DIGITAL PCR FOR THE MOLECULAR ANALYSIS OF UVEAL MELANOMA

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Pieter holds a position at the LUMC in the department of Ophthalmology as PI in the field of uveal melanoma genetics. By applying the most recent technical advances, the heterogeneity of UM is studied. Genetic and cellular heterogeneity provides information on molecular pathogenesis and may ultimately offer chances for treatment. Pieter is part of UM Cure, an European consortium of preclinical researchers that aims to provide treatment for metastatic uveal melanoma

Why is there so much excitement surrounding digital PCR (dPCR)? Especially for the molecular analysis of uveal melanoma?

We are specifically very enthusiastic about dPCR and analysis of tumours because that's bulk tissue with all kinds of different cells and different clones in there. With dPCR we can dissect all the different parts mathematically and accurately, so that's a great improvement. We can then go back to our NGS data and have a meaningful analysis of our expression data. If you have bulk expression data on your tissue, there are different cells in the different tumour clumps. They all work together, so the complete profile is a mixture of all those different cells. With dPCR we can enumerate all the different parts.

What are your key priorities in this space currently?

The key priority is to get our assays, thoughts and ideas to the bigger public. That's an effort that we're trying to make. I was very enthusiastic to present at this conference with all the possibilities to express what we have been researching. We want to spread our message of molecular and mathematical dissection of complex tissues, that is our top priority. We have different levels for assays and analysis which are very specific and technical. But the bigger picture is that we want the public and scientific circles to know what we are doing.

Going a little bit more into this technical side you just explained. What would be the biggest priorities on the technical side?

Previously we managed to analyse tumours, and we analysed those tumours for the T cells. During that process, we could count exactly how many T cells were there. As we knew how many there were, we could extract from the expression profile of the T cells. This gave us a lot of information, for example how the microenvironment is composed in there. The next big challenge is that we want to know about the different tumour clones. We also want to extract specific expression profiles with specific clumps to determine which clump is metastatic, and may kill the patient.

What are the recurring challenges you come up against in your work?

Sample quality is always a very technical challenge. If you have FFPE fixed tissue and badly managed tissue, the DNA is very small. Therefore, it is hard to do good measurements. So that is a technical challenge, you need really good quality DNA from paraffin-embedded tissue. The kind of tissue that you find at pathology departments. So, of course, we would like to pick tissues from the pathology departments.

What are the next steps for this research?

The next steps are that we want to integrate our methodology with a single cell analysis; that would be perfect. We would like to have one cell in each droplet for multiplex analysis, that would allow to accurately count on each cell which chromosome is changed. From this, you have proof of what individual cells look like. These genomic profiles are very detailed, so whether you will have two or three copies of a certain chromosome can be very important data in our analysis.

In uveal melanoma, around 50% of patients will develop metastases with fatal results. Conversely, around 50% of patients will have good prognosis, which can be predicted accurately with genetic tests. That's why we must know exactly if there is a cell somewhere in the tumour with this very malignant characteristic. It is important to ensure detection is as sensitive and accurate as possible so that you don't worry people unnecessarily. That is a very important aspect of our research.

What are the top three takeaways from your presentation?

Besides blood analysis we also perform tissue analysis, dissection of the tumours and the deconvolution of the microenvironments. I want to emphasise that the message I am conveying is that we are essentially digitalizing histology. So, we count cells and have markers for specific cell types. From this, we can calculate exactly how many T cells for example are present in any given tissue. If you manage to evoke an immune reaction to a tumour It can be very important to screen the tumour, it's very important to monitor how many immune cells are there and if they are effective. Counting is done by dPCR to see how many cells came into your tumour. Furthermore, the gene expression will tell you what kind of T cells were there, whether they are killer cells or just cells that were not powerful enough to kill your tumour. That's the combination of the two things that we want to do.

What do you think are the most important areas impacting the dPCR landscape currently?

Most talks that are happening currently are about ctDNA analysis. I personally think that's only 5% of the possibilities. So, there is a lot to learn and many possibilities to explore.

What current innovations are happening in the industry now? And are these innovations coming from the technological side, or more from the research being conducted?

A few developments are happening now. The technique that I previously mentioned, where you want to analyse cell by cell. The next thing is that for real clinical use, you need good data management and analysis software. This is a very big improvement that should be made, and many manufacturers are not aware of this. They should also provide data management and quality management besides machines that will allow us to implement techniques in a clinic. We are also investing quite heavily to make a good database system that's searchable and can be validated so that you always have the raw data available. You must always do the quality assessments to be sure as a clinician that the information you give patients is correct. This is to avoid making false diagnoses or prognoses.

What do you see as the biggest story of this year so far in your area of research?

The big story for my area of research is that there is more technical development in droplet digital PCR. The multiplex analysis is getting larger and more important, that is a development I think is growing in importance. Another big story is that pathologists managed to dissect mixed melanoma tissue and get different progression stages for separate cell analysis. This has proved that within a tumour you may have different tumour cells with different genetics. Despite histology being an old-fashioned approach, it was scientifically very important that they managed to do this. This was good for showing how the development of the tumour proceeded. Our approach aims at the same goal but with a completely different methodology.

We are only at the beginning of the 'genomics revolution'. In your opinion what key trends and enabling technologies will have an impact from next year?

As I said previously, NGS is very important, but I hope it will also be integrated with dPCR. That is something I'm looking forward to. I guess this provides the biggest challenge for multiplexing, as you have limited DNA available. For example, if you do to this to ctDNA, you only have one chance so you should do a lot of tests at the same time. That is the other important thing.