

Bob Barrett: This is the podcast from '*Clinical Chemistry*'. I am Bob Barrett. Since the discovery that the numbers of circulating tumor cells can predict survival rates in metastatic breast cancer, there has been great enthusiasm for their use as a marker for cancer progression.

However, isolating these rare cells while maintaining the viability of the captured cells has proven to be difficult. In an editorial published in the May 2012 issue of '*Clinical Chemistry*' Dr. Keith Neeves, an Assistant Professor and Boettcher Investigator in the Department of Chemical and Biological Engineering at the Colorado School of Mines took a look at several techniques that have sought to capture circulating tumor cells without damage; including a new technique published in the same issue. Dr. Neeves is our guest in this podcast.

Doctor, what exactly are circulating tumor cells and how do they reach the peripheral blood?

Dr. Keith Neeves: Circulating tumor cells are the cells that metastasize from primary tumor. So these are the cells that come off a tumor and then get into the into the blood stream so that they can go to other parts of the body and form of secondary tumors, and these are kind of the hallmark of epithelial cancer. So there are about 85% of cancers which include breast, colorectal and prostate cancers.

Bob Barrett: How can circulating tumor cells be useful in monitoring cancer progression?

Dr. Keith Neeves: Well, it's really the secondary tumor that cause 90% of mortality in cancer, so primary tumors rarely cause death. So while imaging methods like MRI and CT can identify larger tumor, they really can identify these circulating tumor cells because they are small and they are fairly rare, and so you can get at the very early stages of secondary tumors by trying to count how many of these circulating tumor cells are in the blood stream.

Bob Barrett: What are some of the current methods and the technical challenges in isolating circulating tumor cells?

Dr. Keith Neeves: So the technical challenges is really that these are extremely rare. So you can have one tumor cell within anywhere from a million to a billion normal blood cells, so trying to capture that one tumor cell and get it out.

Now the big challenge is just coming up with techniques that can either isolate those tumor cells in sort of sensitive and specific way.

And so the current method tend to rely on immunocapture, so that's the most common method. And so what I mean by immunocapture is taking an antibody against a certain protein that's highly expressed on the surface of these circulating tumor cells, and then pulling those out that way and then selecting them against other cells with a couple other different types of markers.

So this has been a clinically approved approach and that's shown that the number of these CTCs in a bloodstream is a good predictor of survival in something like metastatic breast cancer.

Bob Barrett: Well, let's talk about the approach taken by Mike King and his colleagues, what's novel about their approach?

Dr. Keith Neeves: So what's novel here in what Mike King and colleagues have addressed as a throughput of these techniques. So what I mean by throughput is how much volume of blood can be processed in a given amount of time.

So the issue with using immunocapture to grab these circulating tumor cells is that the time it takes for the bonds to form is relatively slow. So if you can about think about blood flowing along and trying to pull these cells out, you can only flow the blood so quickly, if you fill it too fast, you can't form the bonds.

So it's kind of like you can think of each cell is, it's kind of like an airplane. When airplane has to land it can't just come to a complete stop, drop out of the sky and come to a complete stop. And so the way that blood cells sort of white cells, leukocytes and platelets, the way that they come to the stop on a vessel is that they roll.

So this rolling process is what Mike King and his group have kind of integrated into the circulating tumor cell isolation.

So they use two types of ligands. So these are the molecule that they are going to put on the surface of the wall of their device. And so one of them called the Selectin forms really fast but weak bonds. So these are the rolling bonds and so this allows the circulating tumor cells to roll first and then they come to a stop and they bind to one of those antibodies that we would use for immunocapture.

So they use that paradigm of how the endogenous blood cells come to a stop, then use that for isolating circulating tumor cells.

Bob Barrett: And what are some of the potential factors that circulating tumor cell isolation might overlook?

Dr. Keith Neeves: Well, so the big question is are we selecting for the right population, and so it's estimated that only one in about a hundred of these circulating tumor cells will actually stay alive long enough in the bloodstream and form secondary tumor.

So that kind of raises the question of whether these techniques select preferentially for the 99 loser cells, and how can we identify that one really robust cell. And so that's one issue.

The other issue is -- and this comes up within any type of immunotherapy or immunocapture that, even though, antibodies are very specific, they are not specific just for circulating tumor cells. So some of these markers that we are looking at or expressed on others cells, and some circulating tumor cells actually don't express those certain markers at a very high-level.

So one example is that, some of these cells undergo what's called a epithelial-mesenchymal transition, which leads to the down regulation of one of the markers used to identify circulating tumor cells. And so those cells that go into that transition actually are quite aggressive in terms of spreading tumor and so you wouldn't pick those up from an immuno type of technique.

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Bob Barrett: Doctor, where do you see this field going in the future?

Dr. Keith Neeves: I think the most exciting research in this field are techniques that couple these immunologic techniques, these markers that we've been talking about, with other physical properties that's circulating tumor cells. So there are some technologies out there that looked at, for instance, the mechanical properties of how elastic a cell is and its electrical properties, the capacitance of a cell, and we can use those to differentiate, not only circulating tumor cells from other blood cells, but also sub populations in the circulating tumor cell population.

And so these things can all be integrated using lab-on-a-chip type of technologies into a single device that uses fairly low volumes of blood. So that's really, where the field is moving.

Bob Barrett: Well, finally doctor, the bottomline, how will these technologies improve outcomes for individuals with cancer?

Dr. Keith Neeves: So the mantra in cancer treatment is that early detection leads to the best outcomes. And so it's certainly possible that CTC isolation and enumeration can catch metastasis

much earlier than imaging techniques, but clearly this has to be balanced with the sensitivity and specificity of the assay.

So as we have recently seen in the Popular Press with screening of prostate and breast cancers that earlier and more frequent screening does not necessarily lead to better outcomes, because of the -- sometimes the high rate of false positive.

So there is little bit of a balance here and there is still some work to be done in terms of just the clinical studies in these types of assay, but they are certainly promising.

Bob Barrett:

Dr. Keith Neeves is an Assistant Professor and Boettcher Investigator in the Department of Chemical and Biological Engineering at the Colorado School of Mines. He has been our guest in this podcast from '*Clinical Chemistry*'. I'm Bob Barrett, thanks for listening.

Total Duration: 7 Minutes