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Uncovering New Biomarkers for RCC: A Review of Emerging Techniques

**Dr. Takemoto:**

Welcome to *Project Oncology* on ReachMD. I'm Dr. Jody Takemoto, and joining me to share essential updates on biomarkers for renal cell carcinoma is Dr. David Braun, who's an Assistant Professor of Medicine and a member of the Center of Molecular and Cellular Oncology at Yale Cancer Center. Dr. Braun, thanks for being here.

**Dr. Braun:**

Thank you so much for having me.

**Dr. Takemoto:**

To start us off, Dr. Braun, can you tell us why identifying biomarkers for renal cell carcinoma is so important?

**Dr. Braun:**

Absolutely. I think the idea of biomarkers has a pretty far reach. I think, classically, it helps us to think about should patients receive treatment one or treatment two? Is one treatment more or less likely to work? But I think it really extends beyond that. We have settings, for instance, the adjuvant setting where the question might be, does the patient need treatment at all after surgery, or was surgery likely sufficient? There's a question of beyond whether treatment one or treatment two, choosing between them is an option. Even for a given treatment, prognostically, is something likely or unlikely to work? And that information is helpful. And then we maybe think of it a little bit less, but also thinking about biomarkers in the context of toxicity. Is this patient highly likely to get a side effect or less likely? All of those are really critical things, not just in the laboratory but primarily for patients. And so if we're able to develop biomarkers in any and all of those spaces, I think that really leads to meaningful improvements for patients in our clinic.

**Dr. Takemoto:**

Given that importance, where are we currently when it comes down to the known biomarkers for renal cell carcinoma?

**Dr. Braun:**

Yeah, it's a great question. And I think about it from maybe the most conventional, the most classic, all the way to evolving in newer technologies. And so the first most conventional is looking at it under the microscope, so the oldest tool that we have, but actually, the histology is probably the most active biomarker that we have in kidney cancer. And then we can move on to things like what is the immunophenotype of the tumor, so using tools like immunohistochemistry or immunofluorescence, to what are the genetics of the tumor, all the way to what are the transcriptomic features—the gene expression features that might impact response. And then finally, we have a bunch of emerging things like circulating biomarkers that are all important. And so each of those have areas of excitement, but actually, the one that's the most developed, the one that I would say is the only one, frankly, that we can use in the clinic today is the most conventional, is looking under the microscope, and that's if a pathologist looks under the microscope and sees sarcomatoid histology, so clear cell kidney cancer, but there's a sarcomatoid component, that's actually probably the most informative thing that we have today, and that really pushes us to the use of immunotherapy.

**Dr. Takemoto:**

For those just tuning in, you're listening to *Project Oncology* on ReachMD. I'm Dr. Jody Takemoto, and I'm speaking with Dr. David Braun about biomarkers for renal cell carcinoma. So now that we know why it's so important to discover new biomarkers, Dr. Braun, let's

zero in on some emerging research that aim to address this gap. What can you tell us about the high-dimensional spatial analysis technique?

**Dr. Braun:**

Yeah, absolutely. And so the idea behind this is in the past, when you looked at something under the microscope, you could describe essentially the morphology, what it looks like, and that's been the tool for a long time, pathologists staining with H&E, and they look at what does it look like; what is the papillary architecture. The next level—and frankly, the one that's in clinical use—is immunohistochemistry where you can look at one protein marker at a time. So they'll stain a tumor for PAX 8 and identify it's probably renal cell origin, maybe CA 9 to hint towards clear cell, or CK7 to hint towards papillary. All of these things are looking at one marker at a time. But we know the tumor microenvironment is incredibly complex. There's dozens of different cell types, lots of different T cell phenotypes, macrophages, B cells, plasma cells, obviously tumor cells, endothelial cells, fibroblasts. There's a huge range. And so if we really want to fully understand that complexity and understand the neighborhoods, the cellular neighborhoods that form within a tumor, you have to look at a lot of these things simultaneously, so not just one parameter at a time, not just CA 9, but many things at the same time, markers that are going to capture all of those different populations.

And that's been just technically not possible up until very recently, and there's a few emerging technologies that have really enabled both in the lab, and I think ultimately, in the clinic—though we're not there yet—to look at many populations at the same. One of those, broadly, is spatial transcriptomics, so the idea of RNA sequencing looking at the gene expression profiles, but rather than doing that in a tumor that you've mushed up, that you've put in the blender, or tumors where you've picked out one cell at a time but lost its spatial arrangement, actually being able to do this in space, so on a slide, understanding what is the gene expression pattern of each individual spot of each individual cell. And that's something that was science fiction even five, 10 years ago, but there's many technologies that enable you to do that today. And so that's one aspect, which is spatial transcriptomics.

The other is actually high-dimensional protein analysis. And so rather than looking at one thing at a time—CK7 expression at the protein level, things that IHC allows you to do—there are technologies, such as called PhenoCycler-Fusion now—used to be called CODEX—CycIF. There's a variety of technologies now that enable you to look at dozens, even low hundreds of markers at the same time, and so now you get not only these artistic images of the tumor microenvironment, but you can actually look—because you're looking at different cell compartments—actually find spatial organization, spatial architecture that might be really important for not only making a diagnosis but ultimately thinking about things like response to a given therapy.

**Dr. Takemoto:**

And how about circulating tumor DNA? What do we need to know about this technique?

**Dr. Braun:**

Yeah, so this has gained a lot of prominence in a lot of different types, and I feel like one of the poster trials for this is really bladder cancer. And this was amazing work, again led by the Genentech team and Dr. Tom Powles where they looked at an adjuvant immunotherapy trial, adjuvant atezolizumab for bladder cancer, and well overall the results were not incredibly encouraging when it was subdivided into patients that had detectable circulating tumor DNA, ctDNA-positive versus patients that had no detectable DNA. That's where the clear pattern emerged. So those patients that had no detectable ctDNA, tumor DNA, they really didn't look like they had any benefit from the addition of an adjuvant therapy, whereas those patients that had residual tumors of a molecular marker of minimal residual disease, those are the patients that had tremendous benefit from adjuvant atezolizumab.

And so understandably, there's been a lot of excitement about these sorts of circulating tumor DNA, whether it's panel-based approaches, whether it's bespoke approaches where it's customized to each patient's tumor, like the Signatera Test, or whether it's more whole exome or even whole genome-based approaches integrated with machine learning. All of those are the spectrum of things that are available.

But kidney cancer has been really hard. It's a very low-shedding tumor, and so in comparison to bladder cancer and lung cancer, which sheds not a huge amount but a reasonable amount of tumor DNA into the periphery, kidney cancer or RCC has the lowest amount of any extracranial tumor. The one it's actually closest to is glioblastoma in terms of the amount of DNA that it shed. And so the sensitivity of these tests is much lower than for other tumor types.

I think the reason for excitement is there's technologies that aim to overcome this, so even things like Signatera where it's a bespoke test, so it's able to pick up just under 20 different targets At the same time. There's efforts to even increase that number to not just 20 targets but hundreds of targets, even thousands of targets. I think that's really promising. But there's also newer-based approaches that look at not just whether there's a mutation or not but whether the DNA is altered in some way, it's methylated or not methylated—and

those patterns can be really helpful—whether the histones that wind the DNA, whether those are different in their modifications in terms of their acetylation or not. All of these things can now be integrated together. And so while each one might not have the perfect sensitivity, as you start to build on mutations, methylation, circulating epigenomic profiling, now all of a sudden you have potentially a better predictor.

**Dr. Takemoto:**

Now if we could look at one more technique, how might single-cell analysis help uncover biomarkers?

**Dr. Braun:**

Yeah, absolutely. And for single-cell, it sounds silly, but I have a particular analogy in my mind, which is how we conventionally do things versus how single-cell works. So imagine we have a fruit salad that has bananas and kiwis and blueberries and strawberries, and all these different components. Now conventional sequencing, the way it works is we take that fruit salad, we throw it into a blender—essentially, literally, we mush up a tumor—and then we end up with a fruit smoothie, and then we try to look at that fruit smoothie and say, "Why did that fruit smoothie either have a good response or a bad response to therapy?" It's really hard to tell what are the individual components, what were the fruit that went into it once you have the smoothie. Can you really tell that there is one kiwi in this blend versus one grapefruit piece in that blend? You can't. Or at least it's really challenging. But that's what we've done with conventional bulk RNA sequencing for many years. So the idea behind single-cell approaches is to go back and actually be able to look at inventory; what are all of the components of that fruit salad? What are all the components of the tumor microenvironment? And once you have that, all of a sudden it becomes much, much easier to say, "Ah, this fruit salad, this tumor that responded had the kiwi, had this particular cell type. This fruit salad that didn't respond had a banana, and that's why it didn't respond." And so single-cell RNA sequencing really allows you to pick apart the components of the tumor microenvironment, which gives you a resolution that just wasn't possible before. And so we can now use these approaches. They're still technically challenging, they're costly, but they're still in a laboratory setting. And we've done a large amount of single-cell RNA sequencing at our center but also have started to integrate it into phase 2 and partially to phase 3 clinical trials, and the idea is to really use that in-depth knowledge to understand what are the cell types, the phenotypes, and ultimately, the cell-cell interactions that might influence response or resistance to a certain drug.

**Dr. Takemoto:**

Lastly, Dr. Braun, any final thoughts on the kind of impact this can have on the way we manage renal cell carcinoma?

**Dr. Braun:**

Absolutely. I think right now—at least my hope is that—we're at an inflection point. We have these incredible emerging technologies that we can use in the laboratory, and we have a lot of amazing hypotheses that have come from them, but now they need pretty rigorous validation. They need to be validated in multiple data sets, and ultimately, prospectively. And the ones that succeed, the ones that make it through that validation process, I think they could have the potential, at least, for big impacts and helping us to figure out not just what is the right combination of drugs for our patients but how our patients will do overall, how likely or not likely they are to get side effects, a really important thing, and sometimes whether they need treatment at all. And I think that's the moment where I think we'll start to see this really cool science that's done in the laboratory, actually on a more routine basis help our patients in the clinic.

**Dr. Takemoto:**

Well, given the impacts these interesting research efforts might have on patients with renal cell carcinoma and the clinicians treating them, I want to thank my guest, Dr. David Braun, for joining me to discuss these key updates on biomarkers. Dr. Braun, it was great having you on the program.

**Dr. Braun:**

Thank you again. I really enjoyed it.

**Dr. Takemoto:**

For ReachMD, I'm Dr. Jody Takemoto. To access this and other episodes in our series, visit *Project Oncology* on ReachMD.com, where you can Be Part of the Knowledge. Thanks for listening.