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Chair’s Foreword


This Report highlights some of the ground-breaking research undertaken by the MCRC over the past year and provides real evidence for the progress that can be made when world-class organisations take a collaborative approach to tackling cancer in order to improve patient care.

The MCRC is a strategic alliance between The University of Manchester, The Christie NHS Foundation Trust and Cancer Research UK, an alliance that brings together a dedicated team of research scientists, clinicians and sophisticated facilities to better understand the causes and natural history of cancer, in order to develop and evaluate more effective treatment strategies.

The 2008 Research Assessment Exercise (RAE) confirmed The University of Manchester as amongst the UK’s research elite. In the 2008 RAE, 65% of all research activity at The University of Manchester, was judged to be ‘world-leading’ or ‘internationally excellent’, highlighting its internationally significant research profile over a wide range of subject areas. Within the specific area of Cancer Studies, 90% of all activity was judged world-leading or internationally excellent.

The Christie has been a specialist cancer hospital for over a century and continues to build on its global reputation for excellence in cancer research and treatment. Having been awarded NHS Foundation Trust status in April 2007, The Christie is able to respond more effectively to the needs and wishes of the community it serves. As the leading cancer centre for the Greater Manchester and Cheshire Cancer Network The Christie covers a population of 3.2 million and treats around 40,000 cancer patients every year.

Cancer Research UK is the world’s leading independent organisation dedicated to cancer research, supporting the work of over 4,500 scientists, doctors and nurses. In the year ending March 2008, Cancer Research UK raised a record £420 million, £333 million of which was spent on cancer research.

The MCRC represents a successful and powerful alliance of organisations with the experience, personnel and resources that will drive tangible improvements in cancer research and patient treatment. We have a Centre to be proud of and hope you enjoy reading about some of the achievements made over the year which underpins future progress for cancer patients.

Michael Oglesby
Chair
Manchester Cancer Research Centre
Introduction

The MCRC was established to exploit and build on the expertise and resources of its three partner organisations – The University of Manchester (incorporating the Paterson Institute for Cancer Research), The Christie NHS Foundation Trust and Cancer Research UK. Since the formation of the MCRC in 2006, we have developed successful strategic collaborations, bringing together internationally-recognised research scientists and clinicians to improve our understanding and treatment of cancer.

The MCRC Annual Research Report 2008 highlights some of the major achievements of the past 12 months. These achievements validate the approach we have taken to dovetail laboratory-based research with clinical trials by supporting and developing new ways of working together in multidisciplinary teams. Collaboration is not limited to academic and clinical departments but also embraces industrial partners. This is exemplified by the MCRC-AstraZeneca Alliance which was initiated in recognition of the new era of cancer medicine and the emergence of mechanism-based therapies.

A unique challenge of these new agents over conventional therapy is identifying markers of response or drug activity (biomarkers) that enable tailoring and individualisation of treatment. Working in partnership to develop and validate biomarkers that will expedite the availability of new treatments for patients, the Alliance has set up several initiatives. These include an innovative training scheme for Medical Oncologists that aims to meet the high and growing need for oncologists with expertise in clinical pharmacology, and a cell-death focused biomarker analysis programme which makes full use of some 14,000 blood samples collected annually in AstraZeneca trials world-wide. After a very fruitful two years, the programme has now been extended for an additional three years and will include analysis of biomarkers of circulating tumour cells and angiogenesis – the development of tumour vasculature essential for tumour growth and proliferation.

The ultimate goal of biomarker programmes is to optimise treatment for individual patients based on a solid knowledge of how they are likely to respond to a particular therapy and how their cancer is likely to behave. A second and complementary theme highlighted in this Report is the work being undertaken to define the genetic characteristics of individual patient tumours using microarray technology. MCRC researchers have pioneered a new technique to take advantage of the valuable repository of fixed biological samples which hitherto have not been amenable to analysis using conventional micro-array technology which generally requires fresh tissue samples. The new possibility of using fixed tissue means that researchers can acquire a genetic profile of even rare cancers and also track patient outcome.
in relation to the past and present characteristics of their cancer, helping to identify patients with poor prognostic genetic profiles who may benefit from more aggressive or targeted approaches early in the management of their disease.

We know that cancer is an aetiologically complex disease, characterised by abnormal differentiation and proliferation. Stem cell research undertaken by MCRC researchers is beginning to shed light on early differentiation in uncommitted, pluripotent cell lineages. One recent finding by the MCRC’s Stem Cell Biology Group, published early this year in Nature, has led to a radical revision in our understanding of the earliest steps of normal haematopoietic development. In tandem, the Stem Cell Haematopoiesis Group have discovered genes that promote self-renewal of early haematopoietic stem or progenitor cells in a manner similar to development of leukaemia.

The Breast Biology Group has found evidence that breast cancer stem cells are inherently resistant to current treatments. Identifying novel stem cell regulatory pathways that may overcome this resistance is a major focus of the Group’s work. Recent research by the Leukaemia Biology Group suggests that leukaemic stem cells are biologically distinct from the normal haematopoietic stem cells. The Group is also studying how these aberrant stem cell populations are maintained to identify key factors that promote self-renewal. These biological differences and key maintenance factors may provide rational therapeutic targets for novel anticancer agents.

Cell adhesion is essential in maintaining normal proliferation and survival, processes that are characteristically abnormal in cancer cells, and work by the Streuli laboratory focuses on understanding cell adhesion. In the past year, the team have made notable advances in identifying the genetic importance of integrins in determining cell fate. The group has found that integrin signalling proteins become elevated or activated in a high proportion of breast cancers, and more recently, that different adhesion proteins are involved in controlling the survival of distinct cell types. A major goal now is to identify epithelial-specific survival proteins that represent targets for novel anticancer therapies.

Cancer cells are also characterised by their autonomy – they are able to ignore external environmental stimuli which are usually passed from outside to inside the cell through signal transduction. Work by MCRC researchers is focussing on the phosphoinositide signalling system and how this is hijacked by cancer cells. The Inositide Laboratory have developed a high throughput assay for PIP5K, a kinase that plays a pivotal role in the regulation of a key phosphoinositide signalling molecule. In collaboration with Cancer Research
Technology, this assay has been used to screen approximately 100,000 compounds for their ability to inhibit PIP5K. Promising lead compounds are now being tested to assess their anticancer activity and their effect on normal cells.

Angiogenesis, the formation of new blood vessels, is an essential process for growth of solid tumours and has therefore attracted considerable attention as a potential target for therapeutic intervention. The process is driven by pro-angiogenic cytokines and the work of MCRC scientists has demonstrated a dependency on heparan sulfate (HS) for their biological activity. Understanding HS-cytokine interactions is therefore crucial and could lead to the development of new approaches to inhibit angiogenesis. Indeed, MCRC researchers featured in this Report have developed new inhibitors based on the chemical synthesis of fragments of HS which interfere with the normal function of HS in facilitating cytokine activity.

Studies on specific tumour types are a strategic priority for the MCRC and in last years report we highlighted the progress being made in breast cancer research. In this report we focus on studies in melanoma which have identified a crucial transcription factor in the signalling pathway most frequently aberrantly regulated in this cancer type. MCRC researchers have now developed experimental models for investigating cell signalling and melanoma formation and progression. They are using sophisticated somatic mutagenesis and screening technology to identify novel genes implicated in disease progression, generating \textit{in vitro} data for \textit{in vivo} evaluation, and \textit{vice versa}.

Along with development and evaluation of novel therapies, MCRC researchers focus on optimisation of conventional treatments and a particular area of expertise is radiation therapy – an important tool in the management of cancer with approximately two in five patients receiving this treatment. A major challenge is to identify characteristics which predict how a tumour will respond to therapy and this is being addressed by the Translational Radiobiology Group. Over the past 12 months the team have made real progress towards identification of a clinically useful, hypoxia-associated gene expression signature, and have secured a three-year 2008 MRC Biomarker grant to investigate whether the signature can be used to predict benefit from hypoxia modification therapy.

The establishment of a central Tissue Biobank has been a key aim of the MCRC as highlighted in last years report. This report provides a timely update on the progress of this ambitious programme. In the past 12 months over 200 patients have donated samples and these are now being characterised within a clinically-useful database. In addition, a comprehensive facility for construction and analyses of tissue microarrays is being developed within the Breakthrough Breast Cancer Research Unit at the Paterson institute for Cancer Research. These complementary facilities will provide researchers with resources to support a diverse range of research activity including biomarker validation and evaluation of potential therapeutic targets.
Advances in cancer therapy intrinsically rely on robust, well-conducted clinical trials to assess efficacy and safety of new treatments in adequate numbers of patients and volunteers. The expansion of clinical trials and related activities within the region is well underway with the development of a £35 million Cancer Treatment Centre, providing comprehensive facilities for clinical trials, experimental treatment, and service chemotherapy and due to open at the end of 2010. This new Centre will provide for significant expansion of early phase clinical trial activity and will be one of the biggest such facilities world-wide. We look forward to giving an update on the new Centre in forthcoming reports. In addition, in response to the increasingly complex logistical considerations and new procedural requirements when conducting clinical trials, a Clinical Trials Co-ordination Unit (CTU) has been established. The CTU will manage the full range of clinical trials activity within the MCRC to optimise trial conduct, ensure robustness of trial outputs and thereby facilitate availability of new treatments for patients.

The achievements and continued success of the MCRC are founded on the excellence of the scientists, clinicians and support service providers we are able to attract and retain, and on proactive succession planning. Over the period covered by this report, we have recruited a number of experienced new staff members. Professor Goran Landberg joined us from the University of Lund, Sweden, as Group Leader in the Breakthrough Breast Cancer Unit and also has a Chair in Molecular Pathology. He will play a prominent role in the MCRC Tissue Biobank programme and the development of a dedicated tissue microarray resource. Dr Ivan Ahel joined the MCRC from the Cancer Research UK London Research Institute, as a Junior Group Leader at the Paterson Institute to further strengthen research on DNA repair mechanisms and enhance radiation-related research activity within the MCRC. Dr Donald Ogilvie joined the MCRC from AstraZeneca and will be using his expertise and considerable experience to head the development of a new Drug Discovery Centre at the Paterson Institute. This is an exciting and important new venture funded by Cancer Research UK and will play a crucial role in our goal of translating our laboratory-based research into patient benefit. The Centre will be developed over the coming year.

We also say a highly appreciative farewell to Professor John Gallagher, head of the Glyco-Oncology group, who is retiring after over 30 years at the Paterson Institute and School of Cancer and Imaging Sciences. As a world expert in glyco-biology Professor Gallagher has made numerous important discoveries which, as described in this report, are now being exploited for potential therapeutic intervention.

2008 has been another exciting and fruitful year for the MCRC – we hope that this report gives you a flavour of some of the highlights over the past 12 months so that you can share in our excitement and optimism for the next 12 months and beyond.

Professor Nic Jones
Director
Manchester Cancer Research Centre
The University of Manchester

In less than two years, the partnership between The University of Manchester, The Christie NHS Foundation Trust and Cancer Research UK has established the MCRC as one of the largest research centres of its kind in Europe. There is now a genuine prospect of establishing Manchester as a world-leading centre for cancer research.

In the past year alone, the MCRC has strengthened its capability across the full spectrum of cancer research activity and has recruited a number of eminent researchers to lead its basic science portfolio. The real strength of the MCRC is evident in the University’s closer working relationship with The Christie. Building on that relationship will be vital to expanding our basic research effort and fostering the translation of the knowledge we gain into better patient care.

Professor Alan Gilbert
President & Vice-Chancellor

Professor Dame Nancy Rothwell
Deputy President & Deputy Vice-Chancellor

The Christie NHS Foundation Trust

This partnership has maximised the impact of activity in cancer research and is making Manchester one of the largest and most significant centres for cancer research and treatment.

We are driving forward ambitious plans to provide world class services to our patients, and developing our clinical research is a vital part of these plans. The MCRC partnership is enabling us to double our early clinical trial activity, providing us with the largest phase I clinical trials unit in the world in 2010. This will bring huge benefits to patients, which is ultimately what all our efforts are about.

Jim Martin
Chairman

Caroline Shaw
Chief Executive

Cancer Research UK

Cancer Research UK is delighted to be a partner in the MCRC. We are proud to have had a long association with the highest quality cancer research in Manchester, and the development of the MCRC fits perfectly with our vision that ‘Together we will beat cancer’.

Bringing scientists and clinicians closer together will help us to improve our understanding of cancer and find out how to prevent, diagnose and treat the many different kinds of the disease.

We are committed to building strong partnerships to maximize the opportunities for discoveries in the laboratory to translate into benefits for patients. The MCRC is a great example of such a partnership.

David Newbigging
Chairman

Harpal Kumar
Chief Executive
The development of mechanism based therapies (MBTs) mandates a parallel development of biomarkers to define the right drug(s) (predictive biomarkers), at the right dose and schedule (pharmacodynamic biomarkers) for the right patient. In the era of MBTs for cancer treatment, biomarker science is required to help inform the choice not only of Maximum Tolerated Dose (MTD) but also of Minimal Biologically Effective Dose and Optimal Biological Dose (MBED and OBD). Pharmacodynamic biomarkers are needed to show that drug has hit its target (Proof of Mechanism) and that tumour has responded appropriately (Proof of Principle). Intelligent use of biomarkers should promote the required transition from ‘Response Evaluation Criteria In Solid Tumours’ (RECIST)-dependent endpoints to drug mechanism specific endpoints and provoke the adoption of novel biomarker driven clinical trial designs for MBTs in Phase II. Predictive biomarkers are needed to direct patient stratification in order to exclude patients unlikely to benefit. Safety biomarkers should be implemented to report, or better still predict, drug toxicities. The emerging consensus is that judicious implementation of biomarkers early in the drug development process will reduce the common incidence of drugs failing after large and expensive late phase trials.

There are however, substantial challenges: the ongoing revolution in biomarker science now requires specialist training in translational science for the clinical trialists of the future; and access to the three major pharmacodynamic and predictive biomarker platforms, namely tissue, biofluid and tumour-imaging biomarkers. The acquisition of tumour tissue of sufficient quality and quantity for good biomarker science is not always possible in early clinical trial settings, and studies to improve and extend analysis of tumour biopsies are pivotally important. Collection of blood samples before and serially after novel drug administration is routine although there are few circulating biomarkers of proven clinical utility beyond
prognostication. Tumour based imaging continues to pose challenges in both accessibility for more specialised markers, and cost.

The MCRC-AZ alliance: its purpose and anatomy
With these considerations in mind, the MCRC and AstraZeneca have worked together productively on validation and implementation of pharmacodynamic and predictive biofluid biomarkers, on imaging biomarkers, and on tissue biomarkers including tissue banking. These themes together with basic pre-clinical research and radiation biology comprise the research agenda of the MCRC-AZ alliance as depicted pictorially. Each theme has both AZ and MCRC representation (see Figure).

AZ/MCRC Alliance: Anatomy

Figure The anatomy of the MCRC-AZ alliance. MCRC staff are shown in blue text, those from AZ in black.
The overall goal of the Alliance is to develop cutting edge biomarker science and implement multimodality biomarkers in early clinical trials to gain maximum information for successful drug development. Recognising joint goals and clear mutual benefit of increased interactions on biomarker science, the MCRC-AZ Alliance developed two initiatives in 2005 which serve to exemplify this; The Cancer Research UK-AZ Clinical Pharmacology Training Scheme and The AZ/CEP Serological Biomarkers Alliance.

The CR-UK-AZ medical oncology training scheme:
This innovative scheme for medical oncologists was set up in 2006. This was considered timely because a) there is national deficit in clinical pharmacology expertise in oncology, b) there is urgent need for innovative biomarkers of drug response, c) new platform technologies have emerged for clinical exploitation, d) there is a need to determine the molecular phenotype of a tumour through methods other than an invasive tumour biopsy and e) the urgent need to populate the expanded Derek Crowther Unit (DCU) in 2010 with clinical trialists cognisant of the biomarker imperative. Funding (50% CR-UK/Paterson Institute and 50% AZ) was awarded to support two Clinical Pharmacology Research Fellowships per annum each of three-year duration leading to the award of PhD. The post-holders have completed, or are close to obtaining, their Certificate of Completion of Specialist Training in Medical Oncology. They receive training in biomarker discovery, method development/validation and in clinical trial methodology. During tenure at The Christie NHS Foundation Trust and the Paterson Institute the post-holders receive clinical supervision from Malcolm Ranson and laboratory-based training from Caroline Dive in the Clinical and Experimental Pharmacology (CEP) Group in collaboration with MCRC colleagues. At AstraZeneca they receive training in clinical trials management, regulatory interaction, translational research through project management and attendance at investigator meetings, congresses and management meetings. Clinical training includes one research clinic per week, training in clinical trial design and methodology, International Committee on Harmonisation – Good Clinical Practice (ICH-GCP), EU Directives and research governance. Biomarker method development and application takes place on both sites in all projects with mutual benefit as each fellow brings newly acquired knowledge to each site. Regular meetings take place between the fellows, Andrew Hughes, Malcolm Ranson and Caroline Dive with associated appropriate staff ensuring true collaboration and a ‘joined up’ approach. By the first quarter of 2009 six clinical fellows will have been recruited to this scheme studying circulating biomarkers of cell death or of angiogenesis, mutations in circulating free DNA, circulating tumour cells in several cancer types and window trials of a novel AZ drug in prostate cancer. The first two fellows enrolled in 2006 are now completing their PhD and have achieved considerable national and international progress in the field of biomarker research:

Circulating cell death markers: measuring tumour response from a blood sample (Alastair Greystoke)
The first fellowship project focussed on understanding the behaviour of novel circulating biomarkers of cell death in cohorts of cancer patients on standards of care therapies where response rates, overall survival and toxicity profiles of the administered drugs are well established. It is clear that many MBTs will be used ultimately in combination therapies, and as such it will be important to be able to interpret biomarker profiles generated by conventional agents. The M65 and M30 assays had been validated for clinical use by CEP and measure full length and/or caspase-cleaved cytokeratin 18 released into the circulation from dying epithelial cells. Release of nucleosomal DNA (nDNA) from nucleated cells undergoing apoptotic cell death was also evaluated. Within 18 months over 200 patients were recruited and over 4,500 serial samples have been collected and logged into the CEP Good Clinical Laboratory Practice (GCLP) system at over 1000 time points and a series of studies to optimise the biomarker assays for routine clinical use was completed. The
utility of nDNA, M65 and M30 biomarkers has been explored in several cancer types including small cell lung cancer (SCLC), colorectal cancer (CRC) and lymphoma, where the potential clinical utility of these relatively inexpensive blood tests are emerging as early markers of biological activity across a number of cytotoxics and MBTs. For example in lymphoma, our pilot study with Professors Tim Illidge and John Radford at The Christie, suggests that baseline nDNA predicts therapy promoted tumour shrinkage after several months post treatment.

Circulating free DNA- cfDNA: a virtual tumour biopsy (Ruth Board)

A number of tumour-specific mutations, such as EGFR and K-ras in non-SCLC and CRC, predict response to certain MBTs and progression free survival (PFS). Mutation detection is traditionally performed on archival tissue samples which may no longer reflect current tumour biology, and can be difficult to obtain, especially within the context of clinical trials. Reliable detection of tumour-specific mutations within cfDNA provides an alternative that is minimally invasive, can be performed on serial samples and potentially provides a ‘real time’ assessment of the tumour mutation status to guide clinical decision making. Our second fellowship project is investigating the utility of detecting mutations in cfDNA. Methods for extracting circulating DNA were optimised and sample collection is ongoing of matched tumour and blood samples for DNA mutation detection for EGFR, K-Ras, B-raf and PIK3CA in relevant tumour contexts. Deregulation of the PI-3K pathway in cancer is prevalent, most notably with inactivating mutations of the tumour suppressor gene PTEN or activating mutations in the PIK3CA gene that encodes the catalytic p110α subunit of PI-3K. In order to begin to address the clinical utility of PIK3CA mutation detection as a biomarker, and in collaboration with experts at Manchester based DxS Diagnostics, we participated in the generation of a novel assay based on Amplification Refractory Mutation System (ARMS) allele-specific PCR and Scorpion primers (DxS Diagnostics) to detect the four most common mutations in the PIK3CA gene. The resultant high throughput, multiplexed ARMS assays of these PIKC3A mutations are more sensitive than sequencing. Our first clinical study was of patients with metastatic breast cancer where we obtained plasma, serum and matched archival paraffin embedded tumour samples. So far there is a >96% concordance rate between
plasma and tumour PIK3CA incidence suggesting that plasma derived cfDNA can be used as an alternative to tissue DNA for PIK3CA mutation detection at least in breast cancer. To our knowledge, we are the first group to demonstrate circulating PIK3CA mutations in cancer patients and it will be exciting to determine in the future the predictive potential of this circulating biomarker in patients administered novel agents targeted at the PI-3K signalling pathway. Further work will be needed to validate this approach in other disease settings and for other mutations.

The AstraZeneca serological biomarkers alliance with CEP
From 2006, CEP has undertaken to analyse circulating biomarkers of cell death on up to 14,000 blood samples per annum from AZ clinical trials conducted worldwide and to participate in the interpretation of the data generated and reported back to AZ. The analyses are performed to GCLP standards. This joint working facilitates faster progression to biomarker qualification (proving clinical utility of a biomarker). The funding model allows for significant ‘blue sky’ funding into CEP for additional biomarker research in addition to the contractual assay volume provision. Two successful years of the alliance confirmed that the CEP GCLP laboratory is capable of meeting industry standards for biomarker analysis. Quality Management Systems have been implemented for high throughput analysis and which are also adaptable to benefit the wider CEP group, and for large scale trials in the future. Importantly, recent completion of the first early clinical trial of the aurora kinase inhibitor on which CEP ran M30 and M65 serological biomarkers revealed that the M65 data (interpreted as tumour burden) could contribute to dose selection going forward to Phase II. Furthermore, the ability for samples acquired throughout the globe during dose setting trials to be sent to a “central laboratory” to enable standardisation and quality minimises the variability and thus challenges to interpretation should samples be analysed through a local laboratory model. The juxtaposition of CEP with AZ’s main oncology development site at Alderley Park, Macclesfield is a major benefit to iterative science - enabling researchers to review, discuss and conclude on data interpretation. The success of the initial two years of this alliance was demonstrated in the recent decision to extend for the alliance for a further three years and double in size and capacity to include circulating biomarkers of angiogenesis and circulating tumour cells.

Other themes: building for the future
The “partnership working” and “team science” ethos exemplified above from the “blood-borne biomarkers” theme have very much provided the foundation for the remaining five themes. Within the “Phase 0/1 theme”, the timelines for setting up a new clinical trial have halved during 2008; with currently some seven separate investigational agents from AZ undergoing over a dozen clinical trials within MCRC. A new scheme aims to provide clinical researchers at MCRC with access to investigational agents which have passed Phase I testing to conduct investigator-designed studies under the sponsorship of The Christie NHS Foundation Trust. Under the “human cancer tissue” theme, tissue collectors based at Manchester’s five main hospitals who care for cancer patients ensure that consented, high quality cancer tissue is brought into MCRC’s tissue bank to facilitate basic research into the mechanistic understanding of cancer. New technologies of multiplexing novel markers in human tissue, including the use of Q-dots, have also been explored. Cancer research “imaging” with its state-of-the-art PET and MRI capability at the MCRC Wolfson Molecular Imaging Centre (WMIC) facility has resulted in projects comparing the reproducibility and sensitivity of DCE-MRI - the research gold-standard for assessing the dynamic effects of anti-angiogenic agents - with DCE-CT which
The MCRC-AstraZeneca Alliance

is more widely available; and has provided opportunities for joint research placements at post-doctoral and professorial level between MCRC and AZ. A pre-clinical study with FDG-PET conducted under the AZ-MCRC alliance was one of the first pre-clinical experiments conducted in the new WMIC facilities. The “radiation-related research” theme builds on a successful pre-clinical collaboration which has screened \textit{in vivo} the potential radio-enhancing activity of a number of AZ investigative agents now in Phase III testing. One example is a MEK inhibitor which is the basis for a Phase II investigational study in combination with radiotherapy in NSCLC being conducted by an inaugural fellow to the CR-UK-AZ clinical oncology scheme which parallels the successful medical oncology fellowship scheme described above. Finally, the “pre-clinical” theme which has oversight of work conducted in both the Paterson Institute and the Faculty of Life Sciences pre-clinical centres is planning to hold a science day during 2009 to expand on previously successful collaborations centred particularly around regulation of cell cycle control and invasion.

Complementing these initiatives, a MCRC-AZ Biomarkers Club run by Dive and Hughes has been running quarterly between the two sites (nine half-day symposia over two years since inception each with up to 100 attendees) and has covered the following topics: PD Biomarkers of angiogenesis, of apoptosis, of invasion, hypoxia, methodology to qualify a biomarker, establishing linkage of target to disease, personalised medicine, drug combinations, and optimising scheduling. These meetings serve to ensure regular contact between respective biomarker communities and to progress the original joint mission of embedding biomarkers into clinical trials to facilitate the delivery of the right drug at the right dose to the right patient.
Affymetrix gene expression profiling of archival formalin-fixed and paraffin embedded tissues

By Kim Linton, Yvonne Hey, Stuart Pepper and John Radford

The successful development of reliable protocols for microarray analysis of formalin-fixed paraffin embedded (FFPE) tissues has eluded scientists for years. Building on exciting recent developments in the fields of RNA extraction, microarray platform technology and bioinformatic expertise, MCRC scientists and clinicians have become one of first groups in the world to successfully develop this technology using the Affymetrix platform, thereby opening the way to the molecular biology investigation of rare tumours that until now have been impossible to understand using standard microarray approaches. This short report elaborates our progress to date and outlines our future plans for protocol development.

Whole genome expression microarrays are powerful chips containing short representative sequences of all known and predicted genes in the genome. They have very successfully been used to probe cancer tissues (or more specifically the messenger RNA isolated from these tissues) as a means of discovering which genes and/or expression patterns drive tumour development and behaviour. This information is already being used to aid the management of common cancers such as breast and colon cancers and lymphoma. Microarrays work best using RNA obtained from unfixed cells or tissue; despite years of scientific research, results from fixed tissues such as FFPE (formalin-fixed and paraffin embedded tissue - the most common method of preserving tissue) have been disappointing. The main reason for this is that formalin fixation modifies and fragments RNA to such an extent that it cannot easily be isolated from tissues or recognised by microarray probes. As a result, the overwhelming majority of microarray work to date has been conducted on common cancers, where fresh tissues can be obtained in a timely manner, and which as a consequence are now far better understood at a molecular level than rare cancers.
Being able to make use of the vast archives of FFPE tissues for molecular biology research would make it possible not only to discover which genes are important in the development of rare cancers, where fresh-tissue supplies may be limited or non-existent, but also to retrospectively profile original biopsy specimens and relate this to patients’ clinical outcomes after many years of follow-up. In this way it will be possible to discover which gene expression patterns or signatures predict outcome and most importantly to identify patients, at the time of their diagnosis, who are more likely to have a poor prognosis. These patients may benefit from individualised or more aggressive treatment strategies, including the use of novel experimental agents in clinical trials.

Technological advances in the past few decades have made it possible to adapt laboratory methods such as polymerase chain reaction (PCR) to suit FFPE tissues - to measure a few known genes at a time - but, until now, extension to whole genome microarray analysis of all known genes in a single experiment has been impossible with FFPE. Thanks to several major advances in the fields of RNA extraction, microarray design and more sophisticated bioinformatic analysis of microarray data, MCRC researchers at the Paterson Institute for Cancer Research, The Christie NHS Foundation Trust and The University of Manchester were among the first to successfully develop a reliable and robust approach to the microarray analysis of archival FFPE tissues. This short report will explain how we achieved this success and how we intend to develop this technology further.

**Gene expression analysis using unmodified state-of-the art approaches**

Our earliest work focussed on improving RNA extraction methods. We were able to improve RNA yields from archival FFPE samples by adding higher concentrations of digesting enzymes, subjecting tissues to longer digestion periods and more mechanical disruption, and heating to high temperatures...
to help break down the fixative-induced chemical bonds that trap RNA. The isolated RNA was nevertheless highly fragmented, a phenomenon that cannot be reversed even with the best extraction protocols. In order to compensate for this, we chose Affymetrix Plus 2.0 microarrays to interrogate FFPE RNA as they contain multiple short probesets for each gene, which are more likely to find their match in fragmented samples than arrays containing long probes. We had previously gained considerable experience using the Plus 2.0 platform to profile unfixed tissues. Another reason for choosing Affymetrix is that the presence of several inbuilt quality control features places them amongst the most reliable and robust expression microarrays on the market today, thus inspiring confidence in the validity of the data.

Using Plus 2.0 arrays, we profiled a series of 34 archival FFPE soft tissue sarcoma (STS) samples ranging between 1-5 years old, choosing cases based on their yield and purity results as we had no other means to predict array success. About half of the arrays met the strict Affymetrix quality control parameters, hence only these arrays were deemed reliable enough for data analysis. Using supervised hierarchical clustering, an analytical tool used to find the closest associations among gene profiles to known parameters, we generated a prognostic signature of 500 differentially regulated genes for cases with or without metastatic recurrence (distant dissemination of disease) within 18 months of diagnosis. These gene signatures depicted in Figure 1 clearly separated patients according to metastatic outcome and Interestingly were found to contain many genes already known to be important in cancer biology, a fact that supports the validity of gene selection over and above statistical chance.

Encouraged by these data, we proceeded to test the technical reliability of the Plus 2.0 microarray by comparing data obtained from microarrays with that from real-time PCR, which can be considered as the gold standard assay for measuring gene expression in FFPE samples. Figure 2 summarises these results: the amount of gene expressed in metastatic versus non-metastatic outcome cases was similar by both methods in nearly 80% of cases (19 of 24 genes tested, overall R2=0.4662), a promising result considering that real-time PCR and microarray analyses on a given sample do not always correlate even when RNA quality is optimal.
The final proof needed before a gene can be prospectively evaluated as a prognostic biomarker is that it performs equally well in test and independent cases. Using real-time PCR, we measured the expression of the above 24 genes in 69 independent samples. Approximately one third of the genes retained prognostic value, including RECQL4, FRRS1, CFH and MET. Most importantly, the combined expression of these four genes in this dataset carried significantly greater prognostic value than tumour grade, currently the best prognostic indicator for STS. This finding alone strongly supports the hypothesis that genes identified by expression microarrays on archival FFPE contain valuable prognostic information. Since cellular function is determined by protein expression, we also examined protein expression patterns for three of the identified candidate genes using immunohistochemistry in 85 independent samples. Significantly higher expression was seen in malignant versus benign, and metastatic versus non-metastatic cases for two well-known tyrosine kinase receptors: TRKB and cmet. We concluded from these results that biologically relevant genes can be identified from archival FFPE using adapted microarray protocols and, moreover, found several genes and proteins worthy of further investigation in soft tissue sarcoma.

Modification of gene expression protocols for archival FFPE

Notwithstanding the clinical utility of data obtained from microarray analysis of archival FFPE RNA, the above approach detected only half of the transcripts (genes) that were detectable, using the same microarray platform, in paired unfixed frozen tissue from the same tumour. Relative to full specificity in unfixed tissue (which we have assumed), few genes were found exclusively in FFPE, corresponding with a high specificity of 95% and suggesting that those genes that are being detected are reliable. Others have shown that FFPE profiles mirror those in unfixed tissues, supporting the notion that FFPE faithfully retains the majority of inter-gene expression relationships.

In order to ensure that all potentially interesting and important genes are captured in FFPE assays, it is important to improve gene microarray detection rates from FFPE. In the next series of experiments, we improved gene detection rates by focussing on two important areas: RNA labelling technique and choice of microarray platform.
RNA labelling refers to the laboratory method used to amplify and label RNA to enable it to be detected once it has hybridised to the corresponding probe on the microarray. The earlier protocols we used employed oligo(dT) primers, which anneal to the adenylated regions of a transcript, the richest being the so-called polyA tail. Being highly fragmented, labelled FFPE RNA preparations may therefore only contain the transcript fragments with a polyA tail, as most fragments discontinuous with the tail will not be labelled. Another disadvantage of using oligo(dT) primers is that adenylated regions are preferentially modified by formalin such that primer annealing may be problematic even in the presence of an intact tail. Others have shown that random hexamers improve gene detection in FFPE because these primers are not restricted to any particular sequence. We tested the recently launched NuGEN WT-Ovation amplification and labelling systems, which uses both random and oligo(dT) primers, and found that this method improved the number of transcripts detected in the same FFPE sample by an average 15-20 percentage points compared with our earlier method (the one-cycle Affymetrix approach), giving gene detection rates of 32-55% from archival FFPE. This closely resembles that expected with intact RNA from unfixed cells and tissues. Whilst we were previously able to detect in FFPE only half of the genes detected in paired unfixed tissue, we can now detect over 80% of the transcripts using the NuGEN labelling method.

Our next aim was to identify the best microarray platform for FFPE applications. The recently launched Affymetrix Exon arrays differ markedly from other expression arrays in having multiple independent probesets for every known and predicted exon in the genome (instead of a generally one-one mapping between probeset and gene) and in employing a combined oligo(dT) and random hexamer labelling and amplification approach for whole transcript reporting. We hypothesised that multiple interrogation points (around 40 probes per gene) would prove advantageous for FFPE samples where degradation between samples is not consistent. Running the same sample pairs of FFPE and unfixed frozen tissues as used on Plus 2.0 arrays, the sensitivity of gene detection on Exon arrays improved dramatically to 93% on average. Specificity remained high, suggesting, as before, that false positive rates are low and therefore that detected genes are reliable.

![Figure 3. Increased transcript detection with Exon arrays.](image)
We also found that significantly more transcripts were detected on Exon arrays compared to Plus 2.0 arrays; whilst almost 95% of genes detected on Plus 2.0 were present on Exon arrays, in some cases Exon-detected transcripts were simply not present on the Plus 2.0 array. In order to enumerate the absolute increase in gene detection on Exon arrays compared to Plus 2.0, we created a list of 5383 genes which had no probesets called present on any of the four Plus 2.0 technical replicates, and mapped this list back to exon probesets (Figure 3A). As shown in Figure 3B, 3943 of these genes had at least one probeset detected on the Exon array. By setting confidence limits to genes detected by at least five probesets, Exon arrays detected an additional 609 genes compared to Plus 2.0 (Figure 3C).

Conclusions
The results of these experiments confirm that Affymetrix microarray analysis of archival FFPE is both technically reliable and capable of identifying biologically relevant information. By improving gene detection rates from FFPE, we can now interrogate FFPE to a similar standard achievable from unfixed tissues using either Plus 2.0 or Exon arrays, with the latter performing especially well with FFPE. The next important steps are to confirm these results in larger series and to examine the relevance of genes selected from FFPE against known information by studying a tumour that is well understood at a molecular level (as opposed to the studies we have conducted to date on STS, a rare tumour that is poorly understood at a molecular level). Our next project will compare microarray profiles from FFPE and paired unfixed tissues of diffuse large B cell lymphoma (DLBCL) and is being conducted in collaboration with scientists at the University of Nebraska who have been instrumental in the detailed molecular characterisation of DLBCL using Plus 2.0 microarrays. In this way we hope to confidently establish whether all known or important genes are detectable in FFPE (relative to unfixed tissues) and whether known inter-gene relationships and pathways are faithfully retained in FFPE. This important work, which has been possible only through the close collaborations facilitated by the MCRF, will complete a series validation experiments necessary to develop a highly robust, reliable and reproducible method for gene expression profiling of archival FFPE – and will thereby herald the arrival of a valuable and much-awaited new research tool that will cause a paradigm shift in the way we approach molecular biology investigation, especially of rare tumours.
Stem Cell Research at the Manchester Cancer Research Centre

By Tim Somervaille, Valerie Kouskoff, Georges Lacaud and Rob Clarke

At its simplest, a stem cell has two properties: a capacity to self-renew and a capacity to differentiate. When a stem cell divides and produces a daughter cell with the same proliferative potential and differentiation state as its parent, the cell is said to have undergone self-renewal. In this case the daughter cell is biologically identical to the parent cell. Alternatively, stem cell division may result in the production of a daughter cell committed to becoming a more specialised cell type in a process called differentiation (Figure 1).

Two important types of stem cell are embryonic stem cells (ESCs) and adult-type stem cells (ASCs). ESCs are found in the inner cell mass of the blastocyst and ultimately have the capacity to generate all the different tissues of an embryo. By contrast ASCs are responsible for sustaining certain specialised tissues. Some examples include (i) haematopoietic stem cells (HSCs), which sustain haematopoiesis for a lifetime through the generation of progeny that differentiate into erythrocytes, lymphocytes, granulocytes and megakaryocytes, (ii) intestinal stem cells that differentiate into enterocytes, goblet cells and Paneth cells, (iii) breast stem cells that differentiate into luminal epithelial cells and basal myoepithelial cells and (iv) neural stem cells that differentiate into neurons, astrocytes and oligodendrocytes.

Understanding the biology of normal stem cells is important because many of the genes and cellular pathways that promote self-renewal under normal circumstances are disordered in human cancers. Indeed, a key novel concept for human cancer is that some malignancies are sustained by sub-populations of cells that have aberrantly acquired an ability to undergo self-renewal. These so-called cancer stem cells (CSCs) represent a third distinct kind of stem cell. In an analogous fashion to the organisation of normal tissues, CSCs sit at the apex of a disordered and often dysplastic
cellular hierarchy with the ability both to self-renew and to differentiate into cells that lack self-renewal potential. The concept of CSCs is important therapeutically because in order for malignancies to be cured CSCs must be eradicated. Failure to achieve this results in disease relapse. For these reasons, there is currently considerable interest in improving the understanding of the biology of all types of stem cell and within the Manchester Cancer Research Centre four groups are working in this area.

Georges Lacaud: Stem Cell Biology Group
The transcription factors RUNX1 (AML1) and MYST3 (MOZ) are frequent targets of gene rearrangements in human acute leukaemia and are also important in normal haematopoietic development. The group’s work focuses on the role of these genes in the development and maintenance of HSCs, with the aim of better understanding how novel oncogenic fusions such as RUNX1-RUNX1T1 (AML1-ETO) or MYST3-NCOA2 (MOZ-TIF2) induce and sustain leukaemia stem cells.

In work recently published by the group in Nature, the understanding of the earliest steps of normal haematopoietic development has been revised. Previously it was thought that yolk sac haematopoietic cells and endothelial cells arise directly from a common precursor called a haemangioblast. Using methodology including in vitro culture of ESCs and in vivo analyses, it has now been discovered that haemangioblasts first undergo phenotypic differentiation along the endothelial cell lineage. A set of the resulting cells, collectively called haemogenic endothelium, then produce

Figure 2: Haematopoietic cells generated in vitro through ESC differentiation.
haematopoietic cells. Critically, while RUNX1 is essential for the generation of haematopoietic cells from the haemogenic endothelium, another transcription factor TAL1 is essential for the establishment of haemogenic endothelium from the haemangioblast.

RUNX1 is likely to regulate the expression of an important set of genes at this early stage of normal haematopoiesis and ongoing studies using Runx1 deficient ESCs, promoter assays, microarray analyses and chromatin immunoprecipitation aim to identify and functionally validate these. Furthermore, the group is also working to uncover the function of the different naturally occurring spliced isoforms of Runx1 through studies involving genetic knockin and knockout strategies.

RUNX1-RUNX1T1 (or AML1-ETO), which is frequently associated with human acute myeloid leukaemia (AML), is unable by itself to induce acute leukaemia in experimental mouse models. By contrast, an alternatively spliced isoform induces rapid development of leukaemia. To further investigate how the alternate splicing leads so effectively to the generation of leukaemia stem cells, a mouse model has been developed where this novel isoform can be inducibly expressed and analyses are now underway.

MYST3 is mutated by chromosomal translocation in human AML, resulting in novel oncogenic fusions with CREBBP (CBP), EP300 (p300) or NCOA2 (TIF2). All these fusion partners encode enzymes containing a histone acetyltransferase (HAT) domain, suggesting that aberrant modification of histones could be essential in initiating a leukaemia stem cell hierarchy. To study the role of MYST3 in normal HSCs, a knockin mouse has been generated where MYST3 HAT activity is deleted due to a single amino acid mutation. The resulting mice have a profound reduction in HSCs demonstrating the critical role of MYST3 mediated histone acetylation in promoting normal HSC self-renewal. Current efforts aim to identify the precise mechanisms affected in the absence of the HAT activity of MYST3.

![Figure 3. Schematic representation of the differentiation steps of an ESC into haematopoietic cells](image)
Valerie Kouskoff: Stem Cell Haematopoiesis Group

The emergence of haematopoietic cells during embryonic life occurs soon after gastrulation. A tight coordination of proliferation, differentiation and migration during the generation of these first blood cells ensures the proper survival and growth of the developing embryo. Understanding the molecular mechanisms that control the formation of these blood precursors from the mesodermal germ layer is the major focus of our laboratory.

Several lines of evidence suggest that during adult life leukaemogenesis can result from the inappropriate expression of genes controlling critical steps of this embryonic program. A clear understanding of the molecular mechanisms orchestrating the onset of haematopoietic specification should help us to better define the basis of deregulated self-renewal observed in haematological malignancies.

The in vitro differentiation of ESCs along the haematopoietic lineage accurately recapitulates in vivo haematopoietic development. During differentiation, distinct cell types may be isolated and characterised (Figure 3). We have performed comparative transcriptional profiling of several of these populations to define novel cell markers and the key genes and pathways that regulate them.

Our experiments have identified Cd40 and Icam2 as up-regulated at haemangioblast commitment. Low levels of Cd40 expression specifically define the Flk1+ haemangioblast subpopulation. As haemangioblasts differentiate to haematopoietic precursors Icam2 expression is switched on while Cd40 expression becomes significantly higher. In multi-parameter analysis, integration of these markers into the previously defined pathway of blood differentiation has allowed us to further refine and identify discrete steps during the specification of mesoderm into fully restricted blood cells.

Microarray analyses also demonstrated that two transcription factor genes, Sox7 and Sox18, were sharply but transiently up-regulated at the onset of haematopoietic development. Using various experimental systems, we found that these genes act to promote self-renewal of early haematopoietic stem or progenitor cells through activation of the Wnt signalling pathway, in an analogous manner to leukaemogenesis. Current experiments aim to dissect the key components of this molecular program.

Rob Clarke: Breast Biology Group

The Breast Biology group is working to identify and characterise stem cells in both normal mammary epithelium and human breast cancer. Understanding the function of normal mammary epithelial stem cells is important because they may be targeted by cancer-initiating mutations. Furthermore, genes and pathways that regulate their normal function are often deregulated in breast cancer stem cells. Identification of breast CSCs may also prove critical for the identification of treatment-resistant cells and micrometastases which can re-initiate tumours. Characterisation of the molecular mechanisms by which CSCs undergo self-renewal may result in the discovery of novel targets for effective therapeutic intervention.
The group is currently working to improve identification of breast stem cells using gene expression and proteomic studies. *In vitro* assays such as the mammosphere forming assay (Figure 4) or *in vivo* transfer of primary or cell line material into immune deficient mice are used to assay stem cell potential.

The group has evidence that breast cancer stem cells are inherently resistant to current treatments, including radio-, chemo- and endocrine therapies. Thus, targeting stem cell regulatory pathways, inducing differentiation or interfering with other resistance mechanisms such as survival signals and DNA damage and repair are important areas of research. Stem cell regulation by developmental signalling pathways such as Notch, Wnt, Hedgehog, prolactin, steroid hormones and growth factors are also being studied.

A second aim of the group is to understand how steroid hormones such as oestrogen regulate cellular hierarchies in normal and malignant breast tissue, because both are hormone dependent. Since mammary development and breast tumourigenesis are promoted by the ovarian steroids oestrogen and progesterone, the mechanisms by which these hormones control stem cells and proliferative progeny in normal breast tissue is of interest. The group was the first to demonstrate that steroid receptors are limited to a non-proliferative sub-population, often found adjacent to dividing cells, suggesting that proliferation may be controlled via paracrine factors.

**Tim Somervaille: Leukaemia Biology Group**

The cancer stem cell (CSC) model suggests that many human malignancies consist of two functionally distinct cell types: (i) CSCs, which are self-renewing cells with the capacity to initiate, sustain and expand the disease, and (ii) non-self-renewing progeny cells, derived from CSCs through differentiation, which may make up the bulk of the tumour and account for disease symptoms. In order for malignancies to be cured, it may be necessary and sufficient to exclusively eliminate CSCs.
Leukaemia stem cells (LSCs) represent one type of CSC and the Leukaemia Biology group is working to understand the biology and molecular properties of LSCs by comparison with both their non-self-renewing downstream progeny and their normal HSC counterparts. Modelling human leukaemias in mice represents a powerful way in which LSCs and the aberrant cellular hierarchies they generate can be accurately studied (Figure 5).

In work recently published in Cell Stem Cell by the group, a mouse model of human acute myeloid leukaemia (AML) initiated by oncogenic fusions involving the MLL gene was used to discover that the frequency of self-renewing LSCs in mice with leukaemia varies substantially according to the initiating oncogenic fusion. Thus CSC frequency may not necessarily be a fixed or prospectively definable sub-population when different tumour types are compared with one another.

The observation of variable LSC frequency across cohorts of mice, and the finding that LSC frequency varies within any individual leukaemia according to the extent of c-kit expression, enabled the derivation of a transcriptional program representative of the LSC and also associated with the maintenance of LSCs in a self-renewing state. Comparison of this program with the transcriptional programs of other stem cell types showed that it was more akin to that of ESCs rather than adult-type stem cells, including HSCs. Furthermore, it demonstrated that LSCs in this model are proliferating, metabolically active, aberrantly self-renewing, downstream myeloid cells rather than quiescent cells. This finding has important therapeutic implications because it suggests that LSCs are quite distinct in much of their biology from the normal HSCs required to reconstitute bone marrow function following chemotherapy. These differences represent novel potential therapeutic targets.

Within the LSC maintenance signature, the transcription or chromatin regulatory factors Myb, Hmgb3 and Cbx5 were critical components of the self-renewal program and when co-expressed were able to induce immortalization of myeloid progenitors without up regulation of Hoxa/Meis expression.

Enriched expression of LSC maintenance and ESC-like program genes in normal myeloid progenitors and poor prognosis human malignancies links the frequency of aberrantly self-renewing progenitor-like cancer stem cells to prognosis in human cancer.

Future work in the Leukaemia Biology group will focus on investigating whether targeting expression of MLL LSC maintenance signature genes abrogates LSC potential in human leukaemias in the same way it does in mice.
Cell adhesion

By Charles Streuli

All cellular processes are determined by adhesive interactions between cells and their local microenvironment. For example, the adhesion of cells to the extracellular matrix controls processes such as cell proliferation and survival. These aspects of cell behaviour are some of the most recognisable features to be altered in cancer cells, and understanding precisely how proliferation and survival are controlled underpins future advances for treating cancer patients. The Streuli laboratory is studying how the extracellular matrix regulates the behaviour of breast epithelial cells, with the aim of discovering new possibilities to treat breast cancer.

Humans are multi-cellular and we are largely made up of a collection of tissues, each containing a highly organised set of cells that carry out very specialised functions. One of the most important features of multi-cellularity is that cells exist in communities, often of different types, and they communicate with each other for example through growth factors. During the progression of cancer, problems can arise in the way that cells are organised together, and tissues contain too many cells. Eventually some of the cells escape and start growing elsewhere. In fact, cancer is largely a disease of tissue disorganisation, coupled with excessive numbers of cells.

To be organised properly in tissues, cells bind to each other, and they also interact with an extracellular structural material that forms the bulk of tissues, called the extracellular matrix (ECM). Over the years, it has become apparent that cell adhesion to the ECM is crucial for the way that cells behave. Indeed, some of the specific receptor molecules that bind cells to the ECM, called integrins, are essential for controlling cell survival, proliferation, differentiation (which is the term used to describe what specific cell types actually do), their organisation within tissues, and their ability to migrate. It turns out that one of the central problems in cancer is that cell adhesion becomes altered. Either the integrin receptors change subtly, or the enzymes that they control become altered or mutated, so that the cells don’t know how to behave properly.
**Cell adhesion controls intracellular signalling**

The overall interest of the Streuli laboratory is to determine how cell adhesion to the ECM controls the way that cells work. Although many of the proteins that are involved in cell adhesion are known, there is still much to be learnt, particularly about how these proteins are involved in cancer. It is known that integrins bind to ECM proteins on the outside of the cell, and on the inside of the cell they assemble numerous adapter proteins and enzymes into what are known as adhesion complexes (Figure 1). These are mini-organelles attached to the plasma membrane at ECM attachment sites, which have two main functions. One of these is to organise the cytoskeleton and thereby the internal architecture of the cell. The other is to control signalling enzymes. Several well-known intracellular pathways, for example Src family kinases, MAP kinases and Rho-family kinases are all activated by integrin adhesions. These enzymes then affect cell proliferation, apoptosis (i.e. cell death), and differentiation, as well as the assembly and disassembly of the cytoskeleton, which in turn affects cell shape and movement.

The framework for understanding how adhesion controls cell behaviour has been established, but many of the details remain unknown. For example, precisely how most of the proteins are organised within adhesion complexes is not known, nor is the exact composition, nor how it varies between cells.
or even in the same cells when they interact with different ECMs. Also, the details of how integrin adhesion complexes actually signal to control cell cycle, survival, and differentiation (otherwise called cell-fate decisions), are far from being understood. One thing is certain, though, which is that integrins work together with growth factors, with the integrins providing spatial signals from the tissue microenvironment, and growth factors delivering temporal instructions to influence cell-fate decisions (Figure 2).

Because normal cell behaviour requires teamwork between adhesion signals and other receptor systems, dissecting the molecular mechanisms governing this cooperation is key to understanding diseases such as cancer. For example, one hallmark of cancers is ‘self-sufficiency in growth signals’. Here, some types of growth factor receptors become deregulated so that they are permanently switched on. This results in cancer cells becoming independent of integrins, so that they survive or proliferate within ECM environments that the cells do not normally contact. Additionally, some cancer cells alter certain integrins, which allows them to migrate inappropriately and form metastases.

Figure 2 Integrin adhesion controls cell fate decisions. (a) Soluble factors control cell behaviour temporally. However, cells only interpret their signals in the context of (b) integrin adhesions, and (c) cell-cell interactions. (d) These signals work together to control cell-fate (i.e. behaviour) decisions.

Figure 3 Integrins control cell-fate in breast epithelia. (a) Cells of the normal mammary gland make milk proteins and milk fat (red) when they are lactating (arrow), but cannot do so in mammary glands lacking integrins. (b,c) The alveoli of the normal mammary gland appear a bit like bunches of grapes (top). They are hollow, with the lumens being full of milk (black arrows). In mammary glands lacking integrins, the cells become disorganized and fill up the lumens (blue arrow). This morphology is similar to some early cancerous breast lesions.
Integrins control breast epithelial cell function

The Streuli laboratory is studying how integrins actually signal to control cell-fate in breast epithelial cells. The reason for choosing this cell type is that they are the ones that become altered in breast cancer, eventually losing all their organisational controls, and forming lethal distant metastases. By understanding precisely how integrins control normal processes, the team aims to identify novel targets for treating cancers. It is expected that new drugs against integrin signalling enzymes would work in a different way to conventional therapies, which largely consist of chemotherapeutics and smart drugs against oestrogen receptor and the Her2 receptors.

The lab recently conducted genetic analysis on the function of one specific subunit of integrin, which is expressed in normal breast epithelia. To determine the importance of the β1-subunit of integrin in mammary gland development, they deleted its gene just in mammary epithelial cells, using the Cre-LoxP system. They identified three major problems that arose in the tissue, which combine to prevent mothers producing sufficient milk for their young (Figure 3). They found first, that there was a proliferation defect; second, that the organisation of cells within the alveoli (which are the structures that make milk) was disrupted dramatically; and third, that the epithelial cells were unable to differentiate and make milk proteins. These were profound observations, which showed genetically how important integrins are for several of the main cell-fate decisions described above.

As a result of these findings, much of the Streuli lab is now concentrating on the molecular links between integrins and the downstream mechanisms that control cell-fate. One area of interest regards the connection between integrins and differentiation. During the last year a postdoc in the lab, Nasreen Akhtar, two PhD students, Becky Marlow and Franziska Schatzmann, and several others combined forces to dissect the molecular components of the integrin adhesion complex that controls milk production. They discovered that one adapter protein called integrin-linked kinase (ILK) acts downstream of integrins to control lactational differentiation. They also found that another protein that is located in the same adhesion complex...
complexes, focal adhesion kinase (FAK), does not act in this manner. This was an important finding, because it suggested for the first time that the circuitry within integrin-adhesion complexes is wired specifically for different cell fate decisions.

Previously, Akhtar had discovered that the small GTPase, Rac1, links integrins with the differentiation response (incidentally, this was the first demonstration of the involvement of a small GTPase in the expression of tissue-specific genes in epithelium), and last year she found that Rac1 acts downstream of ILK as well. The lab therefore postulated that the adhesion of breast cells to ECM sets up a signalling pathway encompassing a linear sequence of β1-integrin to ILK to Rac1, which is needed for differentiation to occur (Figure 4a). Interestingly this pathway acts by allowing the endocrine lactogenic hormone, prolactin, to work efficiently. So the integrin system may act a bit like a rheostat, controlling the signaling output from the prolactin receptor. This actually fits within a general (and new) concept for the regulation of signal transduction, which is that integrin adhesions control the signaling output strength and duration of a wide variety of growth factor receptors (including many of the common receptor tyrosine kinases, Figure 4b). This idea now explains how cell adhesion provides the localized instructions for cell behaviour, while growth factors deliver the temporal cues. It also says that cells need to be in the right place (i.e. in contact with the correct ECM) in order to work properly. The discovery of this concept has far-reaching implications for cancer. For example, one inference is that when cancer cells migrate away from their normal ‘home’ during the life-threatening process of metastasis, they activate adhesion signals to keep themselves alive.
Adhesion controls breast cell survival and death

This is indeed the case, and it is now established that integrin signaling proteins, including ILK and FAK, become elevated or activated in a high proportion of breast cancers. Importantly, the extent of these changes easily surpasses the percentage of tumours with over-expressed breast cancer oncogenes that are currently being targeted in the clinic, such as Her2. The discovery that cell adhesion is a rheostat to control growth factor receptors (e.g. prolactin receptor and the IGF receptor) suggests how altered adhesion might be detrimental in cancer. The lab is therefore trying to find out more about how ILK controls the behaviour of breast cells, in order to identify adhesion-signalling enzymes that might provide novel targets for treatment. In collaboration with Dr Andrew Gilmore (Faculty of Life Sciences), the lab is also studying FAK in breast cancer, examining its role in organ-like culture models, and in animals.

Streuli’s collaboration with Gilmore goes back several years, where they have made major advances about how cell adhesion and FAK control apoptosis. Following an initial discovery that the survival of breast epithelial cells is both ECM-dependent and ECM-specific, they identified a completely novel function for integrins. This is to regulate protein trafficking between the cytosol and mitochondria. They found that integrins, via FAK, maintain the death-promoting protein Bax in the cytosol, while altered adhesion leads to translocation of the protein to mitochondria. This results in a conformational change in Bax’s structure, causing it to activate apoptosis. The latest advance in this story came last year, when they discovered that the intracellular survival signals immediately downstream of FAK diverge in epithelial cells from those of the mesenchymal lineage of cells. This means that different adhesion proteins are involved with controlling the survival of these two types of cells. By unravelling the precise molecular details of integrin survival signalling, the two teams hope to identify epithelial-specific survival proteins that might eventually be targeted to treat advanced breast cancers.
Inositol lipids

By Nullin Divecha

The cells of our tissues constantly monitor their environment and respond to any changes they sense. For example, after eating food the glucose levels in the blood rise which is monitored by the cells in the pancreas that release insulin. The increase in insulin is sensed by muscle cells and they respond by taking the glucose out of the blood. However, the presence of an impermeable barrier (plasma membrane) around the cell means that changes on the outside (extracellular) of the cell, for example increased insulin, are not easily detected on the inside of a cell. Therefore cells have developed a number of methods of communication to allow them to detect extracellular changes and to transfer these into signals within the cell (signal transduction). The signals inside the cell are then interpreted and consequently lead to changes in cell behaviour. This system of factors outside stimulating signals inside the cell has enabled the orchestration of cell behaviour to generate complex organised tissues. However in pathological conditions such as the development of a tumour, cancer cells act autonomously and cease to behave in accordance with the extracellular environment. In order to do this cancer cells often misuse signal transduction mechanisms to enable them to grow out of control and metastasise to new tissues.

There are many different signal transduction mechanisms used by the cell and our laboratory is focussed on the phosphoinositide signalling system. Phosphoinositides are a family of lipids that have a hydrophobic (does not like water) tail that sequesters them into the membranes of cells (Figure 1A). Membrane localisation of phosphoinositides is a key feature of how they function as signals inside the cell. Another key feature of phosphoinositides is their hydrophilic (likes water) sugar head group (inositol) that sits out of the membrane and faces into the cytoplasm. The inositol head group has five positions (labelled 2-6) of which the 3, 4 and 5 position can become modified with a small chemical group called a phosphate residue
(phosphorylation). This modification is carried out by a family of enzymes (proteins) called kinases that use ATP, the energy source of the cell, to transfer a phosphate residue onto the inositol group. The addition of a phosphate group to the parent lipid phosphatidylinositol (PtdIns) generates a new phosphoinositide called phosphatidylinositol phosphate (PtdInsP). The inositol head group can be phosphorylated on the 3 or the 4 or the 5 position to generate three new PtdInsPs designated PtdIns3P, PtdIns4P and PtdIns5P.
These PtdInsPs can be further phosphorylated to produce phosphoinositides that have two phosphate groups attached (PtdInsP) and are designated PtdIns(3,4)P₂, PtdIns(3,5)P₂, and PtdIns(4,5)P₂. Finally the inositol head group can be phosphorylated on three positions to produce PtdIns(3,4,5)P₃. In total seven different phosphoinositides have been discovered that can be generated from the initial building block PtdIns (Figure 1B).

Critically the amounts of the different phosphoinositides are regulated in response to changes in the extracellular environment. For example when insulin is sensed by muscle cells, an enzyme that makes PtdIns(3,4,5)P₃ is dramatically stimulated leading to an increase in PtdIns(3,4,5)P₃ levels. The change in phosphoinositides is then interpreted to induce changes in cell behaviour.

What is so special about these different phosphoinositides and how are changes in them interpreted in order to control cell function? One of the ways in which phosphoinositides work is to act in a manner similar to a key. Consider that there are seven different locks into which only one of seven different keys (phosphoinositides) can fit. In this analogy the lock would represent a specific part (domain) of a protein with the door representing the rest of the protein. The specific part of the protein (the lock) that fits with the phosphoinositide is called the phosphoinositide binding domain. Of course biological life is never so simple and there turn out to be many different locks that can fit the same phosphoinositide, but in general each lock fits one phosphoinositide better than the others (Figure 1B). So what happens when phosphoinositide binding domains and phosphoinositides come together? In the analogy of the lock and key the door can exist in two different states: the first is closed and then when the key fits into the lock the door is now in its second state which is open. In much the same way the fit between the phosphoinositide and the phosphoinositide binding domain changes the protein from one state (conformation) to a new one. It is the change in the state of the protein that leads to changes in cell behaviour. There are also other ways in which phosphoinositides help to change cell behaviour. Imagine the cell as a large hall and around the edge of the hall (the membrane) there are various different types of food stalls, Indian, Italian etc. In this case the food stalls represent domains within the membrane which contain different phosphoinositides. The hall is full of people with different tastes (phosphoinositide binding domains) so that eventually people will move (migrate) to their preferred type of food stall. This illustrates two important aspects of phosphoinositide biology. 1. People move to the edge of the hall and this is exactly what happens to proteins with phosphoinositide binding domains that interact with phosphoinositides. They accumulate at the membrane where the phosphoinositide is localised. 2. Having moved to their favourite food stall each person is now in closer proximity with others enabling better interactions between people with the same tastes. In cells there are many different proteins that interact with the same phosphoinositide. An increase in one phosphoinositide in a specific location will concentrate different proteins together and allow them to interact better in order to enable changes in cellular behaviour.

**PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ and their role in cancer.**

PtdIns(4,5)P₂ is at the centre of phosphoinositide signalling and controls many cancer relevant cellular functions such as migration, cell growth and cell survival through the action of the many different proteins that recognise it. PtdIns(4,5)P₂ can also be further phosphorylated on the 3 position to generate PtdIns(3,4,5)P₃ by an enzyme called PI-3-kinase (PI3K), while the reverse reaction, the removal of the
3’phosphate (dephosphorylation) is carried out by an enzyme called PTEN. The gene sequence which codes for PI-3-kinase is often changed in tumours such that PI-3-kinase produced from this mutated gene is much more active and therefore able to generate more PtdIns(3,4,5)P_3 in the cell (Figure 2A). PTEN is often missing in tumour cells which means that PtdIns(3,4,5)P_3 is not removed leading to its accumulation. PtdIns(3,4,5)P_3 recruits and stimulates a number of different proteins with PtdIns(3,4,5)P_3 specific phosphoinositide binding domains that are involved in changing the behaviour of a normal cell to one that is more cancerous. PtdIns(4,5)P_2 also functions as the fuel for the phospholipase C (PLC) signalling pathway (Figure 2A). The PLC pathway cleaves PtdIns(4,5)P_2 to generate two new signals that can alter cell behaviour. The amount of PtdIns(4,5)P_2 being used by the PLC pathway (flux) is also often higher in tumour cells.

Tumour cells divide uncontrollably and are able to survive in conditions in which normal cells would die. They are also able to leave the site of the original tumour mass and migrate to other organs into which the tumour cell invades (metastasise), grows and eventually prevents the organ from carrying out its vital functions. The enhanced production of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 and flux through the PLC pathway are important to enable tumour cells to function in this aberrant manner.
Understanding PtdIns(4,5)P$_2$ regulation.

To understand the importance of a particular phosphoinositide in a cell we need to understand how it is made, when it is made and which phosphoinositide binding proteins are responsible for signalling changes in cell behaviour. Our lab concentrates mainly on how PtdIns(4,5)P$_2$ is regulated. PtdIns(4,5)P$_2$ can be made by two different kinases which phosphorylate two different phosphoinositides (Figure 2A). We try to understand how these two enzymes are regulated and under what conditions they become activated. For example PI3K is the predominant kinase responsible for producing PtdIns(4,5)P$_2$ and is normally localised at the membrane. However, we have found that the localisation of PI3K at the membrane requires its interaction with another protein called Rac and inhibiting this interaction prevents PI3K from sitting at the membrane (Figure 2B). We also study how these kinases are deregulated in cancer cells and what the outcome of their deregulation is for cancer development. In order to find novel pathways that are regulated by PtdIns(4,5)P$_2$ we are searching for proteins that interact with phosphoinositides using different biophysical and biochemical assays.

**PIP5K - a potential Achilles heel of a tumour cell!**

PtdIns(4,5)P$_2$ feeds many cancer relevant pathways that are strongly stimulated in tumour cells suggesting that tumour cells may have to make PtdIns(4,5)P$_2$ better and faster than a normal cell. If we could prevent tumour cells from generating so much PtdIns(4,5)P$_2$ we might be able to provide a window of opportunity with which to kill cancer cells without killing the normal cells of our bodies (Figure 3). For example, our
Inositol lipids studies suggest that it may be possible to develop tools that prevent the interaction between Rac and PIP$_5$K and therefore inhibit PIP$_5$K from sitting at the membrane and making PtdIns(4,5)P$_2$. Another possibility would be to develop a drug that would stop PIP$_5$K from working (inhibitor). In collaboration with Cancer Research Technology we have developed a high throughput assay for PIP$_5$K which has enabled the screening of 100,000 different chemical compounds for their ability to inhibit PIP$_5$K. This screen identified a number of compounds that have been further analysed and modified to make them better at blocking PIP$_5$K. We are now using these inhibitors to determine if they can kill cancer cells and how toxic they are to normal cells. The development of these inhibitors is not only useful as a potential therapeutic but they provide fantastic research tools with which to uncover new roles for PIP$_5$K and PtdIns(4,5)P$_2$ in regulating cellular function.
Angiogenesis, the formation of new blood vessels, is essential for the growth and vascular metastasis of solid tumours. Inhibition of the process, achieved by targeting the prototypic angiogenic cytokine, Vascular Endothelial Growth Factor (VEGF), has improved response rates and survival in several solid tumours, thereby highlighting for the first time the potential clinical benefit of modulating the cancer tissue microenvironment.

Many of the growth factors that promote angiogenesis bind and/or are dependent on heparan sulfate proteoglycans that are present universally on nucleated cells and tissues both in the cell membrane and extracellular matrix. Over the last twenty years our knowledge of the structure-function relationships by which heparan sulfate activates growth factors has increased, facilitating the development of new strategies that hold potential for generating inhibitors that target this mechanism. Here, we review the data concerning the role of this family of molecules and highlight potential strategies for transforming this knowledge into new treatments for our patients.

Angiogenesis and heparan sulfate proteoglycans
Angiogenesis, the formation of new blood vessels, is essential for tumour growth. Validation of the process as a target for anti-cancer treatment has been demonstrated in several randomized trials of conventional therapy with or without VEGF inhibitors, in the first and second line treatment of colorectal, hepatocellular, renal and breast cancer, which have demonstrated a survival advantage in favour of the combination. Angiogenesis is driven by oncogenic as well as hypoxic and metabolic stimuli; the latter two reflecting characteristics of the tumour microenvironment and thereby identifying the microenvironment as a potential target.
The three angiogenic driving forces result in increased concentrations of angiogenic cytokines, the majority of which, for example VEGF, the Fibroblast Growth Factors (FGF) and Hepatocyte Growth Factor (HGF) bind and are dependent on heparan sulfate for their biological activity. Heparan sulfate (HS) is a linear polysaccharide that is covalently bound to core proteins, forming heparan sulfate proteoglycans (HSPG) on the cell surface and in the extracellular matrix. The backbone of heparan sulfate consists of alternating disaccharide structures that are sulfated in domains, creating clusters of anionic charge along the chain that play a critical role in ligand-binding (see Figure 1). While specific binding sequences have been found for certain protein ligands, such as anti-thrombin III, other ligands have different binding

![Figure 1: Heparan sulfate (HS) proteoglycans on the cell surface membrane. Heparan sulfate (HS) polysaccharide chains are covalently-linked to two classes of cell surface proteins, the transmembrane, dimeric Syndecans and the lipid-anchored Glypicans. The segments shown in red on the HS chains represent the sulfated domains (S-domains) that bind strongly to growth factors.](image)
requirements and our recent studies have demonstrated the cooperative binding of FGFs on heparan sulfate such that a conformational change occurs in the HS chain once one FGF molecule has bound, facilitating the binding of a second molecule, creating complexes capable of activating the signal transducing receptors (Figure 2).

In an attempt to capitalize on the obligate dependence of FGFs on heparan sulfate we investigated the relationship between HS, FGF and angiogenesis. In ovarian cancer tissue we used a novel molecular construct to demonstrate that ovarian cancer endothelium expresses heparan sulfate that can activate FGF2, while the tumour cells were largely denuded of biologically active HS. We investigated this further and showed that the cancer cells make HS but it is lost from the cell surface, through the combined effects of heparanase, an extracellular HS-depolymerising enzyme and sulfatase enzymes. These observations taken in conjunction with the activating effect of heparanase on syndecan 1 ectodomain and its role in depolymerisation of HS in the extracellular matrix, clearly identify heparanase is as an attractive target for drug development.

As FGF is dependent on HS for its biological activity, we and others have investigated the potential of fragments of HS to inhibit activation of the growth factor. We demonstrated that heparin octasaccharides inhibited FGF2-induced mitogenesis in vitro and angiogenesis in different models in vivo. However, a critical obstacle in the development of oligosaccharides as potential drugs is the need to prepare bulk amounts of pure heparin oligosaccharides. This has proved difficult to overcome, but nevertheless after five years synthesis has been elucidated and we are testing the potential of different species to inhibit FGF’s biological activity.
Cancer and the HS proteoglycans (HSPGs)

While the structure-function relationships between HS and the FGFs have been investigated over the last twenty years, abundant evidence has accumulated that highlights the potential role of HS and HSPGs in human cancer. Two genetic cancer syndromes have been identified. The first is hereditary multiple exostoses and is due to mutation in the one of the enzymes that regulate the length of the heparan sulfate chain. In this condition patients can develop exostoses that can undergo malignant degeneration to form chondrosarcoma. Secondly, the Simpson-Golabi-Behmel syndrome is caused by a mutation in the glypican 3 gene and is associated with widespread organ overgrowth and predisposition to renal cancer. Indeed glypican 3 has been implicated in the pathophysiology of multiple cancers (e.g. pancreatic and hepatic) and treatments that target the proteoglycan are in early clinical development.

The syndecan proteoglycans have also been implicated in cancer. In particular syndecan 1 is aberrantly expressed in several cancers and this inappropriate expression is often associated with worse outcome. In a recent study we demonstrated that stromal syndecan 1 was associated with a worse outcome in ovarian cancer; a finding that has been confirmed in breast cancer and which also highlights the potential importance of the tumour microenvironment as a target. The pathophysiological mechanism of syndecan 1 remains unclear. However, scission of the ectodomain by matrix metalloproteases can generate species that inhibit FGF2 activity unless the ectodomain is also exposed to heparanase. Syndecan 4 has also been implicated in signal transduction and focal adhesion formation.
Potential therapeutic strategies

Our increasing knowledge of the contribution of HS and HSPGs to angiogenesis and cancer growth has identified a number of potential therapeutic approaches. As highlighted above, heparanase, because of its capacity to generate biologically active HS from syndecan 1 ectodomain and its role in invasion, is a highly attractive target. However, comparatively minor in-roads have been made in the development of inhibitors of this enzyme. In part this could be due to the difficulties involved in targeting the enzyme rather than its inactive pro-enzyme precursor, while from a clinical perspective, there are difficulties in proving in patients that a new drug truly has anti-invasive properties.

The FGF-HS axis is also an attractive target. In particular the FGFs have been implicated in several tumours including bladder and prostate cancer as well as myeloma. Equally importantly, the FGFs have been implicated as mediating resistance to VEGF inhibitors in the clinic and therefore their clinical validity as targets is increasing. Of all the HS-angiogenic cytokine relationships, our understanding of the FGF-HS relationship is the most advanced. We know that HS undergoes conformational change, facilitating formation of dimerised FGF on HS and thereby signal transduction. This therefore provides a potential route of attack; specific oligosaccharides that lack critical moieties or which do not undergo appropriate conformational change, while preserving affinity, could offer additional approaches.

Significant in vitro and in vivo evidence has also highlighted the moiety 6-O-sulfate on glucosamine in the HS chain as being critical for the formation of the tri-molecular (signalling receptor, HS and cytokine) signal transducing complex. Deletion or suppression of intracellular 6-O-sulfotransferase significantly inhibits FGF-induced signalling in vitro and angiogenesis in vivo; again highlighting the enzyme as a potential target.

As mentioned above, a number of proteoglycans (syndecan 1 and glypican 3) have been highlighted in several clinical studies as being of prognostic importance. Glypican 3 antagonists are in early clinical development. However, critical data concerning the proposed mechanism of action of these proteoglycans is missing and this will confound further drug development and optimization.
Caveats

Most of the key angiogenic cytokines bind and/or are dependent on heparan sulfate, highlighting the mechanism as a potential target for therapy. However, there are some caveats to such an approach. Although the majority of VEGF-A isoforms bind HS, one isoform, VEGF121, does not. In addition, the recent discovery of VEGFXXXb isoforms, which are endogenous inhibitors of VEGF, raises the possibility that inhibition of HS function might also inhibit endogenous inhibitors; an hypothesis corroborated by our recent studies of the endogenous anti-angiogenic, heparin-binding protein, endostatin. Thus further development of inhibitors of HS mandates a degree of specificity if we are to avoid antagonising endogenous inhibitors.

Conclusion

Over the last five years we have seen the results of randomised studies of anti-angiogenic agents that have conferred a survival advantage to our patients. As angiogenesis is driven by tumour micro-environmental factors these data offer the first evidence to suggest that manipulation of the tumour microenvironment could offer a therapeutic advantage. As many of the angiogenic cytokines are dependent on heparan sulfate for their biological activity, and in view of the abundant evidence that implicates HS and the proteoglycans in human cancer, this is an area of greatly expanding interest. We have greatest understanding of the relationship between HS and angiogenic cytokines but it is also clear that HS plays an important role in regulation of endogenous anti-angiogenic molecules, highlighting the importance of developing specific inhibitors. It is also clear that the proteoglycans play an important role in cancer but the mechanisms by which they achieve this are not clear and further studies are essential if we are to develop new drugs that target this family of molecules.
Tackling melanoma: new insights from combined approaches

By Claudia Wellbrock and Adam Hurlstone

Melanoma skin-cancer has the fastest growing incidence for cancer worldwide and is highly fatal if surgery alone fails to cure the patient. This is mainly due to the high metastatic potential of melanoma cells and their notorious resistance towards current therapies. The median survival of patients with metastatic melanoma is only 6 months, and the five-year survival rate is less than 5%. A molecular understanding of disease mechanisms in melanoma is essential in order to improve current therapeutic approaches. Moreover, since melanoma cells clearly possess activities that favour invasion and metastasis, studying this cancer type may provide a better understanding of the mechanisms contributing to tumour progression in general.

Already early on during melanoma development, transformed cells adopt changes in adhesion-dependent differentiation, proliferation, survival and motility. This facilitates uncontrolled tumour cell proliferation within the epidermis, invasion into the dermis and eventually colonisation to distant sites (see Figure 1). Recent approaches such as comparative genomic hybridisation and mutation analysis by genome-wide sequencing have provided greater understanding of the biology that underlies melanoma and this is crucial for the development of improved therapeutic approaches.

The genetics of melanoma

Melanoma originates from melanocytes, which are highly specialised pigment cells. They are located at the basement membrane of the epidermis, where their homeostasis is strictly controlled by the surrounding keratinocytes (Figure 1). UV radiation can induce the production of growth factors that trigger the benign proliferation of melanocytes. However the cells will eventually senesce, partly due to the over-expression of cell cycle inhibitor p16<sup>CDKN2A</sup>. Macroscopically such a ‘clone’ of senescent melanocytes is visible as a nevus or common mole (Figure 1). Strikingly, the serine
Threonine kinase BRAF, which is mutationally activated in 50-70% of melanomas (V600E BRAF), is also found mutated in up to 80% of benign nevi. Moreover, activated BRAF can induce the expression of p16<sup>CDKN2A</sup> in melanocytes and hence is thought to contribute to the induction of senescence in benign nevi. This suggests that although mutated BRAF is the most prominent oncogene in melanoma, and acquiring an activating mutation in BRAF clearly seems to create an advantage in the propagation of melanocytic cells, yet signalling from activated BRAF is not sufficient to fully transform normal human melanocytes. Some but not all benign nevi progress to an intra-epidermal lesion called the radial growth-phase (RGP) melanoma, and this is correlated with a loss of p16<sup>CDKN2A</sup> (Figure 1). RGP cells can progress to the vertical growth-phase (VGP), a more dangerous stage where the cells have metastatic potential with nodules or nests of cells invading the dermis (Figure 1). The transition from RGP to VGP melanoma is accompanied by changes in the expression of adhesion receptors such as integrin α<sub>V</sub>β<sub>3</sub> and E-cadherin, which in turn affects adhesion-related downstream effectors such as SRC kinases or beta-catenin. However, not all melanomas pass through each of these phases.
individual phases, as RGP or VGP can both develop directly from isolated melanocytes or nevi, and both can progress directly to metastatic malignant melanoma.

The focus in the last five years has been very much on BRAF, but importantly the small G-protein NRAS, an upstream activator of BRAF is mutated in 15-30% of melanomas. NRAS not only regulates BRAF, but it also stimulates activation of the phosphoinositide 3-kinase (PI3-kinase). PI3-kinase induced signalling is hyper-activated in melanoma. This is however not only due to mutationally activated NRAS, but frequently (5-20% of late stage melanomas) based on the loss of function of the PI3-kinase antagonist PTEN. Furthermore, over-expression of the PI3-kinase effector protein kinase B (PKB/AKT) is proposed to contribute to PI3-kinase activation in melanomas.

Notably, in melanoma, NRAS and BRAF mutations are mutually exclusive, as are NRAS and PTEN mutations, whereas BRAF and PTEN mutations are co-incident in approximately 20% of cases. Thus, the genetics clearly reflect the functional connection of the respective proteins at the molecular level.

Another important regulator of melanoma cell proliferation and survival is the microphthalmia-associated transcription factor (MITF). MITF is specifically expressed in melanocytic cells, and it is the onset of MITF expression that commits neural crest cells to the melanocyte lineage in early development, suggesting that MITF initiates the cellular program that ‘makes’ a melanocyte. The gene is amplified in 5-16% of melanomas, and increasing evidence suggests that MITF expression levels, which show intratumoural variations, determine the fate of the individual cells within a tumour.

Cellular signalling in melanoma

RAS/RAF/MEK/ERK signalling. The RAS/RAF/MEK/ERK pathway is a key regulator of melanoma cell proliferation, with ERK appearing to be hyper-activated in up to 90% of human melanomas. The most common mechanism of ERK activation is through mutationally activated NRAS or BRAF (Figure 2). Oncogenic BRAF activates constitutive ERK signalling, stimulating proliferation and survival and providing essential tumour maintenance functions. It also contributes to neo-angiogenesis by inducing autocrine VEGF secretion. Numerous studies have identified tumour relevant genes in melanoma downstream of oncogenic BRAF, such as the cell cycle regulators cyclin D1 and p16\(^{CDKN2A}\), and the tumour maintenance enzymes MMP-1 matrix metalloproteinase and inducible nitric oxide synthase, iNOS. Thus, BRAF is regulating multiple aspects of melanoma induction and progression.

Because of its relevance to melanoma the RAF/MEK/ERK pathway is a target of great interest for melanoma therapy. Although the first attempts using the kinase inhibitor Nexavar (sorafenib) were rather disappointing, (probably due to insufficient potency in vivo), the MEK inhibitor AZD6244 shows slightly more promising results.

Microphthalmia-associated Transcription Factor (MITF) regulates melanocyte differentiation and survival as well as melanoma cell proliferation (Figure 2). Numerous genes required for the differentiation of melanocytic cells are MITF-target genes, but importantly MITF also regulates the expression of cell cycle regulators such as CDK2, p16\(^{CDKN2A}\) and p21\(^{CDKN1A}\). Recently MITF has also been implicated in the regulation of the actin cytoskeleton through the formin mDia. Furthermore, the Hepatocyte Growth Factor receptor c-MET, involved in melanoma metastasis is an MITF target gene, and MITF induces VEGF expression through
Tackling melanoma: new insights from combined approaches

direct transcriptional regulation of HIF1α. MITF’s crucial role in melanoma biology is reflected in its transcriptional and post-translational regulation by many proteins that are commonly deregulated in melanoma, including BRAF and beta-catenin (Figure 2). Thus, MITF represents a central node in which essential cellular signals are transmitted into a cell type specific transcriptional response.

**NRAS and PI3-kinase signalling.** Another signalling pathway that is emerging as important in melanoma is the PI3-kinase pathway (Figure 2). PI3-kinase signalling regulates cell survival, proliferation, growth (increase in cell mass) and motility. Agents that target PI3-kinase, PKB/AKT and other downstream components of the pathway such as mTOR are being developed and tested in the clinic.

**The importance of MITF levels for melanoma**

MITF expression can be detected in melanocytes as well as in most melanomas and it is used as highly specific melanoma marker. However, its function as a prognostic maker is still a matter of debate; some studies describe the loss of MITF expression to be correlated with progression, whereas other studies suggest that MITF expression is required for melanoma progression. This discrepancy is probably due to the fact the MITF expression levels show intratumoural variation and appear to be linked to tumour cells with different features. This is based on the fact that accurate MITF protein levels are crucial to its function and to cell fate; very high protein expression levels are growth-inhibitory, while only lower/intermediate levels are pro-proliferative and very low levels are linked to invasion and metastasis. Accordingly, MITF protein levels are much lower in melanoma cells than in melanocytes (see Figure 1). Recent studies demonstrate that MITF expression is modified by the tumour microenvironment, which results in individual cells with very low MITF expression levels and high metastatic potential. Since MITF regulation is crucial to the metastatic behaviour of melanoma, it is important to identify the mechanisms regulating MITF expression not only by melanoma relevant signalling pathways but also in the context of microenvironment derived signals.

Dr Wellbrock’s work has shown that MITF expression is tightly regulated by MEK/ERK signalling downstream of oncogenic BRAF and this is crucial for melanoma cell proliferation and survival. This finding links MITF to the signalling pathway most frequently deregulated in melanoma.

**Figure 2. Cellular signalling in melanoma. Major selected pathways involved in melanoma initiation, progression and maintenance. These include the three-tiered ERK/MAPK signalling module, the PI3K pathway, and p16CDKN1A/Cdk4 signalling. Adhesion-mediated signalling is closely linked to the family of SRC kinases, which also regulates cellular pools of beta-catenin. MITF is a central node in which essential cellular signals are transmitted into a cell type specific transcriptional response.**
Current work in the Wellbrock laboratory is investigating the role of SRC kinases and beta-catenin, and the involvement of extracellular microenvironment-induced adhesion signalling in the regulation of MITF, and consequently in melanoma progression (Figure 2).

**Experimental systems for probing cell signalling and melanoma formation and progression**

Animal models have been developed to assess the effect of a targeted intervention influencing cell signalling on melanoma progression. Models include animals with a naturally increased susceptibility to develop spontaneous melanoma, such as certain interspecies hybrids of fish of the *Xiphophorus* genus, Sinclair swine, and gray horses, as well as mice deliberately genetically engineered to develop melanoma. Xenografting human melanoma cells into an immunocompromised mouse host is another popular model. Subsequent targeted interventions can be in the form of application of a pharmacological agent (typically a small molecule), or in human and mouse cells gene ablation or gene knockdown. Recently, a new model organism has been conscripted to the war on melanoma: the zebrafish, opening up new avenues of research. Using a transgenic strategy, the Hurlstone laboratory recently targeted expression of oncogenic Ras to zebrafish melanocytes and obtained melanocytic neoplasms resembling human melanoma (Figure 3). Moreover, by simultaneously expressing a protein inhibitor of endogenous PI3-kinase signalling, they were able to demonstrate the requirement for PI3-kinase in driving the malignant progression of transformed melanocytes (Figure 3). Zebrafish is an attractive organism for investigations in the laboratory due to its small size, short generation time, large number of offspring, low cost, and simple standardized husbandry. Other features also promote its use in medical research including conserved disease mechanisms, rapid, inexpensive and facile genetic manipulation, and optical clarity of tissues, which

![Figure 3](image-url)
promotes light microscopy imaging. Harnessing these virtues, the Hurlstone laboratory is now exploiting their zebrafish model to gain further insight into melanoma genetics and cell signalling (see future approaches below).

Future approaches
The major cause of mortality in patients with cancer is the formation of distant metastases, and there is an urgent need to fully comprehend the molecular mechanisms underlying this process. Melanoma is notorious for its early acquisition of invasive behaviour and its high potential to metastasise, which indicates that melanoma cells possess many activities that allow them to interact with their microenvironment in a way that favours their propagation at distant sites. Studying melanoma therefore will not only help to improve the treatment of this deadly disease in particular, but will also contribute to a better understanding of metastasis related processes in general.

Within the Molecular Cancer Studies group, a platform of experimental systems has been developed that will allow investigation into various aspects of melanoma progression. In vitro studies in the Wellbrock laboratory using reconstituted co-culture models and 3D live imaging are addressing how tumour microenvironment affects melanoma cell behaviour (Figure 3). At the molecular level the influence of adhesion-mediated signalling on the regulation of MITF will be analysed, and furthermore the group is screening for downstream effectors of SRC and BRAF, which are relevant for MITF directed melanoma cell invasion. Subsequent translational studies with identified MITF/invasion regulators will include tissue microarrays of different stages of melanoma progression, with the aim of developing new biomarkers for melanoma.

The zebrafish is renowned for its use in forward genetic screens. The Hurlstone laboratory wishes to bring this powerful gene discovery tool to bear on melanoma progression. By combining their melanoma models with methods for efficient somatic mutagenesis and subsequent screening for progression to VGP melanoma, they hope to identify novel genes implicated in disease progression. In parallel, they have begun analysing changes in whole genome expression accompanying transition from benign melanocytic neoplasia to melanoma (Figure 3). Again it is hoped that among the differentially expressed genes, we will find candidates for master regulators of melanoma progression that may subsequently be validated as therapeutic targets. Additionally, we have recently been exploring the potential of our zebrafish melanoma model for screening small molecule libraries for entities capable of suppressing melanoma.

Findings acquired from the in vitro studies described above subsequently can be further investigated in vivo using the zebrafish model, and - vice versa - knowledge obtained from the fish melanoma model will feed into the design of experiments performed in the in vitro systems (Figure 3). Thus, by combining approaches established within the Molecular Cancer Studies group we hope to provide a better understanding of the mechanisms underlying melanoma development and progression. Moreover, the expertise and the infrastructure within MCRC will help with translating findings from the bench into clinical approaches, which ultimately should lead to improved therapies in the treatment of malignant melanoma.
Translational Radiobiology - taking laboratory advances into the clinic - includes development of novel radiotherapy-related therapeutic approaches and exploitation of scientific progress to develop methods for predicting how cancer patients respond to radiation. Towards the latter goal, new high throughput technologies are being used to derive gene signatures associated with patient response to radiotherapy. These signatures are being developed to reflect hypoxia and sensitivity to radiation with the goal of producing biomarkers that can be used to individualise radiotherapy and improve cancer outcomes.

Translational Radiobiology

The Translational Radiobiology group of the MCRC is drawing on developments in array technology to develop gene signatures that reflect biological factors that influence how patients respond to radiotherapy. After surgery, radiotherapy is the second most important curative treatment for cancer and around 40% of cancer survivors receive radiation at some point in their treatment. Advances in the machines used to deliver radiation are improving the targeting of treatment but ultimately it is the genetic variation of individuals and their tumours that underlies heterogeneity of response and determines who will be cured and who will develop toxicity. Developing gene profiles that predict how patients respond is important as they could determine who should receive additional treatment to improve their chances of surviving. They should also identify who is likely to respond well and would benefit from lower doses of radiation to minimise toxicity and improve the quality of life of long-term cancer survivors. A number of biological factors dominate how cancers and individuals respond to radiation. In comparison with well-oxygenated cells, hypoxic cells are around three times more resistant to the effects of radiation. The rate of tumour cell proliferation is important, as is how sensitive cells are to radiation. The focus of this group's work is to develop profiles associated with tumour and normal tissue radiosensitivity, and also tumour hypoxia. The work involves whole genome
arrays to assess both gene expression at the RNA level and genetic variation reported as single nucleotide polymorphisms (SNPs). This report focuses on work carried out by the group over the past year aimed at deriving a clinically useful, hypoxia-associated gene expression signature.

**Tumour hypoxia**

The radiobiologist Hal Gray highlighted the importance of tumour hypoxia as a potential factor limiting the success of radiotherapy in the 1950s. His observations led to hypoxia dominating radiotherapy-related research for many years. Hypoxia is also now recognised as a key factor driving cancer development, progression, metastasis formation and resistance to other forms of therapy. Numerous studies showed tumour oxygenation status, measured using oxygen electrodes, provides prognostic information. Cancer patients with hypoxic rather than well-oxygenated tumours have a decreased probability of surviving following treatment. Although there are approaches for modifying tumour hypoxia that have been shown to be clinically effective, none has entered routine clinical use within the UK. Progress in this area is hampered by the lack of a method for measuring tumour hypoxia suitable for use on a routine clinical basis. The development of such a method would be a significant advance in cancer research. One of the reasons for this is because there is evidence that assessment of tumour hypoxia predicts benefit from hypoxia modification therapy.

**Approaches for assessing tumour hypoxia**

There are many different approaches for assessing tumour hypoxia. Fine-needle oxygen electrodes have provided the best evidence for hypoxia limiting cancer curability but, although often referred to as the gold standard, they are not widely available and are suitable only for accessible tumours. There are hypoxia-associated markers that are preferentially reduced in hypoxic cells to form stable adducts that can be stained using immunohistochemistry (e.g.
There are insufficient data to know yet whether such markers will play a role in routine clinical use. Imaging approaches including positron emission tomography (PET) and magnetic resonance imaging (MRI) are also of interest. The most widely used method, however, is to study the expression of hypoxia-associated proteins such as carbonic anhydrase 9 (CA9; Figure 1) and hypoxia-inducible factor (HIF)-1α as surrogate markers of hypoxia. Although the approach using immunohistochemistry is straightforward and widely applicable to cancers where pre-treatment biopsy is taken routinely, it is not hypoxia specific as the proteins can be induced by other factors.

**Assessment of tumour hypoxia predicts benefit from hypoxia modification therapy**

There is evidence from three independent trials that hypoxic tumours gain the greatest benefit from hypoxia-modifying therapy. The first study showed the level of pimonidazole binding in head and neck tumours predicted likely benefit from hypoxia-modifying ARCON – accelerated radiotherapy plus carbogen and nicotinamide – with survival rates of ~60% and ~18% for hypoxic tumours receiving ARCON versus conventional radiotherapy, respectively. The second study was linked to a phase III head and neck cancer trial (DAHANCA 5), which showed addition of hypoxia-modifying nimorazole to conventional radiotherapy was associated with an increase in loco-regional control (49% vs 33%) and overall survival (26% vs 16%). Patients
in the DAHANCA 5 trial with high plasma osteopontin levels (associated with tumour hypoxia) were most likely to benefit from nimorazole. Disease-specific survival rates were 51% and 21% for patients with high osteopontin levels undergoing hypoxia-modifying vs radiotherapy alone. A third study showed patients with hypoxic tumours identified using ¹⁸F-FMISO PET had an improved outcome following chemoradiotherapy plus the hypoxia-targeting, bioreductive agent tirapazamine compared with hypoxic tumours that received chemoradiotherapy alone (100% vs 39% loco-regional control rate).

**Development of a hypoxia-associated gene expression signature**

As described above, the most widely studied approach for assessing tumour hypoxia involves measuring the expression of hypoxia inducible proteins in tumour biopsy specimens. One approach to deal with the lack of hypoxia specificity of individual proteins is to use gene expression profiling. This involves developing a signature to provide a more comprehensive cover of a cell’s complex molecular pathway response to decreasing oxygen levels. This is attractive for tumour hypoxia because 1-5% of the human genome is transcriptionally responsive to
changing oxygen levels. In collaboration with the Paterson Institute’s Applied Computational Biology & Bioinformatics group (Dr Crispin Miller) and Professor Adrian Harris’ group in Oxford (Prof Adrian Harris, Dr Francesca Buffa) Affymetrix U133plus2 GeneChips were used to profile 59 prospectively collected head and neck squamous cell cancers. A hypoxia-associated gene signature was obtained by identifying genes the expression of which correlated with ten well-known hypoxia-regulated genes (e.g. CA9, GLUT1, VEGF; Figure 2). Genes that correlated with at least five of the ten genes defined a signature comprising 99 genes. The median expression of the 99 genes was an independent prognostic factor for recurrence-free survival in a publicly available head and neck cancer dataset, outperforming the original trained classifier in multivariate analysis. The signature was also prognostic for overall survival in a well-known breast cancer dataset, independent of clinico-pathological factors and a trained profile.

Development of a reduced hypoxia-associated gene expression signature
Gene expression signatures often lack transferability. Published profiles can have no prognostic significance in independent datasets, and many similarly prognostic signatures can be produced from gene expression datasets. It is, therefore, important to derive inter-study validated meta-signatures. There are also advantages in having a small signature so that RNA expression can be measured using real-time polymerase chain reaction (RT-PCR) for a more sensitive, specific and reproducible approach to quantify gene expression than possible with gene expression microarrays. Applied Biosystems’ TaqMan® Low Density Arrays (TLDA) cards can be custom-built to rapidly assess gene signatures across multiple samples. Therefore, over the past year we have worked towards defining a reduced and more stable hypoxia metasignature. This work was done by Dr Francesca Buffa in Oxford who compared results from different cancer sites where gene expression data were available either through collaboration with other groups or from public repositories. Figure 3 shows the prognostic significance of the 26-gene meta-signature, which is similar to the original 99-gene signature.
The group led a successful application for the 2008 MRC Biomarker call with a proposal to develop a hypoxia biomarker based on the reduced RNA expression signature. The 3-year grant will investigate whether the signature predicts benefit from hypoxia modification therapy in several national and international trials. The MRC Biomarker Programme Grant aims to validate and qualify the 26-gene hypoxia biomarker taking it to the final stage of qualification i.e. prospective evaluation in a clinical trial. The work will involve extracting RNA from tumour samples and determining RNA expression using RT-PCR and Applied Biosystems’ custom-built TLDA cards. Over the past year, Dr Guy Betts in the group has designed the cards and selected five reference genes in discussion with Dr Francesca Buffa and Dr Carla Möller-Levet, who works jointly in the Translational Radiobiology and the Applied Computational Biology & Bioinformatics groups. Samples will be taken from a number of studies: (i) the UK BCON trial randomizing bladder cancer patients to radiotherapy alone or with carbogen and nicotinamide; (ii) the Dutch ARCON trial of carbogen and nicotinamide in head and neck cancer patients; (iii) the Danish DAHANCA5 trial that randomized head and neck patients to radiotherapy alone or with nimorazole. The collaborative effort will draw on expertise within the MCRC: Mr Jarrod Homer, a head and neck surgeon involved in the prospective collection of tumour samples to assess reliability and reproducibility; and Professor Caroline Dive, whose group has the expertise in biomarker validation and qualification. Given the large number of cancer patients who undergo radiotherapy each year, the introduction in clinical practice of tumour hypoxia testing and the biological individualisation of radiotherapy would have a significant benefit on cancer survival in the UK.
MCRC Biobank and future developments in Tissue Microarrays

By Jane Rogan, Noel Clarke and Goran Landberg

Developments in genomics and proteomics have meant that new molecular biomarkers for cancer diagnosis and prognosis and targets for treatment are being identified. To exploit these developments, large numbers of biological samples need to be made available to facilitate the conduct of high quality cancer research. In addition, new regulations surrounding human tissue (The Human Tissue Act 2004) have stimulated the need to centralise human tissue collections within institutions ensuring accurate monitoring of sample collection, use and disposal. In the summer of 2007, a working group was established to set up a cancer Biobank within the MCRC. At the end of 2008, the MCRC Biobank had been actively collecting samples for ten months with donations from over 200 patients.

For the MCRC Biobank to be a tool for all areas of cancer research, samples need to be collected for all possible cancer disease groups. To target these different cancer groups, the MCRC Biobank collects samples from five collaborating Trusts across the region. These are; The Christie NHS Foundation Trust (CFT), Salford Royal NHS Foundation Trust (SRFT), University Hospital of South Manchester Foundation Trust (SMUHT), Central Manchester and Manchester Children’s Hospital NHS Foundation Trust (CMMC) and Pennine Acute Hospitals Trust (PAT). Tapping into the expertise at each of these Trusts ensures the MCRC Biobank captures as many samples from all different cancer disease groups as possible.

![Figure 1: Cancer disease group locations](image)
Sample collection and processing
To facilitate collection of samples, each participating Trust is assigned a Biobank technician who is responsible for seeing sample collection through from beginning to end. The first step in the sample collection pathway is taking informed consent from patients, which is a legal requirement under the new Human Tissue Act (2004).

Once the patient has consented to the study, the Biobank technician aims to collect what is known as a sample “six pack” for each patient per operation event. A “six pack” comprises of:

1. Fresh frozen tumour tissue
2. Fresh frozen paired normal tissue
3. Formalin fixed paraffin embedded tumour tissue
4. Formalin fixed paraffin embedded paired normal tissue
5. Pre-operative blood
6. Pre-operative urine

The blood and urine are collected from the patient either in their pre-operative appointment, on the ward before their operation or in theatres during the operation. To obtain tissue samples, technicians collect the whole excised specimen fresh from theatre and transport it to pathology where sample cut-up takes place. Pathology is involved in the whole process to ensure patient diagnosis is unaffected. Only tissue surplus to diagnostic requirements is sampled for the MCRC Biobank. Frozen tissue samples, blood and urine are all processed by the Biobank technician at the host Trust and fixed samples are placed in pre-filled formalin pots. Once processed, all samples are transported back to the Paterson Institute for Cancer Research for banking or further processing. Processing of the fixed samples is done in the Histology facility, which has recently been extended to incorporate the MCRC Biobank.
All Biobank staff work to standard operating procedures (SOPs) to ensure that sample collection is done in a uniform manner and is of highest standard, and the quality of samples is checked by the MCRC Biobank pathologist who works for the Biobank one day each week.

**Ethics and Legislation**

The MCRC Biobank is governed by the Human Tissue Act 2004 and all storage of human material is covered under The Christie Human Tissue Authority Licence for Research. The Biobank also has a specialist generic ethics approval from the National Research Ethics Service (NRES), which is unique to research tissue banks. This tissue bank generic ethics approval confers approval to anyone using samples from the MCRC Biobank.
so researchers do not need to go and get their own ethics approval for relevant projects using Biobank samples.

This centralised management of all ethics and regulatory requirements is of great advantage to researchers. Recent changes in human tissue research legislation mean that researchers are no longer allowed to have their own tissue collections under broad ethics approval and they must either be collected and used under project-specific ethics approval with defined endpoints, with a new ethics approval for each project, or through a research tissue bank under licence from the HTA requiring a robust infrastructure that takes a significant amount of time and effort. The MCRC Biobank addresses this problem by taking care of all governance and administration centrally; ensuring researchers have easy access to biological research samples that are of guaranteed high quality.

**Management Board**
The MCRC Biobank Management Board was first assembled in December 2007 and meetings are held quarterly. Board members comprise of experts in each cancer type, representatives from each collaborating Trust, a pathologist and a patient representative. The Board’s role is to feedback on and addresses any practical issues on sample collection or storage.

The Biobank is also close to approving a sample access policy so that researchers can access banked samples. All researchers wishing to access Biobank samples will be required to submit a scientific proposal to the Biobank. Members of the Management Board will be responsible for reviewing and approving Biobank applications. It is hoped that the first applications will be submitted by Spring 2009.

![Figure 3: Structure and organisation of the MCRC Biobank](image)
Informatics
The Biobank currently uses an in-house database to track patient samples and work is taking place to ensure the ideal informatics solution is sought for the long-term. As well as a sample tracking tool, it is key that the cancer informatics platform is built with the capability of linking to the wider primary care population databases in order for clinical researchers to explore population based research hypotheses (e.g. link between obesity and cancer risk), and to investigate the effects of new agents to understand more fully the genetic influence upon drug or radiation responsiveness.

Each disease group will specify which information they would like to collect about their patients to form a core informatics framework for each cancer speciality. As well as population of information from other databases, technicians will be responsible for obtaining some of this core information which will be uploaded onto the database system. The aim is that for each sample there will be a comprehensive set of background informatics which will make the samples even more valuable and useful in cancer research.

It is important for Manchester, and in particular the Manchester Cancer Research Centre, to build a cancer informatics platform for the future to exploit the wealth of data available from their patient population, from clinical trials, and from basic science exploration of new targets identified from human tissue. It is hoped that the system will be in place and operational by spring 2009.

Mining the archives and tissue microarrays
In addition to prospectively collected tissue samples, archival tissue blocks both in pathology departments and old research collections are a largely untapped resource which can also be used for cancer research. These samples, which are largely unused, can be assimilated into the Biobank set-up and can become part of the Biobank resource. Archive samples collected before 1st September 2006 are exempt from the consent
provisions of The Human Tissue Act 2004; therefore, bringing the samples into the bank is fairly simple provided there are resources available to do it. For samples collected after this time, the MCRC Biobank allows patients to offer their retrospective consent for surplus samples from operations they have had before the Biobank was up and running.

In order to efficiently study large collections of archival material, a method called tissue microarray (TMA) can be utilised. Within a TMA, several hundred small core biopsies from paraffin blocked tissues can be arranged permitting stringent and high-throughput analyses of large collections of tumours using immunohistochemistry (IHC) or in situ hybridisation (ISH) based methods. Unique and extensive Biobank material of cancer tissues forms the backbone of the molecular pathology platform, and TMA provides the infrastructure for structured and rapid analyses of formalin fixed Biobank material. Today, TMAs with thousands of cancer samples representing either consecutive series of patients, or patients that have been enrolled in randomised treatment trials are extremely important tools in cancer translational research projects. Their key advantages include analyses of gene products in clinical samples and validating the relevance of certain gene products in relation to tumour properties as aggressiveness, patient outcome or response to treatment.

In the process of constructing TMAs, the areas of interest are initially marked on a Haematoxylin-eosin stained slide; these may include representative areas of tumour cells or other areas of interest that will be studied later on. Core biopsies measuring around 1 mm in diameter are then punched from the donor block from an area that corresponds to the earlier marked area using either a manual arrayer or a more automated robotic system. The biopsy is then mounted in a recipient block that could harbour several hundred biopsies. The TMA system keeps track of the localisation of the biopsies and all information is kept on a database. In order to compensate for heterogeneity within the tumour, or in order to better target a specific area of interest, several biopsies from the same tumour are often mounted in the recipient block. The system is flexible and the amount of donor and recipient block and the arrangement of the biopsies can be customized for each specific TMA. With the basic TMA construction methods, all different formalin-fixed material can be arranged in TMAs. Tumour materials and Biobank collections are commonly used but materials from normal tissues as well from mouse model systems or cell lines can also be arranged in TMAs.

The TMA can then be sliced into several hundred thin sections, each including small cores representing the tumours that were included in the TMA. The sections can be stained using conventional IHC or ISH protocols and proteins, RNA or DNA can consequently be quantified in large sets of clinical samples in a standardised way. By including sets of control tissue in each TMA, the reproducibility of specific analyses can be optimised and the variation between staining can be minimised.
The advantages of using TMAs are that precious materials will last longer as several TMAs can be constructed from even a small lesion and each TMA can be sliced hundreds of times instead of an entire block being used up for a limited amount of studies. Using pathology-supervised targeting of representative areas, the quality of the TMA and the relevance of the following research studies will increase. By limiting the areas that will be studied, it will also be easier to adopt an automated analysis system of the TMA which will probably be a prerequisite for future high-throughput analyses of biomarkers in large collections of formalin-fixed clinical samples.
MCRC Biobank and Tissue Microarrays

TMAs have been used in many research projects and have clearly influenced the availability of clinical materials for researchers. However, an important aspect of TMAs is that they need to be linked to clinical information in order to be useful for studies, for example prognostic or treatment predictive features of cancer. It is therefore essential to arrange TMAs from randomised treatment trials with a high quality database of clinical information or to use a standardised collection of Biobank materials as described within the MCRC Biobank program.

Within the Molecular Pathology focus at the Breakthrough Breast Cancer Research Unit at the Paterson Institute for Cancer Research, and in collaboration with the Histology facility, we are establishing a state of the art facility for TMA work including all necessary equipment for TMA construction as well as staining, digital scanning and analyses. Primarily we will focus on breast cancer but within the centre we will also construct and analyse other tumour types such as lung cancer and renal cell carcinomas as well as constructing TMAs from different tumours within the MCRC Biobank program. The expected outcome is that we should construct and distribute easy accessible research packages of tumours arranged in TMAs linked to clinical information. These TMAs will be important in validation of biomarkers in different treatment schedules as well as be essential for basic researchers to extend and validate novel tumour biological relevant targets and key proteins in large collections of high quality clinical samples.
Clinical trials coordination unit

By Angela Ball

The regulatory requirements that govern the management of clinical trials have changed significantly in recent years, and this together with changes in the management arrangements for non-commercial clinical research in the UK has created a need for formal management structures to support researchers in the conduct of the trials that they lead. To address this, the MCRC has developed a clinical trials unit led by The Christie NHS Foundation Trust. The Christie has a long history of managing clinical trials and many of the trials unit staff have nearly 30 years experience in supporting clinical trials in The Christie. The development and progress of the trials unit is discussed in this section.

Background

The purpose of clinical trials is to evaluate alternative therapies in a controlled manner so that evidence is created to guide future clinical care. The basis of the clinical question is formalised in a written protocol which is subjected to scientific, regulatory and ethics review before the study can commence. Operationally the management of clinical trials is complex, it is essential that every step in the process is carefully controlled so that the data generated are robust. To support these processes a number of specialists are involved, these specialists and activities include;

- **Chief investigator** has primary responsibility for the trials, leads the study from a clinical perspective, is responsible for ensuring that the protocol is robust, clinically sound and meets the requirements of the Declaration of Helsinki regarding the protection of trial participants.

- **Statisticians** give advice on methodology and calculate the minimum number of participants required to give confidence in the trial outcomes, they generate the information systems to support data collection and analyse the data as required during the course of the study.
• **Trial managers** support the overall coordination of the study, working with the Chief investigator to identify the number of sites needed to deliver the study in an optimal fashion. They are responsible for ensuring that each site has the information and documentation needed to conduct the study within regulatory and protocol requirements.

• **Data managers** are responsible for the quality of data collected for the trial.

• The drugs or ‘Investigational Medicinal Products’ used in clinical trials are managed separately from other supplies; they are labelled specifically for the study and released for use by recognised practitioners called ‘Qualified Persons’. Many of these activities are contracted out to specialist providers; the process is overseen by the Trials pharmacist. Clinical trials that are investigating medications and devices are subject to the controls of the Medicines and Health Care Products Regulatory Agency.

The overall objective of the trial management team is to optimise the conduct of the study; ensuring that the study is delivered in a timely manner but with minimal administrative workload; i.e. balancing timeliness of delivery versus the number of participant sites.

**Clinical research environment**

There have been changes in the regulations that govern clinical trials, within the European Union (EU) there has been an attempt to standardise these with the introduction of the EU Clinical Trials Directive. This was enacted in the UK with the Medicines for Humans (Clinical Trials) Regulations 2006. These regulations control the conduct of clinical trials in the UK, other regulations that are relevant to clinical trials include the Human Tissue and Data Protection Acts. There are also international guidelines such as the Declaration of Helsinki and Good Clinical Practice (ICH GCP).
The funding arrangements for non-commercial clinical research have changed with the development of the National Institute for Health Research (NIHR). The NIHR provides funding for much of the non-commercial clinical research within the UK, either through direct grant awards that fund the cost of conducting the research itself or through the funding of the NHS ‘support costs’ incurred through the conduct of these trials, these support costs cover additional patient tests such as CT scans etc. The NIHR has stipulated that only registered clinical trials coordination units are permitted to run studies funded by the various NIHR funding routes.

The Christie has a long history of managing clinical trials, however, in the past these were conducted within the disease orientated teams with The Christie. The changing environment for clinical research created a need for the development of formal infrastructure to support trials led by MCRC researchers. To address this, the MCRC has developed a clinical trials unit led by The Christie NHS Foundation Trust. The unit has achieved registration status with the NIHR/UKCRC and is the only registered unit in Manchester.

**Clinical Trials Unit Activities**

The services and activities of the trials unit include the coordination of international trials, provision of support services such as statistical advice and operation of a randomisation line. The unit is also involved in the development of NIHR/UKCRC trials units and has representatives on a number of the working groups. Examples of some current and planned clinical trials are given below.

**Current clinical trials - International studies**

**CONVERT** – Concurrent Once-Daily versus Twice-Daily Radiotherapy – A 2-arm randomised controlled trial of concurrent chemo-radiotherapy comparing twice-daily and once-daily radiotherapy schedules in patients with limited stage small cell lung cancer (LS SCLC) and good performance status.
Chief Investigator; Dr Corrine Faivre-Finn

CONVERT is a multicentre, international, randomised phase III trial. At least 532 patients are to be recruited. Recruitment commenced in April 2008 and is expected to take approximately four years. Seventeen patients have been enrolled onto the trial to date and ten centres are open to recruitment (8 in the UK and 2 in Belgium). 96 sites have expressed an interest in participating in the trial (37 in the UK, 11 in Spain, 26 in France, 12 in Canada, 5 in the Netherlands, 3 in Belgium, 1 in Poland and 1 in Slovenia) and we are currently working towards opening as many sites to recruitment as possible.

The study is sponsored by The Christie NHS Foundation Trust, in collaboration with the EORTC (European Organisation for Research and Treatment of Cancer), NCIC (National Cancer Institute of Canada), Grupo Español de Cáncer de Pulmón and Groupe Français de Pneumo-Cancérologie. CONVERT is the only phase III trial currently open looking at the optimisation of chemo-radiotherapy in LS SCLC. Following on from the Turrisi trial of once vs. twice daily concurrent chemoradiotherapy (published in 1999), there is a real need for a new phase III trial using modern conformal radiotherapy techniques and an increased once-daily radiation dose. This trial has the potential to define a new standard chemoradiotherapy regimen for patients with LS SCLC and good performance status.
Clinical trials coordination unit

**FIZZ** - Phase II Study of Fractionated $^{90}$Y Ibritumomab tiuxetan (Zevalin™) Radioimmunotherapy as an initial therapy of Follicular Lymphoma  
Chief Investigator: Professor Tim Illidge

FIZZ is a multicentre, international, phase II trial which is sponsored by The Christie NHS Foundation Trust. Recruitment commenced in June 2007 and the trial is expected to complete in 2013, to date 25 out of 80 patients have been recruited across 5 sites – The Christie (Manchester), Southampton General Hospital, Dorset Cancer Centre (Poole), St George’s Hospital (London) and two sites in France at hospitals in Lille and Nantes. The trial is also in set-up in Milan, Italy and we hope to commence recruitment there as soon as possible.

**Current clinical trials - National studies**

**Manuka honey** and radiation induced mucositis  
Chief Investigator: Dr. N.J. Slevin

This single centre trial sponsored by The Christie NHS Foundation Trust and funded by the Booth fund completed follow-up in 2008. The trial randomised 131 patients to active manuka honey or a placebo plus standard oral care in the treatment of radiation-induced oral/oropharyngeal mucositis. Data analysis has been completed and publication is anticipated for 2009.

**ACU.FATIGUE** – comparing standard care with or without acupuncture in the relief of fatigue  
Chief Investigator: Professor Alex Molassiotis

This multicentre trial sponsored by The University of Manchester and funded by Breakthrough Breast Cancer opened to recruitment in 2008. It is the largest study of its kind aiming to recruit 320 patients and randomise them to standard care +/- acupuncture with fatigue related measures as the primary outcome. 46 patients were entered into the trial in 2008.

**ANCHoR** – evaluating the efficacy of acupressure wristbands in alleviating chemotherapy-induced nausea  
Chief Investigator: Professor Alex Molassiotis

This multicentre trial sponsored by The University of Manchester and funded by the Health Technology Assessment funding is currently in set-up with recruitment due to commence in early 2009. Around 700 patients will be randomised.
Clinical trials and other studies published in 2008:

ACE/PE - a Phase III randomised trial of doxorubicin-based chemotherapy compared with platinum-based chemotherapy in small-cell lung cancer involving a total of 280 patients (139 ACE, 141 PE).
Chief Investigator: Professor Nick Thatcher

Response rates for the two groups of patients were comparable, but more grades 3 and 4 neutropenia (90 vs 57%, \( P < 0.005 \)) and grades 3 and 4 infections (73 vs 29%, \( P < 0.005 \)) occurred with ACE, resulting in more days of hospitalisation and greater i.v. antibiotic use. ACE was associated with a higher risk of neutropenic sepsis than PE and with a trend towards worse outcome in patients with limited disease, and should not be studied further in this group of patients.

HIF-1α - this study examined the prognostic significance of tumour hypoxia inducible factor-1alpha expression for outcome after radiotherapy for oropharyngeal cancer.
Chief Investigator: Professor Catharine West

HIF-1α expression was examined in a subset of 79 cases in which expression data on HIF-1α were available (from a larger consecutive series of 133 cases receiving primary radiotherapy at The Christie between 1996 and 2001). HIF-1α expression was found to be of prognostic importance with a tentative finding of a greater effect in the tonsil sub-site compared to tongue.

GEM Hospitalisation study – a pragmatic randomised study to compare the hospitalisation rates of two platinum-based outpatient regimes.
Chief Investigator: Professor Nick Thatcher

401 patients were randomised between Nov 2002 and Aug 2006. The number of overnight stays required for chemotherapy administration did not differ. This study demonstrates that fractionated cisplatin treatment with gemcitabine can be given safely to an outpatient and is well tolerated, with significantly less requirement for blood and platelet transfusions, and similar survival outcome to that of the Gemcitabine/Carboplatin regimen.

Non-Hodgkin’s lymphoma - a study which examined long-term (median follow-up 15.7 years in survivors) outcomes from a randomised trial of 80 patients conducted at The Christie.
Chief Investigator: Professor John Radford

The trial was powered for dose-intensity measures rather than survival but these unique long term outcome data generate hypotheses about causes of death that warrant further investigation.

Chemo radiotherapy - a study looking at a consecutive series of 39 patients with locally advanced pancreatic cancer.
Chief Investigator: Professor Pat Price
Fifteen patients treated from 1993 to 1997 received 50 Gy in 20 fractions (group I) and the remaining 24 treated from 1997 to 2003 received an escalated dose of 55 Gy in 25 fractions (group II). The study demonstrated that dose-escalation in pancreatic cancer is feasible along with a suggestion of an improved survival outcome.

**VAC/PC** – a randomized Phase III study of carboplatin and paclitaxel (PC) versus vincristine, doxorubicin and cyclophosphamide (CAV) chemotherapy in intermediate and poor prognosis small cell lung cancer  
Chief Investigator: Professor Nick Thatcher

219 patients (110 for CAV and 109 for PC) patients were randomized. Significantly more grade 3 and 4 infections were observed on CAV arm. Hospitalization required for severe neutropenia and infections was less with PC compared to CAV. Response rates for CAV and PC were similar. The intention to treat analysis concluded that there is statistical difference on the overall survival time in favour of PC ($P = 0.014$). CAV chemotherapy in SCLC patients is associated with higher risk of neutropenic sepsis than taxane-based PC chemotherapy with no significant difference on the response rate. The overall survival was better for the PC arm.

**ACTOR study** - a feasibility and toxicity report from a phase II study of accelerated twice-daily (BDRT) versus high dose once-daily thoracic radiotherapy (ODRT) with concurrent chemotherapy for limited stage small cell lung cancer (LS-SCLC).  
Chief Investigator: Dr Corinne Faivre-Finn

Our preliminary findings are that these regimens are feasible and well tolerated with a lower than expected rate of acute grade 3+ esophagitis. The CONVERT trial will evaluate these regimens in 532 patients for the primary endpoint of survival.
Author Biographies

Caroline Dive and Andrew Hughes - The MCRC-AstraZeneca Alliance – page 10

Professor Caroline Dive is a Senior Group Leader at the Paterson Institute for Cancer Research and Professor of Pharmacology in the School of Cancer and Imaging Sciences at The University of Manchester. She is a co-director of the Clinical and Experimental Pharmacology Group (CEP) within the Paterson Institute for Cancer Research. After completing her PhD studies in Cambridge, Caroline moved to Aston University's School of Pharmaceutical Sciences in Birmingham where she started her own group studying mechanisms of drug induced tumour cell death. She then moved to what became the Faculty of Life Sciences at The University of Manchester to continue this research and was awarded a Lister Institute of Preventative Medicine Research Fellowship. Caroline joined the Paterson Institute for Cancer Research in 2003 to set up the CEP, interfacing with the Derek Crowther Unit for early clinical trials at The Christie. The CEP aims to drive early clinical evaluation of novel mechanism-based targeted therapies by providing biomarkers expertise, analysis of which acts as a readout of drug activity within the body.

Professor Andrew Hughes holds a chair of Translational Medicine in the School of Cancer and Imaging Sciences at The University of Manchester and is head of the Translational Medicine Group, which is part of the Cancer Studies Research Group of the Research School of Cancer and Imaging Sciences within the Faculty of Medical and Human Sciences. Andrew is also accountable for the early phase clinical development of AstraZeneca’s oncology compounds in volunteers and patients and has been a clinical investigator on over 150 clinical trials. His research interests include the evaluation of pharmacodynamic and predictive biomarkers which can be applied to clinical trials, understanding the strength of target linkage with human disease and early clinical trials of novel cancer treatments.

Kim Linton, Yvonne Hey, Stuart Pepper and John Radford - Affymetrix gene expression profiling of archival formalin-fixed and paraffin embedded tissues – page 16

Dr Kim Linton joined The Christie in 2000 as a Specialist Registrar in Medical Oncology and is now working on the Lymphoma Team. She undertook her medical education at The University of Cape Town between 1989 and 1994, and gained her medical qualifications in Cape Town and from the University of Edinburgh. Kim was also awarded a PhD from The University of Manchester in 2007, for her work on the evaluation of novel approaches to prognostic biomarker discovery in soft tissue sarcoma using gene expression microarrays. Kim’s current research interests include optimisation of methods for gene expression profiling of archival formalin-fixed paraffin embedded tissue, molecular-based diagnosis of lymphoma using needle core biopsies, and translational lymphoma and sarcoma research. She is also involved in the development and evaluation of serological and imaging biomarkers of response and prognosis in lymphoma, evaluation of second cancer breast cancer after treatment for Hodgkin Lymphoma and in lymphoma clinical trials.
Yvonne Hey has worked at the Paterson Institute for Cancer Research for 24 years and has spent the last six years in the Molecular Biology Core Facility. She manages the day-to-day running of the Cancer Research UK GeneChip Microarray Facility providing access to cutting-edge microarray technology for all Cancer Research UK grantees. To keep abreast of the latest developments in the field, Yvonne assesses new technologies and introduces them into the service. The team collaborates effectively with both local and national researchers and as a result has helped develop and drive publications that incorporate the use of the most up-to-date microarray protocols.

Stuart Pepper has run the Molecular Biology Core Facility for the Paterson Institute for Cancer Research for the last eight years. The Core Facility provides a range of services including DNA sequencing, DNA extraction and quantitative polymerase chain reaction (PCR) analysis. The Core Facility also houses the Paterson’s Mass Spectrometry facility, which provides state-of-the-art protein analysis services. During the last six years, Stuart has also managed the Cancer Research UK GeneChip Microarray Facility, providing access to cutting edge microarray technology for all Cancer Research UK grantees. The Core Facility team have collaborated widely with local and national researchers and have produced several publications using the latest microarray protocols available.

Professor John Radford is the Research and Development Director at The Christie and Professor/Honorary Consultant at the Cancer Research UK Department of Medical Oncology within the Faculty of Medical & Human Sciences in the School of Cancer and Imaging Sciences at The University of Manchester. He is involved in developing and testing new ways of diagnosing and treating lymphoma and monitoring the effectiveness of these interventions. John is also interested in the late effects of treatment on fertility, heart function and the risk of secondary cancers and investigates how best to prevent or minimise the impact of these in patients cured of lymphoma.
Dr Tim Somervaille leads the Leukaemia Biology group at the Paterson Institute for Cancer Research and is also Honorary Consultant in Haematology at The Christie NHS Foundation Trust. He studied Medicine at St Mary’s Hospital Medical School (now part of Imperial College London). Following postgraduate training in General Medicine, he undertook specialist training in Clinical Haematology at University College London, where he also studied for a PhD as a Medical Research Council Clinical Training Fellow. Subsequently as Leukaemia Research Fund Senior Clinical Fellow, he spent four years as a postdoctoral researcher at Stanford University. His current research focuses on understanding the biology of both normal haematopoietic stem cells and disordered leukaemia stem cells (LSCs) in haematological malignancies in order to identify the genes and cellular pathways critical for LSC function.

Dr Valerie Kouskoff joined the Paterson Institute for Cancer Research in 2003 and is Group Leader of the Institute’s Stem Cell Haematopoiesis Group. She obtained her MSc in 1988 from the Universite Louis Pasteur, Strasbourg, France and was a post-graduate student at the Laboratoire de Genetique des Eucaryotes du CNRS in Strasbourg between 1988 and 1994. Valerie undertook her post-doctoral training at the National Jewish Center, Denver, USA between 1994 and 1999, before becoming an Instructor (1999-2002) then Assistant Professor at the Mount Sinai School of Medicine in New York, USA (2002-2003). Her research focuses on identifying the cascades of gene expression that regulate either initial formation of the haemangioblast, the first blood precursor, or its subsequent decisions to differentiate. Valerie’s studies should provide new insights into the mechanism of diseases that affect haematopoietic subpopulations in the adult.

Dr Georges Lacaud is Group Leader of the Stem Cell Biology group at the Paterson Institute for Cancer Research, a post he has held since 2003. He graduated from the European Biotechnology School of Strasbourg, France, and received his PhD from the University Louis Pasteur of Strasbourg working on gene therapy and HIV. He then completed a postdoctoral fellowship at the National Jewish Medical Center in Denver, Colorado, USA and then worked at the Mount Sinai School of Medicine in New York, USA as an Assistant Professor. Georges’ research focuses on the molecular and cellular mechanisms of haematopoietic development and how alterations of these processes lead to leukaemia.
Dr Robert Clarke is a Breast Cancer Campaign Research Fellow, Group Leader and Lecturer within the School of Cancer and Imaging Sciences and is based at the Paterson Institute for Cancer Research, The University of Manchester. He obtained his PhD in 1995 for studies on the control of proliferation in the normal and neoplastic human mammary gland at The University of Manchester and undertook his post-doctoral training with Dr Liz Anderson in the Clinical Research Department of The Christie, Manchester. He returned to The University of Manchester as a Cancer Research UK Research Fellow in 2001, becoming a Group Leader in the Division of Cancer Studies at the Paterson Institute for Cancer Research. His research aims to understand the hierarchical relationship between cells in breast epithelium – characterising stem and differentiated cells, investigating their regulation and how they interact or communicate – in order to elucidate how cancer is initiated and what underlies its recurrence.

Charles Streuli - Cell adhesion – page 28

Professor Charles Streuli is Research Director of the Breakthrough Research Unit in Manchester, Professor of Cell Biology, and is associated with the Wellcome Trust Centre for Cell-Matrix Research. He is also a founding member of the Manchester Breast Centre. Charles obtained his PhD from the University of Leicester, and has held several postdoctoral positions including research posts at the Imperial Cancer Research Fund Laboratories (then Royal Postgraduate Medical School) London, and the Lawrence Berkeley Laboratories, California. He joined The University of Manchester in 1992 with a Wellcome Senior Research Fellowship in Basic Biomedical Science to establish his own research group. His research focuses on understanding how signalling pathways regulate the behaviour and function of breast epithelial cells, and how apoptosis (programmed cell death) is controlled in breast epithelia. Charles' group is currently exploiting their work in the normal breast to elucidate the mechanisms underlying how altered adhesion and apoptosis contribute to breast cancer, and are identifying potential targets for therapeutic intervention.

Nullin Divecha - Inositol lipids – page 34

Dr Nullin Divecha is a senior Group Leader at the Paterson Institute for Cancer Research and heads the Inositide Laboratory. He received his BSc in Biochemistry from The University of Manchester in 1985 before undertaking a PhD at the University of Sheffield in 1988, focussing on proline-rich proteins. Nullin joined the Babraham Institute in Cambridge (UK) in 1988, first as a Cancer Research Fellow then as a Babraham Fellow, running his own laboratory. He accepted an AVL Fellowship at The Netherlands Cancer Institute in Amsterdam in 1997 before returning to the UK in 2007 to join the Paterson Institute. Nullin's research focuses on cell signalling. His group aim to understand how phosphoinositides, a family of lipid second messengers intimately involved in signal transduction, regulate cancer-relevant cellular pathways and to identify novel targets for anticancer therapy.
Professor John Gallagher is head of the Glyco-Oncology Group in the School of Cancer and Imaging Sciences at The University of Manchester. He received his DPhil at the University of York in 1970, and then worked as a post-doctoral fellow at the Universities of Oxford and Durham before joining the Department of Medical Oncology at The University of Manchester in 1976. John was elected as a Fellow of the Academy of Medical Sciences in 2002 and in 2005 launched a new start-up company, Iduron, which supplies research reagents for glycoscience research. His research is mainly focused on the structure and function of cell surface heparan sulfate proteoglycans (HSPGs) that play fundamental roles in the regulation of cell growth, adhesion and differentiation. He is particularly interested in the basic molecular structure of the HS-polysaccharide chain and the identification of intra-chain active sites that promote the assembly of signalling complexes of growth factors and their receptors. John is also interested in the role of HSPGs in the regulation of self-renewal and lineage commitment of embryonic stem cells and is involved in identifying HS-saccharide inhibitors of angiogenic growth factors that stimulate the growth of tumour blood vessels essential for tumour growth and spread.

Professor Gordon Jayson is Professor of Medical Oncology and leads the glycoangiogenesis research undertaken by the School of Cancer and Imaging Sciences at The University of Manchester. He qualified in Medicine at the University of Oxford before undertaking his medical and oncology training in Manchester and The Christie. Following his PhD, he conducted post-doctoral research that aimed to translate new data and understanding of heparan sulfate biology gained from fundamental research into the clinic. Gordon’s current research interests are largely centred on ovarian cancer, the disease that he oversees within The Christie. He focuses on laboratory development of novel oligosaccharides as anti-angiogenic agents and the early and late clinical trial evaluation of this class of drugs. The aim is to identify blood-borne and imaging based biomarkers that will identify patients most likely to benefit from treatment antiangiogenics, and to develop rational combination regimens of biological agents – identifying therapies that may work together to eliminate cancer cells.

Dr Claudia Wellbrock leads the Cell and Adhesion Signalling group at The University of Manchester. Claudia received her PhD in Chemistry from the University of Wuerzburg, Germany in 1995, for studies on the oncogenic function of receptor tyrosine kinases and stayed at the University to work on the signal transduction involved in pigment-cell transformation and melanoma development. In 2002 she joined the Institute of Cancer Research in London, UK, studying the role of the BRAF gene in the initiation and development of melanoma, before joining The University of Manchester / Manchester Cancer Research Centre in 2007. Claudia’s research focuses on two major aspects of melanoma biology: the adhesion-dependent signalling that is induced by the tumour-microenvironment and the cellular signalling that provides tumour-specificity. She aims to understand the crosstalk between particular cellular signalling cascades in melanoma initiation and progression in order to identify new targets for cancer therapy.
Dr Adam Hurlstone leads a group studying pharmacogenetics and tumourigenesis in zebrafish at The University of Manchester. He obtained his BA from the University of Cambridge in 1994, before undertaking post-graduate research at the Beatson Institute in Glasgow, identifying genes implicated in human cancer, and obtaining a PhD in 1998. Adam subsequently undertook post-doctoral research in the field of Wnt-signalling and cancer in The Netherlands then became a lecturer at The University of Manchester in 2004. His group uses the zebrafish as a model organism to better understand tumour biology and to identify factors which result in malignant progression as well as chemotherapy resistance. These models are also tools for drug discovery and development and his group is studying the efficacy of small molecules in suppressing cancer, and screening for novel anti-cancer drugs.

Catharine West – Translational Radiobiology – page 52

Professor Catharine West established and leads the Translational Radiobiology Group in the School of Cancer and Imaging Sciences at The University of Manchester. She holds a BA in Biology from York University and studied radiobiology as a postgraduate at the Institute of Cancer Research, Sutton. After postdoctoral work at the University of Rochester Cancer Centre, New York, Catharine joined the Paterson Institute for Cancer Research in 1986 and The University of Manchester in 2002. She is an honorary member of the Royal College of Radiology and in 2007 became an honorary fellow of the British Institute of Radiology. Since 2007, she has also been Postgraduate Director for the School of Cancer and Imaging Sciences at The University of Manchester. Her research interest lies in the characterisation of molecular profiles that reflect relevant biological characteristics and predict how tumour and normal tissue respond to radiation. Her group focuses on hypoxia (oxygen deprivation common in solid tumours), why and how different individuals show different sensitivity to radiation, and the optimisation of non-invasive imaging techniques.

Jane Rogan, Noel Clarke and Goran Landberg - MCRC Biobank and future developments in Tissue Microarrays – page 58

Jane Rogan is the MCRC Biobank Business Manager. She is responsible for the day-to-day management and administration of the Biobank. With over five and a half year’s service in the NHS, she has extensive experience of human tissue research and its accompanying regulation and has been involved in tissue banking for over four years. Her background is in Biological Sciences and she is currently studying for an MBA at the University of Huddersfield.
**Professor Noel Clarke** is a Consultant Urological Surgeon at The Christie and Salford Royal Hospitals in Manchester and Professor of Urological Oncology in the School of Cancer and Imaging Sciences at The University of Manchester. He qualified in medicine at Charing Cross Hospital, London, in 1981 and gained full accreditation as a Urological Surgeon in 1993. Noel is Chairman of the National Cancer Research Institute (NCRI) Prostate Clinical Studies Group, Chairs the European Organisation for Research and Treatment of Cancer (EORTC) Prostate Disease Orientated Group and is Chair of the Greater Manchester and Cheshire Urology Network Clinical Studies Group. He directs the Genito-Urinary (GU) Research Group and is the Principal Investigator of the Manchester arm of the Medical Research Council (MRC) Northern Prostate Cancer Collaborative. Noel’s research has focused on cancer stem cell biology and the pathophysiology of metastatic behaviour, with translational studies directed towards analysis of new biomarkers, advanced cancer imaging and the evaluation of novel therapies.

**Professor Göran Landberg** is the first Team Leader to be appointed at the Breakthrough Breast Cancer Research Unit in Manchester and heads the Molecular Pathology Group in the School of Cancer and Imaging Sciences at The University of Manchester. His laboratory is based within the Paterson Institute for Cancer Research and his clinical work at The University Hospital of South Manchester. Goran obtained his medical degree and his PhD from Umeå University, Sweden, and then moved to The Scripps Research Institute in San Diego, USA, to work on cell cycle related auto antigens as a postdoctoral fellow. He then established his own research group at Umeå University and moved to Lund University, Sweden, as a Professor in Pathology with affiliation to Malmö University Hospital, Sweden in 2000. He is the founder of the Swedish Tissue Micro Array Centre and a member of the executive committee of the Swedish Cancer Foundation. Goran’s research focuses on cell cycle regulators in relation to the resistance of breast cancer to therapy, and the interplay between proliferation and migration control in breast cancer as well as in other tumour model systems such as basal cell carcinomas. These studies will provide the potential for discovering novel diagnostic markers of resistance and new therapeutic targets.

**Angela Ball**

Angela Ball is Research and Development Manager at The Christie, a post she has held since 2002. She is responsible for the development and delivery of The Christie’s research strategy. As General Manager for the Research and Development Division, Angela is tasked with ensuring delivery of the Division’s performance objectives. She is also responsible for the business and operational management of all clinical research including research facilities and business units (the Phase I Clinical Trials Unit, the Wade Radiotherapy Research Centre, Tissue Bank and the Clinical Trials Coordination Unit), for the management of a multidisciplinary research team of approximately 140 staff and for the management of research funding/income of around £8 million per annum, approximately £2.2 million if which is commercial income. Angela also works to ensure regulatory compliance of research projects including the Medicines for Humans (Clinical Trials) regulations and the Human Tissue Act and is Designated Individual for the Trust’s research license.