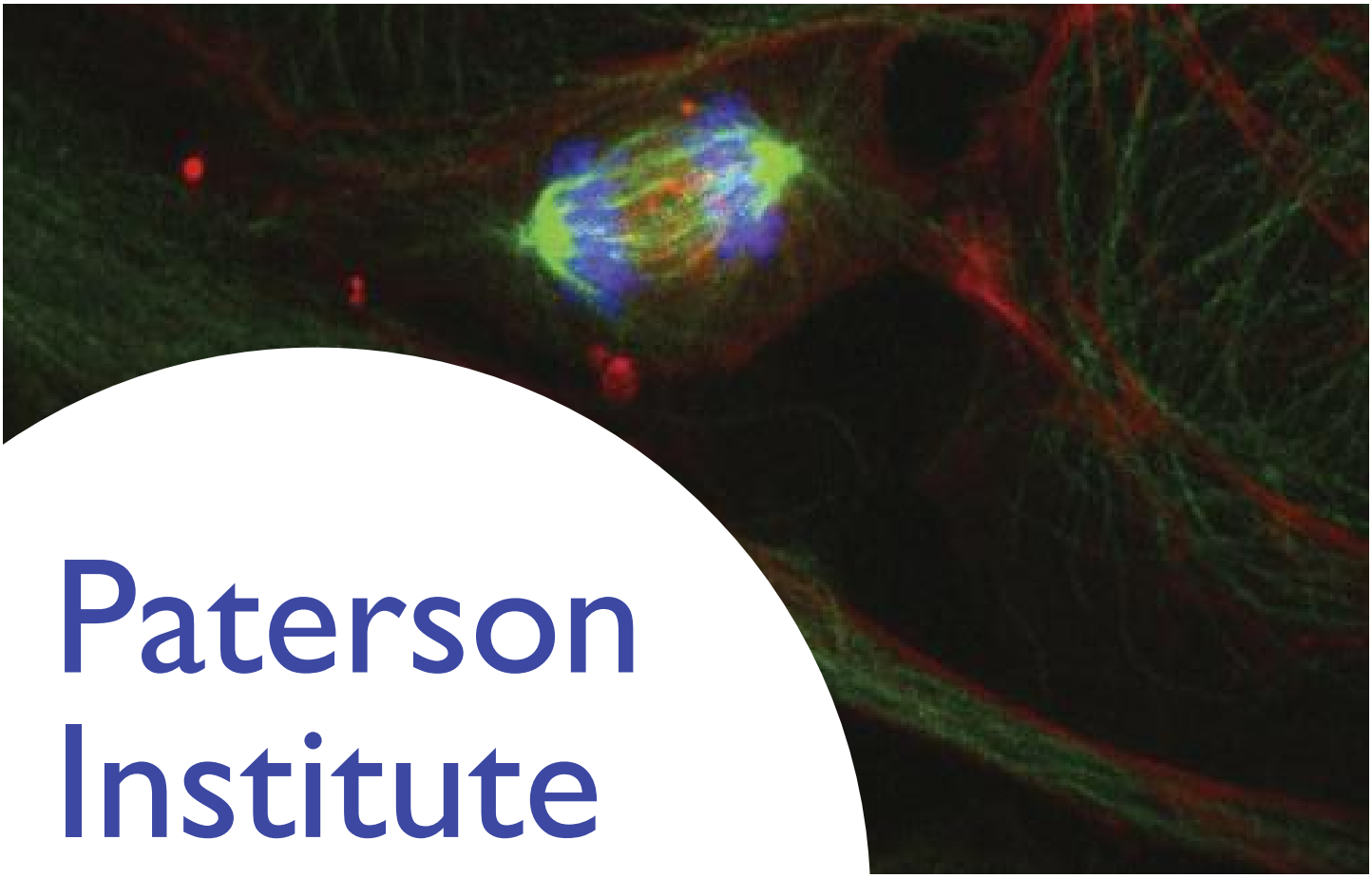

Paterson Institute for Cancer Research

Scientific Report 2009

Together we will beat cancer

CANCER RESEARCH UK

Cover images

Top

Mitotic BPAE cells in anaphase. F-actin is labelled with Texas Red-x phalloidin. Microtubules, in green, are labelled with mouse anti-α-tubulin BODIPY FL goat anti-mouse IgG. Blue nuclear staining with DAPI. Imaged on the Spinning Disk Confocal microscope.
Image provided by Achille Dunn, Advanced Imaging Facility.

Bottom

Immunostaining demonstrating blood vessels surrounding a tumour. Glut1 immunostaining (red) specifically labels veinous structures whereas arterial structures are Glut1 negative. Red blood cells in the vessels were detected by inherent autofluorescence (green) and cell nuclei were labelled with DAPI (blue).
Image provided by Darren Roberts, Clinical and Experimental Pharmacology Group.

Scientific Report 2009

Paterson Institute for Cancer Research

Paterson
Institute for Cancer Research

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The University of Manchester

CANCER RESEARCH UK

Contents

Director's introduction

Nic Jones

Welcome to the 2009 Paterson Institute Annual Scientific Report. It has been a particularly important year with the completion of the Institute's Quinquennial Review but also with the initiation of the new Drug Discovery Centre.

The Institute is very privileged to receive such strong financial core support from Cancer Research UK. In return the Institute has to ensure that it is undertaking research of the highest international quality and that the research that we do will impact significantly towards achievement of CR-UK's major research goals as outlined in their 5-year strategic plan. There are a number of ways of measuring our success which includes periodic review of all the research programmes. However, perhaps the most important process is the Quinquennial Institute Review which is commissioned by CR-UK and involves assembling a team of world-leading scientists experienced in running research organisations or institutes. The review team visits the Institute over a two-day period and assesses in depth the Institute's performance over the last five years and also reviews the strategic direction and plans for the next five-year period. For CR-UK, such reviews are essential for them to ensure that the 'return on investment' is meeting or exceeding their expectations and thereby justifies the long-term commitment they make to the Institute and to the Institute Director. For us, it is a strong reminder of the competitive environment we work in and the duty we have to deliver high-quality and relevant research.

The review took place at the end of June with a very successful outcome. The review party praised the continuing progress we had made over the last five years and were especially supportive of the establishment of the Manchester Cancer Research Centre (MCRC), the progress it had made in such a short-period of time since it was initiated and the significant role the Institute plays in delivering the MCRC's goals and ambitions. I was obviously very pleased that the Institute received such strong support and that developments over the last few years were positively recognised. However, these reviews are not just about judgement of past activities but are also about assessing and advising on future directions and in this respect

the review was also supportive and provided valuable, constructive input into the development of future programmes which will ensure that we can build on the progress we have made over the next five years. Cancer research is at a very exciting stage and driven by new technologies, new and exciting avenues are opening up especially at the laboratory/clinical interface. Given our proximity to The Christie NHS Foundation Trust and our involvement in the MCRC, we are well placed to take advantage of these new opportunities.

An exciting development that really began to take off this last year is the Drug Discovery Centre, an initiative that is a key component of CR-UK's strategic plan to increase its capability and activity in the development of small molecule drugs. Two new centres are being developed linked to the Paterson Institute and our sister institute in Glasgow, the Beatson Institute. CR-UK is providing significant new funding in order to develop the centre at the Paterson Institute. Linking the centre to the Institute has a number of advantages including the potential interactions with already existing groups involved in biomarker research and the testing of new therapies in early clinical trials, the exploitation of the cancer biology research ongoing with the Institute and the wider interactions facilitated through the MCRC. There are real opportunities for academic drug discovery programmes to exploit and add value to the research that we do and to consider areas of real clinical need that for a variety of reasons might not be attractive to the pharmaceutical industry. Developing new drugs is very challenging and takes many years but the benefits to us of having such a Centre within the Institute will be tangible from the start – it will greatly enhance the multidisciplinary environment of the Institute, provide leading–edge chemical tools to enhance our research efforts and instil within the Institute a drug hunting culture. Donald Ogilvie joined us in February 2009 to lead this new development. He previously acquired considerable experience in leading drug discovery programmes at AstraZeneca having overseen the development of at least eight candidates from target identification to clinical trials. A chemistry lead (Allan Jordan) has also been recruited as well as a number of chemistry and biology team members and refurbishment of laboratory facilities suitable for this type of activity has just been completed. Thus in 2010 a number of discovery programmes will be initiated and we look forward to experiencing the success of the Centre over the years to come.

Recruitment and retention of internationally competitive scientific leaders is essential for maintaining research excellence and building areas of research strength. Many of our recruits are at the Junior Group Leader level and after six years they undergo a rigorous evaluation to consider promotion to Senior Group Leader level and as a consequence long-term and increased commitment to research support. This is a very important quality-control step and the opinions of outside experts in the field are crucial to the decision that is made. Only those leaders who have a demonstrable international profile and have contributed significantly to the field are expected to be successful. During the last year, two group leaders were evaluated for such promotion – Georges Lacaud and Valerie Kouskoff. We are delighted that both were successful and will continue their careers and productive research programmes in the Institute. They both work on the differentiation of embryonic stem cells especially down the haematopoietic lineage. Understanding in detail how this process is regulated is important to understanding a number of haematological malignancies. Another indicator of research

success and reputation is winning individual awards and this year two of our group leaders received an award. Iain Hagan was elected as an EMBO member. Membership is a lifelong honour with new members nominated and elected annually based on proven excellence in research. Karim Labib was awarded the Hooke Medal by the British Society for Cell Biology. The medal is awarded to an emerging leader in cell biology and will be presented to Karim at the annual spring meeting in 2010.

We continue to develop our research services – they are a vital component of the infrastructure of the Institute providing cutting-edge capabilities and technologies. The quality of these services was especially praised during the site visit and there is no doubt that their availability profoundly changes the nature of the experimental approaches that can be adopted within the various research programmes. As part of our continuing investment we established a next generation sequencing platform and the additional computation and analysis infrastructure that supports this technology. This technology is incredibly powerful and is being used to address a number of important biological questions and will increasingly in the future be essential for addressing questions of high clinical importance. This will be an area that will require continuous investment over the coming years as the applications of the technology grows and new generation platforms developed.

Inevitably the MCRC was an important theme considered by the Institute review party and there was great enthusiasm for the partnership and in particular the potential that is has for promoting translational research and ensuring that research funding can ultimately benefit cancer patients. Much progress within the MCRC has been made over the last year: investment in breast cancer research continued with the appointment to the University Medical School of Professor Jonas Bergh from the Karolinska Institute; further investment was made to the tumour biobank initiative recognising its impressive success; investment in biomarker research by AstraZeneca through the AZ/MCRC alliance was doubled in a new three-year agreement; development of a strategic plan for investment and development of lung cancer research. These are just a few examples of developments in the MCRC. We are building for the future and 2009 saw great progress in the development of a new clinical treatment centre by The Christie NHS Foundation Trust. A third of this new £35 million facility will be devoted to early phase clinical trials leading to one of the biggest dedicated trial centres of its kind worldwide. Completion of this exciting development is expected in 2010. In addition, work is expected to begin soon on the detailed planning of a new MCRC research building co-funded by CR-UK and The University of Manchester. This will provide great opportunities to increase our overall research efforts in key areas of cancer research. So there is much to look forward to. I hope you enjoy reading this annual report and seeing the advances we are making.

Research Highlights

In this section we are highlighting some research publications from 2009 which report significant advances in specific areas. The selected papers demonstrate the breadth and the quality of the research being undertaken by Cancer Research UK-funded groups in the Paterson Institute.

Woodcock, S.A., Rooney, C., Liontos, M., Connolly, Y., Zoumpourlis, V., Whetton, A.D., Gorgoulis, V.G. and Malliri, A.
SRC-induced disassembly of adherens junctions requires localized phosphorylation and degradation of the rac activator tiam1.
Mol Cell 2009; 33: 639-653.

The Rac activator Tiam1 is required for adherens junction (AJ) maintenance and its depletion results in AJ disassembly. Conversely, the oncoprotein Src potently induces AJ disassembly and epithelial–mesenchymal transition (EMT). In this study it was shown that Tiam1 is phosphorylated on Y384 by Src. This occurs predominantly at AJs, is required for Src-induced AJ disassembly and cell migration, and creates a docking site on Tiam1 for Grb2. It was found that Tiam1 is associated with ERK. Following recruitment of the Grb2-Sos1 complex, ERK becomes activated and triggers the localised degradation of Tiam1 at AJs, likely involving calpain proteases. Furthermore it was demonstrated that in human tumours Y384 phosphorylation positively correlates with Src activity, while total Tiam1 levels are inversely correlated. Therefore, these data implicated Tiam1 phosphorylation and consequent degradation in Src-mediated EMT and resultant cell motility, and established a new paradigm for regulating local concentrations of Rho-GEFs.

Tubbs, J.L., Latypov, V., Kanugula, S., Butt, A., Melikishvili, M., Kraehenbuehl, R., Fleck, O., Marriott, A., Watson, A.J., Verbeek, B., McGown, G., Thorncroft, M., Santibanez-Koref, M.F., Millington, C., Arvai, A.S., Kroeger, M.D., Peterson, L.A., Williams, D.M., Fried, M.G., Margison, G.P., Pegg, A.E. and Tainer, J.A.
Flipping of alkylated DNA damage bridges base and nucleotide excision repair. *Nature 2009; 459: 808-813.*

A few years ago, the Carcinogenesis Group discovered a new family of proteins that recognise certain types of damage in DNA bases. Collaborating with groups in Newcastle, Sheffield, Bangor, Hershey, Minneapolis, Lexington and La Jolla, the crystal structure of the protein from *Schizosaccharomyces pombe*, bound to a short oligonucleotide containing such damage, has now been published in Nature. The protein clamps around the damaged base and flips it out of the helix into a binding pocket, generating a kink in the DNA. This results in the elimination of the lesion from DNA, but defining the detailed molecular mechanism of this process is proving rather a challenge. Nevertheless, if a similar mechanism occurred in human cells, it could have important implications not only in cancer causation, but also in cancer chemotherapy, where the sensitivity of normal cells to the toxic side effects of treatment, and the resistance of tumour cells to drugs, are recurrent problems. The search is on.

Ivanov, A., Beers, S.A., Walshe, C.A., Honeychurch, J., Alduaij, W., Cox, K.L., Potter, K.N., Murray, S., Chan, C.H., Klymenko, T., Erenpreisa, J., Glennie, M.J., Illidge, T.M. and Cragg, M.S.
Monoclonal antibodies directed to CD20 and HLA-DR can elicit homotypic adhesion followed by lysosome-mediated cell death in human lymphoma and leukemia cells.
J Clin Invest 2009; 119: 2143-2159.

After the initial success with Rituximab (anti-CD20) monoclonal antibody (mAb) which has improved outcomes for patients with in B cell

malignancies, mAb are increasingly utilized in the treatment of many cancers. Although the Fc-FcgR interactions with recruitment of immune effector cells such as macrophages and NK cells are thought to explain much of the therapeutic effect seen with some mAb like Rituximab, this does not explain why certain mAb specificities are more potent than others. An additional effector mechanism available to mAb is the direct induction of cell death. Previously, we demonstrated that Type II anti-CD20 mAb were able to evoke a non-apoptotic mode of cell death that appeared linked with the induction of homotypic adhesion and furthermore was able to overcome resistance to apoptosis in tumour cells. In this publication we reveal that peripheral re-localization of actin is critical for the adhesion and cell death induced by both Type II anti-CD20 mAb and HLA DR Class II mAb in both lymphoma cell lines and primary CLL cells. The mode of cell death engaged is rapid, non-apoptotic, non-autophagic and dependent on both the integrity of plasma membrane cholesterol and activation of the V-type ATPase. This cytoplasmic cell death involves lysosomes which swell and then disperse their contents, including cathepsin B, into the cytoplasm and surrounding environment. The resulting loss of plasma membrane integrity occurs in the absence of DNA fragmentation and is independent of caspase and Bcl-2 control. These experiments provide new insights into how two clinically relevant mAb elicit cell death and show for the first time that this occurs through a previously unrecognized lysosome-dependent pathway.

Somervaille, T.C., Matheny, C.J., Spencer, G.J., Iwasaki, M., Rinn, J.L., Witten, D.M., Chang, H.Y., Shurtleff, S.A., Downing, J.R. and Cleary, M.L.
Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. *Cell Stem Cell 2009; 4: 129-140.*
Highlighted in:
Cell Stem Cell Preview.
Cell Stem Cell 2009; 4: 97-98.

An important question in the biology of acute myeloid leukaemia is whether the leukaemia stem cells (LSCs) that drive expansion of the disease and which trigger relapse are closer in nature to normal haematopoietic stem cells (HSCs) or alternatively more like downstream myeloid lineage cells that have inappropriately acquired an ability to undergo self-renewal. In a mouse model of human leukaemia initiated by MLL fusion oncogenes LSCs have biological properties quite distinct from HSCs: they are metabolically active, proliferating, aberrantly self-renewing, downstream myeloid cells which have a transcriptional programme more akin to that of embryonic stem cells than adult tissue stem cells. This observation suggests that genes and pathways important in LSCs could be selectively targeted by therapies that spare normal HSCs.

Patel, N., Krishnan, S., Offman, M.N., Krol, M., Moss, C.X., Leighton, C., van Delft, F.W., Holland, M., Liu, J., Alexander, S., Dempsey, C., Ariffin, H., Essink, M., Eden, T.O., Watts, C., Bates, P.A. and Saha, V.
A dyad of lymphoblastic lysosomal cysteine proteases degrades the antileukemic drug L-asparaginase. *J Clin Invest 2009; 119: 1964-1973.*

We are now in an unprecedented era where ~90% of children with acute lymphoblastic leukaemia can be cured with combination cytotoxic chemotherapy. The drugs used are non-specific in action, show a wide interpatient variability and associated with considerable toxicity. This makes tailoring therapy difficult. This paper shows for the first time how leukaemic cells from some patients produce proteases that degrade and inactivate a key antileukaemic drug L-Asparaginase, suggesting that early screening may identify patients who do not benefit from this drug. By pinpointing and then modifying the exact sites of cleavage, the investigators were able to produce a protease resistant active L-Asparaginase. In the process, they identified key structural details that will allow the engineering of a safer and better drug for all patients. While current focus is on identifying smart molecules for targeted therapy, this paper shows there is still life in the old drug yet.

Morohashi, H., Maculins, T. and Labib, K.
The Amino-Terminal TPR Domain of Dia2 Tethers SCF(Dia2) to the Replisome Progression Complex. *Curr Biol 2009;19: 1943-1949.*

E3 ligases for ubiquitin and Sumo play a key role in preserving genome stability during chromosome replication, by activating or repressing particular pathways of DNA repair. This study reported that a specific form of the SCF ubiquitin ligase associates with the replisome in budding yeast. All eukaryotes have multiple forms of the SCF E3 ligase, distinguished from each other by different 'F-box' subunits that target the ligase to specific substrates. In addition to the substrate-binding domain, around one third of F-box proteins have additional domains at the amino terminus of unknown function. The association of SCFDia2 with the replisome was found to be mediated by a unique TPR domain at the amino terminus of Dia2, which binds two particular components of the replisome. The TPR domain of Dia2 tethers SCFDia2 to the replisome, probably increasing the local concentration of the ligase at forks. This represents a novel form of regulation of SCF E3 ligases, and becomes important when cells accumulate a specific class of stalled fork. It now seems likely that the amino terminal domains of other F-box proteins might also control the localisation of their cognate SCF ligases.

Lawrence, C.L., Jones, N. and Wilkinson, C.R.
Stress-Induced Phosphorylation of S. pombe Atf1 Abrogates Its Interaction with F Box Protein Fbh1. *Curr Biol 2009; 19: 1907-1911.*

The Atf1 transcription factor is critical for directing stress-induced gene expression in fission yeast. Previously we found that upon exposure to stress, Atf1 is hyper-phosphorylated by the MAP kinase, Sty1, which results in its stabilization. The resulting increase in Atf1 is vital for a robust response to stress. Here, we investigated the mechanism by which phosphorylation stabilizes Atf1 and found that this protein is a target for the ubiquitin-proteasome system with its degradation dependent upon an SCF E3 ligase containing the F-box protein Fbh1. F-box proteins usually target phosphorylated substrates for ubiquitination. However, stress-induced phosphorylation serves to inhibit the binding of Atf1 to Fbh1, thus representing a novel means of regulating the interaction between an F-box protein and its substrate. Atf1 is the first example of a substrate for any SCFFbh1 complex but it seems likely that Fbh1, in common with other F-box proteins, will direct multiple targets for ubiquitination via the SCFFbh1. Potential substrates are proteins involved in the homologous recombination pathway of DNA repair, as others have shown that Fbh1 acts downstream of Rad51 in this process. Moreover, the mechanism we have described for regulating Atf1-Fbh1 binding may apply to other substrates of Fbh1.

Dean, E., Jodrell, D., Connolly, K., Danson, S., Jolivet, J., Durkin, J., Morris, S., Jowle, D., Ward, T., Cummings, J., Dickinson, G., Aarons, L., Lacasse, E., Robson, L., Dive, C. and Ranson, M.
Phase I trial of AEG35156 administered as a 7-day and 3-day continuous intravenous infusion in patients with advanced refractory cancer.
J Clin Oncol 2009; 27: 1660-1666.

This paper demonstrates synergistic working between the DCU Early Clinical Trials Unit and the Clinical and Experimental Pharmacology Group and reports the 'first into man' study of AEG35156, a second generation antisense to X-linked inhibitor of apoptosis protein (XIAP). The clinical hypothesis tested was that XIAP inhibition reduces the threshold for apoptosis in tumour, exploiting inherent cellular stresses in the tumour micro-environment. This CR-UK sponsored trial was the first undertaken worldwide for a XIAP targeted drug. We determined the maximum tolerated dose of AEG35156, and examined a number of pharmacodynamic circulating and imaging biomarkers. Knock down of XIAP mRNA was demonstrated in PBMCs and drug-induced changes in circulating cell death biomarkers were observed. The study showed that the drug was well tolerated with clinical evidence of activity in refractory lymphoma, melanoma and breast cancer. Dr Dean has since taken up a Clinical Lectureship to continue her research on apoptosis targeted drugs.

Lancrin, C., Sroczynska, P., Stephenson, C., Allen, T., Kouskoff, V. and Lacaud, G.
The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage.
Nature 2009; 457: 892-895.
Highlighted in:
Nature News & Views.
Nature 2009; 457: 801-803.
Cell Stem Cell Preview.
Cell Stem Cell 2009; 4:189-190.
Nature Reports Stem Cells 2009; Mar 12; doi:10.1038/stemcells.2009.35.
selected by F1000

The cellular origin of blood cells is controversial. One first model proposes that haematopoietic and endothelial cells arise from a common mesodermal precursor called the haemangioblast. A conflicting theory instead associates the first haematopoietic cells to a

differentiated endothelial cell with haematopoietic potential, i.e. a haemogenic endothelium. In this paper, we demonstrated that the emergence of blood cells from the haemangioblast precursor proceeds through a haemogenic endothelium intermediate. These results unite the two theories on the origin of haematopoietic development into a single linear developmental process. This finding strongly supports the endothelial origin of some, if not all, haematopoietic cells.

Gandillet, A., Serrano, A.G., Pearson, S., Lie, A.L.M., Lacaud, G. and Kouskoff, V.
Sox7-sustained expression alters the balance between proliferation and differentiation of hematopoietic progenitors at the onset of blood specification. *Blood 2009; 114: 4813-4822.*

The molecular mechanisms that regulate the balance between proliferation and differentiation of precursors at the onset of haematopoiesis specification are poorly understood. We show in this study that Sox7 is transiently expressed at the onset of blood specification. While Sox7 knockdown decreases the formation of haematopoietic progenitors, the enforced expression of this transcription factor promotes the maintenance of multi-potency and self-renewal. Our data demonstrate that the sustained expression of Sox7 is sufficient to completely alter the balance between proliferation and differentiation of haematopoietic precursors. Removal of Sox7-enforced expression fully restores this equilibrium and leads to the efficient differentiation of haematopoietic progenitors. This represent a very attractive characteristic of Sox7 function and might in the future become a powerful molecular tool to allow the expansion of haematopoietic progenitors to be used for potential cell replacem-ent therapy. From a fundamental perspective, it will be very interesting to explore the molecular programme that is either maintained or initiated by Sox7 expression.

Drug Discovery in the Manchester Cancer Research Centre

by Donald Ogilvie and Allan Jordan

Donald Ogilvie & Allan Jordan

In their recent strategy review, Cancer Research UK decided to increase significantly their long term investment in small molecule drug discovery and to align this additional resource with the core-funded cancer research institutes in Glasgow (Beatson) and Manchester (Paterson).

The purpose of co-locating these activities is, of course, to maximise the opportunity for translating the ground-breaking basic cancer research from these centres of excellence into novel therapeutic opportunities.

In this article, we will outline our vision for the drug discovery centre in Manchester and how we intend to deliver maximum value from this new investment.

Our ultimate aim is to identify novel drug therapies to satisfy the unmet clinical needs of cancer patients. However, drug discovery and clinical development are long and complex processes and we will need to engage with many partners to achieve this goal.

The first key partner is of course Cancer Research UK who are providing the crucial funding - £8 million for the first five years. But Cancer Research UK is more than just a source of funding for this new venture. As well as individual programme grants, Cancer Research UK already supports major drug discovery centres in London, Sutton and Newcastle providing a broad portfolio of projects. The new centres in Manchester and Glasgow will be seeking to complement one another in extending this portfolio into new areas of breaking cancer science and drug discovery technology. The leaders of these drug discovery centres are now meeting regularly to share expertise, coordinate their activities and identify areas of cooperation and collaboration in order to maximise the effectiveness of Cancer Research UK drug discovery. As one example of this cooperation, we will be accessing the compound collection and screening technology in the London centre to support our hit identification projects.

Another important part of the Cancer Research UK "family" is Cancer Research Technology (CRT) who provide us with intellectual property and business development support. This is particularly important for the protection of drug discovery inventions and, in the longer term, for identification of partners to take our candidate drugs into clinical trials.

Our major source of local partnerships is the Manchester Cancer Research Centre (MCRC). Within this environment there is a rich pool of basic and translational cancer science, cutting edge technology and clinical expertise.

A key component of the MCRC is the breadth of clinical and drug development expertise at The Christie Hospital. This provides direct insight into the areas of unmet clinical need and the hypotheses to address them but also brings a tangible connection with our ultimate customer, the cancer patient. At the other end of the MCRC spectrum are the basic scientists in the Paterson Institute and more broadly in Manchester University who provide insights into the mechanisms of cancer and how to measure these in preclinical models. In the middle are the translational scientists and clinicians, particularly in the Clinical and Experimental Pharmacology Group at the Paterson, who provide the roadmap for initial clinical development, particularly in the validation of novel biomarkers. We are also exploring opportunities to access other key technologies (e.g. biophysical and computational chemistry, biochemistry and protein structural analysis) through experts in Manchester University.

Since drug discovery and development takes such a long time (10+ years) and many projects do not make it to the clinic we need to need to be able to demonstrate that we are making

progress in the shorter term. In the first five years, this will be primarily through the generation of a unique (within Cancer Research UK) portfolio of attractive drug discovery projects.

During the last six months we have spent a lot of time developing our target selection strategy into a "roadshow" that we have been presenting to groups of cancer researchers in the MCRC. These presentations have been followed up with more detailed target discussions and this has identified the highest priority projects that are already underway (through collaborations). Target review will be an ongoing activity so that we can keep abreast of new developments in cancer science and fuel the drug discovery "pipeline" with the best opportunities.

Once a target is identified, the aim of the drug discovery process is to identify compounds that modulate its activity in order to deliver clinical benefit. This is an iterative process involving the identification of initial chemical "hits", the exploration of their drug potential to create "leads" and then the optimisation of these leads to create a clinical candidate for testing in cancer patients.

In parallel with our target selection activities, we have designed, built and equipped a new laboratory and have recruited a highly skilled team of drug discovery biologists and chemists, all of whom have had industrial experience in the large or smaller (Biotech) pharmaceutical sectors. This core of expertise will enable us to hit the ground running when the new laboratory opens in January 2010. An unusual but deliberate feature of the new facility is the co-location of chemistry and biological science activities in the same laboratory. We believe that this will foster closer teamwork between those who design and make novel compounds and those who test their activity.

In 2009 we laid the foundations of this new and exciting venture. By the end of 2010 we will have a fully functioning team and laboratory and will have started our first home-grown MCRC drug discovery projects.

Figure 1
The newly completed Drug Discovery laboratories


Carl Zeiss

Research groups
Paterson Institute for Cancer Research

Applied Computational Biology and Bioinformatics Group

http://www.paterson.man.ac.uk/bioinformatics

Group Leader
Crispin Miller

Postdoctoral Fellows
James Bradford
John Hall (joint with Translational Radiobiology Group)
Hui Sun Leong
Yaoyong Li
Carla Möller-Levet (joint with Translational Radiobiology Group; to September 2009)

Scientific Officer
Paul Scutt

Research Applications Programmers
Tim Yates
Chris Wirth

Graduate Students
Danny Bitton
Sharmin Naaz (joint with Stem Cell and Haematopoeisis Group)
Andrzej Rutkowski (joint with Immunology Group)

System Administrator
Zhi Cheng Wang (joint with IT department)

The Applied Computational Biology and Bioinformatics group is a computational genomics group focused on developing a better understanding of the genome and the role it plays in cancer. Much of the group's work is directed at exploring the complexities that arise through processes such as alternative splicing and the expression of non-coding RNAs. We do this through a combination of bench science, computer science, mathematics and statistics, and our work is highly dependent on analysing and integrating the data arising from technologies such as next generation sequencing, microarrays and proteomics.

Alternative Splicing

Although generally the most well characterised parts of the genome, many protein-coding loci are still not fully understood, not least because of the additional complexities caused by alternative splicing. This is the process by which cells can selectively remove different sections of pre-mRNA during RNA processing. It allows the expression of a set of closely related, but different transcripts from a single locus, is prevalent, and tightly controlled. The majority of human genes are alternatively spliced, increasing the molecular repertoire of a cell substantially. Given its prevalence, it is not surprising that it is intimately involved in many of the key processes associated with cancer, including angiogenesis, differentiation and apoptosis, and it has been shown to be disrupted in many cancers.

Until relatively recently, it has been impossible to study alternative splicing in a systematic manner, due to our inability to generate global surveys of transcription at sufficient levels of detail. However, advances in technology have now started to make this possible. Affymetrix Exon 1.0ST arrays aim, for example, to separately target every known and predicted exon in the entire genome by featuring individual probesets placed at strategic intervals across each gene. In collaboration with Professor Adrian Harris in Oxford and the Translational Radiobiology Group at The University of Manchester, we have been using these arrays to consider changes in splicing as a consequence of tumour hypoxia in Head and Neck Squamous Cell Carcinomas (HNSCCs). To do this, Carla Möller-Levet has developed novel algorithms for analysing the signals from each individual exon probeset targeting a given gene in order to identify differential splicing events. This work has built on earlier efforts in the group to develop annotation databases (http://xmap.picr.man.ac.uk; Yates *et al*., Nucleic Acids Res 2008; D780) and analysis software in R/BioConductor (exonmap; Okoniewski *et al*., Genome Biol 2007; 8: R79).

Through these studies, we were able to identify a set of characteristic splicing events, a subset of which were subsequently validated using real time PCR (Guy Betts; Translational Radiobiology). This included an isoform of the gene Laminin $\alpha 3$, LAMA3-A, which we were able to show was prognostic for overall survival, while an alternate isoform of the same gene, LAMA3-B, was not (Möller-Levet *et al*., 2009).

Massively Parallel Nucleotide Sequencing (MPNS) and RNA-Seq

A substantial amount of the group's effort has been directed at handling the billions of nucleotides generated each week by our AB SOLiD Next Generation Sequencing platform. James Bradford has been exploring how it can be used to generate global surveys of transcription through the analysis of total RNA and generating, in collaboration with Yaoyong Li,

Figure 1
RNA-Seq data generated from AB SOLiD and displayed in the X:Map genome browser. Millions of short 50mer RNA sequences were generated using the AB SOLiD sequencer, and aligned to the genome (green peaks) in order to provide a very fine-grained measure of gene expression. These data can be placed alongside genome annotation using the X:Map browser. The blue box in the figure corresponds to a gene, with individual exons shown by the smaller red boxes. The 3' UTR is shown at the end of the gene in white, and the locations of known protein domains are shown in the orange box below. Below this, protein domain annotations are shown.

the analysis techniques and statistical filters needed to routinely use the platform for RNA-Seq applications. This has involved extensive use of genome annotation supplied through our database, X:Map, and BioConductor package, exonmap.

Non coding RNAs

The human genome consists of approximately 3 billion nucleotides, of which only about 2% actually code for proteins. This raises a fundamental question as to how much of the remaining 98% of the genome is functional, and the additional roles it might play within a cell. Recent technological developments including tiling microarrays and next-generation sequencing have led to a substantial increase in our understanding of the non protein-coding complement of the human genome, and it is now known that the majority of it is actually transcribed. Many of these loci are now known to express RNA sequences that are functional within their own right, even though they are never translated into proteins. A key focus of the group is to develop a better understanding of these non-coding RNAs (ncRNAs) and the roles they play in cancer. We are currently using MPNS technology to generate RNA-Seq datasets to support these analyses.

Genome annotation

As technology advances further, a common theme is the ability to generate unbiased surveys of the entire genome at increasingly fine levels of granularity. In order to make sense of these data, it is necessary to have access to genome annotation in a form that makes it amenable to algorithmic analysis and the application of appropriately robust statistics. We have been developing annotation tools that make these data available in the statistical software R, and have been extending this work to provide integrated access to DNA, RNA and protein level annotation.

A further challenge with MPNS datasets is the need to visualise the results of an experiment, which in its raw form consists of millions of individual short nucleotide sequences. We are developing extensions to our Google Maps based genome browser (Tim Yates, X:Map; http://xmap.picr.man.ac.uk) that allows MPNS data to be presented alongside genome annotations (see Figure).

Formalin-Fixed Paraffin-Embedded Tissue

Vast archives of well-annotated clinical material exist as Formalin-Fixed Paraffin-Embedded (FFPE) tissue. However, this approach to tissue preservation, which was developed before techniques such as microarrays were invented, poses significant challenges if these samples are to be used for expression profiling experiments. In collaboration with Kim Linton (The University of Manchester) and Stuart Pepper (Molecular Biology Core Facility) we have been able to show that the material can be successfully profiled on Affymetrix Exon arrays (Linton *et al.*, 2009), and work is also underway in collaboration with the Translational Radiobiology Group to apply these arrays to the analysis of cervix tumours (see also the Translational Radiobiology report).

Publications listed on page 66

Carcinogenesis Group

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We have been involved in investigating the mechanism of the biological effects of a class of chemical agents called the alkylating agents. Our interest is based on the observations that agents of this type are mutagenic, and are probably human carcinogens, and on the fact that they are toxic, a characteristic that is exploited in their use in the treatment of certain types of cancer. Both mutation and toxicity can be explained by the reaction of these agents with the purine and pyrimidine bases in DNA. Although there are more than a dozen known types of DNA damage that can be generated, one of these, O^6-alkylguanine, which often constitutes only about 6% of the total damage, seems to be the most important. Our current focus is on how this damage is processed and the impact that this has on the biological effects of these agents.

Background

The simplest representatives of the alkylating agents are the methylating agents. These include potent toxins and mutagens such as N-methyl-N-nitrosoguanidine (MNNG) and chemotherapeutic agents such as dacarbazine, which is used in the treatment of malignant melanoma and the Cancer Research UK drug Temozolomide, which is used in the treatment of melanoma and glioma. All of these agents generate O^6-methylguanine in DNA and this appears to be responsible for their biological effects. Our current perception of the mechanisms of these effects is summarised in Figure 1. The most critical factor in whether or not these effects are manifested is probably the damage reversal protein O^6-methylguanine-DNA methyltransferase (MGMT), which can simply remove the methyl group and restore the DNA to its predamaged state (Figure 1) in a reaction that also results in the inactivation of the protein. If this does not happen, the DNA can be replicated and a mispair, either O^6meG:T or O^6meG:C, can be generated. If the former undergoes further replication, a G:C to A:T transition mutation is generated, and this is the most characteristic mutational hallmark of these agents. However, both mismatches can be recognised by the post replication mismatch repair system, which results in a series of events that can culminate in cell death or DNA recombination. MGMT can therefore protect cells against both the mutagenic (both point mutations and recombinations) and toxic effects of alkylating agents.

In relation to cancer chemotherapy, that some tumours do not respond to dacarbazine or Temozolomide treatment has been attributed to the protective effect of MGMT. In our attempts to circumvent this, we have previously described the drug Lomeguatrib (LM), originally known as PaTrin-2 and one of the products of a very fruitful collaboration with Prof Brian McMurry and the late Dr Stanley McElhinney (and their group at the Chemistry Department, Trinity College, Dublin). LM is a very potent inactivator of MGMT and in a range of preclinical studies it effectively sensitised human cells and human tumour xenografts to the killing effect of Temozolomide and other agents of that type. Clinical trials of LM in combination with Temozolomide have been completed and the dose required to inactivate MGMT in several tumour types has been established.

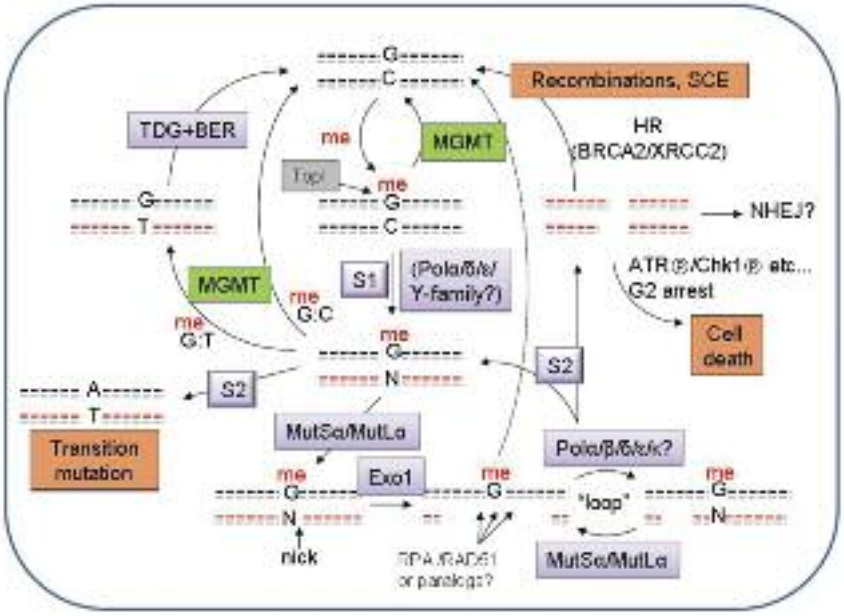

Figure 1
Possible fates of O^6-methylguanine in DNA. SCE, sister chromatid exchange; TDG, thymine-DNA glycosylase; BER, base excision repair; MGMT, O^6-methylguanine-DNA methyltransferase; me, methyl group; Top1, indicates binding of topoisomerase 1 to O^6-methylguanine present in Top1 cleavage sites; HR, homologous recombination; NHEJ, non-homologous end joining; Y-family, translesion DNA polymerases; N, Cytosine or Thymine; Ⓟ, phosphorylation; S1, S2, first and second S-phase following exposure to methylating agents and formation of DNA damage; Exo1, Exonuclease 1. Black and red horizontal dashed lines generally indicate parent and template DNA strands. Question marks indicate a degree of uncertainty.

Some organisms do not have an MGMT gene, and based on our original amino acid sequence homology searches we have established that these possess a different mechanism for dealing with O^6-alkylguanine damage in their DNA. Efforts directed towards the characterisation of this novel repair pathway are ongoing.

Clinical trials of Lomeguatrib

Lomeguatrib, has now completed phase II clinical trials in combination with Temozolomide in malignant melanoma. Unfortunately, LM did not improve the response of melanoma to Temozolomide, but actually this is not unusual, melanoma being one of the most difficult and chemotherapy-resistant tumours to treat successfully. Further work in this area has involved defining the dose of LM that is necessary to inactivate MGMT in other human tumour types including glioma, prostate and colorectal tumours. This study involved administration of LM to patients who were about to undergo surgical removal of their primary tumour. Resected tumours were analysed for active and inactivated MGMT and this allowed calculation of the amount of MGMT that was inactivated. In subsequent patients, doses were escalated until complete inactivation was obtained. In principle this knowledge can now be exploited to design phase II trials in these diseases.

In addition, we have published an assessment of the levels of MGMT in peripheral blood cells in relation to the toxic effects of Temozolomide in the bone marrow. This has highlighted the possibility that blood samples might be used to indicate which patients may be more affected by the myelosuppressive effects of Temozolomide, and hence be more closely monitored, but also which patients should be more resistant, and hence might tolerate higher doses of the drug.

These possibilities have yet to be put into practice clinically.

Alkyltransferase-like proteins

MGMT genes are present in prokaryotes, archea and eukaryotes and are characterised by the presence in the active site domain of a cysteine residue that accepts the alkyl group from the O^6-position of guanine. A few years ago we reported the presence of what we called alkyltransferase-like (ATL) proteins in prokaryotes and some simple eukaryotes. The key difference is that ATL proteins do not have cysteine, but usually tryptophan in this position. The evolution of these proteins is itself intriguing, inasmuch as *E. coli* expresses in fact two MGMT-like and one ATL protein, that we have named eATL, whereas *Saccharomyces cerevisiae* expresses only an MGMT protein and *Schizosaccharomyces pombe* expresses only an ATL protein, that we have named Atl1. ATL proteins are much smaller than MGMT proteins and the tryptophan residue is not the only reason for their inability to transfer alkyl groups: in our initial studies, we showed that mutation of tryptophan to cysteine in the Atl1 protein did not confer MGMT activity.

Work in this area continues and in one collaboration with David Williams (University of Sheffield) and John Tainer (Scripp's Research Institute, La Jolla), crystal structures of Atl1 bound to short duplex oligonucleotides containing O^6-methylguanine or O^6-pyridyloxobutylguanine have been obtained. From these, the similarity in the ways that both Atl1 and MGMT bind to DNA, flip out the O^6-alkylguanine from the base stack using an arginine "finger" and accommodate the base in a binding pocket are very clear. We have also provided further evidence, in the form of additional epistasis analysis, to support our previous suggestion that processing of the damage proceeds following the binding of Atl1, via the nucleotide excision repair pathway, and not the base excision repair or double strand break repair pathways. Thus there is increasing evidence that Atl1 is a damage sensing protein that signals to downstream factors. We are currently exploring if this is the global genome or transcription-coupled branch of this mechanism and, using various methodologies, what proteins might be involved in these interactions.

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Cell Cycle Group

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Our group studies the mechanisms that drive the eukaryotic cell cycle. During 2009 we described novel aspects of the structure and function of the eukaryotic replisome, the multi-protein machine that mediates chromosome replication at DNA replication forks. Co-ordination between the replicative helicase that unwinds the parental duplex, and the DNA polymerases that act on the leading and lagging strands, is important to minimise the exposure of single-strand DNA and thus preserve genome integrity. We found that the Ctf4 protein and the GINS complex play a key role in coupling the helicase complex to DNA polymerase alpha that acts on the lagging strand. In addition, we found that two proteins associated with the helicase at forks serve to recruit a particular E3 ubiquitin ligase, which acts to preserve genome integrity during chromosome replication.

Chromosome replication is a highly complex process in eukaryotic cells, about which much remains to be discovered. Part of the complexity comes from the fact that replication is regulated very carefully to try to ensure that a single perfect copy of the genome is made in each round of the cell cycle. Additional complexity comes from the fact that DNA synthesis at replication forks is coupled to other interesting processes such as the reproduction of epigenetic chromatin marks throughout the genome, the establishment of cohesion between the nascent sister chromatids, and the activation of checkpoint signaling pathways in response to problems at forks (e.g. caused by DNA damage).

By analogy with prokaryotes it seems very likely that a subset of replication factors acting at DNA replication forks will interact to form a large multi-protein machine called the replisome. The formation of the replisome ensures that unwinding of the DNA duplex by the replicative helicase is co-ordinated with synthesis of the leading and lagging strands. This co-ordination is very important as it serves to reduce the exposure of single-strand DNA that might otherwise be attacked by nucleases and lead to breakage of chromosomes during replication. The eukaryotic replisome is poorly characterised, and is likely to be significantly more complex than its prokaryotic counterparts. It seems likely that the formation of the replisome will involve factors that physically link the MCM2-7 helicase to the three replicative polymerases that mediate DNA synthesis on the leading and lagging strands.

Previously we found that a set of regulatory factors assemble around the MCM2-7 helicase at replication forks to form what we called the Replisome Progression Complex or RPC (Gambus *et al*., Nat Cell Biol 2006; 8: 358). Formation and maintenance of the RPC requires the GINS complex, which together with Cdc45 is likely to be an essential component of the active MCM2-7 helicase (Cdc45-MCM-GINS together form the CMG complex). This year we reported that the Ctf4 protein plays a key role in connecting MCM2-7 helicase at forks to DNA polymerase alpha that acts on the lagging strand (Gambus *et al*., 2009). We found that Ctf4 binds directly to the amino terminus of the catalytic subunit of DNA polymerase alpha, as well as binding directly to the GINS complex that is

associated at forks with the MCM2-7 helicase (Figure 1). In the absence of Ctf4, DNA polymerase alpha is no longer able to associate stably with the replisome. Under such conditions cells are still viable but have a greatly increased rate of genome instability. Simultaneous removal of Ctf4 and Mrc1 (another component of the RPC) causes a sustained and lethal DNA damage response during chromosome replication. These findings indicate that replisome formation is needed not simply to allow DNA synthesis to proceed when the parental DNA duplex is unwound, but is also crucial to allow cells to survive the process of chromosome replication without incurring permanent damage to the chromosomes.

By isolating the RPC from budding yeast cells we also found that it is associated with a specific E3 ubiquitin ligase, called SCF^{Dia2} (Morohashi *et al.*, 2009). All eukaryotes have multiple forms of the SCF ligase (SCF = Skp1, Cullin, F-box protein), which are distinguished from each other by different F-box proteins that represent the substrate binding subunits of the SCF. Each F-box protein is connected to the rest of the ligase by the F-box motif, on the carboxy-terminal side of which is located the substrate binding domain. In addition, around a third of the 20 F-box proteins encoded by the budding yeast genome contain an additional domain of unknown function at the amino terminus of the protein. We found that a unique TPR domain at the amino terminal end of Dia2 links SCF^{Dia2} to the Mrc1 and Ctf4 components of the RPC, thereby tethering SCF^{Dia2} to the replisome (Figure 2). It thus appears that the TPR domain controls the localisation of SCF^{Dia2}, probably increasing the local concentration of the ligase at DNA replication forks. We found that the TPR domain is required in cells that accumulate stalled DNA replication forks at sites in the genome where non-nucleosomal proteins are bound very tightly to DNA. Such paused forks do not activate a checkpoint response, and tethering of SCF^{Dia2} might help the ligase interact more effectively with substrates under such conditions. As many other F-box proteins have amino terminal domains of unknown function, it now seems likely that these too might regulate the localisation of the cognate forms of the SCF.

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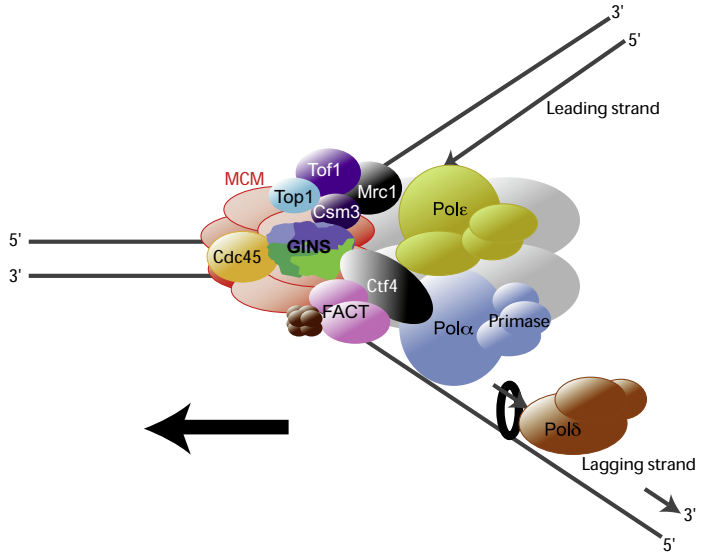

Figure 1
A complex of Ctf4 and GINS plays a key role in connecting DNA polymerase alpha to the MCM2-7 helicase at DNA replication forks. The replisome is still poorly understood in eukaryotic cells and it is likely that further components remain to be characterised (indicated in grey in the figure). See text for further details.

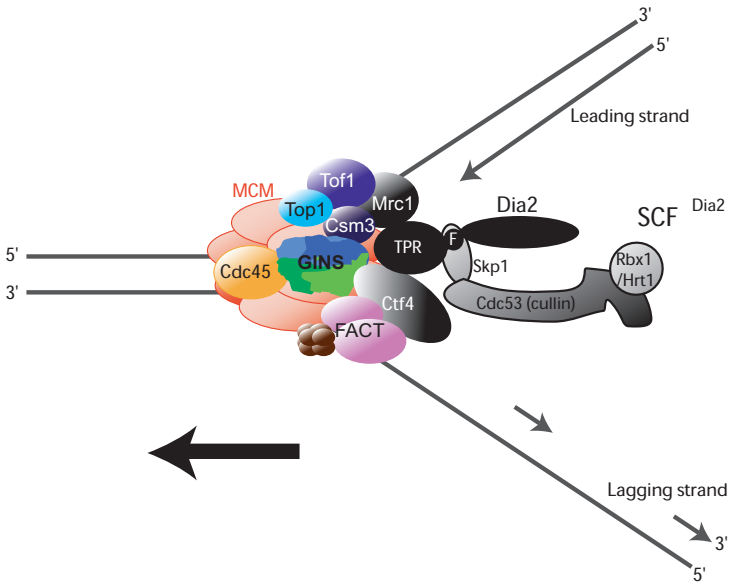

Figure 2
SCF^{Dia2} associates with the Replisome Progression Complex at DNA replication forks. The amino terminal TPR domain of Dia2 binds Ctf4 and Mrc1, thereby tethering SCF^{Dia2} to the RPC at DNA replication forks. For the sake of simplicity, other replisome components are not included in the figure.

Cell Division Group

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Development and health rely upon the controlled balance of cell growth and division. The size of each organ at each stage of our lives is a product of the number of cells in that tissue and the size of each cell. Cancer arises from an imbalance of cell proliferation. It is becoming increasingly apparent that errors in the ability to integrate growth control and cell division can lead to this imbalance. Thus, understanding the co-ordination between growth and division lies at the heart of understanding the basis of many cancers. Because the regulatory networks that control the timing of cell division and chromosome segregation are highly conserved, studying the complexities of cell division in the relatively simple unicellular yeasts greatly accelerates the analysis of the more complex issue of the control of cell division in man.

Activation of Mitosis Promoting Factor (MPF) regulates the timing of cell division

We study cell division in the fission yeast *Schizosaccharomyces pombe* because it is a simple, unicellular organism with excellent genetics that is cheap to grow and divides rapidly. Commitment to mitosis in *S. pombe*, as in all eukaryotes, is regulated by the activity of a protein kinase complex called MPF. MPF is composed of a catalytic sub-unit encoded by the $cdc2^+$ gene and a regulatory sub-unit called cyclin B. Prior to mitosis MPF is inhibited via phosphorylation by the protein kinase Wee1 on a residue (tyrosine 15) that lies in the ATP binding pocket of Cdc2. This phosphate can be removed by a protein phosphatase encoded by the $cdc25^+$ gene. The balance of activity between Cdc25 and Wee1 is the critical factor in determining when MPF will be activated to drive mitotic commitment. Once a critical threshold level of MPF is activated a positive feedback loop is promoted to boost Cdc25 activity and suppress Wee1 activity, thereby driving full-scale commitment to mitosis (figure 1). Fully activated MPF then activates a number of highly conserved kinases that are named after the founder members of each group Polo, aurora and NIMA. These kinases participate in the positive feedback loop to promote the further activation of Cdc25 and inhibition of Wee1 to drive complete commitment to mitosis.

Chromosome segregation by the mitotic spindle

In addition to their role in the feedback activation of MPF, the mitotic kinases promote the assembly of the bipolar mitotic spindle that physically segregates the duplicated genomes into each daughter cell. The principal components of the mitotic spindle are the two sets of microtubules that extend from the two spindle poles. After attachment to spindle microtubules, the chromosomes align midway between the two poles (figure 2) before each chromosome splits in two and each half moves to either pole. Subsequent ingression of the plasma membrane between these segregated genomes (figure 3) completes cell division as each daughter cell inherits one genome and one spindle pole.

Figure 1

Figure 2
Microtubules extend from the two spindle poles (purple spheres) to either interdigitate with microtubules extending from the opposite poles (black lines) or bind to specialised regions on the chromosomes (brown bars) called kinetochores (green circles).

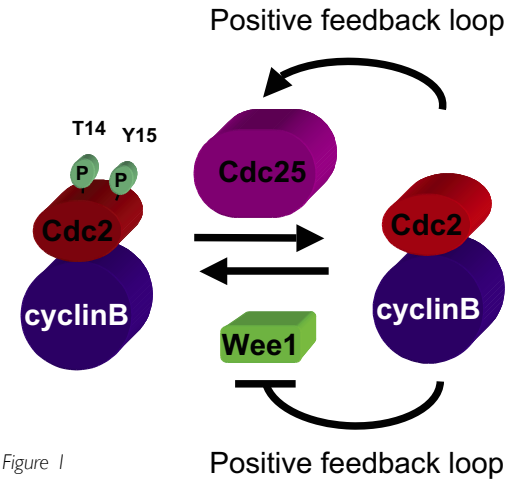


Figure 1

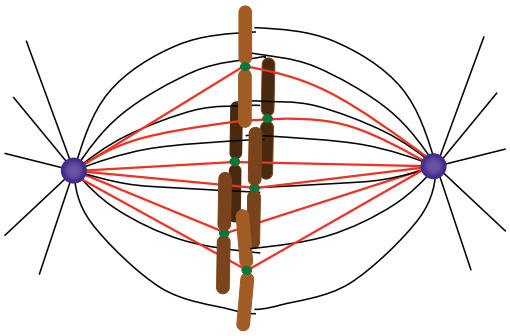


Figure 2

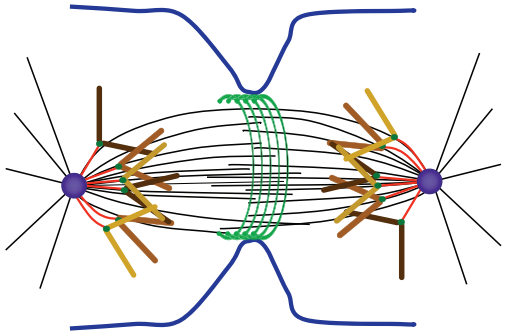
Characterisation of Cut12 suggests that events on the spindle pole regulate MPF activation during mitotic commitment

The characterisation of the *cut12.1* mutation in our laboratory a number of years ago uncovered an unanticipated link between the spindle pole and MPF activation. We found that this loss of function mutation in the spindle pole component Cut12 blocked spindle formation as cells formed monopolar, rather than bipolar spindles. Surprisingly, we also found that the *cut12*$^{+}$ gene was identical to the *stf1*$^{+}$ gene. The gain-of-function *stf1* mutations had been identified because they enabled cells to live without the mitotic promoter Cdc25 (suppressor of twenty five). Subsequent work in our laboratory had established that this influence of Cut12 upon mitotic control involved modulation of the activity of the feedback loop kinase polo at the spindle pole, however, the basis for the *cut12.1* monopolar spindle phenotype has remained obscure. By exploiting the light- and electron-microscopy core facilities within the Paterson Institute we have established that *cut12.1* cells are unable to form a bipolar spindle because they fail to activate the newer of the two mitotic spindle poles. Thus, the *cut12.1* loss-of-function phenotype arises from a local deficiency in MPF activation while the gain-of-function *stf1* mutations lead to inappropriate global activation of MPF throughout the entire cell. These observations put the Cut12/Stf1 molecule and the spindle pole at the heart of the controls that determine the timing of cell division.

From yeast to man

Our findings in fission yeast suggest that the feedback loop that provides the priming impetus to promote global MPF activation emanates from the spindle pole. The demonstration that active MPF first appears on human spindle poles in human cells by Jon Pines' group (CR-UK funded, Gurdon Institute Cambridge) supports the view that the networks we are studying in yeast are representative of those operating in man. Thus, continued exploitation of the malleability of fission yeast to interrogate Cut12 function will shed light on conserved mechanisms operating in the more intractable human cells.

Figure 3
After the chromosomes have moved towards the poles along kinetochore associated microtubules the plasma membrane (thick blue line) is pulled in by the cytokinetic, actomyosin ring (green hoops) to initiate the separation of the cell into two daughters.



Lessons from yeast

The ability to manipulate genes at will in a simple organism whose primary purpose is simply to grow and divide is enabling us to explore the finer points of the pathways that co-ordinate growth with spatial and environmental cues. This information informs studies in higher systems that, in turn, raise models that can be most readily tested in yeast. This re-iterative cycle of comparative studies ensures that great strides are being made in understanding the molecular basis of cell division and growth.

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The AP-1 transcription factor is activated in response to many extracellular signals including growth factors, cytokines and various stress conditions. As a result it is essential for a wide variety of biological activities which in mammalian cells range from cell proliferation and differentiation to regulation of apoptosis. Deregulation of AP-1 activity has been associated with numerous disease conditions such as inflammation and cancer.

The AP-1 factor comprises a diverse array of homo- and heterodimeric complex combinations involving proteins from the Jun, Fos, ATF and Maf transcription factor families. These combinations vary from one cell type to another and different combinations recognise distinct DNA elements and are differentially regulated. Much of the work in our laboratory has focused on two of these AP-1 proteins, the transcription factors ATF2 and ATF7, which are both activated by phosphorylation mediated by the stress activated MAP (mitogen activated protein) Kinases p38 and JNK. ATF2 and ATF7 have been specifically implicated in stress responses, cell cycle progression, apoptosis and DNA damage response. Germ line mutation of *Atf2* leads to post-natal lethality and simultaneous deletion of both *Atf2* and *Atf7* leads to embryonic lethality as a result of massive apoptosis in the embryonic liver involving both developing hepatocytes and haematopoietic cells.

Evidence is accumulating that ATF2 can promote oncogene activation or tumour suppression activity depending on the tissue context. We are using different mouse models to investigate the potential role of ATF2 and ATF7 in tumourigenesis. A tumour suppressor role has been revealed in a skin tumourigenesis model using a mutant mouse where ATF2 is specifically deleted in keratinocytes (in collaboration with Z Ronai, Burnham Institute for Medical Research). Upon tumour initiation and promotion, the mutant animals demonstrate a significantly earlier onset of papillomas as well as greater numbers. Likewise we have shown that irradiation of mice with ATF2 specifically deleted in T-cells results in earlier onset of T-cell lymphomas.

We are characterising the potential role of ATF2 in B-cell lymphomas since a number of reports have shown that JNK is highly active in cultured B lymphoma cell lines, and that JNK is critical for tumour cell growth and survival. Interestingly we found that the levels of active ATF2 is also elevated in B lymphoma lines (e.g. Burkitt's lymphoma, Follicular lymphoma) and to address the functional significance of this increased activity we currently analyse a set of tumour cell lines in which ATF2 has been targeted by RNA knockdown. In a complementary approach we have generated a B-cell specific ATF2 knockout mouse and crossed them to transgenic mice that express the B-cell tumour inducing Eμ-Myc transgene. Differences in the number of Myc induced lymphomas or the timing of lymphoma onset will establish the importance of ATF2 in this tumour type.

We had previously established that ATF2 and ATF7 were essential for the survival of hepatoblasts in the developing embryo through coordinating negative regulating feedback mechanisms that restrict the activity of the stress activated kinase p38. Cultured hepatoblasts can be used to study the onset of hepatocellular carcinoma (HCC) through transformation with oncogenes and reintroduction into recipient livers via orthotopic transplantation. We utilised this technique to address a possible role for ATF2 and ATF7 in HCC. We found that double knockout hepatoblasts transformed with the HRas oncogene (HRasG12D) produced more and significantly larger tumours in recipient livers compared to hepatoblasts that were normal for ATF2. In addition, deletion of ATF2 and ATF7 in cells isolated from established liver tumours

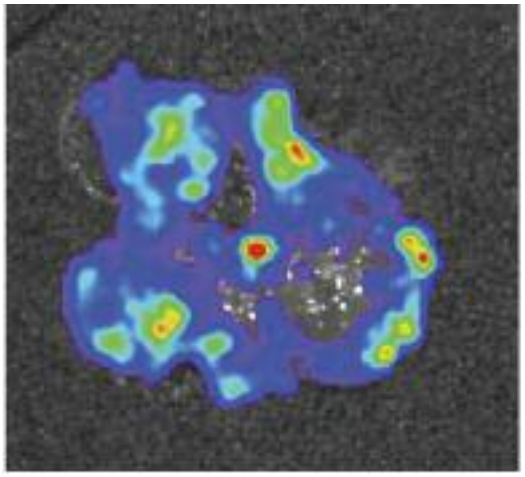

Figure 1
Bioluminescence visualisation of H-Ras transformed hepatoblasts orthotopically transplanted into donor livers.

result in accelerated growth in graft models. The results therefore support a potential role of these factors in suppressing tumour induction or tumour cell growth. The significance of these findings will be further explored.

Homologues of the AP-1 family are found in all eukaryotic organisms and their involvement in the stress response is highly conserved. In fission yeast stress responses are coordinated through the Sty1 signalling pathway which is analogous to the mammalian p38 pathway. Furthermore, many of the changes in the transcriptional profile of cells following stress is orchestrated through the Atf1 and Pap1 transcription factors which are related to mammalian ATF2 and cJun respectively.

Given the central role that Atf1 plays in the stress response, we have characterised in detail its regulation and its interaction with the Sty1 kinase. Previously we found that upon exposure to stress, Atf1 is hyper-phosphorylated by the MAP kinase Sty1 which results in its stabilization. Further studies show that Atf1 is a target for the ubiquitin-proteasome system and that its degradation is dependent upon an SCF E3 ligase containing the F-box protein Fbh1. This F-box protein is unique in that it has its own intrinsic enzymatic activity - in addition to containing an F-box motif, it is also a DNA helicase. Turnover of Atf1 requires an intact F-box but not DNA helicase activity of Fbh1. Atf1 and Fbh1 interact under basal conditions but this binding is lost upon stress-dependent phosphorylation of Atf1.

Most F-box protein-substrate interactions described to date are mediated positively by phosphorylation. Thus our findings represent a novel means of regulating the interaction between an F-box protein and its substrate. Moreover, Atf1 is the first target described, in any organism, for the Fbh1 F-box protein. However in addition to Atf1, it seems likely that Fbh1, in common with other F-box proteins, will direct multiple targets for ubiquitination via the SCF^{Fbh1}. Potential substrates are proteins involved in the homologous recombination pathway of DNA repair, as others have demonstrated that Fbh1 plays a role in this process, downstream of the key recombination factor, Rad51.

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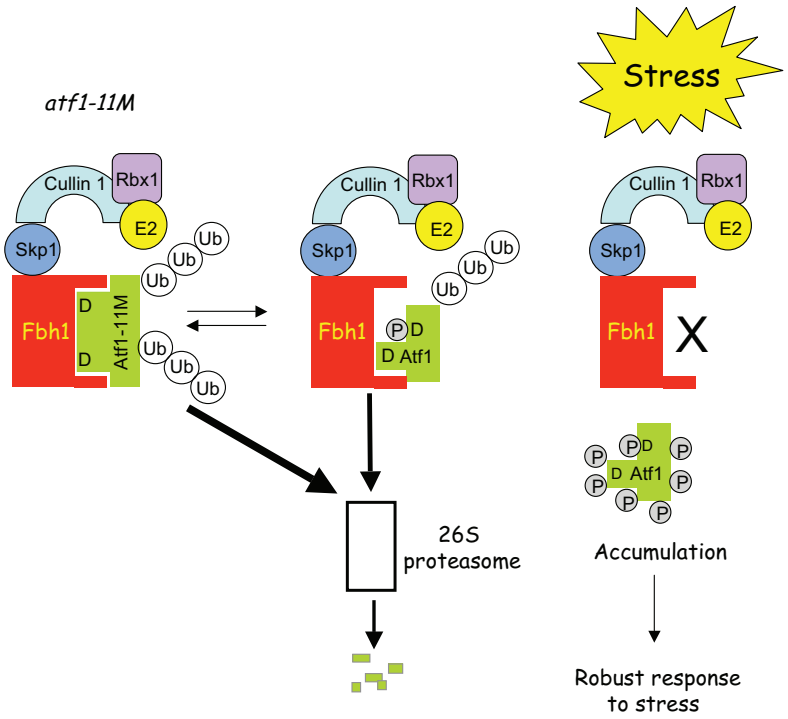

Figure 2
Model explaining the regulation of Atf1 levels by Fbh1. D represents putative degrons in Atf1 which interact with Fbh1. Upon stress, the degrons become phosphorylated which abrogates the interaction with Fbh1 thus promoting stability of Atf1.

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Tumour initiation and progression result from inappropriate activation of intracellular signalling cascades. Rho-like GTPases are molecular switches in signalling pathways that regulate cytoskeletal and junctional organisation, as well as gene transcription. In this way, Rho proteins influence cell morphology, adhesion, motility, as well as cell cycle progression and cell survival. Rho proteins are transforming *in vitro* and are essential for Ras-mediated *in vitro* transformation. Moreover, data have emerged to directly implicate Rho proteins in tumour initiation and progression *in vivo*. Our group investigates how the activities of certain regulators of the Rho protein Rac are controlled. We are also identifying signalling events and cellular processes downstream of Rac that modulate tumour susceptibility and disease progression.

Tiam1 (for T-lymphoma invasion and metastasis protein) is a guanine nucleotide exchange factor (GEF) that selectively activates Rac. Tiam1-deficient cells are resistant to Ras-induced cellular transformation (Malliri *et al*., Nature 2002; 417: 867). Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by chemical carcinogens and consequent oncogenic activation of the c-*Ha-Ras* gene (Malliri *et al*., Nature 2002; 417: 867). Nonetheless, the few skin tumours arising in Tiam1-deficient mice progressed more frequently to malignancy than those in wild-type mice, suggesting that Tiam1 deficiency promotes malignant conversion (Malliri *et al*., Nature 2002; 417: 867). Thus, while Tiam1/Rac co-operate with Ras in establishing tumours, they antagonize Ras during tumour invasion.

Tiam1/Rac signalling is targeted by Src during the epithelial–mesenchymal transition.

One mechanism by which Tiam1 and Rac suppress malignant progression is through promoting cell–cell adhesion. *In vitro* studies have shown that over-expression of activated Rac or Tiam1 promotes the formation of adherens junctions (AJs) and the accompanying induction of an epithelial-like phenotype in a number of mesenchymal cell lines (Malliri & Collard, Curr Opin Cell Biol 2003; 15: 583). Moreover, using both RNA interference and cells derived from Tiam1-deficient mice, it has been shown that endogenous Tiam1 is required for both the formation as well as the maintenance of cadherin-based adhesions (Malliri *et al*., J Biol Chem 2004; 279: 30092). The oncoprotein Src, a non-receptor tyrosine kinase implicated in malignant progression, potently induces epithelial–mesenchymal transition (EMT) by targeting AJs for dissassembly. We recently showed that direct phosphorylation of Tiam1 by Src is required for the initial stages of Src-induced EMT. Moreover, we identified a novel post-translational mechanism of regulating Tiam1 levels. We showed that Src phosphorylates Tiam1 on tyrosine 384 (Y384). This occurs predominantly at AJs during the initial stages of Src-induced EMT and creates a docking site on Tiam1 for Grb2. We found that Tiam1 is constitutively associated with extracellular signal-regulated kinase (ERK). Following recruitment of the Grb2-Sos1 complex, ERK becomes activated and triggers the localised degradation of Tiam1 at AJs through activating calpain proteases. Significantly, we demonstrated that in human lung, colon, and head and neck cancers

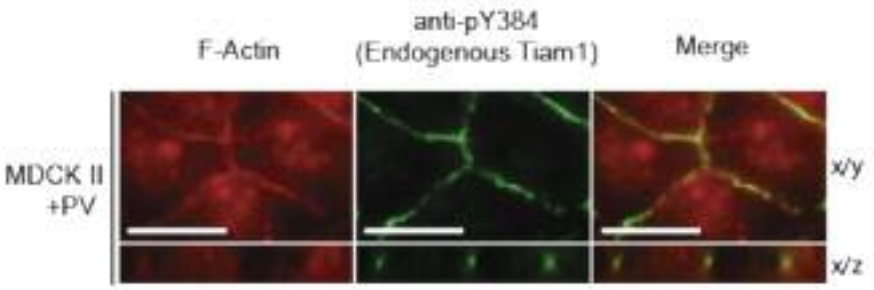

Figure 1
MDCK cells treated with sodium pervanadate (PV), an irreversible protein-tyrosine phosphatase inhibitor, display endogenous Tiam1 phosphorylated at Tyrosine 384 specifically at cell-cell adhesions.

phosphorylation of Y384 of Tiam1 positively correlated with Src activity, while total levels of Tiam1 were inversely correlated with Src activity, consistent with the above-mentioned post-translational regulatory mechanism operating in malignancies. Abrogating Tiam1 phosphorylation and degradation suppressesed Src-induced AJ disassembly. As a consequence, cells expressing a non-phosphorylatable Tiam1 showed a marked decrease in wound closure in response to Src (Woodcock *et al*., 2009b).

A distinct role for the homologue of Tiam1, STEF, in regulating focal adhesions.

The mechanisms underlying focal adhesion disassembly, required for optimal cell migration, are poorly understood. Microtubules are critical mediators of this process; direct targeting of focal adhesions by microtubules coincides with their disassembly. Re-growth of microtubules, induced by removal of the microtubule destabiliser nocodazole, activates the Rho-like GTPase Rac, concomitant with focal adhesion disassembly. Recently we have shown that the Rac guanine nucleotide exchange factor (GEF) STEF (for Sif and Tiam1-like exchange factor) is responsible for activation of Rac during microtubule re-growth. Importantly we also showed that STEF is required for multiple targeting of focal adhesions by microtubules. As a result, focal adhesions in STEF knock-down cells have a reduced rate of disassembly and are consequently enlarged. This leads to a reduced speed of migration in these cells. Taken together, these findings reveal a novel role for the Rac-GEF STEF in focal adhesion disassembly and cell migration via microtubule-mediated mechanisms.

Tiam1 interacting proteins.

It is increasingly apparent that Rho GEFs do more than simply activate Rho molecules; several studies now point to their role in influencing the choice of biological response elicited by a given Rho protein. GEFs have been shown to bind to effectors directly or to scaffold proteins that complex with components of effector pathways. Thus Tiam1 interacts with IB2/JIP2, a scaffold that promotes Rac activation of p38 kinase cascade over JNK MAP kinase cascade (Buchsbaum *et al*., Mol Cell Biol 2002; 22: 4073), and also with spinophilin, a scaffold that promotes Rac activation of p70 S6K over Pak1, a different Rac effector (Buchsbaum *et al*., J Biol Chem 2003; 278: 18833). In our lab, we are using biochemical approaches to identify Rac and Rac GEF interacting proteins involved in different aspects of transformation including malignant progression (acquisition of invasiveness). Toward this end, we recently reported a modified tandem affinity purification method that enriches for transient protein interactions. Using this technique, we identified 14-3-3 proteins as Tiam1 binding partners. The interaction of Tiam1 with 14-3-3 proteins was largely dependent on the N-terminal region of Tiam1; within this region, there are four putative phospho-serine-containing 14-3-3 binding motifs, and we confirmed that two of them (Ser172 and Ser231) are phosphorylated in cells using mass spectrometry. Moreover, we showed that phosphorylation at three of these motifs (containing Ser60, Ser172 and Ser231) is required for the binding of 14-3-3 proteins to this region of Tiam1. We showed also that phosphorylation of these sites does not affect Tiam1 activity; significantly however, we demonstrated that phosphorylation of the Ser60-containing motif is required for the degradation of Tiam1 (Woodcock *et al*., 2009a).

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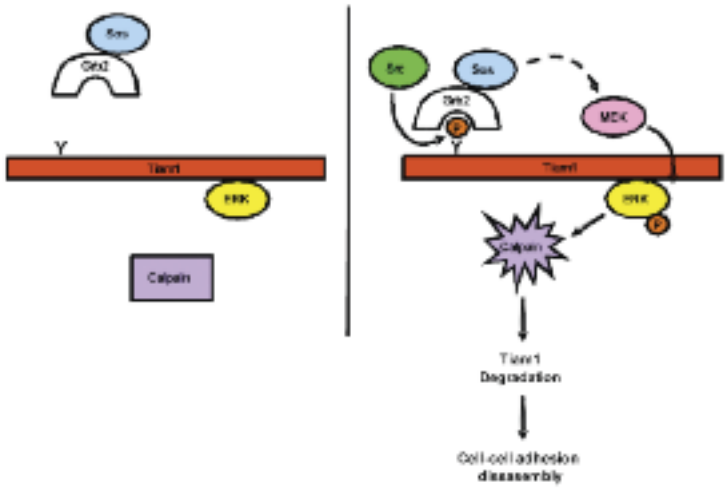

Figure 2
Model: Src phosphorylates Tiam1 at sites of cell-cell adhesions. Phosphorylated Tiam1 recruits the Grb2-Sos complex and, via MEK, increases activation of the ERK associated with Tiam1, and hence the local activation of calpain proteases at cell–cell adhesions. Calpain mediated proteolysis of Tiam1 results in its inactivation, reducing the activity of Rac that is necessary to maintain cadherin adhesions.

Clinical and Experimental Pharmacology Group

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Development of molecularly targeted anticancer drugs mandates parallel development of biomarkers to achieve the right drug at the right dose for the right patient. CEP research is predicated on novel agents entering clinical trial within the Derek Crowther Early Clinical Trials Unit (DCU) at The Christie Hospital. DCU typically supports c100-120 trials with c6400 patient visits p.a. A new £35M Cancer Treatment Centre will open in 2010 to provide comprehensive clinical trials facilities, one of the largest early clinical trials centres worldwide, and the incorporated and enhanced biomarker laboratory facilities will strengthen further the CEP-DCU axis. In 2009, biomarker research highlights included investigations of the collective migration of circulating tumour cells, the utility of circulating cell death biomarkers in B lymphoma, drug combination studies for BH-3 mimetic drug that target apoptosis control, and developments in clinical proteomics, tissue biomarkers and biostatistics approaches related to biomarker driven clinical trials.

Circulating Tumour Cells (CTCs).

Within the Lung Cancer Disease Focus Group (Lead, F Blackhall), a filter-based approach was tested to isolate CTCs. CTCs were found circulating both as tumour microemboli (clusters of cells, Figure 1) and as single cells that expressed the lung diagnostic biomarker TTF1. Considerable cellular heterogeneity was apparent regarding epithelial (cytokeratins) and mesenchymal (vimentin, N-cadherin) markers in both single CTCs and microemboli suggestive of partial Epithelial Mesenchymal Transition (EMT). Initial analysis suggests that CTC survival is enhanced during collective migration (as microemboli) and the relevance of this to patient's response to therapy and survival is being sought.

CK18 based circulating biomarkers of cell death in lymphoma

Despite recent improvement in lymphoma treatment, chemoresistance and toxicity risk are hard to predict. In collaboration with Prof John Radford and Dr Kim Linton, circulating biomarkers of cell death (nucleosomal DNA (nDNA) and cytokeratin 18 (CK18)), together with circulating FLT3 ligand, a potential myelosuppression biomarker, were assessed at baseline and serially after standard chemotherapy. CK18 is not expressed in lymphoma cells and thus reports drug-induced epithelial toxicity. Baseline nDNA level was higher in all lymphoma subtypes compared to healthy controls and was prognostic for overall survival in Diffuse Large B Cell Lymphoma. Baseline nDNA also predicted extent of tumour shrinkage post chemotherapy. Circulating CK18 increased within 48h of chemotherapy and was significantly higher in patients experiencing epithelial toxicity graded >3 by CTCAE scoring criteria. FLT3 ligand was elevated within 3-8 days of chemotherapy and predicted subsequent development of neutropenic sepsis. These early promising data suggest that inexpensive circulating biomarkers could contribute useful information regarding tumour response and

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toxicity for lymphoma patients on standard chemotherapy and have potential utility in development of mechanism-based therapeutics. These biomarkers are being incorporated within multicentre phase III trials in order to progress to biomarker qualification.

The Colorectal (CRC) Disease Focus Group

Our focus on CRC (lead: Dr Andrew Renehan) has developed in 2009 with respect to biomarker-driven trials (and their statistical considerations) and the influence of obesity on anti-cancer therapy response. Key landmarks were commencement of the biomarker-linked E-SCOUT trial of cetuximab combination therapy (encompassing CTCs, detection of *KRAS* mutations in circulating free DNA and circulating biomarkers of cell death); expansion of our blood and tissue collection portfolio (to include Stepping Hill Hospital); appointment of a CR-UK AZ clinical fellow with specific interest in anti-angiogenic therapies and biomarkers in CRC; and establishment of the Manchester-Birmingham Biostatistical Biomarkers Collaboration (MBBBC) with Professors Cindy Billingham and Philip Johnson. With the appointment of a further postdoctoral scientist, studies commenced using an *in vitro* model of K-Ras dependent chemoresistance to chronic insulin exposure and of CRC progression in obese mice.

Clinical proteomics

In collaboration with Prof Tony Whetton, the clinical proteomics team substantially improved its capability to detect and relatively quantify, low abundance proteins in human plasma following upgrade of our MALDI-ToFToF mass spectrometer. The new ABI 5800 instrument enables 10x faster data acquisition with improved resolution and sensitivity. Various operating methods involved in ion selection and data processing were optimised for maximal protein identification with minimal false discovery rates. The throughput of MALDI plate spotting was increased and processing of 8-channel iTRAQ samples of human plasma is now twice as fast with 2-3 times the number of protein identifications. Performing at internationally competitive levels to detect and quantify ~400 proteins in human plasma, we now detect proteins in the low abundance range, a probable source of tissue leakage molecules that includes disease-specific biomarkers. Our first clinical study assessing the intra-and inter-person reproducibility and experimental variation of samples from the PACER-TRANS clinical trial in pancreatic cancer is underway in collaboration with Prof. Catherine West.

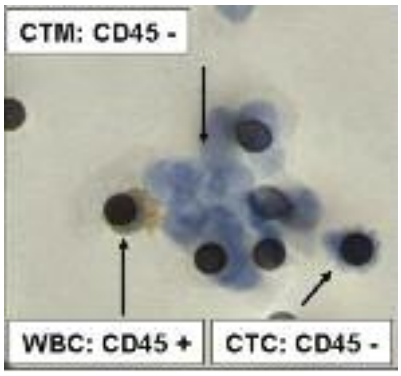

Figure 1
The collective migration of SCLC circulating tumour microemboli (CTM) identified using ISET technology. Dark circles are pores in the filter, brown staining for CD45 identifies contaminant white blood cells (WBC).

BH-3 Mimetics, Hypoxia and Drug Combinations

The preclinical pharmacology team examines effects of tumour micro-environment on drug sensitivity and evaluates drug combinations, with a particular emphasis on apoptosis-targeted drugs. Hypoxia occurs in regions of solid tumours distant from blood supply where cells exhibit reduced sensitivity to standard chemotherapy and radiotherapy. However, hypoxia sensitised CRC and SCLC cells to apoptosis induced by the Bcl-2 family targeted BH-3 mimetic ABT-737 via reduced expression of Mcl-1, an established ABT-737 resistance factor. Increased ABT-737-induced apoptosis occurred in hypoxic regions of SCLC tumour xenografts. This suggests that, if delivered sufficiently, ABT-737 could target hypoxic tumour cells that are chemo-refractive that in patients cause tumour repopulation during relapse. In CRC cancer cells, ABT-737 induced cell death was also enhanced when combined with inhibitors of PI3-kinase signalling. This was not recapitulated by inhibition of PKB or mTOR that act downstream of PI3-kinase. PI3-kinase inhibition was associated with increased binding of the pro-apoptotic BH-3 only protein BIM to the ABT-737 target Bcl-x_L, suggesting a possible mechanism for this drug interaction.

Tissue Biomarkers

Our validated tissue biomarker assay portfolio increased in 2009 to include Ca IX, GLUT-1 1 and pimonidazole to report hypoxia. With tissue biomarker studies increasing, automated histology has been incorporated into the GCLP laboratory base. With ECMC funding, an Ariol image analysis platform (Applied Imaging Genetix) has been installed and is being customised for CEP GCLP activity allowing high-throughput and automated image analysis for biomarker quantification. This system supports 'slide-link' a software package linking serial tissue sections. CEP has scanned and analysed >800 slides from a number of preclinical *in vivo* studies This approach allows analysis of co-localised multiple markers without the need for complex multiple staining techniques and was used to this approach to quantify cell death biomarkers in both normoxic and hypoxic regions of tumour xenografts treated with ABT-737.

Publications listed on page 67

DNA Damage Response Group

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The genetic material (DNA) is constantly exposed to damage, both from endogenous and exogenous sources, and the living organisms had to evolve a variety of DNA repair mechanisms to maintain the genome stability. Inadequate or abnormal DNA repair can cause diseases that are in man associated with cancer, neurodegeneration, immunodeficiency or developmental abnormalities. Our laboratory is studying molecular pathways employed in mammalian DNA damage response in order to gain a better understanding of the mechanisms underlying human disease, which could potentially provide a basis for the development of new therapies.

Genomic instability is the driving force of cancer development, which requires multiple DNA mutations resulting in loss of cellular growth control. To accelerate the accumulation of genetic changes, cancers often sacrifice specific DNA repair pathways, which in turn can be exploited as an Achilles heel of cancer. This means that the genetic damage that builds up in cancer cells makes them susceptible to treatment with certain DNA-damaging agents, or to inhibitors that block alternative DNA repair pathways, while normal cells with a full repair repertoire are readily much less sensitive. One specific class of anticancer drugs that work in this way are inhibitors of family of enzymes called poly(ADP-ribose) polymerases (PARPs). PARPs use cellular NAD as a substrate to synthesize a peculiar type of posttranslational protein modification consisting of long chains of repeating ADP-ribose units (poly(ADP-ribose) or PAR) and regulate a variety of important processes including DNA repair, chromatin structure, mitosis, transcription, checkpoint and apoptosis (Hakme *et al*., EMBO rep 2009; 9: 1094).

Poly(ADP-ribosyl)ation in regulation of DNA repair

The role of poly(ADP-ribosyl)ation is best understood in the regulation of DNA repair, which is controlled by the two PARPs responsive to DNA strand breaks (PARP1 and PARP2). Poly(ADP-ribose) arising at the sites of damaged DNA serves as a platform for specific recruitment and scaffolding of DNA repair complexes. In addition, the damage-induced poly(ADP-ribosyl)ation has been known to have a role in relaxation of the chromatin structure, as well as in apoptotic signalling. The recent development of potent PARP1/2 inhibitors provided powerful tools to study pathways regulated by poly(ADP-ribose), as well as providing one of the very promising class of drugs for cancer treatment. Specifically, selective inhibition of the single-strand break repair pathway using permeable PARP inhibitors has been proven highly effective against breast and ovarian cancers (Bryant *et al*., Nature 2005; 434: 913). Thus, understanding the molecular basis of PAR-dependent DNA repair processes is likely of vital importance for selecting and developing efficient therapies.

Identification and characterization of novel poly(ADP-ribose)-regulated factors

Our laboratory is particularly interested in identification of new pathways and protein functions regulated by poly(ADP-ribosyl)ation. Recently, in screening for proteins with the ability to bind poly(ADP-ribose), we discovered a poly(ADP-ribose)-binding zinc finger motif (PBZ). PBZ is a structurally distinctive, atypical type of zinc finger that is associated with several proteins involved in response to DNA damage (Ahel *et al*., Nature, 2008; 451: 81) (Figure 1). One of the human proteins containing a PBZ

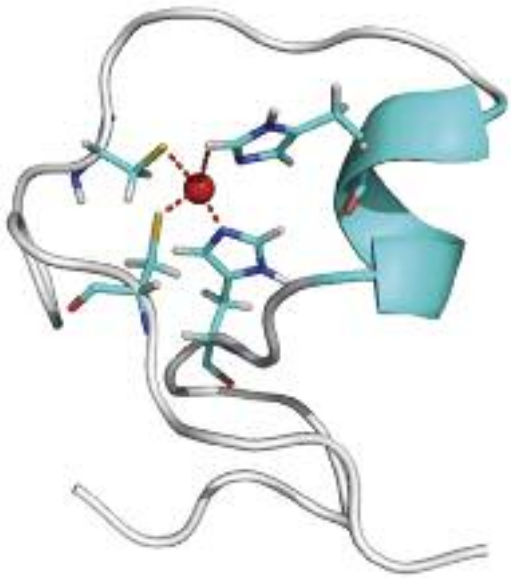

Figure 1
NMR structure of APLF poly(ADP-ribose)-binding zinc finger.

motif is a protein called Checkpoint protein with FHA and RING domains (CHFR). CHFR is an ubiquitin ligase frequently inactivated in human epithelial tumours, which acts as a key regulator of the poorly understood early mitotic checkpoint that transiently delays chromosome condensation and nuclear envelope breakdown in response to variety of stresses. The elucidation of the function of the PBZ motif gave us a vital clue to discover that the CHFR-dependent checkpoint is regulated by PARPs and that the PBZ motif in CHFR protein is critical for checkpoint activation. Another PBZ-regulated protein we are studying is a protein called Aprataxin-PNK-like factor (APLF). APLF is a protein of unknown function, which uses tandem PBZ repeats for direct interaction with poly(ADP-ribosyl)ated PARP1, which allows APLF's timely localization to the sites of DNA damage (Figure 2).

Another class of proteins in focus of our research are macro-domain proteins. Macro-domain is another module with the capacity to bind poly(ADP-ribose) and we recently identified two human macro-domain chromatin-associated factors that are recruited to broken DNA ends in a poly(ADP-ribose)-dependent manner. One of these factors is a histone H2A variant called macroH2A, which has been previously implicated in heterochromatin maintenance, but its function in DNA damage response is still unclear. Another macro-domain factor we are studying is a chromatin remodeler of the Snf2 helicase family called ALC1 (Amplified in Liver Cancer; also know as CHD1L). ALC1 is frequently overexpressed in human hepatocellular carcinoma (HCC) and it has been shown that ALC1 misregulation triggers tumorigenesis (Chen *et al*., Plos One, 2009; 4: e6727). Our recent data show that ALC1 chromatin remodeling activity is critical for efficiency of DNA repair reactions and that this activity is stimulated by the interaction with poly(ADP-ribosyl)ated PARP1 (Ahel *et al*., 2009). Furthermore, we showed that overexpression of ALC1 results in specific sensitivity to certain types of DNA-damaging drugs, which suggests a potential basis for developing a targeted therapy for ALC1-overexpressing tumours.

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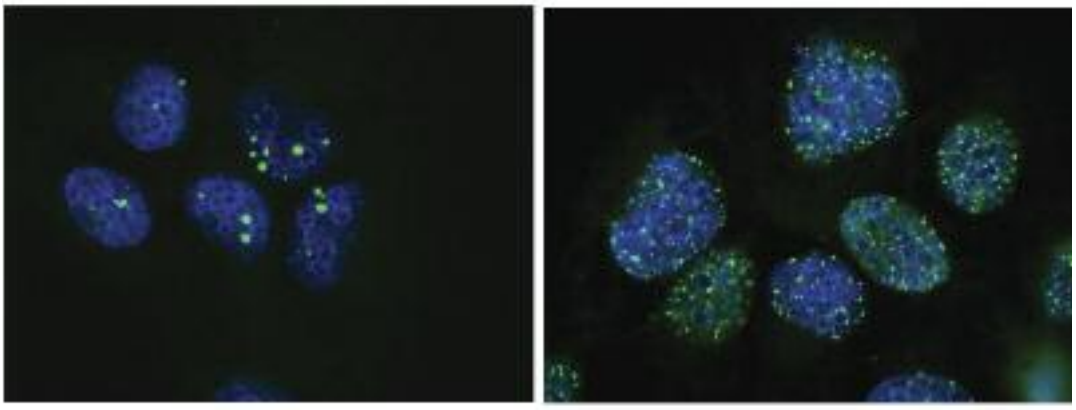

Figure 2
Recruitment of APLF to repair foci after DNA damage. Immunostaining of endogenous APLF in untreated U20S cells (left panel) and in 5-Gy irradiated U20S cells (right panel). DNA is stained with DAPI.

Immunology Group

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The basic research of the Immunology Group is directed at understanding and exploiting the function and/or expression of 5T4 oncofoetal molecules in the context of cancer associated changes in motility and adhesion contributing to metastasis. We also have significant collaborations within the MCRC focused on more translational objectives for 5T4-directed immunotherapies with the Cellular Therapy Group (RE Hawkins), Gynaecological Oncology (HC Kitchener, St Mary's Hospital) and the Children's Cancer Group (V Saha). Some of our studies relating to successful translation of 5T4 directed immunotherapies are reported elsewhere (BIGT). This report will focus on our work utilizing our 5T4 KO mouse and the investigation of modulation of immune regulatory processes in 5T4 tumour immunity.

5T4 knock out (KO) mouse

We have constructed a 5T4 KO mouse by replacing the second exon of 5T4, which encodes the entire protein, with an IRES-LacZneo reporter gene in ES cells. These cells were used to produce chimaeric mice and germline progeny; 5T4 KO heterozygote mice were backcrossed to the C57BL/6 background (> 10 generations). KO 5T4 C57BL/6 animals are viable but adult animals show some structural disorganization within the brain and exhibit a high frequency of hydrocephalus (13%, at median age 49 days). Preliminary studies suggest that lack of 5T4 expression *in vivo* can influence the rate of wound healing and that polyp formation by null 5T4-MIN mice is significantly reduced. Previous studies have shown a role for 5T4 influencing the organization of the actin cytoskeleton relating to altered motility relevant in development and cancer. We have now used primary KO 5T4 mouse embryonic fibroblasts (MEF) from day 13 embryos as a tool for further investigation of 5T4 function. For example, Figure 1 shows that in 5T4KO MEFs, the organization of the actin cytoskeleton is disrupted compared to the WT-MEF. Current studies are focused on the molecular mechanisms underpinning 5T4 influence on motility in embryonic and cancer cells.

5T4 immunotherapy

We have utilized the 5T4 KO mice to generate several monoclonal antibodies specific for m5T4 (B3F1 (IgG_{2a}); B5C9 (IgG_1); B1C3 (IgG_{2a}); P1C9 (IgG_{2b}) and P1H10 (IgG_{2b}), mapped the location of the different epitopes recognized within the m5T4 glycoprotein and measured their affinities. One aim is to assess the functional and possibly therapeutic impact of treatment with these antibodies in different autologous tumour therapy models (e.g. m5T4B16, CT26). The focus on antibodies has been prompted by results from several clinical studies of the 5T4-MVA vaccine (TroVax) which have associated prolonged survival with an antibody response to 5T4, and an immunotherapy based on targeting of a modified bacterial superantigen with antibodies against 5T4 with efficacy in renal carcinoma patients (Elkord *et al*., 2009b). Model systems have also been established investigating chimaeric immune receptor (CIR) T-cells directed to human 5T4 in combination with vaccination (Jiang *et al*., J Immunol 2006; 177: 4288). We are now exploring aspects of T-cell and antibody therapy in our C57BL/6 m5T4 tumour model system utilizing the 5T4 null mouse to generate

cytotoxic T lymphocytes and the KO mAbs for direct therapy and for construction of CIR T cells. To generate gene modified m5T4-specific T-cells, single chain variable fragments (ScFvs) were isolated from two hybridoma cell-lines producing anti-m5T4 mAbs and the sequences were validated. CIR T-cell populations specific for m5T4 will be generated using retroviral construct transfection of polyclonally activated T-cells. For comparison, natural m5T4-specific T-cells are primed in 5T4 KO mice by immunization with Adm5T4 and expanded *in vitro* by weekly restimulation with MHC class I or class II restricted m5T4 peptides for CD4 or CD8 T cells respectively and IL2/IL7. The goal is to examine any potential toxicity and relative efficacy of B16m5T4 tumour therapies based on either 5T4 specific antibody or T-cell treatments in C57BL/6 compared to 5T4 KO mice. One potential limitation to the T-cell therapeutic approaches is our demonstration that during T-cell development, high affinity 5T4 specific T-cells are functionally deleted in the WT animals unlike the 5T4KO mice which are able to evoke strong immunity against 5T4. We have explored the hypothesis that an important mechanism controlling natural recognition of 5T4 is the presence of 5T4 specific T regulatory cells (T reg). We have now shown that WT mice have greater 5T4 specific T reg activity than 5T4 KO mice. It is apparent that treatment with antibodies targeting immuno-modulatory proteins on T reg can augment natural and/or vaccine induced tumour immunity in murine models and this has led to a number of early phase clinical trials. Cytotoxic T lymphocyte antigen 4 (CTLA4) is a key negative regulator of T-cell activation and can be targeted by antibody to release potentially useful anti-tumour activity. Folate receptor 4 (FR4) is a new cell surface marker of T reg where specific antibody can be used to modulate T reg activity *in vivo*. We have used anti-CTLA4 or anti-FR4 antibodies for modulating controlling T reg activity in C57Bl/6 mice in order to potentiate 5T4 specific immunity following Adm5T4 vaccination and assessment of autologous m5T4 tumour therapy. FR4 antibodies were shown to improve the efficacy of the 5T4 vaccination protocol in our B16m5T4 tumour model but anti-CTLA4 failed to improve tumour therapy in the Balb/c CT26 tumour model. An anti-CTLA4 antibody (tremelimumab) has been also been investigated in a phase II trial in patients with advanced gastric and oesophageal adenocarcinoma.

Anti-CTLA4 Clinical Trial

We conducted a phase II trial of tremelimumab as second-line treatment for patients with metastatic gastric and oesophageal adenocarcinomas. Tremelimumab was given every 3 months until symptomatic disease progression. Safety, clinical efficacy and immunologic activity were evaluated. Eighteen patients received tremelimumab. Most drug-related toxicity was mild, but there was a single toxic death due to bowel perforation complicating colitis. Four patients had stable disease with clinical benefit; a single patient achieved a partial response after 8 cycles (25.4 months) and remains well on study at 32.7 months. Markers of T reg phenotype, forkhead box protein 3 (FoxP3) and CTLA4, doubled transiently in $CD4^{+}CD25^{high}$ lymphocytes in the first month after tremelimumab before returning to baseline. In contrast CTLA4 increased in $CD4^{+}CD25^{low/negative}$ lymphocytes throughout the cycle of treatment. *De novo* or boosted proliferative responses were detected to tumour-associated antigens (TAA) 5T4 (8 of 18 patients) and CEA (5 of 13). Patients with a post-treatment CEA proliferative response had median survival of 17.1 months compared to 4.7 months for non-responders ($p=0.004$). Despite the disappointing objective response rate of tremelimumab, one patient has remarkably durable benefit for this poor prognosis disease. *In vitro* evidence of enhanced proliferative responses to relevant TAA suggests that combining CTLA4 blockade with antigen-targeted therapy may warrant further investigation in this challenging setting.

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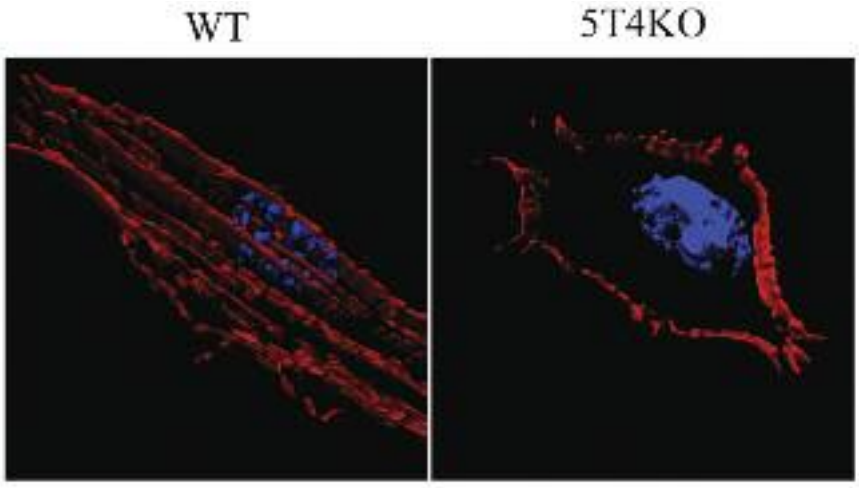

Figure 1
In differentiating 5T4KO ES cells, the F-actin filaments are principally at the cell periphery compared to the typical radial cytoskeletal pattern in WT differentiating ES cells. F-Actin was labelled using Phaloidin-633 Alexa Fluor (Invitrogen) and nuclei by DAPI. The images are presented as 3D surparsed projections.

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Phosphoinositides are a family of lipid second messengers that are regulated by a network of kinases and phosphatases in response to environmental changes. Alterations in phosphoinositide levels can regulate many different cancer-relevant pathways including survival, proliferation, migration, cell substratum interactions and transcription. PtdIns(4,5)P_2 is at the heart of phosphoinositide signalling in cancer as it is the substrate for phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PIC) both of which are deregulated in human tumours. Furthermore PtdIns(4,5)P_2 is itself a regulator of cytoskeletal dynamics, cell survival and cell polarity.

PIP5Ks and PtdIns(4,5)P_2

PtdIns(4,5)P_2 is present in the plasma membrane and in the nucleus where each pool can be regulated separately. PtdIns(4,5)P_2 can be synthesised by two different families of kinases using two different substrates (see figure 1). It is likely that PIP5Ks are the major regulators of PtdIns(4,5)P_2 synthesis while the PIP4Ks regulate PtdIns5P levels and perhaps a minor pool of PtdIns(4,5)P_2.

There are four genes coding for PIP5K (α, β and γ and L) of which α, β and γ are active while L is inactive but can interact with and may regulate the localisation and activity of other PIP5K isoforms. We are using RNAi mediated suppression of PIP5Ks to define isoform specific regulation of PtdIns(4,5)P_2 pools and whether PIP5K isoforms are linked to specific downstream pathways. For example suppression of α or β expression in N1E-115 neuroblastoma cells leads to their differentiation and a cessation in proliferation, while the suppression of γ does not. Furthermore suppression of α reduced PtdIns(4,5)P_2 levels by approximately 50% while suppression of β and γ had little effect on cellular PtdIns(4,5)P_2 levels. How a cell maintains different pools of PtdIns(4,5)P_2 and how these pools signal differentially is not clear. PIP5Kβ is upregulated in a variety of cancer cell lines and overexpression of PIP5Kβ can induce dramatic changes in cell morphology, increase migratory capacity and attenuate apoptosis in response to cellular stressors. PIP5Kβ is modified by phosphorylation at multiple sites and one of these sites is phosphorylated in response to PKC activation, although it is not clear if this is direct. PKC is stimulated in response to PLC signalling, suggesting that phosphorylation of PIP5K may regulate the its activity or localisation downstream of phospholipase signalling. To understand how PIP5K isoforms regulate specific signalling pathways we are identifying proteins that interact with them using affinity purification and mass spectrometry and yeast two hybrid analysis. To define how PtdIns(4,5)P_2 regulates cellular function we have identified nuclear and cytosolic proteins, that interact specifically with this lipid (collaboration with Dr. C. D'Santos CRI Cambridge) and will study how their interaction with PtdIns(4,5)P_2 is able to modulate their function *in vivo*.

PIP4K and PtdIns5*P*

There are three isoforms of PIP4Ks (α, β and γ) of which α is cytosolic, β is cytosolic and nuclear and γ localises to internal membrane compartments. PIP4Ks can regulate the cellular levels of PtdIns5*P* however *in vitro* studies measuring their activity showed that α>>>β>>>>γ. Analysis of the molecular size of PIP4Kβ showed that it exists as a dimer. PIP4Kβ forms homodimers and heterodimers with the α isoform and heterodimerisation can target the α isoform to the nucleus. Furthermore PIP4Kα is required for functions

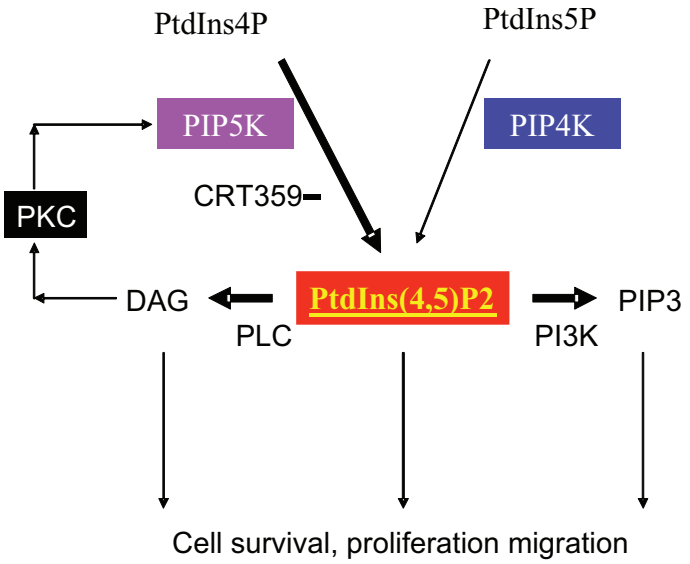

Figure 1
There are two pathways for PtdIns(4,5)P2 synthesis however the major pathway is probably through PIP5K. PIP4K probably regulates the levels of PtdIns5P. CRT359 is an inhibitor of PIP5K which should inhibit the PI3K and the phospholipase (PLC) pathway. Diacylglycerol (DAG) activates protein kinase C (PKC) which regulates the phosphorylation of PIP5K.

previously associated with PIP4Kβ. Interestingly heterodimerisation is more prevalent in the nucleus than in the cytosol suggesting that it may be a regulated process.

We have developed an antibody to PIP4Kβ, and in collaboration with Prof Landberg (Breakthrough Breast Biology and University of Manchester) have interrogated a tissue array of advanced breast tumour samples. Our initial study shows that PIP4Kβ is often upregulated in human breast tumours (figure 2). This study will be extended to a larger tissue array which will enable us to correlate PIP4Kβ overexpression with specific human tumour profiles.

In response to oxidative stress the cellular levels of PtdIns5*P* are dramatically increased through the modulation of a variety of important regulatory circuits within the cell. To deduce how PtdIns5*P* modulates responses to oxidative stress we have generated a cell line in which increased PtdIns5*P* can be specifically attenuated. Signalling pathways and gene expression profiles are being interrogated to uncover PtdIns5*P* specific signalling. To define how PtdIns5*P* regulates cellular and nuclear function, proteins that specifically interact with PtdIns5*P* have been purified and identified (collaboration with Dr. C. D'Santos CRI Cambridge). For example PtdIns5*P* interacts with the PHD finger of inhibitor of growth protein 2 (ING2) and modulates the ability of ING2 to regulate p53 acetylation and transcriptional activity. PHD fingers can also interact with histones, both methylated and non methylated, although the relationship between phosphoinositide interaction and histone binding is not clear. We have identified a variety of other PHD finger containing proteins that interact with phosphoinositides, one of which is involved in the regulation of the basal transcription. We have generated mutants that no longer interact with phosphoinositides which will delineate the role of phosphoinositides in basal transcription. In collaboration with Prof. Z. Avramova (UNL Center for Biotechnology and Plant Science Initiative, UNL, Lincoln, NE, USA) we have analysed the function of ATX1, a PHD finger containing plant homologue of a mammalian trithorax gene. ATX1 controls developmental transcription programmes through its ability to control histone methylation. We have found that the nuclear localisation of ATX1 and therefore its nuclear activity is regulated by interaction with PtdIns5*P*. Mammalian trithorax genes also contain PHD fingers that can interact with phosphoinositides and these genes often undergo chromosomal translocations and deregulation that can induce human leukaemias.

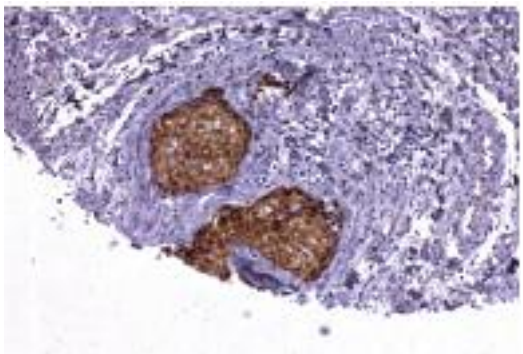

Figure 2
A tissue array of advanced human breast tumours (180 samples) was stained for PIP4Kβ. The figure shows specific staining (brown area) of the tumour but not in the stroma.

PIP5K as a target for drug development.

As PtdIns(4,5)P_2 is the substrate for both the PI-3-kinase which regulates the oncogenic activity of PKB and for PLC which regulates calcium dynamics and PKC activity, inhibition of PtdIns(4,5)P_2 synthesis may be useful to inhibit cancer cell growth (figure 1). PIP5K inhibitors, identified and developed by Cancer Research Technology (CRT), attenuate PKB signalling and are able to inhibit cell growth. Migration of cancer cells from the tumour and their dissemination into new tissues (metastasis) is the major cause of death in human cancers. We have found that both RNAi mediated suppression of PIP5K isoforms or treatment with CRT359 inhibits migration.

Publications listed on page 69

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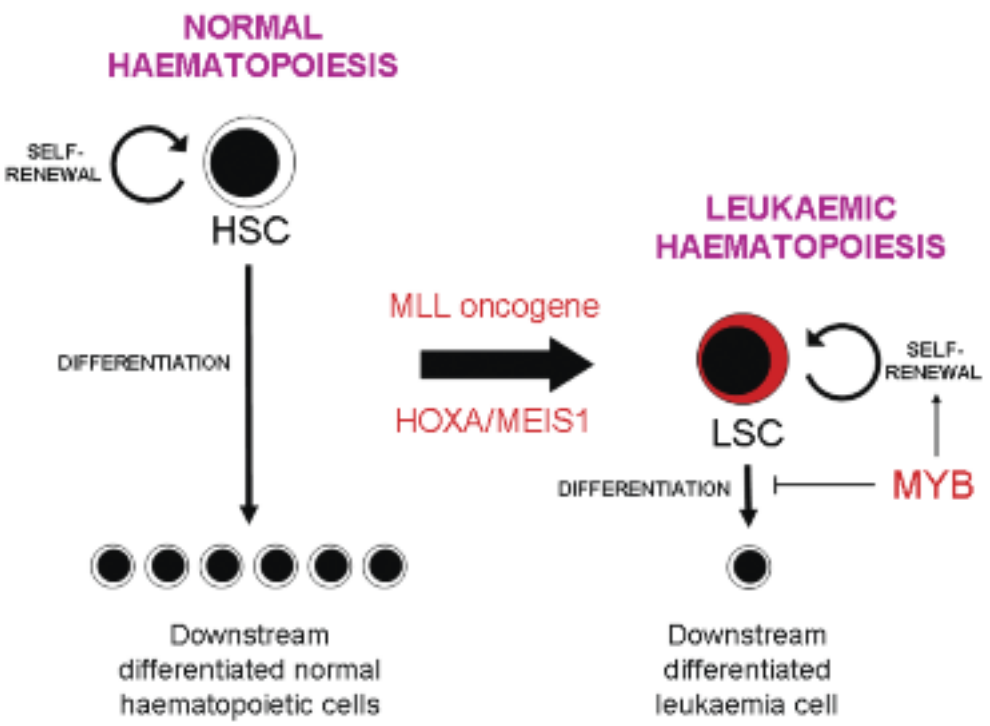
The development of malignant disease is thought to require the accumulation of multiple collaborating genetic mutations in a long lived cell. In leukaemogenesis, one hypothesis is that mutations accumulate over time in the self-renewing haematopoietic stem cell (HSC) compartment, perhaps conferring a selective proliferative or survival advantage in the mutated HSCs by comparison with unmutated ones. This eventually results in full-fledged leukaemia stem cells (LSCs) that share many of the properties of their normal HSC counterparts. A complementary hypothesis is that leukaemogenic mutations confer the capacity to undergo self-renewal on progenitor cells downstream of the HSC, which under normal circumstances are irreversibly committed to terminal differentiation. Aberrantly self-renewing downstream progenitor cells, which may proliferate more frequently that the relatively quiescent HSC pool, represent an important potential reservoir for the acquisition and fixation of further cooperating mutations required for development of full potency LSCs.

In work published in Cell Stem Cell earlier this year, members of the Leukaemia Biology Group (Tim Somervaille and Gary Spencer) reported findings pertinent to this question. A retroviral transduction and transplantation model that faithfully recapitulates many of the pathological features of human acute myeloid leukaemias (AMLs) associated with chromosomal translocations of the MLL gene was used in the study. The MLL gene is mutated by chromosomal translocation in approximately 5-10% of human acute leukaemias. The study lead to the conclusion that MLL fusion oncogenes convert a normal haematopoietic hierarchy into a leukaemia cell hierarchy, sustained at its apex by a population of LSCs most similar in nature to inappropriately self-renewing downstream myeloid cells (Figure 1). Critically, MLL LSCs retain the capacity to undergo spontaneous differentiation *in vivo* to terminally differentiated neutrophils, emphasising that the differentiation block is incomplete. Interestingly terminal differentiation of MLL transformed cells takes place in the presence of continued high level expression of *Hoxa/Meis1* suggesting that, while inappropriate expression of these genes may be sufficient to establish the leukaemia cell hierarchy, alternative mechanisms may regulate exit of LSCs from the self-renewing compartment. It is interesting to speculate that the cellular effect of *Hoxa/Meis1* expression in MLL leukaemias may be to introduce at the level of the GMP or myeloblast a finite probability of a self-renewal division where none previously existed, although the molecular circuitry of this phenomenon remains unclear. The accumulation of an inappropriately self-renewing, proliferating population of pre-LSC downstream of the HSC would provide a long-lived target population of cells receptive to secondary mutations in, for example, Ras or Flt3 which are often mutated in human MLL leukaemias, or for accretion of

Figure 1
MLL fusion oncogenes convert a normal haematopoietic hierarchy into a leukaemia cell hierarchy sustained at its apex by self-renewing leukaemia stem cells.



epigenetic dysfunction, which may permit adaptation of the LSC to the prevailing microenvironment (e.g. Somervaille and Cleary, Cancer Cell 2006; 10: 257).

Comparison of the relative frequencies of LSCs in mice with AML initiated by distinct MLL fusion oncogenes has enabled the derivation of a transcriptional program closely associated with the LSC (Somervaille *et al*., 2009). The program is concordantly regulated between high and low LSC frequency populations, both within and between individual leukaemias. Interestingly, the global transcriptional profile of MLL LSCs is much more akin to that of ESCs rather than adult stem cells, emphasising that even though MLL LSCs share some transcriptional features with HSCs, substantial differences exist. Within the core gene set that regulates the self-renewal of the LSC, *Myb*, *Cbx5* (HP1α) and *Hmgb3* are critical components, as demonstrated by abrogation of LSC potential upon genetic knockdown. Conversely, when over expressed together *Myb*, *Cbx5* and *Hmgb3* robustly immortalise murine haematopoietic stem and progenitor cells without inducing up regulation of *Hoxa* or *Meis1*. This suggests that downstream of the critical *MLL/Hoxa/Meis1* leukaemia initiation program lies a distinct LSC maintenance program that regulates exit of LSCs from the self-renewing compartment, and which is influenced but not directly controlled by the prevailing levels of *Hoxa* or *Meis1* (Figure 1).

Now the Leukaemia Biology Laboratory is fully staffed, new experimental approaches are being developed and refined, all with the aim of trying to define the genes, pathways and cellular mechanisms that sustain leukaemia stem cells in a self-renewing state. These include experiments using adult or neonatal immune deficient mice where the capacity of human AML cells to initiate xenografts can be tested (Figure 2). Our close proximity to The Christie and links with the Department of Haematology facilitate both the acquisition of human tissue and the development of such experimental approaches.

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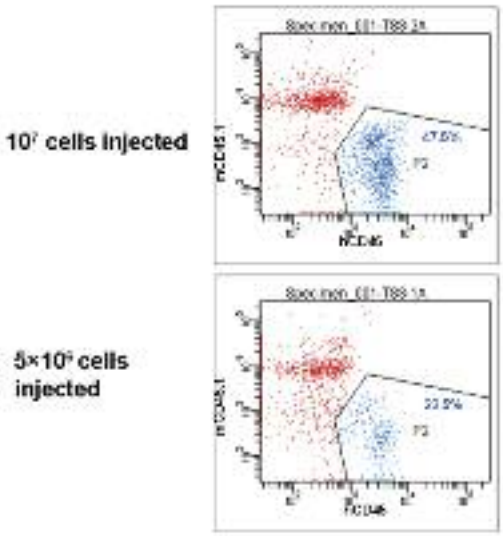
Figure 2
Human acute myeloid leukaemia cells may engraft immune deficient mice. FACS plots show bone marrow cells from NSG mice. Human AML cells (in blue) share the marrow space with recipient normal haematopoiesis (in red) six to eight weeks following transplantation (experimental work performed by Xu Huang).



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The major interest of our lab is to decipher the cellular and molecular mechanisms controlling the development and maintenance of the haematopoietic system. In this context, we study the functions of the transcription factor AML1/RUNX1 and the transcriptional co-activator MOZ. *AML1/RUNX1* is one of the most frequent targets of gene rearrangements and mutations in acute leukaemia. Similarly the gene *MOZ* is involved in myeloid chromosomal translocations. Understanding the function of these transcription regulators during normal haematopoiesis should result in a better comprehension of how perturbations of their functions lead the development of leukaemia.

Generation of blood cells

The earliest site of blood cell development in the mouse embryo is the yolk sac where blood islands, consisting of haematopoietic cells surrounded by a layer of angioblasts, develop at approximately day 7.5 of gestation. The parallel development of these two lineages in close association provided the basis for the hypothesis that they arise from a common precursor, a cell called the haemangioblast. A conflicting theory however associates the first haematopoietic cells to a phenotypically differentiated endothelial cell with haematopoietic potential, i.e. a haemogenic endothelium. Support for the haemangioblast concept was initially provided by the identification during embryonic stem (ES) cells differentiation of a clonal precursor, the blast colony-forming cell (BL-CFC), which gives rise after 4 days to blast colonies with both endothelial and haematopoietic potential. Although recent studies have now provided evidence for the presence of this bipotential precursor *in vivo*, the precise mechanism of generation of haematopoietic cells from the haemangioblast still remains completely unknown.

We performed a series of studies to determine the cellular and molecular events leading to the generation of blast colony from BL-CFC. Our data demonstrate that the haemangioblast generates haematopoietic cells through the formation of a haemogenic endothelium intermediate, providing the first direct link between these two precursor populations. This haemogenic endothelial cell population is transiently generated during blast development and is also detected in gastrulating embryos. At the molecular level, we have demonstrated that the transcription factor SCL/TAL1 is indispensable for the establishment of this haemogenic endothelium cell population from the haemangioblast whereas RUNX1/AML1 is critical for the generation of haematopoietic cells from this haemogenic endothelium. These results indicate that the two *a priori* conflicting theories on the origin of haematopoietic development, haemangioblast and haemogenic endothelium, can be merged into a single linear developmental process leading to the formation of the first committed haematopoietic precursors.

Transcriptional targets of Runx1/AML1:AI467606

Our initial studies revealed a profound defect in the potential of the *Runx1*$^{-/-}$ ES cells to generate blast colonies and therefore RUNX1 is likely to regulate the expression of an important set of genes at this stage of development. To identify these genes, we compared gene expression in haemangioblast-derived cell populations

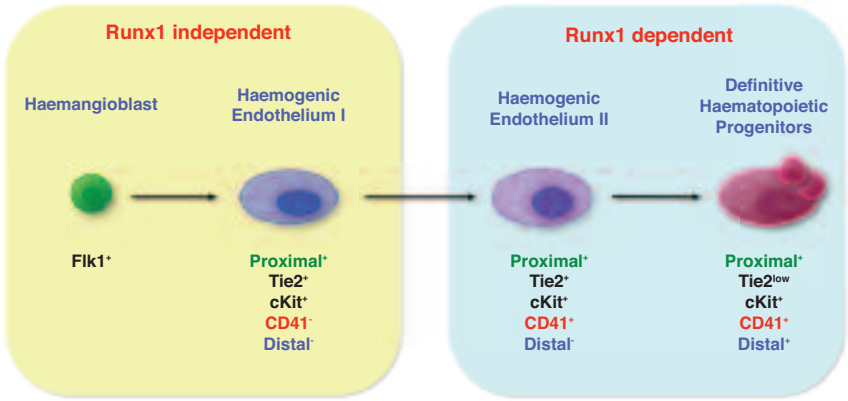

Figure 1
Model of sequential activation of Runx1 promoters.

generated from either *Runx1* deficient or *Runx1* competent ES cells. Such comparisons highlighted a candidate, *AI467606* (*AI*), a previously uncharacterized gene, for further studies. This novel gene encodes a protein of 225 amino acids with a putative trans-membrane domain. We first demonstrated by chromatin immuno-precipitation the binding of *Runx1* to the promoter of *AI* during blast colony development and by luciferase reporter assay, the regulation of *AI* transcription by *Runx1*. Furthermore we observed that re-expression of *Runx1* in *Runx1*$^{-/-}$ cells led to a rapid upregulation of *AI* expression. Altogether these results suggest that *AI* is a direct transcriptional target of *Runx1*. To track the cells expressing *AI*, we generated a transgenic ES cell line with a modified bacterial artificial chromosome (BAC) containing a *GFP* reporter gene knocked-in the *AI* locus. We demonstrated *in vivo* and *in vitro* the emergence of *AI* expressing cells from the CD41^{+} cell population. In foetal and adult mice, *AI* was specifically and highly expressed in haematopoietic organs as indicated by immuno-histochemistry and flow cytometry analysis. Furthermore, the *AI*$^{+}$ cell population contained all haematopoietic potential. Altogether these results suggest that *AI* expression marks haematopoietic cells throughout development, from their emergence in the yolk sac, through foetal liver and finally to adult haematopoietic organs. We are currently establishing knock-out ES cells and conditional knock-out mouse line to evaluate the potential function of this gene during haematopoiesis.

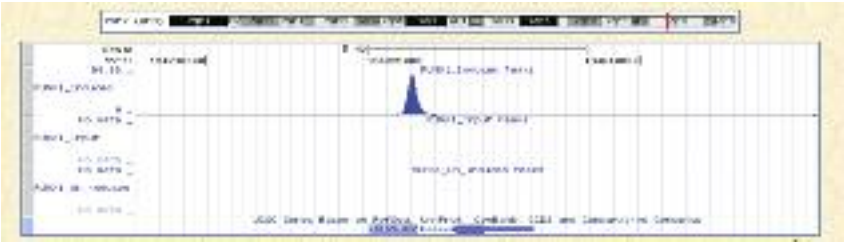

Figure 2
Binding of Runx1 to the promoter of AI467606 detected by ChIP-seq.

Transcriptional activities of Runx1 promoters

In vertebrates, the transcription of the *Runx1* gene is under the control of two alternative promoters, distal P1 and proximal P2, which generate specific *Runx1* transcripts. We investigated the activities of distal and proximal *Runx1* promoters at the single cell level and tracked the cell populations expressing the two respective isoforms. We demonstrated that at the onset of haematopoiesis both *in vitro* and *in vivo* the activity of the proximal promoter marks a haemogenic endothelium cell population, whereas the subsequent activation of the distal *Runx1* promoter defines fully committed definitive haematopoietic progenitors. Interestingly, haematopoietic commitment in distal *Runx1* knock-out embryos appears normal, suggesting that the proximal isoform plays a critical role in the generation of haematopoietic cells from haemogenic endothelium. Altogether, our data demonstrate that the differential activities of the two *Runx1* promoters define milestones of haematopoietic development. Identification and access to the discrete stages of haematopoietic development defined by the activities of the *Runx1* promoters will provide the opportunity to further explore the cellular and molecular mechanisms of blood development.

Chromatin remodelling and *Runx1* at the onset of haematopoietic development

We have examined in collaboration with the group of Prof. Bonifer (Institute for Molecular Medicine, Leeds University) the molecular events mediated by *Runx1* leading to the transcriptional activation of two master myeloid genes *Pu.1* and *Csf1r*. We demonstrated that although *Runx1* binds to both loci, it is sufficient to initiate chromatin remodelling events at the *Pu.1* locus but not at the *Csf1r* locus. We also found that *Runx1* directly interacts with its target sequences in haemangioblasts, but that this interaction is initially transient ("hit and run" fashion). Accordingly, a single pulse of *Runx1* expression in developing *Runx1*$^{-/-}$ blast colony is sufficient to rescue the expression of these genes and the development of definitive haematopoietic precursors. These results suggest that *Runx1* is crucial for the initial chromatin remodeling events at loci encoding transcription factors that are regulators of specific blood cell lineages. Our data suggest that once these transcription factors are expressed, stable transcription factor complexes are formed on these genes, active chromatin is maintained and an epigenetic memory for active gene expression is established. Once this has occurred, *Runx1* becomes less important and is used only in certain genomic contexts as one of many other transcription factors. This could explain why *Runx1* is not generally essential for adult haematopoiesis.

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Understanding the molecular mechanisms that control the formation of blood precursors from the mesodermal germ layer is the major focus of our laboratory. Several lines of evidence suggest that leukemogenesis may result from the re-initiation of an embryonic programme during adult life or from the inappropriate expression of genes controlling critical steps of the haematopoietic embryonic programme. A clear understanding of the molecular mechanisms orchestrating the onset of haematopoietic specification should help us to better define the basis of de-regulated proliferation and differentiation observed in haematological malignancies.

Sox7-sustained expression alters the balance between proliferation and differentiation at the onset of blood development

Based on its differential expression profile between mesoderm and haemangioblast, we selected *Sox7* as a potential candidate gene involved in the regulation of blood lineage formation. A thorough analysis of *Sox7* expression at the onset of blood specification revealed that this transcription factor was transiently expressed in Flk1$^+$ precursors as they underwent specification to the haematopoietic programme. To identify the cells that transiently express *Sox7*, we established an ES cell line carrying a bacterial artificial chromosome (BAC) transgene that contains the complete *Sox7* locus with the first exon of *Sox7* replaced by a GFP-reporter cassette. Upon the *in vitro* differentiation of these ES cells, we were able to track and analyse *Sox7*-expressing cells. Results obtained using this ES cell line revealed that *Sox7* was transiently expressed in a small subset of Flk1$^+$ cells that subsequently became committed to CD41$^+$ haematopoietic precursors of both primitive and definitive lineages. The expression of CD41 and *Sox7* was inversely correlated as *Sox7* expression was down regulated as soon as the cells acquired CD41 expression. Using a mouse transgenic line derived from these *Sox7*-GFP BAC ES cells, we also showed that a small subpopulation of Flk1$^+$ cells co-expressed *Sox7* transiently in gastrulating embryos. Altogether, these data suggest that *Sox7* expression defines a narrow window of development between haemangioblast specification and haematopoietic commitment.

We also undertook classical loss- and gain-of-function approaches to define a possible role for *Sox7* in haematopoiesis. As a mean to achieve loss of function, we made use of the shRNA technology to knockdown *Sox7* expression *in vitro*. Knocking down *Sox7* expression during the specification of mesodermal precursors to haematopoiesis significantly decreased the formation of haematopoietic progenitors as well as endothelial progenitors. *Sox7* knockdown led to a substantial increase in precursor cell death, pointing toward a possible role for *Sox7* in cell survival during haematopoiesis specification. *Sox7* expression is naturally down regulated as haematopoietic differentiation progresses to the formation mature blood lineages. It seemed therefore of interest to address the biological relevance of this down-regulation for haematopoietic maturation. Gain-of-function studies were performed using an inducible system to generate ES cell lines in which the expression of *Sox7* was inducible upon doxycycline addition. Surprisingly, *Sox7*-sustained expression in the earliest committed haematopoietic precursors resulted in the maintenance of their multi-potent and self-renewing status. *Sox7*-expressing cells rapidly accumulated in the culture, showing extensive proliferative potential but no sign of maturation.

Removal of this differentiation block driven by *Sox7*-enforced expression led to the efficient differentiation of haematopoietic progenitors to all erythroid and myeloid lineages.

Altogether, our study identify *Sox7* as a novel and important player in the molecular regulation of the first committed blood precursors. Our data demonstrate that decreased *Sox7* expression affects the formation of blood precursors while its sustained expression is sufficient to alter drastically the balance between proliferation and differentiation.

Contrasting effects of Sox17 and Sox18-sustained expression at the onset of blood specification

Given the known redundant roles of *Sox7*, *Sox17* and *Sox18* in cardiovascular development and the importance of *Sox7* at the onset of haematopoiesis, we decided to explore how Sox17 and Sox18 enforced expression may influence blood specification. To determine the impact of *Sox17* and *Sox18* ectopic expression on blood precursor development, we established ES clones carrying either *Sox17* or *Sox18* inducible constructs using the doxycycline inducible system described for Sox7. Without doxycycline, cells derived from day 5 EB formed primitive and definitive haematopoietic colonies, whereas with doxycycline few primitive or definitive colonies could be detected. Upon *Sox18*-enforced expression, a large number of colonies, blastic in appearance, developed whereas very few blastic colonies were observed upon *Sox17*-enforced expression. Little evidence of maturation was detected in *Sox18*-expressing blast colonies, with most cells retaining an immature immuno-phenotype and morphology. Upon removal of doxycycline and down-regulation of *Sox18* expression, individual Sox18^{+} colonies gave rise to all erythroid and myeloid lineages. Overall, the outcome of *Sox18*-enforced expression was reminiscent of the phenotype observed upon *Sox7*-enforced expression: in both cases, we observed a block in blood lineage maturation and the accumulation of blast-like colonies containing immature multi-potent blood progenitors.

Considering the high degree of homology among the three Sox F genes and their known functional redundancy, it was surprising that *Sox17*-enforced expression led to an almost complete absence of colonies, either mature or immature. Intrigued by these results, we set out to define the basis for such a difference. We previously showed that *Sox7*-enforced expression led to the enhanced proliferation of blood precursors. Similarly, *Sox18* induced the massive proliferation of CD41^{+} blood precursors. In contrast, *Sox17* did not confer any proliferative advantage. Indeed, cell death analysis revealed that *Sox17*-enforced expression caused an increase in apoptosis. However, this apoptosis was cell-context dependent and specific to early haematopoietic precursors, as *Sox17* induction in undifferentiated ES cells did not impair proliferation or increased apoptosis. To compare and contrast enforced-expression phenotype to expression pattern, we analysed endogenous expression of *Sox7*, *Sox17* and *Sox18* at the onset of haematopoietic specification. Absolute quantification of the three transcripts during haemangioblast differentiation revealed that *Sox7* was the most abundant transcript in Flk1^{+} cells and at day 1 of blast development. *Sox18* expression was predominant at day 2 and 3 while *Sox17* expression was only marginally present at all time points analyzed.

Overall, our data suggest that despite the redundant roles generally observed for Sox F factors in several aspects of cardiovascular development, their expression patterns and function upon enforced expression at the onset of blood development are rather different. *Sox7* and *Sox18* have partially overlapping expression patterns and their sustained expression promotes the proliferation of early blood precursors while blocking further differentiation. In contrast, *Sox17* expression is marginal at the onset of blood development and its ectopic expression appears detrimental to the survival of these early precursors.

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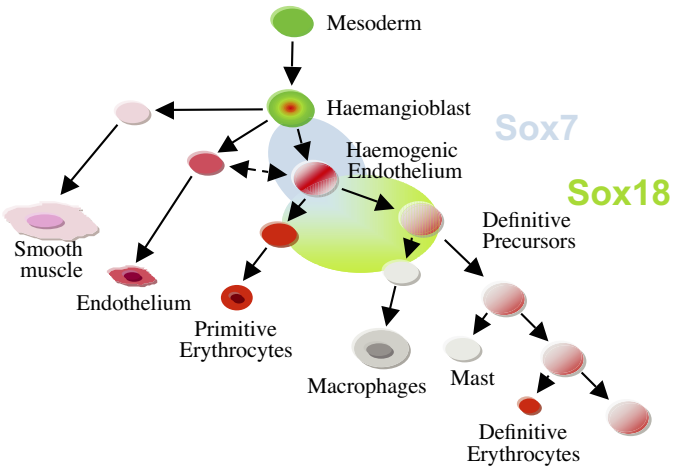
Figure 1



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Human tumours are highly complex tissues and the non-neoplastic cell compartment of tumours, which is often termed the "stroma", is itself quite complex histologically. Carcinoma cells initially recruit and/or activate these various stromal non-neoplastic cells, including fibroblasts, myofibroblasts, immune cells, endothelial cells and bone marrow-derived cells. The resulting stromal cells reciprocate by fostering carcinoma cell growth and survival during the course of tumour progression. Studying the heterotypic interactions between the neoplastic cells and the supporting stroma is believed to be essential for understanding nature of a bulk of carcinoma mass. We focus on studying 1) how tumour-associated stromal fibroblasts become altered and co-evolve with tumour cells during the course of tumour progression, 2) how the stromal fibroblasts facilitate tumour progression, and 3) what specific stroma-derived signal is crucial in promoting tumour invasion and metastasis.

Tumour-promoting roles of carcinoma-associated fibroblasts (CAFs)

Neoplastic epithelial cells coexist in carcinomas with a stroma composed of various types of mesenchymal cells as well as extracellular matrix (ECM), both of which create the complexity of the tumour microenvironment. Noticeable numbers of myofibroblasts, which are characterized by their production of α-smooth muscle actin (α-SMA), have been observed repeatedly in the stroma of the majority of invasive human breast cancers (Figure 1). However, the specific contributions of these cells to tumour progression are poorly defined. Myofibroblasts also exist in areas of wound healing and chronic inflammation, and are often portrayed as "activated fibroblasts" that play crucial roles in wound repair; myofibroblasts possess greatly increased contractile ability, promote angiogenesis, and stimulate epithelial cell growth through the production of ECM and the secretion of growth factor and cytokines. The striking histological resemblance is observed between tumour stroma and the stroma present in sites of wound healing, both of which contain considerable numbers of myofibroblasts.

Stromal fibroblasts and myofibroblasts, collectively termed carcinoma-associated fibroblasts (CAFs), were extracted from various human carcinomas. CAFs, in comparison with their control fibroblasts, when coinjected with carcinoma cells into immunodeficient mice, are known to substantially promote carcinoma growth and neoangiogenesis.

Evolution of tumour stromal myofibroblasts in tumour

CAFs retain their myofibroblastic properties and tumour-promoting phenotypes, after they have been passaged for ten population doublings (PDs) *in vitro* in the absence of ongoing contact with carcinoma cells. Accordingly, even though the CAFs appear to have initially acquired their unique phenotypes under the influence of carcinoma cells, once it is acquired, they might

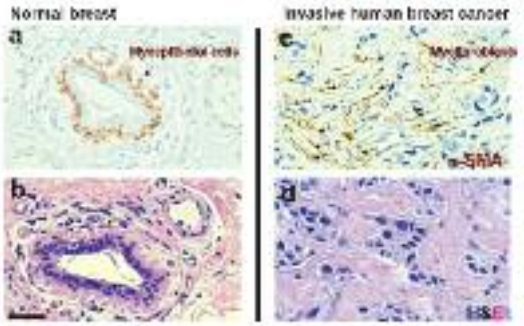

Figure 1.
Tumour-associated stroma includes large numbers of α-smooth muscle actin (α-SMA)-positive myofibroblasts in human breast cancers. Immunostaining of human breast tissues by an anti-α-SMA antibody (a, c) and also staining with H&E (b, d). The tumour region (c, d) and non-tumour region (a, b) dissected from the breast tissue of the human breast cancer patient are shown. Myofibroblasts (indicated by an arrow) in the tumour region (c) and myoepithelial cells in non-tumour breast region (a) are strongly positive for α-SMA. Scale bar, 75 μm.

display this trait independent of further signalling from the carcinoma cells. Unanswered by these observations are (i) how do CAFs acquire and maintain their activated, tumour-enhancing phenotypes? (ii) might CAFs harbour genetic and/or epigenetic alterations that act to confer the unique phenotypes?

Some reports indicate that stromal regions microdissected from human breast cancers exhibit a high frequency of genetic alterations, such as chromosomal regions of loss of heterozygosity (LOH) and somatic mutations. A recent report also suggests that stromal fibroblasts that have undergone p53 loss are clonally selected during tumour progression, yielding a highly proliferative stroma. However, others indicate that tumour-associated stroma and CAFs exhibit no detectable genetic alterations, as gauged by array CGH and SNP array analyses; this suggests that any stably maintained phenotype may depend on epigenetic modifications of the genome, such as DNA methylation. Alternatively, the stabilization of their phenotype may depend on some type of positive-feedback signalling of the sort created by autocrine signalling loops. We note that our CAFs show no detectable aneuploidy as determined by karyotype analysis, no anchorage-independent growth in culture, and no tumourigenicity *in vivo*. Moreover, some of the CAFs begin to senesce after 15 population doublings (PDs) in culture, similar to the behaviour of normal human stromal fibroblasts.

Various cell types are thought to act as a source for the emergence of tumour-promoting CAF myofibroblasts (Figure 2). Resident fibroblasts are likely a major source of myofibroblasts, whilst other mesenchymal cell types including endothelial cells, pericytes, smooth muscle cells and preadipocytes are also capable of converting into myofibroblasts. Bone marrow-derived progenitors, such as fibrocytes and mesenchymal stem cells (MSCs), are also reported to differentiate into myofibroblasts within the tumour. It is also possible that a small number of myofibroblasts present within the normal fibroblast populations are clonally expanded in response to selective pressure imposed by the tumour microenvironment. In addition, the stromal cells that have acquired epigenetic or genetic alterations may differentiate into myofibroblasts and these cells are then also likely to undergo clonal expansion.

The tumour stroma continues to remodel itself during tumour progression and actively recruits various cell types into the tumour mass where they act as different sources for myofibroblasts. It is not known, however, if different cells of origins in the myofibroblast populations in the stroma of tumour exhibit different tumour-promoting properties. Once generated, myofibroblasts could maintain their ability to substantially promote tumour growth and progression in many aspects via their interactions with carcinoma cells and other host stromal cells.

Studying cross-talk between tumour cells and mesenchymal cells during tumour progression could help understand nature of biology of human carcinomas and facilitate to develop novel stroma-targeted therapeutic approaches.

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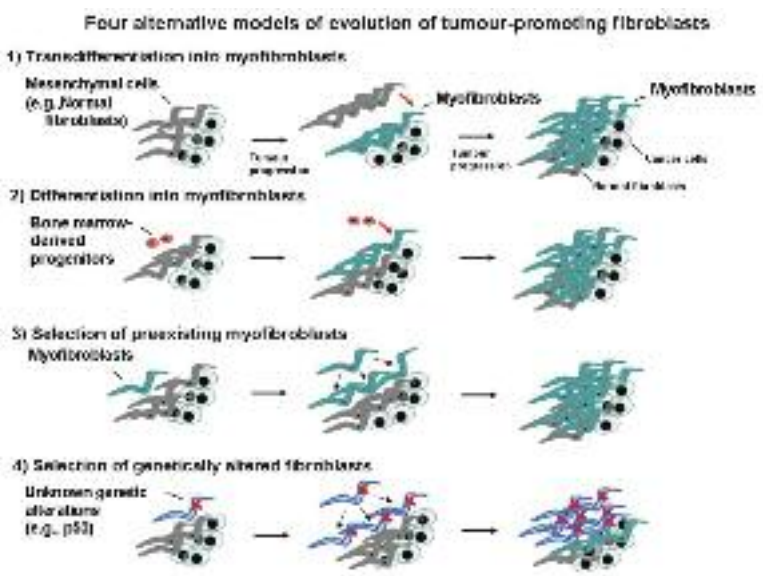
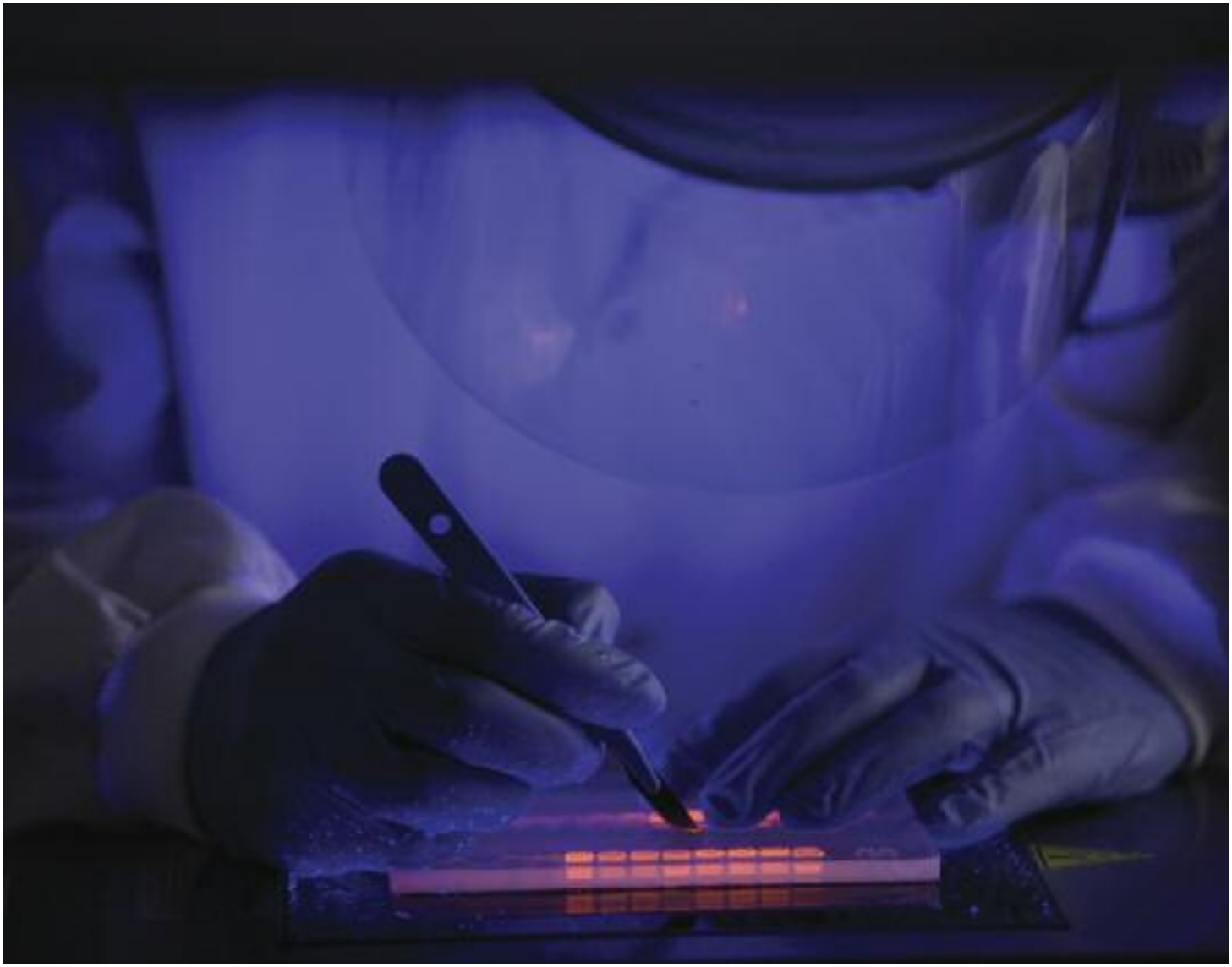


Figure 2.
Schematic representation of cellular origins of CAF myofibroblasts during tumour progression; 1) myofibroblasts are proposed to be derived from various mesenchymal cell types, such as pre-existing fibroblasts, pericytes and/or endothelial cells through trans-differentiation. 2) Bone marrow-derived progenitor cells (BMDCs) including mesenchymal stem cells (MSCs) and fibrocytes also differentiate into myofibroblasts. 3 and 4) A small population of residual myofibroblasts or mesenchymal cells that carry genetic alterations may be clonally selected during tumour progression. The genetically altered mesenchymal cells may or may not differentiate into myofibroblasts.



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Christy Ralph (to Dec 2009)
Alaaeldin Shablak
Saladin Sawan (with Catherine Holland)
Smita Sharma (from Oct 2009)

Research Student
Sameena Khan

The Biological Immune and Gene Therapy (BIGT) group focuses on translational and clinical research in this field. The Clinical Immune and Molecular Monitoring Laboratory (CIMML) in TRF2 was established at the end of 2008 and is now functional in respect to GCLP evaluation. A range of validated immune and molecular based assays for monitoring clinical trials have been established including primary, secondary and research related assays in several different studies. There is a separate but linked development of a GMP cell processing facility which will open in March 2010 at the Incubator Building.

GCLP validation

The CIMML was built through Christie Gene Therapy appeal funding and the staffing is supported by CR-UK following our 2005 site visit. The GCLP compliance of CIMML was audited in November 2009 by CR-UK with an extremely positive outcome. The GCLP primary and secondary assays of the ongoing gene modified T-cell clinical trials have now been transferred from the National Blood Service. Molecular assays developed include DNA/RNA isolation from clinical samples, T-cell spectratyping, real-time PCR, LAM-PCR, SNP analysis, mutational analysis and gene expression studies. Immunological assays developed include PBMC isolation, multicolour flow cytometric assays for detection of surface and intracellular markers, ELISPOT, cellular proliferation, cellular cytotoxicity, ELISA and cytokine release assays. These are broadly applicable to a range of trials and are critical for engineered T-cell therapy trials (see below).

Trials targeting 5T4

5T4 is a trophoblast antigen found to be selectively expressed on many common cancers. It was discovered by Professor Stern and he and the BIGT group have been involved at all stages of pre-clinical and clinical development. This work links with companies who have licensed the antigen from CR-UK. There are two current approaches to targeting 5T4:

- TroVax® is a recombinant viral vaccine based on MVA delivering 5T4
- Anyara® is an antibody targeted super-antigen which acts to target T cells to tumours

Both are progressing through Phase II to Phase III studies and the BIGT group continues to play a major role in their evaluation. Both studies were adopted by the UK NCRN.

Vaccination of metastatic renal cancer patients with TroVax given alongside interferon-alpha

We conducted an open-label phase I/II trial in which TroVax was administered alongside interferon-alpha (IFNα) to 11 patients with metastatic renal cell carcinoma. Antigen-specific cellular and humoral responses were monitored throughout the study, and clinical responses were assessed by measuring the changes in tumor burden. Treatment with TroVax plus IFNα was well tolerated with no serious adverse events attributed to TroVax. All 11 patients mounted 5T4-specific antibody responses and 5 (45%) mounted cellular responses. No objective tumour responses were seen, but the overall median time to progression of 9 months was longer than expected for IFNα alone and the median overall survival was >26 months which is also much longer than expected. The high frequency of 5T4-specific immune responses and apparent long term benefit provided part of the justification for a large Phase III trial (TRIST).

Scientific Officers
Debbie Burt (seconded from Immunology)
Hayley Batha
Natalia Krillova (at GMP Cellular Therapy Unit)
Sam Mowbray (at GMP Cellular Therapy Unit)
Lidan Chrisitie (at GMP Cellular Therapy Unit)

Research Nurse
Andrea Byatt

Administration
Anvi Wadke – Clinical Trials Administration
Nicola Hudson – ATTACK/ATTRACT project manager

A randomised, double-blind, placebo-controlled phase III study of MVA-5T4 (TroVax) in metastatic renal cancer patients

The TRIST trial (TroVax Renal Immunotherapy Survival Trial; 2006-001246-13), which investigated whether TroVax added to first-line standard of care, prolonged the survival of patients with metastatic clear cell renal cancer, has completed recruitment. 700 patients were randomised 1:1 to receive up to 13 immunisations of MVA-5T4 or placebo in combination with either Sunitinib, low-dose IL-2 or IFN-α. Only preliminary results are available at present but Professor Hawkins presented these for the first time at ECCO 2009 in Berlin. The treatment was generally well tolerated with no significant toxicity related to the vaccine. Early survival analysis demonstrated no significant improvement in overall survival (20.1 vs 19.2 months; HR: 1.07; 95% CI: 0.86, 1.33; $p = 0.55$). However, a prospectively planned analysis demonstrated a significant survival advantage in good prognosis patients treated with IL-2 + Trovax (not reached vs 19.5 months; HR: 0.54; 95% CI: 0.30, 0.98; $p = 0.04$). Antibody responses against 5T4 were induced in most MVA-5T4-treated patients and were associated with enhanced survival. The group of good prognosis patients comprises those who benefit most from immunotherapy and consideration is being given how to take this positive finding forward in the context of changing standard treatments for renal cancer.

5T4 antibody targeted superantigen therapy

A Phase II/III study of ABR-217620 (ANYARA) combined with IFN-α compared to IFN-α alone in patients with advanced renal cell cancer has been undertaken. The interim analysis satisfied pre-defined criteria and the study was expanded from a Phase II to a Phase III study. Recruitment is now completed and CIMML has completed the validation and undertaken an extended immune analysis of a subset the patients treated with ANYARA and IFNα. The analysis includes flow cytometric assays for the expansion of specific Vβ TCR cells driven by the ANYARA, and also the evaluation of different lymphocyte subpopulations.

Cellular Therapy

Adoptive cellular therapy for cancer is a very promising and potentially general approach to the treatment of cancer. The Manchester Group is the leading European Centre and co-ordinates two large European programmes in this area and is the only centre with two engineered cell therapy trials on-going. The ATTACK consortium (www.ATTACK-cancer.org; Coordinator: Robert Hawkins) is a €12M FP6 preclinical project which has had a major impact in technology development in this field. An FP7 training network (ATTRACT) is commencing (end 2009) with a more clinical/translational focus. The overall aim is to efficiently evaluate this promising approach to cancer treatment.

Engineered T-cell Trials

Two products developed by the Cellular Therapy Group are in CR-UK-NAC and Kay Kendall programme funded Phase I/II trials targeting CEA in gastrointestinal and CD19 in B-cell malignancies. They both combine preconditioning chemotherapy and IL-2 in addition to cell therapy as pre-clinical models and clinical trials at the NCI in melanoma patients suggest this is advantageous. The trial targeting CD19 is at early stage but already encouraging clinical effects have been seen and treatment is well tolerated. The trial targeting CEA is nearing the maximum doses and we hope to expand these cohorts to look for efficacy and to optimize the cellular production methods. These trials are clinically complex and NHS R&D funding supports ward facilities where these types of specialist treatment can be undertaken. The clinical team led by Professor Hawkins and Dr Fiona Thistlethwaite is supported by experienced research nurse/trials coordinators to ensure effective management of these complex patients. Recruitment to these trials will continue next year.

GMP Cell Therapy Unit

To further develop this area we are developing a new GMP Cell Therapy Unit. This is being fitted and equipped with funding (£700,000) from The Christie Charity and has University and EU support via the ATTRACT training network as well as specific clinical trials grants. The unit is anticipated to produce its first GMP product for the current CR-UK trial in March 2010.

Based on the strategy defined at our ATTACK organized international workshop we are also developing clinical trials in melanoma linking with the NCI. After initial national feasibility studies multi-centre European studies are planned. Initial melanoma studies should commence mid 2010 and already key preliminary studies have been completed with the melanoma group.

Publications listed on page 70

Figure 1

Figure 1
Specifically designed isolator being installed in the Cellular Therapy Unit at The University of Manchester Incubator Building. The innovative design of the unit, devised with MHRA and CR-UK involvement, will facilitate the ambitious plans to develop a core multi-user facility which can deliver multiple cellular therapy products in an efficient manner.

Children's Cancer Group

Group Leader
Vaskar Saha

Postdoctoral Fellows
John Bridgeman
Clare Dempsey
Mark Holland
Jizhong Liu

Clinical Research Fellows
Shekhar Krishnan
Ashish Masurekar

Scientific Officers
Seema Alexander
Naina Patel

Graduate Student
Eva Diffner

Clinical Trials Manager
Catriona Parker

Administrator
Charlotte O'Horo

Our research focuses on understanding the biological basis for the differences in the therapeutic response in children with acute lymphoblastic leukaemia (ALL). Our clinical data is obtained from a number of international clinical trials which we coordinate and we obtain clinical material nationwide from children with ALL undergoing therapy on national protocols. The material linked with clinical data, provides the basis for our laboratory research. This has been a busy year for us as the group is finally at full strength. We moved into new premises last summer and the laboratory is now fully kitted and expanding.

Clinical Trials

We reported on the results of the clinical trials, managed by Catriona Parker, last year. While therapy is highly successful in childhood ALL, relapses within the central nervous system (CNS) are a poorly understood peculiarity of the disease. Opinion is divided as to the mechanisms of these relapses. As this feature is therapy related, Shekhar Krishnan investigated the temporal changes and incidence in the pattern of CNS relapses in children with ALL treated on four consecutive national trials from 1985 – 2001. During this period we abandoned cranial irradiation and progressively intensified systemic therapy. Shekhar's critical analysis showed that not only have overall relapses halved but both isolated and combined (with bone marrow) CNS relapses have dramatically decreased since 1985 (Krishnan *et al*., Leukaemia in press). This suggests that systemic therapy rather than directed CNS therapy has been the key to the decline in all types of relapses. However, over time the proportionate number of CNS relapses has increased and bone marrow relapses have declined. This supports the view that cells which have an increased ability to migrate into extramedullary sites may have a survival advantage giving rise to relapses later on. These findings have influenced the design of the forthcoming frontline trial, ALL2010.

Laboratory

We reported last year lymphoblasts from patients with high risk ALL express the lysosomal cysteine proteases Cathepsin B (CTSB) and asparaginyl endopeptidase (AEP) and degrade the drug asparaginase (2). Ashish Masurekar is investigating this in context of a prospective national biomarker trial in patients undergoing therapy. AEP has been reported to be overexpressed at the leading edge of epithelial cancers of poor prognosis with increased invasive and metastatic potential. In our observations active AEP was localised to a discrete endocytic compartment contiguous with the plasma membrane (Patel *et al*., 2009). We speculated that lymphoblast adhesion and invasion into the CNS could be facilitated by AEP. In our initial experiments we found *in vitro* evidence of increased invasive potential in AEP producing leukaemic cell lines. However, such invasion was only modestly inhibited by an AEP inhibitor or by knocking down AEP. To further investigate the novel changes in the plasma membrane proteome of invasive cells, Mark Holland used a comparative semiquantitative mass spectrometry (MS) approach. Almost half the proteins on the plasma membrane of the invasive cell type were associated with adhesion, invasion and cytoskeleton re-organisation. Pathway analyses suggested a pivotal role for RAC. Seema Alexander showed that specific inhibition of RAC completely abrogated invasion

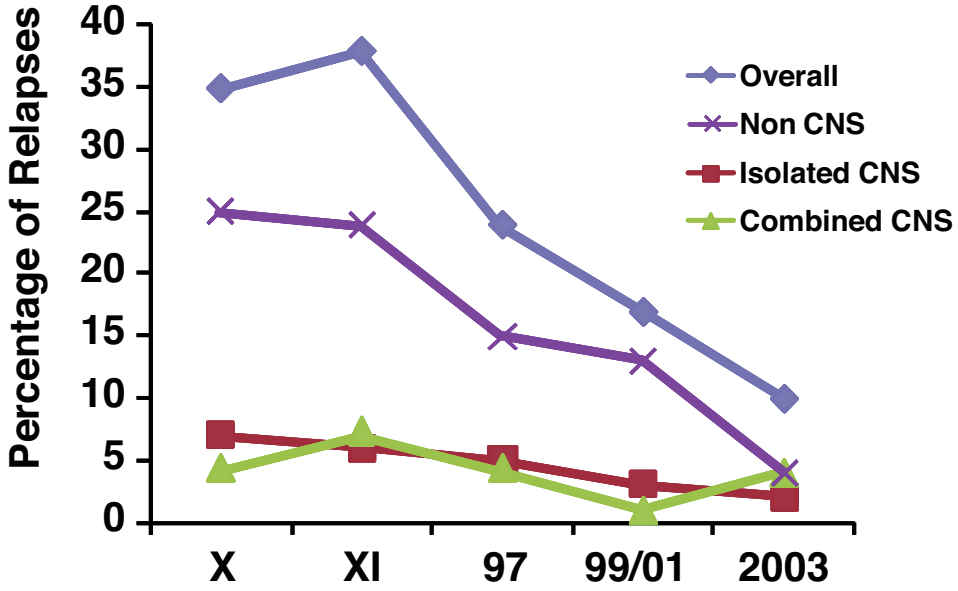


Figure 1
Progressive improvement in outcome on consecutive clinical trials in childhood ALL. The trials were UKALL X (1985-90); UKALL XI (1991-7); ALL97 (1997-99); ALL 99(1999-2002) and the current trial ALL2003 (2003 till present).

and that the invasive cell lines had a well-organised filamentous actin structure. Along with Fernanda Castro (Peter Stern's Immunology Group), she has also shown that subpopulations of leukaemic blast cells carry proteomic signatures similar to that identified in invasive cell lines. Using a NOD-SCID model Fernanda and Mark were then able to show that while non-invasive cell lines produced leukaemia in mice, the invasive cell lines led not only to leukaemia but the establishment of CNS disease, which was partially inhibited by a RAC inhibitor. Overall the work suggests a model by which cells migrate to extramedullary sites, adhere to the host cells then are able to invade across the blood and CSF brain barriers into the central nervous system.

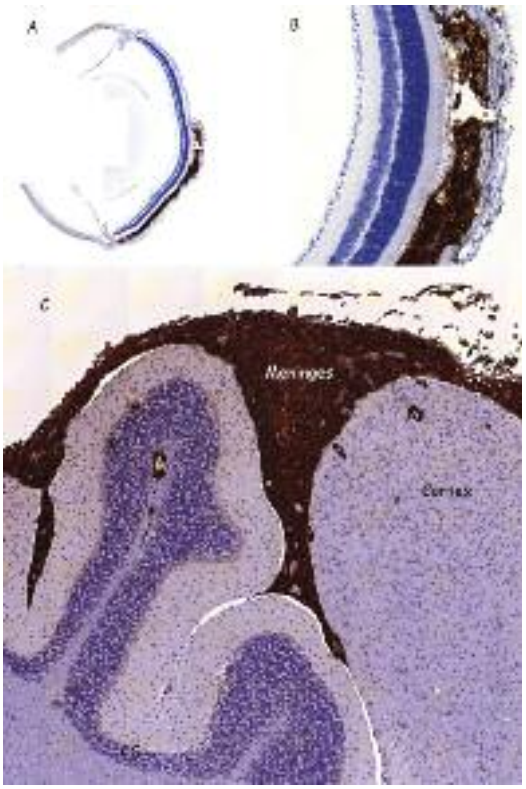


Figure 2
A murine model of CNS disease in childhood ALL. Histological sections through the eye (A and B) and brain (C) of mouse with CNS leukaemia after engraftment with an invasive cell line. Human cells are GFP positive and stain brown. (A) Shows infiltration into the choroid with no evidence of disease within the vitreous or retina. (B) A close up of A. Disease lies within the layers of the choroid and could only have tracked along the optic nerve. (C) Shows heavy infiltration in the meninges and presence of disease within vessels supplying the cortex.

Observations by others and our own experiments suggest that the ability to overcome the chemotherapy is not innate to all lymphoblasts, but that stromal and mesenchymal cells, which are relatively chemoresistant, are also able to protect leukaemic cells. Using the 3D *in vitro* system we described last year, Jizhong Liu has now demonstrated that the mesenchymal cells produce soluble factor(s) that are able to protect leukaemic cells, not in physical contact with the stroma. It is highly likely that the soluble factor(s) induce this by signalling through receptors identified in our previous proteomic analysis. Such signalling could well be processed by the increased endosomal/lysosomal trafficking we have observed in lymphoblasts. John Bridgeman is developing techniques for the subfractionation and proteomic analyses of these subcellular organelles. The poor risk CALM-AF10 cytogenetic sub type of childhood ALL disrupts clathrin mediated endocytosis. Clare Dempsey along with Eva Diffner have introduced variations of this fusion gene into lentiviral vectors to create stable cell lines. They are now in the process of transducing early haematopoietic cell progenitors to study the changes in behaviour in both *in vitro* and *in vivo* experiments.

Overall as a translational research group, we continue to conduct hypothesis directed researched based on observations made on patients undergoing clinical trials. In 2009 we bid farewell to Naina Patel who came up with the group from London and was principally responsible for re-establishing the laboratory from scratch and supervising its subsequent expansion. We wish her well in her new job at University College London. We are pleased to report that both Hany Ariffin and David Samuel successfully defended their doctoral dissertations in 2009.

Publications listed on page 71

Targeted Therapy Group

Group Leader
Tim Illidge

Preclinical Group
Postdoctoral Fellows
Jamie Honeychurch
Ellie Cheadle

Academic Clinical Fellow
Nick Brown

Graduate Students
Waleed Alduaij
Monique Melis

Senior Lecturer
Yong Du

Clinical Radioimmunotherapy Group
Senior Clinical Scientist
Maureen Zivanovic

Clinical Scientist (ECMC funded)
Jill Tipping

Research Nurses
Susan Neeson
Caroline Hamer

The past year has been a highly successful one for the Targeted Therapy Group. The highlights have included a successful quinquennial review of the CR-UK programme grant, the award of a Leukaemia Research Fund project grant, high impact factor publications, the appointment of two new post-doctoral research fellows namely Drs Ellie Cheadle and Simon Dovedi (arriving in January 2010) and the development of CR-UK/AZ clinical training fellowships in conjunction with Professor Stratford (School of Pharmacy).

The overarching goal of the Targeted Therapy Group is to define the optimal way to combine radiotherapy (RT) with immunotherapy in the treatment of cancer by enhancing our understanding of the underlying mechanisms of action. The specific objectives of the group are i) to investigate the mechanisms of action of radioimmunotherapy ii) to investigate how the recognition of RT-induced tumour cell death by different antigen presenting cells in the tumour microenvironment can impact on the ensuing immune response; iii) to investigate the role of myeloid derived suppressor cells (MDSC) in tumour regrowth after RT and to develop strategies to enhance RT tumour control by modifying host immune response after RT and iii) to translate our experimental research findings into developing early phase clinical trials. Aspects of progress made with these research projects are outlined below.

Mechanisms of action of radioimmunotherapy

Our recent work, in collaboration with Dr Mark Cragg's group in Southampton, has investigated a new form of mAb induced cell death in B-cell leukaemias and lymphomas. Using both lymphoma cell lines and primary chronic lymphocytic leukaemia (CLL) cells, we have demonstrated for the first time the importance of lysosome-mediated cell death for antibody therapy elicited using clinically relevant mAb directed against two different target antigens namely CD20 and HLA DR. We have determined that death is preceded by homotypic adhesion with both adhesion and death being dependent upon actin redistribution (Figure 1).

The ability of functionally different mAb targeted to different antigens namely, CD20 and HLA DR antigens suggests that the phenomena observed may be a more general mechanism of lymphoma cell killing by activating antibodies. The latter is potentially of great interest as it provides a potential means to bypass the often dysregulated apoptotic death pathways of tumour cells allowing for effective tumour cell killing in the presence of apoptotic inhibition. Interestingly both of the mAbs studied evoke lysosomal non-apoptotic cell death pathway and this is likely to go some way to explain their efficacy *in vivo*. This work was recently published in the Journal of Clinical Investigation. (Ivanov *et al.,* 2009). We have built on this success recently and Waleed Alduaij in collaboration with Andrei Ivanov and Mark Cragg's group has focused on the new-generation humanised anti-CD20 Monoclonal Antibody (GA101). GA101 has been shown to initiate large amounts of this non-apoptotic mode of cell death in B-lymphoma cell lines in contrast to rituximab. Inhibitors of actin polymerization (latrunculin B and cytochalasin D) inhibited cell death elicited by GA101. The role of lysosomal activity in GA101-induced programmed cell death (PCD) was assessed using the inhibitor of the lysosomal protease, cathepsin B, which significantly inhibited cell death induced by GA101 (Figure 2). To confirm that this mode of death is non-apoptotic, we

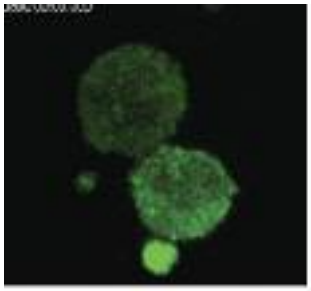


Figure 1
Actin relocalization and cell death following treatment with GA101

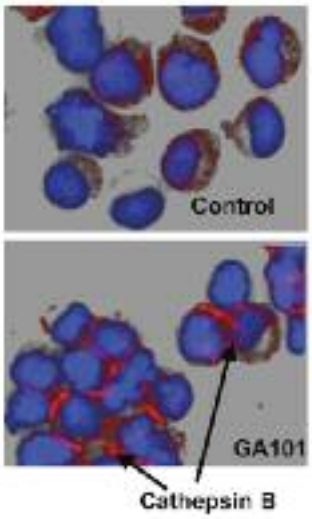


Figure 2
GA101 induced-PCD is dependent on lysosomes

demonstrated that GA101-induced PCD was independent of the antiapoptotic BCL-2 oncoprotein and caspases. Taken together, these findings demonstrate that GA101 is the first humanized anti-CD20 mAb with Type II properties, potently eliciting a novel mode of cell death in B-cell malignancies, which potentially can lead to improved B-cell depletion over rituximab. We are currently investigating this *in vivo* using human CD20 transgenic mice and this work will be presented as an oral presentation at the American Society of Hematologists 2009.

Immune response to RT-induced dying tumour cells

Our recent work in this area has focused on understanding the nature of the host immune response to RT-induced tumour cell death. We have focused our attentions on two types of Antigen Presenting Cells (APC), namely macrophages (MΦ) and Dendritic cells (DC). This work, carried out by Jamie Honeychurch, has demonstrated that by manipulating MΦ within the tumour microenvironment protective anti-tumour CD8 T-cell responses with anti-CD40 against irradiated lymphoma cells can be induced. In these studies the potential importance of MΦ in cellular vaccination has been demonstrated. Depletion of MΦ using clodronate-encapsulated liposomes has been shown to considerably enhance primary vaccination efficacy in the presence of adjuvant anti-CD40 mAb. Our results demonstrate that in order to induce a protective immune response, additional host immune stimulation is required and that depletion of MΦ populations can improve tumour cellular vaccination strategies.

Monique Melis in collaboration with Kathryn Simpson (Clinical Experimental Pharmacology) has developed a Doxycycline-regulated Caspase-3 death switch in a number of tumour models. These models will enable us to titrate the proportion of 'death switch' cells and define the levels of apoptotic cell death required in tumour to provoke immune responses as well as assessing the T-cell responses to apoptotic cell death and how this changes with depletion of selective APC.

Clinical translational applications of the laboratory research programme

There have been a number of major successes in translational research that have resulted directly from, or are closely related to this CR-UK laboratory programme of work.

A serum rituximab ELISA assay has now been validated to GCLP in collaboration with the Clinical and Experimental Pharmacology Group (CEP), through a CR-UK TRICC grant (in collaboration with Professor Dive) to fund Grace Hampson. A robust, reliable and reproducible ELISA which can accurately establish serum rituximab concentrations accurately has been established as a national reference laboratory resource (Hampson *et al.*, – submitted). Work is ongoing analyzing serum rituximab levels from samples collected as part of the NCRI Phase II SCHRIFT study.

Early phase clinical trials of radioimmunotherapy (RIT)

The clinical RIT group has made considerable progress over the last few years in leading early phase clinical trial design both nationally and internationally, and has a substantial portfolio of early phase clinical trials. A highlight of the year was the publication of the Phase I/II dose escalation RIT study using ^{131}-rituximab. This study was the first to investigate the effect that induction therapy (4 weekly infusions of 375 mg/m^2 rituximab) and the subsequent efficacy and toxicity of anti-CD20 RIT in relapsed indolent B cell Lymphoma (Illidge *et al.*, 2009). Induction therapy with rituximab was found to significantly increase the effective half-life of ^{131}I-rituximab. An important observation from this work was that induction therapy with multiple doses of rituximab did not compromise the clinical efficacy or increase toxicity of subsequent ^{131}I-rituximab RIT. The overall response rate (ORR) was 94%, with a complete response (CR) rate 50%. The median time to progression was 20 months, significantly longer than for the last qualifying chemotherapy with ongoing durable remission of more than 60 months.

The phase II trial of Fractionated Zevalin (FIZZ study) is the first study to be performed using two fractions of RIT in previously untreated follicular lymphoma. It is a multicentre investigator-led study using fractionated RIT and is progressing well and 54 of the 70 patients required have been recruited. A further novel investigator-led trial uses abbreviated chemotherapy followed by RIT in relapsed follicular lymphoma. The SCHRIFT study (**S**hort **CH**emotherapy **R**adioimmunotherapy **I**n **F**ollicular lymphoma **T**rial) is a NCRI lymphoma group study and has recruited 32 of the 60 required patients with widespread interest across the UK. The SCHRIFT study is the first RIT study ever to be conducted across the UK making RIT widely available to more patients than has ever been previously possible within the UK. Translational science associated with all of these trials include the measurement of rituximab pharmacokinetics, Human Anti-Mouse Antibody (HAMA) responses and circulating biomarkers of cell death nucleosomal DNA (nDNA) in collaboration with Professor Dive's CEP group.

Publications listed on page 71

Translational Radiobiology Group

Group Leader
Catharine M.L. West

Postdoctoral Fellows
John Hall (joint with Applied Computational Biology & Bioinformatics)
Carla Möller Levet (joint with Applied Computational Biology & Bioinformatics; to September 2009)
Fabian Zanella (from August 2009)

Clinical Research Fellows
Guy Betts
Ahmad Mirza (from February 2009)

Scientific Officers
Joely Irlam-Jones
Helen Valentine

Graduate Student
Stephanie Donaldson (joint with Imaging Science)

Scientific Administrator
Rebecca Elliott

The expansion of knowledge through sequencing the human genome has been accompanied by the growth of high throughput technologies for genome-wide analyses. These developments increase the possibility of future personalised therapy based on molecular profiling. The Translational Radiobiology Group aims to exploit high throughput technologies to develop molecular profiles that predict the response of cancer patients to radiation therapy.

Tumour radiosensitivity

Some tumours respond well to radiotherapy, whereas others do not. The underlying biology that account for differences in response to radiotherapy is poorly understood. There is evidence that intrinsic sensitivity to radiation, hypoxia and proliferation are important. Work carried out by the group several years ago showed measurements of tumour radiosensitivity determined as the surviving fraction of cells after 2 Gy of irradiation (SF2) in an *in vitro* clonogenic assay was an independent prognostic factor for the outcome of radiotherapy. However, the clonogenic assay took four weeks to perform and was technically demanding, precluding its use as a clinical test. John Hall is carrying out a project exploring the potential of using Affymetrix Human Exon 1.0 arrays in conjunction with archival specimens (aged 15-23 years) to derive a molecular profile associated with tumour radiosensitivity (SF2). In collaboration with Carla Möller-Levet, a tumour radiosensitivity associated gene expression signature was generated using 49 formalin-fixed, paraffin-embedded (FFPE) cervix tumours for which SF2 data were available. Differential expression analysis compared 11 samples with high and 11 with low SF2 values. A signature was derived comprising 1073 probesets targeting 1025 genes (false discovery rate, FDR 0.1; figure A).

One of the genes predicted to be linked with radiosensitive tumours from the array analysis ($p=0.001$, FDR-adj.) was verified at the protein level by immunohistochemistry in 74 cervix tumours (Helen Valentine). High protein expression of the gene was associated with low SF2 ($p=0.0038$) and a good outcome following radiotherapy ($p=0.024$). This work demonstrates that RNA extracted from archival FFPE samples (up to 23 years old) can be used to accurately identify genes which are differentially expressed at the protein level. These initial findings endorse the methods used and allay fears concerning sample degradation. Continuing work is focussing on validating the gene signature and investigating the role candidate genes play in determining the intrinsic radiosensitivity of cervix tumours.

Tumour hypoxia

In collaboration with Prof Adrian Harris and Dr Francesca Buffa in Oxford and the Applied Computational Biology and Bioinformatics (ACBB) Group, a tumour hypoxia-associated gene signature was derived. Last year the signature was streamlined and reduced from 99 to 26 genes, and an MRC grant obtained to validate and qualify the multiplex 26-gene hypoxia biomarker. Guy Betts liaised with Applied Biosystems to produce customised 384-well TaqMan Low Density Array (TLDA) cards for a quantitative real-time PCR (qRT-PCR) application. An endogenous control (18S ribosomal RNA) and five reference genes are included with the signature so that four samples can be run in triplicate per array. One of the first experiments carried out by Guy Betts and Fabian Zanella showed an excellent correlation between TLDA and Affymetrix HGU133 Plus 2 array data ($r=0.91$; figure B) in a series of human head and neck squamous cell carcinomas (HNSCCs). An achievement for 2009 was

setting up Good Clinical Laboratory Practice (GCLP) methods to take the work forward (Joely Irlam-Jones) and starting RNA extraction from FFPE blocks from the BCON bladder cancer trial that randomised patients to receive radiotherapy alone or with hypoxia modification.

Work by the group deriving gene signatures to predict the response of cancer patients to radiotherapy is also increasing knowledge of the biological processes that underlie variation in radioresponse in cancer patients. Alternative splicing is an increasingly recognised method for obtaining condition-specific differential protein expression. Although several hypoxia-associated genes are known to be alternatively spliced (e.g. *CA9, VEGF, SLC2A1*), no genes are known to be alternatively spliced in response to hypoxia. Again in collaboration with the ACCB group, 10 of the 59 HNSCC samples arrayed previously using Affymetrix HGU133 Plus 2 arrays were interrogated using exon arrays. Five samples had high and five low hypoxia scores as determined by the median RNA expression of 99 hypoxia-associated genes. Analysis by Carla Möller-Levet identified individual gene transcripts (exons) likely to be differentially expressed in response to hypoxia and Guy Betts validated the potential alternatively spliced transcripts using qRT-PCR. Using a high stringency level for detecting alternative splicing associated with hypoxia, four genes were identified: *LAMA3, WDR66, SLCO1B3* and *COL4A6*. Of the four genes, *LAMA3* (laminin α-3) was selected for validation due to the availability of discriminatory, reliable RT-PCR primers. The Exon 1.0 array findings for *LAMA3* were confirmed using qRT-PCR. *SLC2A1* (Glut-1) was used as a control as there was no evidence for hypoxia-associated alternative splicing from the Exon 1.0 array analysis but it is known to be highly induced under hypoxia. *SLC2A1* served not only as a positive control detecting hypoxic transcriptional activation, but also as a negative control showing no differential transcript expression between two primers targeting different transcripts of *SLC2A1*. Subsequent interrogation of the Affymetrix HGU133 Plus 2 microarray data (n=59) showed specific microarray probes for both transcripts of LAMA3 (LAMA3-A and LAMA3-B). Differences in individual probe expression within one gene would not have been identified using conventional microarray analysis. Outcome analysis, based on different transcript expression, was performed on the series of 59 HNSCCs. LAMA3-B had no prognostic significance (figure C) but expression of LAMA3-A was prognostic (figure D) for overall survival. The work shows that splice variants may be more phenotype specific than generic gene assays and are thus an important consideration in biomarker development.

Normal tissue radiosensitivity

The Translational Radiobiology group (Rebecca Elliott) co-ordinates the UK RAPPER study (**R**adiogenomics: **A**ssessment of **P**olymorphisms for **P**redicting the **E**ffects of **R**adiotherapy). The multi-centre study has collected over 2,600 blood samples from radiotherapy patients and the first genotyping work was carried out 2009. Collaborators in Cambridge (Drs Gill Barnett and Alison Dunning) looked at polymorphisms in the *TGFB1* gene in relation to toxicity in 778 breast cancer patients included in the RAPPER study. The large study failed to confirm previously reported associations in smaller studies, which highlights the need to analyse sufficient sample sizes to have rigorous statistical power. Highlights during 2009 were securing funding to carry out a genome wide association study on the RAPPER samples and the group leading the organisation of a meeting in Manchester that established an international Radiogenomics Consortium.

Publications listed on page 72

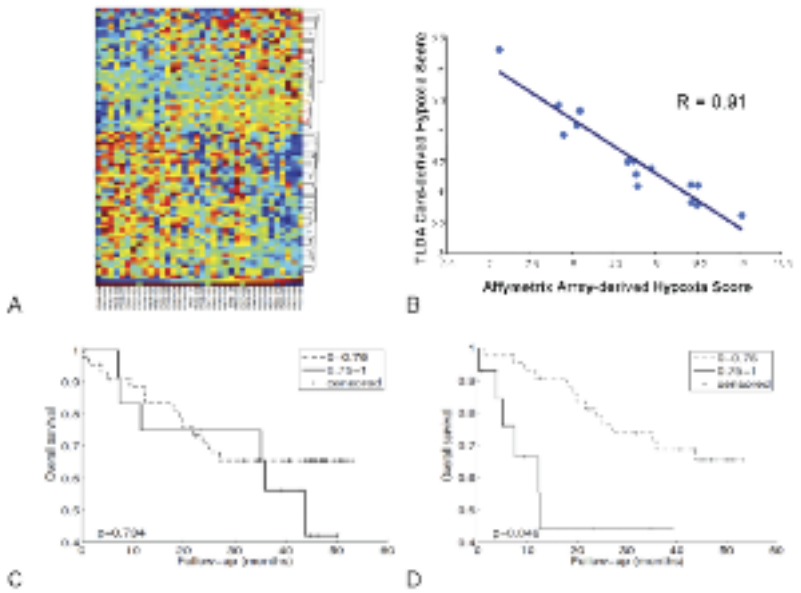


Figure A
Derivation of a gene signature associated with tumour radiosensitivity.

Figure B
Validation of a 26-gene hypoxia biomarker: relationship between TLDA and Affymetrix array data in HNSCC.

Figure C,D
Prognostic significance of LAMA3-B (C) and LAMA3-A (D) in 59 HNSCC. Patients were stratified by highest quartile of RNA expression (0.75-1.00) versus lowest three quartiles (0-0.75).

Medical Oncology: Cell Therapy Group

Group Leader
Robert Hawkins

Senior Research Fellow
David Gilham

Postdoctoral Fellows
Vivien Hanson
John Bridgeman (until October)
Eleanor Cheadle (until October)
Jennifer Loconto (until October)

Scientific Officers
Vicky Sheard
Marzieh Kamjoo (from November)
Allison Robinson (until October)

Graduate Students
Grazyna Lipowska-Bhalla
Erik Alcantar Orozco
Mariam Al-Muftah (with Immunology)
Anissa Cucchi (visiting student)

ATTACK Project Officer
Nikki Hudson

Tumours actively avoid recognition by the immune system. This avoidance takes many forms but includes the loss of key immune cell recognition proteins on the tumour cell surface effectively making the cancer invisible to T-cells, a critical part of the immune system. We have been working on methods to modify T-cells so that they can recognise and then specifically target tumours.

Chimeric Antigen Receptors

Our central approach has been the expression of a chimeric antigen receptor (CAR) on T-cells which uses antibody-based technology to target tumour cells directly thereby circumventing some of the immune avoidance mechanisms employed by the tumour. The CAR also consists of a T-cell signalling domain which, as a result of binding of the antibody to tumour, results in activation of the T-cell and subsequent immune mediated destruction of the target tumour cell. Whilst the approach is feasible and now being tested in clinical trials in Manchester (see BIGT report), there are significant improvements that are required in order to increase the therapeutic potential of this approach. Delivering these improvements is the focus of the research activity of the Cell Therapy group.

Improving the expression of CAR's in T-cells: Gene-transfer and vector engineering.

For the CAR T-cell approach to succeed, the genetic material encoding the receptor needs to be efficiently introduced and strongly expressed in primary T-cells. The most commonly used approach involves retroviral gene transfer. However, to achieve high level genetic modification, T-cells need to be activated – a process that may reduce the ability of the T-cell to optimally function within the patient. Lentiviral vectors can transduce non-dividing cells; however, these vectors are less efficient in T-cells. During the past year, we have optimised methods that permit lentiviral vectors to transduce primary mouse T-cells (Gilham *et al.*, J Gene Med, *in press*) thereby allowing us to fully explore the benefits of this vector in model systems. As an alternative approach, we are also testing non-viral nucleofection systems for their ability to modify primary T-cells. Our preliminary studies indicate that the approach is feasible and T-cells expressing CAR's can be generated using this approach.

In order to select the gene-modified T-cells, we have employed multi-gene expression systems. To this end, a selection marker (e.g. the truncated CD34 protein) and the CAR are co-expressed from a single vector by means of bi-cistronic expression motifs. We have tested the effect that one such motif (the Foot and Mouth Disease virus 2A proteocleavage sequence) and identified that the orientation of the transgenes relative to the 2A sequence may be important and especially so with respect to secreted proteins (Rothwell *et al.*, submitted to Hum Gene Ther). Further optimisation of the 2A and CAR sequence has now generated vectors that drive higher levels of protein expression and these are now being tested in primary T-cells to determine whether this enhanced expression correlates with improved tumour killing.

Identification of CAR – host protein interactions on the T-cell surface: implications for optimal CAR function.

In last years report, we documented our preliminary observations showing that the CAR associates with the host T-cell receptor and that this interaction was important for the optimal function for CAR's bearing a CD3ζ signalling domain. These observations have been strengthened during this past year (Bridgeman *et al.*, J Immunol, *under revision*) with the technical development of a novel bead-based flow cytometry immuno-precipitation method (Bridgeman, Blaylock *et al.*, Cytometry part A, *in press*; the result of a collaborative effort with

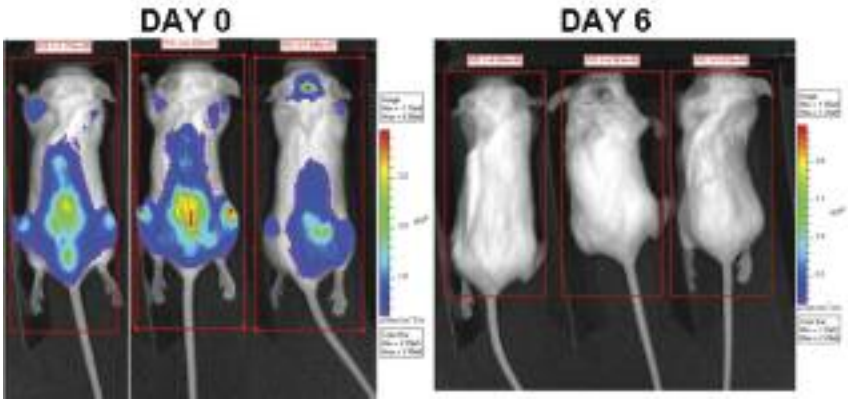


Figure 1
In vivo *imaging of mice bearing systemic A20 B-cell lymphoma labelled with a luciferase marker gene (day 0) were treated with a single of CD19z specific T-cells. Six days later, imaging confirmed that the B-cell lymphoma had been eradicated due to targeted T-cell activity.*

Morgan Blaylock) being critical to proving the biochemical interaction of CAR and T-cell receptor.

Mouse models of CAR T-cell function: the role of the autologous antigen.

In order to understand how CAR T-cells function and to prove that the modifications we introduce result in an increased anti-tumour potency, mouse models are critical. We have previously shown that human T-cells can challenge the growth of B-cell lymphoma in immuno-compromised models when combined with chemotherapy. Furthermore, mouse T-cells bearing a CD19 specific CAR can eradicate a 13 day established mouse B-cell lymphoma expressing human CD19 (Cheadle *et al*., 2009).

However, these systems do not accurately model the natural situation where the tumour antigen may be expressed on normal healthy tissues. We have generated a mouse CD19 specific CAR and used this to target mouse cell lymphoma in the immuno-compromised mouse. In this system, the tumour antigen is naturally expressed at high level on host B-cells. As such, this model questions whether the CAR T-cells can target tumour cells while examining any potential side-effects on normal cells. Similar to our previous studies, these T-cells can efficiently reject long-term (13 day) established syngeneic lymphoma; however, the CAR T-cells persist within the animal's peripheral circulation for a period of 40 – 50 days after which they disappear. During this time, the number of circulating B-cells remains depressed but returns to normal levels shortly after the CAR T-cells disappear (Cheadle *et al*., J Immunol, *in press*, Figure 1). Taken together, these observations suggest that the CAR T-cells are eliminating host B-cells and finally become exhausted in the face of the continuous re-population of B-cells by the host and are themselves eliminated. In this model, there is no apparent toxicity associated with this transient absence of B-cells. Questions remain concerning the potential impact of CAR T-cells that possess more powerful signalling domains and whether targeting of tumour antigens that are expressed on 'more sensitive' organs by CAR T-cells results in more severe toxicities remains an important area to investigate. As such, the development of these autologous model systems is important and a major focus of the group's work.

Malignant Melanoma: pre-clinical development of cell therapy.

Following the lead of workers in the USA (Dr S Rosenberg), we are looking to develop cell therapy protocols to treat malignant melanoma in Manchester. Our initial work focused upon isolating tumour infiltrating lymphocytes (TIL) for use in adoptive cell therapy and this work is now moving on to clinical development with proposals to test the approach both at a small scale local level and also to test TIL therapy in multi-centre EU phase III trials as the major impetus of a major EU grant initiative lead by our group. We are also working on a gene therapy approach using a T-cell receptor provided by Dr Rosenberg to re-direct T-cells to melanoma tumours.

The 'ATTACK' consortium

The ATTACK consortium is an EU FP6 funded integrated project (lead by Professor Hawkins) involving 16 laboratories focusing upon the pre-clinical optimisation of gene-modified T-cell therapy. The second ATTACK-organised 'Cellular Therapy of Cancer' symposium was held in Milan, Italy this year and attended by over 150 delegates from the EU, USA and Australia. A report summarised the meeting has been published (Bridgeman *et al*., 2009); however, a critical output of the meeting was a clinically focused discussion meeting where it was agreed that multi-centre clinical trials of cell therapy were clearly needed in order to test the ability to deliver and the potency of cell therapies in the wider context than that currently achieved within small-scale phase I clinical trials. All participants agreed that this was an important focus for the community to work towards delivering in the near future.

Publications listed on page 72

Medical Oncology: Translational Anti-Angiogenesis Group

Group Leader
Gordon Jayson

Senior Fellow
Egle Avizienyte

Postdoctoral fellows
Alison Backen
Claire Cole
Steen Hansen
Gavin Miller

Clinical Research Fellows
Ying Kiat Zee
Gireesh Kumaran
Claire Mitchell
Nish Murukesh

Scientific Officers
Graham Rushton
Karen Brookes

Heparan sulfate (HS) is essential for the biological activity of the majority of angiogenic cytokines. Using novel synthetic chemistry we have generated a range of oligosaccharides that have allowed us to determine the structures that inhibit the activity of several angiogenic growth factors. One issue that is critical to the development of these molecules is the elucidation of proof of mechanism biomarkers and to that end we are developing novel assays of HS and HS proteoglycans, which will complement our existing portfolio of serological and imaging biomarkers that have been implemented in the clinic.

Heparan sulfate and angiogenesis

Heparan sulfate (HS) is a linear glycosaminoglycan that is essential for the biological activity of most angiogenic cytokines. Previously we demonstrated that heparin oligosaccharides inhibited angiogenesis *in vivo* and therefore undertook an organic chemistry programme to synthesise HS oligosaccharides to determine structure-activity relationships. We have generated a range of HS oligosaccharides containing up to 12 saccharide residues and have demonstrated that specific sulfation patterns are associated with a broader range of activity against several angiogenic cytokines that have been implicated in ovarian cancer (see figure). These molecules demonstrate the capacity to inhibit cytokine-induced endothelial migration, proliferation and tube formation and further *in vivo* evaluation is planned.

FGF and ovarian cancer

We previously demonstrated that human epithelial ovarian cancer (EOC) endothelium expresses heparan sulfate that can activate FGF2. We therefore undertook a comprehensive evaluation of FGFs in EOC and have identified a receptor isotype switch where transformation is associated with the expression of FGFR2 IIIb; an isoform that confers on EOC cells the ability to respond to FGFs 3, 7 and 10. Further *in vitro* and subsequently *in vivo* evaluation demonstrated that RNAi-mediated knock down of FGFR2 IIIb in human EOC xenografts impaired tumour growth and significantly enhanced the sensitivity of the xenografts to platinum chemotherapy through enhanced apoptosis and reductions in the number of cells entering the cell cycle. These data identify FGFR2IIIb and its ligands as new targets for the treatment of EOC. However, in preliminary studies we have demonstrated that inhibition of other FGF receptors can have the converse effect implying that caution should be exhibited when introducing broad spectrum FGF receptor inhibitors into the clinic.

Imaging biomarkers

As the above programme moves towards the clinic it is critical that we develop biomarkers that can detect proof of mechanism and principle in phase I evaluation. In a longstanding programme that aims to identify imaging biomarkers that could be used to evaluate anti-angiogenic agents we have demonstrated in several different clinical trials and clinical settings that the vascular enhancing fraction is of prognostic significance. We therefore sought to determine whether anti-angiogenic agents impact on enhancing fraction and have demonstrated that bevacizumab does reduce enhancing fraction and subsequently that cediranib maintains a reduction in enhancing fraction in patients who continue to benefit from VEGF inhibitors.

We have reported the potential of oxygen-enhanced imaging as a novel biomarker for the function of the vasculature in tumours allowing

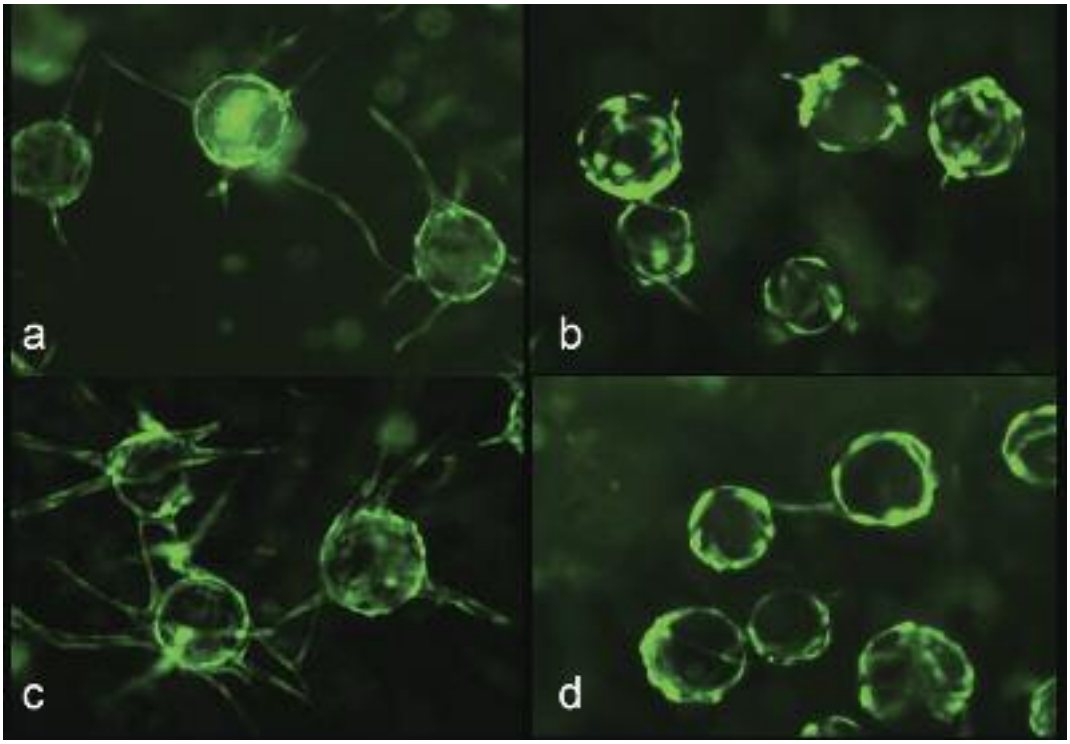


Figure 1
HUVEC coated beads were embedded in fibrin gels and treated with either FGF2 (a: 10 ng/ml) or VEGF (c: 20 ng/ml) in the absence (a, c) or presence of defined oligosaccharides (b: FGF2 + oligosaccharide 50μg/ml. d: VEGF + oligosaccharide 50μg/ml). Tubules were visualised by staining with Calcein AM.

us to build on our recently published time course trial of the impact of bevacizumab on several imaging parameters in patients with colorectal cancer liver metastases. These data led to the construction of the trial described below where we will test the predictive value of imaging parameters in patients with metastatic colon cancer.

Having evaluated a series of dynamic contrast enhanced magnetic resonance imaging parameters over the last few years we have demonstrated that the findings from this methodology compare well with those derived from dynamic contrast-enhanced computed tomography and, for the first time, evaluated the relationship between tumour growth and dynamic imaging in patients with residual ovarian cancer following initial treatment. This study was complemented by simultaneous evaluation of serological biomarkers that revealed novel relationships between imaging and circulating parameters. The combination of imaging and circulating parameters demonstrated that at very low levels of vp (an index of tumour perfusion), higher concentrations of VEGFR1 and 2 were found in plasma suggesting that hypoxia was driving this relationship.

Serological biomarkers

In collaboration with colleagues at the University of Leeds (Banks, Hall) and the MRC Clinical Trials Unit we have completed the first academic international sample collection within the context of a randomised trial (ICON7). The trial examines the potential value of bevacizumab in patients with EOC receiving platinum based chemotherapy. The concentration of a number of angiogenesis-related cytokines in the samples will be determined towards the end of this year. Additional international studies will be facilitated through our role as the lead site for translational research for ICON7.

The combination of imaging and serological biomarkers is a powerful means of biologically evaluating anti-angiogenic agents. We have therefore initiated a trial in colorectal cancer where we are combining serological and imaging biomarkers to determine which patients with metastatic colorectal cancer benefit from bevacizumab. The trial, which will run over the next two years, will bring state of the art biomarker studies to address this question and will incorporate novel statistical analyses to identify the critical suite of biomarkers.

Publications listed on page 73

Research Services

http://www.paterson.man.ac.uk/research/

Head of Research Services
Jenny Varley

The quality of our Research Services was rightly highlighted for praise during the Institute Site Visit in 2009 and the enthusiasm and knowledge of all the service unit heads was acknowledged. The Research Services underpin the investigations of all groups in the Paterson, and we continue to invest in both the staff and equipment to ensure the services are properly resourced.

Advanced Imaging Facility
Head: Steve Bagley

The Advanced Imaging Facility provides access to state-of-the-art imaging tools, analysis of images, training researchers and consultancy in experimental design, all of which is undertaken with an array of microscopes and computational tools for both imaging live cellular processes and tissues. To achieve this staff within the facility develop and integrate a range of techniques for detecting cellular and molecular interactions, to improve the spatial and temporal resolution of the microscope and to progress environmental control so that cells remain in a physiologically viable state whilst imaging.

One of the major advances this year has been to investigate and refine how the cells under study are presented to the microscope. There is a requirement for changing the aqueous environment around the cells which is essential when setting up drug screening investigations or when considering how the cells respond to changes in the surrounding environment, for example when they undergo a stress response. Conventionally, to image biological activity over time, cells in media are imaged between two glass coverslips, on a multi-well plate, Petri dish or culture flask. The coverslip/cell chamber method, which is by far the most accurate method of both imaging and environmental control, has a major flaw in that if a liquid such as cell media is introduced over time, a small change in pressure occurs which leads to one of the coverslips acting as a diaphragm. This axial movement of the coverslip leads to the object under study going in and out of focus which in turn requires the biological sample to be sampled more frequently. As biology occurs in four dimensions, in 3D space over time, cell chamber systems are not ideal as they are not amenable to allowing the researcher to alter the environment around the cell whilst maintaining constant focus of the protein or cell structure under study. Over this year, micro-fluidic systems have been examined as a viable technique for the researcher to change the liquid phase around cells for tasks such as cell maintenance and drug screening. Both the cell media and treatment can be altered around the cell in a controlled manner whilst maintaining a fixed point of focus. These techniques are in the process of being introduced into the laboratory for both short and long term time lapse and are suitable for quantifying changes in cell content and response in both mammalian cell culture and model cell systems.

The Förster Resonance Energy Transfer (FRET) technique allows the researcher to investigate molecular interactions at the level of protein/protein interaction (4-6nm resolution).

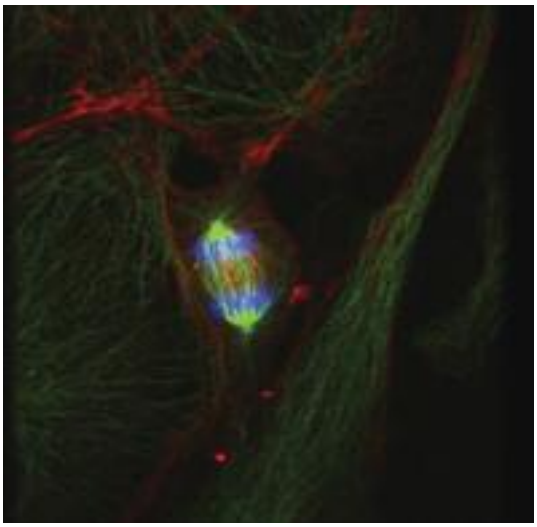


Figure 1
Mitotic BPAE cells in anaphase. F-actin is labelled with Texas Red-x phalloidin. Microtubules, in green, are labelled with mouse anti-α-tubulin BODIPY FL goat anti-mouse IgG. Blue nuclear staining with DAPI. Imaged on the Spinning Disk Confocal microscope.

Currently in the laboratory, filters are utilised to record three waveforms and then a calculation is made of the level of interaction between two proteins. An examination of spectral unmixing techniques for the analysis of FRET has been undertaken; a spectrophotometer attached to a microscope records across a field of view, not just for intensity as with camera based systems, but also for wavelength (from 500-1000nm), and then by gating the resultant wavelengths a level of the amount of interaction between two proteins can be assessed. Over the coming year this will be developed further to allow standardisation of the technique and development of computation analysis. The spectrophotometer is also be utilised for quantum dot multiplexing and for spectrally unmixing fluorescent proteins which will allow for the visualisation of up to five molecular interactions.

Over the last year several approaches have been made to improve both the image quality and the standardisation of the equipment. With the appointment of a new member of staff it is now possible for all of the equipment to be monitored on a weekly basis for defects in the way the equipment is set up that would lead to changes in photo-sensitivity, faithfulness of the imaging method and highlights any problems that could arise in the future. Additional checks are also made to the quality of data being produced by histological imaging. As many of the techniques utilised as standard are becoming more complex and multifaceted, support and advice at the microscope is now easier to obtain.

The facility is working closer than ever with the IT department, consequently storage and archive are now maintained and managed in a more suitable fashion. In 2009 15-17 Terabytes of raw data were generated. Analysis of images (volumes of data over time) is becoming more prevalent and at the end of the year a separate computer room has been provided with 64-bit workstations running a variety of software tools to achieve visualisation and numerical analysis. Due to the amount of data being generated there are problems that ensue when trying to locate data and to marry both the image with the information about how the cells were treated in the laboratory, consequently image database techniques have been examined as a technique for the archival of raw data and laboratory method. Over the coming there will be a trial of the software in the laboratory environment.

The advances in techniques, equipment and training have allowed more scientists to use the microscopes as a tool in their research. Over the last year forty five scientists have been trained to use the microscopes and to analyse their results in an appropriate manner. Ten research papers have been published by members of the Institute where equipment from the facility has been integral part of the study which cover diverse subjects such as defects in cell division, proliferation, differentiation, haematopoiesis, hypoxia, drug resistance, cell adhesion and cell migration.

During 2009, 150 members of the public been shown the imaging techniques used in the laboratory. Over two days, school leavers and sixth form students have used the microscopes to investigate cell division and had hands on experience in the analysis of histological images.

Over the coming year priority will be given to refinement of the FRET technique, introduction of micro-fluidics as a routine practice, controlling lasers for inducing DNA damage in live cells to study the DNA repair and stress responses, and developing analysis software.

Biological Resources Unit

The Paterson animal facility is now running at full capacity with all 3000-plus cages in use, and we have seen a steady increase in both the transgenic and experimental areas which supports 13 project licence holders and 65 personal licence holders.

Transgenic production remains at the forefront with approximate 140 lines in active breeding programmes. To ensure that stocks are available for experimental use, transgenic mice are produced under a centralised service licence which provides strict control with respect to minimum wastage, batch production and optimal animal husbandry.

The facility is regulated by the Home Office in strict accordance with the Animal Scientific Procedures Act 1986 and locally the Ethical Review Process is managed through a committee which reviews new applications, amendments and grant applications.

Transgenic Services

During 2009 we have been developing techniques for *in vitro* fertilisation (IVF) using fresh sperm. We are achieving a 50-60% success rate which is comparable with the Jackson Laboratories. We have also continued to optimise the IVF technique using cryopreserved sperm – a technique which can be problematic. When using cryopreserved sperm the thaw rate of the straws is critical as is the use of the additive methyl-β-cyclodextrin (MBCD) in the human tubule fluid (HTF) media. MBCD is used to sequester cholesterol from the sperm

membrane which aids capacitation before the addition of the oocytes to the IVF culture dish. Chemical thinning of the oocyte zona pellucida and selection of motile sperm is also employed to increase fertilisation rates. Through refinement of these techniques our cryopreserved sperm fertilisation rates have achieved rates as high as 40% which is comparable to other transgenic facilities within the UK.

The microinjection service has carried out over 25 Embryonic Stem cell injections and associated techniques such as critically timed superovulation regimes in female mice, and the production of sterile male mice. To date eleven genetically altered mouse strains have been produced, with some others still in the pipeline.

Resource sharing of genetically altered animals has resulted in a number of lines being transferred and received to facilitate the research both within the UK and worldwide. Sharing strains in this way ensures the application of the 3R's and as such the IVF technique will lead to a major refinement, reduction and replacement for archiving of strains.

The rederivation process of new strains at the Paterson has included the following

- MC4R and tubby strains. These are both well characterised models of obesity and will be studied to better understand the biological mechanisms underpinning the clinical observation that obesity is associated with increased cancer risk and adverse treatment outcome
- MLL conditional mice. This strain will be used to ascertain whether MLL is required for leukemogenesis by MLL fusion oncogenes or other leukaemia-initiating oncogenes
- B6 FVB Tg (ITGAM) mice. These provide a model system for the conditional ablation of macrophages and will be used to understand how macrophages influence the immune response to radiation-induced tumour cell death and contribute to the activity of anti-CD20 monoclonal antibody therapy
- Rt TA2S-M2 mice. This line ubiquitously expresses rtTA which is controlled by an 8kb genomic fragment from the methylation-free CpG island of the human hnRNPA2B1-CBX3 housekeeping gene locus. This strain will be crossed with a number of established lines that carry the tetracycline reverse transcriptional activator rtTA

A dedicated Quarantine area using flexible film isolators running at negative pressure accommodates 'live mice' with unknown health status to ensure the specific pathogen free (SPF) status of the facility is maintained.

Experimental Services

In vivo technical support has been provided offering a range of surgical and non surgical procedures. Many of these procedures are well established, and range from routine oral dosing, subcutaneous cell implantation, venepuncture via the lateral tail vein, intra-tumoural injections, radiolabelled antibody delivery, intramuscular injections and X-ray irradiations. Health and welfare for animals under procedure remains paramount and extensive monitoring has been performed including calliper measurements for subcutaneous tumours, body weights, abdominal palpations and routine tissue sampling *post mortem* at the completion of studies.

New *in vivo* techniques developed have included,

- Intra-facial vein injection of mouse neonates following irradiation
- Bone marrow cells transplanted to form heterotopic ossicles
- Subcutaneous wound healing

Equipment Purchase

- Two replacement cage changing stations for carrying out routine husbandry tasks and animal manipulation

Cancer Research UK GeneChip Microarray Service
Head: Stuart Pepper

The Molecular Biology Core Facility at the Paterson hosts a microarray service which is available not just to staff on site but to all Cancer Research UK funded groups. The facility has two full Affymetrix systems which allow us to handle a high throughput of samples. Despite the appearance of competing technologies, demand for expression profiling has remained high and this year we have processed several hundred samples.

The main difference we have seen over the last year is that more samples require special handling: small samples resulting from FACS protocols have become more common as have archival samples extracted from formalin-fixed paraffin-embedded material.

Last year we reported a collaboration with Dr Kim Linton (Christie Hospital) determining gene expression profiles in archival sarcoma samples. This year we have published a follow-up paper showing that by using exon arrays and newly released reagents, it is now possible to obtain better results than previous methods allowed (Linton *et al*., Biotechniques 2009; 47: 587).

Figure 2
A snapshot of sorting in action - each of the droplets contains a single cell, the sorter decides on their fate.

Since this work was completed we have had several large projects exploiting this application brought to the facility.

Samples derived from FACS sorting are often limited in the number of cells available. Using the latest amplification protocols we have been able to return good quality data to users where populations have been limited to a few hundred cells rather than the more usual several million used as a start point. Ideally we would want to offer single-cell profiling, but whilst this is possible there is no currently available protocol that would allow it to be offered as a routine service. In the meantime we will continue to evaluate any approaches to single-cell profiling that become available.

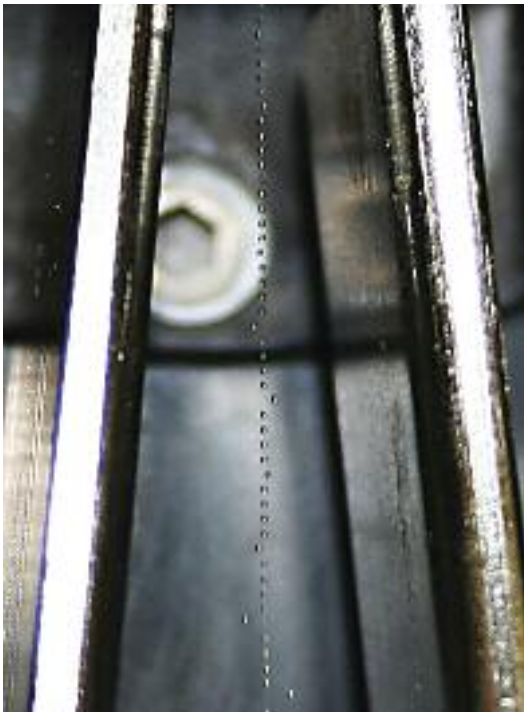
Flow Cytometry Facility
Head: Morgan Blaylock

The Flow Cytometry Facility at the Paterson Institute provides state-of-the-art instrumentation, education and expert technical assistance to investigators for the successful performance of flow cytometry-based studies. The goal of the facility is both to support current research applications and to continuously extend the repertoire of flow cytometric methods available to users to facilitate researchers in finding answers for the treatment, prevention and understanding of cancer.

Flow cytometry can be viewed as a specialised form of fluorescence microscopy and is a means of measuring the physical and chemical characteristics of cells or particles. Any aspect of a cell which can be labelled or detected with a fluorescent marker can be identified and quantified by flow cytometry. We can assess: cell phenotype by looking for expression of cell surface, cytoplasmic or nuclear antigens, cellular DNA or RNA content, fluorescent protein expression, functional aspects of the cell such as enzyme activity, apoptotic status, ion flux or pH. In addition, any population identified on an analytical flow cytometer can be retrieved by using a cell sorter which has the ability to physically separate cells of interest from a mixed population.

Analytical Cytometry

Early this year we relocated analytical cytometry to our new dedicated lab which offers a very stable optimal environment for the systems. The facility currently has four bench top cytometers including one plate-based bead reader. These are all user-operated systems which we offer basic training in a group setting which is supplemented with one to one training for specific applications.



BD FACScan - 3 colour single laser (blue)
BD FACSCalibur - 4 colours dual laser (blue and red)
BD FACSArray - 4 colours, dual laser (green and red)
BD LSRII - 17 colours, quadruple laser (UV, violet, blue and red)

Cell sorters

With the removal of the analytical cytometers from the main cytometry lab we have established a sorting suite which has minimised the traffic through the room providing a much more stable environment for the sorters. The sorting suite currently houses three cell sorters which are able to retrieve up to four specifically defined populations so that cells may be recovered for further study including re-culture, RNA or DNA extraction or use in functional cell assays. The cell sorters are operated solely by the Flow Cytometry team on a daily basis

BD FACSVantage SE - 2 way sorting, 5 colours, dual laser (blue and red)
BD FACSAria - 4 way sorting 12 colours, triple laser (violet, blue and red)
BD InFlux - 4 way sorting, 14 colours, quadruple laser (UV, violet, blue red or orange)

Other services

Our lab offers a full range of educational and cytometric services. We are able to advise on a wide variety of cytometry related subjects including experimental design, selection of reagents, data analysis and interpretation, we can act as a beta test site for novel cytometry applications and we also advise on data

presentation. The latter is becoming more and more important as journals require cytometric data to be more transparent.

We have been involved in the continuation and development of a number of projects this year. We have continued our collaboration with the Leukaemia Biology Group, who are studying the developmental control of human haematopoietic stem cells and their progenitors. They are using human bone marrow to isolate different populations of human haematopoietic cells. They have been using flow cytometry to immunophenotype and sort cells, producing purified populations highly enriched for previously defined cellular potentials. By applying microarray and proteomic analysis to these cells they are expecting to generate a detailed gene and protein expression map in human haematopoiesis. In addition we have been involved in studies which have yielded novel applications of flow cytometry such as work conducted with John Bridgeman and David Gilham. Together we have developed a flow cytometric immunoprecipitation method capable of investigating protein-protein interactions specifically the analysis of the T-cell receptor.

Histology
Head: Garry Ashton

Workloads have continued to increase, but the recruitment and training of a new scientific officer has given the unit the capacity to continue to offer a comprehensive and flexible service in all of our heavily used key services. We have also been able to continue to focus on the unit's development over the last twelve months.

In early 2009 the existing Arcturus PixCell II laser capture microdissection (LCM) system was replaced with the Leica LMD6000 system. The system relies on gravity for sample collection and a UV optics driven laser. Evaluation of various sample preparation techniques has continued. At present the Clinical and Experimental Pharmacology Group are looking at the feasibility of utilising LCM for the purification of circulating tumour cells (CTCs) enriched by either the CellSearch or ISET technologies, and the subsequent RNA evaluation for gene expression analysis. Proof of principle spiking experiments have shown promising results. Several other groups are using the system including the Breast Biology Group who are looking for differences in gene expression within breast tissue from patients before and after an intermittent diet. In particular, gene expression differences between microdissected stromal and epithelial cells are being investigated.

Arrival of the automated tissue microarray platform (Beecher ATA27) is imminent. Speed of construction will be increased, whilst mapping of an H&E to the donor block will allow more accurate core acquisition. Chemically-induced mouse liver tumours, head/neck, gastric, lung and breast tumour TMA's have been constructed manually over the last 12 months. Numerous TMA's have also been constructed for antibody validation studies and the evaluation of image analysis systems.

The Manchester Cancer Research Centre Biobank has continued to expand, collecting matched blood, urine and tissue samples from five collaborating Trusts across Manchester. All the samples are centrally processed within the lab. To date, samples from almost 800 patients have been collected with over 40% containing both fixed and frozen tumour and matched normal pairs.

Last year the Biobank was expanded to include the collection of blood and bone marrow from haematological malignancies including acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), myelodysplasia (MDS) and chronic lymphocytic leukaemia (CLL). This methodology has now been fully optimised and samples from 52 patients (with consent from a further 50), representing 100 bone marrow and peripheral blood samples have been banked and available for use by researchers. To date, 10 applications have been made to the Biobank for samples.

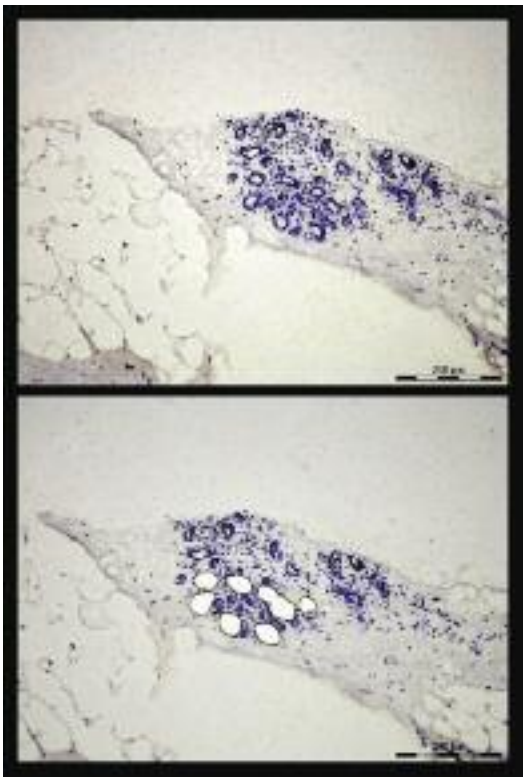


Figure 3
The photomicrographs show normal human breast tissue before (upper panel) and after (lower panel) laser capture microdissection of epithelial cells. The frozen section has been stained with Gills haematoxylin which highlights the terminal ductal lobular unit (centre of view) from which epithelial cells are captured for RNA isolation in order to study gene expression.

Development of the immunohistochemistry (IHC) service has continued. The i6000 automated platform and the antigen retrieval stations allow for high throughput and standardisation. These are multi-user pieces of equipment currently running at full capacity. Optimisation and multiple labelling studies together with alternative fixation and decalcification regimes have continued to be explored.

In collaboration with the Stromal Tumour Interaction Group, IHC has been used in order to understand the paracrine and/or autocrine mechanisms responsible for trans-differentiation of normal fibroblasts into tumour-promoting stably maintained carcinoma-associated fibroblasts (CAFs). The characterisation of α-SMA-positive myofibroblasts and the validated myofibroblast-associated markers from *in vitro* findings in human ductal invasive breast carcinoma samples have been performed.

IHC has also been used in the development a mouse model for acute lymphoblastic leukaemia (ALL). To achieve this GFP/luciferase-positive human leukaemic cell lines were injected into immunodeficient mice and once metastasised tissue samples were isolated. A rapid decalcification and extended retrieval method was developed to allow visualisation in whole head samples without destroying the morphology of fragile structures.
The Translational Radiobiology Group is using IHC to investigate the expression of genes which are implicated in radiosensitivity. This is a key avenue of exploration as validation at the protein level has substantiated Affymetrix data generated from RNA extracted from 15-25 year old FFPE samples. The protein expression of a number of different markers which can potentially be used to define histological subgroups such as squamous carcinoma and adenocarcinoma of the cervix is also being studied.

Kostoris Library
Head: Steve Glover

Working in partnership with The University of Manchester, the Kostoris Library provides on site and online access to resources for all Paterson staff and students. In 2009, the library launched a DSpace Institutional Repository capturing the research output of The Christie and Paterson Institute for Cancer Research. The repository presents the information within an organisational context allowing papers to be mapped to groups and departments. The database is fully searchable by both keyword and free text searching and also has a comprehensive author index which allows the work of the individual scientist to be captured. The repository holds records from 2009 dating back to 2001 and it is planned to add historical records dating back as far as 1950.

The library offers a comprehensive portfolio to Institute members including access to online journals and databases provided jointly by the University and The Christie. Databases include PubMed, EMBASE, BIOSIS, Scopus, Science Citation Index, and Journal Citation Reports from Thomson ISI's Web of Knowledge.

The library also offers a number of services around literature searching, training and alerting services. Literature search requests will aim to be turned around within two working days and results can be delivered in a variety of formats. In addition to *ad hoc* over the counter search requests the librarians will also set-up a number of automated systematic searches of the databases on a monthly or bi-weekly schedule. These will help users to keep on top of the latest published research on a particular topic, gene, protein or molecule. Training is also available and the library staff can deliver training in small groups, one-to-one, or even outreach sessions to suit the clients' needs.

Where papers, textbooks or documents cannot be readily accessed online or via the library a fast and efficient inter-library lending and document supply service is available. Urgent papers can usually be sourced same-day if the request is received before 3pm and the library has a 98% success rate on sourcing papers in the public domain.

In addition to access to resources the library can provide a place to study and has recently embarked on an upgrade to the study environment which will include a new reading area, classroom, IT training room, and computer drop-in suite. This work should be taking place in early 2010 and is expected to be completed by April.

Laboratory Services
Head: Mark Craven

The Laboratory Services department has three main roles within the Institute. Firstly we provide the Institute with a bulk liquid media and a separate Agar plate pouring service. We can produce over 1000 litres of sterile liquid media a month and we work with the laboratory groups and can produce new types of media as and when required. We aim to deliver the media within 3 days of the initial request

Secondly, we supply sterile glassware and plastics to the laboratories each day and arrange for the return of dirty glassware back to Laboratory Services for washing and autoclaving. We also supply sterile water and PBS.

Finally, Laboratory Services provide the laboratories with a Laboratory Aide to perform a range of housekeeping duties requested by each laboratory at fixed time points each week.

During 2009 the Institute has expanded to take on new groups. This has increased our workload and we have increased our staff numbers to enable us to meet this higher demand whilst maintaining the flexibility to provide all of the above requirements.

Logistics
Head: Maurice Cowell

A modern and efficient Logistics facility provides a comprehensive and vital role in supporting the research carried out at the institute. This group undertakes a wide range of duties including the accurate and efficient receipt, checking, booking in and distribution of goods ordered by personnel in the Institute. The logistics team is also responsible for the collection and removal of waste, be it general rubbish, yellow bags or GM waste, and for the collection of liquid nitrogen containers from laboratories and transportation to the loading bay for refilling and returning.

Ordering and distribution of the Central Stores stock via the intranet email has been updated to become more user-friendly and it is our duty to ensure adequate stock levels are maintained at all times. This also includes maintaining and monitoring the media and enzymes stored in the Institute freezers (Sigma, Invitrogen, Roche, Promega and Qiagen), and recently a New England Biolab freezer has been introduced to save on delivery charges and number of deliveries, again the Logistics department is responsible for the ordering, distribution and stock levels of these items. Currently we are also looking at the gas cylinder usage and are awaiting estimates for the installation of tanks (to save money and avoid handling cylinders). The department works closely with all groups and helps out where necessary, be it tracing and confirming delivery of goods with suppliers, and dealing with missing, damaged or wrong items. We also assist or manage the moving of heavy equipment or furniture, and setting up various meeting rooms for numerous events.

Molecular Biology Core Facility
Head: Stuart Pepper

The Molecular Biology Core Facility covers a wide range of technologies including two self contained service areas - advanced mass spectrometric analysis of proteins and microarray based expression profiling - which are detailed separately in this section.

There are three core services that have a continuous level of demand; these are plasmid DNA preparation, DNA sequencing and PCR-based genotyping. These services have all continued this year with similar or slightly increased throughput. Typically we run around 2000 PCR reactions, 600-800 DNA extractions and anywhere from 400 to 800 sequences per week.

The core facility also provides support for expression profiling by quantitative PCR. An ABI7900 in conjunction with an Eppendorf epMotion allows qPCR to be carried out in 384-well formats, allowing large projects to be processed efficiently. Demand for this service has been particularly high over the last few months and as the year draws to a close we are looking into adding more equipment to support this application.

The most exciting development in the core facility this year has been the arrival of a high throughput clonal sequencer. These platforms were initially designed to facilitate whole genome sequencing projects and have the capacity to generate a staggering amount of data; we are generating around 300 million sequences per run. Although these systems were designed for DNA sequencing they are now proving to be flexible tools for many other types of analysis. Our main interest is in evaluating the use of sequence data sets for high resolution expression analysis as this may have some advantages over traditional microarray profiling. These systems are highly complex, presenting challenges both for the laboratory work and data analysis. MBCF is working very closely with the Crispin Miller's Applied Computational Biology and Bioinformatics Group to ensure that research groups within the Paterson will be able to access this cutting edge technology.

Biological Mass Spectrometry Facility
Head: Duncan Smith

The Biological Mass Spectrometry Facility at the Paterson Institute enables the use of cutting-edge LCMS technology to groups for a multitude of protein characterisation needs. Our applications encompass both protein identification and post-translational modification

analysis in both qualitative and quantitative environments. Our remit involves both routine service provision and novel application development in conjunction with research groups in the Institute. These application developments are key contributors to the maintenance of the Institute's presence at the research cutting-edge. Our routine service portfolio has benefitted from the addition of four new applications (delivered from our development pipeline in 2009) and these are discussed below.

In the area of post-translational modification analysis, we have developed and implemented a linear ion trap based approach to allow researchers to reliably and confidently map sites of ubiquitination. This approach has involved significant tuning of both the mass spectrometric and database searching protocols to facilitate the successful analysis of isopeptides which carry the analytically useful information about the site of Ubl modification. This work was carried out in collaboration with the Cell Signalling Group who have now successfully mapped sites of Ubl modifications on Tiam1 utilising our new approach. With respect to mapping sites of phosphorylation, we have developed a set of protocols which has massively improved typical sequence coverage of protein molecules key in comprehensive PTM analysis. This has been achieved by utilising 'low specificity' proteases with the intention of generating overlapping peptide fragments not usually associated with 'high specificity' digest agents. The work has focussed on both the generation of these 'low specificity' fragments and the most appropriate informatics route to ensure efficient database searching parameters are able to map sites of phosphorylation. This development work has been a collaborative effort with the Cell Division Group who have recently been able to double the number of phosphorylation sites mapped on a target as a consequence of the utility of this approach.

In the area of protein quantitation, we have developed a 'label-free- Fourier Transform MS' based approach to facilitate the relative quantitation of specific phosphopeptides defined in previous qualitative mapping experiments. This work has been performed in collaboration with the Cell Division Group and has facilitated the quantitative comparison of multiple sites of phosphorylation on Cut12 between different genetic background strains, helping to define which sites of phosphorylation are likely implicated in key biological processes. A similar 'label-free- Fourier Transform MS' protocol has also been applied to the quantitative study of complex proteomes (whole human cell extracts) in collaboration with the Molecular Pathology and Genito-Urinary Groups. This has demanded bespoke developments of (1) ultra high performance nano-LC (ultra high resolution separations), (2) gas phase fractionation (essentially approximating to 2 dimensional performance from a 1 dimensional LCMS run) and (3) advanced informatics tools for data analysis (purchase of LC-MS data package).

All the new LCMS applications (Ubl mapping, phospho-mapping with overlapping fragments, label-free quantitation of phosphopeptides and complex proteome label-free quantitation) are now available to all groups.

Research publications

Crispin Miller (page 16)
Applied Computational Biology and Bioinformatics Group

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Geoff Margison (page 18)
Carcinogenesis Group

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Karim Labib (page 20)
Cell Cycle Group

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Iain Hagan (page 22)
Cell Division group

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Nic Jones (page 24)
Cell Regulation Group

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Angeliki Malliri (page 26)
Cell Signalling Group

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Caroline Dive and Malcolm Ranson (page 28)
Clinical and Experimental Pharmacology

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Ivan Ahel (page 30)

DNA Damage Response Group

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Peter Stern (page 32)
Immunology Group

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Nullin Divecha (page 34)
Inositide Laboratory

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Tim Somervaille (page 36)
Leukaemia Biology Group

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Somervaille, T.C. and Cleary, M.L. (2009)
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Georges Lacaud (page 38)
Stem Cell Biology Group

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Valerie Kouskoff (page 40)

Stem Cell and Haematopoiesis Group

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Akira Orimo (page 42)

Stromal-Tumour Interaction Group

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Robert Hawkins and Peter Stern (page 46)

Biological, Immune and Gene Therapy

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Vaskar Saha (page 48)

Children's Cancer Group

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Tim Illidge (page 50)

Targeted Therapy Group

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Catharine West (page 52)
Translational Radiobiology Group

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Robert Hawkins (page 54)
Medical Oncology: Cell Therapy Group

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Cheadle, E.J., Hawkins, R.E., Batha, H., Rothwell, D.G., Ashton, G. and Gilham, D.E. (2009)
Eradication of established B-cell lymphoma by CD19-specific murine T cells is dependent on host lymphopenic environment and can be mediated by CD4+ and CD8+ T cells. *J Immunother*, **32**, 207-218.

Elkord, E., Dangoor, A., Burt, D.J., Southgate, T.D., Daayana, S., Harrop, R., Drijfhout, J.W., Sherlock, D., Hawkins, R.E. and Stern, P.L. (2009)
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Gordon Jayson (page 56)

Medical Oncology: Translational Anti-Angiogenesis Group

Refereed Research Papers

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Jayson G, Gardiner J, Hansen S.
Synthesis route for multioligomeric heparan sulfate molecules, PCT Application: PCT/ GB2009/ 000300, 2009-02-04

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Interesting times for microarray expression profiling. *Brief Funct Genomic Proteomic*, **8**, 170-173.

Seminar Series 2009

A variety of national and international speakers visited the Institute over the year, to make the seminar series the most varied it has been. The series is complimented by seminars held within The Christie and at The University of Manchester. For the first time the series was also merged with speakers arranged through the Breakthrough Breast Cancer Unit. The postdoctoral weekly seminar series also proved to be popular.

Kari Alitalo
University of Helsinki, Finland

Facundo Batista
CR-UK London Research Institute, London

Spencer Collis
University of Sheffield Medical School

Janine Erler
The Institute of Cancer Research, London

Martin Glennie
University of Southampton

Frank Grosveld
University Medical Centre Rotterdam, Netherlands

Thanos Halazonetis
University of Geneva, Switzerland

Martin Humphries
University of Manchester

Masato Kanemaki
Osaka University, Japan

Nicholas Ktistakis
Babraham Institute, Cambridge

Rolf Marschalek
Goethe University, Frankfurt, Germany

Fernando Martin-Belmonte
Centro de Biologia Molecular Severo Ochoa, Madrid

Angel Nebreda
Spanish National Cancer Research Centre (CNIO), Madrid

Chris Proud
University of Southampton

Tim Schroeder
German Research Centre for Environmental Health, Munich

Len Stephens
Babraham Institute, Cambridge

Charles Swanton
CR-UK London Research Institute (LRI), London

Simon Tavare
University of Cambridge

Marc Timmers
University Medical Centre, Utrecht, Netherlands

Marcos Vidal
CR-UK Beatson Institute, Glasgow, Scotland

Paresh Vyas
University of Oxford

Breakthrough Breast Cancer Research Unit Seminar Series 2009

John Bartlett
Edinburgh Cancer Research Centre

Gabriela Dontu
Department of Academic Oncology, Guy's Hospital

Doug Easton
University of Cambridge

Nadia Harbeck
Technical University of Munich, Germany

Rita Falcioni
Regina Elena Cancer Institute, Rome

Jorge Reis-Filho
Breakthrough Breast Cancer, London

Margaret C Frame
Institute of Genetics & Molecular Medicine, University of Edinburgh

Andy Gescher
Department of Cancer Studies, University of Leicester

Rudolf Kaaks
Division of Cancer Epidemiology, German Cancer Research Centre

Charlotte Kuperwasser
Tufts University School of Medicine, Boston

Rob Michalides
Professor of Cell Biology, The Netherlands Cancer Institute

Bill Muller
McGill University, Canada

Salvatore Pece
University of Milan

Derek Radisky
Mayo Clinic Cancer Center, USA

Postgraduate Education

http://www.paterson.man.ac.uk/education

Postgraduate Education Manager
Julie Edwards

Postgraduate Tutor
Crispin Miller

A thriving Graduate Programme is a fundamental aspect of a research Institute such as the Paterson, not simply to train the researchers of tomorrow, but also for the valuable contribution made by our students to the labs they are working in. In 2009, we welcomed another ten graduate students from around the world to join our four-year PhD programme, working in fields as diverse as yeast genetics, stem cells DNA repair, computational genomics and clinical research.

The Paterson Graduate Programme

The goal of the graduate programme is for each student to receive training in scientific research, through a project that is both achievable and intellectually demanding. Each project is peer-reviewed in advance, and monitored throughout the PhD through a mixture of talks, written reports and progress and planning meetings. These are designed not only to provide formal points at which progress (of both the student and the project) can be assessed and goals discussed, but also to help develop the presentation skills that are so fundamental to the majority of careers in science. Graduate training is monitored by an Education Committee, which features Group Leaders, senior clinicians and scientists, and student representatives. Each student is also assigned an advisor (similar to a personal tutor on an undergraduate programme) whose role is to provide impartial support and advice, while further support is also available from the Postgraduate Tutor and a Student Welfare Group.

The Paterson runs an external seminar series featuring talks from many of the key players in cancer research, and students are also expected to attend postdoctoral research seminars and to present their own work in lab meetings within the institute.

PhD Studentships

All our CR-UK funded studentships are four years long, and consist of an extended research project in one or more of our research groups. We also offer rotation projects in which the first year consists of three shorter projects, each in a different lab, before focusing on a more extended project for the final three years of the PhD. Recruitment is highly competitive and the majority of interviews are typically conducted over a 2-day period in late November or early December.

Students benefit from access to state-of-the-art-facilities including Advanced Imaging, Mass Spectrometry, Microarrays, Flow Cytometry, Histology and Next Generation Sequencing (see section on Research Services). All our research groups offer PhD studentships, and projects cover the entire breadth of research within the institute.

Fellowships in Clinical Pharmacology Research

In order to help train the next generation of clinical pharmacologists with expertise in oncology, in 2007 the Paterson Institute, in collaboration with the MCRC and AstraZeneca, established a fellowship scheme in Clinical Pharmacology Research. The fellowships are open to applicants who have obtained, or are close to obtaining, their Completed Certificate of Specialist Training (CCST) in Medical Oncology.

Each Clinical Pharmacology Research Fellow undertakes a three-year PhD project, which provides training in biomarker discovery, method development/validation, and in clinical

trial methodology. During tenure at The Christie/Paterson the post holders receive clinical supervision from Malcolm Ranson, and laboratory-based training from Caroline Dive in CEP (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, ICH-GCP, EU Directives and research governance. Biomarker method development and application take 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, their supervisors, as well as other staff members involved in the project, ensuring true collaboration and a 'joined up' approach.

Education Committee 2009
Iain Hagan – Chair
Fiona Blackhall
Richard Cowan
Caroline Dive
Julie Edwards
David Gilham
Ian Hampson
Tim Illidge
Gordon Jayson
Valerie Kouskoff
Karim Labib
Crispin Miller
Vaskar Saha
Tim Somervaille
Jenny Varley
Catharine West
Caroline Wilkinson

Student Representatives
Monique Melis
Andrzej Rutkowski

Operations

Director of Operations
Pippa McNichol

The first half of 2009 was focused upon the Paterson's Quinquennial Review (Site Visit) which took place at the end of June. Discussions for the review started eighteen months earlier with the planning of the refurbishment of the Paterson's corridors, stairways and facilities.

The aim of the work was to brighten up the Institute and give staff a sense of pride in their workplace, whilst at the same time ensuring a greater degree of sustainability. The corridors were given new daylight lighting and a fresh coat of paint and artistic scientific images were blown up and created on acrylic and then affixed to the stairwells. An artwork project – 'the Art meets Science Challenge' was created in conjunction with Sale Grammar School which culminated in a prize-giving ceremony for the winning pupils attended by the local press. The School was so pleased with the project that they have suggested refreshing the art in two years time.

The Site Visit itself lasted over 2 days and was gruelling for all concerned but the behind-the-scenes organisation was second to none and this was due to the smooth organisational skills of Amy Weatheritt and Esther Walker. The Review party commended the Paterson for its organisation of the visit.

The Annual Staff Meeting was particularly well attended this year as staff came to listen to the Director and Director of Operations talk about the Institute's financial situation. The budget received from CR-UK had been cut by 5%, which did not take into account the annual pay award and incremental rises, resulting in real terms of a cut of 8%. Various cost savings sub-committees were formed and recommendations were actioned to ensure that savings were made. The Scientific Officers Working Group (SOWG) proved to be invaluable, sitting on some of the cost-saving sub-committees and working closely with the Procurement department to negotiate the best deals for the Institute. It is gratifying to see that prudent management of the budgets has resulted in a slight surplus being projected for the year end. This money is going to be used to improve the ventilation facilities in two of the laboratories that are located in the oldest part of the building. The real test of good budget management will be in 2010/2011 when the Paterson will be faced with more real term cuts.

The Operations team was joined by some new staff during the year – Julie Jarratt, a part-time recruitment administrator who is based within the HR department; Martyn Bottomley, a Business Manager employed by Cancer Research Technology (CRT), CR-UK's oncology focused development and commercialisation company and Julia Wright, CR-UK's new High Value Donor Manager. Julia's post is a new one and it is the first time that CR-UK has employed a fundraiser to be directly based within one of its Institutes. Amy Weatheritt, who had previously been employed as an administration assistant was very deservedly promoted into the post of PA to the Director and Director of Operations. The Reception/security function of the Paterson had been undertaken by a national company but it was decided to bring the service back in-house and so negotiations are currently underway to ensure that the new service becomes operational at the beginning of January 2010.

The HR department reorganised the staff induction programme, resulting in a more streamlined process and are going to be working on introducing a mentoring scheme for new starters during 2010.

The Paterson's website was redesigned during the year, providing a more up-to-date website which is easily accessible. The IT team have also

been working with an external supplier to provide a new intranet (it has been named PICRaboo after a competition was held amongst the staff and students to name it). It is anticipated that the intranet will launch in the New Year and will provide much functionality for staff to manage their own leave, look up polices and procedures and each area/interest group will have its own section; for example there will be sections for the students, the union, the Scientific Officers Working Group, each of the Service Units and Operations departments.

CR-UK introduced a new pay and grading scheme for non-scientific staff towards the end of 2009 and so the Paterson has started the formal consultation process with the unions. It is anticipated that the scheme will be implemented in the New Year.

It has been a really productive year and I would like to extend my thanks to every member of the Operations team for their hard work and dedication. Particular thanks must go to Margaret Lowe, my deputy, who has combined managing a very busy department, with implementing the University's new Procurement to Pay scheme, whilst ably assisting me in the running of the operations department.

Admin and Reception Services
Manager: Amy Weatheritt

Over the year the department's focus has shifted from providing a catering and document production facility to spending more time and energies on event support and organisation. The administration team played a key role in the organisation of the EMBO course which was held in Manchester throughout June and also the annual Paterson Colloquium in September.

The Site Visit has been the main focus of the department, working alongside the rest of the operations team to ensure that all 'behind the scenes' details ran smoothly. The department continues to evolve and improve, providing the best service possible to the Institute. Over the coming year we hope to maintain the standards and increase confidence in the stability of the department.

The Director's Office
PA to the Director and Director of Operations: Amy Weatheritt

Throughout 2009, the Director's office has continued to organise the Paterson Seminar Series to provide a varied programme of national and international speakers, serving to foster collaboration and encourage positive interaction within the wider scientific community. The 2010 series has now been confirmed and will again provide great opportunities for the staff within the Institute. A list of speakers for 2010 can be found at www.paterson.man.ac.uk/seminars

The department, like the rest of the Institute has been occupied with the Site Visit which was a great success. It involved a lot of hard work over the few days the review party were at the Institute and also in the run up to the event. After the Site Visit the office was finally able to move into the new office in TRF2, this has been a positive change and enabled a fresh new start for the office coinciding with the new term.

The office is currently working on recruitment and the forthcoming tenure reviews due to be held at the end of December. The Director's office will

carry on providing a supportive role to the Institute and its Group Leaders as well as aiding the MCRC with various meeting arrangements and hopes to continue building on relationships throughout the Institute and manage the busy workload.

Estates
Manager: Steve Alcock

June 2009 saw the successful completion of the refurbishment of the Institute's thoroughfares and main stairwells (including the toilet facilities) in time for the Institute Site Visit. The dated facilities have been given a professional look and designed to be more sustainable with presence detectors installed to lower the lighting levels when the area has not been occupied for a while.

Since the review, two capital projects are progressing and are due to be handed over to the Institute in December 2009. These are the new Drug Discovery laboratory, which is located on the ground floor opposite the administration offices, and the replacement generator, (a 1.5MVA unit), which will eventually be able to supply the whole of the Institute in the event of mains failure.

A number of minor projects have also been completed during 2009 which have improved the environment and the services for the Biological Research Unit, the DNA Damage Response Group, Breast Breakthrough, the Clinical and Experimental Pharmacology Group, the Logistics Department, Medical Oncology and the Director's accommodation.

The Estates team has been pro-active in 2009, keeping the backlog maintenance in the Institute to a manageable level. The team has also identified areas around the Institute that will require some refurbishment to be carried out in the near future.

Steve Alcock has collaborated with Richard Sandland from the Estates team in the Faculty of Medical and Human Sciences to write a report on the activities and procedures that the Paterson's Estates team undertake. This is to ensure that the Paterson's Estates department is adhering to The University of Manchester's rules and regulations. A line manager from the University's Estates department will be providing professional guidance to the Paterson's Estates' department. This will also satisfy the University's governance requirements.

The team as always endeavour to provide a quality service that is value for money within budget constraints in these challenging times of recession.

Finance and Purchasing
Manager: Margaret Lowe

Last year the University, in partnership with Deloitte Consulting, undertook a review of its procurement and payment processes. This review made a range of recommendations, with each one aimed at improving the purchasing experience both for

requestors of goods and services and for the University's suppliers. The Procurement to Payment (P2P) Project has been implemented throughout the University over the second half of this year. Procurement has made a number of changes to the way that goods and services are bought and Accounts Payable have moved to a single shared function.

As the Institute was already using internet procurement the end users would have noticed little or no difference and additional staff have received training as required. However, as with all new systems this has brought its own problems and the staff are working hard to resolve the queries that are coming through from the University to ensure suppliers are paid promptly.

In light of the current economic climate and reduced funding the Procurement department and the Scientific Officer Working Group (SOWG) have been working together to identify savings in consumable spending. It is envisaged that these meetings will continue over the coming year.

Apart from the everyday purchasing and finance procedures the Finance department has continued to support the research groups by providing effective and efficient professional advice when costing new research proposals and administering existing grants. As the funding to the Institute has been reduced we have strived to ensure that accurate management information is provided on a timely basis to assist group leaders with budget control.

Health and Safety
Manager: Colin Gleeson

Safeguarding the health and safety of the Paterson's staff is a continuing priority. Initiatives have included the improvement of the building access control system with the introduction of proximity card readers and restriction of access to some areas of the Institute. Also the provision of Occupational Health was transferred to The University of Manchester, identifying employees and students and the health surveillance programmes in which they are enrolled.

Health and safety training has been provided in a number of key areas including induction, risk assessment, work with biological agents and genetically modified (GM) organisms, and COSHH. These are presented quarterly and are well attended. Local laboratory induction training was reviewed and developed with employee representatives in the form of the Scientific Officers Working Group (SOWG), to standardise laboratory induction across the Institute. Other work with the same group has lead to the development of an improved user friendly risk assessment form. This will be rolled out in early 2010. It is hoped that employee participation in health and safety matters will develop further, encouraging participation and ownership of health and safety issues and improve the Institute's health and safety culture.

Informal and formal safety inspections have been undertaken in a number of areas, in a rolling programme covering the Institute. There has also been a review of the Institute's transgenic work. Both projects have revealed relatively minor issues of non-compliance which have been or will be addressed. The Environment Agency inspected the Institute's arrangements for accumulation and disposal of radioactive substances. The Institute demonstrated compliance with the legislative requirements and there were no recommendations for any remedial action.

Some early work has been carried out in order to prepare the Institute for the introduction of the Single Regulatory Framework for work with biological agents. No doubt this will continue at some pace in 2010.

HR
Manager: Rachel Powell

Over the past year the HR Department has continued to provide a professional proactive HR service which has been delivered through the use of effective systems and processes.

The main focus for this year has been on streamlining the recruitment and selection process. The department has expanded with the recruitment of an HR Administrator which has enabled the department to continue to meet its objectives in terms of providing a high quality, effective recruitment service to the Institute. This has resulted in the successful recruitment of 32 highly skilled individuals throughout the year. The department has also had to become familiar with the new points-based system for immigration in order to advise and provide guidance to managers and employees on the new legislation.

Joint partnership working with the unions has continued throughout the year which has resulted in the agreement of several revised policies including Pay Protection Policy and Probation Policy.

The HR department has recently been involved with the successful transfer of the Paterson's Occupational Health services to The University of Manchester. This involved consulting with the Occupational Health providers and the staff to ensure a smooth transition without any interruption to the service.

Moving forward, the department is due to launch a new HR section on the Institute's Intranet which will enhance and complement the high class service that the HR department currently provides.

IT
Manager: Malik Pervez

Information technology, electronic communication and network connectivity have become crucial to the success of the Paterson Institute. Over the years a substantial investment in IT has been made to provide a world class IT infrastructure. The Institute has an enviable platform and range of technologies.

The IT team are highly trained to support all approved systems and provide excellent support to the researchers within the organisation. The systems are protected 24/7 by robust technologies ensuring that data is not compromised and a disaster recovery solution ensures that in the event of a catastrophic event systems can be reinstated rapidly.

Continuing to build upon this success year on year ensures that the Institute stays ahead of the game in terms of the technology required to support the research and development and 2009 has seen the Institute add to the level of technology via a number of investments and developments.

A wireless network has been implemented across the organisation to access and provide a much more flexible working environment. Furthermore, additional storage and archiving facilities have been procured and deployed to meet the growth in data requirement. The Institute's website has recently undergone a major facelift. The Institute also has plans to develop a new Intranet for staff to improve internal communication and access to shared documents which will be developed during 2010.

A further development planned for 2010 will be to implement video conferencing facilities to improve external communication with other scientific organisations. This feature will also create savings as it will reduce the need for staff to travel to some meetings as these can be undertaken from the Institute.

Cancer Research Technology (CRT)
Manager: Martyn Bottomley

Cancer Research Technology is a specialist oncology-focused development and commercialisation company. CRT aims to maximise patient benefit from publicly funded research worldwide by advancing research discoveries into development with pharmaceutical and biotechnology parties. CRT is wholly owned by Cancer Research UK.

CRT bridges the fundamental gap between cutting edge academic research and industrial development of cancer therapeutics and diagnostics. This is achieved by working closely with prestigious, international research institutes, such as the Paterson, and funding bodies to develop, protect and commercialise oncology related discoveries. Core activities of business development and drug discovery are supported by specialists, integrated in the business with expertise in patents, legal, finance and marketing. CRT's exclusive focus in oncology

provides an unrivalled depth of knowledge and experience in cancer-specific translational development and commercialisation. By arrangement with The University of Manchester, CRT owns and is responsible for the development and commercialisation of intellectual property arising from Cancer Research UK funded research at The University of Manchester (including the Paterson). Proceeds are shared with inventors and the University, whilst surplus CRT revenues are returned to Cancer Research UK to support further cancer research. The company is well resourced to achieve its mission, with headquarters and established drug discovery labs in London and Cambridge (UK), a US subsidiary in Boston and a collaborative drug discovery vehicle in Australia.

CRT has an enviable reputation for the productive nature of its academic and commercial partnerships. CRT partnered therapeutics are currently marketed by AstraZeneca (Tomudex), Schering-Plough (Temozolomide) and a third product was progressed to market by Pfizer (Zinecard). More than 20 partnered therapeutics are currently in clinical development, with 5 in Phase III clinical trials including ANYARA, being developed by Active Biotech (Phase III) and stemming from Cancer Research UK funded work at the Paterson (Professor Peter Stern).

CRT's relationship with the Paterson reflects the specific requirements of the scientist, the Paterson, Cancer Research UK and the individual project. To effectively facilitate these requirements and interactions CRT has a Business Manager (Martyn Bottomley) based at the Paterson dedicated to working closely with the staff there. CRT has a large and broad portfolio of development programmes and robust licensing opportunities (including some originating from the Paterson) that continue to attract commercial partners who excel in their field.

CR-UK Fundraising Manager: Julia Wright

This year Cancer Research UK expanded its fundraising capacity in the North West by introducing a new fundraising role, based at the Paterson, to generate large private donations for cancer research projects in the North West. This new role will focus on raising money from local people and charitable trusts as part of CR-UK's initiative to establish 20 'Centres of Excellence' across the UK. The Paterson has been fortunate to receive a number of high-value donations from individuals and organisations in 2009 and this new post will enable CR-UK to secure more support of this kind.

Cancer Research UK's Local Engagement and Development

LEAD Manager
James Dunphy

Researchers at the Paterson Institute have once again excelled in engaging the public with science. Through a series of lab tours, supporter open days and other activities in support of Cancer Research UK's fundraising events, researchers talked to and inspired thousands of people about their work and how it impacts on cancer.

In Manchester the Local Engagement and Development (LEAD) department has worked hard to support our fundraising teams by facilitating interactions between the Institute and Cancer Research UK's supporters. LEAD plays an important role in helping to retain and build continuing loyalty with these supporters by showing them tangible examples of how their money is being spent and the impact that it is having.

Over the last 12 months there has been around fifty engagement activities supported by researchers from the Institute. These include attendance at a wide variety of external fundraising events (such as Race for Life, Relay for Life and fundraising committee AGMs) as well as hosting events within the Institute. Following one of our monthly lab tours Roslyn Burgess (a Cancer Research UK shop manager) said:

"It has made me more determined to beat our shop target each week in order to be able to fund such brilliant work".

The Institute also hosts an annual schools day and the aim of this is to engage with local schools and colleges, giving students a practical insight into a potential career in cancer research. This is the fifth year the event has taken place with eight local schools bringing fifty students to the Institute. Nick Snowden, biology head at Manchester College School said: "I think it's fantastic for the students to have the opportunity to visit such a research facility and it provided excellent support for various parts of the A-Level syllabus. A most useful day."

Involvement in Cancer Research UK activities also extended to hands-on fundraising with the Institute raising £4,000 through their participation in Stockport Relay for Life and

Figure 1
Cancer Research UK Chief Executive Harpal Kumar with Andrew Lansley MP, Shadow Secretary of State for Health, and Mark Simmonds MP, Shadow Minister of State for Health at the Paterson Institute

Figure 2
A team of Paterson researchers helping out at Manchester's Race for Life

the arduous 40 mile Keswick to Barrow walk. The Relay team donned lab coats for the full 24 hours of the event and thoroughly enjoyed their experience. During the afternoon they took time to conduct strawberry DNA extraction experiments for the other participants. This is an innovative way of engaging a wide audience in science and provides an opportunity for people to hear more about the work at the Institute. This successful engagement activity was replicated at Manchester Race for Life in Heaton Park. Here a team of postdoctoral fellows and graduate students delivered an impressive 110 demonstrations to around 450 supporters - including a four year old and the Lord Mayor of Manchester.

This year the Institute also helped Cancer Research UK's Policy and Public Affairs team organise a tour for two senior Conservative MPs (Andrew Lansley MP, Shadow Secretary of State for Health, and Mark Simmonds MP, Shadow Minister of State for Health). They visited during the Conservative Party Conference in Manchester and met with Harpal Kumar, Chief Executive of Cancer Research UK, and Professor Nic Jones. Harpal Kumar said:

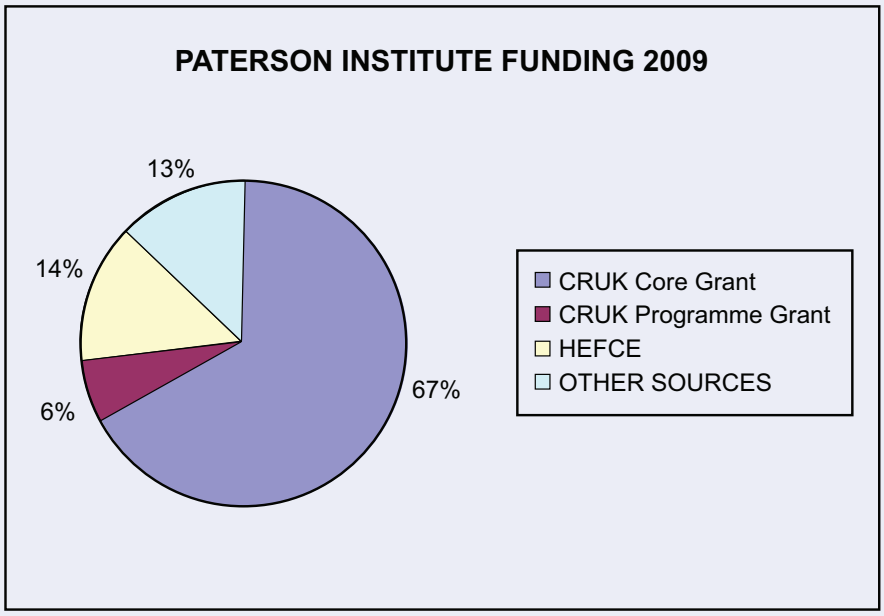
"It is crucial that politicians and decision makers are aware of Britain's place at the forefront of medical research worldwide and that they help us maintain our research excellence. I am very pleased therefore that Andrew Lansley and Mark Simmonds have been able to visit our Institute to find out not just about the work we undertake but also about how we're planning for the future."

The success of local engagement activity in Manchester has resulted in the city being selected to host an exciting new fundraising initiative. In 2010, Manchester will be hosting Cancer Research UK's first ever night-time walking marathon called 'Shine'. It is expected 5000 men and women will take part to raise over £1 million for the Charity. The route will take participants past the Institute and plans are in place to ensure that all participants are given the opportunity to engage with their local researchers and learn more about the research undertaken in Manchester.

LEAD is a key strand in Cancer Research UK's Centres Initiative, which is an important strategic component of the Charity's drive to achieve its 2020 Goals. The excellent activity undertaken in Manchester is now being replicated across the UK, with LEAD Managers now in Glasgow, Belfast, Cardiff, Birmingham, Southampton and Newcastle.

Acknowledgement for Funding of the Paterson Institute

The total funding of the Paterson Institute for 2009 was £15.8M. The major source of this funding (67%) was through a core grant from Cancer Research UK (CR-UK). The actual value of this award in 2009 was £10.5m. This is divided between the various scientific groups and service units within the Institute to enable them to carry out their research. In addition to this the CR-UK awarded us £1m to set up the Drug Discovery unit (6%).



The infrastructure of The Paterson Institute is funded by HEFCE-generated income at a cost of £2.3m (14%).

The final 13% of the Institute's funding is received from a number of additional sources. The research carried out through these additional projects enhances and supports the research undertaken by the core funding.

These sources are as follows:

- AstraZeneca
- Roche
- European Commission
- ECMC
- BBSRC
- Leukaemia Research Fund

We are immensely grateful to all our sponsors.

Career Opportunities at the Paterson Institute

The Paterson Institute is located alongside The Christie NHS Foundation Trust, and has a strong programme of basic and translational research. There are very close links with clinical and translational research groups throughout The Christie Hospital site.

The Manchester Cancer Research Centre (MCRC) was created nearly four years ago with partners including the Paterson Institute, The Christie Hospital NHS Foundation Trust, The University of Manchester and Cancer Research UK. This is an extremely exciting development which is enhancing all aspects of cancer research, education and treatment. The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular services, a microarray platform, proteomics, flow cytometry, histology, the production of knock-in/knock-out animal models, real-time PCR and advanced imaging. Details of all groups and facilities are given throughout this report, and can guide interested parties to the appropriate contacts. Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education. We encourage applications from suitable qualified graduates to apply to join either the PhD or MD programmes. Graduates with a first or 2.1 honours degree in a biological science can apply each year to train for a four-year PhD in one of our research laboratories. First year students will complement their laboratory skills by attending a small number of specialised postgraduate taught and training courses allowing them to gain a sound knowledge base of the latest developments in cancer treatment and research. The Institute also has a well-developed process for ensuring suitable pastoral care and mentoring for all students.

Postdoctoral applicants of high calibre are regularly sought. Although post docs will be encouraged to apply for their own fellowships, funded positions are available for outstanding candidates. Interested applicants should contact the Group Leaders directly, with details of their area of interest and recent experience.

In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates to the positions of Junior and Senior Group Leaders. The packages provided are extremely attractive and commensurate with the experience of the applicant, with significant funding for personnel, recurrent expenditure and equipment. Junior Group Leaders are appointed for an initial six-year period with a review at five years for consideration for promotion to Senior Group Leader, with Senior Group Leaders appointed to non-time limited positions.

Specific vacancies can be found on our web pages (http://www.paterson.man.ac.uk/jobs/index.asp), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.

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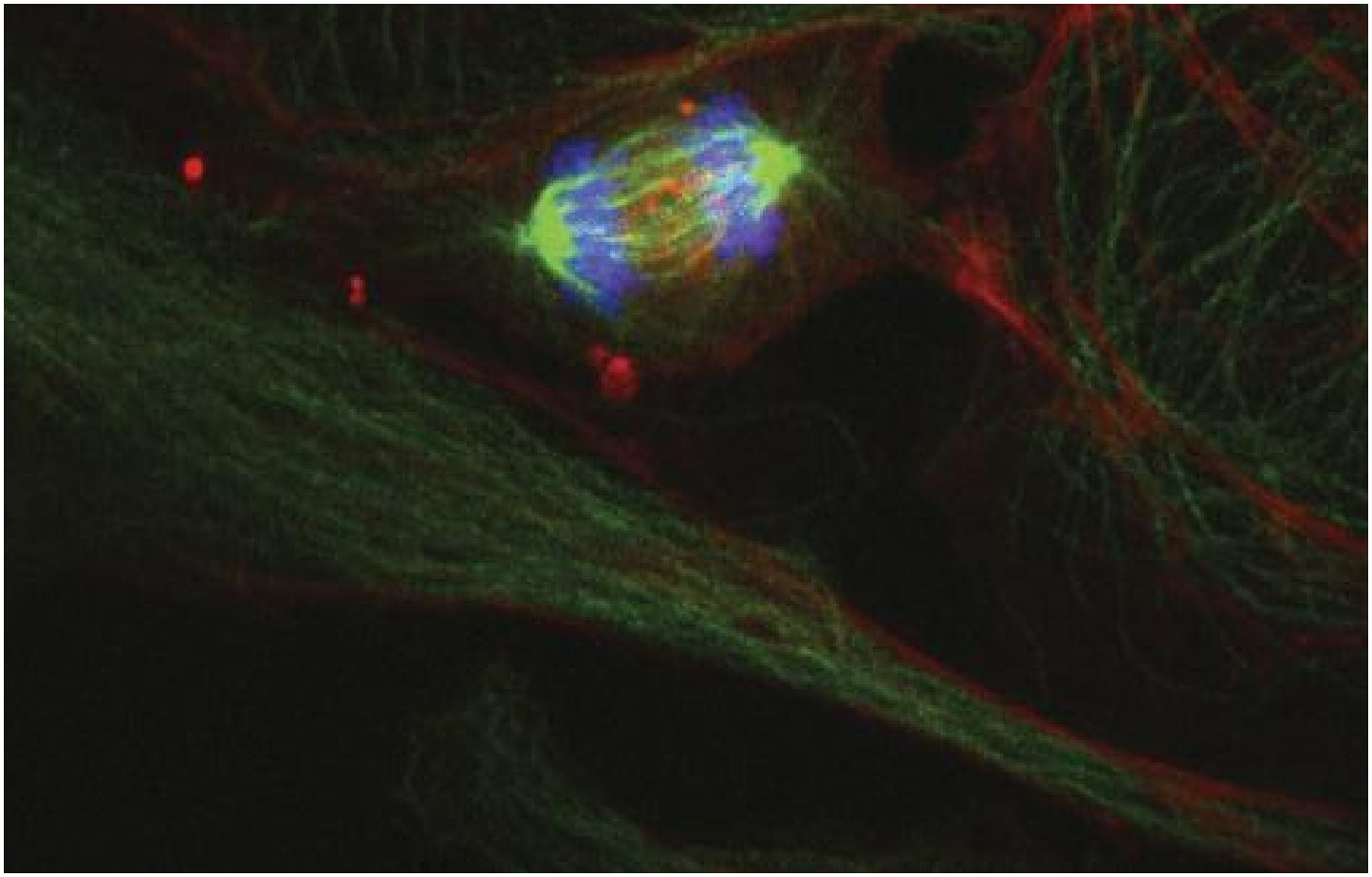

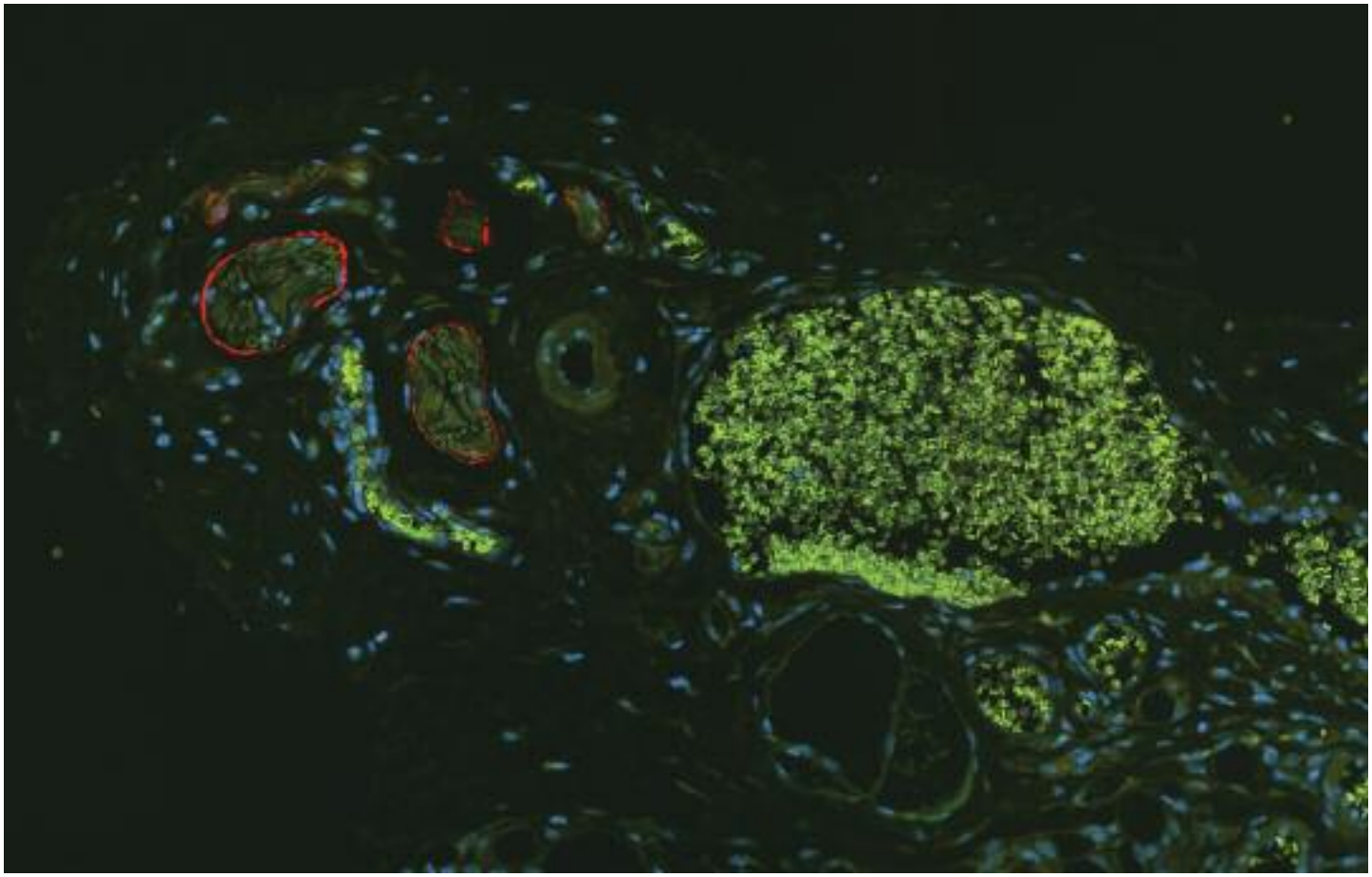

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