THE 2017 EARLY DETECTION OF CANCER CONFERENCE REPORT

CANCER RESEARCH UK

OHSU KNIGHT CANCER Institute

EXECUTIVE SUMMARY

Early detection is one of the most powerful ways that we can improve cancer survival. Improving the discovery of lethal cancers as they form is fundamental to treating patients more effectively and improving outcomes. Early detection is a new and complex field, requiring a multinational, multidisciplinary effort to address the many challenges and to accelerate progress.

The second Cancer Research UK and OHSU Knight Cancer Institute conference on Early Detection of Cancer was held between 20 and 22 September 2017 in Cambridge, UK. The conference brought experts from a wide range of disciplines together to discuss ongoing research and future challenges in the emerging field of early detection.

Over four themed sessions - Innovative Imaging, the Tumour Environment, Liquid Biopsies and ctDNA, and Technology - our speakers presented cutting-edge research, stimulating wide-ranging discussion.

The **Innovative Imaging** session highlighted a need to develop a diagnostic funnel, as MRI and some other imaging techniques are too expensive and time consuming to be used in first line screening. At risk populations could be identified through cheaper alternatives, such as liquid biopsy testing or photoacoustic imaging techniques, before referral to more costly testing.

A major concern highlighted in the imaging field was the lack of standardisation in image collection, resulting in high variability and difficulties in comparing and harmonising results and datasets. In addition, images are not available through a public database, making data collection from existing images a challenge. Although there is no clear solution to these issues at the moment, efforts are being made to move forward in these areas.

Speakers covering the **Tumour Environment** emphasised how the induction of stromal remodelling by nascent cancers is poorly understood. In addition to lacking a complete picture of how the immune system interacts with cancer, how genome wide association study-determined risk factors and ethnic diversity affect the environmental 'soil' into which cancers seed themselves is almost wholly unexplored.

Recruitment of developmental and evolutionary biologists and immunologists to work on cancer-related problems is also a priority, as understanding the normal environment is vital for discovering how cancers develop and thrive. Communication and collaboration between biologists and clinicians also needs to be improved, so that studies using human tissue are better focused and resourced. Finally, there is a pressing need for bioinformatics tools for integration of tumour and environment biology into a complex model.

The **Liquid Biopsies and ctDNA** session concerned the many challenges of establishing clinical benefit from early detection by liquid biopsy; lead time bias is clearly a problem here, meaning that increased survival rates may not be a good endpoint. Large-scale multivariable clinical trials are required, and for this, different disciplines need to communicate effectively to agree a joint approach. However, the biomarkers that might be useful are yet to be fully established. For example, using immune cells as one component of an early detection biomarker panel would be risky, as current knowledge of cancer immunology is insufficient.

Discussions during the **Technology** session emphasised the need for a common language for communication between academia and industry, as well as a business model that reflects shared goals. Any future model should be more collaborative, to allow for a relationship beyond 'fee for service.' As technology is sometimes advancing ahead of the current state of research, there is an opportunity for industry to shape better informed experiments and fill-in knowledge gaps. To move early detection research forward, we should be clear about the differences in culture and drivers for academia and industry, and work to build a platform for collaboration that supports everyone.

New technologies and discoveries will drive improvements in our ability to detect, stratify and treat cancer. New markers and methodologies need to be integrated into clinical trials and practice as quickly as possible. However, the current system of clinical trials is not fit for purpose, partly because the multi-centre large-scale global trials required to appropriately power the studies are very difficult to fund and pursue.

What follows is a report describing the conference proceedings, together with a summary of the overarching challenges identified by the participants.

UPCOMING MEETINGS:

OHSU KNIGHT CANCER INSTITUTE, CANARY CENTER AT STANFORD AND CANCER RESEARCH UK PRESENT THE EARLY DETECTION OF CANCER CONFERENCE

2-4 OCTOBER 2018, PORTLAND, OREGON

24-26 SEPTEMBER 2019, STANFORD, CALIFORNIA

SESSION 1: INNOVATIVE IMAGING

One of the greatest scientific challenges facing early detection research is the problem of how to discriminate between high- and low-risk lesions at early stages of disease. Imaging has a key role to play in making this distinction between the 'tigers' and the 'pussycats' but many issues remain to be solved. Improving current practice through the modification of existing technologies (PET/CT, MRI) and the development of new techniques that employ optical or acoustic modalities were discussed in the session. Several themes emerged that cut across the methods, which were explored further during the panel discussion.

John Kurhanewicz discussed the power of advanced imaging techniques for better diagnosis, risk stratification and treatment of prostate cancer. Due to problems with sampling and heterogeneity even a biopsy cannot fully predict the risk of progression of a tumour; 20-30% of those classified as low risk end up progressing. Multiparametric ^{1}H MRI (mpMRI) can detect lesions very efficiently in a single staging exam of the whole prostate, allowing for MRI/ultrasound guided biopsies and a lower false negative rate. An additional benefit to using this approach in prostate cancer detection is that it can give a measure of aggressiveness of the tumour, which in turn can reduce the number of false positive diagnoses made.

The biggest recent advance in the field is the advent of hyperpolarized ^{13}C (HP^{13}C) imaging, a technique that exploits the high rates of glycolysis seen in cancer cells. Imaging using HP^{13}C pyruvate, a metabolite involved in lactate synthesis via aerobic glycolysis, can potentially be used to stage cancers and determine the aggressiveness of the tumour. A completed phase I trial showed that the most aggressive prostate cancers have the highest flux from pyruvate to lactate, and in an ongoing phase II trial, preliminary data indicates that high flux maps precisely onto high grade disease. A combination of HP^{13}C pyruvate and HP^{13}C urea is also being trialled to monitor progression. With far better spatial and temporal resolution than ^{1}H MRI and greatly enhanced signal strength, it can be used for diagnosis, risk stratification and importantly, for monitoring treatment response in real time.

Radiomics is an approach to extract quantitative mineable data from routine medical images, including those in a screening setting. **Robert Gillies** discussed the potential of radiomics as a way of distinguishing between the many indeterminate pulmonary nodules (IPNs) detected during lung cancer screening.

Radiomic features include descriptors of size, shape, location, and texture. These were extracted from CT scans of participants in the US National Lung Screening Trial (NLST) to determine if features at baseline could predict subsequent development of cancer. This resulted in a radiomics risk score, that was 93% accurate for predicting high- and low-risk, encompassing more than half of the subjects. The radiomics risk score could therefore be used to suggest the length of time between follow-up screens (longer for low risk nodules), or if the patient should go to surgery immediately. As not all cancers are life-threatening, radiomics has also been trialled as a method to distinguish indolent from aggressive screen-detected cancers and can predict the post-diagnosis survival of NLST participants with non-small cell lung adenocarcinoma.

OPTICAL IMAGING TECHNOLOGIES OFFER OPPORTUNITIES FOR DIAGNOSIS AND THERANOSTICS, USING TARGETED IMAGING TO ENABLE IMAGE-GUIDED SURGERY

Moving to more localised imaging modalities, **Sarah Bohndiek** discussed the diagnosis and monitoring of Barrett's oesophagus, the precursor lesion to oesophageal adenocarcinoma, describing the development of optical imaging methods to differentiate between low- and high-grade dysplasia, the intermediate stages between Barrett's and full-blown adenocarcinoma.

Standard white light endoscopy coupled with autofluorescence imaging can detect the major structural and functional changes in oesophageal tissue during the transition from Barrett's through dysplasia to adenocarcinoma, but is less effective at discriminating between low and high-grade dysplasia. In contrast, optical coherence tomography gives cross-sectional information at 7-10μm revealing the abnormalities indicating progression from Barrett's to dysplasia and beyond. It is now possible to image the whole length of the oesophagus by swallowing a tethered capsule carrying a fibre optic array, and see the difference between healthy and dysplastic tissue. Recent developments include multispectral and hyperspectral (100+ colours) imaging methods, which will allow even better colour painting, giving a far more detailed image.

There are also exciting developments in molecular imaging using fluorescent contrast agents. Fluorescently-conjugated markers have been developed together with near-infrared fluorescence imaging, to eliminate the problem of background autofluorescence. Such imaging can be co-registered with morphological information to identify dysplastic areas and assess their risk with even more precision.

The optical imaging techniques outlined by Bohndiek offers opportunities for diagnosis and theranostics, using targeted imaging of tumour bulk and margins to enable image-guided surgery. The technique is also safe and relatively cheap, and likely to become cheaper in the future, potentially making it suitable for use in a primary care setting.

Ultrasound and photoacoustic imaging are currently in a phase of rapid technological development for use in early detection, as discussed by **Jeff Bamber.** Multiparametric ultrasound tomography (UST) gives exquisitely detailed functional imaging information about soft tissue; UST can be used for detection of anomalous masses, and its ability to measure tissue stiffness, a strong indicator of an aggressive tumour, allows it to discriminate between benign and malignant cancers. It offers an attractive alternative to breast mammography, as it can quantitate breast density, one of the strongest risk factors for breast cancer, with an accuracy on par with MRI.

A WEARABLE ULTRASOUND PATCH IS IN DEVELOPMENT

In melanoma, UST can measure the extent and depth of skin lesions before, during and after treatment. A wearable ultrasound patch able to wirelessly transmit data is now in development, with the aim of monitoring response to therapy during melanoma treatment. Similar simplified devices could be used in primary care for early detection of cancerous changes in suspicious skin lesions.

Instead of pulsing sound into a tissue, photoacoustic imaging sends a transient pulse of light. This causes a pressure and temperature rise in the tissue, which generates sound waves detectable by standard ultrasound equipment. The hybrid technique offers the high contrast of optical imaging combined with the high resolution of ultrasound, allowing structural, functional and molecular imaging; molecules detected can be endogenous, such as melanin, or exogenous chromophores, meaning that existing FDA-approved agents can be easily used. However, whilst photoacoustic imaging can penetrate several centimetres of tissue, there is still scope to extend the distance of propagation.

Applications of photoacoustic imaging in early detection include measuring hypoxia. Microbubbles and nanocontrast agents make it possible to model the average oxygenation near a cancer cell. In melanoma, differential diagnosis of pigmented lesions and management of suspicious cases using photoacoustic techniques may mean that in future, the majority of patients with benign lesions can be diagnosed without the need for further referral.

GATHERING THE EVIDENCE THAT IMAGING-BASED SCREENING TECHNIQUES LEAD TO REDUCED MORTALITY WILL REQUIRE ENORMOUS, APPROPRIATELY POWERED CLINICAL TRIALS

Hybrid modalities such as photoacoustic imaging are exciting, and as research and development in this area is driven by interest in mobile phone technology and surveillance, major technological advances are likely. However, before introduction into primary care, several major issues need to be addressed: there is no gold standard; there are no good in vivo models, and standardised quality assurance is lacking.

Participants made a number of points in general discussion. Firstly, it is clear that MRI and other expensive and slow whole-body methods cannot be used as a first line diagnostic. There has to be a diagnosis funnel, whereby other criteria, probably drawn from liquid biopsy biomarkers and the cheaper photo and acoustic imaging techniques, are used to stratify the general population, with high-risk groups proceeding into more complex and expensive testing. In future, if implementation of early detection imaging techniques becomes widespread, it will also be necessary to think about pan-cancer or even pan-disease multi-organ imaging, to avoid multiple single-issue scans.

A number of challenges lie ahead. There has to be clinical evidence that such imaging-based screening techniques would lead to reduced mortality and this would require enormous, appropriately powered clinical trials. In the imaging field, this is fraught with difficulties as there is a high level of inter-operator and inter-machine variation in imaging data collection, meaning that co-registration of images and correlation with pathology is very difficult when attempting to analyse imaging data from multiple sources. Furthermore, there is no centralised image repository on a common open access platform, so data analysis will be hard. Whilst no solution currently exists, the imaging community is well aware that it lags behind other fields such as genome science and structural biology in standardising and sharing data, and is actively searching for ways forward.

SESSION 2: THE TUMOUR ENVIRONMENT (EXTRINSIC AND INTRINSIC)

Regulation of cancer growth depends not only on the classical intrinsic hallmarks of the cancer cells themselves, but also on their surroundings. Understanding and exploiting the interactions between the early tumour and its microenvironment will be critical to facilitate early detection.

Tumours contain many ostensibly normal cell types which can all contribute to the cancer cells' acquisition of hallmark traits. Therapeutic targeting of the tumour microenvironment is already widespread, but there is still much to learn, both in terms of the geography of the tumours as they progress, and the identity and function of the non-cancer cells. The switch from benign to aggressive disease is likely to depend in part on how permissive the microenvironment is to growth, so this is obviously a key area for early detection research. Further, the non-cancer cells might display detectable signals (for example in immune response) of the presence of a tumour before the cancer itself is detectable.

THE SWITCH FROM BENIGN TO AGGRESSIVE DISEASE IS LIKELY TO DEPEND ON HOW PERMISSIVE THE MICROENVIRONMENT IS TO GROWTH

Many of these themes were covered during the session and the discussion highlighted how greater communication between cancer biologists and immunologists working on microenvironment and those exploring markers for earlier detection of cancer is crucial moving forward.

Lisa Coussens opened the session with a discussion of what is appropriate to target in the tumour environment. Using partially penetrant genetically engineered mouse models (GEMMs) of cancer, her lab has shown that the biggest changes in the surrounding vasculature and extracellular matrix actually occur during the transition from normal to benign hyperplasia, rather than later in tumourigenesis. Similarly, immune cells are perturbed early on as part of a chronic inflammatory response. Tumour vasculature leakiness and immune cell recruitment suggests that the body may be detecting early cancer as a wound that needs to be healed. Studying the tissue-based programmes regulating chronic versus acute inflammation and tissue remodelling may therefore yield valuable clues to how cancers progress.

Focusing on the role of the immune system in tumour progression demonstrates that innate immunity plays a vital role; GEMM models of skin, pancreatic and lung cancer lacking mast cells only ever develop benign lesions. The role of B cells is also becoming clearer; in GEMM models of pancreatic ductal adenocarcinoma, reduction of B cell activity stimulates immune activation and inhibits tumorigenesis.

SIGNATURE PATTERNS OF CHANGES IN LEUKOCYTE POPULATIONS ARE CHARACTERISTIC OF VERY EARLY RESPONSES TO NASCENT TUMOURS

Using the powerful new technology of mass cytometry, **Matt Spitzer** provided further insight into how immune behaviour in different organs coordinates the response to a tumour. Mass cytometry uses elemental isotopes to tag cells, allowing sorting by mass of the isotope tag rather than light emitted by the fluorophore tags traditionally used in flow cytometry analysis. Using elemental isotope tags is favourable because the number of tags that can be used in any given experiment is not limited by the spectral overlap, as is the case with flurophore tags.

Using mass cytometry, the Spitzer lab can watch the entire immune system adapting to different conditions during cancer growth and therapy. As proof of principle, they used a mouse breast cancer model to track the immune response in untreated animals versus either an ineffective or an effective anti-tumour immunotherapy. By modelling increases and decreases in the prevalence of immune cells infiltrating tumours, and also their proliferative rate, they showed that tumour shrinkage is not accompanied by increased immune cell proliferation in the tumour. Rather, the immune response is driven by increased proliferation in the lymph nodes, spleen, blood and bone marrow. Blockade of egress from the lymph nodes and spleen results in treatment failure, demonstrating that systemic immunity is absolutely required for tumour eradication.

Whilst it still unclear whether the patterns of leukocyte flux also occur in response to infectious or automimmune disease, and therefore whether they are truly cancer-specific, this new technique has important implications for understanding the early development of cancer. In mouse models, there are signature patterns of changes in particular leukocyte populations which are characteristic of very early responses to nascent tumours, and signatures differ depending on which organ is harbouring the cancer.

Jenny Ting highlighted the importance of a deeper understanding of how innate immunity is involved in cancer. Inflammasomes are innate immune system receptors and sensors that regulate pro-inflammatory responses to host protein fragments and infectious microbes. The inflammasomes can activate the adaptive immune system and thus can enhance the body's anti-tumour immune response. However, the chronic activation of the inflammasome can promote carcinogenesis. Chronic inflammation augments the proliferative, anti-apoptotic, and pro-fibrogenic signals in the tumour microenvironment as well as in the tumour cells.

The formation of the inflammasome is driven by pattern recognition receptors (PRRs). Ting's talk focused on AIM2 and NLRX1, two non-canonical members of the NOD-like receptor (NLR) protein family of PRRs that behave as negative regulators and are potential therapeutic targets. Mouse models lacking AIM2 or NLRX1 have increased incidence of colitis-associated cancer, suggesting that both molecules are tumour suppressors. This finding is consistent with the findings from human cancers. There is potential for AIM2 and NLRX1 mutations as biomarkers for risk stratification in future early detection screening programmes.

Intriguingly, NLRP12, yet another non-canonical NLR family member, can attenuate colon inflammation by maintaining colonic microbial diversity and promoting commensal bacteria growth, reducing polyp formation. Data on the gut microbiome suggest that early detection of colon cancer will be enhanced by an understanding of how the gut flora interacts with the genetics of the host to give colitis.

EARLY DETECTION OF COLON CANCER WILL BE ENHANCED BY UNDERSTANDING HOW THE GUT FLORA INTERACTS WITH THE GENETICS OF THE HOST IN COLITIS

We know the individual cancer cells that make up a tumour are genetically heterogeneous, and their abilities to develop resistance to targeted therapies by rapid evolution is one of the major problems for successful cancer treatment. **Phil Jones** and colleagues (including **Inigo Martincorena**) showed that in samples of aged human eyelid skin, over 25% of cells are already carrying cancer-causing mutations despite behaving normally. However, these cells never out-compete their normal neighbours, suggesting that a restraining mechanism exists.

Mouse oesophagus is a good model for tracing cell fate, as all cells make the same binary decision: to divide, or to stop dividing and differentiate. It is the balance between these two outcomes that regulates tissue homeostasis, and an imbalance causing net cell division can result in transition into dysplasia. In a model of mouse oesophageal carcinogenesis, lineage tracing shows that the rate of cell division is not significantly different between lesions and surrounding normal epithelium. Instead, dividing tumour cells have a small bias in cell fate so that on average, the odds of a tumour cells producing dividing rather than non-dividing daughters is slightly higher than for normal cells. Over successive rounds of cycling, tumour cells will therefore begin to predominate. This bias in cell fate is even more evident in mutant KRas invasive cancers. Therefore, agents that can restore the balance of cell fate may be effective therapeutics, and might also promote a return to normality if used as a first-line intervention following early detection of pre-cancers.

RECRUITMENT OF DEVELOPMENTAL AND EVOLUTIONARY BIOLOGISTS AND IMMUNOLOGISTS TO WORK ON CANCER-RELATED PROBLEMS IS A PRIORITY

The wide-ranging nature of the discussions – concerning changes in cellular DNA right through to whole-organ and whole-body events – illustrated the breadth of the challenge in understanding the tumour environment. How nascent cancers induce stromal remodelling is poorly understood, as are the roles of ion homeostasis, pH changes and mutations in ion channel function. Further, in addition to lacking a complete picture of how the immune system interacts with cancer, very little is known about the role of platelets, recently shown to be strongly predictive of cancer when their levels are raised in blood; is there a possibility that platelet infiltration and activity could be a useful marker for early detection? Finally, how GWAS-determined risk factors and ethnic diversity affect the environmental 'soil' into which cancers seed themselves is almost wholly unexplored.

Creating next generation partially penetrant GEMMs which better mimic human biology will allow investigation of tumour-environment interactions, subversion of normal processes, and normal tissue evolution; in turn, such investigations could identify novel markers for early detection of cancers. Recruitment of developmental and evolutionary biologists and immunologists to work on cancer-related problems is also a priority, as understanding the normal environment is vital for understanding how cancers develop and thrive. At the other end of the spectrum, communication and collaboration between biologists and clinicians needs to be improved, so that studies using human tissue are better focussed and resourced. There is also a pressing need for bioinformatics tools for integration of tumour and environment biology into a complex model.

SESSION 3: LIQUID BIOPSIES AND CTDNA

To make blood biomarkers feasible for earlier detection, there are many problems to solve: apart from ensuring that signal-to-noise ratio is sufficient for accurate and sensitive detection, we need to know what we are detecting if individuals are mixtures of clonal populations, what the non-genetic constraints are on mutations, and finally, how immunological biomarkers will inform diagnosis.

The session explored these challenges and presented the latest strategies and understanding in the area. In the discussion that followed, the practicalities of implementing liquid biopsy in the clinic and the biological understanding that will be necessary to make early detection a reality were debated.

Looking at changes in immune activity may be one answer to the signal-to-noise problem. By virtue of the exponential nature of a primary immune response, the adaptive immune system acts as an amplifier and an early warning of disease. The challenge is how to recognise which T cell receptors (TCRs) are specific to particular tumours.

The antigenic specificity of T cells is largely determined by the amino acid sequence of the hypervariable CDR3 region of the TCR, which can therefore be used as a neutral marker to track the prevalence of a T cell clone within the total population. **Harlan Robins** described how clonoSEQ, which relies on next generation sequencing to rapidly sequence the CDR3 regions of an individual's entire T cell repertoire, can detect minimal residual disease, and hence early detection of relapse, prior to the existence of clinical symptoms. The presence of a cancer-specific T cell clone can be tracked following treatment at a frequency of less than one in a million cells, outperforming flow cytometry.

A CLONOSEQ APPROACH COULD HAVE POTENTIAL AS A PRIMARY DETECTION TECHNIQUE IN A LIQUID BIOPSY

T cell and B cell repertoires are specific for an individual and their cancer, facilitating the above approach in relapse when the antigen and TCR sequences are already known; TCRs specific for EGFR neo-antigens, which are common to many patients, have already been identified. Currently, a TCR antigen map is being created to enable rapid identification of TCR clones specific to any neo-antigen, something of intense interest in the immunotherapy field. With further research into early disease and understanding of the changes in the TCR repertoire occurring in early stages of cancer, this approach could have potential as primary detection technique as a liquid biopsy.

Jeff Pollard spoke about another important component of the tumour microenvironment: the monocyte-macrophage lineage. Innate immune cells are highly represented in the tumour microenvironment, with the most abundant cell type being tumour-associated macrophages (TAMs). In tumours where inflammation is a causal factor, TAMs are involved in tumour initiation, and they are also tumour promoters, supporting angiogenesis, invasion, migration and extravasation, as well as suppressing the immune response to the primary tumour. The closely related metastasis-associated macrophages (MAMs) are involved in almost all steps of the metastatic cascade.

TAMs and MAMs differentiate from distinct types of monocytes, but not all monocyte subspecies become macrophages. Some monocytes are functional cells in their own right, and have a role in tumour promotion; for example, monocytes produce VEGF, leading to vascular permeability. Given this functionality, it is highly likely that there is a cancer-specific monocyte signature detectable in tumours, and more importantly for early detection, in blood.

Inigo Martincorena discussed the challenges for genome-based diagnostics posed by his recent collaboration with **Phil Jones** showing that driver cancer mutations are extremely frequent in normal skin cells. Building on that work, deep sequencing of 1,000 samples of the upper and middle oesophagus (the tissue of origin of oesophageal squamous carcinoma) showed that whilst the mutation rate was lower than in skin, there were still 1,000-3,000 mutations per genome per cell, mostly in genes related to ageing. The enrichment of cancer driver mutations was even greater than in skin, with NOTCH1 and TP53 being particularly prominent.

LONGITUDINAL MONITORING TO DETECT DEVIATIONS FROM A BASELINE MUTATION RATE WOULD BE THE BEST STRATEGY TO DETECT CRITICAL INITIATING STEPS... BUT THIS IS EXPENSIVE

This work underlines the fact that mutation is necessary but not sufficient for cancer (although it is likely that multiple driver mutations in a single cell will be a predisposing factor), implying that other factors are needed, for example from the microenvironment. The bulk sequencing approach used means that it was not possible to distinguish between many cells with few mutations, and few cells with many mutations, so the mutational load of individual normal cells is not yet known. However, given the high background level of driver mutations in normal cells, using a single mutation as a circulating tumour DNA (ctDNA) diagnostic will be unlikely to specifically identify early neoplasia; a panel-based approach is more likely to be effective. One possibility is to use higher variant allele frequency combined with deeper copy number variant detection as a measure of excessive mutation, and hence a greater likelihood of cancer. There is also a need to determine the liquid biopsy source with the smallest driver burden, to try to reduce the mutational background; for example, should urine, or cerebrospinal fluid, be considered? To detect critical initiating steps in the transition from communities of mutated normal cells to aggressive cancer, longitudinal monitoring of individuals to detect deviations from a baseline mutation rate would be the best strategy, but this is expensive.

Such a personalised longitudinal approach to early detection is being explored as part of the TracerX study, as described by **Chris Abbosh**. TracerX is collecting longitudinal clinical samples from non-small cell lung cancer (NSLC) patients before, during and after treatment, and analysing them for mutational change. Blood samples are being used to try to intercept minimal residual disease (MRD) and predict relapse.

Not all primary NSLCs shed detectable amounts of ctDNA into the blood; whilst squamous cell carcinomas were all detectable, only 11/58 adenocarcinomas had a ctDNA signature. Adenocarcinomas could be subtyped to show that those with solid histology, necrosis, high levels of proliferation and metabolism, and lympho-vascular invasion were most likely to be shedding ctDNA.

There are several challenges for using ctDNA for early detection in NSCLC. Firstly, the prognostic impact of ctDNA is not yet clear – can detection of ctDNA in at-risk populations predict the aggressiveness of a subsequent NSCLC? Once clonal evolution in early NSCLC is better understood, fixed-gene capture panels need to be designed and validated, and analytical sensitivity must be sufficient to detect very early stage disease, where the concentration of ctDNAs will be extremely low in comparison to non-tumour circulating DNA; if tumour cell-free DNA is less than 0.1% of the total cell-free DNA, it becomes extremely hard to detect due to limitations of the technology, and very small early tumours result in proportions much lower than this.

TRACERx is also evaluating whether ctDNA can be used for early detection of relapse post-therapy. Early results indicate that ctDNA is detectable many months before clinically overt relapse can be diagnosed by CT scans, and that it may eventually be possible to identify potentially lethal clones and eliminate them in a targeted fashion. Further, if the lag time to sequencing the primary tumour material post-operatively could be reduced to just a few weeks, ctDNA could be used to inform treatment decisions. Again, clinical trials are required to determine whether earlier knowledge of MRD status both pre- and post-relapse will improve outcome.

Discussions centred on the problems of establishing clinical benefit from early detection by liquid biopsy; lead time bias is clearly a problem here, meaning that increased survival rates may not be a good endpoint. Earlier detection is valuable if it can lead to earlier and potentially milder interventions, thus improving patient outcomes. Success in early detection is also dependent on the parallel development of treatment strategies, to facilitate this.

It has yet to be established which biomarkers will be the most informative in a liquid biopsy. Although progress is being made in this area, it is likely that a combination of multiple different markers will be necessary to identify early cancers with specificity and sensitivity. Identifying the right makers is a challenge. For example, using immune cells as one component of an early detection biomarker panel would be risky, as current knowledge of cancer immunology is insufficient. ctDNA is another tempting target signal for a liquid biopsy, however its use if currently limited by the detection limit of the technology used. Very low amounts of ctDNA present in the blood of patients with early stage cancers, and further difficulties in detection is added by the background noise from circulating DNA from normal tissues. Exploring which fluids and markers would be most amenable to a liquid biopsy will be key questions to address when moving forward in this area. Large-scale multivariable clinical trials will be required to start addressing these issues, and for this, different disciplines need to communicate effectively to agree a joint approach.

IT IS LIKELY THAT A COMBINATION OF BIOMARKERS WILL BE NECESSARY TO IDENTIFY EARLY CANCERS WITH SPECIFICITY AND SENSITIVITY

SESSION 4: TECHNOLOGY

To be feasible, large-scale early detection measures must be cheap, available at point-of-care, preferably in the primary care setting or in all hospitals, and patient-friendly. There is a great deal of interest in both the academic and commercial sectors in developing the equipment to realise this ambition, and the speakers in the technology session provided a snapshot of what might be possible. The discussion at the end of this session focused on the interactions between academia and industry and ways to improve working for mutual benefit of both areas.

Billy Boyle discussed a promising method for patient-friendly diagnosis – using a breathalyser to detect volatile organic compounds (VOCs) as signatures of early disease. Although all bodily fluids contain thousands of VOCs, those contained in blood can be transferred into breath. Cancer's altered metabolism – the Warburg effect – means that VOCs may differ in cancer patients relative to a normal population. This has been validated in lung cancer, where VOCs present only in lung cancer patients were shown to drop post-surgery; VOCs specific to other cancers are currently being defined.

Boyle's company has developed a miniaturised mobile chemical detection system on a chip, which can be removed from a breathalyser and sent for diagnosis at a central lab. Using this technology, the phase II LuCID trial is currently running, with the aim of determining whether VOC biomarkers are effective in detecting lesions in patients under clinical suspicion of lung cancer, and whether indeterminate nodules can be stratified to identify those at risk of progressing. Phase III trials will involve population-based screening.

Boyle estimated that successful introduction of a lung cancer screening programme using VOC biomarkers could save approximately 3,200 lives per year and £82m in treatment costs in the UK alone. The technology, with its ability to detect multiple VOCs and hence multiple disease signatures, could be an effective early warning system of disease in general. Patients scoring positive on a VOC test could then be referred for further, more specialised diagnosis.

Jonas Korlach described a novel method for long-read sequencing, which is necessary to accurately sequence so-called NGS 'dead zones' – regions of high sequence homology which are currently not resolvable into the sequences of individual genes. Long read sequencing also means that individual alleles can be sequenced as a discrete entity, which allows one to determine how many mutations are present on each. Other uses include the identification of structural genetic variation such as rearrangements, and splice variants, and BCR and TCR repertoire sequencing, as the entire V(D)J region can be covered in a single read.

Long-read sequencing is the backbone of deep mutational functional screens – a high throughput approach to sequencing millions of variants of a gene and then assaying how the different variants perform in standard functional assays. Such genotype-phenotype screens generate large-scale datasets with information about protein properties and behaviour, and offer a rapid method of determining the functional relevance of the many mutations picked up in cancer cells. In a paper due out shortly, Korlach's company has used the technique to scan the BRCA1 gene mutational profile.

From sequencing technologies, the session moved on to **Utkan Demirci,** whose innovative technology applies a magnetic field to levitate cells, separating them based on their density, rather than their size. Cancer cells are abnormally light compared to normal cells in the blood, meaning that circulating tumour cells (CTCs) and CTC aggregates can be readily separated from other cells. So far, the technique has worked for colon, lung, renal and breast cancer cells. Currently, the sort rate is rather slow (2ml blood an hour), but the technique is scalable, and there is also potential for multiplexing. Platform validation with clinical samples shows that rare CTCs can be detected in renal and lung cancer patients.

CTC aggregates especially are proving to be extremely informative with respect to relapse and metastasis. Assessment of markers specific to epithelial and mesenchymal cells show that a 'double positive' population exists in the blood of cancer patients, probably matching an intermediate undergoing epithelial-mesenchymal transition (EMT). Quantitation of epithelial, mesenchymal and double positive intermediates shows that the latter only appear during relapse.

TO MOVE EARLY DETECTION RESEARCH FORWARD, WE SHOULD BE CLEAR ABOUT THE DIFFERENCES IN CULTURE AND DRIVERS FOR ACADEMIA AND INDUSTRY, AND WORK TO BUILD A PLATFORM FOR COLLABORATION THAT SUPPORTS EVERYONE.

Moving from CTCs to cell-free biomarkers of cancer, **Raj Krishnan** showed how putting samples into an A/C asymmetric electrical (ACE) field allows rapid isolation based on size, this means that cell-free (cf) DNA, RNA in exosomes, and aggregated protein biomarkers, which all lie in the 0.01µm - 1µm size range, distinct from the larger cells and smaller debris, can be captured directly from liquid biopsy. ACE is configured for on-chip analysis with marker staining, and has been miniaturised such that it can be run from a smartphone using a few microlitres of blood. Potentially, such devices could be used for easy longitudinal monitoring of disease, and early detection of relapse.

Cologuard, a stool test to detect the early signs of colorectal cancer (CRC), has been used by 800,000 people in the US, of whom 51% had never before been screened. Cologuard works by sampling a DNA/protein biomarker panel specific for polyps and CRC in stool, which normally contains almost no human DNA, and therefore has a very low background. Markers were identified first in CRC tissue, and then tested to see whether they were also found in polyps. Quantitative allele-specific target and signal amplification (Quart) assays are used to detect signals, and the system was refined using a 50,000-strong set of normal samples, and then trained on a 1,000-patient sample set.

Crucially, Cologuard is packaged in a way that minimises user exposure to the stool sample, probably the reason that it has higher uptake than less fastidious methods. The test is particularly attractive to health services, as it could replace colonoscopy with a cheaper, easier and more patient friendly alternative.

Rounding out the session, **Jasmin Fisher** discussed how oncogenic signalling can be deconstructed using computational cancer models. Her aim is to identify the mechanistic rules by which onset, regression and all the stages in between of cancer are regulated by oncogenic signalling. If successful, it may be possible to use in silico models to predict treatment options on a personalised basis.

During the discussions that followed the technology session, many delegates pointed out that academia working with industry can sometimes be a challenge. The need for a common language for communication, as well as a business model that reflects shared goals of both parties is apparent. Any future model should be more collaborative, to allow for a relationship beyond 'fee for service.' As technology is sometimes advancing ahead of the current state of research, there is an opportunity for industry to shape better informed experiments and fill-in knowledge gaps. To move early detection research forward, we should be clear about the differences in culture and drivers for academia and industry, and work to build a platform for collaboration that supports everyone.

FUTURE CHALLENGES

In addition to the issues described by the speakers, some overarching general challenges became apparent during the meeting. The first is a logistical one: the large amount of archived clinical, epidemiological and imaging data and the human tissue banks around the world are invaluable resources, but they are maintained by different disciplines, and inter- and intra-disciplinary information is not well-disseminated. For example, population/at-risk cohorts have been studied widely, but there is currently no easy method of finding those most relevant to early detection research. The challenge is to find the best way of cataloguing and using them, and to ensure that future collection of samples and data is done intelligently, with consideration given to the needs of all potential users.

In the imaging field, image harmonisation is a major problem; different institutes and technology platforms capture and process images in different ways (even within a single modality), and these must be harmonised in order to integrate the data and analyse across them. How this can be done is not yet clear, but such integration is necessary to develop accurate diagnostic tools for early detection (parenthetically, computer-aided diagnosis, perhaps the ultimate goal, may not be achievable in the short term; imaging data in the US is being used to develop clinical decision support tools, rather than computer-assisted diagnosis, partly because the former do not require a phase III trial to be approved by the FDA, but also because health insurance companies wish to retain control of deciding what risks they consider acceptable).

The second challenge concerns indeterminate diagnoses. When considering aggressive versus indolent tumours, extreme phenotypes are easy to distinguish, but the 'grey area' of potentially risky lesions is still very large. This problem can only increase; as implementation of early detection approaches becomes more widespread, many more people will fall into this grey area, not just for one cancer, but for many, and perhaps for more than one disease.

For the grey area to narrow, which is economically necessary if early detection is to succeed as a health intervention measure, it is essential that researchers and clinicians commit to using data from multiple modalities and sources in order to give more accurate prognoses. Further, longitudinal monitoring of the population, for example by a biannual wellness check, will in time become part of a normal health regime, and consideration must therefore be given to ensuring biopsies are as cheap, kind and information-rich as possible.

Developing efficient ways of rapidly proving that novel detection regimes are better than the current gold standards will be one of the rate-limiting steps to widespread adoption; it will be essential to ensure that trials have the appropriate multidisciplinary input to make them maximally informative, and to develop surrogates for mortality rates, so that the ability of the new intervention to discriminate between aggressive and indolent disease can be determined as quickly as possible.


CREW

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APPENDIX

Agenda

DAY 1 – Wednesday 20 September 2017

09:00 – 09:30	WELCOME
09:30 – 10:40	INNOVATIVE IMAGING (PART 1) Chairs: Kevin Brindle, Cancer Research UK Cambridge Institute Charles Springer, Oregon Health & Sciences University John Kurhanewicz, University of California San Francisco Robert Gillies, Moffitt Cancer Centre
10:40 – 11:10	BREAK
11:10 – 12:30	INNOVATIVE IMAGING (PART 2) Chair and Speaker: Sarah Bohndiek, Cancer Research UK Cambridge Institute Jeffrey Bamber, The Institute of Cancer Research
12:30 – 14:00	LUNCH
14:00 – 15:10	TUMOUR ENVIRONMENT (EXTRINSIC & INTRINSIC) (PART 1) Chair: Rebecca Fitzgerald, Medical Research Council Cancer Unit, University of Cambridge Chair and Speaker: Lisa Coussens, OHSU Knight Cancer Institute Phil Jones, Medical Research Council Cancer Unit & Wellcome Trust Sanger Institute
15:10 – 15:40	BREAK
15:40 – 17:00	TUMOUR ENVIRONMENT (EXTRINSIC & INTRINSIC) (PART 2) Matthew Spitzer, University of California San Francisco Jenny Ting, University of North Carolina
17:00 – 19:00	RECEPTION Cambridge Museum of Archaeology and Anthropology

DAY 2 – Thursday 21 September 2017

09:20 – 09:30	WELCOME
09:30 – 10:40	LIQUID BIOPSIES AND CTDNA (PART 1) Chairs: James Brenton, Cancer Research UK Cambridge Institute Paul Spellman, Oregon Health & Sciences University Harlan Robins, Fred Hutchinson Cancer Research Center Jeffrey Pollard, University of Edinburgh
10:40 – 11:10	BREAK
11:10 – 12:30	LIQUID BIOPSIES AND CTDNA (PART 2) Inigo Martincorena, Wellcome Trust Sanger Institute Chris Abbosh, University College London
12:30 – 14:00	LUNCH AND NETWORKING
14:00 – 17:00	NETWORKING ACTIVITIES Speed networking Punting – Granta Moorings, The River Cam
19:30 – 23:00	GALA DINNER King's College, Cambridge

DAY 3 – Friday 22 September 2017

09:20 – 09:30	WELCOME
09:30 – 10:40	TECHNOLOGY (PART 1) Chair: Sadik Esener, OHSU Knight Cancer Institute Chair and Speaker: Billy Boyle, Owlstone Medical Jonas Korlach, Pacific Biosciences Utkan Demirci, Canary Centre at Stanford Raj Krishnan, Biological Dynamics
10:40 – 11:10	BREAK
11:10 – 12:30	TECHNOLOGY (PART 2) Barry Berger, Exact Sciences Jasmin Fisher, University of Cambridge & Microsoft Research
12:30 – 13:30	LUNCH
13:30 – 17:00	DEFINING MAJOR CHALLENGES IN EARLY DETECTION RESEARCH AND FUNDING Chairs: Richard Gilbertson, Cancer Research UK Cambridge Centre Sara A. Courtneidge, OHSU Knight Cancer Institute A discussion on the themes emerging from the conference: • Challenges and barriers to progress • Gaps in our knowledge and expertise • Defining calls-to-action for the cancer research community • Exploring collaboration, multiple disciplines, research areas and sectors • Achieving meaningful progress towards health impact • Influencing funding strategy in early detection research.
17:00	MEETING CLOSE

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