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Plasma-Derived Tumor DNA Analysis at Whole-Genome Resolution

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Guest:
Professor Charles Swanton is the Director of the Translational Cancer Therapeutics Laboratory of the London Research Institute.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. The analysis of circulating free tumor DNA has been thought to offer substantial advantages over invasive biopsies. However, until now circulating free tumor DNA analysis has not been able to determine distinct tumor copy number events or resolve single nucleotide variants at the genome-wide scale.

In the January 2013 issue of *Clinical Chemistry*, Dennis Lo, and his colleagues in Hong Kong provide long awaited evidence that massively parallel sequencing analysis of circulating free tumor DNA can resolve DNA structure elaborations across the entire genome.

An accompanying editorial by Professor Charles Swanton of the London Research Institute Translational Cancer Therapeutics Laboratory appeared in the same issue of the journal; one devoted to cancer diagnostics. Professor Swanton is our guest in this podcast.

Professor, what are some of the major challenges in understanding tumor metastasis and drug resistance?

Charles Swanton: So one of the emerging and most important problems facing cancer oncologists in the modern era is the changing nature of solid tumors over time. And by that I mean tumor heterogeneity that drives Darwinian evolution through selection of subclones that likely drive the evolution of resistance to therapy over time.

So we are left with a problem where tumors are essentially dynamic states changing both in space and in time, such that potentially spatially separated biopsies may harbor subtly or perhaps more dramatic differences in their

genomic structure or somatic mutational profile, and those mutations can change and potentially adapt over time.

And the question is how can we accurately measure the somatic landscape of a tumor without necessarily having to biopsy every site of metastatic disease, and indeed how similar are the sites of metastatic disease and we still don't have robust answers to those questions across all solid tumors currently.

Bob Barrett: What about intratumor heterogeneity, what challenges do we find there?

Charles Swanton: So intratumor heterogeneity really reflects what I have just said in many ways, that this is the sort of observation that there can be many subclones of a tumor within one individual biopsy, or indeed there can be differences between biopsies from the same tumor, both in terms of comparisons between the primary metastatic sites. And emerging evidence suggests in renal cancer, at least within one primary tumor, there can be differences in the somatic mutational spectrum between biopsies of the same tumor.

Now, that's not to say that each biopsy is completely different within a tumor from monoclonal disease like cancer. There are shared common events, so-called ubiquitous events which we liken to sort of earlier events in tumor development, the so-called trunk of the tumor if you like.

It's quite helpful to think about this in terms of Darwin's tree diagram speciation drawn in 1837. So you have the sort of trunk of the tumor which are the common mutations present in every clone and every subclone. But then you have the phenomenon of branched evolution in some tumors, which we are increasingly seeing, where you get mutations present in some subclones but not others, and those relate to the sort of the heterogeneous events within tumors that can be within one subclone but not another in a single biopsy, or spatially separated between subclones in one tumor.

And that is really the sort of essence of intratumor heterogeneity. And I guess it doesn't just relate to somatic mutations either, it can relate to transcriptomic aberrations that can differ from one region to the next. And it can relate to whole chromosome aberrations that differ from one cell to the next or different focal copy number amplification or deletion events that can differ from cell to cell or region to region.

In essence, intratumor heterogeneity can affect many genetic and epigenetic levels, and indeed transcriptomic

levels and probably also proteomic levels, where different cells from the same tumor can harbor different behavioral patterns potentially and different genetic somatic mutations or chromosomal copy number events.

Bob Barrett: How does the paper by Dennis Lo begin to address some of these challenges?

Charles Swanton: So one of the key problems with dealing and treating solid tumors is that we can't ask ethically a patient to allow us to biopsy every site of disease. So it can be very difficult with what we are seeing in the literature emerging, where there are differences between primary and metastatic sites, and possibly between metastatic sites, to reliably profile potentially the entire somatic mutational landscape of the tumor.

So we desperately need better sampling techniques that more reliably give us a general measure of somatic mutational profiles and copy number events that might be aberrant within a typical tumor without having to biopsy every site of disease.

Now, circulating tumor DNA has shown promise for certainly a year or two now, where there has been intense study and investigation of the potential for somatic mutations identifiable in plasma DNA from tumors to give us some guide as to outcome potentially and potentially failure of therapy.

And the Cambridge Research Institute has done some very elegant work in this regard in ovarian cancer. I think the next step here from Professor Lo's paper is to demonstrate that massively parallel sequencing analysis of circulating tumor DNA can begin to resolve DNA structural aberrations across the entire genome.

And they have shown for patients with hepatocellular carcinoma, the representation of copy number events in the tumor with events detected in circulating tumor DNA, and when those tumors are resected, the almost complete loss of those events from plasma following resection of the hepatocellular carcinomas.

In other words, what we are seeing is that the copy number events detectable in the tumor are very nicely reflected in circulating tumor DNA using this technology of massively parallel sequencing.

Now, clearly, it's a very expensive technology, but one would imagine the technology cost would drop dramatically over the course of the next two to five years, such that this

could become potentially a routine test for monitoring outcome, for monitoring minimal residual disease potentially, and hopefully, and this is speculative at the moment, potentially for a diagnostic tool.

They then go on to show that when they study a patient, a unique patient with synchronous breast and ovarian primaries, the structural DNA copy number aberrations that are detectable in circulating plasma DNA was actually a hybrid of both the ovarian and breast cancer primaries.

And then they showed attenuation of those DNA copy number signals in circulating tumor DNA following the sequential resection of those tumors over the course of an operation in one day really mirrored the copy number profiles derived from those surgically resected tumors, which I think is elegant, interesting, and potentially very informative for the future of monitoring disease over time.

Going back to the point I made earlier that we are seeing tumors adapt and evolve over time, if we can potentially detect those adaptations over time using this type of technology, I think the future is potentially quite bright in understanding how tumors adapt to therapy perhaps more readily than we can at the moment using just conventional imaging technologies, which tell us nothing about how the tumor's DNA or somatic aberrations are changing over time.

And then when they look more closely at some of the heterogeneous events in ovarian cancer that differ between the bilateral ovarian tumors, they show that you can actually detect those at low frequency in circulating tumor DNA as well, which begin to shed light on the potential for this technology to really understand tumor heterogeneity between perhaps spatially separated tumors or its changing nature over time.

So at a number of levels this paper begins to address some of the major problems facing oncologists, academic oncologists interested in understanding how disease changes, both spatially and temporally during the disease course, using potentially minimally invasive approaches that overcome the need to -- we would hope in due course -- to sample multiple metastatic sites using CT-guided biopsies or multiple biopsies in the same tumor, that you get, if you like, a sort of a liquid biopsy from just a blood draw that can perhaps be, we hope in the future, particularly informative when understanding tumor evolution and drug resistance.

Bob Barrett: What way do you think that this paper advances the field?

Charles Swanton: Well, it's really, as far as I am aware, one of the first, if not the first, paper to show that you can use a liquid biopsy,

circulating tumor DNA analysis to begin to resolve the underlying structures of tumors, both in terms of the tumor heterogeneity and in terms of the common copy number events.

So I think that is potentially a major step forward, that I think certainly from my perspective is fascinating, because it provides the potential now to, I would hope, avoid the need for multiple biopsies of metastatic sites, and one could potentially see a future where we are relying on the analysis, intense and in-depth analysis of circulating tumor DNA to profile a patient's tumor.

Now, it must be said that this paper, unsurprisingly, has had to rely on the profiling of the solid tumor to derive meaningful information from circulating tumor DNA analysis. The next step is going to be really moving beyond that and saying, well, can we just analyze the circulating tumor DNA without having to sequence the primary tumors. And I think that the field is now primed for that big step, which I suspect will be possible in due course.

Bob Barrett: Well, finally Professor Swanton, what's next? When are we likely to see this technology in routine clinical practice?

Charles Swanton: I think we are some way from that in all honesty, partly because it's so expensive at the moment. I think the cost would have to come down, both in terms of sequencing cost, but also labor cost. I mean, you cannot underestimate the amount of work that would be required for a paper such as this and others in the field.

There is a lot of technology development in terms of developing assay suitable for clinical implementation, any biomarker type approach. There are very sort of intense qualification steps that are required to make sure these assays are reproducible within individual patients and sampling processing is absolutely stringently validated before this would be fit for clinical implementation.

But I think certainly in the research setting we will see in the course of the next 18-24 months a series of papers like this, but now really begin to explore the dynamics of tumor evolution over time, really the next major frontier, in my view, in cancer medicine, and this paper very much sets the field for that type of analysis.

In terms of clinical implementation, I think in the next 10-15 years, if not before, we will be seeing this type of technology, I would hope, being routinely implemented in the clinical setting when considering switching patients on therapy and perhaps relying more on the changing nature of the tumor over time at a genetic level rather than perhaps

relying, as we do at the moment in the oncology setting, on a crude measurement of a changing size of the tumor based on CT imaging.

That perhaps I could see a future where we are using both CT-guided imaging, as well as the clonal dynamics of the tumor derived from genetic technology, such as this, guiding therapy and guiding clinical trial stratification. So very exciting times all in all.

Bob Barrett: Professor Charles Swanton is the Director of the Translational Cancer Therapeutics Laboratory of the London Research Institute. He has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening!