Clinical Chemistry

Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from plasma of colorectal cancer patients

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Guest:
Valerie Taly is a researcher working on applying microfluidic-based strategies for cancer research at the University of Paris.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Multiplex digital PCR can be used for the sensitive detection of circulating tumor DNA with performance unachievable by other current molecular detection approaches.

A paper in the December 2013 issue of *Clinical Chemistry* demonstrated the clinical utility of multiplex digital PCR to screen for multiple mutation simultaneously with sufficient sensitivity to detect mutations in circulating tumor DNA obtained by noninvasive blood collection.

Valerie Taly, a researcher working on applying microfluidic based strategies for cancer research at the University of Paris was lead author of that study, and she joins us today in this podcast.

Valerie, could you summarize the findings of your work? Who is involved in this project and what were their backgrounds?

Valerie Taly: So this work is in direct line with previous works that has demonstrated that digital PCR in general and droplet digital PCR in particular could represent a great tool for clinical research.

We have been using a procedure that uses multiplex droplet Digital PCR to perform the sensitive and quantitative detection of different biomarkers in multiplex. These biomarkers are known to be involved in resistance to targeted EGFR, namely cetuximab and panitumumab.

When the procedure is actually used in clinical practice consists on analyzing the primitive tumor with low sensitivity procedures, we demonstrate the possibility to perform this analysis directly on DNA extracted from plasma of patient with advanced colorectal cancer.

We believe that this work contributes to demonstrate the possibility to perform noninvasive biopsies for patient follow up and treatment management. So in that work many scientists have been involved from CNRS, INSERM, Université Paris Descartes, European Georges Pompidou Hospital, University of Strasbourg, ESPCI, and RainDance Technologies.

In its early developments it has also involved researcher from the Max Planck Institute in Goettingen. More importantly, it has required the involvement of scientists from various backgrounds, namely biologists, microphysicists, clinicians, physicists, and chemists.

Our main area of expertise being the development of biomarker developments using droplet-based microfluidics.

Bob Barrett: Are there any differences between the procedures published in *Clinical Chemistry* and the procedures used in clinical practice?

Valerie Taly: The classic procedure that I use in clinical practice will analyze in sampling its globality and could just miss lower presented DNA, like the one that comes from the tumor.

Indeed, the clinical samples are generally composed of mixture of DNA coming from both normal and tumoral cells, and the DNA coming from the tumor could be very diluted. And it has been described that it could represent as low as 0.01% of the total circulating DNA in early stage cancer.

Moreover, the classically used procedures are not quantitative. Our procedure by using millions of strictly identical droplets allow us to analyze each DNA independently, each DNA that is present in the sample, and test and allow to detect and quantify tumor DNA. The sensitivities here are just limited by the number of compartments that you could analyze and the high number of droplets that we use in this study, which is 3-5 million, in that range, allows us to work over a wide dynamic range.

Moreover, the use of single molecular reactions allow to perform core multiplexing and to screen and quantify the seven mutation of the KRAS oncogene in just two experiments.

To summarize, I would say that the main difference here are the high sensitivity and quantitativity of the procedures added to true multiplexing.

Bob Barrett: Could such procedures be easily implemented in clinical settings?

Valerie Taly: So as every new concept, the use of droplet digital PCR in clinical practices will require validation values for prospective and prospective studies. It will firstly serve as a highly useful tool for cancer research. However, if positive, the validation will lead to rapid acceptation of this procedure for clinical testing and the used procedure is being compatible with clinical settings, both in terms of workflow simplicity and the limitation of cross-contamination.

Bob Barrett: What can the possibility to perform circulating tumor DNA analysis offer to the patient and to patient treatment?

Valerie Taly: So the procedures that I use in today's practices relies on the analysis of archive primary tumor tissues to make a therapeutic decision. The time delay between the collection of this tissue sample and the therapeutic decision could just be several months to a year.

Consequently, if you are able to monitor directly in blood the mutational status of the patient, this is really appealing. Indeed, this could represent a more realistic picture of the patient's status at the time where the therapeutic decision has to be made.

Moreover, it has also been suggested by many works that the use of liquid biopsies would give information about the whole status of the patient with comparison to classic solid biopsies that are much more localized and represent just a little part of the tumor.

And in general the use of liquid biopsy would represent ideal noninvasive procedure where the patient's participation is facilitated. It could allow monitoring of patients or follow up during treatment and also the follow up during remission.

In the longer term it could also be used for patient diagnostic if a sufficient set of biomarker could be screened.

Bob Barrett: Well, finally Dr. Taly, what applications are you looking at now or plan to in the near future?

Valerie Taly: As we just mentioned above, droplet digital PCR as a highly sensitive and quantitative procedure would represent a great tool for cancer research. So we are now applying this strategy to try to research more heterogeneity to understand secondary resistance event.

From a clinical perspective, we also aim at continuing this work with a more important quantity of samples to validate its use for advanced colorectal cancer follow up.

In the longer term we would like to apply it for early stage cancer in the aim of detecting cancer recurrence events. That aspect will also be extended to the type of cancer in collaboration of course with clinicians.

Bob Barrett: Valerie Taly is a researcher working on applying microfluidic-based strategies for cancer research at the University of Paris. She has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening!