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NCRI Biomarkers & Imaging CSG Cell-free DNA workshop

Workshop Report

Christie Education Centre, Manchester
30th January 2014

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Workshop summary

86 delegates from a variety of specialities attended the one-day workshop, held at The Christie in Manchester.

Chaired by Prof. Robert (Bob) Brown (Imperial College London), Prof. Caroline Dive and Dr Ged Brady (both, CRUK Manchester Institute), the workshop aimed to:

1. Disseminate information about technologies available and robustness in clinical samples.

2. Communicate the current status of cfDNA analysis.

3. Address the important clinical questions and how they can be implemented.

4. Provide information on the use of cfDNA as a biomarker in future clinical trials.

The workshop was CPD-accredited by the Royal College of Physicians.

This report is a summary of the workshop, capturing the main messages that came out of the sessions and an outline of the general discussion.

Introduction

On 30th January 2014, the NCRI Biomarkers & Imaging Clinical Studies Group (BICSG) hosted a cell-free DNA (cfDNA) workshop at the Education Centre, Christie Hospital, Manchester. Aimed at members of the research community who are focusing on cfDNA in their research or wishing to learn more, the workshop was attended by 86 delegates from technicians to PhD students, clinical fellows to group leaders. The main aim of the day was to discuss the importance and current status of cfDNA research with a view to set out guidelines for the future use of cfDNA in a clinical setting.

Prof. Bob Brown opened the meeting, welcomed delegates, introduced the BICSG and their work streams, and explained the importance of the workshop. Prof. Brown also spoke about promoting biomarker technologies and clarified the aims of the workshop was also to discuss issues around biomarker analysis to be used by the cfDNA community and work towards a consensus regarding best practice.

Introduction sessions

cfDNA as a biomarker

Dr James Brenton (CRUK Cambridge Institute) introduced the field of cfDNA; the concept, plus examples of cfDNA use from his research in breast and ovarian cancer. Dr Brenton also discussed parallels with chronic myeloid leukaemia (CML), explaining how lessons can be learnt from CML, such as minimal residual disease (MRD)-testing. Dr Brenton went on to compare cfDNA vs. circulating tumour cells (CTCs) in breast cancer, and cfDNA vs. CA125 in ovarian cancer.

cfDNA as a biomarker in breast cancer

Prof. Jacqui Shaw (University of Leicester) began her talk by discussing how the field has exploded with the advent of next generation sequencing, and how cfDNA can be described as a liquid biopsy as well as discussing the challenges of using cfDNA as a biomarker. Prof. Shaw compared cfDNA extractions using QIAamp® DNA Blood Mini Kit vs. QIAamp® Circulating Nucleic Acid Kit, demonstrating that the kits give comparable sequence profiles. Also covered, was the possibility of using cfDNA to assist with breast cancer screening, in individuals who had been recalled for suspicious mammograms. Post-talk discussions included the origin of cfDNA, how to stabilise samples, given the short half-life, and whether patients should fast prior to sample collection.

cfDNA assays, GCP-L criteria and implications

Dr Jonathan Tugwood (CRUK Manchester Institute) talked through the conventional workflow for plasma preparation and cfDNA isolation. He considered the issues around sample handling and the impact on sample stability. Self-posting experiments were discussed, addressing the issue of degradation in the post and an in-house validation project highlighted an advantage of using CellSaveTM tubes over conventional EDTA tubes.

Dr Jeff Cummings (CRUK Manchester Institute) debated the challenges of meeting innovation and standards set out following the publication of the MHRA GCP 'Grey Guide', and introduced 'fit-for-purpose biomarker method validation. Post-talk discussions resulted in an agreement that the right balance between innovation and standards must be struck. However, with the revision of the EU directive on GCP underway and due for publication in 2016, there is likely to be even more regulatory burden placed on Clinicians and Scientists conducting Clinical Trial related research

Molecular analysis at different scales of resolution

Dr Nitzan Rosenfeld (CRUK Cambridge Institute) outlined the idea of tracking the cancer genome non-invasively via plasma DNA and probing cfDNA at different scales of resolution, increasing sensitivity for rare mutations and increasing genomic coverage. Dr Rosenfeld also introduced the concept of Tagged-Amplicon Sequencing (TAm-Seq) – sensitive identification and accurate quantification of mutations, in fragmented FFPE specimens vs. fragmented cfDNA. Also discussed was the possibility of resistance-conferring mutations being identified non-invasively, conceivably leading to the discovery of novel resistance mechanisms.

Industry perspective

Dr Gillian Ellison (AstraZeneca) discussed how cfDNA can aid drug development and allow more patients to access the most appropriate treatments. Advantages of using cfDNA over tissue were expanded on, namely the difficulty of obtaining tissue samples, which subsequently must be fixed. Dr Ellison also presented work to demonstrate that mutation analysis of cfDNA correlates with the tumour in terms of positivity and response to therapy, but not tumour status or burden. The use of cfDNA to study the emergence of resistance biomarkers was also considered; the main advantage being that it is less invasive that using tissue, especially as the original diagnostic sample may not be suitable for such studies.

Discussion sessions

cfDNA – trials to clinical application

Prof. Jacqui Shaw (University of Leicester) and Prof. Charles Coombes (Imperial College London) discussed clinical applications of cfDNA, particularly in the setting of breast and lung cancer.

Prof. Coombes led a discussion on the importance of monitoring patients who have had breast cancer, with particular emphasis on a test for micrometastases before the development of overt metastatic disease, as well as introducing the IES trial, DEVA trial and the NeoCENT trial. Also discussed was the potential for cfDNA technology to replace biopsies in woman with abnormal mammograms. Unanswered questions include whether the time and site of relapse can be predicted in those treated for breast cancer; whether survival can be improved by treating individuals with a sustained rise in CNVs; and whether plasma DNA can be used to identify and target molecular abnormalities. Increased levels of plasma DNA following neoadjuvant treatment was discussed. It is currently unclear why sustained levels exist and this doesn't correlate with tumour shrinkage or response.

Prof. Shaw outlined tissue versus liquid biopsy for surveying tumour heterogeneity and monitoring tumour burden by using cfDNA as a marker of tumour dynamics over time. She also introduced the 'Tracking non small cell lung Cancer Evolution through therapy (Rx;TRACERx)' study, which involves monitoring genetic changes that occur over time from diagnosis and throughout the treatment period, and blood samples collected will be analysed for cfDNA as well as CTCs.

cfDNA vs Circulating Tumour Cells

Prof. Caroline Dive (CRUK Manchester Institute) and Dr James Brenton (CRUK Cambridge Institute) discussed the issues and complementarities of cfDNA and circulating tumour cells (CTCs), comparing the advantages and disadvantages of both, depending on what is required. For example, whether a biomarker is being sought rather than the biology; identification of a drug target; investigation of tumour response or mechanisms of drug resistance and analysis of tumour heterogeneity and evolution. There was a discussion around enrichment vs. pure CTCs and Prof. Dive also described current technologies for the detection and enumeration of CTCs, such as CELLSEARCH®, CTC-iChip, CellSieveTM, SpiralChip, Parsortix, Gilupi CellCollectorTM, DEPArrayTM and ImageStream, to name a few. In SCLC, there are lots of CTCs but no change after treatments. In vivo experiments of SCLC with CTCs have been carried out, and molecular profiling after CTC isolation using the DEPArrayTM. Dr Dive also compared CDX and CTC genomic landscapes, and single CTC sequencing (Tam-Seq – Dr Rosenfeld) and discussed the issue of CTC heterogeneity.

Dr Brenton outlined the advantages of cfDNA over CTCs; the major advantages being that cfDNA analysis is cheaper and technically less challenging. However, the field of cfDNA is still new.

Putative tumour stem cells were discussed. As they are thought to not be hit by therapy, leading to drug resistance, it is possible. Markers need to be identified as relapsed tumours are different to the primary tumour. Profiling at a diagnostic level was also considered. For SCLC, most patients are already advanced at diagnosis, and this is the same for ovarian cancer. Screening heterogeneity does not help. Rolling back targeting therapy to those at a high risk of relapse; if a clinician could predict those with a risk of recurrence, what could they do with that information?

General Discussion

The day ended with a final discussion, involving all the speakers. The discussion session is summarised below:

1) Prof. Dive spoke about the Biomarker Expert Review Panel (BMERP); encouraging applications in circulating biomarkers and spoke about importance of the 4 S's: Science, Stats, Samples & Assays and Study Team. BMERP primarily reviews and funds biomarker and imaging research in an academic setting, but will consider collaborations between academia and industry.

2) The clinical questions to be implemented were discussed:

3) Prof. Coombes spoke about CA125 and the requirement for targeted therapy. Other reliable, reproducible markers would be extremely valuable. Endocrine therapy, HER2 amplification, FGFR amplification are areas that are being investigated, but work is still needed. For example, we need to know that patients showing an increase in HER2 expression will respond to HER2-targeted therapy (research validity vs. clinical validity). Standardisation steps will be needed (translation) and funding is also key.

4) All members of the discussion panel agreed that there is potential in a UK-wide cfDNA consortium. The role of the consortium would be, for example, to compare technologies, share practise and agree on protocols for standardisation. The consortium would benefit from input from industry (both financial and influential), and Qiagen agreed on this.

5) Prof. Brown mentioned that an initiative like this does not yet exist in the UK and would be happy to help kick-start this, with the help of the NCRI BICSG and ECMC network. Prof. Shaw offered to host a consortium meeting in Leicester.

5) Dr Rosenfeld suggested testing the same protocol at three different centres – standardise outputs and decided what can be measured.

6) Dr Brenton highlighted the importance of learning lessons from those working in haematological oncology. A UK standardisation group exists for CML. Instead of starting from scratch, the cfDNA consortium could follow on from CML.

7) A realistic starting point would be to look at 2-3 clinically actionable cancers, liaise with the respective scientists and identify 1-2 mutations. Speak to those in regulatory affairs and have general guidelines; looking to scientists to standardise/validate.

8) With respect to funding the consortia, CRUK and pharma should be approached to share costs.