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BIG Research in Focus
Issue 1
September 2014

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The clinical potential of liquid biopsy

What is the potential of circulating tumour cell (CTC) and circulating tumour DNA (ctDNA) analysis techniques for monitoring disease progression and tailoring treatment? How soon could they become part of daily oncology practice?

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International plasma harmonisation project

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Editorial

On behalf of the entire editorial team and the Breast International Group (BIG) Headquarters, I am pleased to share with you the very first issue of *BIG Research in Focus*.

With this new publication – which will be issued twice a year – BIG wants to address key and timely breast cancer research themes in depth. Bringing to the table different expert opinions, we hope to offer readers a broad perspective and an improved understanding of topics that are relevant to the breast cancer research community and are changing the way we work.

In this issue, we focus on liquid biopsies. Today's technologies are enabling rapid progress in this domain, and researchers within the BIG network and at other major breast cancer research institutions have started to assess the clinical utility of circulating tumour cell (CTC) and circulating tumour DNA (ctDNA) analysis for monitoring disease progression and tailoring treatment.

We asked Drs Ben Ho Park (Sidney Kimmel Comprehensive Cancer Center, USA), Sarah-Jane Dawson (Peter MacCallum Cancer Centre, Australia) and Michail Ignatiadis (Institut Jules Bordet, Belgium) about the potential of these new techniques and how soon they could become a part of daily oncology practice.

In addition, an interview with Dr Françoise Rothé brings us into her daily work and research on liquid biopsies in breast cancer at the Breast Cancer Translational Laboratory of the Institut Jules Bordet, Belgium.

I hope you enjoy the reading.

Martine Piccart-Gebhart
Breast International Group (BIG) Chair

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The clinical potential of liquid biopsy

By Jenny Bryan

Rapid progress in liquid biopsy technology is enabling researchers within the Breast International Group (BIG) network and at other major breast cancer research institutions to use the new techniques to assess their clinical utility for monitoring of disease progression and tailoring treatment. Jenny Bryan asked researchers in Europe, the USA and Australia about the potential of these new techniques – namely, circulating tumour cell (CTC) and circulating tumour DNA (ctDNA) analysis – and how soon they could become a part of daily oncology practice.

Replacing tissue biopsy with blood tests would be a huge advance for patients with breast cancer. As well as avoiding the need for an invasive procedure, it could provide a more accurate, real-time indication of dormant or progressive disease than is possible with current biopsy techniques, and enable treatment to be personalised to the molecular profile of each patient's tumour.

"A tissue biopsy is an invasive test and isn't always possible. It's also limited by the heterogeneity of tumours. By sampling one small part of the tumour, we may be missing a significant amount of information," explains Dr Sarah-Jane Dawson, from the Peter MacCallum Cancer Centre, Melbourne, Australia. "The new techniques are very exciting, with significant potential to enter practice and help with clinical decision making."

At present, CTC technology is more established, with data available from around 2800 patients treated in clinical trials. But ctDNA is catching up fast.

"ctDNA has potential for greater clinical utility than CTCs because of its sensitivity and ability to look directly at mutations instead of having to capture rare cancer cells and separate them from large numbers of normal ones. ctDNA results can reflect different clones of cells, for example in the liver or brain, which may have different mutational profiles," says Professor Ben Park, from the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA.

CTC numbers can predict survival

CTCs were first recognised nearly 150 years ago, when cells similar to those of a patient's tumour were found in their blood after death[1]. But it is only the technical advances of the last decade that have made it possible routinely to detect, count and assess their biological properties[2].

The presence of low CTC numbers in a background of 10^6-10^7 nucleated blood cells has led investigators to enrich samples before CTC detection. Enrichment methods are based on either the physical properties of the CTCs, such as size (filter technologies), density (ficoll centrifugation) and electrical charges (dielectrophoresis), or biological properties (enrichment by targeting cell surface markers)[2].

CellSearch® – the only semi automated system approved by the US Food and Drug Administration for CTC analysis – uses magnetic particles coated with antibodies against epithelial cell adhesion molecule (EpCAM) to select out EpCAM-bearing cells[3]. Immunofluorescence staining is performed using a cytokeratin (CK)-8,18,19 epithelial marker, the nuclear dye, 4',6-diamidino-2-phenylindole (DAPI) and the leucocyte marker CD45. According to the CellSearch® definition, a CTC is any intact cell with at least 4µm in size that is CK+/DAPI+/CD45- with a nucleus that is 50% inside the cytoplasm.

In 2004, US researchers reported that the number of CTCs identified by CellSearch® in whole blood before treatment was an independent predictor of progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast cancer[4]. In the study, 49% of 177 women had 5 of more CTCs per 7.5 ml of whole blood at baseline, and they had a shorter median PFS (2.7 months vs 7 months, p<0.001) and OS (10.1 months vs >18 months, p<0.001).

A pooled analysis of data on CTCs detected by CellSearch® from almost 2000 patients with metastatic breast cancer recently confirmed that CTC levels are an independent predictor for PFS and OS[5]. Nearly 50% of patients had a CTC count of > 5 per 7.5 ml at baseline, which was associated with decreased PFS (HR: 1.92, p<0.0001) and OS (HR 2.78, p<0.0001) compared with patients with a CTC count of < 5 per 7.5 ml. Survival prediction was significantly improved by adding baseline CTC count to clinicopathological models, while carcinoembryonic antigen (CEA) and cancer antigen (CA) 15-3 concentrations at baseline added little.

In women with large, locally advanced breast cancer, research has shown that pre-chemotherapy CTC levels predict early metastatic relapse and OS after neoadjuvant chemotherapy[6]. At baseline, 23% of patients were CTC positive, but only 10% of this population with earlier stage disease had >1 CTC per 7.5 ml of blood. Similar baseline CTC levels were reported in other studies in early breast cancer and were also linked to worse post-treatment outcomes[7-9].

> "A tissue biopsy is an invasive test and isn't always possible. It's also limited by the heterogeneity of tumours. By sampling one small part of the tumour, we may be missing a significant amount of information."

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In the large SUCCESS study of 2026 women with early breast cancer, 21.5% of patients had ≥1 CTC per 30 ml before adjuvant chemotherapy and this was associated with reduced disease free survival (DFS) and OS[8]. The study showed that quantity of CTCs was important, not just their presence. Women with higher numbers of CTCs had a greater risk of recurrence or tumour-related death (≥5 CTCs: DFS: HR 4.51 and OS: HR 3.6 vs > 1CTC: DFS: HR = 2.11; OS: HR = 2.18). Persisting CTCs after chemotherapy correlated with reduced DFS (HR = 1.12; p= 0.02) and OS (HR = 1.16, p = 0.06).

ctDNA shows superior sensitivity

Detection of tumour DNA in plasma doesn't go back as far as observations of CTCs. Cell free DNA (cfDNA) in the blood of cancer patients was first reported in 1977, with higher levels in patients with metastatic than non-metastatic disease, and decreases in those with good response to anti-cancer treatment[10]. However, it is only in the last few years that rapid progress has been made in developing digital polymerase chain reaction (PCR) and next generation sequencing techniques for measuring tumour specific circulating cell free DNA (ctDNA) in cancer patients.

> **"We've known about free floating plasma DNA for decades but foetal medicine was about 10 years ahead of us when they developed plasma DNA tests to replace amniocentesis and chorionic villus sampling for prenatal screening."**

Professor Park points out that oncologists can thank colleagues in foetal medicine for early developments in plasma DNA technology:

"We've known about free floating plasma DNA for decades but foetal medicine was about 10 years ahead of us when they developed plasma DNA tests to replace amniocentesis and chorionic villus sampling for prenatal screening."

Initial breast cancer studies showed higher levels of circulating cell-free DNA (cfDNA) in women with breast tumours than healthy individuals, and subsequent research has focused on identifying specific mutations that can differentiate ctDNA from cfDNA[11].

In 2012, Professor Park and colleagues at Johns Hopkins and Massachusetts General Hospital Cancer Centre reported mutations in *PIK3CA* – the most common genomic mutation in women with breast cancer – in plasma of nearly 30% of patients with metastatic disease[12]. They also showed how mutations can change with time. While there was 100% concordance in *PIK3CA* status for formalin-fixed, paraffin embedded tissue samples and plasma samples taken contemporaneously, this fell to only 72.5% when *PIK3CA* status in patients' current ctDNA samples was compared with that from archival tissue.

"We know that using old plasma samples is unreliable. We often cannot base treatment of metastatic breast cancer on *PIK3CA* or other specific mutations present at initial diagnosis as these can change by the time a woman comes back with recurrent or advanced disease," says Professor Park.

In 2013, Dr Dawson and colleagues reported results of a proof-of-concept analysis which showed that ctDNA sequencing for *PIK3CA* and a second common mutation found in breast cancer, TP53, was more sensitive than CTCs or the tumour marker, CA 15-3, and correlated better with tumour burden and prognosis[13]. ctDNA was detected in 97% of women with known genomic alterations in breast tissue samples, while CA 15-3 and CTCs were found in 78% and 87%, respectively.

The team at the University of Cambridge investigated the effectiveness of the biomarkers for tumour monitoring compared with CT imaging. Serial changes in ctDNA were seen in 95% of patients tested and these generally correlated with CT changes that occurred with treatment. Serial changes in CTCs correlated with CT changes, but only when levels were > 5 per 7.5 ml blood.

Women with high levels of CA 15-3 also showed fluctuations in line with changes on CT, but with a smaller dynamic range. When CA 15-3 levels were < 50 U per ml, no consistent serial changes were seen. In half of women, increases in ctDNA preceded signs of progression on CT imaging, by a median of five months.

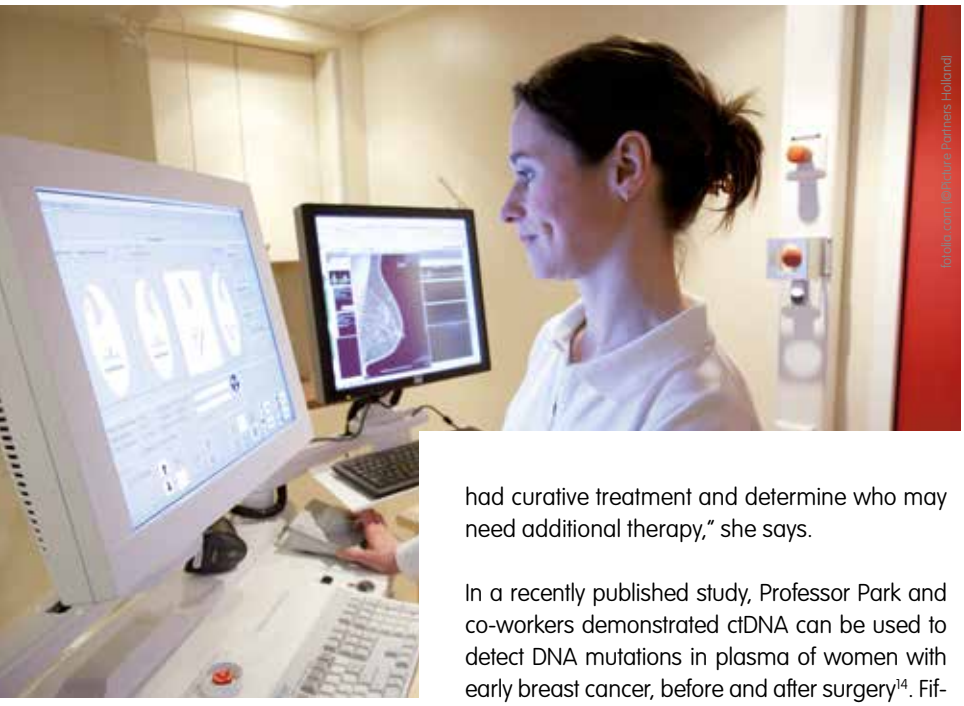
Increasing levels of ctDNA were associated with worse survival ($p<0.001$) as were increasing CTC levels ($p=0.03$), but CA 15-3 did not have prognostic value.

Dr Dawson explains that about 50% of breast cancers have a mutation in one or both of PIK-3CA and TP53, and that plasma levels of these mutations correlate closely with tumour burden.

"We showed that ctDNA is more sensitive than CTCs, and it is also much easier to collect and analyse plasma samples," she says.

But she stresses that ctDNA is currently a research tool and considerable technical work is needed before it is ready for routine clinical use.

"Potential clinical applications include following a specific therapeutic target in plasma, such as *PIK3CA*, with a view to guiding treatment decisions and monitoring treatment response. But we still need to understand how ctDNA may help us in early disease to assess patients who have



had curative treatment and determine who may need additional therapy," she says.

In a recently published study, Professor Park and co-workers demonstrated ctDNA can be used to detect DNA mutations in plasma of women with early breast cancer, before and after surgery[14]. Fifteen mutations of *PIK3CA* were identified in breast tumour samples from 29 women with early stage breast cancer. Of these 15 mutations, 14 were detected in presurgical plasma tumour DNA, and no mutations were found in plasma from patients with *PIK3CA* wild-type tumours (sensitivity 93.3%, specificity 100%). Ten patients shown to have *PIK-3CA* mutations in pre-surgery plasma DNA tests also had post-surgery tests, and five of these still had detectable plasma tumour DNA.

"It's essential to do these tests before surgery as well as afterwards because we are putting so much weight on a negative result after surgery in determining further treatment. We need to be sure that it is not just a technical anomaly," says Professor Park.

His next study will focus on neoadjuvant therapy to shrink tumours prior to surgery, to investigate whether ctDNA can be used to determine which women may be able to avoid surgery altogether:

"When we shrink tumours before surgery, about 30% of these women have no evidence of cancer in their breast or their nodes but we still have to operate and take out healthy tissue in case there are micrometastases. We want to be able to show that a negative liquid biopsy result is equivalent to a pathologically complete response in breast cancer because that would enable some women who have neoadjuvant chemotherapy to avoid surgery."

ctDNA techniques: digital PCR and next generation techniques

Digital polymerase chain reaction (PCR) forms the basis of the novel techniques being developed for ctDNA analysis.

Conventional PCR amplifies a DNA sample so that it can be probed for genetic mutations. But the exponential amplification that occurs in PCR can make it hard to probe for rare mutations within the resulting DNA product. In digital PCR, the DNA sample is diluted and partitioned into multiple compartments. Each contains a single DNA template with either a normal or mutated allele. Once these are amplified and probed for mutations, such as those of *PIK3CA*, it is possible to produce a more accurate, quantitative analysis than with standard PCR.

Even so, explains Professor Park, unmodified digital PCR is impractical because so many reactions need to be analysed. BEAMing (beams, emulsions, amplification and magnetic) was developed to simplify the process of digital PCR while maintaining its accuracy. Individual DNA molecules are attached to magnetic beads in water-in-oil emulsions and subjected to compartmentalised PCR[11]. The bead-bound DNA can then be removed from solution with magnets or by centrifugation and checked for mutations[15]. Flow cytometry is used to quantify the level of mutant DNA present in the plasma[11].

> "BEAMing enabled us to go from looking for a needle in a haystack to isolating each straw and checking it for the needle. The drawback is that it is very specialised and expensive."

"BEAMing enabled us to go from looking for a needle in a haystack to isolating each straw and checking it for the needle. The drawback is that it is very specialised and expensive," explains Professor Park.

In an effort to make the BEAMing approach more affordable, a second generation, automated version – droplet digital PCR (ddPCR) – was developed. Professor Park likens it to "BEAMing on steroids". According to one manufacturer's website ddPCR technology analyses 20000 water-in-oil droplets per 20µl sample – or nearly 2 million partitioned PCR reactions in a 96-well plate, compared to the hundred or thousand partitions in a standard digital PCR system. And all this in just 3½ hours.

Professor Park explains that the main challenge for researchers using next generation PCR techniques,

such as ddPCR, is that patient levels of plasma DNA are very variable, especially in early stage disease.

"With metastatic disease, the tumour burden is higher but, in early disease, we often need to draw three tubes of blood instead of one. Research is also looking at the impact of diurnal variations and exercise on ctDNA levels as more cancer DNA may get pushed out into the plasma at these times and make it easier to collect," he explains.

As well as focusing on common breast cancer mutations, such as *PIK3CA* and *TP53*, next generation ctDNA research is investigating the potential for wider genomic screening.

Dr Dawson points out that about 50% of women do not have *PIK3CA* or *TP53* mutations, and other mutations associated with breast cancer occur at low frequencies. So there is a role for targeted deep sequencing to look for a broader panel of mutations in plasma DNA.

"If you want to follow ctDNA levels and see how they correlate with tumour burden and treatment response, then you need to understand something about the mutations in an individual's tumour. In many cases, you probably need to track a broader number of mutations than just *PIK3CA* or *TP53*," she says.

In a recently published study, Dr Michail Ignatiadis, from the Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium, and colleagues reported results of a pilot study of next generation sequencing for a commercially available 50-gene panel in which 69 primary and metastatic tumour samples and 31 plasma samples were tested from 17 women with metastatic breast cancer[16].

Results of tumour sample analysis showed that 12 of 17 (71%) of patients had ≥1 mutation in *p53*, *PIK3CA*, *PTEN*, *AKT1* or *IDH2* genes. When plasma samples were analysed, 12 of 17 (71%) of patients had ≥1 mutation in *p53*, *PIK3CA*, *PTEN*, *AKT1*, *IDH2* and *SMAD4*. When tumour and plasma samples collected at the same time point were analysed, there was 76% concordance between tumour and plasma in 13 of 17 (76%, 95%CI: 50-93%) patients. There was discordance in 4 of 17 (24%, 95%CI:7-50%) patients. In two cases a mutation was found in tumour but not in plasma, and in two cases a mutation was found in plasma but not in tumour.

"Although smaller gene panels have been tested, to our knowledge this is the first study to demonstrate that plasma can be an alternative tissue source for targeted genetic screening using a commercially available panel of 50 cancer genes," says Dr Ignatiadis.

He explains that the whole process was performed at OncoDNA, Gosselies, Belgium, under the ISO15189 accreditation to simulate real-time, and appropriate analytical validation was demonstrated. He suggests that this approach may allow increased patient access to molecular screening programmes and should be prospectively tested.

"Next generation sequencing allows us to search much larger regions of DNA but for all these techniques it's essential that we develop assays that are analytically validated and show clinical validity and ultimately clinical utility," he adds. "Each technique needs to do what it says, to demonstrate clinical value and to help us improve the outlook for patients."

International plasma harmonisation project

There is increasing evidence to support enormous heterogeneity in breast cancer, both spatial (i.e., different anatomic deposits of the disease exhibiting different molecular aberrations) as well as temporal (i.e., molecular evolution of the disease during its life cycle). Of note, this heterogeneity can mediate resistance to anticancer treatments, since cancer cells with different aberrations can show different sensitivity profiles. Serial and comprehensive biopsies of metastatic disease is not clinically feasible; thus the collection of liquid biopsies and the characterisation of circulating tumour DNA (ctDNA) could be a non-invasive, safe and efficient way to tackle this problem. However, it is felt that the analytical validity of these procedures should be further proven before being applied in the clinical setting to avoid the waste of precious biospecimens.

Recognising this critical issue, **Breast International Group (BIG) and the North American Breast Cancer Group (NABCG) have recently formed a working group of internationally renowned researchers holding great expertise in the field of plasma-based biomarkers.** With the generous financial support of the Breast Cancer Research Foundation, this collaborative effort will serve a two-fold aim: first, the procedures for ctDNA collection and characterisation in a few key research laboratories in the context of the BIG-NABCG collaboration will be harmonised; second, once a common protocol and standard operating procedures are in place, the ctDNA collected from metastatic breast cancer patients enrolled in AURORA (NCT02102165) will be characterised. Comparison of the ctDNA profile with that of the primary and metastatic tissue collected from the same patients at the same time will enable us to understand whether ctDNA can be used as surrogate for biopsies to characterise and monitor breast cancer disease evolution. This will greatly facilitate our understanding of the biology of metastatic disease and, if validated, will spare patients risky and painful procedures in the future.

> **Each technique needs to do what it says, to demonstrate clinical value and to help us improve the outlook for patients.**

Liquid biopsy in BIG research

Treat CTC (NCT01548677) is the first, multicentre, European trial using CTC detection by CellSearch® to test the role of trastuzumab in non-HER2+, non-metastatic breast cancer. It is being conducted by the European Organisation for Research and Treatment of Cancer (EORTC) with the participation of the German SUCCESS and the French Unicancer groups under the BIG umbrella. Treat CTC is screening women with breast cancer for CTCs after they have completed surgery and (neo)-adjuvant chemotherapy. Those with at least 1 CTC per 15ml blood will then be randomised to six cycles of trastuzumab or observation. The primary endpoint is CTC detection rate at week 18 for the two trial arms, and the secondary endpoint is disease free survival (DFS).

Dr Ignatiadis explains that CTC was chosen for the Treat CTC instead of ctDNA because most published data came from CTC studies when the study was planned.

"Even today, most data are from CTC detection. The only results on ctDNA and clinical outcome in early breast cancer are from small studies. There is still work to be done to standardise procedures and technologies and larger studies on the prognostic value of ctDNA in the early disease setting are needed. We are working on the Treat ctDNA trial which will be an observational study to generate such data from women who are not entering the Treat CTC trial because no CTCs could be identified in their samples," he says.

For Treat CTC, a total of 2175 women will be screened in 100 hospitals in six countries (Germany, France, Belgium, UK, Greece and Austria) and blood samples analysed at seven national laboratories. The aim is to identify 174 women with detectable CTCs, i.e., 8%.

"It is the only trial worldwide that is using CTCs to make treatment decisions for women with early breast cancer. One of the challenges in early disease is the low proportion of women who have CTCs after treatment which is why we will be screening such a large number of women," explains Dr Ignatiadis.

With so many women being screened at so many centres and a low CTC count expected in women with early breast cancer, it was important to ensure consistent methodology across laboratories. Before Treat CTC started, an inter-reader variability study was carried out on 272 CellSearch® images of either CTCs or white blood cells or artefacts from 109 non-metastatic (M0) and 22 metastatic (M1) breast cancer patients[3]. These were sent to 22 readers from 15 academic laboratories and 8 readers from two Veridex laboratories. Each image was scored as no CTC vs CTC HER2- vs CTC HER2+, and results from the academic laboratories were compared with those from a consensus index derived from findings from the Veridex laboratories.

There was 92% median agreement for distinguishing between no CTC and CTC images, though this was slightly lower for M0 than M1 samples (91% vs 98%, $p<0.001$). Agreement was also slightly lower for samples < 3 CTCs vs > 3 CTCs in M0 samples (87% vs 95%, $p<0.001$).

"Having shown that the greatest variability occurs in women with non-metastatic disease with very few CTCs, we have established, within the EORTC, a system of central image review and continuous training for all labs in the Treat CTC study," explains Dr Ignatiadis.

After the first 40 patients were screened, results were reviewed and further training provided, as needed. Real-time review of samples is continuing and regular teleconferences are held with participating centres to discuss findings. Current-

ly, CTC screening rates to identify women eligible for randomisation are in line with predictions.

"In 2015, an independent data monitoring committee (IDMC) will review findings from Treat CTC to check the CTC detection rate and the overall feasibility of the study. It's very challenging to carry out such a large study but very rewarding to see the results coming in and the laboratories performing to a high standard," adds Dr Ignatiadis.

In another initiative, AURORA (NCT02102165), BIG's landmark programme in metastatic breast cancer, ctDNA analysis will be carried out on all plasma samples that are collected, and additional samples will be collected for CTC analysis at participating centres in Belgium and Italy. This will enable results from metastatic tissue biopsy to be compared with those from CTC and ctDNA analyses.

Liquid biopsy in everyday practice?

At a handful of centres, such as Johns Hopkins, ctDNA testing in breast cancer patients is being incorporated into clinical decision-making, with the aim of targeting therapy according to changing DNA mutations.

Professor Park predicts that the use of ctDNA testing will one day enable clinicians to stop chemotherapy that isn't working for a patient at an earlier stage than is currently possible.

"At present, we have to wait and see if changes appear on scans to see if there has been a response, but ctDNA may enable us to see if treatment is working after just one or two weeks of chemotherapy," he says.

As more centres start using liquid biopsy, Professor Park hopes that it will become a viable option for routine care. He believes that initial results of ctDNA testing in early breast cancer are compelling because testing could change treatment of early breast cancer.

"Seventy per cent of patients are cured by surgery, but 30% aren't, and that's a big number. At present, we have to make educated guesses about who should get chemotherapy and who doesn't need it. We end up giving chemotherapy to patients who don't need it, and over treatment has its own problems, such as increased risk of leukaemias and peripheral neuropathies and, rarely, even death. We need a way to identify microscopic disease so we can determine who is likely to relapse and only treat those patients," he says.

Dr Dawson agrees:

"It's more difficult to follow ctDNA in early disease because levels are lower than in metastatic breast cancer. But, as our techniques improve, that's changing and it's becoming feasible to do analyses at all stages of disease."

She believes that ctDNA could be used to monitor genomic changes as tumours become resistant to different treatments, and provide new insights into mechanisms of resistance to help tailor therapies more effectively[17].

Another opportunity for liquid biopsy would be to monitor women at risk of late relapse so that treatment could be adapted accordingly. Professor Park points out that the majority of women with ER-positive breast cancer use hormone treatment after surgery but there is debate over how long this should continue. For some women, five years of treatment may be enough to eradicate microscopic disease, but others may need longer, and liquid biopsy could help to identify them.

> **"Seventy per cent of patients are cured by surgery, but 30% aren't, and that's a big number. At present, we have to make educated guesses about who should get chemotherapy and who doesn't need it. We end up giving chemotherapy to patients who don't need it, and over treatment has its own problems."**

"Hormone therapy doesn't eradicate micrometastases in some women, it just makes the cancer dormant, but if we can monitor their ctDNA levels we can identify those who need to continue treatment," he says.

A number of challenges remain before ctDNA testing becomes part of routine cancer care, including the limited time window for processing ctDNA samples.

"Plasma has to be processed within 2-3 hours of blood being drawn as DNAases chew up the DNA, and cell lysis ensues, which contaminates the plasma with large quantities of normal DNA," explains Professor Park. "Protocols are being

Meet the interviewees

Professor Ben Ho Park
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA

Dr Sarah-Jane Dawson
Peter MacCallum Cancer Centre, Melbourne, Australia

Dr Michail Ignatiadis
Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium

drawn up to ensure rapid analysis of plasma samples, though methods are being developed to extend the time during which we can analyse samples. The hardest part is to ensure the quality of the plasma and DNA because we're using very small amounts and need to be hyper cautious about contamination," he adds.

Dr Dawson also points to the need for optimisation and standardisation of methodology.

"At present, ctDNA is still a research tool but, in a few years, it could be possible to do routine serial testing of women undergoing breast cancer treatment," she says.

She predicts that CTC and ctDNA technology will have complementary uses:

> "At present, ctDNA is still a research tool but, in a few years, it could be possible to do routine serial testing of women undergoing breast cancer treatment."

"The power of CTC analysis is that you're analysing a whole cell and can apply DNA, RNA or protein-based tests. After all, CTCs are the tumour cells that enter the circulation and seed distant metastatic sites, so they still have a lot to tell us about the biology of metastatic disease," she says.

But she concludes that it is the simplicity and sensitivity of ctDNA analysis that is exciting most interest:

"Until now, we haven't had the genomic technology that was sensitive enough to look for the very small fraction of ctDNA present amongst background levels of DNA being released from healthy cells. But recent advances are now making liquid biopsies one of the most exciting breakthroughs in the biomarker field that we've had for a long time."

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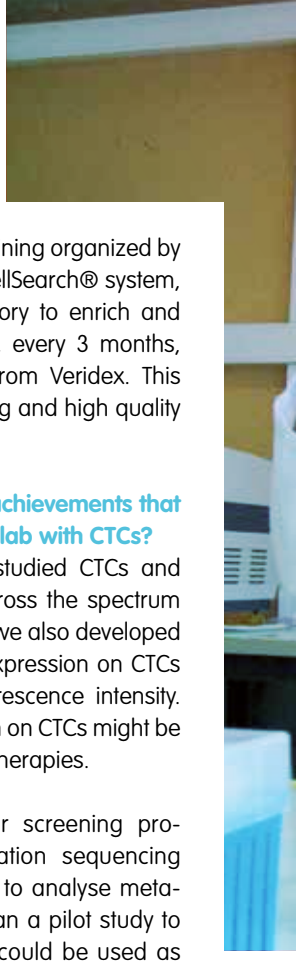
A talk with…
Dr Françoise Rothé

Françoise Rothé's current research focuses on liquid biopsies in breast cancer, studying both circulating tumour DNA and circulating tumour cells, at the Institut Jules Bordet, Breast Cancer Translational Research Laboratory (BCTL) in Brussels, Belgium, where she works as Researcher and Lab Manager.

Françoise Rothé, 37 years old, earned a master's degree in molecular and cellular biology from the Université Libre de Bruxelles in 1999, followed by a PhD in basic research in 2005. Since 2006, she has been working at the BCTL, where she first started her post-doc focusing on the role of microRNAs in the biology of breast cancer. Françoise has published 13 articles in peer reviewed journals. She dedicates her free time to her family; she likes visiting museums and playing with her two children.

BIG Research in Focus: What do you do in your daily work? Describe a typical working day.
Francoise Rothé: I work at the BCTL as a researcher and lab manager. In my daily work, I supervise and train our students and lab technicians. Liaising with other researchers and scientists is also part of my daily work. Most of my time is of course dedicated to research itself. My current projects focus on liquid biopsies in breast cancer, studying both circulating tumour DNA and circulating tumour cells (CTCs). For the latter, I'm working in collaboration with Dr Michail Ignatiadis, oncologist at Institut Jules Bordet and Principal Investigator of the Treat CTC study (NCT01548677). With BCTL serving as a central lab for the Treat CTC study responsible for the detection of CTCs, I also review all images of the patients enrolled in the study.

Your primary area of research is thus circulating tumour cells. What is a circulating tumour cell, and how will your research benefit patients?
CTCs are cells that have detached from the primary tumour or from established metastatic lesions or both, and that move through the blood-



> **The promises of liquid biopsies still need to be confirmed and a lot of studies and analyses are still needed. However, I believe that liquid biopsies will become part of daily clinical practice in the coming five years.**

stream to a new site, to form metastases. CTCs are different from plasma circulating cell free DNA (ctDNA), which consists of DNA that has been released during the necrosis or apoptosis of the primary tumour cells or the metastasis cells.

My area of research covers the detection and molecular characterisation of CTCs isolated from the blood of breast cancer patients, whether with metastatic or early disease. A liquid biopsy is the collection of blood (through a blood test), as compared to a solid biopsy, which represents the collection of a sample from the primary tumour and/or from a metastatic site (using a more invasive technique). Liquid biopsies provide real time evidence of cancer in the blood circulation thanks to tumour CTCs and tumour ctDNA. We hope that in the future we will be able to closely monitor the disease with regular blood collection and analysis, as well as follow the response to a treatment or detect a potential recurrence. Liquid biopsies are easy and safe to perform. They represent a relatively non-invasive procedure that will benefit patients in several ways. Another advantage of liquid biopsies is that they could be useful when metastatic lesions are not accessible or when insufficient quantity or quality of tumour material is available for analysis. Liquid biopsies could also be useful for patients who do not want to undergo a standard (solid) and invasive biopsy.

How did you end up working on CTCs? Did you follow any training to be able to work on CTCs?

I started working on CTC research projects in 2009 in collaboration with Dr Ignatiadis.

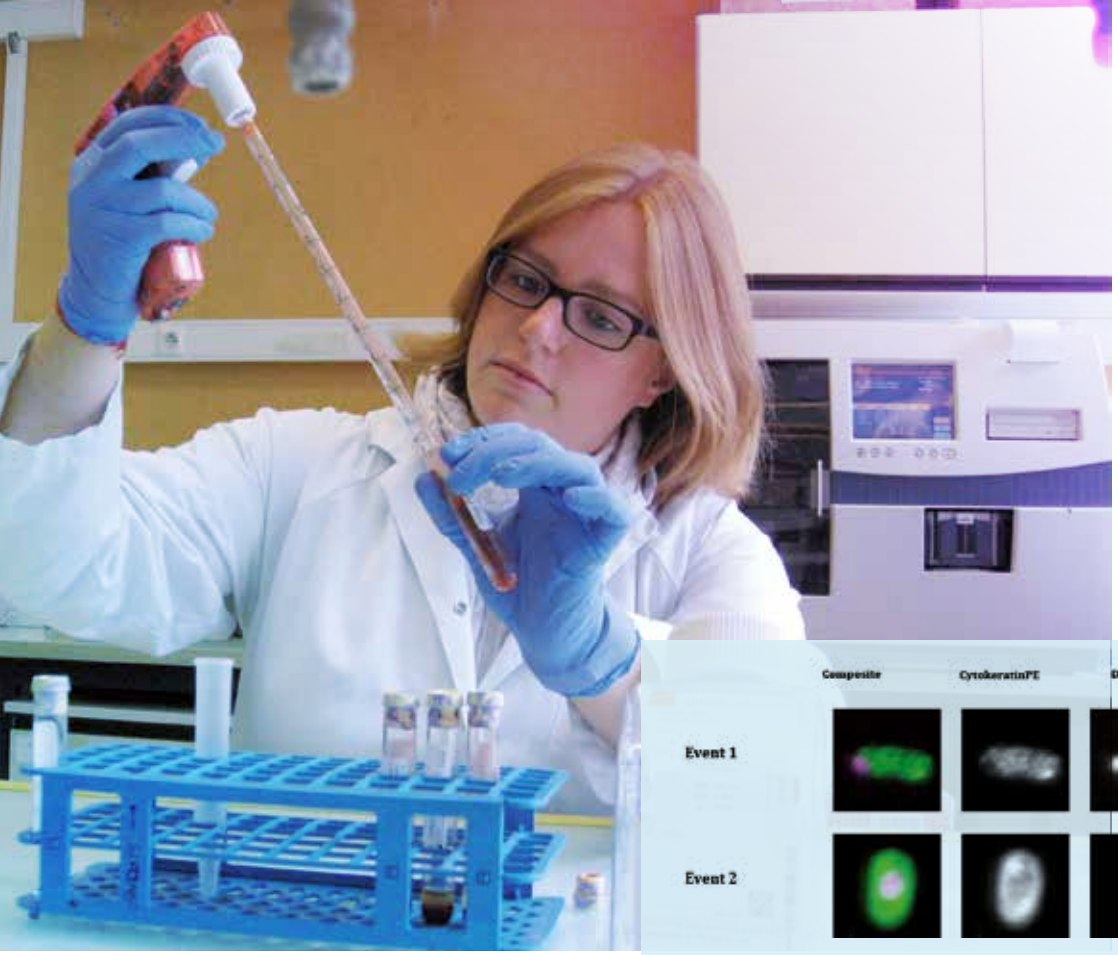
Yes, I had a well-structured training organized by Veridex, the provider of the CellSearch® system, which is used in our laboratory to enrich and enumerate CTCs). Since then, every 3 months, I receive images to review from Veridex. This guarantees continuous training and high quality CTC images review.

Can you share some of the achievements that have been obtained by your lab with CTCs?

In one of our projects, we studied CTCs and HER2 expression on CTCs across the spectrum of breast cancer. In this study we also developed a method to quantify HER2 expression on CTCs based on HER2 immunofluorescence intensity. Monitoring of HER2 expression on CTCs might be useful in trials with anti-HER2 therapies.

We also work on molecular screening programmes using next-generation sequencing (NGS) of cancer gene panels to analyse metastatic biopsies. We recently ran a pilot study to interrogate whether plasma could be used as an alternative to metastatic biopsies. We used NGS for a commercially available 50-gene panel tested separately on tumour (primary and/or metastatic) lesions and serial plasma samples, totalling 100 samples from 17 patients with metastatic BC. We showed that in 76% of the patients, tumour and plasma provided concordant results. To our knowledge, our study is the first to show the feasibility of performing NGS for a 50-cancer gene panel in plasma samples from metastatic breast cancer patients. This approach might increase patient access to molecular screening programmes and should be prospectively tested[1].

Our team has also led an international study to assess the inter-reader variability of CTCs enriched from breast cancer samples. In our lab, CTCs are enriched using the CellSearch® machine, after which images of potential CTCs are read by scientists to determine the exact number of CTCs present in the blood sample. It is important to study the variability of results depending on the individual laboratory and on different readers. Image interpretation is crucial, especially if CTC detection is to be used in the context of clinical trials. Our study resulted in consensus guidelines for image interpretation for CTC detection in non-metastatic breast cancer. It emphasized the importance of continuous training, independent image review, and adherence of CTC experts to these consensus guidelines.



Example of intact circulating tumour cells from a patient with breast cancer

What are the challenges you face?

The detection and genomic characterisation of CTCs still involves a lot of difficulties and technical obstacles. The rarity of CTCs also represents a challenge: their number in a blood sample can be very small, especially in the adjuvant setting. Since their number is small, CTC genomic DNA has to be amplified before genomic analysis. The analysis of single CTCs using next generation sequencing is still a major challenge. The inter-reader variability is also a problem we face.

To date, the liquid biopsy has generated a lot of excitement in the lab but little in the clinic. Do you think this will change? How and when?

Yes, I definitely think that this will change. The promises of liquid biopsies still need to be confirmed and a lot of studies and analyses are still needed. However, I believe that liquid biopsies will become part of daily clinical practice in the coming five years.

Which are the main steps in the collection and analysis of circulating tumour cells?

In the lab, we are using the CellSearch® machine in order to enrich and enumerate CTCs. The first step consists of the blood draw in a dedicated tube. The blood is then mixed with a buffer before being processed by the machine. The enrichment method is based on the biological properties of the CTCs: specific antibodies bind to the CTCs, which will then be magnetically captured and separated from the other blood cells. CTCs are then stained with cytokeratin antibodies. The cartridge containing the enriched cells is then scanned in order to visualize and review all the images of potential CTCs.

What are the next projects you will be working on?

We are currently running a study on the genomic characterisation of CTCs isolated from patients with metastatic breast cancer from Institut Jules Bordet. This study aims to characterise CTCs using NGS and compare them with the primary tumour, the metastatic lesions and the tumour ctDNA isolated from the plasma. We aim to better understand the colonization and progression of the disease. There is also a running project in collaboration with a laboratory in Italy aiming to characterise CTCs isolated from patients with non-metastatic breast cancer using NGS. With these two projects, we aim to explore if CTC genomic analysis can provide additional information to the genomic analysis of the primary tumour and the metastases.

Reference

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BIG

Breast International Group

We will find a cure for breast cancer through global research and collaboration

The **Breast International Group (BIG)** is a non-profit organisation for academic breast cancer research groups from around the world.

Founded by leading European breast cancer experts in 1999, BIG now constitutes a network of 55 groups based in Europe, Canada, Latin America, Asia and Australasia. These entities are tied to several thousand specialised hospitals and research centres worldwide. About 30 clinical trials and several research programmes are run or are under development under the BIG umbrella at any one time. BIG also works closely with the US National Cancer Institute and the North American Breast Cancer Group, so that together they act as a strong integrating force in the breast cancer research arena.

www.BIGagainstbreastcancer.org

BIG means:

Truly international reach

BIG is a truly international body focused exclusively on conducting and coordinating breast cancer research, primarily through clinical trials and innovative research programmes. To test new treatments with enough patients to be confident about the results, most research cannot be limited to one institution, or even to one country.

Real research

BIG designs and conducts its own research through its member groups and their extended network of hospitals and investigators – BIG does not simply redistribute funding to other third parties. BIG trials that are conducted in collaboration with the pharmaceutical industry are done so in a manner designed to maintain independence and eliminate bias, keeping patients' interests at the heart.

Research principles

BIG facilitates academic research but also works closely with the pharmaceutical industry in a way that is "win-win" for all. BIG trials respect specific principles of research conduct to ensure that data collected are handled and analysed independently, generating highly credible results. Moreover, patients are followed long after treatment ends, with the aim to detect long-term side effects. BIG studies are also governed by committees and policies designed to reduce bias and protect the patient. Finally, the processes surrounding access by scientists to precious tumour and other tissues donated by patients for future research are subject to strict rules to ensure that only the best research ideas are supported.

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BIG has the ability to achieve faster results and greater patient benefits by enrolling larger numbers of patients into clinical trials more quickly, and doing so in many countries around the world.

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