReachMD. I'm Ryan Quigley, and today I'm joined by two of my ReachMD colleagues, Drs. Hallie Blevins and Mimi Maeusli, to discuss a paper focused on a noninvasive test that may aid in the early detection of colorectal cancer.

Hallie, Mimi, it's great to have you both joining me today.

Dr. Blevins:
Thank you for having me. Excited to be here.

Dr. Maeusli:
Looking forward to the conversation. Thanks.

Mr. Quigley:
Now, to start us off, I do want to dive into the background of this study. Hallie, let's start with you. Where can people find this study? And what about it did you find particularly interesting?

Dr. Blevins:
Well, this paper was published in *ACS Sensors,* so it is a chemistry-based paper, and this paper focuses on colorimetric aptasensing. It uses aptamers, and aptamers are not necessarily new. They were used early in the '90s, and colorimetric assays are by no means new either, but the combination of these things to detect a bacteria in the gut for CRC detection is an exciting technique.

Mr. Quigley:
As a follow-up to that, I want to ask: what makes this test different from some of the currently existing tests out there for CRC?

Dr. Maeusli:
Well, to start, I think it's important to provide a little bit of background about CRC. Colorectal cancer is the second-most common cause of cancer-related deaths when combining males and females. And while deaths due to CRC have been declining due to increased testing for precancerous polyps and earlier detection of CRC in general, we still have about one in three people in the United States who should be screened for CRC who have never been tested. So what that means is that the current methods could be improved to get more people screened. And as you guys had mentioned earlier, this is a noninvasive test. Some of the current screening methods can be invasive and costly, like colonoscopy.

And so what was really interesting about this study that Hallie alluded to is that they essentially leverage the field of the gut microbiome. And so, more specifically, they were looking at *Parvimonas micra* as a biomarker. So it's *P. micra*, which is a gram-positive obligate anaerobe and it's commensal in the body, meaning that it's found in healthy individuals, but its increased abundance has been associated with oral pathogens. But now, evidence is also suggesting that an elevated *P. micra* level is also associated with colorectal cancer and potentially tumorigenesis. So this makes *P. micra* an interesting target for biomarker development.

Mr. Quigley:
Now, one thing I want to ask is—and, Hallie, you mentioned this earlier—can you dive in a little bit deeper on what an aptamer is?

Dr. Blevins:
Yeah. I like to compare them to antibodies in the way they function, but they are not antibodies. So aptamers are made up of oligonucleotides, so they can be DNA- or RNA-based. This paper used DNA-based aptamers. They're designed to recognize and bind

to specific targets, so things like proteins. They recognize whole cells, which is what this study is looking at today. But they can also get down to small molecules. I know that they've been used in testing for drugs and things like that, so they've got a wide range of applicability, which is really nice. So in that way, they are similar to antibodies, but they are structurally and chemically different.

But this paper used an aptamer library, so they purchased a bunch of different synthetic aptamers and they looked at I think over 450 unique single-stranded DNA aptamers.

Mr. Quigley:
Okay. So with that in mind, how did the investigators identify the best aptamer for the study?

Dr. Maeusli:
Right. That's a really good question. What they ended up doing was 17 rounds of selection. And so what I mean by that is they started with four rounds of positive selection, meaning that they ran the aptamers and tested them against the *P. micra* target microbe over and over for several rounds. And what they do is they test for binding of the aptamer with the bacteria and then they enrich for that binding. And so they'll take PCR and amplify the aptamers. And then after four rounds of that, they wanted to ensure that they don't have false positives so that they don't bind to bacteria that are off target or that may be native to the gut or present in those complex microbiomes. And so in doing that, they did 13 rounds of negative selection against three gut bacteria that have been associated with colorectal cancer and then E. coli as well. And after that they performed binding experiments to ensure the specificity of that *P. micra* and tested it again with six common intestinal bacteria and nontarget bacteria associated with CRC. I think what would be interesting to talk about as well is the designing of the nanoparticles, and I'll let Hallie answer that because this is truly her jam as a medicinal chemist.

Dr. Blevins:
So this study used magnetic nanoparticles made of iron oxide, and then they coated them with gold. And both of the components of the nanoparticle are both intentional and important to the assay and the readout of the assay. So then they covalently bound the aptasensor to the nanoparticle to make one solid unit. Now, when it gets to the assay and how they detected this *P. micra*, the study used 36 fecal samples. 18 were from CRC patients, and 18 were from healthy patients. And they combined the nanoparticle with the fecal samples, let them incubate for a little bit because it's asked to attach and recognize the *P. micra*, and then they used magnets to separate the nanoparticles from the non-bound—the stuff we don't care about so much in the fecal sample. So then they washed the magnetic beads a little bit just to get everything off so they only have what's bound to the nanoparticle, and then they added two reagents called TMB and hydrogen peroxide. And these reagents are traditionally used in colorimetric reactions, but they're usually used with an enzyme called HRP. And anyone who's worked at the bench in colorimetric assays knows this is a very traditional use of these reagents, but the iron oxide nanoparticles have the same capabilities as this HRP enzyme, so the reaction can still take place. So what happens is, when you add all those things together, it creates this deep blue color, but when *P. micra* is present and the nanoparticles are bound to this bacteria, it essentially covers the surface of the nanoparticle and it blocks this reaction from happening. So the more *P micra* that's present, the more the nanoparticles are covered and the less of this blue color that will happen.

Ryan:
So this is obviously very early in the testing process. From your perspectives, with such a strong background on the scientific end of these things, how would you both approach taking this testing a little bit further down the road?

Dr. Maeusli:
Yeah, that's a really good question, and I can start us off here. So I think that one of the first things that I would try to address is that at least in this publication, there was no staging data for the stool samples from the colorectal cancer patients. And so what I mean by that is that we need to evaluate whether the *P. micra* itself can be detected in precancerous or early-stage colorectal cancer. And we need to identify what type of absorbance reading of these nanoparticles are reflective of a positive diagnosis or an intermediate or a negative. But that said, I think this research was really interesting and very exciting in the sense that these aptamers are easy to produce and have low manufacturing costs.

Dr. Blevins:
Yeah, Mimi, I agree with you. Optimizing the biomarker is definitely a priority. We want to make sure that we're using the best biomarker for detecting CRC—the one that's most correlated with CRC—so that's definitely the priority here. But I do think there's also an opportunity to optimize lab methods or the readout of these experiments. Nanoparticles and this colorimetric assay is not the only way to do it, so there might be some opportunity there. But no matter what CRC methods or detection methods are coming out, they're going to need to withstand some clinical trials, and we'll have to be able to compare performance to the standard methods that we use in the hospitals.

Mr. Quigley:

Yeah, this is a very interesting study, and I'm so glad that you both joined me to dive a little bit deeper into it.

I want to again thank my guests, Dr. Mimi Maeusli and Dr. Hallie Blevins, my colleagues here at ReachMD, for joining me to discuss this.

Mimi, thank you very much for joining me.

Dr. Maeusli:
It was a pleasure.

Ryan:
And, Hallie, thank you as well. I really appreciate you doing this.

Dr. Blevins:
Yeah, this was great. Thank you.

Ryan:
For ReachMD, I'm Ryan Quigley. To access this and other episodes in our series, visit *Project Oncology* on ReachMD or go to ReachMD.com, where you can Be Part of the Knowledge. Thank you for listening and watching.