The Liquid Biopsy: ctDNA, Circulating Tumour Cells and Bloodborne Biomarkers

Max Rayne Auditorium, Royal Society of Medicine, 1 Wimpole Street, London, W1G 0AE. Thursday 8th March 2018, 09.00 - 17.00

Meeting report by Dr Susan Richman

This was the second in a series of biomarker workshops organised by CM-Path's Discovery Workstream, where invited speakers gave an overview on circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) and discussed recent advances in technology, which have led to their application both in research and more recently, in clinical trials. During very lively panel discussion sessions, the barriers to widespread adoption within clinical practise was discussed.

CM-Path introduced three exciting new innovations at this workshop; firstly, four travel bursaries and free workshop registration, were awarded to Pathology trainees, who are currently undertaking out-of-programme research. Secondly a 'Collaboration Corner' was set up, where delegates were encouraged to meet and discuss potential ways of working together on research projects. Finally two £100 book vouchers were on offer to the delegates who asked the 'best question', as judged by the CM-Path organising committee, on the basis of them stimulating discussion and showing insightful knowledge of the research area.

The meeting was opened by **Ian Lewis**, Head of Clinical Research Groups at the NCRI. Ian gave an overview of the NCRI and its partner organisations, and also drew attention to the work of the 18 clinical studies groups (CSGs) and highlighted three NCRI initiatives; CT-Rad, Living with and Beyond Cancer and of course, CM-Path.

The first invited speaker in the morning plenary session was **Dr David Guttery**, a Lecturer in Early Cancer Detection at the University of Leicester (dsg6@le.ac.uk). David highlighted the current issues surrounding the collection of tissue biopsies, namely and most relevant to patients, is the invasive nature of sample collection. Furthermore a biopsy is merely a 'snapshot' of the tumour at one particular time, and longitudinal sampling is, in most cases, challenging to say the least. Additionally following resection, just how does one acquire follow-up samples, when the tumour has been surgically removed? The liquid biopsy provides the answer to these issues, being less invasive, more representative of tumour heterogeneity, and readily lends itself to longitudinal sampling. David provided an excellent overview of the different types of liquid biopsies; CTCs, cfDNA and exosomes (adapted from *Wan et al, Nat Rev Cancer 2017*). Starting with ctDNA, he went on the show the complete lack of consensus there is when it comes to blood processing, then the subsequent isolation, processing and quantification of ctDNA. David then covered the potential, and indeed current uses of ctDNA; a biomarker for early stage cancer detection (*Bettegowda et al, Sci Trans Med 2014*); a marker of minimal residual disease (*Shaw et al, Genome Res 2012*); as a predictive biomarker of response to therapy (*Fribbens et al, JCO 2017*) and as a monitoring tool (*Guttery et al, Clin Chem 2015*). Moving onto CTCs, David discussed the rarity of these cells (with often less than 5 being isolated per ml of blood). The

technologies used to isolate CTCs come at a vast cost, (*Joose et al, EMBO Mol Med 2014*) but once isolated, CTCs can be used in a plethora of different assay platforms, including protein expression, phosphorylation, and are capable of being grown *in vitro*. Additionally, pre-clinical studies from Professor Caroline Dive's group have demonstrated that CTCs grown in either culture or mice, behave identically when treated with drug therapies, as was seen in the patient they were originally isolated from (*Lallo et al, TLCR 2017*). Moving forward, David highlighted the potential of using both ctDNA and CTCs in patient management, where ctDNA would be used as an initial diagnostic and monitoring tool, then CTCs, isolated from patients would be used to develop an *in vitro* model to identify drug therapies, and finally ctDNA would be used subsequently to follow the patient's response to treatment.

The second invited of the morning plenary session speaker was **Dr Andrew Wallace**, a Consultant Clinical Scientist from the Manchester Centre for Genomic Medicine, who gave a fascinating overview of the trials and tribulations involved in his lab's validation of EGFR ctDNA testing, where they took, what was initially a research assay and, developed a routine clinical diagnostic test. The journey to UKAS accreditation took in excess of 18 months, as each part of the process had to be rigorously evaluated and validated.

The third invited speaker of the morning plenary session was **Dr Chris Abbosh**, a Senior Clinical Fellow in Medical Oncology at UCL and also the Clinical Fellow on the Lung TRACERx (TRAcking lung Cancer Evolution through treatment Rx) study. This is a UK-wide prospective observational cohort study aiming to increase knowledge and transform current understanding of non-small cell lung cancer (NSCLC). The study will run for 9 years, recruiting 842 patients from 18 UK hospitals and aims to determine how clonal heterogeneity affects the risk of tumour recurrence and survival. This will then guide the analysis of intra-tumoural heterogeneity to develop novel targeted and immune-based therapies. Chris described one of the sub-studies he lead on; 'Phylogenetic ctDNA analysis depicts early stage lung cancer evolution' (*Abbosh et al, Nature 2017*). ctDNA was isolated and used to follow the evolution of NSCLCs in the first 100 patients enrolled onto TRACERx. They were able to demonstrate that the amount of detectable ctDNA in tumour samples was tumour sub-type specific, with significantly more ctDNA being detected in the plasma taken from squamous cell carcinoma patients, compared to adenocarcinoma patients. An additional sub-study involved the analysis of longitudinal samples in the adjuvant setting, where blood was taken from 24 patients; half of who had relapsed and half who had not. ctDNA was detected in the samples from the patients who recurred, and in some patients, this was several months before recurrence was determined clinically. This was an excellent example of the use of ctDNA in the monitoring of minimal residual disease.

The first Keynote speaker of the day was **Professor Nicholas Turner,** an Honorary Consultant in Medical Oncology at ICR, who gave a fantastic presentation entitled '**ctDNA in clinical trials and breast cancer**'. Nick concentrated on three particular areas of his research; the use of ctDNA as 1) a biomarker for selecting therapy in metastatic breast cancer; 2) a means of monitoring response to therapy and 3) its use as a tool for detecting residual disease.
Firstly delegates were given a comprehensive overview of a review paper (*Nature. 2012 October 4; 490(7418): 61–70*) where primary breast cancers were analysed

by DNA copy number arrays, methylation, exome sequencing, mRNA arrays, miRNA sequencing and reverse-phase protein arrays. This work identified the challenge facing treating clinicians; the fact that there are multiple targetable genetic drivers, and these drivers differ between primary and secondary tumours. Nick also described the 'plasmaMATCH' trial, a phase IIa multicentre trial for patients with advanced breast cancer, which aims to analyse whether ctDNA could act as a suitable alternative to standard biopsies for the identification of actionable mutations in the tumours and subsequently whether these patients would benefit from targeted therapies. Nick then moved to a discussion of the use of ctDNA to monitor response to therapy, giving three superb examples of studies where this has been done (*Dawson et al, NEJM 2013; O'Leary et al, Nature Comms 2017; Fribbens et al, Ann Oncol 2017*). All these studies clearly demonstrated that the genetic heterogeneity in advanced breast cancer is extremely challenging. Nick then introduced the c-TRAK-TN trial, which is a phase II study, assessing the utility of ctDNA samples as a suitable tool to screen for the presence of residual disease in triple negative breast cancer patients. If ctDNA is identified in their plasma sample, patients are randomised between an observation arm and a pembrolizumab treatment arm. If they are ctDNA negative, follow-up screening will be carried out every three months for two years. Finally Nick touched on current issues when it comes to identifying residual disease including assay sensitivity. As he pointed out, when you have 'Sanctuary' or 'Dark' metastatic sites, such as in the brain, detection is difficult. Equally where you have solitary metastatic sites, such as in an ovary, these will be equally difficult to detect. Furthermore, assays are limited and may only pick up 2/3 of mutations present in a baseline sample, so methods have to be adapted to improve sensitivity. Whole genome sequencing may be one way to do this.

The afternoon sessions were opened by **Professor Mark Arends**, Professor of Pathology at the University of Edinburgh. Mark gave an overview of the funding opportunities available to Pathology Trainees though the Pathological Society of Great Britain and Ireland. These included research schemes funded jointly by the Jean Shanks Foundation and Pathological Society, where £4-5m are available.
Mark then chaired the second Plenary session, where Trainees had the opportunity to showcase their current research. **Dr Philip Elliott** (Barts Cancer Institute) gave a presentation on his plans to carry out integrated genomic analysis of DCIS tissue, with the aim of defining a signature for stratified response to therapy. This research will generate pilot data to carry forward into a clinical PhD. **Dr Sara Waise** (University of Southampton) showed her research using a microfluidic system called DropSeq and multi-plex IHC to aid sub-classification of cancer associated fibroblasts (CAFs). **Dr Alex Lee** (ICR) discussed the mobilisation of FFPE tissue from archives for soft tissue sarcoma research, with a particular focus on modelling Pazopanib resistance. Finally, **Dr Kalnisha Naidoo** (ICR) described her work, where she has successfully been able to perfuse axillary nodes outside ex-vivo, complete with a fascinating short video of her model in practise.

Professor Fiona Blackhall, an Honorary Consultant in Medical Oncology at the Christie NHS Foundation Trust gave the next plenary lecture. She discussed the clinical application of liquid biopsy detection in lung cancer, but this presentation focussed on CTCs, rather than ctDNA. Who knew that CTCs were firstly described back in 1869 by Thomas Ashworth (*T.R. Ashworth, "A case of cancer in which cells similar to those in the tumours were seen in the blood after death," Australasian*

Medical Journal, vol. 14, pp. 146–147, 1869)?! Fiona described the Cell Search platform, known as the 'gold standard' for detection and enumeration of CTCs and also the ISET (Isolation by Size of Tumour cells) test, but stressed that only Cell Search is FDA-approved and validated for the routine diagnostic analysis of CTCs. As Fiona pointed out, SCLC is a very challenging disease to study, especially given the fact that serial biopsy collection is difficult, the rate of relapse within 6 months is high, and targeted agents are very rare. That being said, it lends itself perfectly to using CTCs to monitor disease progression, and gave an example of a study where the prognostic significance of CTCs was demonstrated, where the presence of >50 CTCs was used as a cut-off to identify patients with a poor prognosis. This was true for both PFS and OS. In NSCLC, another study showed a cut-off value of 5 CTCs was significant for prognosis. Fiona explained that CTCs can now be used to study the biology of SCLC through the course of the disease and at the time of study treatment (2013 US Recalcitrant Cancer Act & 2014 US National Cancer Institute Scientific Research Framework). As an example of this work, she told the audience about a study where CTC-derived explants (CDX) tumours showed both a similar SCLC morphology and IHC characteristics and mirrored the cisplatin and etoposide-responses, as seen in the original patients. CDX models are proving themselves as the ideal system in which to carry out pre-clinical drug testing, and in fact are currently being used in phase I/II studies of olaparib and temozolomide.

The second Keynote speaker, and indeed final speaker of the workshop was **Professor Nitzan Rosenfeld**, a Senior Group Leader at the CR-UK Cambridge Research Institute, who gave an excellent presentation entitled 'Future implications of liquid biopsy and ctDNA in clinical practice'. (*Wan et al, Nature Reviews Cancer 2017*). Nitzan summarised very nicely many of the points that the days' other speakers had mentioned. It is indeed a huge challenge to move from a tissue diagnosis to a liquid biopsy classification. It can take years to go from a proof of concept research study to use in the clinic. As an example of this, a non-invasive pre-natal test (NIPT) for Downs Syndrome will be rolled out in the NHS later this year, having been first worked up in a laboratory over 10 years ago! Nitzen also talked about some of the work carried out by Inivata, a global clinical cancer genomics company (www.inivata.com), which has developed gene panel assays for liquid biopsies, which are now in clinical use. To finish his presentation, Nitzen pointed his audience to an excellent review paper 'Circulating Tumor DNA analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review' by *Merker et al, JCO 2018*).

Travel bursary winners:

1. Katherine Quiohilag
2. Matthew Ahearne
3. Rachel Bowden
4. Sasagu Kurozumi

'Best question' winners:

1. Sarah Hargreaves, Clinical Research Fellow, Velindre NHS Trust.
2. Alison Reid, Consultant Medical Oncologist, Royal Marsden Hospital.