Paterson Institute for Cancer Research

Scientific Report 2010

Together we will beat cancer

CANCER RESEARCH UK

Cover images

Top
Multicolour immunofluorescence images of lung cancer cells spiked into human blood, see Figure 1 in the Clinical and Experimental Pharmacology report (page 27) for details.

Bottom
Imaging podosomes on acute lymphoblastic leukaemia cells in culture, see the Children's Cancer Group report on page 49 for details.

Scientific Report 2010

Paterson Institute for Cancer Research

Paterson
Institute for Cancer Research

MANCHESTER 1824
The University of Manchester

CANCER RESEARCH UK

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Director's introduction

Nic Jones

Welcome to the 2010 Paterson Institute Annual Scientific Report. This is the last report from me as Director of the Institute and therefore provides an opportunity not only to look back on the events and successes of the last year but also on the progress we have made over the last ten years and its aspirations for the future.

The last ten years has been a period of great change within the Institute. During this time the research focus has been re-prioritised with a complete reorganisation of the research programmes facilitated through the recruitment of many new group leaders – in fact over the last few years over twenty new group leaders have joined the Institute and helped to ensure that the Institute is now internationally recognised for the high quality of research that it supports. The research services have also developed significantly and are now extensive, state-of-the-art and crucial to the success of the Institute. New laboratory facilities – such as TRF1, TRF2 and the Drug Discovery Centre – have been developed to allow expansion of our research base. The training programmes within the Institute have expanded greatly and increased in quality fulfilling an important remit of the Institute to train the researchers of the future. All of these changes and developments have lead to a great increase in international recognition and reputation, an increase in scientific output and quality and maximising the potential and opportunities of the core funding we receive from Cancer Research UK. Over the

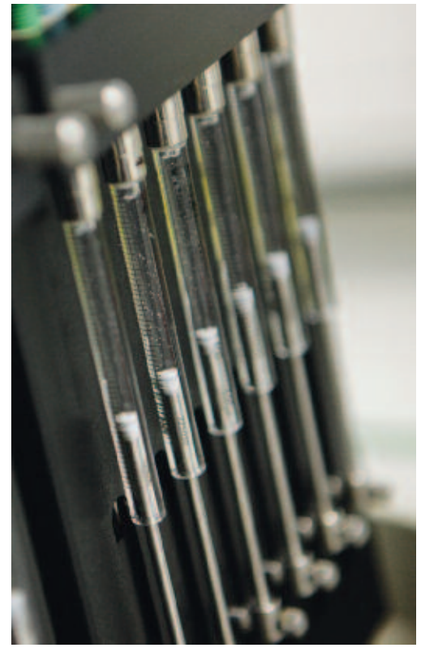
Figure 1
Architects cartoon showing the proposed new MCRC building

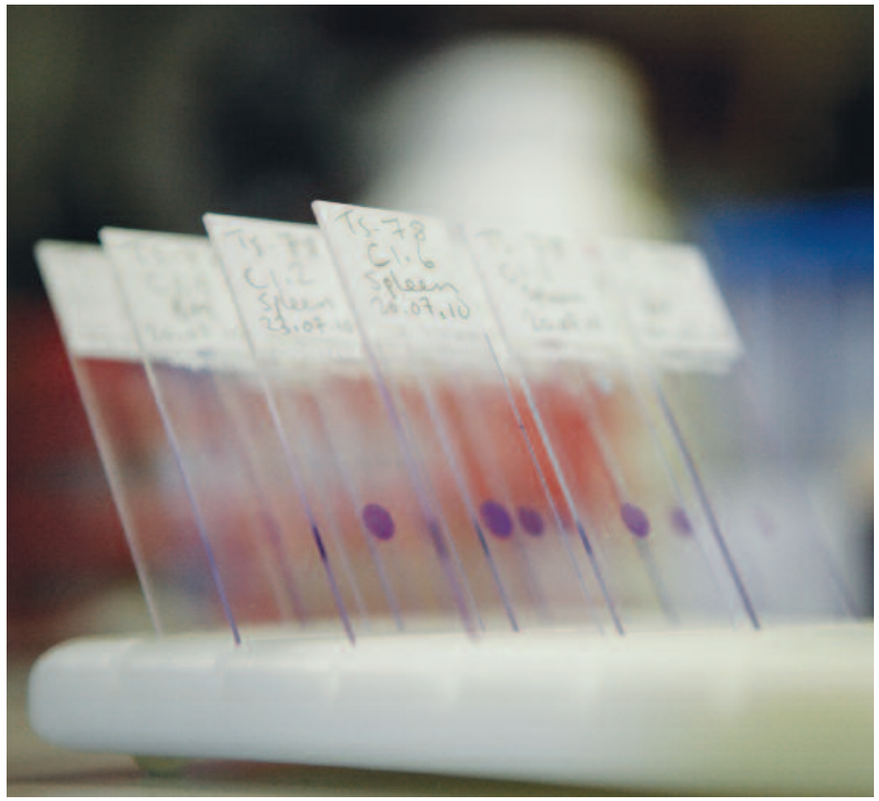
last ten years, the Institute has been reviewed twice by high level, international panels and in both cases was praised for the positive developments that had taken place and the plans for the future.

Another significant development has been the creation of the Manchester Cancer Research Centre (MCRC) with the Paterson Institute at its core. The MCRC, with its mission of co-ordinating cancer research in Manchester, is a very exciting and important development and of great benefit to the Institute. It provides the means by which the Institute can contribute across the research spectrum from basic to translational to clinical research and thereby gain considerable 'added value'. Within its brief five year tenure it has already made a big difference with significant advances in a number of research areas (for example biomarker and early phase clinical trial research, radiation-related research, breast and lung cancer research) and development of research infrastructure (for example the new early-phase clinical trials unit, one of the biggest worldwide). The Centre is still very much in its infancy but provides a wonderful platform for future development.

Looking back over the last ten years, we can I think feel very satisfied of the achievements that have been made. There is a very strong platform for which to build further and ensure that the Institute continues its upward trajectory. This will be the task for my successor!

Focussing on the last year, a number of positive developments have taken place. John Brognard joined us as a Junior Group Leader. John was a postdoctoral fellow in the laboratory of Tony Hunter at the Salk Institute in California and has initiated an exciting research programme that aims to identify and characterise novel kinases or signalling networks that are altered in tumours and are essential for driving tumourigenesis. It is exactly this type of research programme that can provide novel targets for our Drug Discovery Centre which, over the last year, has reached its full complement of research staff. A number of exciting discovery projects have been initiated and the Centre has already had a major influence on the Institute by promoting discussions around a number of potential targets and instilling a 'drug hunting' culture. Junior Group Leaders are core-funded for six years in the first instance to provide sufficient time to build up a dynamic, successful and productive research programme. At the end of this period there is a rigorous evaluation to consider promotion to Senior Group Leader and the prospect of long term support. Only those leaders that have really demonstrated significant output and success and who have gained international recognition are expected to be successful in this process. This was the case with Angeliki Malliri who was promoted to Senior Group Leader on the basis of her excellent work on the role of regulators of Rho-like GTPases in cancer. Karim Labib was elected as an EMBO member. Our research services also continued to develop and, in particular, this year saw the Histology service expand and increase its capabilities by providing access to tissue microarrays. One of the major reasons why the research services are so excellent is the leadership provided by Jenny Varley, Assistant Director of Research, who oversees all the research services. Jenny has decided to retire in 2011 – she will be missed but leaves behind a strong legacy.





Plans for the new MCRC building are now well advanced and we anticipate that work can begin early in 2012 with a likely handover date in early 2014. The iconic building will allow essential expansion of research activities with accommodation for approximately 150 laboratory-based researchers and 90 clinical trials unit staff. The building has been designed to reflect and further embed the cross-disciplinary research approach of the MCRC.

The search for a new Director of the Paterson Institute has now been instigated and the expectation is that a new incumbent will be in place during the coming year. Being Director of a core-funded Institute is a very exciting and rewarding job. I have been privileged to lead the Institute for the last eleven years and to steer it through a period of great change and development. Institutes are critical to the success of CR-UK – they represent approximately 40% of CR-UK's total research spend and are therefore vital to the delivery of the organisation's research strategy. I am confident that the Paterson will continue to strengthen and thus play its role in realising the ambitions and goals of CR-UK.

Research Highlights

In this section we are highlighting some research publications from 2010 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.

Bitton, D.A., Smith, D.L., Connolly, Y., Scutt, P.J. and Miller, C.J.
An integrated mass-spectrometry pipeline identifies novel protein coding-regions in the human genome.
PLoS One 2010; 5: e8949.

Protein identification by mass spectrometry is a fundamental component of the modern molecular biology toolkit. The approach works by fragmenting peptides and then accurately measuring the mass/charge ratio of each individual fragment using a mass spectrometer. The resultant mass spectrum provides a diagnostic fingerprint that can be used to identify each peptide when compared against a computer-generated database of predicted spectra created from the set of known proteins. A limitation of the technique is that it is reliant on a database of candidate proteins against which to search, preventing its use in finding novel proteins. We therefore extended the technique by creating a much larger database comprising all possible protein sequences that might be expressed in the human genome, derived by translating the entire genome sequence in all three forward and all three reverse strands. We then searched existing mass spectrometry data against this much larger database to identify sequences that matched to novel regions outside known protein coding genes. Through this approach we identified hundreds of novel proteins, allowing us not only to predict new genes, but also to predict novel isoforms of existing proteins.

Daayana, S., Elkord, E., Winters, U., Pawlita, M., Roden, R., Stern, P.L. and Kitchener, H.C.
Phase II trial of imiquimod and HPV therapeutic vaccination in patients with vulval intraepithelial neoplasia.
Br J Cancer 2010; 102: 1129-1136 (featured article).
Highlighted in:
Nature Medicine 2010; 16: 499.

Vulval intraepithelial neoplasia (VIN) is a premalignant condition, which is frequently associated with type HPV 16 infection, and multifocal disease has high rates of surgical treatment failure. This study treated the high grade VIN lesions of 19 women topically for 8 weeks with imiquimod (an immunostimulatory cream) followed by three monthly vaccinations with TA-CIN, a fusion of the HPV 16 L2 minor capsid, E6 and E7 oncogenic proteins. The rationale was that the imiquimod would altered the local balance between CD8 T cells, which can destroy the HPV infected premalignant cells, and regulatory T cells which suppress immune activity, with the vaccination boosting the effective HPV 16 oncogene T cell immunity. A majority of women had objective clinical responses and no symptoms one year after receiving the treatment and this therapeutic effect was associated with both increased CD8/Treg ratios locally and boosted E6/E7 T cell responses systemically. The potential for increased vaccine immunogenicity by addition of an adjuvant has been established in preclinical models (Karanam *et al.*, Vaccine 2009; 27: 1040) so a future goal is to test the adjuvanted vaccine in combination with local immune stimulation to further increase patient response rates.

Kojima, Y., Acar, A., Eaton, E.N., Mellody, K.T., Scheel, C., Ben-Porath, I., Onder, T.T., Wang, Z.C., Richardson, A.L., Weinberg, R.A. and Orimo, A.
Autocrine TGF-β and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts.
Proc Natl Acad Sci U S A 2010; 107: 20009-20014.

Much interest is currently focused on the emerging role of tumour-stroma interactions essential for supporting tumour progression. Carcinoma-associated fibroblasts (CAFs), rich in myofibroblasts, are predominantly present in the stroma of human carcinomas and substantially contribute to promoting tumorigenesis. However, the precise cellular origins of these cells and the molecular mechanisms by which they evolve into tumour-promoting myofibroblasts remain unclear. Using a co-implantation tumour xenograft model, we experimentally generated CAFs from preexisting human mammary fibroblasts that have been extracted from human breast carcinomas. These cells recapitulate the tumour-promoting myofibroblastic phenotypes of CAFs prepared from breast cancer patients. During the course of tumour progression, resident fibroblasts progressively increase cell-autonomous and self-sustaining TGF-β and SDF-1 autocrine signaling that promotes their differentiation into tumour-promoting myofibroblastic CAFs. This autocrine signalling may prove to be an attractive drug target to block the evolution of tumour-promoting CAFs.

Castillo-Lluva, S., Tatham, M.H., Jones, R.C., Jaffray, E.G., Edmondson, R.D., Hay, R.T. and Malliri, A.
SUMOylation of the GTPase Rac1 is required for optimal cell migration.
Nat Cell Biol 2010; 12: 1078-1085.

The Rho-like GTPase Rac1 is well known for its role in cytoskeletal rearrangements and cell migration. Rac activation is regulated through a number of mechanisms, including control of nucleotide exchange and hydrolysis, regulation of subcellular localization or modulation of protein-expression levels. In this study it was shown that the small ubiquitin-like modifier (SUMO) E3-ligase, PIAS3, interacts with Rac1 and enhances levels of active (GTP-bound) Rac, promoting cell migration in response to hepatocyte growth factor (HGF). Significantly, it was demonstrated that Rac1 can be conjugated to SUMO-1 in response to HGF treatment and that SUMOylation is enhanced by PIAS3. Moreover it was shown that this modification increases the levels of GTP-bound Rac1 and promotes membrane ruffling, cell migration and invasion. SUMOylation of Rac1 seems to be required for maintaining, rather than inducing, activation, and may strengthen interactions with guanine nucleotide-exchange factors or inhibit interactions with GTPase-activating proteins. These results point to the existence of a novel mechanism, through SUMOylation, for regulating GTPases.

Parker, C., Waters, R., Leighton, C., Hancock, J., Sutton, R., Moorman, A.V., Ancliff, P., Morgan, M., Masurekar, A., Goulden, N., Green, N., Revesz, T., Darbyshire, P., Love, S. and Saha, V.
Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial.
Lancet, 2010; 376: 2009-2017

This reports the conclusion of a randomised study comparing the effect of mitoxantrone with idarubicin in children with first relapse of acute lymphoblastic leukaemia (ALL). A clear survival advantage of >20% was noted in those who received mitoxantrone and the randomisation stopped early. This is one of the largest improvements by a single modification to treatment ever reported in childhood ALL. Curiously, the speed of clearance of disease, as measured by minimal residual disease (MRD) techniques was similar in both arms. Thus this serves a caveat for the use of MRD as a surrogate marker of response in evaluating new agents. Though overall toxicity was less in mitoxantrone patients, they exhibited delayed count recovery 5-12 months after receiving the drug, suggesting a noxious effect on the haematopoietic stem cell niche. Thus the clinical data contends that the microenvironment plays a major role in disease recurrence and the effect of chemotherapy may be indirect by disrupting interaction between host and tumour cell rather than by direct cytotoxicity.

Serrano, A.G., Gandillet, A., Pearson, S., Lacaud, G. and Kouskoff, V.
Contrasting effects of Sox17- and Sox18-sustained expression at the onset of blood specification.
Blood, 2010; 115: 3895-3898.

We have previously shown that Sox7 is transiently expressed at the onset of blood specification and is implicated in the regulation of cell survival, proliferation and maturation of haematopoietic precursors. In this manuscript, we have assessed, using embryonic stem cell differentiation as a model system, whether Sox17 and Sox18, two close homologues of Sox7, may act similarly to Sox7 at the onset of haematopoietic development. Sox18-enforced expression led to the enhanced proliferation of early haematopoietic precursors while blocking their maturation, a phenotype highly reminiscent of Sox7-enforced expression. In striking contrast, Sox17-enforced expression dramatically increased the apoptosis of these early precursors. Similarly to Sox7, Sox18 was transiently expressed during early haematopoiesis, but its expression was predominantly observed in CD41$^+$ cells, contrasting with Sox7, mostly expressed in Flk1$^+$ cells. Conversely, Sox17 remained marginally expressed during blood specification. Overall, our data uncover contrasting effect and expression pattern for Sox18 and Sox17 at the onset of haematopoiesis specification.

Pearson, S., Lancrin, C., Lacaud, G. and Kouskoff, V.
The sequential expression of CD40 and Icam2 defines progressive steps in the formation of blood precursors from the mesoderm germ layer.
Stem Cells, 2010; 28: 1089-1098.

During embryogenesis, the haematopoietic programme is specified from the mesodermal germ layer through the formation of haemangioblast. This precursor gives rise to a haemogenic endothelium that later on mature to generate haematopoietic precursors. A major hurdle in the quest to further understand blood formation is the lack of specific cell surface markers to identify cells with discrete developmental potential. In the present study, we identify CD40 and Icam2, two markers typically associated with the adult immunological compartment, as expressed at the earliest stages of blood specification. We show that the sequential expression of CD40 and Icam2 delineates a transition in the acquisition of the blood potential from haemangioblast to haemogenic endothelium leading to the formation of haematopoietic progenitors. Taken together, our data identify novel cell surface markers allowing us to further refine our understanding of the events marking progressive haematopoietic commitment from the mesoderm germ layer.

Morrow, C.J., Ghattas, M., Smith, C., Bonisch, H., Bryce, R.A., Hickinson, D.M., Green, T.P. and Dive, C.
Src Family Kinase Inhibitor Saracatinib (AZD0530) Impairs Oxaliplatin Uptake in Colorectal Cancer Cells and Blocks Organic Cation Transporters.
Cancer Res 2010; 70: 5931-5941.

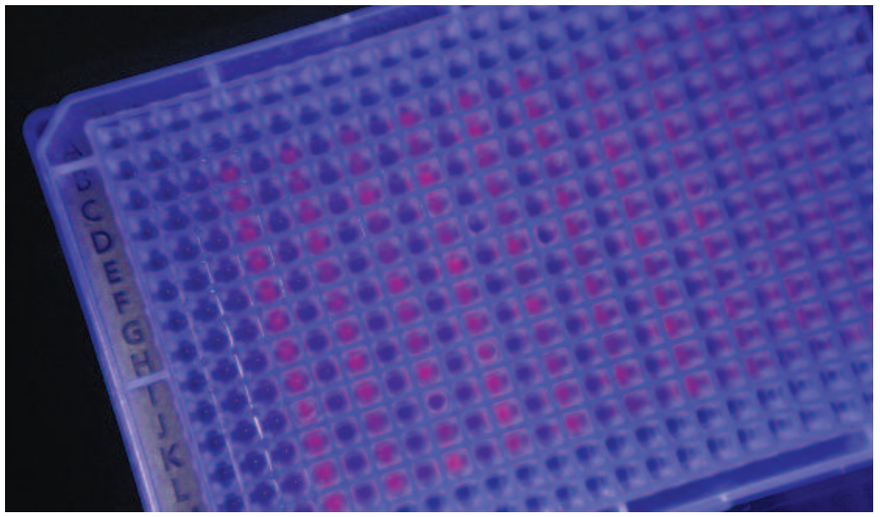
The preclinical evaluation of drug combinations is crucial, not only to identify combinations that may have clinical utility but also to determine whether a drug combination should be avoided. In this study the effect of combining the novel Src family kinase inhibitor saracatinib with colorectal cancer standard of care cytotoxic agents was investigated in colorectal cancer cell lines. The main finding was that saracatinib impaired the efficacy of the DNA-damaging agent oxaliplatin by reducing the amount of oxaliplatin taken up by the cells. This was because saracatinib prevented the drug transporter responsible for carrying oxaliplatin into cells, Organic Cation Transporter 2, from functioning effectively, resulting in reduced oxaliplatin uptake. Therefore, this work suggests that combining saracatinib with oxaliplatin in the clinic should be treated with caution, with the schedule the two drugs are given and the effect on oxaliplatin cytotoxicity being of upmost importance.

Ndamukong, I*., Jones, D.R.*, Lapko, H., Divecha, N.$^+$ and Avramova, Z.$^+$
***joint first authors; $^+$joint communicating authors**
Phosphatidylinositol 5-phosphate links dehydration stress to the activity of *ARABIDOPSIS* TRITHORAX-LIKE factor ATX1.
PLoS One 2010; 5: e13396.

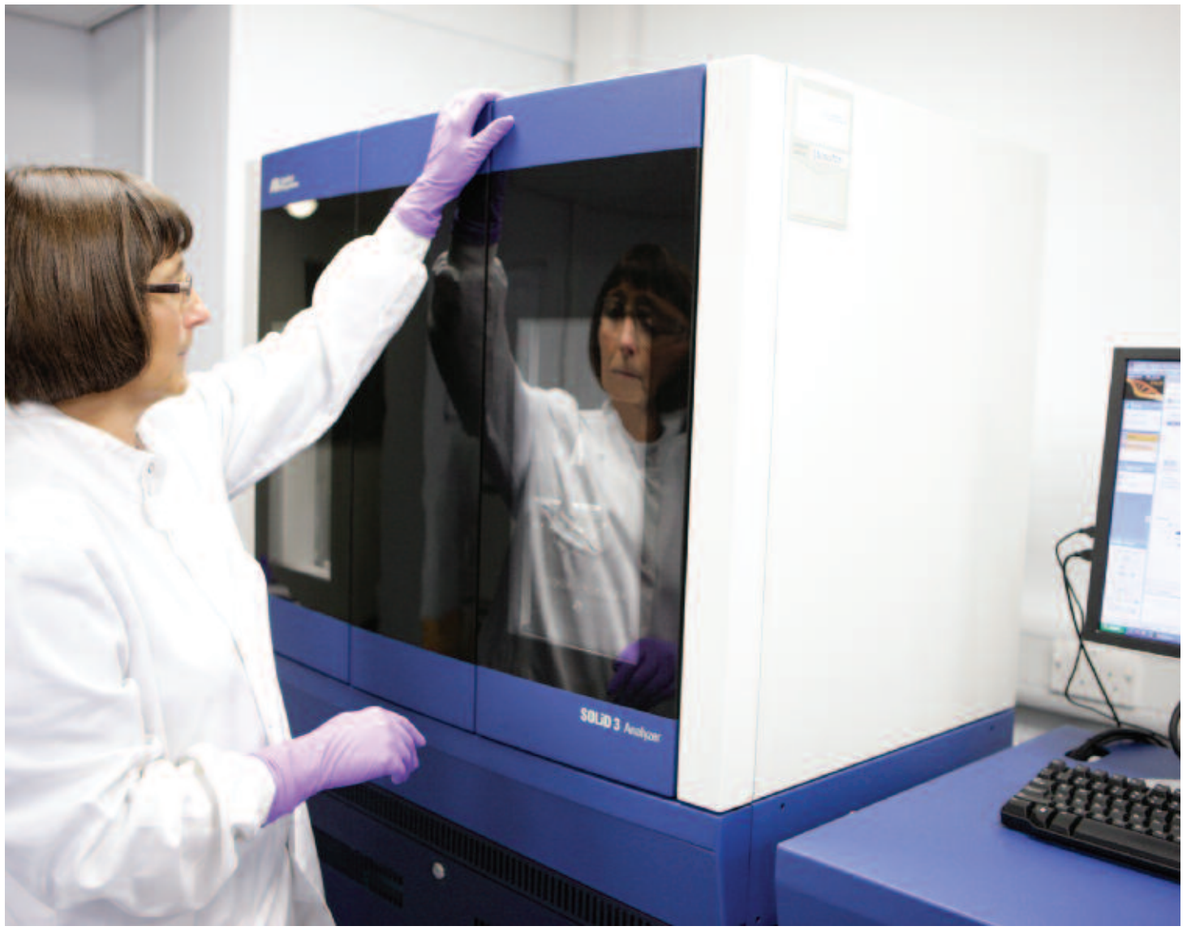
In advanced breast tumours the expression of PIP4Kβ may be a prognostic indicator of patient survival, suggesting that PIP4Kβ plays a role in tumour progression. PIP4Kβ phosphorylates and controls the levels of PtdIns5*P* a phosphoinositide that interacts with proteins possessing a PHD Zinc finger motif. Post-translational modification of histone proteins is important in the epigenetic control of gene expression patterns which modulates proliferation and differentiation and organismal adaptation to the environment. Tri-methylation at lysine 4 of histone H3 (H3K4Me3) occurs at active promoters and can enhance gene transcription. ATX1 is an *Arabidopsis* trithorax-like protein that controls H3K4Me3 and has a PHD motif which binds PtdIns5*P*. In response to environmental stress, we showed that the activity and localisation of ATX1 is controlled by changes in PtdIns5*P* levels through PtdIns5*P* interaction with the PHD finger of ATX1. Changes in PtdIns5*P* lead to a decrease in the levels of ATX1 and H3K4Me3 at the promoter of a target gene and to a decrease in its transcription. These data provide a mechanistic link between PtdIns5*P* and H3K4Me3 through the regulation of trithorax methylase activity which is often deregulated in human tumours.

Eustermann, S., Brockmann, C., Mehrotra, P.V., Yang, J.C., Loakes, D., West, S.C., Ahel, I. and Neuhaus, D.
Solution structures of the two PBZ domains from human APLF and their interaction with poly(ADP-ribose).
Nat Struct Mol Biol 2010; 17: 241-243.

Posttranslational modification of proteins by poly(ADP-ribosyl)ation in response to DNA damage acts as an important cellular signal for the recruitment of DNA repair factors and thus for the efficient restoration of genome integrity. Several eukaryotic DNA repair proteins, including the histone chaperone Aprataxin and PNK-Like Factor (APLF) possess a specific poly(ADP-ribose)-binding zinc-finger (PBZ) element in their structure that enables their timely recruitment to the sites of DNA damage *via* direct interaction with poly(ADP-ribose). In this study, we present the solution structures of PBZ modules of APLF protein in the complex with the fragments of poly(ADP-ribose), revealing for the first time the structural basis of how this novel type of zinc finger recognizes poly(ADP-ribose



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

Software Architect
Tim Yates

Bioinformatics Programmer
Chris Wirth

Bioinformatician
Jan Taylor (joint with Translational Radiobiology Group)

Scientific Officer
Paul Scutt

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

Systems Administrator
Zhi Cheng Wang (joint with IT department)

The Applied Computational Biology and Bioinformatics (ACBB) group is focused on developing an improved understanding of the 98% of the human genome that does not code for proteins, and the role they play in cancer. To do this, the group applies a mixture of computer science, software engineering and mathematics to the analysis of high-throughput data arising from proteomics, microarrays and high-throughput sequencing. We then test our hypotheses at the bench in collaboration with other research groups. Work in the group is increasingly multidisciplinary, and projects span from wet-bench molecular biology through computational analysis to more clinically focused studies.

Next generation sequencing

The human genome contains over 3 billion residues. Generation of the first sequence involved many years of collaboration between sequencing centres around the world. Progress in the field has been remarkable, and recent advances in technology now make it possible to perform equivalent amounts of sequencing in a few weeks using a single high-throughput machine. This year, the Molecular Biology Core Facility (MBCF) took delivery of one such machine, an AB SOLiD™, which is capable of generating hundreds of millions of short 50-mer reads in a single machine run over a few days. Although these machines are often used for DNA sequencing, they can also be used to sequence RNA from cells, an approach called RNA-Seq. When these data are aligned to a reference copy of the genome they can provide a highly sensitive measure of gene expression, since the number of reads aligning to a particular locus provides an estimate of the amount of RNA originating from that region. Clearly, aligning hundreds of millions of short 50-mer sequences to the 3 billion residues that make up the human genome, and then mapping them to the location of known genes, is a computationally intensive task, and a major focus of the group has been to develop the analysis pipelines and strategies necessary to use this technology routinely as part of our studies. Chris Wirth from the ACBB group, and Mark Wappett from the MBCF have been working closely together to develop automated pipelines that take data from the sequencer and onto our computer cluster, where the preliminary alignments and read-counting tasks that form the first steps of any RNA-Seq data analysis take place. To do this we are making heavy use of the open source software project BioConductor, which provides access to a wide range of statistical tools and software libraries for handling the data from our sequencer. Tim Yates has continued to develop our annotation database and genome browser, X:Map (http://xmap.picr.man.ac.uk), and we have been using our BioConductor package, xmapcore, to supply the genome annotation we need to interpret the RNA-Seq data. 2010 saw our first paper using data from the platform, and we have many other projects currently underway in collaboration with other groups in the Institute, these include work analysing ChIP Seq data and consideration of DNA level changes.

Proteomics

Although only a small part of the genome codes for proteins, a substantial proportion is transcribed, even if it does not result in protein expression. Increasing numbers of non-coding RNAs are being found to be functional, and a significant proportion of work within the group is focused on exploring their role in cancer. However, the large amount of transcription observed in the genome raises the possibility that some of these transcripts correspond to novel protein coding genes, which have yet to be identified using conventional gene-prediction techniques. In collaboration with the Biological Mass Spectrometry Facility a graduate student in the group, Danny Bitton, has been using Mass Spectrometry to identify novel proteins. In a typical proteomics identification experiment, proteins are first cleaved into sets of peptides, which are then submitted to the mass spectrometer for analysis (Bitton *et al.*, 2010). The peptides are further fragmented, and the mass/charge ratio of each of the fragments is measured. This yields a characteristic mass-spectrum for each peptide that can then be identified by searching against a database of candidate mass spectra derived from a catalogue of known proteins. Although powerful, the reliance on a database of known targets makes it impossible to identify novel peptides using this technique. We therefore generated a database of candidate proteins by translating the entire genome in all three forward and all three reverse reading frames, to yield a set of all possible proteins that could be expressed. By searching mass spectrometry data against this expanded dataset, we have been able to identify a set of additional peptides corresponding to novel protein coding genes. A challenge with this approach is that the vast majority of candidate protein sequences would never be expressed, increasing the likelihood of spurious matches by chance. Consequently, additional filtering steps were necessary to reduce the error rate to something more manageable. Using these approaches, Danny has been able to identify hundreds of novel proteins.

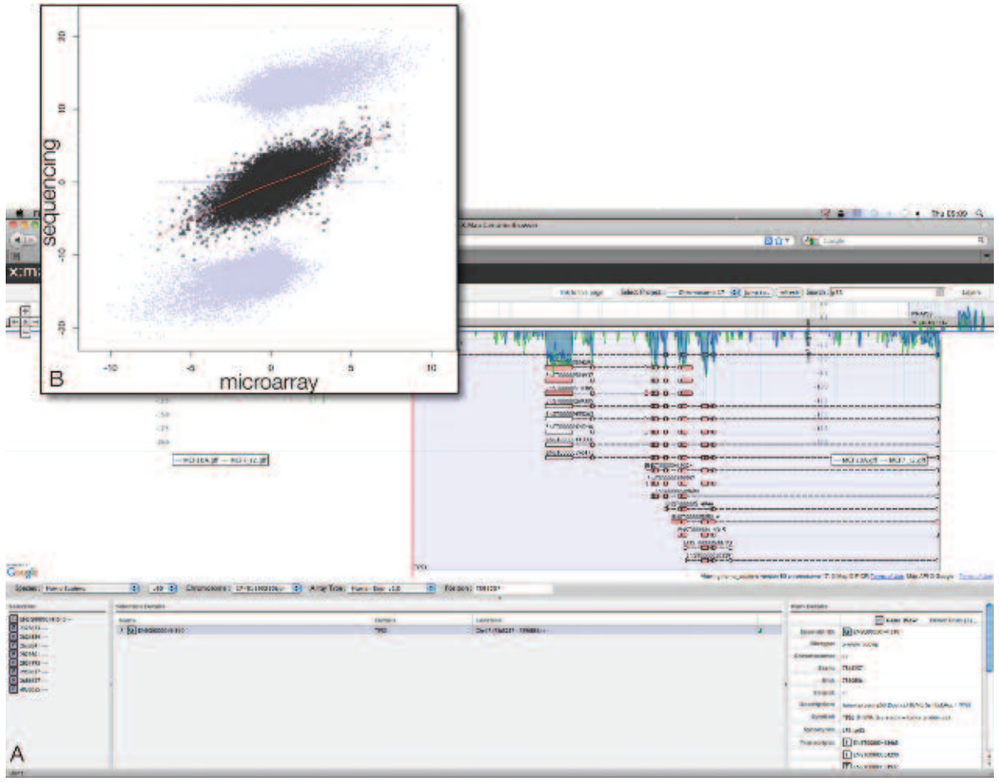


Figure 1
A) RNA-Seq data aligned to the genome and visualised in the X:Map genome browser. The image shows the region of chromosome 17 containing the gene TP53 (large purple box). Each of the individual tracks within the gene represents a different transcript, as annotated by ENSEMBL (Flicek et al., Nucleic Acids Res 2010; epub Nov 2), with the locations of exons identified by the smaller red boxes in each track. White boxes correspond to UTRs. The blue and green graphs overlayed represent RNA-Seq data for two cell lines, MCF7 and MCF10A aligned to the genome, such that the height of the peaks correspond to the $\log_2$ of the number of reads matching at that location, and thus an estimate of transcript abundance. These data can then be grouped by the exons they match using xmapcore and the R/BioConductor libraries to yield expression levels (B).

(B) Fold-change plot of log2 exon level expression data, showing high correspondence for statistically significant loci (black points). Purple clouds offset on the y-axis correspond to exons for which no-reads matched in the seq data in one sample or the other (Bradford et al., 2010).

Formalin-fixed paraffin embedded (FFPE) tissue

We are continuing to collaborate with the MBCF and the Translational Radiobiology Group in the analysis of microarray data from FFPE tissue. This material is important because vast archives of well-annotated clinical material have been preserved in this way. They present a challenge, because this method of tissue preservation was developed before techniques such as microarrays were developed, and fixation in formalin can lead to chemical modification and increased degradation of the mRNA. However, with the right protocols and data analysis methods, we are finding that it is possible to extract useful data from archival samples.

Publications listed on page 66

Carcinogenesis Group

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

Group Leader
Geoff Margison

Postdoctoral Fellow
Vitaly Latypov

Scientific Officers
Gail McGown
Mary Thorncroft
Mandy Watson

Graduate Students
Andy Marriott (until Sept)
Pat Senthong (Jointly with Dr Andy Povey, Health Sciences group at The University of Manchester)

Undergraduate Students
James Ding (Aug-Sept)
Sonia McNichol (until May)
Ali Bennett (until Sept)
Jo Kelly (from June)
Vitaly Sukhinin (from Sept)

The group's work focuses on the mechanism of action of a group of chemical compounds called alkylating agents. Agents of this type display a wide range of biological effects in living organisms all of which are attributed to the introduction of various types of DNA damage. The ability of these agents to kill cells is exploited in their use as anti-tumour agents in the treatment of certain types of cancer. Although a number of different lesions can be generated in DNA, one of these, *O*^{6}-alkylguanine, seems to be the most important. We are trying to establish precisely how cells respond to this damage and the impact that this has on the biological effects of these agents.

Background

Chemotherapeutic alkylating agents include drugs 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. These and other agents such as steptozotocin, used in the treatment of pancreatic cancer, damage cellular macromolecules *via* the formation of highly reactive methyl groups. Attack on the DNA bases generates more than a dozen different types of damage but one of the minor lesions, *O*^{6}-methylguanine which constitutes just 6% of the total damage, appears to be the product that is responsible for the majority of the biological effects. Thus, if DNA containing this lesion is replicated, an *O*^{6}-methylguanine-thymine mispair can occur, and further replication of this mispair can result in the formation of a permanent change in the form of an adenine-thymine transition mutation. Such mutations are a classical effect of the alkylating agents and have been seen in oncogenes and tumour suppressor genes in human tumours, suggesting that alkylating agents could be involved in human cancer aetiology. The *O*^{6}-methylguanine-thymine mispair can undergo an alternative fate if it is recognised by the post replication mismatch repair system, which can result in cell death or, if cells survive, chromosomal rearrangement induced by DNA recombination. The most critical factor in whether or not any of these biological effects of *O*^{6}-methylguanine effects are manifested is the damage reversal protein *O*^{6}-methylguanine-DNA methyltransferase (MGMT), which can transfer the methyl group from *O*^{6}-methylguanine and restore the DNA to its pre-damaged state in a reaction that also results in the inactivation of the protein. MGMT and other alkyltransferases can therefore protect cells against both the mutagenic and toxic effects of alkylating agents.

In some patients, the very poor response of tumours to dacarbazine or Temozolomide treatment has been attributed to this protective effect of MGMT. However, our attempts to circumvent this, using the potent MGMT inactivating drug, Lomeguatrib (LM), developed in collaboration with Prof Brian McMurry and the late Dr Stanley McElhinney (and their group at the Chemistry Department, Trinity College, Dublin) did not result in improved tumour responses. This was despite preclinical data demonstrating that LM effectively sensitised human cells and human tumour xenografts to the killing effect of Temozolomide and other agents of that type, and clinical data showing that LM very effectively ablated MGMT activity in tumours. The reasons for the lack of effectiveness of LM may include the existence of alternative repair pathways for *O*^{6}-methylguanine, and this is being investigated.

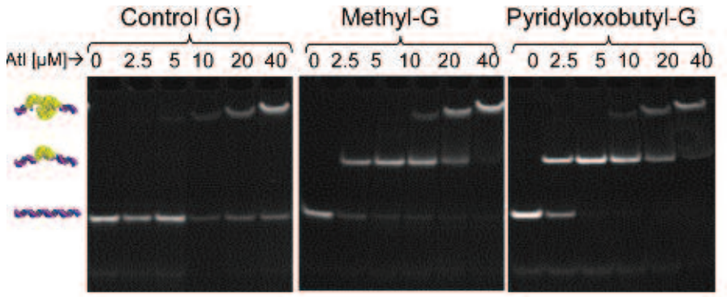


Figure 1
Electrophoretic mobility shift assay of binding of increasing concentrations of Atl1 to short oligonucleotides containing guanine (G, left panel), O^6-methylguanine (central panel) or O^6-pyridyloxobutylguanine (right panel). On the left is shown the structure of the target double stranded oligonucleotide and the suggested structures of the complexes.

Some organisms do not have an alkyltransferase gene, and we have established that these possess a different mechanism for dealing with *O*^{6}-alkylguanine damage in their DNA. Because of the possible implications to repair of alkylation damage in DNA in man, we are currently attempting to establish the precise details of how this mechanism operates using the fission yeast *S. pombe* as a model organism.

Alkyltransferase-like proteins

Alkyltransferase genes are present in prokaryotes, archea and eukaryotes and the encoded proteins share an active site domain characterized by a cysteine residue, usually within the sequence PCHRV, that accepts the alkyl group from the *O*^{6}-position of guanine. In some organisms the cysteine residue is replaced by a tryptophan residue and we have previously cloned yeast (*S. pombe*) and bacterial (*E. coli*) genes encoding these proteins and named them alkyltransferase-like (ATL) proteins. ATL genes are present in a number of organisms, but interestingly, while budding yeast only have an alkyltransferase, and *S. pombe* only has an ATL, *E. coli* expresses both alkyltransferase genes (*ada* and *ogt*) and an ATL gene (eATL) so the evolution of these proteins and why some organisms seem to need both functions is itself intriguing.

One of the characteristics of the ATL proteins is their ability to bind very strongly to alkylguanine lesions in DNA, and in our original *in vitro* studies this was shown to effectively block the repair of the lesion by MGMT. In collaboration with David Williams and his group (University of Sheffield) binding to these lesions in short oligonucleotides has also been shown and in collaboration with John Tainer and his group (Scripps Research Institute, La Jolla), crystal structures of Atl1 bound to short duplex oligonucleotides containing *O*^{6}-methylguanine and the much larger *O*^{6}-pyridyloxobutylguanine were published last year. *O*^{6}-pyridyloxobutylguanine is generated in DNA by the metabolite of a tobacco-specific nitrosamine. We suspect that rather than *S. pombe* being exposed to, or endogenously generating such agents, this reflects the size of the lesion-binding domain in ATL proteins, and this is being further examined. The crystal structures strongly suggest that both Atl1 and MGMT bind to DNA, "flip" out the *O*^{6}-alkylguanine from the base stack using an arginine "finger", rotate the phosphodiester bond by means of a tyrosine residue, and accommodate the base in the binding pocket. In the case of MGMT, this allows alkyl group transfer and rapid dissociation of the MGMT, but ATL, unable to transfer the alkyl group, remains bound.

The current evidence suggests that the bound ATL is a substrate for and/or recruits, components of the nucleotide excision repair system which ultimately results in the elimination of the lesion from DNA. Establishing precisely how this occurs and what proteins are involved is one of the major tasks of the group.

CHEMORES

Over the past few years we have been one of the members of a European Union Framework 6 programme-supported Consortium, the main objective of which is to investigate the mechanisms of chemotherapy resistance (hence the acronym "CHEMORES") in melanoma and lung cancer. Dr Paul Lorigan from The Christie Hospital Foundation Trust is co-Principal Investigator on Work Package 10, which focuses on DNA repair aspects of chemotherapy resistance, of this programme. As we have reported previously, despite promising results from *in vitro* and preclinical studies of the MGMT inactivator, LM in combination with Temozolomide, the clinical trials of this combination in late stage melanoma have shown no clinical benefit. Prolonging the treatment schedule did not improve this situation suggesting that there may be other DNA repair-based mechanisms of resistance to this agent. Studies we undertook many years ago showed that nucleotide excision repair (NER) might act on the same type of damage as MGMT in certain cell lines, but since then, the vast majority of evidence had supported the critical role for MGMT in resistance. To pursue the possibility of alternative repair processes, we have been examining melanoma cell lines from the Karolinska Institute and our own stocks for evidence of such a pathway. We are using biochemical assays of functional activity of DNA repair proteins along with highly sensitive methods of quantifying DNA lesions generated by treatment with Temozolomide. The idea is that this pathway is likely to resemble the Atl1 pathway in *S. pombe*, so the information and methodology that we have from this research is being applied to human cells.

Publications listed on page 66

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Our group studies the mechanisms and regulation of chromosome replication and cytokinesis. In animal cells and yeasts, cell division is driven by the assembly at the cleavage site of a contractile ring of actin, type II myosin and many other factors. Work with fission yeast showed that the Cdc15 protein is essential for assembly of the contractile ring and contains an amino terminal F-BAR domain that induces membrane curvature as well as an SH3 domain at the carboxyl terminus that recruits other cytokinesis factors to the cleavage site. The budding yeast Hof1 protein is orthologous to Cdc15 but is not essential, and we have screened systematically for other SH3 proteins that become essential for cytokinesis in the absence of Hof1. In this way we have identified a novel role during cytokinesis for Rvs167, the yeast orthologue of Amphiphysin in higher eukaryotes.

The BAR domain (Bin1/Amphiphysin/Rvs167) forms an alpha-helical dimer in the shape of a banana, which is present in a diverse range of eukaryotic proteins. The best characterized role of the BAR domain is to induce or sense membrane curvature by binding along the length of the dimer to phospholipids. In diverse eukaryotic species, proteins containing BAR domains have been shown to play a key role in endocytosis and other aspects of membrane trafficking. In addition, some BAR domains are known to bind a range of small GTPases, indicating that BAR domains can have other roles as well as sensing or inducing membrane curvature.

The F-BAR domain is a related module found in diverse eukaryotic proteins, and also forms curved alpha-helical dimers. F-BAR domains can induce membrane curvature *in vitro* or *in vivo*, analogous to the action of BAR domains, and have also been found to mediate key protein-protein interactions. One of the best characterized F-BAR proteins is fission yeast Cdc15, which is essential for cytokinesis. The F-BAR domain of Cdc15 is thought to play a key role in assembly of the contractile actomyosin ring at the cleavage site, in part through binding of F-BAR to the formin Cdc12. In addition to its amino terminal F-BAR domain, Cdc15 also has an SH3 domain at the carboxyl terminus that recruits other cytokinesis proteins to the cleavage site. The SH3 domain of Cdc15 is not essential, but a recent study found that this reflects redundancy with the SH3 domain of a related F-BAR protein called Imp2 (Roberts-Galbraith *et al*., J Cell Biol 2009; 184: 113).

It is not yet clear whether analogous F-BAR proteins play a similar role to Cdc15 during cytokinesis in animal cells. Nevertheless, the budding yeast orthologue of Cdc15 is known as Hof1 ('Homologue of Fifteen') and also contributes to cytokinesis, but the molecular function of Hof1 is understood less well, partly due to the fact that Hof1 is not an essential protein. Budding yeast also contains another closely related F-BAR protein called Bzz1, but there is no evidence to suggest that this shares an essential role during cytokinesis with Hof1.

We found previously that Hof1 is the major binding partner in budding yeast cell extracts of the Inn1 protein, which we showed is essential and Sho1. In addition, cells lacking Hof1 grew more slowly in the absence of a fourth factor, Sla1 (previously shown to have a related function to Rvs167).

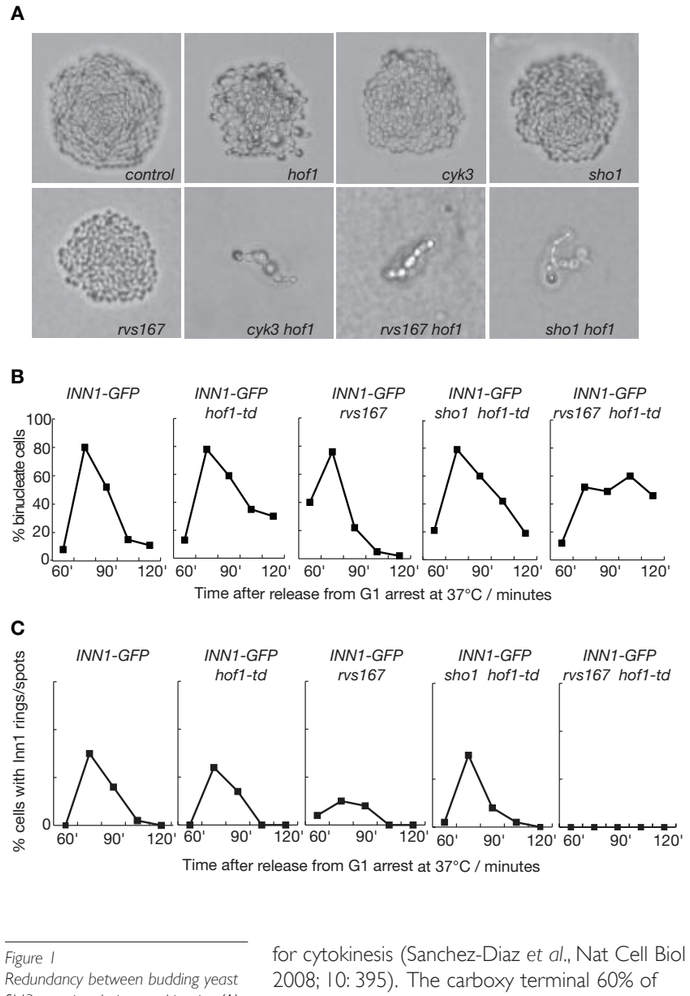

Figure 1
Redundancy between budding yeast SH3 proteins during cytokinesis. (A) Cells of the indicated genotypes were examined 24 hours after germination of spores. (B) Cells combining the heat-sensitive hof1-td allele and deletion of the indicated genes were synchronised in G1 phase at 24°C, and then monitored as they completed mitosis at 37°C. (C) Recruitment of Inn1 to the cleavage site was monitored in the same experiment.

for cytokinesis (Sanchez-Diaz *et al*., Nat Cell Biol 2008; 10: 395). The carboxy terminal 60% of Inn1 is very rich in proline and contains many PXXP motifs that are typically found in the binding sites of SH3 proteins. As Inn1 is essential for cytokinesis in contrast to Hof1, we speculated that recruitment of Inn1 (and probably other factors) to the cleavage site might be shared in a redundant fashion between Hof1 and other SH3 proteins. These need not necessarily have an F-BAR domain like Hof1 or Bzz1, as previous work showed that Hof1 becomes essential in the absence of another SH3 protein called Cyk3.

To address in a systematic and unbiased fashion the ability of other yeast SH3 proteins to play a redundant role with Hof1 during cytokinesis, we created a series of diploid yeast strains lacking one copy of the *HOF1* gene and with only one copy of each of the other 23 genes encoding proteins with SH3 domains. Upon sporulation we separated the meiotic progeny by tetrad analysis, and examined the growth of the resultant colonies. In this way we found that Hof1 becomes essential in the absence of any one of three other SH3 proteins: Cyk3, Rvs167 and Sho1. In addition, cells lacking Hof1 grew more slowly in the absence of a fourth factor, Sla1 (previously shown to have a related function to Rvs167).

Sho1 is a transmembrane protein that activates a signaling pathway culminating in the Hog1 MAP kinase, in response to osmotic stress. We found that other components of this pathway also become essential in the absence of Hof1, suggesting that *hof1*Δ cells might suffer from osmotic stress. It is interesting to note, however, that previous work indicated that Sho1 might contribute to activation of the Mitotic Exit Network that controls cytokinesis, and so it remains possible that Sho1 plays a more direct role in cell division in cells lacking Hof1.

Rvs167 is the yeast orthologue of the Amphiphysin protein of higher eukaryotes and is a regulator of the actin cytoskeleton that plays a key role during endocytosis. Whereas Hof1 is a member of the F-BAR family, Rvs167 has a conventional BAR domain at its amino terminus (an additional alpha helix distinguishes such 'N-BAR' domains from the F-BAR sub-family) and was one of the founding members of the BAR domain family. Although Rvs167 is not closely related to Hof1 and has not previously been shown to act during cytokinesis, both proteins share an analogous structure, with an amino terminal BAR or F-BAR domain, and an SH3 domain at the carboxyl terminus. We have found that cytokinesis is blocked upon simultaneous inactivation of either Rvs167 and Hof1, or Cyk3 and Hof1. Our data indicate that assembly of the contractile ring is defective in cells lacking both Rvs167 and Hof1, as is recruitment of Inn1 to the cleavage site. In addition we have shown that Inn1 can interact directly via its proline-rich carboxy terminal domain with Rvs167 Hof1, and Cyk3, in a manner dependent upon the SH3 domains of the latter proteins. Hof1 becomes essential in cells lacking the SH3 domain of Rvs167, and our data indicate that a network of SH3 proteins collaborate to recruit key factors to the cleavage site during cytokinesis in budding yeast (Targosz *et al*., in preparation). Together with the previous studies of Cdc15 and Imp2 in fission yeast, it now seems possible that diverse eukaryotes might use a variety of SH3 proteins to recruit cytokinesis factors to the cleavage site, and these SH3 proteins can have either F-BAR or N-BAR domains that are capable of inducing membrane curvature. It is possible that the high potential for redundancy between such factors might have masked the role of orthologous factors during cytokinesis in previous studies of animal cells, and this issue might be addressed by more systematic studies in the future.

Publications listed on page 67

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Errors in chromosome transmission alter the balance of tumour suppressor and tumour promoter genes. This imbalance favours changes in genome composition in the ensuing cell divisions that can lead to cancer. Chromosome segregation during mitosis is initiated by the attachment of the microtubules of the mitotic spindle to the chromosomes. Once all chromosomes have become attached to both spindle poles the chromosomes split into two identical chromatids that then move to the poles. Because the regulatory networks that regulate mitotic progression 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.

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* is regulated by the activity of a protein kinase 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 $p34^{cdc2}$. 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. 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.

Cut12, the spindle pole and mitotic commitment

Our studies of the spindle pole body (SPB) component Cut12 have uncovered a critical role for events on the spindle pole in mitotic control. Specifically, they suggest that the MPF amplifying positive feedback loop is primed from the SPB. The core data that prompted the work leading to this view are reciprocal genetic interactions between cut12 and cdc25. The *cut12.s11* gain of function mutation suppresses loss of function mutations in cdc25. Conversely, mutational enhancement of Cdc25 activity suppresses loss of Cut12 function. Consistently, combining conditional loss of function mutations within cdc25 and cut12 in the same strain generates synthetic lethality.

Cut12 and polo in mitotic commitment

In seeking ways to understand how an SPB component could compensate for loss of Cdc25, we exploited the key genetic relationship uncovered by Peter Fantes in 1979: removal of Wee1 function enables cells to survive without Cdc25. The molecular basis for this genetic observation is now very clear: without the kinase that puts the phosphate in the catalytic pocket of Cdc2, there is no requirement for a phosphatase to remove this phosphate. Thus, MPF activation and mitotic commitment will occur in the absence of Cdc25 when Wee1 is inhibited. A second cue for the direction for our studies came from the key role played by Polo kinase in the MPF positive feedback loop in higher eukaryotes. We therefore considered the possibility that Cut12 suppresses ablation of Cdc25 because it inappropriately prompts Polo to shut down Wee1. This line of work uncovered a direct relationship between polo activity and Cut12 status; Polo activity was elevated when Cut12 function was enhanced and severely reduced when Cut12 function was compromised. Furthermore, Polo association the SPB was modified by promotion of Cut12 function. Polo normally associates with the SPB for 30 minutes prior to mitosis. In *cut12.s11* cells this association occurred earlier, starting an hour before mitosis.

Polo and the environmental control of mitotic commitment

Phosphorylation of Polo on serine 402 promotes its association with the SPB thereby promoting commitment to mitosis. The reliance of serine 402 phosphorylation upon signalling from the Sty1 MAPK kinase links changes in the extracellular environment to the timing of cell division. For example, Sty1-dependent phosphorylation of serine 402 is promoted by a reduction in the quality of nutrients to accelerate division. Similarly MAPK driven phosphorylation at this site promotes re-entry into cell division after it has been blocked by a rapid heat shock.

Loops within loops

Our recent efforts have focused upon assessing the impact of phosphorylation upon the control of Cut12 function and addressing the means by which events on the interphase SPB influence the subsequent commitment to mitosis. Surprisingly, we have found that factors that were previously considered to be strictly mitotic are required for the interphase control of Cut12 function at the SPB to promote mitosis. Furthermore, assessment of the functional significance of phosphorylation on Cut12 at 25 mitotic different sites has identified different subsets of sites that influence Cut12's ability to modify mitotic commitment in different ways. Mutations to acidic residues, in an attempt to mimic the phosphorylated state in one subset, mirror the ability of *cut12.s11* to promote mitosis when Cdc25 activity is compromised. This suggests that the activation of Cut12 helps to flip the mitotic switch into a "pro-mitotic state" that drives commitment to mitosis.

Initiation of mitotic commitment at the pole in humans

The investigations into the initiation of mitosis in human cells by the group of Prof. Jon Pines (Gurdon Institute, Cambridge) have revealed that active MPF first appears on the centrosomes (spindle poles). This strongly suggests that the networks we are studying in yeast occur in human cells. In other words, key decisions about whether to divide or not do not arise from the gradual accumulation of a "pro mitosis" state, rather, they are taken at a discrete location, the spindle pole. This concentration of signalling to a limited subset of molecules at a discrete location facilitates rapid and highly sensitive cross talk between different signalling networks.

Lessons from yeast

The ability to manipulate genes at will in a simple organism, whose primary purpose is to divide, is enabling us to explore the finer points of the pathways that co-ordinate a successful cell division. 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.

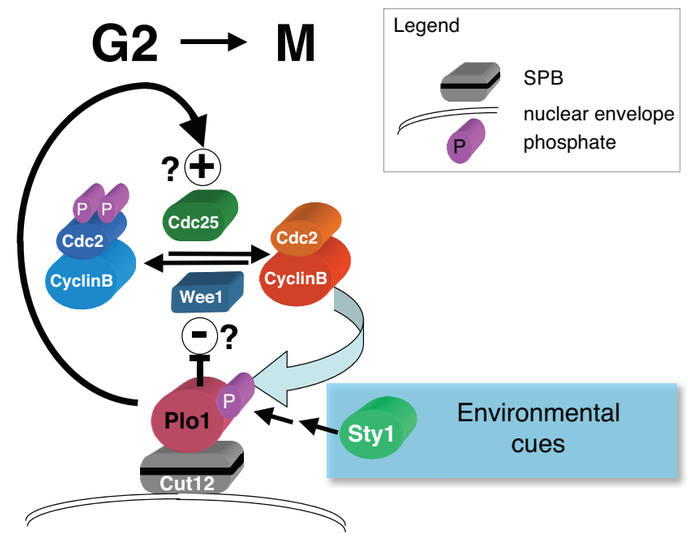

Figure 1

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MAP kinase signalling pathways are frequently deregulated in cancer. However, distinct MAP kinase families regulate different and diverse cellular programmes. While oncogene-mediated activation of the ERK MAP kinase family is commonly associated with cell survival and growth promotion, the p38 MAP kinase family can induce cell cycle arrest and apoptosis and is generally regarded as tumour suppressive. In contrast, the JNK MAP kinase family has been associated with both tumour promoting as well as tumour suppressing activities. For example, in hepatocellular carcinoma (HCC), JNKs have been found to be frequently hyperactive and in a mouse model for HCC the germline loss of JNK1 resulted in reduced tumour formation suggesting that JNK1 is a major tumour driver in liver cancer.

Among the critical targets of MAP kinase activities are members of the AP-1 transcription factor, a dimeric transcription factor consisting of Jun, Fos, and ATF family DNA binding proteins. Depending on the cellular context the composition of the dimeric complexes determines the regulation of growth, survival or apoptosis. Using genetic model systems our group is exploring the functions and molecular activities of MAP kinase pathways and in particular their targets among the ATF family, in stress and oncogenic transformation.

Exploring the role of ATF2 in cancer models

Among the ATF family, ATF2 and ATF7 are closely related proteins with a wide spectrum of transcriptional targets. To understand potential roles for ATF2, and ATF7, we have adapted different mouse models of oncogenic transformation. To explore their role in oncogenic Ras-mediated transformation and tumorigenesis in the liver, we adopted a model which is based on the explantation of embryonic hepatocyte precursors (hepatoblasts), and their oncogenic transformation *in vitro* followed by their reintroduction into recipient mice through orthotopic transplantation. We found that introduction of activated H-Ras into ATF2/7 knockout hepatoblasts resulted in early onset and significantly accelerated development of tumours in recipient livers compared to ATF2 wild type control cells. Furthermore, this model also allowed us to induce ATF2 deletion into cells that were derived from established HCC. When these cells were reintroduced into recipient mice as grafts, the ATF2-deleted tumour cells grew markedly faster compared to controls suggesting that tumours which had developed in the presence of ATF2 gained significant growth potential once ATF2 has been inactivated. *In vitro* assays, for example colony formation in soft agar, confirmed the growth advantage of ATF2/7 mutant tumour cells observed in animals. Interestingly, both double knockout hepatoblasts and tumour cells had markedly elevated levels of activated JNK. In addition experiments using chemical inhibitors showed that while colony formation was largely independent of ERK and p38, it was strongly dependent on JNK activity. In conclusion, this suggested that JNK is a crucial mediator of hepatoblast growth and HCC development and that ATF2/7 may be critical in limiting the activated levels of JNK, possibly through regulating negative acting feedback mechanisms. We are currently investigating the potential

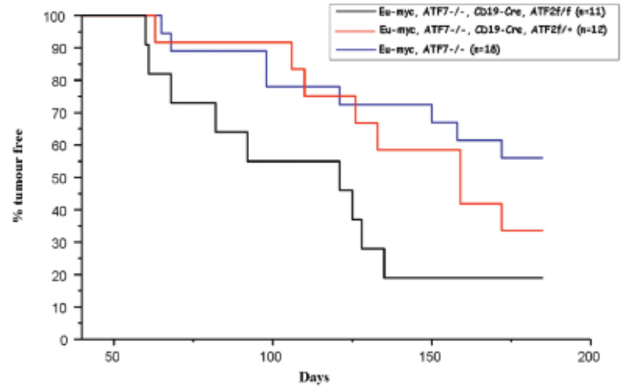


Figure 1
ATF2 B-cell specific knockout mice display significantly accelerated Eμ-Myc induced B lymphoma onset compared to ATF2 heterozygote or wild type controls (red, blue lines). (All mice were on an ATF7 knockout background.)

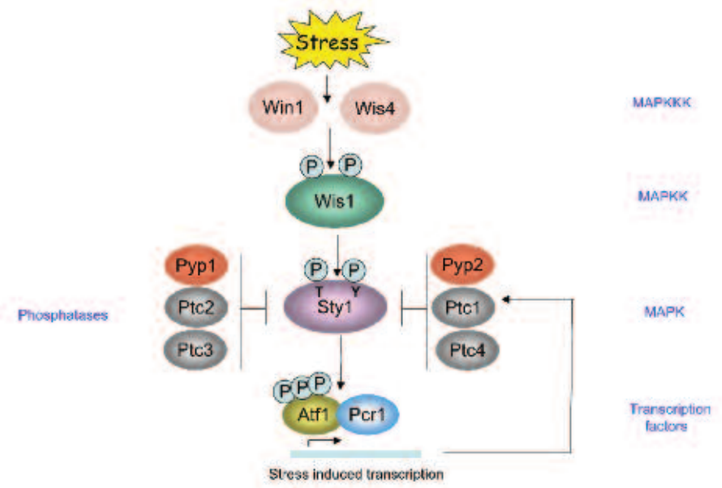
regulation by ATF2 of specific JNK specific phosphatases in hepatoblasts and HCC.

In an independent mouse model we found that in the absence of ATF2/7 in B-cells, cMyc (Eμ-Myc) dependent B lymphoma development was significantly accelerated. Further experiments *in vitro* suggested that B-lymphoma cells deficient for ATF2/7 are also less prone to increased spontaneous and genotoxic drug-induced apoptosis compared to ATF2 proficient tumour cells. Chemical inhibition experiments further showed that DNA damage induced apoptosis in cMyc transformed B cells is also JNK dependent. It is therefore likely that JNK mediated induction of apoptosis depends, at least in part, on ATF2 dependent gene expression.

Figure 2
The fission yeast stress-activated MAP kinase pathway. The genes encoding some of the phosphatases that down-regulate Sty1 are induced upon stress in a Sty1/Atf1 dependent manner and hence form part of a negative feedback loop.

Stress responses in fission yeast

The Cell Regulation Group also uses fission yeast as a convenient model to gain insights into the nature and regulation of stress responses. As these processes are conserved in eukaryotes, it is anticipated that these insights will be relevant to mammalian cells. In *Schizosaccharomyces pombe*, the Sty1 MAP kinase plays a key role in mediating a general stress response. Like its mammalian counterpart, p38, Sty1 is phosphorylated and activated by a variety of stress stimuli and inactivation of the kinase results in pleiotropic stress sensitivity.



Sty1 phosphorylates a number of targets and co-ordinates an integrated response to stress by affecting the activities of proteins involved in a range of cellular activities; an example being the Atf1 transcription factor which directs the expression of many stress-induced genes. We have developed a novel biochemical approach to identify targets of Sty1 and are currently characterising a number of previously unidentified substrates for this kinase. These include proteins involved in processes such as transcription, cell cycle control and signal transduction.

For any MAP kinase, both the amplitude and duration of signalling is carefully controlled. These parameters can greatly influence the cellular response to the input signal and their control is orchestrated through positive- and negative-acting signals, as well as through the spatial distribution of the pathway's components. The negative-acting signals include phosphatases which dephosphorylate and thereby inactivate the MAPK. Two classes of phosphatase act upon Sty1: the Pyp and the Ptc phosphatases which dephosphorylate P-tyr and P-thr residues respectively. The tyrosine-specific phosphatase, Pyp1, is constitutively expressed and is largely responsible for maintaining low levels of active Sty1 under basal conditions. We have found that Pyp1 is a phospho-protein that becomes hyper-phosphorylated upon stress. Many of the phosphorylation sites have been mapped and a kinase responsible for a subset of them has been identified. We have generated both non-phosphorylatable and phospho-mimic mutants of Pyp1 and are using these to study how phosphorylation of the phosphatase affects its ability to modulate Sty1 activation.

Ptc4 is a PP2C family phosphatase and we have found that it regulates both the magnitude and duration of Sty1 activation in response to hydroperoxides, but not to other stress conditions. Furthermore, Ptc4 undergoes a post-translational modification specifically in response to hydroperoxides. The oxidative stress-generated isoform has increased affinity for Sty1 thus providing an explanation for the stress-specific nature of Ptc4's regulation of Sty1. We are currently investigating the mechanism that underpins the modification of Ptc4 under these conditions.

Publications listed on page 67

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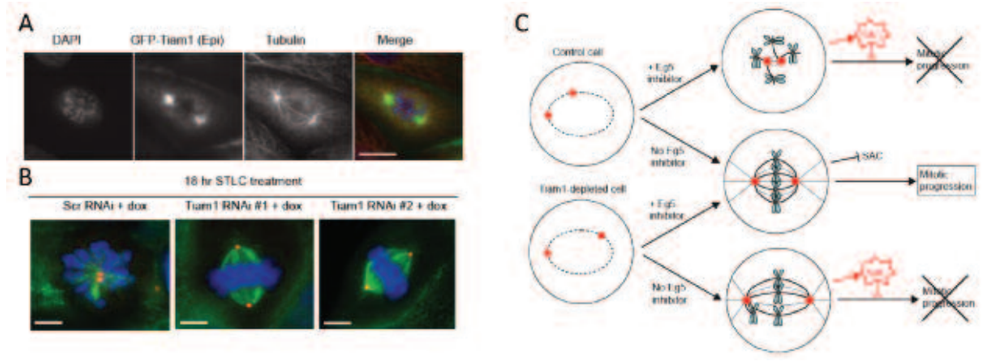
Tumour initiation and progression result from inappropriate activation of intracellular signalling cascades. Rho-like GTPases are molecular switches in signalling pathways that regulate cytoskeletal and junctional organisation, as well as gene transcription. In this way, Rho proteins influence cell morphology, 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 the mechanisms by which certain regulators of the Rho protein Rac control cell cycle progression and cell adhesion and how their activities, as well as activity of Rac itself, 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. 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. Nonetheless, the few skin tumours arising in these 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).

One mechanism by which Tiam1 and Rac suppress malignant progression is through promoting cell–cell adhesion. 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 and Collard, Curr Opin Cell Biol 2003; 15: 583). Moreover, 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 disassembly. 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. Abrogating Tiam1 phosphorylation and degradation suppressed 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*., Mol Cell 2009; 33: 639).

Figure 1
(A) MDCKII cells stably expressing GFP-tagged Tiam1 (green) stained also for tubulin (red) and DNA (blue) reveal Tiam1 localization to centrosomes at prophase. (B) Cells treated with low concentrations of the Kinesin-5 (Eg5) inhibitor- STLC- and expressing control siRNA (Scr RNAi) display monopolar mitotic spindles while cells treated with STLC and expressing siRNA selectively targeting Tiam1 (RNAi #1 and #2) have bipolar spindles. (C) Model: Suppression of Eg5 in control cells leads to a monopolar spindle, resulting in the activation of the spindle assembly checkpoint (SAC). Conversely, Tiam1-depletion results in a greater intercentrosomal separation in the early stages of mitosis, leading to mis-aligned chromosomes and activation of the SAC. However, Eg5 suppression within a Tiam1-depleted cell now restores the inter-centrosomal distance to that of a 'control' Eg5 inhibitor-untreated mitotic cell, thus allowing efficient chromosome congression and therefore progression through mitosis.



Novel role of Tiam1-Rac signalling during bipolar spindle assembly that facilitates chromosome congression and mitotic progression

Centrosome separation, critical for bipolar spindle formation and subsequent chromosome segregation during mitosis, occurs via distinct prophase and prometaphase pathways. Kinesin-5 (Eg5), a microtubule (MT) motor, pushes centrosomes apart during bipolar spindle assembly and its suppression results in monopolar spindles and mitotic arrest. Forces that antagonize Eg5 in prophase are unknown. We identified a new force-generating mechanism mediated by Tiam1, dependent on its ability to activate Rac. We revealed that Tiam1 and Rac localize to centrosomes during prophase and prometaphase, and Tiam1, acting through Rac, ordinarily retards centrosome separation. Importantly, both Tiam1-depleted cells in culture and Rac1-deficient epithelial cells *in vivo* escape the mitotic arrest induced by Eg5 suppression. Moreover, Tiam1-depleted cells transit more slowly through prometaphase and display increased chromosome congression errors. Significantly, Eg5 suppression in Tiam1-depleted cells rectifies not only their increased centrosome separation but also their chromosome congression errors and mitotic delay. These findings identified Tiam1-Rac signalling as the first antagonist of centrosome separation during prophase, demonstrated its requirement in balancing Eg5-induced forces during bipolar spindle assembly *in vitro* and *in vivo*, and showed that proper centrosome separation in prophase facilitates subsequent chromosome congression (Woodcock *et al*., 2010).

SUMOylation of the GTPase Rac1 is required for optimal cell migration

It is well established that Rac1 induces cytoskeletal rearrangements required for cell migration. Rac activation is regulated through a number of mechanisms, including control of nucleotide exchange and hydrolysis, regulation of subcellular localization or modulation of protein-expression levels. We performed a screen for proteins that interact with Rac under conditions of migration and identified the small ubiquitin-like modifier (SUMO) E3-ligase, PIAS3, as a novel Rac interacting protein. We found that PIAS3 interacts better with the GTP-bound form of Rac. We then showed that PIAS3 is required for increased Rac activation and optimal cell migration in response to hepatocyte growth factor (HGF) signalling. Subsequently we demonstrated that Rac1 can be conjugated to SUMO-1 in response to hepatocyte growth factor treatment and that SUMOylation is enhanced by PIAS3. We also showed that the GTP-bound form of Rac is a better substrate for SUMOylation. Furthermore, we identified non-consensus sites within the polybasic region of Rac1 as the main location for SUMO conjugation. We demonstrated that PIAS3-mediated SUMOylation of Rac1 controls the levels of Rac1-GTP and the ability of Rac1 to stimulate lamellipodia, cell migration and invasion. The finding that a Ras superfamily member can be SUMOylated provides an insight into the regulation of these critical mediators of cell behaviour. Moreover, our data revealed a role for SUMO in the regulation of cell migration and invasion (Castillo-Lluva *et al*., 2010).

A distinct role for the Tiam1 family member, 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 STEF is responsible for Rac activation 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 (Rooney *et al*., 2010).

Publications listed on page 67

Clinical and Experimental Pharmacology Group

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

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CEP validates and implements pharmacodynamic, prognostic and predictive biomarkers working in tandem with the Early Clinical Trials Unit at the Christie Hospital. This year witnessed the opening of the new £35M Cancer Treatment Centre incorporating one of the largest early clinical trials units worldwide. In parallel, CEP opened its third Good Clinical Practice (GCP) biomarker laboratory reflecting the current and future significant increases in clinical trials incorporating biomarkers of cell death and of angiogenesis, mutation detection in circulating free DNA (cfDNA) and Circulating Tumour Cells (CTCs). Biomarker research highlights reported this year include preclinical pharmacology of PI-3K inhibition, studies of CTC number in lung cancer, a proteomic approach to define early changes in the micro-environment of a cell destined for apoptosis and pilot studies with colleagues at the Wolfson Molecular Imaging Centre (WMIC) to examine changes in imaging biomarkers that accompany apoptosis or cytostasis.

Preclinical models of induced tumour cell death and cytostasis as tools for circulating biomarker discovery and characterisation of imaging biomarkers

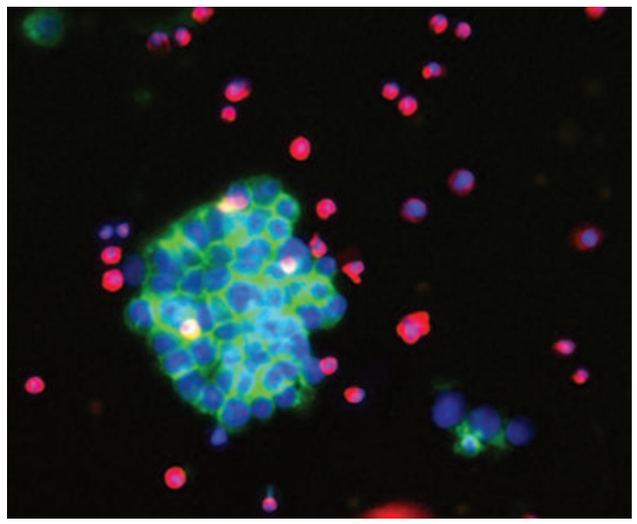
We developed and validated colorectal cancer cell lines engineered to express a robust doxycycline (dox) inducible constitutively active mutant of effector caspase 3. This allows induction of synchronous apoptosis where changes in released/secreted proteins early and late after 'death switching' are being identified using quantitative proteomics both in monolayer culture, and in the blood stream of mice bearing 'death switched' tumour xenografts in collaboration with Prof Tony Whetton's proteomics team. Proteins released into cell media from 'death switched' cells *in vitro* were also identified in the bloodstream of mice bearing 'death switched' tumours. Interestingly, early after dox addition and before loss of plasma membrane integrity, most changes in secreted/released proteins were decreases suggesting reduction of molecules normally released by viable tumour cells into their microenvironment might be an early consequence of triggering tumour apoptosis. A panel of cell death biomarkers is now under validation for future clinical implementation.

In collaboration with Dr Kaye Williams and Dr Chris Cawthorne at the WMIC, pilot studies in pre-clinical PET imaging, using [^{18}F]FLT (a biomarker of proliferation) or [^{18}F]FDG (a biomarker of tumour metabolism) are being conducted in mice bearing colorectal cancer tumour xenografts in which cytostasis or apoptosis are synchronously dox-induced. Changes in tumour cell population dynamics can be induced in all or a defined percentage of cells within the 'switched' tumour allowing us to explore the impact of changes in cell death or proliferation on these commonly used PET imaging biomarkers. This approach is allowing us to define the dynamic range of the imaging biomarkers and notably, the smallest percentage cells induced to die or to stop proliferating that can be detected. The imaging studies are conducted with parallel collection of tumour and blood samples allowing circulating, tissue and imaging biomarkers to be correlated with the horizon of improved interpretation of multi modality biomarker data in the clinic. Further collaborations have recently been initiated to utilise these 'cell fate switch' models further in MRI/S imaging studies to investigate the effects of tumour cell death or proliferation on the extracellular and extravascular space.

Preclinical pharmacology of PI-3K inhibition

There are compelling links between the activity of phosphatidylinositol-3 kinase (PI3K) and common human cancers and great interest in targeting PI3K as an anti-cancer therapy. By inducible over-expression of a dominant negative PI3K subunit or addition of commercially available PI3K inhibitors in colorectal cancer (CRC) cell lines, we demonstrated that PI3K inhibition reduced cellular proliferation *via* delay in G1 phase of the cell cycle without increased apoptosis. This occurred *in vitro* and *in vivo* and to the same degree in cells with mutant and wild-type *PIK3CA* (the gene encoding the PI3K subunit p110**α**). PI3K inhibition did not modulate the sensitivity of CRC cell lines to the CRC standard of care cytotoxic agents oxaliplatin or 5FU, however there was a cell line dependent increase in the sensitivity of cells to SN38 (the active metabolite of Irinotecan). The inducible dominant negative PI3K system was also exploited in a pilot phospho-proteomics study aimed at the identification of novel downstream targets of PI3K signalling. This has revealed several potential new direct or indirect targets of PI3K signalling which are now being verified.

Figure 1
Multicolour immunofluoresence of H526 lung cancer cells spiked into volunteer blood and recovered by ISET filtration onto a track edged polycarbonate membrane perforated by 8μM pores. Tumour cells were stained for E-cadherin (green), white blood cells were stained for CD45 (red) and cell nuclei were stained with DAPI (blue).



Circulating tumour cells

Clinical studies involving CTCs are currently are underway in melanoma, lung, colorectal prostate and pancreatic cancers. CTC number and in some cases assessment of drug target levels, and/or potential biomarkers of drug responsiveness are being assessed. Using the CellSearch technology platform, we demonstrated in a cohort of 101 Non Small Cell Lung Cancer patients that those with ≥5 CTCs per 7.5ml blood had a significantly worse prognosis than those with fewer CTCs (Krebs *et al*., JCO in press). We observed that tumour cells within circulating tumour microemboli (clusters of CTCs) in lung cancer patients were out of cycle and appeared to avoid apoptosis whereas some subpopulations of single CTCs were proliferative and others were apoptotic (Hou *et al*., Am J Path, in press). We recently developed a multi-colour immunofluorescence assay that can now be applied to explore the process of epithelial to mesenchyme transition in CTCs (Figure 1).

Progress on the CEP quality management system

Quality Assurance is vital to success of the three GCP laboratories and the new Biomarker Laboratory in the expanded clinical trials Unit at the Christie Hospital. The CEP QA team was expanded this year and continues to be a driving force within the Paterson, the Manchester ECMC, the national ECMC network and the Manchester Cancer Research Centre for quality assurance (QA), regulatory affairs and expertise in biomarker method validation. Twenty eight new biomarker projects were initiated this year, 25 protocols were written, reviewed and approved; 31 study reports were QA checked and signed off and 56 Standard Operating Procedures were issued. Significant QA input was invested in three major undertakings in 2010 a) the implementation of a Laboratory Information Management System (LIMS), b) achieving compliance to the MHRA guidance document on GCP for laboratories, and c) conducting a comprehensive programme to validate all the software systems within CEP.

Publications listed on page 67

DNA Damage Response Group

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

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Dea Slade

DNA is constantly exposed to damage and living organisms have evolved a variety of DNA repair mechanisms to maintain genome stability. Inadequate or abnormal DNA repair can cause diseases that in humans are associated with cancer, neurodegeneration, immunodeficiency or developmental abnormalities. Therefore, furthering our understanding of the molecular pathways employed in the mammalian response to DNA damage potentially provides a basis for the development of new therapies in the treatment of human disease.

Many cancer therapy procedures, such as radiotherapy and certain types of chemotherapy, work by overwhelming the capacity of the cell to repair DNA damage, resulting in cell death. Most rapidly dividing cells - cancer cells - are preferentially affected by such treatments, providing the opportunity to use DNA damaging agents to selectively kill cancer cells. In addition, genomic instability is the driving force of cancer development, which requires multiple DNA mutations resulting in the loss of cellular growth control. In order to accelerate the accumulation of these genetic changes, cancers often sacrifice specific DNA repair pathways. This can make cancer cells additionally susceptible to DNA damaging agents and/or to inhibitors that block alternative repair pathways that allow cancers to thrive without the full DNA repair repertoire. For these reasons, studying the protein components involved in the repair of damaged DNA has been proven to be a valuable strategy in searching for novel approaches and targets in cancer therapy.

Poly(ADP-ribosyl)ation in the 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 poly ADP ribose polymerases (PARPs) responsive to DNA strand breaks (PARP1 and PARP2). Poly(ADP-ribosyl)ation arising at the sites of damaged DNA serves as a platform for the specific recruitment and scaffolding of DNA repair complexes. In addition, damage-induced poly(ADP-ribosyl)ation has been known to have a role in the relaxation of 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 a very promising novel 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 poly(ADP-ribose)-dependent DNA repair processes is likely to be 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 the 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). One of the human proteins containing a PBZ motif is a protein called Checkpoint protein with FHA and RING domains (CHFR). CHFR is a 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 a 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 the CHFR protein is critical for checkpoint activation. Another PBZ-regulated protein that we are studying is a protein called Aprataxin-PNK-like factor (APLF). APLF 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 1). We recently discovered that the role of APLF is to act as a histone chaperone to modulate chromatin structure and facilitate DNA repair reactions in response to poly(ADP-ribose) signalling (Mehrotra *et al*., Mol Cell 2011; in press).

Another class of proteins in focus of our research are macro-domain proteins (Figure 2). The macro-domain is another module with the capacity to bind poly(ADP-ribose) and we recently identified several human macro-domain chromatin-associated protein factors that are recruited to broken DNA ends in a poly(ADP-ribose)-dependent manner. These include a histone H2A variant called MacroH2A and chromatin remodeler called ALC1 (Amplified in Liver Cancer; also know as CHD1L), as well as several other uncharacterized macro-domain proteins.

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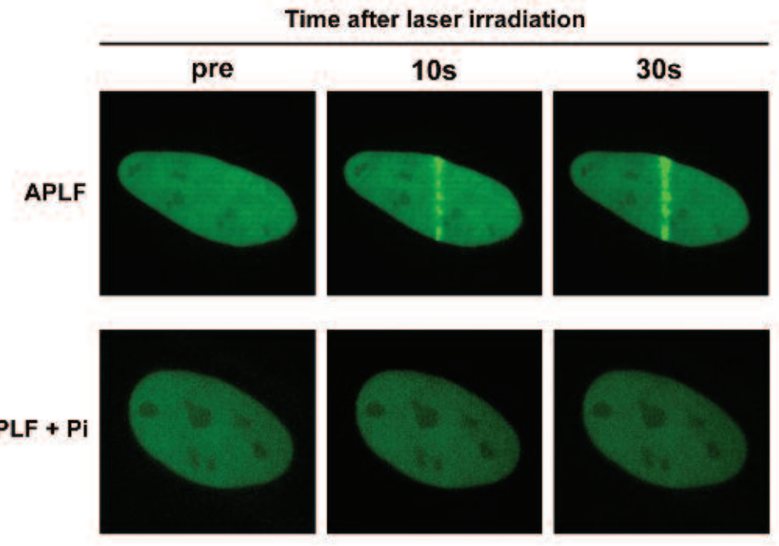

Figure 1
Recruitment of fluorescently labelled APLF protein to the laser-induced DNA break sites in the cell nucleus. The recruitment is blocked by treatment of cells with a specific PARP inhibitor (lower panel).

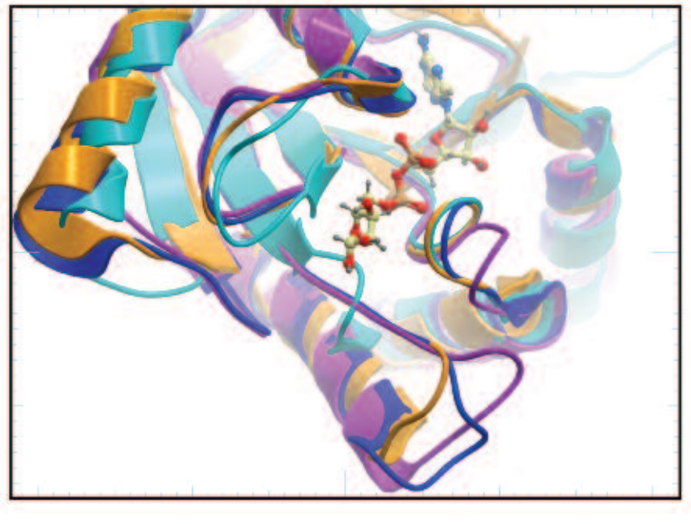

Figure 2
3D structural overlay of the 4 different macro-domain protein active sites with bound ADP-ribose.

Drug Discovery Group

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

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During 2010 we have opened and equipped a new Drug Discovery laboratory, recruited a highly skilled team and have initiated the Manchester Cancer Research Centre drug discovery portfolio. Four hit-to-lead phase projects are now underway targeting a variety of cancer targets. These activities are underpinned by multiple collaborations and significant investments in infrastructure and informatics.

Infrastructure

Following completion of the building phase, on time and within budget, in December 2009, the refurbished laboratory was equipped with capital items and open for the arrival of the first new recruits in January 2010. By the end of April, the team was up to full strength with five bioscientists and five medicinal chemists. In the laboratory design, we have sought to integrate closely the making, by chemists, and testing, by bioscientists, of novel compounds. A particularly important feature of this "make-test" workflow is the ability to handle accurately nanolitre quantities of compounds in solution using state-of-the-art acoustic dispensing technology. This has significant benefits both for chemical synthesis (less compound required) and biological data quality (accurate and consistent dilution and dispensing).

Informatics

Apart from the facilities described above, we have also made major investments in integrated informatics platforms to support the drug discovery process. As a key part of this, we have purchased in an in-house "Dotmatics" database to keep accurate records of the compounds synthesised in the laboratory and all the biological information accrued on them. This informatics capability allows our project teams to access, process and visualise complex datasets and make optimum decisions around project strategy and progression.

We have also generated an internal database of some three million commercially available, drug-like compounds which can be used to support and complement our internal synthetic efforts. These compounds, carefully selected to remove any potentially troublesome or toxic chemical features, also form the basis of our "virtual screening collection", the use of which is described in more detail below. In collaboration with an expert chemoinformatics partner, we have completed the major task of formatting this database for use in generating startpoints for novel targets and are now actively employing this information to progress two drug discovery projects.

In mid-2010, we began a phased move from paper-based laboratory records toward a newly established electronic laboratory notebook (ELN) system. ELN integrates closely with both our databases of chemical reagents and our database of compounds for screening, allowing increases in our efficiency and productivity alongside greater accuracy of recorded data. Moreover, when fully implemented, it will allow rapid searching of all our laboratory experiments across the group, giving every user rapid access to stored historical data and technical information. This ELN functionality is being developed in close conjunction with Dotmatics, and our early adoption allows the precise tailoring of the software to meet our specific needs and requirements within the Drug Discovery group.

Projects

In order to kick start our capabilities and project portfolio we have engaged in a major multifaceted collaboration with the Cancer Research Technology Drug Discovery Laboratory (DL) in London. The first component of this collaboration was to deploy our new medicinal chemistry group onto one of the cancer lead identification projects in the DL. In just six months, our chemistry team designed and synthesised over 350 novel compounds. This has significantly advanced this project, and one of the chemical series embellished by our medicinal chemists has been selected for further optimisation by the DL team. In addition to the benefits for this cancer drug discovery project, this collaboration also allowed our chemistry capability to be fully established and immediately deployed onto our first in house hit-to-lead project in September. The second vital component of the DL collaboration has been the running of high throughput screens (HTS) for novel cancer drug targets initiated in our laboratory. In HTS, a simple drug target-related assay is interfaced with a collection of ~100,000 diverse chemicals in order to try and identify compounds ("hits") which interfere with the functional activity of the target. The first HTS campaign with an MCRC target was completed in July 2010 and has identified useful start points for our initial hit-to-lead project. At the time of writing, a second HTS campaign is also underway against a second target and these efforts are anticipated to generate preliminary data in December 2010.

Whilst HTS campaigns can provide useful chemical startpoints, we have diversified our hit-finding efforts and are also employing virtual screening (VS) on projects within our portfolio. In VS, compounds are computationally assessed *in silico* to calculate how well they interact, in all possible conformations that they can adopt, with the biological target of interest. Those with the most promising calculated affinity can then be purchased and screened in a real biological assay to confirm this activity (or not). The virtual nature of this approach allows the investigation of a higher number and wider diversity of chemical compounds than would be possible, or cost-effective, in a HTS screen. VS is not without its limitations and can only be utilised when the atomic structure of the target protein is known. Despite this caveat, it offers a useful strategy for the generation of starting points for new drug discovery programmes which is complementary to HTS.

Our current drug discovery portfolio consists of four hit-to-lead projects, two of each directed at metabolism and DNA repair biological targets. In support of these projects, we have initiated a range of target biology and technology-related collaborations both within and beyond the MCRC.

The future

In 2011, with the increased funding stream for this programme, we will be doubling the size of the team and progressing the project portfolio from hit identification to the more advanced lead identification phase, in which the potential of a chemical series to develop a drug is explored further.

Figure 1
Views of the laboratory and some of the equipment used in the drug discovery process

Immunology Group

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5T4 oncofetal glycoprotein was discovered while searching for molecules with invasive properties likely to be shared by trophoblast and cancer cells. 5T4 expression has been shown to influence adhesion, cytoskeletal organization and motility, properties which might account for its association with poorer clinical outcome in some cancers. Our recent work reported the unexpected discovery that 5T4 molecules are required for functional expression of CXCR4 at the cell surface of embryonic cells (Southgate *et al*., 2010).

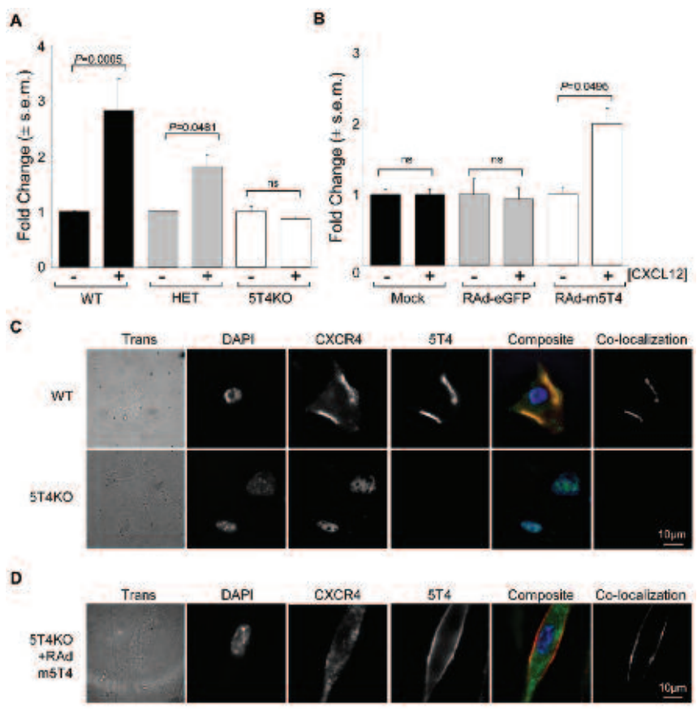
CXCR4 mediated chemotaxis is regulated by 5T4 oncofetal glycoprotein

One significant transcriptional change identified in a microarray analysis of differentiating embryonic stem (ES) cells, stratified by up-regulation of surface 5T4 expression, was the down-regulation of transcripts for the dipeptidyl peptidase IV, CD26, a cell surface protease that cleaves the chemokine CXCL12. Interestingly, differentiating ES cells also showed an up-regulation of CXCL12 transcription. CXCL12, through its receptor CXCR4, regulates many biological processes but also plays an important role in tumorigenesis. Therefore, the inverse correlation between 5T4 and CXCL12 with CD26 transcript levels during mouse ES cell differentiation, and the known roles of these molecules in cell migration/motility, suggested that particular regulatory processes are common to both ES cell differentiation and tumour metastasis.

To examine biological response to CXCL12, wild type (WT) and 5T4 knockout (KO) ES cells were tested for CXCL12 chemotaxis before and after differentiation. Both WT and 5T4KO undifferentiated ES cells showed no chemotaxis towards CXCL12 but upon differentiation WT-ES cells were significantly more chemotactic, while 5T4KO-ES cells remained unresponsive. To test whether 5T4 might play a role in CXCL12-dependent chemotaxis, undifferentiated and differentiating 5T4KO-ES cells were infected with recombinant adenoviral vector encoding mouse 5T4 (RAd-m5T4) or RAd-GFP control vector. There was no change in chemotaxis of either WT or 5T4KO undifferentiated ES cells infected with the different vectors. Expression of m5T4 in differentiating 5T4KO-ES cells restored CXCL12 chemotaxis comparable to that of differentiating WT-ES cells. These data show that 5T4 expression is a necessary cofactor for CXCR4 functional expression and CXCL12 chemotaxis in differentiating ES cells. One mechanism that might account for these results would be if 5T4 molecules facilitate stable cell membrane expression of CXCR4 molecules in differentiating ES cells. Undifferentiated WT-ES cells are 5T4-negative with CXCR4 expression low and intracellular, however following differentiation both molecules can be detected at the cell surface with clear areas of co-localization. By contrast, differentiated 5T4KO ES cells show only intracellular CXCR4 expression. However, when differentiating but not undifferentiated 5T4KO-ES cells are infected with RAd-m5T4, CXCR4 can be detected at the cell surface co-localized with 5T4 molecules. These data are consistent with 5T4 molecules being necessary for the surface expression of the CXCR4 receptor and chemotaxis to CXCL12 in differentiating ES cells.

5T4 dependency for CXCR4-mediated chemotaxis is also apparent in mouse embryo fibroblasts (MEFs) as shown by: (1) a 5T4 gene-dose influence on CXCL12 chemotaxis in WT, heterozygote and 5T4KO MEFs (Figure 1A); (2) the restoration of the chemotactic response of 5T4KO MEFs by RAd-m5T4 (Figure 1B); and (3) the co-localization of some CXCR4 molecules with typical 5T4 cell surface expression in WT MEFs while 5T4KO MEFs show only intracellular CXCR4 (Figure 1C) that can be rescued at the cell surface by RAd-m5T4 (Figure 1D). Upon ligand binding, CXCR4 undergoes a conformational change that facilitates activation of heterotrimeric G proteins and signalling effectors at the plasma membrane. This initiates a signalling cascade resulting in downstream phosphorylation of proteins such as ERK1/2. These activities are dependent on CXCR4 expression at the plasma membrane and cellular events that reduce the latter can abrogate the biological effects. We have shown in MEFs that in the absence of 5T4, the CXCR4 is no longer able to activate this pathway and the phosphorylation status of ERK1/2 is not responsive to CXCL12.

Figure 1
Role of 5T4 expression in the CXC12/CXCR4 axis. (A) 5T4 gene dose related CXCL12 chemotaxis of MEFs. (B) 5T4KO MEF chemotaxis is rescued by RAd-m5T4 infection. (C) CXCR4 (green) and 5T4 (red) co-localize at cell surface (composite yellow or co-localized areas in separate channel) in WT but not 5T4KO MEFs. (D) RAd-m5T4 infected 5T4KO MEFs show co-localized surface expression 5T4 and CXCR4 (Southgate et al., 2010).



The 5T4 gene is highly conserved across different vertebrate species and the transmembrane (TM) region is completely conserved. Chemokine receptors, G-protein-coupled seven TM spanning proteins, are also highly conserved in evolution, with the hydrophobic amino acids of TM domains forming α-helical structures which anchor the receptors in the membrane. Initial interaction between CXCL12 and the CXCR4 extracellular region facilitates rapid binding and efficient extracellular anchoring while the ligand-binding N-terminus remains highly dynamic and searches for the binding cavities buried with the receptor TM helices; this second step interaction triggers conformational changes in the CXCR4 TM to induce G-protein signalling (Kofuku *et al*., J Biol Chem 2009; 284: 35240). Importantly, the chemotactic response of both differentiated ES cells and MEFs can be blocked by some but not all antibodies recognizing distinct parts and epitopes of m5T4 molecules. It seems likely that the inhibition results from allosteric effects on CXCR4 altering the nature of ligand binding or its consequences. It is possible that the 5T4 transmembrane region specifically recognizes intramembrane residues of CXCR4 and contributes not only to the stability of the CXCR4 expression in the plasma membrane but possibly also to conformational changes in the receptor which govern responsiveness to ligand.

The regulation of CXCR4 surface expression by 5T4 molecules is a novel means to control responses to the chemokine CXCL12 for example during embryogenesis but can also be selected to advantage the spread of a 5T4 positive tumour from its primary site. The influence of 5T4 on CXCL12/CXCR4 responses in human cancer is now being explored.

Publications listed on page 69

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Phosphoinositides are a family of lipid second messengers that are regulated in response to environmental changes by the activities of a network of kinases and phosphatases. Alterations in phosphoinositide levels can regulate many different cancer-relevant pathways including cell survival, proliferation, migration, cell substratum interactions and transcription. In cancer PtdIns(4,5)P_2 is at the heart of phosphoinositide signalling as it is the substrate for phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PIC) both of which are deregulated in human tumours (figure 1). 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 its levels in these two compartments can be regulated separately. PtdIns(4,5)P_2 can be synthesised by two different families of kinases using two different substrates (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 isoforms of 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 the other PIP5K isoforms. Using various techniques to study the intracellular localisation of PIP5K, we find that while all isoforms of PIP5Ks localise at the plasma membrane, they also localise to other subcellular compartments, such as the Golgi, focal adhesions or the cytokinetic furrow. These studies suggest that the synthesis of PtdIns(4,5)P_2 at these specific sites may be required to regulate specific signalling pathways. How PIP5Ks localise to specific cellular locations is not clear but is probably dependent on a combination of posttranslational modification together with isoform-specific interacting proteins. Mass spectrometry has shown that PIP5Ks are modified by phosphorylation at multiple sites and we have used affinity purification and yeast two hybrid analysis to identify novel interacting proteins.

To define how PtdIns(4,5)P_2 might regulate nuclear function we have identified nuclear proteins that interact specifically with this lipid (collaboration with Dr. C. D'Santos CRI Cambridge). 168 proteins harbouring phosphoinositide-binding domains were identified. We identified a subset of proteins with known phosphoinositide-binding such as the pleckstrin homology (PH) or plant homeodomain (PHD) modules, although the vast majority of the nuclear proteins that interacted with PtdIns(4,5)P_2 contained lysine/arginine-rich patches (K/R-(Xn=3-7)-K-X-K/R-K/R). For example, toposiomerase IIα helps to unwind DNA by creating transient breaks in the DNA. Toposiomerase IIα interacts with PtdIns(4,5)P_2 and the interaction with phosphoinositides regulates its *in vitro* decantenation activity.

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. We have developed an antibody to PIP4Kβ and in collaboration with Prof Landberg (Breakthrough Breast Biology Group and The University of Manchester) have interrogated tissue microarrays of advanced breast tumour samples. PIP4Kβ expression is both up- and down-regulated in human breast tumours and interestingly, expression is a prognostic indicator of patient survival. Studies

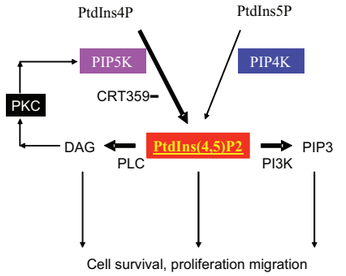

Figure 1

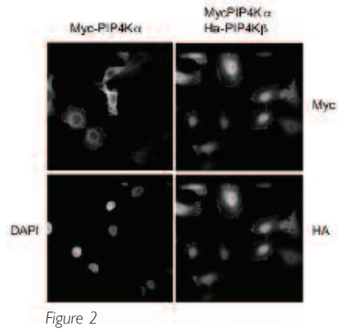

Figure 2

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.

Figure 2
Myc-PIP4Kα was expressed alone or together with HA-PIP4Kβ and cells were fixed and stained for Myc and HA. When expressed alone Myc-PIP4Kα localizes to the cytosol, however, co expression with HA-PIP4Kβ leads to enhanced localization of PIP4Kα both at the membrane and in the nucleus, where it co-localises with PIP4Kβ.

Figure 3
GST-PHD fingers were assessed for interaction with either phosphoinositides (PI) or with a Histone-H3 peptide containing lysine4, which was methylated (H3K4Me). Wild type (WT) PHD finger interacts with PI and H3K4Me, while Mutant M1only interacts with methylated peptides and M2 only interacts with PI. M3 no longer interacts with either.

in human breast tumour cell lines also show that expression of PIP4Kβ may regulate tumour cell growth. As PIP4Ks phosphorylate PtdIns5*P*, we presume that changes in the expression of PIP4Kβ lead to changes in the levels of nuclear PtdIns5*P*. However, PIP4Kβ has 2000 times less activity than PIP4Kα, which has led us to re-evaluate the intracellular localisation of the two isoforms. Unexpectedly, we have found that PIP4Kα and PIP4Kβ interact and that their interaction can relocalise PIP4Kα from the cytosol to the nucleus (Figure 2). Furthermore the ability of PIP4Kβ to regulate PtdIns5*P* levels appears to largely depend on the activity of PIP4Kα.

How PtdIns5*P* regulates nuclear processes is far from clear although some PHD finger-containing proteins are downstream nuclear targets for phosphoinositides. The PHD finger is a cross-braced Zinc finger that can interact with phosphoinositides and can also interact with methylated and non-methylated histone proteins to modulate gene transcription. For example, the PHD finger of TAF3 interacts with both PtdIns5*P* and methylated histones and TAF3 interacts with and regulates the activity of the basal transcriptional machinery. We have identified mutants within the PHD finger of TAF3 that interact only with methylated histone or phosphoinositides or with neither (Figure 3). These mutants will be crucial in defining the relationship between nuclear phosphoinositides, histone interaction and the regulation of basal transcription.

We have also investigated if PtdIns5*P* can modulate the levels of trimethylation of lysine4 of histone H3 (H3K4Me3) at the promoters of active genes. In plants the protein product of the *ATX1* gene can modulate H3K4Me3 at specific gene promoters to regulate developmental gene transcription programmes. In collaboration with Prof. Z. Avramova (UNL Center for Biotechnology and Plant Science Initiative, UNL, Lincoln, NE, USA) we show that the activity of ATX1 can be controlled by PtdIns5*P* levels through the ability of PtdIns5*P* to interact with the PHD finger of ATX1. Changes in PtdIns5*P* lead to a decrease in the levels of H3K4Me3 at the promoter of a downstream target gene and to a decrease in its transcription. ATX1 is a plant homologue of the mammalian trithorax gene family, which often undergo chromosomal translocations and deregulation leading to human leukaemias.

PIP5K as a target for drug development.

As PtdIns(4,5)P_2 is important in the regulation of cancer relevant pathways, inhibition of PtdIns(4,5)P^2 synthesis may be useful to inhibit cancer cell growth (Figure 1). PIP5K inhibitors, were identified using a high throughput PIP5K assay and have been subsequently developed by rational chemistry (Cancer Research Technology). We have developed a 96 well *in vivo* assay to measure PtdIns(4,5)P_2 in living cells, which will be used to asses the structure/function relationships of inhibitors *in vivo* to enhance their specificity and potency.

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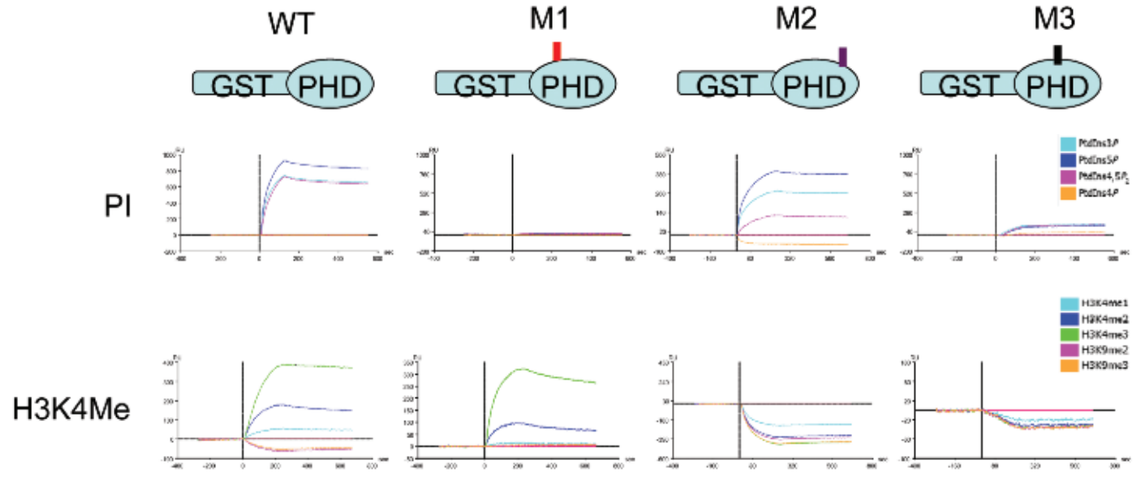

Figure 3

Leukaemia Biology Group

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Human acute myeloid leukaemias (AML) are considered to be hierarchically organised neoplasms in which the cells of the malignant clone exhibit heterogeneous proliferative potentials. At the apex of the hierarchy is a population of leukaemia stem cells (LSC) which has the highest proliferative, or self-renewal, potential. These cells sustain the malignant disease but critically, if not eradicated by chemotherapy or bone marrow transplantation, they initiate leukaemic relapse and consequent treatment failure. Understanding the genes and cellular pathways that LSCs use to undergo self-renewal is the main focus of the Leukaemia Biology Laboratory.

Proteins that regulate chromatin are important mediators of self-renewal in stem cells in general. Through post-translational modifications of histone proteins, regulation of chromatin density and targeted assembly of transcription complexes to promoters, they regulate expression of key genes that specify cell fate and function. For example, the chromatin remodelling factor ISWI is essential for self-renewal of germ-line stem cells in *Drosophila*; the polycomb protein BMI1 is essential for maintaining self-renewal of haematopoietic stem cells; and the histone acetyltransferase HTATIP (TIP60) is a key regulator of embryonic stem cell identity. Relatively little is known, however, of the role such proteins play in LSCs. To address this question, we have generated a library of lentiviral vectors that express shRNAs that target for knockdown over 250 human genes known to regulate chromatin. These include SWI/SNF, ISWI, NuRD and INO80 chromatin remodelling complex components, histone methylases and demethylases, histone acetyltransferases and deacetylases, Polycomb and Trithorax complex genes, histone arginine methylases and deiminases, and genes that regulate DNA methylation. Using both myeloid leukaemia cell lines and primary human leukaemia cells obtained from the Manchester Cancer Research Centre's Biobank, studies performed to date have identified a number of genes that appear to be required for self-renewal of human LSCs. Some genes appear to be required for cell cycle progression, others appear to be required to prevent apoptosis and, as shown in Figure 1, some appear to be required to prevent terminal differentiation. In the months ahead, validation of the roles of these genes as LSC regulators in primary human AML will be performed in further xenograft experiments and additional studies will be performed to identify mechanisms by which these genes promote or sustain self-renewal. A particular emphasis will be placed on genes with enzymatic activity that could function as therapeutic targets.

A similar screening strategy is being pursued for regulators of phosphoinositide signalling in collaboration with Nullin Divecha of the Inositide Laboratory. Proliferation of primary myeloid leukaemia blast cells is known to be dependent upon signalling through the phosphoinositide-3-kinase pathway, however the role of other components of the phosphoinositide pathway in survival, proliferation and differentiation of myeloid leukaemia cells is not known. As for the chromatin library, to date this approach has revealed a number of interesting candidate regulators of LSC function and these are currently being validated in further experiments. As before, a number of these "hits" have enzymatic activity and could be targeted therapeutically.

The bone marrow microenvironment is a critical regulator of self-renewal of both normal and leukaemic haematopoeitic stem cells. Indeed, abnormalities in bone marrow stromal cells, such as absence of *Dicer1* or *Rb1*, can lead to non-cell autonomous haematological disorders such as myelodysplasia or myeloproliferation. To further investigate the role of the haematopoietic microevironment in normal human haematopoiesis and leukaemogenesis, we have developed a heterotopic ossicle mouse model of the human haematopoietic microenvironment. Human mesenchymal stromal cells (MSCs) loaded on to a tricalcium phosphate hydroxyapatite scaffold and implanted sub-cutaneously on to the back of a mouse, undergo differentiation *in vivo* into osteoblasts and osteocytes to form bone and haematopoietic microenvironmental cells that support haematopoiesis. As shown in Figure 2, eight weeks following implantation, both bone and haematopoiesis can be seen within the heterotopic ossicle, both of which are dependent on the presence of the human MSCs. While the haematopoietic cells are murine in origin, osteoblasts, osteocytes and adventitial reticular cells are human. This model will be used to investigate candidate key regulators of the haematopoietic microenvironment which have been identified through whole genome transcriptional profiling of human bone marrow stromal cells. The model will also be used to determine the extent to which human leukaemia cells might be dependent on the presence of human microenvironmental cells, rather than murine ones, which is especially relevant given the typically poor levels of engraftment seen when human AML LSCs are transplanted in to mice.

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Figure 1A

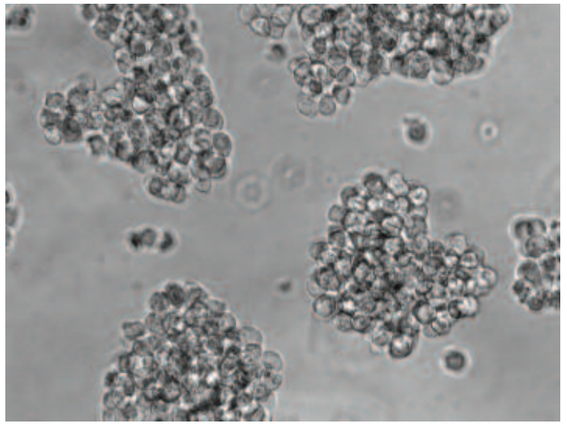

Figure 1B

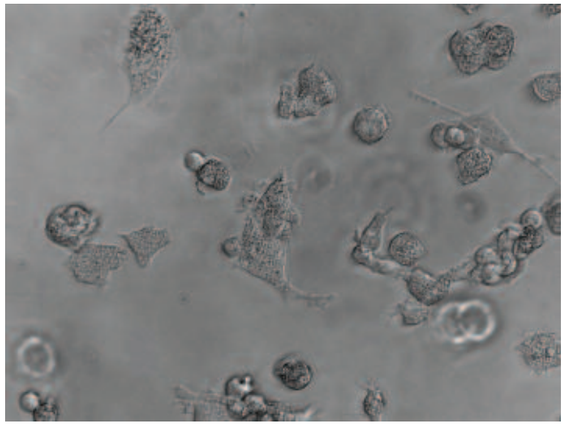

Figure 1
Knockdown of a chromatin regulatory gene induces differentiation of human MLL-AF9 myelomonocytic leukaemia cells. Control THP1 cells normally grow in clumps in semi-solid culture (A) but following knockdown of a chromatin regulatory gene using a lentivirally expressed shRNA, they differentiate into macrophages (B).

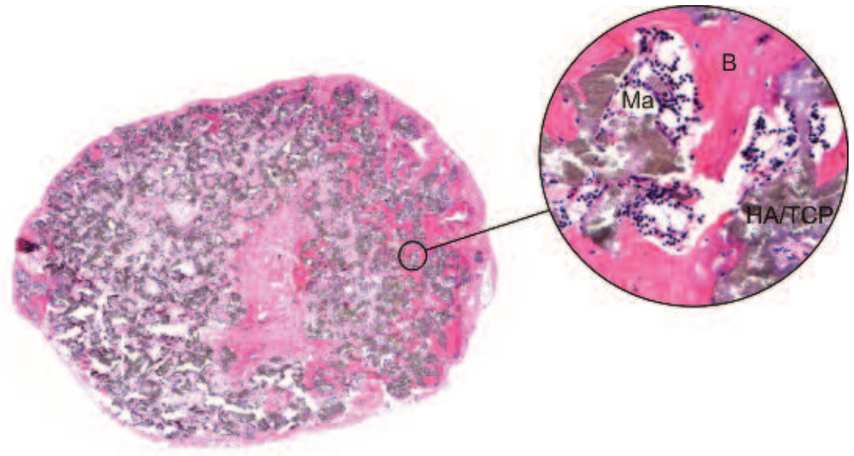

Figure 2
Heterotopic ossicles. Human mesenchymal stromal cells were loaded on to a tricalcium phosphate hydroxyapatite scaffold (HA/TCP) and implanted sub-cutaneously in an immune deficient mouse. Eight weeks later, bone (B) and extramedullary haematopoiesis (Ma) is observed. Osteoblasts, osteocytes and adventitial reticular cells forming a haematopoietic microenvironment are human, haematopoiesis is murine.

Signalling Networks in Cancer Group (since July 2010)

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

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Protein phosphorylation regulates most intracellular processes, and serves a critical role in signal transduction from the membrane to the nucleus. Now, more than ever, in-depth analysis is critical for understanding the function of phosphorylation in both normal and cancer cells, where evidence that perturbation in phosphorylation of several networks plays a causal role in cancer has accumulated at an accelerated pace as a result of genomic sequencing of tumours. Further highlighting the importance of signalling pathways in cancer is the development of small molecule inhibitors specifically targeting activated kinases in cancer patients (B-RAF and ALK), which are achieving unprecedented success in the clinic for historically unresponsive cancers, such as metastatic melanoma and non-small cell lung cancer. These proof-of-principle drugs are ushering in a new era of personalized cancer treatment, portending a future where tumours will be classified and treated based on their genetic aberrations and each cancer patient will be administered a cocktail of specific inhibitors (or possibly activators) tailored to their specific cancer.

Novel cancer associated kinases

The technology to sequence a cancer patient's genome is accelerating at an amazing pace, however our understanding of the many genetic aberrations that contribute to the cancer phenotype lags far behind the development and application of this technology. A primary objective of the laboratory is to identify novel kinases with functional mutations in cancer patients that are essential for tumorigenesis. As an initial step in identifying novel or understudied protein kinases with candidate cancer driver mutations, we used bioinformatic tools to analyze kinases with somatic mutations reported in various cancer kinome sequencing studies for those most likely to contribute to tumorigenesis (primarily lung tumorigenesis). This analysis has led to the identification of several novel kinases as rational targets, including MLK4, MYO3B, ANKK1, and SgK085.

Candidate cancer-associated kinases that our lab will study are determined based on several criteria: (1) the kinase must have limited characterization and a high probability of possessing a driver mutation (Greenman *et al*., Nature 2007; 446: 153); (2) mutations should be predicted to be cancer mutations based on bioinformatic anaylses (CanPredict and PMUT); (3) mutations should occur at evolutionarily conserved amino acids. The main goal of these preliminary studies was to identify kinases where all or almost all of the observed somatic mutations are predicted to be functional driver mutations.

To characterize the mutant kinases, our general strategy is to first assess the functional consequences of somatic mutations on overall kinase activity utilizing *in vivo* and *in vitro* kinase activity assays. We will compare the activity of

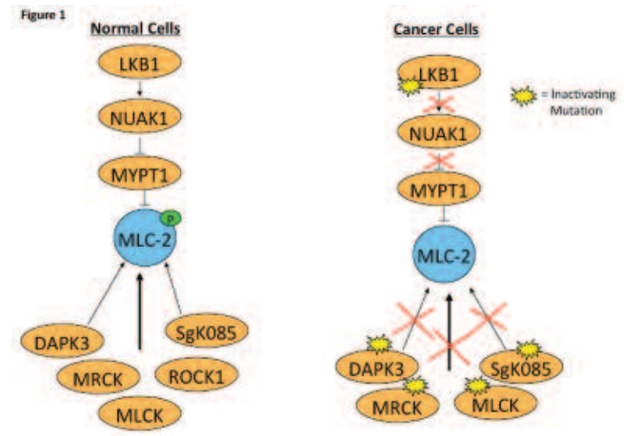
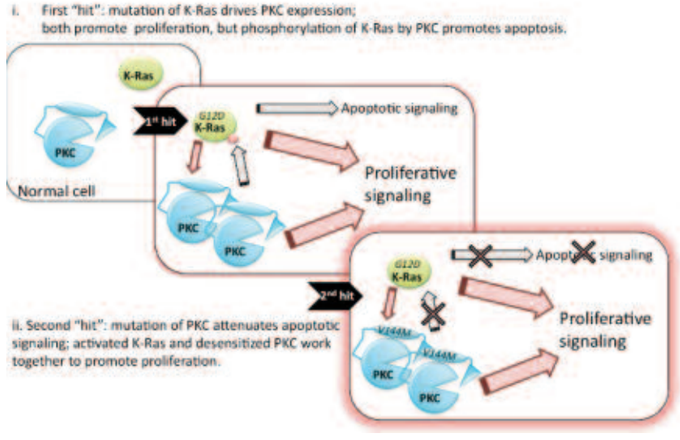


Figure 1
Shedding light on a novel signalling pathway. The recent discovery that LKB1 can activate NUAK1, leading to inactivation of MYPT1 and increased MLC-2 phosphorylation, suggests this pathway may be important in cancers with LKB1 LOF mutations. LOF mutations in other kinases, such as DAPK3, could be a means for a tumour cell to reach the same end point.

the kinases harbouring cancer mutations (engineered through site-directed mutagenesis) to wild type (WT), kinase dead (KD) and hyperactivated forms of the kinase. Next we will determine phenotypic effects of expressing the WT, KD and mutant forms of the target kinase on proliferation, survival and transformed properties of appropriate tumour and normal cell lines. We will verify the function of the kinase using si/shRNA and evaluate the role of the endogenous kinase in regulating cellular phenotypes associated with tumorigenesis. We will also investigate the molecular mechanisms utilized by the cancer mutants to promote tumorigenesis. For example, if the mutation occurs in only one allele and results in loss-of-function (LOF), we will determine if the mutant kinase acts as a dominant negative (DN) to suppress the function of the WT allele. Alternatively, if the mutation is an activating mutation, we will identify cancer relevant substrates that are phosphorylated by the cancer mutants to promote tumorigenesis. Finally we will assess the consequences of somatic mutations utilizing cell lines that harbour endogenous mutations in the target kinase. The overall goal of these studies will be to identify common and convergent pathways utilized by tumour cells to promote tumorigenesis and identify convergent and essential targets that could be exploited for the development of novel therapeutics (for example MLC-2 in Figure 1).

Figure 2
Model describing potential mechanism whereby inactivating PKC mutations contribute to K-Ras mediated tumorigenesis.



PKC signalling and tumorigenesis

The protein kinase Cs (PKC) are an important family of ten signal-transducing enzymes. There are three subfamilies of PKCs defined on the mode of regulation in response to lipid signalling - conventional PKCα, βI/II, and γ, contain a Ca^{2+}-sensitive C2 domain and tandem DAG-sensitive C1a-C1b domains; novel PKCδ, ε, η, and θ contain tandem DAG-sensitive C1a-C1b domains and a C2 domain that does not sense Ca^{2+}; atypical PKCλ and ζ contain a single C1 domain, insensitive to DAG, and are predominantly regulated by scaffolding protein interactions. The PKC family has been intensely investigated for over 25 years in the context of cancer. Historically, this arises from the discovery of PKC as the receptor for the tumour-promoting phorbol esters, such as TPA, which suggested that activation of PKC by phorbol esters promoted tumorigenesis induced by carcinogens. However, this interpretation is now open to question, since long-term treatment with phorbol esters is known to initiate degradation of PKC, thus down-regulating its activity.

Given this debate regarding the necessity of activation or inactivation of PKC family of kinases in cancer, the discovery of non-synonymous point mutations in many PKC family members, primarily in colorectal cancer (CRC) and non-small cell lung cancer (NSCLC), provides an opportunity to address this very pertinent question. We will characterize mutations observed in the PKC family of isozymes in cancer utilizing live-cell imaging techniques. Additionally, we have discovered that all PKC mutations we will be studying in CRC and NSCLC occur in the context of activating K-Ras mutations. K-Ras is a substrate for PKC, where phosphorylation of K-Ras by PKC alters its subcellular targeting and causes K-Ras to promote apoptosis. We will determine if LOF mutations in PKC can promote survival of colon cancer cells harbouring K-Ras mutations, by suppressing a K-Ras-induced apoptosis feedback loop (Figure 2). In the context of other mutations present in a given tumour, evaluation of PKC cancer mutants should begin to shed some light on the role this family of kinases play in tumour progression and provide guidance for therapeutic treatment.

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Stem Cell Biology Group

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The major interest of our lab is to decipher the cellular and molecular mechanisms that control the development and maintenance of the haematopoietic system. In this context, we study the functions of the transcription factor AML1/RUNX1 and of the transcriptional co-activator MOZ. AML1/RUNX1 is one of the most frequent target 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 to 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) cell 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. Recent studies have now provided evidence for the presence of this bipotential precursor *in vivo*.

We have recently demonstrated 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.

Transcriptional targets of RUNX1/AML1 and identification of RUNX1 binding sites

These previous studies indicate that RUNX1 is likely to regulate the expression of a critical set of genes at this stage of development. To identify these genes, we have compared gene expression in haemangioblast-derived cell populations generated from either *Runx1* deficient or *Runx1* competent ES cells. These microarray analyses of *Runx1*$^{-/-}$ and *Runx1*$^{+/-}$ transcriptomes identified a relatively large number of genes differentially expressed. It would be therefore pertinent to correlate these data with the detection of *Runx1* binding by chromatin immunoprecipitation (ChIP) to identify direct transcriptional targets. One limitation of this approach is the large number of cells required and the requisite for a ChIP grade antibody.

As an alternative approach, we have generated constructs based on the DamID strategy, developed by Bas van Steensel, NKI, Amsterdam, which alleviate the need for highly specific antibodies and large quantities of cells. Instead the chromatin associated protein is fused to the DNA adenosine methylase (Dam) of *E. coli* which will create a methyl tag at GATC genomic sequences close to the binding sites of the fused protein. These sequences can be specifically isolated by DpnI digests and amplified. We have now performed these analyses and the products generated have been sequenced in our institute by the Molecular Biology Core Facility on an AB SOLiD™ high throughput sequencing machine. We are currently analyzing these results and validating them in collaboration with James Bradford and Crispin Miller (Applied Computational Biology and Bioinformatics Group). We are also planning to extend the DamID approach to mice to survey Runx1 binding sites at different stages of haematopoietic development and at different levels of the haematopoietic hierarchy.

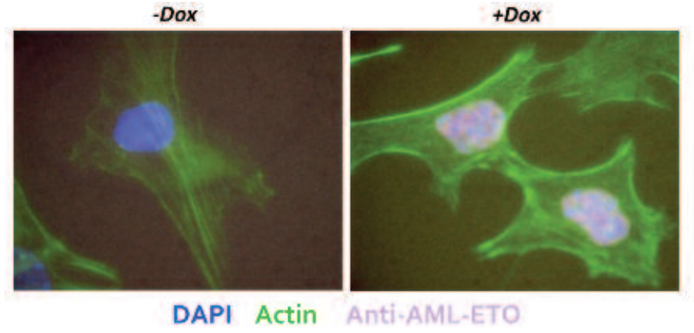

Figure 1
Detection of induced AML-ETO9a expression (+Dox) by immunofluorescence.

Model of leukaemia

To study the aetiology of leukaemia, we initiated the development of a murine model of AML based on a *Runx1* translocation. For this project, we used *AML1-ETO9a (AE9a)*, a natural alternate splice variant of the *AML1-ETO* transcript. *AE9a* was recently found to induce acute myeloid leukaemia (AML) in a mouse retroviral transduction-transplantation model. Using an ES cell line with a doxycycline *AE9a IRES GFP* inducible cassette, we first validated *in vitro* that the induced expression of *AE9a*, thought to act as a dominant inhibitor of *AML1/Runx1*, blocked the generation of hematopoietic cells during blast development. Based on these results, we subsequently generated a mouse line from these ES cells.

To restrict the induction of *AE9a* expression to the haematopoietic system, we transplanted bone marrow cells of these mice into sub-lethally irradiated recipients and subsequently fed them with either normal food or food containing doxycycline. The mice expressing *AML1-ETO9a* developed extramedullary haematopoiesis followed by the development of acute myeloid leukaemia. The disease latency was shorter when *AML1-ETO9a* was induced on a P53$^{+/-}$ background. This result is consistent with the two-hit model of leukemia development in which one genetic alteration will affect haematopoietic differentiation (such as AML-ETO) whereas another is required to alter signal transduction cascades associated with cell proliferation (such as mutations to Flt3, c-Kit or Ras). Accordingly, transplantations of the leukaemic cells led to development of AML in secondary recipients with a shorter latency.

Altogether these results indicate that we have now established a new model of leukaemia development, which will allow us to investigate further the molecular and cellular events associated with t(8;21) leukaemogenesis.

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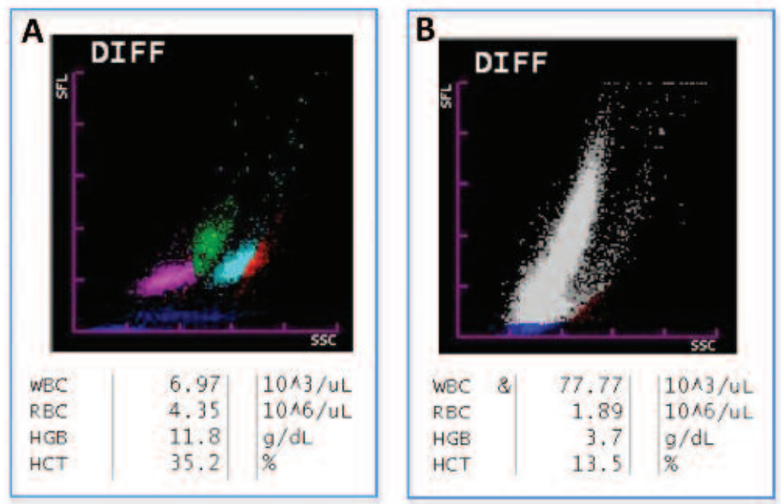

Figure 2
Blood cell analysis of control (A) and leukaemic (B) mice.

Stem Cell and Haematopoiesis Group

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The balance between proliferation and differentiation during haematopoietic development in the embryo is a complex process, the detailed molecular mechanisms of which remain to be fully characterized. Many studies suggest that leukaemia may result from the re-initiation of this embryonic programme during adult life or from the inappropriate expression of specific genes controlling the proliferation and differentiation of embryonic haematopoietic precursors. The main goal of our laboratory is to further our understanding of the transcriptional networks that orchestrate the formation of the haematopoietic system during embryonic development.

Embryonic haematopoiesis is a dynamic process that originates from mesodermal precursors and ultimately gives rise to the haematopoietic stem cells that are the source of blood cell production throughout adult life. The development of blood cells in the embryo takes place in multiple anatomical locations and incorporates a rapid succession of cell fate specification and proliferation events. The earliest precursors are multi-potential haemangioblasts, with the capacity to give rise to blood, endothelial and smooth muscle. Differentiation of endothelial and blood cells from the haemangioblast leads to the formation of yolk sac blood islands, the source of the first haematopoietic cells. Yolk sac haematopoiesis can be modelled *in vitro* by the differentiation of mouse embryonic stem (ES) cells. A number of regulators of haematopoietic specification have already been identified. Some of these regulators, such as Etv2 or Scl are critical for the proper development of all blood cells, while others, such as Runx1 or the EPO signalling network are essential only for definitive haematopoiesis and do not impair the formation of primitive erythrocytes.

In a global gene expression profiling screen for novel haematopoietic regulators, we recently identified Mxd4 as being tightly regulated at the onset of haematopoietic specification. Mxd4 is a transcription factor belonging to the Myc-Max-Mad transcriptional network. The function of c-Myc is essential for embryonic haematopoiesis: c-Myc deficient embryos do not generate definitive haematopoiesis, show impaired primitive haematopoiesis, and die at E9.5. While the widespread role of Myc in normal and tumourigenic development has been extensively studied, less is known about its functional antagonists, the Mad protein family. Like Myc, the four Mad proteins (Mad1, Mxi1, Mad3 and Mxd4) require heterodimerization with Max in order to bind DNA. Mad-Max heterodimers recognize the same consensus sequences as Myc-Max, but with opposing transcriptional effects: while Myc is generally a transcriptional activator, Mad proteins are transcriptional repressors. Most of the information we have on the role of Myc-Max-Mad interaction in embryonic haematopoiesis is from the Myc aspect. Since Mad proteins are antagonists of Myc function, we were intrigued when Mxd4 was detected in a genome-wide expression analysis showing a tightly regulated pattern of expression during embryonic haematopoietic development. We therefore set out to study the role that Mxd4 might play in haematopoietic differentiation, with the aim of establishing a better understanding of the balance between proliferation and differentiation during this process, and the specification of cell lineage type.

Our initial findings established that Mxd4 expression followed a distinctive and tightly regulated pattern during embryonic haematopoiesis *in vitro*, suggesting that the expression level of this transcription factor might play a role in the development of the blood progenitors and might modulate the dynamic of the Myc-Mad-Max regulatory network. Based on this hypothesis, we were interested to gain insight into the potential role of Mxd4 in embryonic haematopoiesis and to study the significance of its down-regulation at the time of haematopoietic commitment. This was performed using an ES cell doxycycline-inducible expression system to analyze the effect of enforced Mxd4 expression upon haematopoietic specification *in vitro*. Data obtained using this inducible ES cell system revealed that modulation of Mxd4 expression interfered with the colony-forming ability of the earliest precursors generated. Further analyses indicated that the enforced expression of Mxd4 during the emergence of haematopoietic progenitors was detrimental to the production of mature blood cells and that this effect was already apparent when Mxd4 expression was maintained only for a short period.

The reduction in colony numbers seen upon Mxd4 induction suggested that Mxd4 might play a part in survival, proliferation or differentiation of the developing progenitors. However, the observed reduction in clonogenicity was not accompanied by significant changes in lineage marker expression, suggesting no direct implication of Mxd4 in the differentiation process. In contrast, the possible implication of Mxd4 in the survival and/or proliferation of progenitors came from the analysis of cell number counts upon culture, which was significantly lower in Mxd4-induced cultures. To address whether this decrease could be linked to a decrease in cell proliferation or an increase in apoptosis, we performed cell cycle analysis on progenitors expressing or not Mxd4. A significant reduction in the S-phase population was observed in the Mxd4-expressing cells, concomitant with an increase in the G0/G1 and G2 subsets. Increase in apoptosis was not detected by either BrdU or AnnexinV staining assays. Altogether, these results suggest that enforced expression of Mxd4 influences the cell-cycle progression of haematopoietic progenitors rather than increasing apoptosis or interfering with differentiation.

To explore further the cell cycle alterations detected upon Mxd4 induction, we determined the protein levels of key cell cycle regulators in cells induced or not to express Mxd4. These analyses revealed that the protein levels of the cell cycle kinases Cdk4 and Cdk6 were markedly decreased in Mxd4-expressing cells. This was accompanied by an increase in protein level of the cell cycle inhibitor p27. Furthermore, total c-Myc, phospho-c-Myc as well as phospho-Rb levels were also consistently reduced in Mxd4-expressing cells. Altogether, these results correlated with the cell cycle profile observed upon Mxd4 induction with an increase in the cell cycle inhibitor p27 and decrease in the cell cycle activators Cdk4, Cdk6 and phospho-Rb, all effect possibly mediated via the modulation of the Myc-Max complex known to directly regulate p27 (Figure 1).

In light of Mxd4 function as an antagonist of c-Myc, we propose that the sharp down-regulation of Mxd4 in newly formed haematopoietic progenitors is necessary to allow Myc to promote proliferation and expansion of the population prior to further differentiation. Further studies will be needed to ascertain how Mxd4 function to antagonise c-Myc in this context. Their interaction might be direct through transcriptional control of the same target genes or indirect by binding to different target promoters. The reported ability of Mxd4 to bind partners other than Max raise the intriguing possibility that it might exert its effect in more than one way in specific cell context and perhaps play a role in other, as yet undetermined functions during embryonic haematopoietic development.

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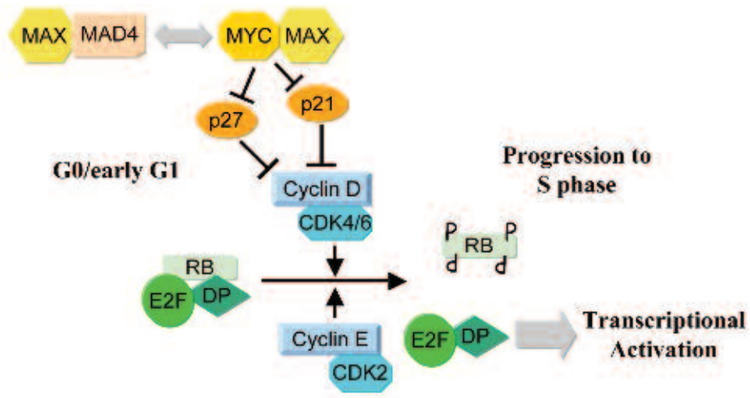
Figure 1



Stromal-Tumour Interaction Group

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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, bone marrow-derived cells etc. 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. 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 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 and (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 (for example in the *TP53* gene). However,

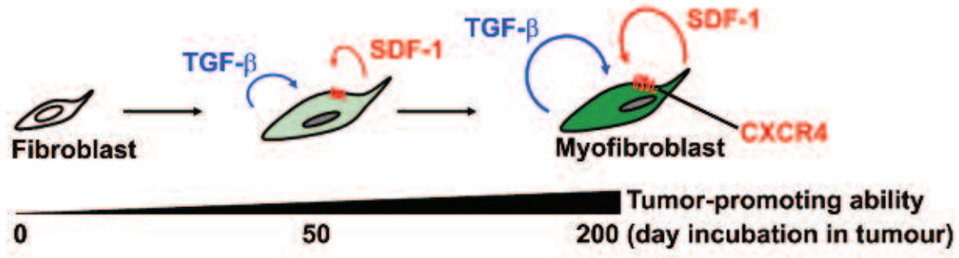

Figure 1
Coevolution of stromal fibroblasts with carcinoma cells during tumour progression. Resident stromal fibroblasts within the tumour increasingly acquire two autocrine signalling loops involving TGF-β and SDF-1 during the series of tumour progression. These autocrine signalling loops mediate transdifferentiation of stromal fibroblasts into tumour-promoting CAF myofibroblasts. (Kojima et al., 2010).

others indicate that tumour-associated stroma and CAFs exhibit no detectable genetic alterations, as gauged by comparative genomic hybridisation (CGH) and single nucleotide polymorphism (SNP) array analyses, and show alterations in epigenetic modifications of the genome, such as DNA methylation. 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 PDs in culture, similar to the behaviour of normal human stromal fibroblasts. Alternatively, the stabilization of their phenotype may depend on some type of positive-feedback signalling of the sort created by autocrine signalling loops.

We find that normal human mammary fibroblasts, when co-inoculated with breast carcinoma cells into immunodeficient mice, convert stably into tumour-promoting myofibroblasts within the resulting tumours. During tumour progression, these fibroblasts progressively elevate two autocrine signalling loops mediated by the TGF-β and SDF-1 cytokines in self-stimulating and cross-communicating fashions, thereby enhancing both their transdifferentiation into myofibroblasts and the associated tumour-promoting capability. Taken together, these findings indicate that the establishment of cross-communicating TGF-β and SDF-1 autocrine signalling gives rise to myofibroblast differentiation and mediates the evolution of residual fibroblasts into tumour-promoting myofibroblasts.

Stroma-derived signalling crucial in promoting tumour metastasis

The tumour invasion-metastasis cascade is a complex multistep process that includes localised invasion of carcinoma cells, entrance into the systemic circulation, survival during transportation, extravasation, the establishment of micrometastases in distal tissues and colonization to form macroscopic metastases. It has long been assumed that dissemination of metastatic carcinoma cells depends largely on their cell-autonomous effects, due to epigenetic and/or genetic alterations that accumulate within these malignant cells. However, emerging evidence now proposes a different schema in which metastatic spread is not totally dependant on the acquisition of additional genetic alterations within carcinoma cells. The tumour microenvironment also serves as an important determinant that encourages carcinoma cells in the primary tumour to become motile and invasive, and to disseminate into distant organs. Interaction of carcinoma cells with the tumour-associated stroma facilitates the invasion-metastasis cascade.

Tumour-associated stromal fibroblasts play a significant role in regulating migratory and invasive behaviours in carcinoma cells. This is supported by evidence indicating that stromal myofibroblasts are frequently present at the invasive front of human carcinomas. In addition, it has been shown that CAF myofibroblasts extracted from human carcinomas increase the migratory and invasive propensity of the cancer cells co-cultured alongside them in collagen gels. Tenascin C, HGF and SDF-1, which are secreted by CAFs, also play a role in mediating CAF-stimulated invasion of cultured carcinoma cells. It remains, however, unclear as to what paracrine signalling from CAFs is essential for facilitation of metastatic dissemination of carcinoma cells *in vivo* and what molecular alteration(s) is provoked in such metastatic carcinoma cells. Studying crosstalk between tumour cells and mesenchymal cells during tumour progression could help understand nature of biology of a bulk of human carcinomas and facilitate the development of novel stroma-targeted therapeutic approaches.

Publications listed on page 70

Research groups
The University of Manchester School of Cancer and Enabling Sciences

Children's Cancer Group

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Undergraduate Student
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Clinical Trials Manager
Catriona Parker

Administrator
Charlotte O'Horo

Our group investigates the biological mechanisms responsible for the variations in the therapeutic response in children with acute lymphoblastic leukaemia (ALL). We conduct a number of international clinical trials which provides us with the data and clinical material for hypotheses based laboratory investigations. This year our studies have produced significant advances in the field of childhood ALL.

Clinical trials

We reported on the results of randomisation of mitoxantrone with idarubicin in the ALL R3 trial (Parker *et al*., 2010). This trial runs in all paediatric oncology centres in UK, Ireland, Netherlands, Australia and New Zealand. The estimated 3-year progression-free survival (PFS) was 35.9% (95% CI, 25.9 – 45.9) in the idarubicin group versus 64.6% (54.2 – 73.2) in the mitoxantrone group (p=0.0004). The differences in progression-free survival (PFS) were mainly related to a decrease in disease events (progression, second relapse, disease-related deaths; HR 0.56, 0.34 – 0.92, p=0.007) rather than an increase in adverse treatment effects (treatment death, second malignancies; HR 0.52, 0.24 – 1.11, p=0.11) (Figure 1). This is the largest improvement ever achieved by a single treatment modification in childhood ALL. Mitoxantrone is a cheap and readily available drug and thus the results of the trials offers possibilities for children world wide.

The trial produced another important finding. The detection of minimal residual disease (MRD) after a block of treatment has been widely reported to be of prognostic value in patients undergoing treatment for cancer. In some studies, MRD is being used as a surrogate marker for outcome. In this trial, there was no difference in MRD levels between the two randomised arms. Thus if MRD and not PFS had been used as a surrogate endpoint, mitoxantrone would not have been further evaluated. Thus the study serves as a caveat to the use of surrogate markers as endpoints in clinical trials. So if the effect of mitoxantrone is unrelated to disease clearance, what then is the mechanism by which it controls disease recurrence? Both mitoxantrone and idarubicin are given on the first two days of therapy. Increased gastrointestinal and hepatic toxicity were seen in the first eight weeks in the idarubicin arm. In contrast those in the mitoxantrone arm, who were not transplanted, showed a delay in haematopoietic recovery while on maintenance therapy more than four months later. This suggests that mitoxantrone affects the normal haematopoietic stem cell (HSC) niche. While *in vitro* and *in vivo* models have long suggested that the bone marrow microenvironment is protective of leukaemic cells, this is the first clinical study to suggest that this is a highly significant factor in the eradication of leukaemic cells. This has thus become a major focus for our laboratory investigations.

In an international collaborative study, we have also published the outcome of 326 children with Philadelphia chromosome positive childhood ALL, treated in nine different countries, prior to the introduction of tyrosine kinase inhibitors (Arico *et al*., 2010). The seven year overall survival of 44.9% was better than reported previously (36%) for this high risk group of patients. The data suggested, as previously, the benefit of matched donor transplantation in maintaining remission.

Laboratory

We have further refined the drug L-asparaginase, from our report of its degradation by leukaemic lysosomal proteases last year (Offman *et al*., 2010). Using molecular modelling and molecular dynamics performed by our collaborator Dr Paul Bates at the London Research Institute, we have developed a protease resistant recombinant L-asparaginase with activity comparable to the wild type. L-asparaginase also has glutaminase activity. The latter is thought to contribute to toxicity, rather than disease control. We have shown that in the absence of glutaminase activity, cell kill is markedly reduced. However, only minimal glutaminase activity is required to restore the cytotoxic effect of the drug, suggesting that there is a synergy between the two enzymatic activities. Thus we have created a new drug which potentially is less toxic and may have a longer half-life than the currently available native product.

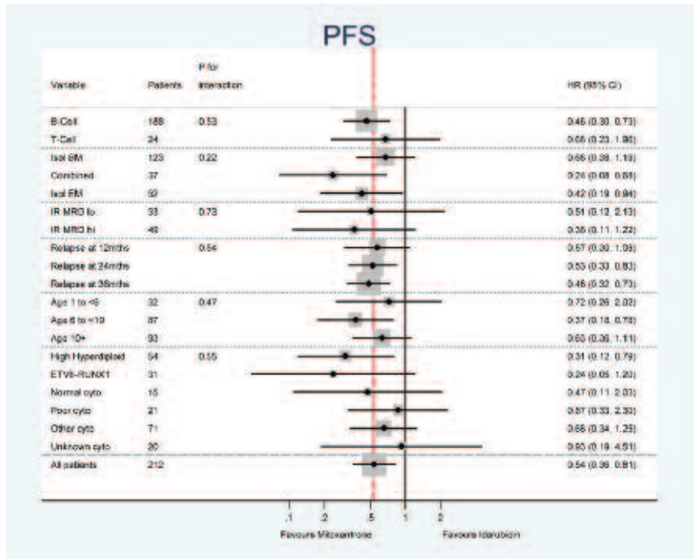


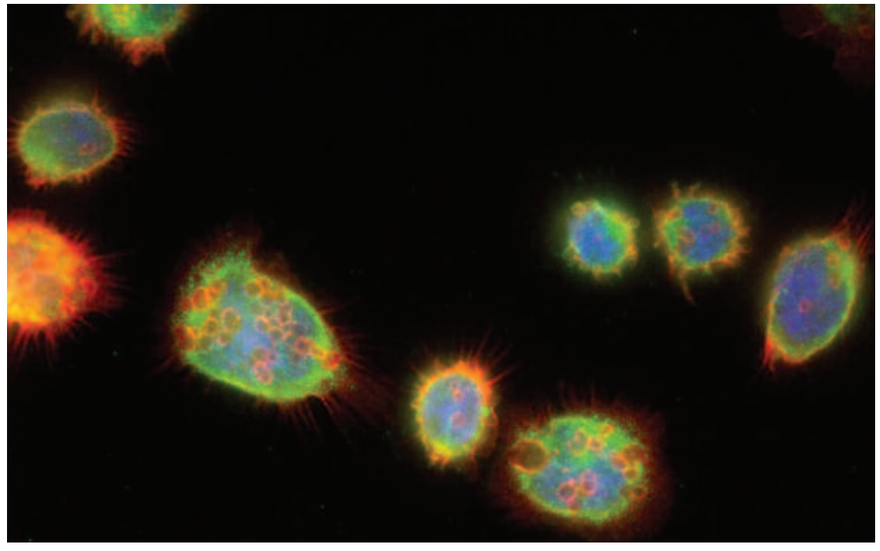
Figure 1
Randomised drug effect on progression-free survival by patient characteristics. P values for interaction test the hypothesis that the randomised treatment effect varies between the subgroups. Hazard ratios indicate that all subgroups show a treatment effect favouring mitoxantrone. HR = hazard ratio. MRD = minimal residual disease. Cyto = cytogenetic subgroups.

Last year we reported on the clinical presentation and outcome of children with ALL who suffered CNS relapse (Krishnan *et al*., 2010). Our clinical data suggested that CNS disease occurred from systemic seeding from leukaemic cells with the ability to transgress blood-brain and blood-CSF barriers. We have created both *in vitro* and *in vivo* models to test this hypothesis. Discovery-based proteomics and murine models suggest that leukaemic blast cells with aberrant peripheral localisation of lysosomes and expressing RAC2, the adhesion dyad of LFA-1 and ICAM1 and the signalling molecule CD70, have the ability to cross the blood brain and CSF barrier in NSG mice. Examination of primary leukaemic blast cells suggest that there is a higher prevalence of ICAM1/CD70$^+$ cells in a CD10$^+$/CD19$^+$ selected population in relapsed compared to *de novo* disease and normal volunteers. Investigations of the mechanism of transgression suggest leukaemic blast cells are able to migrate via transcellular and paracellular methods (Figure 2). Our data suggests that key to the development of extramedullary disease maybe the nature of the adhesion molecule(s) expressed on the surface of the cell. Thus heterotypic contact between host and tumour may be responsible not only for disease recurrence but also for the development of an invasive phenotype. If this can be verified, then this becomes a potential target for therapy.

We have continued to refine our approach to creating suitable *in vitro* and *in vivo* models to understand the disease process and the response to therapy. Overall our clinical and laboratory data suggest that the tumour microenvironment plays a major role in protecting ALL cells. Currently therapy which targets both tumour and host cells provides the best result, with considerable morbidity. If we are able to disrupt the cross talk between host and leukaemic cell, cheaper, simpler and less toxic therapy may become a distinct possibility.

Publications listed on page 71

Figure 2
Podosomes present on the ventral surface of the acute lymphoblastic leukaemia cell line, grown on a CellTak coated coverslip. Cells were fixed with paraformaldehyde and probed for Cortactin (green), polymerised actin (red) and DNA (blue) using fluorescence immunocytochemistry. Podosomes are a structural change observed in transcellular migration.



Targeted Therapy Group

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MRes Student
Khimara Naidoo

MSc Student
Mahsa Azizyan

Senior Lecturer
Yong Du

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 RT-induced tumour cell death is recognized and processed by different antigen presenting cells (APC) in the tumour microenvironment and how this impacts on the subsequent adaptive immune response; iii) to investigate the role of bone marrow derived myeloid cells in tumour regrowth after RT. We aim to translate our experimental research findings into developing early phase clinical trials and over the last year our research highlights have included the areas outlined below.

Novel mechanisms of antibody induced cell death

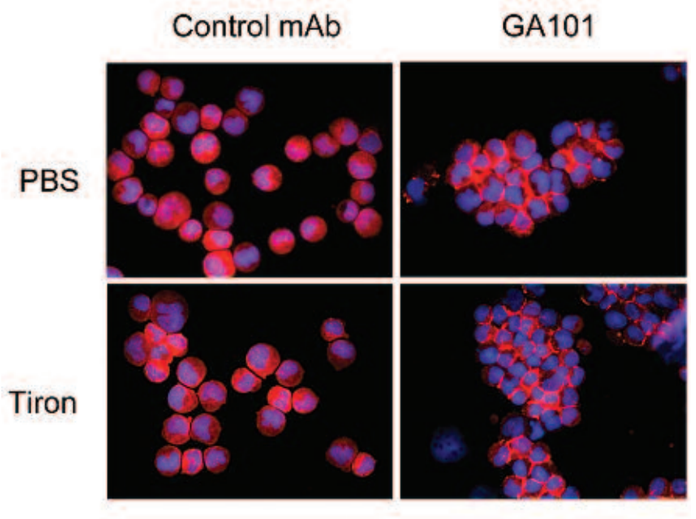
We have focused on investigations into a new form of monoclonal antibody (mAb) induced cell death in B-cell lymphomas and leukaemias in collaboration with Prof Mark Cragg's group in Southampton. Using both lymphoma cell lines and primary chronic lymphocytic leukemia (CLL) cells we demonstrated for the first time the importance of lysosome-mediated cell death for antibody therapy (Ivanov *et al*., J Clin Investig 2009; 119: 2143). We have followed up these initial observations by investigating the mechanisms of action of a novel third generation humanized type II anti-CD20 mAb called GA101 which has a modified Fc region aimed at improving antibody directed toxicity (ADCC). In addition to this potential mechanism of action, we have observed that GA101 can initiate profound non-apoptotic cell death in a range of B-lymphoma cell lines and primary B-cell malignancies. We have gone on to demonstrate that GA101-induced cell death is dependent upon homotypic adhesion, can be abrogated by inhibitors of actin polymerization and is independent of Bcl-2 over-expression and caspase activation. This mAb induced cell death appears to be executed by lysosomes which disperse their contents into the cytoplasm and surrounding environment.

Building on these observations we have gone on to further characterize the mechanisms involved in this cell death and have defined an important role for reactive oxygen species (ROS) in the cell death pathway. Type II anti-CD20 mAb (tositumomab, GA101) but not Type I anti-CD20 mAb (rituximab) or control mAb induce the production of ROS in the Raji cell line, Raji cells over-expressing Bcl-2 (confirming independence from apoptotic pathways) and primary human CLL samples. In addition, both whole IgG and F(ab)^2 fragments of Type II mAb can induce ROS, providing further evidence that the induction of cell death by Type II mAb is independent of the Fc arm of the mAb. ROS scavengers such as tiron, ascorbic acid and tempol sequester superoxide and protect lymphoma cells from Type II anti-CD20 mAb-induced cell death as measured by flow cytometry (Annexin-V/propidium iodide staining) and chromium release assays. ROS scavengers do not affect homotypic adhesion (HA), lysosomal membrane permeability or cathepsin release; all critical determinants of the Type II mAb-mediated cell death pathway. Moreover, pharmacological inhibitors of actin polymerisation (cytochalasin D, latrunculin B), vacuolar ATPase (concanamycin A) and cathepsin activity (cathepsin inhibitor III) all block the induction of ROS by Type II mAb, suggesting that ROS generation lies downstream of cathepsin release. The mitochondrial respiratory chain is an important source of ROS; however, whilst loss of $\Delta\Psi$m occurs in response to Type II mAb, we do not believe mitochondria are the source of ROS in our system. Through a collaboration with Peng Huang and Helene Pelicano at the MD Anderson, University of Texas, experiments have revealed that Type II anti-CD20 mAb induce HA, cell death and ROS in respiratory deficient Raji sub-clones (ϱ^- cells).. Mahsa Azizyan (MSc student) is now working to confirm the source of ROS and the potential contribution to this mechanism of cell death. This work has been accepted for oral presentation at the Keystone Symposium- 'Antibodies as Drugs' on February 2011 and will be presented by PhD student Waleed Alduaij.

Figure 1
Raji Burkitt's lymphoma cells were preincubated with PBS or the ROS scavenger Tiron and subsequently treated with the anti-CD20 mAb GA101 for 4 hours. Fluorescence microscopy of the lysosomal protease Cathepsin B staining (red) was then performed. DNA was counter-stained with DAPI (blue). GA101 induces marked Cathepsin B release into the cytosol and surrounding points of cellular adhesion in the presence of absence of the ROS scavenger Tiron.

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 APC, namely macrophages (MΦ) and Dendritic cells (DC). This work, done by Jamie Honeychurch has demonstrated that by manipulating MΦ within the tumour microenvironment protective anti-tumour CD8 T-cell responses can be induced with anti-CD40 against irradiated lymphoma cells. 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. Recent work done by Simon Dovedi has demonstrated for the first time that the efficacy of external beam radiotherapy (EBRT) can be significantly enhanced by combination with R848, a clinically established TLR7 agonist to elicit a CD8+ T-cell dependent anti-tumour immune response leading to long-term tumour-free survival. We are currently expanding this study to include additional syngeneic models of lymphoma. Work is ongoing to elucidate the role of the DC, B cell and MΦ in the generation of protective anti-tumour immunity post-EBRT.



Monique Melis in collaboration with Kathryn Simpson (Clinical and Experimental Pharmacology Group) has developed a Doxycycline regulated Caspase-3 death switch in a number of tumour models. *In vitro*, Doxycycline induced apoptosis was verified by Annexin V/Propidium Iodide staining and western blotting for cleaved caspase 3 and cleaved PARP. Up to 80% apoptosis was observed at 24 hours which could be inhibited by the pan-caspase inhibiter Q-VD. Apoptotic cell death was confirmed by electron microscopy. Death was associated with release of danger signals including HMGB1. *In vivo*, Doxycycline treatment resulted in pronounced tumour regression and tumour eradication was observed in C57BL/6 immunocompetent mice. However, no tumour eradication was observed in SCID/NOD immunodeficient mice. Preliminary data show that there is increased infiltration of CD11b and F4/80 cells into the tumour microenvironment after Doxycycline treatment and induction of large amounts of tumour cell apoptosis. We are currently investigating the role of the immune system and what regulates immunogenicity of cell death in vivo. The "death switch" which we have developed allows us to explore the relationship between the amount of apoptotic cell death and the host immune response and how this changes with depletion of selective APC.

Publications listed on page 71

Translational Radiobiology Group

Group Leader
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Postdoctoral Fellows
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Amanda Williamson

Clinical Research Fellows
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Rohan Iype (MRes student)

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Dimple Jain
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Bioinformatician
Jan Taylor (joint with Applied Computational Biology and Bioinformatics; from October 2010)

Scientific Administrators
Rebecca Elliott
Sophie Perry

Since the discovery of radiation at the end of the twentieth century, it has become one of the most important modalities for the curative treatment of cancer. Radiotherapy schedules developed somewhat empirically over the years, to maximise tumour kill while minimising damage to surrounding healthy tissue. As there is a direct relationship between radiation dose and tumour control, the development of side-effects in a minority constrains the potentially curative dose that can be safely prescribed to the majority of patients. The ability to predict a patient's likely response to radiotherapy would enable individualised dose prescriptions and decrease the mortality and morbidity associated with cancer. The Translational Radiobiology Group aims to exploit high throughput technologies to develop molecular profiles that predict a cancer patient's response to radiotherapy.

Some tumours respond well to radiotherapy, whereas others do not. The underlying biology that accounts for differences in response to radiotherapy is poorly understood. There is evidence that intrinsic sensitivity to radiation, hypoxia and proliferation are important.

Tumour radiosensitivity

Work carried out by the group several years ago showed measurements of primary human tumour radiosensitivity determined as surviving fraction after 2 Gy (SF2) in an *in vitro* clonogenic assay was an independent prognostic factor for radiotherapy outcome. Ongoing work by the group is deriving a gene expression signature associated with tumour radiosensitivity (John Hall). A challenge has been whether old formalin-fixed paraffin-embedded (FFPE) blocks linked to unique SF2 data can be profiled to generate gene expression signatures associated with measurements of primary human tumour radiosensitivity. Over the last few years the Molecular Biology Core Facility has developed and refined a series of workflows that have supported the laboratory aspects of this approach. FFPE samples contain a wealth of information pertaining to clinical disease, particularly cancer. Degradation and chemical modification of RNA in FFPE tissue prevents its routine use in expression profiling studies. Working with the Applied Computational Biology and Bioinformatics Group (Hui Sun Leong) a unique pipeline was developed employing an ensemble of new techniques. We showed that robust genome-wide signatures with potential for clinical exploitation can be reliably obtained from FFPE material. Affymetrix Human Exon 1.0 ST arrays were used to profile 19 cervical squamous cell carcinoma (SCC) and 9 adenocarcinoma (AC) samples. The gene signature was tested on an independent fresh-frozen non-small cell lung cancer (NSCLC) series. Analysis revealed 1,062 genes higher in SCC compared with AC, and 155 genes higher in AC (FDR $p<0.01$; absolute fold-change >2). This signature of 1,217 genes was capable of correctly separating 58 NSCLC samples into SCC and AC subtypes. Twenty-six genes from the signature were then shown to correctly partition cervix samples using Quantigene 2.0 Plex (a multiplex bead-based alternative to qRT-PCR) into the appropriate histological groups. In the AC a network of genes centred on HNF transcription factors (HNF1B, HNF4A and HNF4G) and GATA6 was also identified, suggesting for the first time, that a HNF-GATA

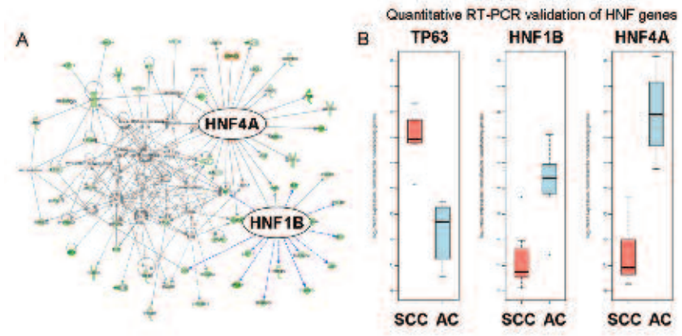
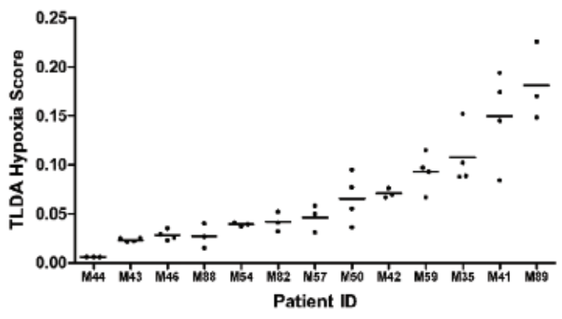


Figure 1
Biological network analysis of FFPE samples identifies hepatic nuclear factor (HNF) regulated transcription in adenocarcinoma. A) Analysis identifies a putative developmental axis centred on HNF. B) qRT-PCR validation of HNF genes. Data are for 7 SCC and 6 AC samples. TP63 is a control expressed only in SCC.

developmental axis plays a role in glandular cell differentiation of the cervix, through the transcriptional regulation of multiple target genes (Figure 1). This work shows that gene expression signatures can be derived from old FFPE material that are sufficiently robust to a) be applied to independent dataset in a different tissue type; b) be used to design assays that can be successfully applied using an independent platform with potential for clinical use, and c) identify potential transcriptional regulation pathways important in biology.

Tumour hypoxia

Hypoxia in tumours limits the efficacy of radiotherapy. However, its accurate measurement is difficult and no technique has been incorporated into routine clinical practice. Towards this goal, we derived a hypoxia metagene using microarray gene expression analysis. A meta-analysis showed that expression of a 25-gene signature was highly prognostic in multiple cancers (Buffa *et al.*, 2010). Customised TaqMan Low Density Arrays (TLDAs) were developed and used to investigate hypoxia metagene expression in head and neck cancer (HNC) samples and cell lines. Median expression of the metagene (Hypoxia Score; HS) was higher in 2 HNC cell lines (CAL-27 and SSC-25) cultured in hypoxia (0.1% O_2) versus normoxia ($n=3$; $p<0.05$). In frozen HNC samples variation in HS between patients was large (CV 74.3%) and not associated with tumour stage or grade. Analysis of multiple biopsies from the same patient showed that the HS is more robust (CV 22.7%; range: 1.6-46.0%) than CA9 mRNA expression alone (CV 58.0%; range: 31.6-154.5%; n=13; Figure 1). Comparison with other measures of hypoxia showed TLDA HSs correlated with microarray-derived HSs ($n=15$; $r=0.91$, $p<0.01$) and the level of pimonidazole binding measured using immunohistochemistry (0.22, $p=0.021$ and $n = 24$). To maximise clinical utility, the customised TLDA has been validated for use with FFPE HNC samples. The correlation between housekeeper gene expression in matched frozen and FFPE HNC samples supports its use with this sample type ($r=0.71$; $p<0.0001$; $n=28$). In summary, we have shown that the TLDA HS is a robust and reliable measure of hypoxia in HNC samples (Amanda Williamson, Guy Betts, Joely Irlam).



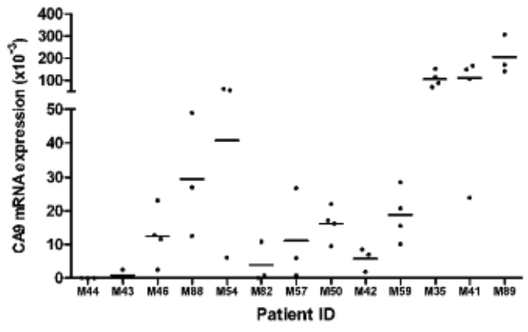


Figure 2
Inter- and intra-tumour variation in TLDA Hypoxia Score (A) and CA9 gene expression (B). Biopsies were taken from 3 or 4 locations within the same HNC sample before analysis.

Normal tissue radiosensitivity

RAPPER - Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy - has now recruited 2,957 samples from patients enrolled in national radiotherapy trials. The Translational Radiobiology Group co-ordinates the sample collection (Rebecca Elliott, Sophie Perry, Helen Valentine) with genotyping and analysis carried out in Cambridge (Drs Gill Barnett and Alison Dunning). Although ~2,000 samples have been genotyped using the Illumina Human CytoSNP-12 GWAS chip, results will not be available until the middle of 2011. However, a candidate gene study was carried out to test all single nucleotide polymorphisms (SNPs) reported in the literature to be linked with the development of radiotherapy toxicity. A multivariate analysis of 99 SNPs in 1,167 DNA samples from breast and prostate cancer patients included clinical co-variables that might influence the development of toxicity. Only one SNP in ATM emerged as significant after Bonferroni correction for multiple comparisons.

Publications listed on page 72

Medical Oncology: Clinical and Experimental Immunotherapy Group

Group Leader
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Eyad Elkord (until Dec)
Ryan Guest (GMP Cell Processing Unit)

Postdoctoral Fellow
Vivien Hanson (until Oct)

Scientific Officers
Debbie Burt (with Immunology) (CIMML)
Natalia Kirillova (GMP Cell Processing Unit)
Sam Mowbray (GMP Cell Processing Unit)
Lidan Christie (GMP Cell Processing Unit)
Vicky Sheard
Marzieh Kamjoo (until Nov)

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Erik Alcantar Orozco
Mariam Al-Muftah (joint with Immunology)
Hannah Gornall (from Oct)

Clinical Fellows
Alaaeldin Shablak (to Sept)
Smita Sharma (CIMML)

Marie-Curie Training Fellow
Vania Baldan (from Oct)

ATTACK Project Managers
Nikki Hudson (until Nov)
Helena Kondryn (from Nov)

Immunotherapy utilises the immune system to control and potentially eradicate cancer. Whilst the group is involved in a number of innovative approaches in clinical trials, at a pre-clinical level we focus on developing adoptive cell therapy.

Clinical trials

We are undertaking two Phase I trials of gene-modified cell therapy and both have shown interesting clinical effects. The initial data were presented at the International Cellular Therapy of Cancer Symposium chaired by Professor Hawkins and organised by ATTACK in Montpellier in September 2010. The trial targeting CEA caused unexpected respiratory toxicity. The scientific basis of this side effect is being further examined but it correlated with high levels of T cells and high levels of cytokine release suggesting specific T cell activation. Whilst the side effects were self limiting the DSMB decided the trial should close rather than attempt to modulate toxicity. In a trial targeting CD19 on B-cell malignancies one patient has had a durable partial remission.

Phase II and subsequent Phase III trials of Trovax (targeting 5T4 –discovered by Peter Stern, Immunology Group) in renal cancer have now been fully reported – overall, there is no benefit for adding vaccine to standard of care but there appears to be a benefit of Trovax with interleukin-2 (IL2) compared to IL2 alone in good prognosis patients (Amato et al., 2010). In this context we have updated our analysis of renal cancer patients treated with high-dose IL2 after prospectively assessing histological features. The outcome for these patients is excellent with around 25% obtaining a complete remission and most of these appear durable (Shablak *et al.*, in press). We now plan an international randomised study to formally confirm the potential advantages over other available treatments for this group of patients.

Targeted therapies are currently the mainstay of renal cancer treatment and we play a leading role in many trials. Notably the Phase III study of pazopanib in renal cancer (Sternberg *et al.*, 2010) is a potentially practice-changing study in renal cancer and lead to the licensing of pazopanib. In upper gastrointestinal cancer our phase II study of an anti-CTLA4 antibody (tremelimumab) was published (Ralph *et al.*, 2010) and the remarkable durable remission in one of the patients continues now at over 3 years.

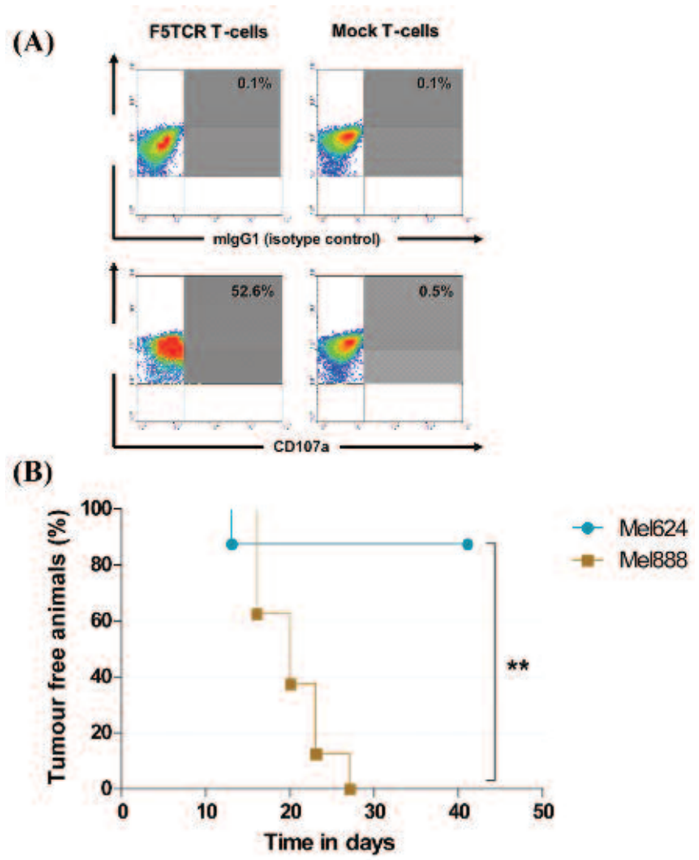
Laboratory aspects of clinical trials

To facilitate development of further trials two new laboratories have been developed. The Clinical Immune and Molecular Monitoring Laboratory (CIMML) is fully functional and has a range of GCLP immune and molecular assays set up. It is a generally available facility and links with a number of clinical groups (eg Haematology, Radiobiology). The Cellular Therapeutics unit was opened in February 2010 and has gone through a process of setup and validation prior to submission of an MHRA licence application. Approval is expected early in 2011 and trials targeting melanoma and other cancers are expected to start after that. Working with the melanoma group and with input from Mark Dudley (NCI) we plan to set up adoptive cell therapy treatment for melanoma.

Pre-clinical research

A key aim is to re-direct the effector functions of T cells towards tumour cells by gene-modifying primary T cells to express targeting receptors. Our predominant focus has been upon the use of Chimeric Antigen Receptors (CAR) which use antibody-type domains to target the T cell to cell surface protein antigens (reviewed in Bridgeman *et al.*, 2010c). However, to target intracellular proteins, natural T cell receptors (TCR) are used – this involves the expression of the α and β chains of a tumour-specific TCR thereby bestowing the T cell with new tumour specificity. Over the past year, we have been working with both types of gene-modified T cell as well as natural T cells to develop adoptive cellular therapy.

Figure 1
Targeting of MART-1$^+$ melanoma cells by T cells expressing the F5 TCR. (A) F5TCR transduced co-cultured for 4 hours with Mel624 melanoma tumour activate by the cell surface translocation of CD107a while mock control T cells fail to respond to the tumour cells. (B) F5 TCR$^+$ T cells can prevent the growth of 7 day established HLA-A2$^+$ Mel624 cells but not HLA-A2$^-$ Mel888 tumour cells.



CARs containing the CD3ζ receptor incorporate into the endogenous T cell receptor complex.

The expression of CARs on the T cell surface enables the potent re-direction of T cell function. However, there is little information concerning the biochemical and structural interactions that the CAR has with endogenous host proteins on the T cell. In particular, does the expression of a CAR affect normal T cell function? To investigate this, we determined the potency of cytokine response of cell lines following mitogenic activation and found that cells expressing a CAR containing the CD3ζ protein showed an increased sensitivity to mitogenic stimulation. We explored the reasons for this and identified that the expression of the CD3ζ CAR resulted in an increased level of TCR expression on the transduced T cell. This modulation of cell surface TCR complexes appeared to be due to the incorporation of the CAR into the TCR complex. Site-directed mutagenesis studies confirmed that charged transmembrane amino-acids within the CAR were driving this interaction. However, proving the interaction through the analysis of co-precipitating proteins proved difficult using standard techniques. To this end, we have developed a novel bead-based flow cytometry based method to examine protein-protein interactions (Bridgeman *et al.*, 2010a). This method conclusively proved that the CD3ζ CAR is indeed present within the TCR complex and, importantly, that this interaction remains critical for the optimal function of the CAR (Bridgeman *et al.*, 2010b).

Improved model systems to investigate CAR T cell function

We have put a considerable effort into developing models that reflect the immunological setting of the cancer patient. Most targets are *tumour associated antigens* where the particular protein is expressed at high level on tumours but is also expressed at lower levels on normal healthy tissue. These models seek to answer whether a tumour specific response can be generated against tumour associated antigens and whether the patient's own immune response can be triggered to respond to tumour but not to over-react by targeting those proteins expressed on healthy tissue.

A key model is CD19 which is found on normal B cells as well as on B-cell malignancies. When using a CAR consisting of the CD3ζ receptor only, tumour is eradicated and mice remain B cell depleted until the CAR T cells eventually disappear at around one month after transfusion (Cheadle *et al.*, 2010) suggesting the CAR T cells become exhausted during this time because of their continuous killing of re-populating normal healthy B cells. More recently experiments have confirmed that using a CAR with greater signalling power results in longer term survival of the CAR T cells and also long term depression of B cell numbers in the treated mice. This is encouraging since it suggests that the engineered T cells can protect against CD19 expressing cells over a long period of time.

Engineered T cells for the treatment of malignant melanoma.

Through collaborations with the NCI (Dr Steven Rosenberg/Dr Paul Robbins) we are also testing T-cell receptor (TCR) engineered T cells. We have TCRs that target both MART-1 and NY-ESO1 which are HLA-A2 restricted epitopes which is expressed to high level on many melanomas and some other tumours. For example, primary human T cells transduced to express the F5 TCR de-granulate in the presence of tumour cells expressing MART-1 and HLA-A2 and these cells can prevent the growth of these tumour cells but fail to affect the growth of cells which lack HLA-A2 thereby confirming therapeutic specificity (Figure 1). We are further optimising this approach prior to potential future trials.

Publications listed on page 73

Medical Oncology: Translational Anti-Angiogenesis Group

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Egle Avizienyte

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Claire Cole
Steen Hansen
Gavin Miller

Clinical Fellows
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Scientific Officer
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Several randomised trials have demonstrated a survival advantage in epithelial malignancies in patients who are treated with conventional therapy supplemented with VEGF inhibitors. However, the effect is modest, and new improved anti-angiogenic agents are required. Heparan sulfate proteoglycans are essential for the biological activity of the majority of angiogenic growth factors. In previous work we demonstrated that heparin oligosaccharides were anti-angiogenic *in vivo*. This year we have completed the chemical synthesis of two families of heparan sulfate oligosaccharides, demonstrating structure-function relationships against different endothelial phenotypes that are essential for angiogenesis.

Heparan sulfate oligosaccharides as anti-angiogenic agents

(Collaborator: Dr. John Gardiner, Manchester Interdisciplinary Biocentre)

Heparan sulfate (HS) is a linear glycosaminoglycan that consists of multiple copies of a disaccharide that contains N-substituted glucosamine and a hexuronic acid; either iduronic or glucuronic acid, depending on the C5 configuration. HS can be sulfated at various positions of each disaccharide although the most common sites are iduronic acid 2-O-sulfate, glucosamine N-sulfate and glucosamine 6-O-sulfate. Sulfation tends to be clustered, creating domains of high anionic charge that facilitate the interaction with protein ligands, the prototype of which are the Fibroblast Growth Factors (FGFs).

HS is covalently bound to a core protein to generate the HS proteoglycans, which have been implicated in binding and activating several angiogenic cytokines, including FGF, VEGF (except $VEGF_{121}$) and chemokines such as IL-8 and SDF-1. The fact that the dependency on HS extends beyond molecules that signal through tyrosine kinase receptors highlights a potential new approach to the development of anti-angiogenic agents.

In 2005 (Hasan *et al*., Clin Cancer Res 2005; 11: 8172) we demonstrated that heparin oligosaccharides, containing 8-10 monosaccharide residues, inhibited angiogenesis in different models *in vivo*. However, the oligosaccharide species involved had anti-coagulant potential as they were derived from heparin and a scalable synthesis was required if the strategy was to be advanced. We therefore established an organic chemistry programme that resulted in the first inexpensive and scalable synthesis of iduronate (Hansen *et al*., Org Lett 2009; 11: 4528) and subsequently led to the elucidation of the complete controlled synthesis of heparan sulfate oligosaccharides containing up to 12 saccharide residues with variable sulfation patterns.

Structure-function studies were performed using endothelial cells. Two families of HS oligosaccharide were investigated: one was sulfated at the iduronic acid 2-O- position only while the other was sulfated at both the iduronic acid 2-O- position and glucosamine N- position. Oligosaccharides were generated that contained between 4 and 12 saccharide residues and their potential to inhibit endothelial cell proliferation, migration and tube formation were evaluated.

The data demonstrated that the ability of the oligosaccharides to inhibit endothelial phenotypes increased with saccharide length and sulfation. Interestingly, the compounds were most potent in assays of migration and tube formation rather than proliferation (Cole *et al*., 2010b). In the case of $VEGF_{165}$ these observations were associated with reduced phosphorylation of VEGF receptor tyrosines implicated in cell migration rather than proliferation. In addition, reduced phosphorylation of FAK and assembly of F-actin at the cellular periphery was observed when cells were treated with the most biologically active oligosaccharide (Figure 1).

In a programme supported by Cancer Research UK and the MRC we have explored *in vivo* activity of size and sulfation-defined oligosaccharide that showed the highest inhibitory activity in endothelial cell-based assays. We demonstrated that the oligosaccharide achieves appropriate concentrations in tumours in vivo and that this is associated with significant reductions in FGF receptor activity (data unpublished).

FGFs are well known angiogenic growth factors but their contribution to the epithelial component of cancer is less clear. We therefore undertook a comprehensive evaluation of FGF and FGF receptor expression in epithelial ovarian cancer and demonstrated that FGFR2 isotype switching occurs upon transformation and that the ligands for the new receptor are expressed in ovarian cancer cell lines and tissue. Knockdown of FGFR2 inhibited proliferation *in vitro* and *in vivo* and increased the sensitivity of cell lines and tumours to cisplatin chemotherapy *in vitro* and *in vivo*, respectively. However, disparate results were seen when FGFR1 expression was reduced suggesting that selective FGFR tyrosine kinase inhibitors might be clinically superior (Cole *et al*., 2010a).

Angiogenesis biomarkers

(Collaborators: Profs. Caroline Dive, Alan Jackson, Geoff Parker; Paterson Institute and The University of Manchester)

The addition of VEGF inhibitors to conventional therapy has improved progression free and/or overall survival in several tumour types. The effect is modest and it is therefore appropriate to identify predictive biomarkers that will allow patient selection thereby reducing toxicity and expense while allowing combination regimens containing VEGF inhibitors to be developed.

We have developed a programme in which imaging and circulating biomarkers are being used to identify those patients who will benefit from anti-angiogenic agents. In addition to CR-UK phase I clinical trials (e.g. GSAO) we are testing the predictive value of Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI), Diffusion Weighted Imaging (DWI) and blood-borne, protein and cellular biomarkers in a large trial in patients with metastatic colorectal cancer with liver metastases who are being treated with cytotoxic chemotherapy and bevacizumab. The complex data set that emerges from this study will be evaluated in collaboration with Professor Cindy Billingham (University of Birmingham).

In a second programme, we lead the translational research programme associated with the MRC trial, ICON7; a randomised trial comparing carboplatin and paclitaxel with or without bevacizumab (an anti-VEGF antibody) in the first line treatment of ovarian cancer. The trial demonstrated a significant but small advantage in progression free survival, highlighting the importance of translational research. The first translational research experiments have been conducted in the Paterson Institute. Additional research programmes will occur around the world over the next year with the aim of identifying predictive biomarkers that will allow us to select patients who are most likely to benefit from anti-angiogenic agents.

Publications listed on page 74

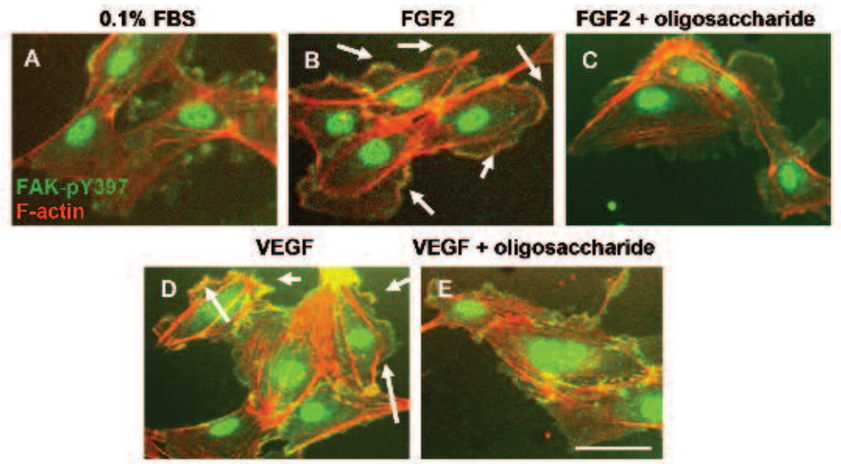


Figure 1
FGF2- and $VEGF_{165}$-induced peripheral accumulation of activated FAK and F-actin is inhibited by oligosaccharide. (A) Lack of peripheral accumulation of FAK phosphorylated on tyrosine 397 and F-actin in serum-starved HUVECs. Cells were co-stained with anti-phospho-FAK (pTyr397) antibody and phalloidin-AlexaFluor568. (B and D) Peripheral FAK phosphorylated at tyrosine 397 and F-actin are detected after 10 min stimulation with FGF2 (B) or $VEGF_{165}$ (D). (C and E) Oligosaccharide prevents peripheral localization of phosphorylated FAK and F-actin in response to FGF2 (C) or $VEGF_{165}$ (E). Scale bar, 75 μm.

Research Services

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

Head of Research Services
Jenny Varley

There have been a number of significant developments in our Research Services in 2010. Appointments have been made in the Biological Mass Spectrometry and Histology Facilities, and we are in the process of recruiting an additional scientific officer to work in the Molecular Biology Core Facility. The support for these posts demonstrates the importance that the Institute places on all of its support facilities. One notable advance during 2010 has come from the implementation of workflows for high throughput sequencing (HTS) using the AB SOLiD^{tm} system. The techniques for HTS are evolving incredibly rapidly but are providing vast amounts of data which would have been unthinkable just a few years ago. Coupling HTS, particularly for transcript analysis, with proteomic data is proving to be particularly fruitful.

All the Research Services provide invaluable support to all researchers in the Institute. Whilst it is easy to pick out modern new technologies and equipment for praise, we should never forget the vital importance of the support given by teams such as Logistics and Laboratory Services. Without such services supporting all groups the Institute would be unable to function.

Advanced Imaging Facility
Head: Steve Bagley

Microscopes within the facility are utilised to impart visual and numerical evidence of localised information concerning functional properties of the cell and to consider the spatial and temporal relationships linking development, phenotype and biochemical interactions. Over the previous year the facility has been developing new methodologies to image more efficiently and with more utility for the researchers within the Institute.

Techniques used in the laboratory for live cell assays have been re-examined and as a consequence precise control and manipulation of fluids (micro-fluidics) has been introduced. Micro-fluidic systems allow for the analysis of how single or populations of cells respond to a switch of fluids or a concentration gradient. This technique can be utilised by shifting the concentration of fluids through a cell population to facilitate the study of how a cell responds to rapid changes in the environment such as drug concentrations. Three research groups within the Institute are now utilising these techniques in diverse studies from examining organelle processes within the cell to the chemotaxic response in populations of cells. In addition, another live cell system, currently in development and which will be introduced in the middle of the coming year, will allow for the gaseous environment around cells to be modified. Hypoxia at the microscope, depriving a population of single cells or tissue of an adequate oxygen supply, allows for the modelling and study of tumour development and growth where oxygen concentrations are lower than that of the surrounding area. Hypoxic tumour cells are usually resistant to radiotherapy and chemotherapy and so by having the ability to study hypoxia and its effect in the live cellular environment under the lens our understanding of protein expression and the tumour micro-environment is enhanced.

To compare the efficacy of newly-developed drugs or the effect of the environment on cell populations there is a requirement to study populations of various cell types in differing environments, and how they respond over time. Over the last fifteen years the accuracy of positioning devices has permitted multiple investigations to be carried out simultaneously which has led to the use of microscopy data to move from the stand-alone image to statistically relevant streams of data. Consequently new technologies are being developed to image not just multiple but thousands of areas, each area with different cellular or environmental conditions, and then to image over long time frames. High-throughput imaging is being developed within the Institute along with the requirement for novel tools for the analysis of the data. Capturing these large data sets is a relatively routine process but being able to derive meaningful numbers becomes a logistical problem which the facility is endeavouring to solve.

There is an ongoing requirement in the facility to increase detection levels so that single molecular events can be revealed. Signals from molecular interaction of what is termed 'low-light' can be detected easily but the cell may not respond as it once did due to the effects of photo-toxicity (the light used to illuminate the cell causing damage to cell processes) thus rendering the study ineffective. To accomplish this task even a 5% increase in detection sensitivity or efficiency has a benefit to the investigations carried out in the laboratory, subsequently all of the systems within the facility are constantly modified to improve efficiency. The requirement for low-light imaging is essential when trying to resolve the interaction of structural and molecular interactions within the cell by techniques such as Förster Resonance Energy Transfer or split-GFP. To realise this aim a new camera has been purchased to push both our light detection limits and spatial resolution to greater levels than formerly possible. The new camera system permits not only presenting the data in the form of intensity but also in units of photons which leads to the analysis of both location and quantity.

Twelve publications have been published with guidance and data derived from the facility over the last year. In addition to training 52 new microscope users and the ongoing advice, experimental design and training of the 121 active users, there have been presentations and demonstrations to over two hundred and fifty members of the public on the techniques used within the laboratory to study oncology in addition to external demonstrations at Cancer Research UK fundraising events. Over the next year the facility, as ever, remains responsive to the imaging and analysis requirements of the researchers within the Institute whilst developing novel techniques for elucidating tissues, populations, single cells and molecular interactions in tandem with other technologies.

Biological Mass Spectrometry Facility
Head: Duncan Smith

Our remit is to provide cutting-edge LCMS workflows to Paterson groups for a multitude of protein characterisation needs. This characterisation predominantly involves protein identification, analysis of the type and position of post-translational modifications (PTM) and peptide based protein quantification. We have a large portfolio of routine services plus a research and development pipeline designed to maintain the portfolio's position at the cutting-edge of cancer research related proteome analyses.

This year has seen recruitment of two new staff into the facility. In January Simon Perkins was appointed as the team's informatician followed by John Griffiths who joined us a senior mass spectrometrist in August. Both the new appointments underline the Institute's commitment to enhancing both routine service provision and research and development (R&D) within the facility. The new appointees increase our ability to support the growing demand for

service provision and are critical to our ability to maintain R&D activity in an increasingly busy facility. Our R&D activity encompasses biochemistry, liquid chromatography, mass spectrometry and bioinformatics.

In the area of protein quantification, we have added dimethyl labelling to our existing toolbox. This chemical labelling strategy facilitates MS based quantification in the same fashion as SILAC without the necessity to metabolically label cells in culture. This opens the potential to perform high quality protein quantification in both preclinical and clinical environments.

In the area of PTM analysis, we have developed an approach to facilitate phospho-site specific quantification (in collaboration with the Cell Regulation Group). Moreover, we are actively developing novel workflows to enhance selectivity of both phosphopeptide and isopeptide enrichment strategies critical to pushing the global phosphoproteomic and Ublproteomic fields forward.

The new informatics research capability of our facility has been instrumental in supporting all our current research projects. Some key current projects include the active development of an exciting global phosphoproteomic pipeline (in collaboration with the Applied Computational Biology & Bioinformatics and Cell Division Groups), automation of phosphosite assignment and novel label free quantification analysis pipeline.

2011 is set to be an exciting and demanding year for the facility. We expect of significant growth in routine service demand and a step-change in the demand for complex quantitative proteome and phosphoproteome analyses within the Institute.

Biological Resources Unit

Once again in 2010 the Paterson animal facility has continued to run at full occupancy with all 3000-plus cages in operation and this has led to the identification of a real need to future-proof to ensure that we continue to deliver a high quality research service. Although the Institute has not seen a huge increase in project licence holders there has been expansion of existing groups and three major users were granted tenure this year which has led to more complex requirements. In addition two new groups have been recruited to the Institute whose research focus has extensive requirements for *in vivo* studies.

September 2010 saw the publication of the European Union Directive 2010/63 which will lead to rigorous debate over the coming months to ensure that the changes to UK legislation in January 2013 are achieved seamlessly. It is clear that this will be the most significant change since the Animal Scientific Procedures Act (1986) was implemented over 20 years ago.

Transgenic services
This year has seen ten new transgenic lines being produced by embryonic stem cell microinjection and we are currently awaiting germline transmission for a further five microinjected lines.

In vitro fertilisation (IVF) from cryopreserved sperm has historically presented many problems due to low fertilisation rates and this has been a particular problem in C57 backcrossed strains. This year however we have established the technique successfully on one new line and expect this will increase considerably in 2011. Further IVF improvements maybe considered in the near future by using a triple gas incubator and associated media which gives a potential 20% increase in fertilisation rates for some C57 backcrossed strains. We have also started to develop Non Surgical Embryo Transfer (NSET) this year with promising results comparable with other NSET-experienced establishments, to date we have achieved a live birth rate/total embryos implanted of ~25% and this technique will continue to be developed through 2011 which will lead to not only a refinement but a major reduction in animal numbers.

Cryopreservation of transgenic strains continues to increase each year with ~30 strains/10,000 embryos being archived in 2010. Cryopreserved embryos are always tested in *in vitro* culture and assessed for viability before cessation of a transgenic line. Sperm cryopreservation has also been employed for the archiving of transgenic strains with a further 13 lines processed and we are expecting this to continue at a higher rate throughout 2011.

With the introduction of a new cryogenic liquid nitrogen freezer for archiving all our transgenic strains we now have more control over freezing profiles, this new freezer supercedes the near 20-year old programmable freezer (which has given sterling service having cryopreserved over 52,000 embryos).

The rederivation process of new strains obtained as live mice in 2010 at the Paterson has included the following:

- ACTFlp
- Flk-1
- Gfil/EGFP-k1
- GfilB/EGFP-k1
- p16/p19
- PDX1 Cre

On arrival these lines are housed in the dedicated Quarantine area using flexible film isolators which run at negative pressure. The rederivation process subsequently involves superovulated specific pathogen free (SPF) females mated to stud males with transfer of collected embryos, thus ensuring that the SPF status of the facility is maintained.

Experimental services
Technical support has been provided by three licenced technicians for a number of established and newer research groups facilitating the scientific goals of the Institute. Planning *in vivo* sessions, assigning stocks, preparing experimental cage labels and observation sheets has all been facilitated before licenced procedures commence. A range of techniques has been delivered for both surgical and non surgical requests and these have included subcutaneous implantation of slow releasing hormone pellets, parenteral injections, gavage, blood sampling with and without recovery, whole body irradiation, local irradiation to subcutaneous tumour and *in vivo* imaging.

Health and welfare for animals under procedure remains paramount and extensive daily health checks including body weight, tumour measurement, dental assessment and abdominal palpations have routinely been carried out.

New *in vivo* techniques developed have included:

- Intra-mammary injections
- Bilateral irradiation
- Supra-spinal injections

The British Journal of Cancer published the Guidelines for the welfare and use of animals in cancer research earlier this year which updates the second edition of the UKCCR guidelines (Workman *et al.*, 2010). The guidelines focus on animal welfare and the use of animals in cancer research demonstrating the need to incorporate the 3Rs: replacement, reduction and refinement, and they also include detailed information on a number of models such as orthotopic and metastatic tumour systems.

Cancer Research UK GeneChip Microarray Service.
Head: Stuart Pepper

For the last nine years the Molecular Biology Core Facility at the Paterson Institute has hosted a microarray service which has been made available to all Cancer Research UK-funded groups. Over that time the service has built up a broad customer base and supported numerous CR-UK groups – a total 189 individual researchers have used the service, submitting 455 projects between them.

Over the last few years there has been a marked shift in the samples that are handled routinely. In the early years of microarrays most work was based on cell lines as the amount of RNA required for microarray labelling was quite high; now we frequently handle samples where less than 10ng of RNA is available. This has opened up opportunities for laser capture microdissection (LCM) of specific populations for array analysis and we have seen a lot more of these types of projects arriving over the last year.

The service has continued to support profiling of archival formalin fixed samples, another area where we are seeing an increase in the number of projects. Profiling of archival samples has moved from an exploratory phase where most papers where concerned with validating the technique to a position now where it is becoming generally accepted that expression profiling of archival samples is a valid, if difficult, approach.

With the advent of clonal sequence platforms there is a lot of speculation about the future for microarrays. Our service has been consistently busier over the last two years then at any time previously, and the number of new projects being discussed has not slowed down. This would suggest that at least for the next year or two at least microarrays will still be in big demand.

Flow Cytometry Facility
Head: Morgan Blaylock

The Flow cytometry Facility at the Paterson 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, providing the tools to help our researchers treat, prevent and understand cancer in its many forms.

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 using fluorescent markers. As the cells flow past a focused laser beam of appropriate wavelength, the probes fluoresce and emit light; this is collected by detectors which in turn translate the light signals into electronic signals proportional to the amount of light collected. 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; cell cycle analysis; fluorescent protein expression; functional aspects of the cell such as enzyme activity; apoptotic status; mitochondrial membrane potential; 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 biologically complex population. Some of the more common research applications include immunology; cell cycle and cell growth; cell function and activation; cell differentiation; apoptosis; toxicology and fluorescent protein detection.

The use of flow cytometry in the Paterson Institute can be divided into two broad categories; analytical cytometry and cell sorting.

Analytical cytometry
The ability of flow cytometers to evaluate cells at an extremely rapid rate (e.g. up to 20,000 events per second) makes this technology ideally suited for the reliable and accurate quantitative analysis of selected physical properties of cells of interest. The sensitivity of these instruments for detecting the presence of molecules expressed at low levels is impressive; given high quality cell preparations and reagents, as few as 50 molecules per cell may be detected. The facility currently has four bench top cytometers including one plate-based bead reader. These are all user-operated systems for which we offer basic training in a group setting which is supplemented with one to one training for specific applications:

- FACS Calibur - 3 colour single laser (blue)
- FACS Calibur - 4 colours dual laser (blue and red)
- FACS Array - 4 colours, dual laser (green and red)
- LSRII - 17 colours, quadruple laser (UV, violet, blue and red)

Cell sorters
One of the properties of the larger flow cytometers is the ability to electronically deflect cells with preset, defined properties into a separate collection tube. For cell purification, flow cytometry is especially well suited for applications requiring high purity. Because multiple fluorochromes (e.g. up to fourteen distinct fluorescent probes reacting with different cell associated molecules) can be assessed simultaneously, cell sorting by flow cytometry can separate complex mixtures of cells on the basis of multiple marker expression. 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:

- Vantage SE - 2 way sorting, 5 colours, dual laser (blue and red)
- FACS Aria II u - 4 way sorting 12 colours, triple laser (violet, blue and red)
- InFlux - 4 way sorting, 14 colours, equipped with 5 lasers (UV, violet, blue, red and orange)

Other services
Our facility 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, presentation, interpretation, we also 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.

Technical developments
This year as part of our internal annual review of our instrument stock we upgraded the computing systems which control the Caliburs which has greatly improved the data acquisition and stability of the systems. Additionally we have upgraded the FACS Aria to the FACS Aria II; this has drastically improved the workflow and stability of the system allowing us to provide a much improved product to our users.

We have been involved in the continuation and development of a number of projects this year. We have been collaborating extensively with a number of researchers in the Institute including Dr Gillian Farnie and Pam Willan who have been investigating the resistance of breast cancer stem cells (BCSCs) to DNA damage by radio- or chemotherapy. There is evidence the BCSCs preferentially survive this type of anti-cancer therapy and Gillian and Pam have been addressing whether the amount of DNA damage caused by therapy is similar in BCSCs vs. non-BCSCs by flow cytometry. They treat cells with radio- or chemotherapy, and stain the cell surface markers to define the BCSC population. Cells are then stained for an intra-nuclear protein which is a marker of double strand DNA breaks and a DNA stain to allow cell cycle analysis, and are then analysed on the LSRII. Their results have shown that the BCSCs have lower levels of DNA damage after radio- and chemotherapy compared to the non-BCSCs. They have also shown that after pre-treatment of breast cancer cells with differentiating agents BCSCs can be re-sensitised to radio- and chemotherapy, indicated by an increase in DNA damage.

We have also continued our close involvement with John Bridgeman and David Gilham. Together we have developed a novel flow cytometric immunoprecipitation method capable of investigating protein-protein interactions specifically the analysis of the T-cell receptor which has yielded a number of publications for the facility (Bridgeman *et al*., 2010a).

Histology
Head: Garry Ashton

Again the year has been exceptionally busy with several key developments taking place. The recruitment and training of another scientific officer has allowed the unit to continue to offer a comprehensive and flexible service whilst also allowing us to continue to focus on our development.

Use of the Leica LMD6000 laser capture microdissection (LCM) system has increased. Projects focusing on the role of heparan sulfate (HS) in ovarian cancer, the effects of intermittent energy restriction on gene expression in breast tissue in women at increased risk of breast cancer and the targeting of tumour cells/normal bronchial epithelium for subsequent nucleic acid extraction in lung cancer patients are examples of current studies.

In anticipation of future demand from the expansion of tissue biomarkers and the requirements of the Manchester Cancer Research Centre (MCRC) Biobank, in early 2010 the lab took delivery of the new ATA27 automated tissue microarray platform. The system allows high throughput, accurate TMA construction of tumour-specific and custom arrays. A large amount of time has been spent optimising this process resulting in the production of extremely high quality TMAs giving true representation. One example is the construction of a small fifty-patient breast tissue array where both tumour and matched normal cores were transplanted to study the role of ATF2 and stress activated protein kinases (SAPK) in breast cancer progression.

The Manchester Cancer Research Centre Biobank has now been running for two and half years. Solid tumour with paired normal tissue is collected and processed centrally by the facility. Blood and bone marrow from haematological malignancy patients is also processed. To date samples from over 1600 patients has been banked. 2010 has seen a significant rise in the

number of applications to use Biobank samples. In total, there have been almost thirty applications (six of which have been for TMAs), with an approval rate for projects of over 90%. Applications have been received from a variety of sample types, including lung, breast and liver.

The routine and troubleshooting immunohistochemistry (IHC) service offered by the facility has seen unprecedented demand recently. In addition the number of research groups using the facilities to perform their own IHC has risen sharply. The lab now houses several epitope retrieval stations and the i6000 automated IHC platform which gives groups the flexibility to optimise and validate their own specific IHC projects. The i6000 platform is regularly oversubscribed and as a result we soon hope to purchase a second fully enclosed IHC platform. Both systems will compliment each other, offering high-throughput antibody validation service availability and unrivalled standardisation and reproducibility. Both these systems will become invaluable with the availability of any new image analysis software.

Specialised techniques have been employed to process hydroxyapatite/tricalcium phosphate biomaterial that has been implanted subcutaneously in NOD/SCID IL2Rγ$^{-/-}$ immunocompromised mice in a study with the Leukaemia Biology Group defining and evaluating biological mechanisms in bone marrow stroma responsible for the regulation of normal and malignant bone marrow haematopoietic stem and progenitor populations using a humanised implantation mouse model.

Work has continued with the Stromal Tumour Interaction Group, where 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 myofibroblast rich carcinoma-associated fibroblasts (CAFs). We have also continued studies with the Children's Cancer Group where IHC has also been used in the development a mouse model for acute lymphoblastic leukaemia (ALL).

Laboratory Services
Head: Mark Craven

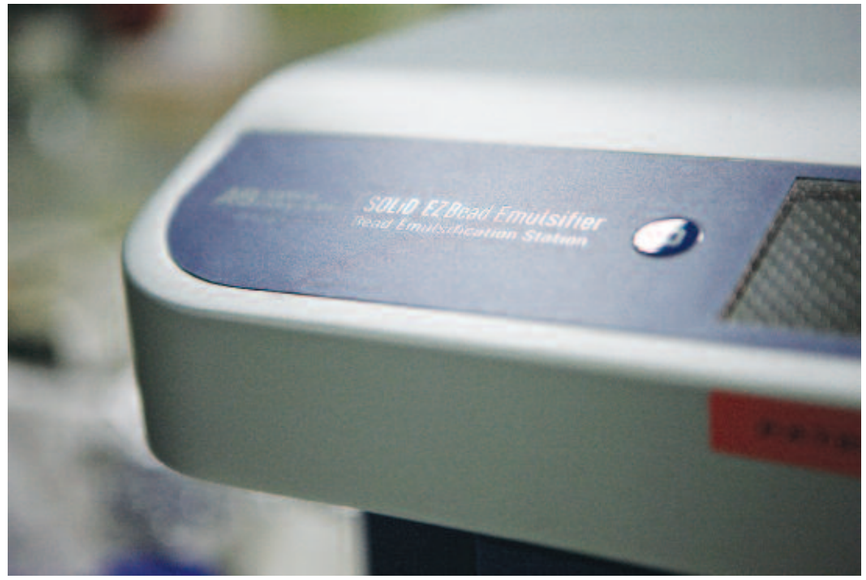
The Laboratory Services department supports all the research labs by supplying them with sterile glass and plastic ware, sterile fluids and media. We will also sterilize non routine items sent up from the labs. We use two industrial glass washers and two autoclaves to provide this service together with a clean room for the production of media and agar plates. We also supply the research labs with a member of the department, a Lab Aide, to perform certain lab duties in order to assist the smooth running of the labs. Each day we collect the dirty glassware and deliver clean glassware and plastics to the labs.

As the number of groups within the Institute has risen over the last year, we have had to process larger amounts of glass and plastics on a daily basis. To absorb this additional workload it has been necessary to adjust the amount of Lab Aide time assigned to individual labs. We have taken on an additional member of staff to help with this increased throughput. The number of labs requesting bulk media has increased and we have increased the range of media we offer whilst keeping to our production deadlines.

Logistics
Head: Maurice Cowell

The Logistics department provides a comprehensive and vital role in supporting the research carried out at the Institute. This group encompasses a wide range of duties including the accurate receipting, checking, booking in and efficient distribution of goods ordered by numerous Personnel in the Institute. The collection and removal of waste, be it general rubbish, yellow bags or GM waste is also the porters responsibility. They are accountable for the collection of liquid nitrogen containers from laboratories, transporting to the loading bay for refilling and returning to the labs. This is done three times a week and dry ice is delivered twice a week.

Ordering and distribution of the Central Stores stock via the intranet E-mail "Order Stock Items" function has been updated to become more user friendly and it is our duty to ensure adequate stock levels are maintained at all times. Included in this are the media and enzymes stored in the Institute freezers (Sigma, Invitrogen, Roche, Promega, New England Biolabs, Fisher kits and Qiagen), again the Logistics department is responsible for the ordering, distribution and stock levels of these items. The Institutes' usage of gas cylinders is looked after by the porters who are in charge of replacement and ordering as and when necessary. 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 movement of heavy equipment or furniture, and the setting up of various meeting rooms for numerous events.



Molecular Biology Core Facility
Head: Stuart Pepper

Over the last year the main focus for development has been on our new clonal sequence platform. At the start of the year we had just completed our purchase of an Applied Biosystems SOLiD™ platform and were starting to work on developing services. Clonal sequencers are bringing a true revolution to biological research as they have made genome sequencing accessible to small core facilities as well as the major sequencing centres such as Sanger. Aside from sequencing entire genomes these systems are also open platforms which can be adapted for many applications, including transcriptome analysis and chromatin IP studies.

Our main interest has been looking at the potential to use sequencing of RNA (RNAseq) to yield both quantitative and qualitative information. For the last decade microarrays have been the leading platform for expression profiling, however RNAseq offers some potential advantages over microarrays. Firstly, to design a microarray it is necessary to have some advance knowledge of the transcripts to be detected whereas RNAseq can be used to characterize novel transcripts. A further benefit of RNAseq is in the characterization of mutations which may cause loss of detection on a microarray. In the first year we have supported several research groups in projects exploring human, mouse and yeast transcriptomes with exciting results. The first publication from this service is already out (Bradford *et al*., 2010) with more on the way for next year.

Aside from technology platforms such as the SOLiD and quantitative PCR (qPCR) systems, the core facility also provides routine screening services. For some time now the facility has offered a regular cell line mycoplasma screen but this year we have expanded to include a cell line authentication service. The standard technology for authentication is to use short tandem repeat profiling, which is the same approach used in forensic labs to identify individuals. As part of this service we have created a database of reference profiles that allows confident validation of test samples. There has been a high uptake for this service resulting in more than 400 samples being analysed. Having completed the first round of screening we will now offer this service on a regular basis so that new cell lines can be authenticated. It is gratifying that the vast majority of our cell lines are correct.

At the end of last year we were facing a bottleneck on our qPCR platform, the Appplied Biosystems 7900 system. We were fortunate to be able to buy a second 7900 system to support qPCR this year thereby avoiding delays to research projects. Along with continued demand to use the 7900s for expression profiling, we have seen an increase in the number of groups who want to study micro RNA expression and so the extra capacity on the qPCR platform has been particularly beneficial.

Research publications

Crispin Miller (page 14)
Applied Computational Biology and Bioinformatics Group

Refereed Research Papers

Bitton, D.A., Smith, D.L., Connolly, Y., Scutt, P.J. and Miller, C.J. (2010)
An integrated mass-spectrometry pipeline identifies novel protein coding-regions in the human genome. *PLoS One*, **5**, e8949.

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A comparison of massively parallel nucleotide sequencing with oligonucleotide microarrays for global transcription profiling. *BMC Genomics*, **11**, 282.

Buffa, F.M., Harris, A.L., West, C.M. and Miller, C.J. (2010)
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CXCR4 mediated chemotaxis is regulated by 5T4 oncofetal glycoprotein in mouse embryonic cells. *PLoS One*, **5**, e9982.

Geoff Margison (page 16)
Carcinogenesis Group

Refereed Research Papers

Duthie, S.J., Grant, G., Pirie, L.P., Watson, A.J. and Margison, G.P. (2010)
Folate deficiency alters hepatic and colon MGMT and OGG-1 DNA repair protein expression in rats but has no effect on genome-wide DNA methylation. *Cancer Prev Res* **3**, 92-100.

Saad, A.A., Kassem, H., Povey, A.C. and Margison, G.P. (2010)
Expression of O^6-Alkylguanine-DNA Alkyltransferase in Normal and Malignant Bladder Tissue of Egyptian Patients. *J Nucleic Acids*, **2010**, 840230.

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Targeting O^6-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. *Cell Mol Life Sci*, **67**, 3663-3681.

Margison, G. (2010)
O^6-methylguanine in DNA: bad penny? *Cell Cycle*, **9**, 441-442.

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O^6-Substituted guanine derivatives, a process for their preparation and their use in treating tumour cells. McMurry, T.B.H., McElhinney, R.S., McCormick, J.E., Elder, R.H., Kelly, J., Margison, G.P., Rafferty, J.A., Watson, A.J. and Willington, M.A.
International Patent Application PCT/IE94/0031, published (WO 94/29312, 70 pages) through CR Technology Ltd. (*Chem. Abs.* 1995, **122**, 239458e).

Pyrimidine derivatives and guanine derivatives, and their use in treating tumour cells. McMurry, T.B.H., McElhinney, R.S., McCormick, J.E., Donnelly, D.J., Murray, P., Carola, C., Elder, R.H., Kelly, J., Margison, G.P., Watson, A.J., Rafferty, J.A., Willington, M.A. and Willington, M.R.
International Patent Application PCT/IE96/00084, published (WO97/20843, 129 pages) through CR Technology Ltd. (*Chem.Abs.*, 1997, **127**, 108802t).

Potentiation of temozolomide in human tumour cells. Baer, J., Freeman, A.A., Newlands, E.S., Watson, A.J., Rafferty, J.A., and Margison, G.P.
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Karim Labib (page 18)
Cell Cycle Group

Other Publication

Labib, K. (2010)
How do Cdc7 and cyclin-dependent kinases trigger the initiation of chromosome replication in eukaryotic cells? *Genes Dev*, **24**, 1208-1219.

Nic Jones (page 22)
Cell Regulation Group

Refereed Research Papers

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Nemoto, N., Udagawa, T., Ohira, T., Jiang, L., Hirota, K., Wilkinson, C.R., Bahler, J., Jones, N., Ohta, K., Wek, R.C. and Asano, K. (2010)
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Shah, M., Bhoumik, A., Goel, V., Dewing, A., Breitwieser, W., Kluger, H., Krajewski, S., Krajewska, M., Dehart, J., Lau, E., Kallenberg, D.M., Jeong, H., Eroshkin, A., Bennett, D.C., Chin, L., Bosenberg, M., Jones, N. and Ronai, Z.A. (2010)
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Angeliki Malliri (page 24)
Cell Signalling Group

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The Rac activator STEF (Tiam2) regulates cell migration by microtubule-mediated focal adhesion disassembly. *EMBO Rep*, **11**, 292-298.

Woodcock, S.A., Rushton, H.J., Castaneda-Saucedo, E., Myant, K., White, G.R., Blyth, K., Sansom, O.J. and Malliri, A. (2010)
Tiam1-Rac signaling counteracts Eg5 during bipolar spindle assembly to facilitate chromosome congression. *Curr Biol*, **20**, 669-675.

Caroline Dive (page 26)
Clinical and Experimental Pharmacology

Refereed Research Papers

Board, R.E., Wardley, A.M., Dixon, J.M., Armstrong, A.C., Howell, S., Renshaw, L., Donald, E., Greystoke, A., Ranson, M., Hughes, A. and Dive, C. (2010)
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Inhibition of FGFR2 and FGFR1 increases cisplatin sensitivity in ovarian cancer. *Cancer Biol Ther*, **10**, 495-504.

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Considerations for the use of plasma cytokeratin 18 as a biomarker in pancreatic cancer. *Br J Cancer*, **102**, 577-582.

Hampson, G., Ward, T.H., Cummings, J., Bayne, M., Tutt, A.L., Cragg, M.S., Dive, C. and Illidge, T.M. (2010)
Validation of an ELISA for the determination of rituximab pharmacokinetics in clinical trials subjects. *J Immunol Methods*, **360**, 30-38.

Kraan, J., Sleijfer, S., Strijbos, M.H., Ignatiadis, M., Peeters, D., Pierga, J.Y., Farace, F., Riethdorf, S., Fehm, T., Zorzino, L., Tibbe, A.G., Maestro, M., Gisbert-Criado, R., Denton, G., de Bono, J.S., Dive, C., Foekens, J.A. and Gratama, J.W. (2010)
External quality assurance of circulating tumor cell enumeration using the CellSearch((R)) system: A feasibility study. *Cytometry B Clin Cytom*, epub Nov 10.

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The development of composite circulating biomarker models for use in anti-cancer drug clinical development. *Int J Cancer*, epub Jun 14.

Mitchell, C.L., O'Connor, J.P., Jackson, A., Parker, G.J., Roberts, C., Watson, Y., Cheung, S., Davies, K., Buonaccorsi, G.A., Clamp, A.R., Hasan, J., Byrd, L., Backen, A., Dive, C. and Jayson, G.C. (2010)
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Moreira-Leite, F.F., Harrison, L.R., Mironov, A., Roberts, R.A. and Dive, C. (2010)
Inducible EGFR T790M-Mediated Gefitinib Resistance in Non-small Cell Lung Cancer Cells Does Not Modulate Sensitivity to PI103 Provoked Autophagy. *J Thorac Oncol*, **5**, 765-777.

Morrow, C.J., Ghattas, M., Smith, C., Bonisch, H., Bryce, R.A., Hickinson, D.M., Green, T.P. and Dive, C. (2010)
Src Family Kinase Inhibitor Saracatinib (AZD0530) Impairs Oxaliplatin Uptake in Colorectal Cancer Cells and Blocks Organic Cation Transporters. *Cancer Res*, **70**, 5931-5941.

Pires, I.M., Ward, T.H. and Dive, C. (2010)
Oxaliplatin responses in colorectal cancer cells are modulated by CHK2 kinase inhibitors. Br J Pharmacol, 159, 1326-1338.

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Cummings, J., Raynaud, F., Jones, L., Sugar, R. and Dive, C. (2010)
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Cummings, J., Ward, T.H. and Dive, C. (2010)
Fit-for-purpose biomarker method validation in anticancer drug development. *Drug Discov Today*, **15**, 816-825.

Murukesh, N., Dive, C. and Jayson, G.C. (2010)
Biomarkers of angiogenesis and their role in the development of VEGF inhibitors. *Br J Cancer*, **102**, 8-18.

Roberts, D.L., Dive, C. and Renehan, A.G. (2010)
Biological Mechanisms Linking Obesity and Cancer Risk: New Perspectives. *Annu Rev Med*, **61**, 301-316.

Ivan Ahel (page 28)

DNA Damage Response Group

Refereed Research Papers

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Solution structures of the two PBZ domains from human APLF and their interaction with poly(ADP-ribose). *Nat Struct Mol Biol*, **17**, 241-243.

Peter Stern (page 32)

Immunology Group

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Daayana, S., Elkord, E., Winters, U., Pawlita, M., Roden, R., Stern, P.L. and Kitchener, H.C. (2010)
Phase II trial of imiquimod and HPV therapeutic vaccination in patients with vulval intraepithelial neoplasia. *Br J Cancer*, **102**, 1129-1136.

Ralph, C., Elkord, E., Burt, D.J., O'Dwyer, J.F., Austin, E.B., Stern, P.L., Hawkins, R.E. and Thistlethwaite, F.C. (2010)
Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. *Clin Cancer Res*, **16**, 1662-1672.

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CXCR4 mediated chemotaxis is regulated by 5T4 oncofetal glycoprotein in mouse embryonic cells. *PLoS One*, **5**, e9982.

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Progress in vaccination against cancer (PIVAC-10), Cambridge, UK. *IDdb Meeting Report*, September 27-30.

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From HPV infection to oncogenesis; a brief review of the complex immunobiological events. Current *Cancer Therapy Reviews*, **6**, 110-116.

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Stern, P.L. and Hole, N. (1999)
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Nullin Divecha (page 34)

Inositide Laboratory

Refereed Research Papers

Bultsma, Y., Keune, W.J. and Divecha, N. (2010)
PIP4Kbeta interacts with and modulates nuclear localization of the high-activity PtdIns5P-4-kinase isoform PIP4Kalpha. *Biochem J*, **430**, 223-235.

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Keune, W.J., Bultsma, Y., Sommer, L., Jones, D. and Divecha, N. (2010)
Phosphoinositide signalling in the nucleus. *Adv Enzyme Regul*, epub Oct 28.

Tim Somervaille (page 36)
Leukaemia Biology Group

Refereed Research Papers
Liedtke, M., Ayton, P.M., Somervaille, T.C., Smith, K.S. and Cleary, M.L. (2010)
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Wong, P., Iwasaki, M., Somervaille, T.C., Ficara, F., Carico, C., Arnold, C., Chen, C.Z. and Cleary, M.L. (2010)
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John Brognard (page 38)
Signalling Networks Group

Other Publication
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Georges Lacaud (page 40)
Stem Cell Biology Group

Refereed Research Papers
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Valerie Kouskoff (page 42)
Stem Cell and Haematopoiesis Group

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Holley, R.J., Pickford, C.E., Rushton, G., Lacaud, G., Gallagher, J.T., Kouskoff, V. and Merry, C.L.R. (2010)
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Serrano, A.G., Gandillet, A., Pearson, S., Lacaud, G. and Kouskoff, V. (2010)
Contrasting effects of Sox17- and Sox18-sustained expression at the onset of blood specification. *Blood*, **115**, 3895-3898.

Akira Orimo (page 44)
Stromal-Tumour Interaction Group

Refereed Research Paper
Kojima, Y., Acar, A., Eaton, E.N., Mellody, K.T., Scheel, C., Ben-Porath, I., Onder, T.T., Wang, Z.C., Richardson, A.L., Weinberg, R.A. and Orimo, A. (2010)
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Vaskar Saha (page 48)
Children's Cancer Group

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Clinical Outcome of Children With Newly Diagnosed Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia Treated Between 1995 and 2005. *J Clin Oncol*, **28**, 4755-4761.

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Tim Illidge (page 50)
Targeted Therapy Group

Refereed Research Papers
Cheadle, E.J., Hawkins, R.E., Batha, H., O'Neill, A.L., Dovedi, S.J. and Gilham, D.E. (2010)
Natural expression of the CD19 antigen impacts the long-term engraftment but not antitumor activity of CD19-specific engineered T cells. *J Immunol*, **184**, 1885-1896.

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Hampson, G., Ward, T.H., Cummings, J., Bayne, M., Tutt, A.L., Cragg, M.S., Dive, C. and Illidge, T.M. (2010)
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YY1 expression predicts favourable outcome in follicular lymphoma. *J Clin Pathol*, epub Nov 25.

Omed, A., Lawrance, J.A., Murphy, G., Laasch, H.U., Wilson, G., Illidge, T., Tipping, J., Zivanovic, M. and Jeans, S. (2010)
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Elkord, E., Alcantar-Orozco, E.M., Dovedi, S.J., Tran, D.Q., Hawkins, R.E. and Gilham, D.E. (2010)
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Catharine West (page 52)

Translational Radiobiology Group

Refereed Research Papers

Barnett, G.C., Coles, C.E., Burnet, N.G., Pharoah, P.D., Wilkinson, J., West, C.M., Elliott, R.M., Baynes, C. and Dunning, A.M. (2010)
No association between SNPs regulating TGF-beta1 secretion and late radiotherapy toxicity to the breast: Results from the RAPPER study. *Radiother Oncol*, **97**, 1295-1296.

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Tumour budding and a low host inflammatory response are associated with a poor prognosis in oesophageal and gastro-oesophageal junction cancers. *Histopathology*, **56**, 893-899.

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Large meta-analysis of multiple cancers reveals a common, compact and highly prognostic hypoxia metagene. *Br J Cancer*, **102**, 428-435.

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Other Publications

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Genetic predictors of normal tissue response to radiotherapy. In: *Pharmacogenetics: making cancer treatment safer and more effective*. Eds Newman, W.G. pp 127-135. Springer Dordrecht, Heidelberg, London, New York

Robert Hawkins (page 54)

Medical Oncology: Clinical and Experimental Immunotherapy Group

Refereed Research Papers

Amato, R.J., Hawkins, R.E., Kaufman, H.L., Thompson, J.A., Tomczak, P., Szczylik, C., McDonald, M., Eastty, S., Shingler, W.H., de Belin, J., Goonewardena, M., Naylor, S. and Harrop, R. (2010)
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Cytokine stimulation and the choice of promoter are critical factors for the efficient transduction of mouse T cells with HIV-1 vectors. *J Gene Med*, **12**, 129-136.

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Sunitinib in metastatic renal cell carcinoma patients with brain metastases. *Cancer*, epub Sep 22.

Khan, S., Burt, D.J., Ralph, C., Thistlethwaite, F.C., Hawkins, R.E. and Elkord, E. (2010)
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A phase I/II study of siltuximab (CNTO 328), an anti-interleukin-6 monoclonal antibody, in metastatic renal cell cancer. *Br J Cancer*, **103**, 1154-1162.

Rothwell, D.G., Crossley, R., Bridgeman, J.S., Sheard, V., Zhang, Y., Sharp, T., Hawkins, R.E., Gilham, D. and McKay, T.R. (2010)
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Management of a New Isolated Metastasis during Sunitinib Treatment in Renal Cell Carcinoma Patients: A Lesson from Two Cases. *Urol Int*, epub Dec 2.

Sternberg, C.N., Davis, I.D., Mardiak, J., Szczylik, C., Lee, E., Wagstaff, J., Barrios, C.H., Salman, P., Gladkov, O.A., Kavina, A., Zarba, J.J., Chen, M., McCann, L., Pandite, L., Roychowdhury, D.F. and Hawkins, R.E. (2010)
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Waddington, S.N., Crossley, R., Sheard, V., Howe, S.J., Buckley, S.M., Coughlan, L., Gilham, D.E., Hawkins, R.E. and McKay, T.R. (2010)
Gene Delivery of a Mutant TGFbeta3 Reduces Markers of Scar Tissue Formation After Cutaneous Wounding. *Mol Ther*, **18**, 2104-2111.

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Building better chimeric antigen receptors for adoptive T cell therapy. *Curr Gene Ther*, **10**, 77-90.

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Do CARs Need a Driver's License? Adoptive Cell Therapy with Chimeric Antigen Receptor-Redirected T Cells Has Caused Serious Adverse Events. *Hum Gene Ther*, **21**, 1039-1042.

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T regulatory cells in cancer: recent advances and therapeutic potential. *Expert Opin Biol Ther*, **10**, 1573-1586.

Hawkins, R.E., Gilham, D.E., Debets, R., Eshhar, Z., Taylor, N., Abken, H., Schumacher, T.N. and Consortium, A. (2010)
Development of adoptive cell therapy for cancer: a clinical perspective. *Hum Gene Ther*, **21**, 665-672.

Gordon Jayson (page 56)

Medical Oncology: Translational Anti-Angiogenesis Group

Refereed Research Papers

Brookes, K., Cummings, J., Backen, A., Greystoke, A., Ward, T., Jayson, G.C. and Dive, C. (2010)
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Cross-visit tumor sub-segmentation and registration with outlier rejection for dynamic contrast-enhanced MRI time series data. *Med Image Comput Comput Assist Interv*, **13**, 121-128.

Cole, C., Lau, S., Backen, A., Clamp, A., Rushton, G., Dive, C., Hodgkinson, C., McVey, R., Kitchener, H. and Jayson, G.C. (2010a)
Inhibition of FGFR2 and FGFR1 increases cisplatin sensitivity in ovarian cancer. *Cancer Biol Ther*, **10**, 495-504.

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Synthetic heparan sulfate oligosaccharides inhibit endothelial cell functions essential for angiogenesis. *PLoS One*, **5**, e11644.

Goncalves, V., Jayson, G. and Tarrier, N. (2010)
A longitudinal investigation of psychological disorders in patients prior and subsequent to a diagnosis of ovarian cancer. *J Clin Psychol Med Settings*, **17**, 167-173.

Jonker, D.J., Rosen, L.S., Sawyer, M.B., de Braud, F., Wilding, G., Sweeney, C.J., Jayson, G.C., McArthur, G.A., Rustin, G., Goss, G., Kantor, J., Velasquez, L., Syed, S., Mokliatchouk, O., Feltquate, D.M., Kollia, G., Nuyten, D.S. and Galbraith, S. (2010)
A phase I study to determine the safety, pharmacokinetics and pharmacodynamics of a dual VEGFR and FGFR inhibitor, brivanib, in patients with advanced or metastatic solid tumors. *Ann Oncol*, epub Dec 3.

Ledermann, J.A., Gabra, H., Jayson, G.C., Spanswick, V.J., Rustin, G.J., Jitlal, M., James, L.E. and Hartley, J.A. (2010)
Inhibition of carboplatin-induced DNA interstrand cross-link repair by gemcitabine in patients receiving these drugs for platinum-resistant ovarian cancer. *Clin Cancer Res*, **16**, 4899-4905.

Mitchell, C.L., O'Connor, J.P., Jackson, A., Parker, G.J., Roberts, C., Watson, Y., Cheung, S., Davies, K., Buonaccorsi, G.A., Clamp, A.R., Hasan, J., Byrd, L., Backen, A., Dive, C. and Jayson, G.C. (2010)
Identification of early predictive imaging biomarkers and their relationship to serological angiogenic markers in patients with ovarian cancer with residual disease following cytotoxic therapy. *Ann Oncol*, **21**, 1982-1989.

Mitchell, C.L., O'Connor, J.P., Roberts, C., Watson, Y., Jackson, A., Cheung, S., Evans, J., Spicer, J., Harris, A., Kelly, C., Rudman, S., Middleton, M., Fielding, A., Tessier, J., Young, H., Parker, G.J. and Jayson, G.C. (2010)
A two-part Phase II study of cediranib in patients with advanced solid tumours: the effect of food on single-dose pharmacokinetics and an evaluation of safety, efficacy and imaging pharmacodynamics. *Cancer Chemother Pharmacol*, epub Dec 1.

Rustin, G.J., van der Burg, M.E., Griffin, C.L., Guthrie, D., Lamont, A., Jayson, G.C., Kristensen, G., Mediola, C., Coens, C., Qian, W., Parmar, M.K. and Swart, A.M. (2010)
Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. *Lancet*, **376**, 1155-1163.

Other Publications

Hasan, J. and Jayson, G.J. (2010)
Novel anti-angiogenic therapies in ovarian cancer. In: *Emerging therapeutic targets in ovarian cancer*. Eds Kaye, S., Brown, R., Gabra, H. and Gore, M. pp 51-72. Springer New York

Murukesh, N., Dive, C. and Jayson, G.C. (2010)
Biomarkers of angiogenesis and their role in the development of VEGF inhibitors. *Br J Cancer*, **102**, 8-18.

Zee, Y.K., Chan, S.W., Harris, J. and Jayson, G.C. (2010)
The ethical and scientific case for phase 2C clinical trials. *Lancet Oncol*, **11**, 410-411.

Zee, Y.K., O'Connor, J.P., Parker, G.J., Jackson, A., Clamp, A.R., Taylor, M.B., Clarke, N.W. and Jayson, G.C. (2010)
Imaging angiogenesis of genitourinary tumors. *Nat Rev Urol*, **7**, 69-82.

Active Patents

Jayson, G., Gardiner, J. and Hansen, S.
Production of L-Iduronate containing polysaccharides, Int. publication: WO 2006/129075 A1: 2006-12-07.

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

Prenant, C., Bailey, J., Gillies, J., Chimon, G., Smith, N., Jayson, G.C. and Zweit, J.
[18F] Fluoroacetaldehyde, a potential new reagent for direct radiofluoroalkylation of proteins and peptides (under review in Journal of Labelled Compounds and Radiopharmaceuticals). Patent by The University of Manchester: U.S. Patent Appln No.60/902060.

Additional Publications

Refereed Research Papers

Betsou, F., Lehmann, S., Ashton, G., Barnes, M., Benson, E.E., Coppola, D., DeSouza, Y., Eliason, J., Glazer, B., Guadagni, F., Harding, K., Horsfall, D.J., Kleeberger, C., Nanni, U., Prasad, A., Shea, K., Skubitz, A., Somiari, S. and Gunter, E. (2010)
Standard preanalytical coding for biospecimens: defining the sample PREanalytical code. *Cancer Epidemiol Biomarkers Prev*, **19**, 1004-1011.

Workman, P., Aboagye, E.O., Balkwill, F., Balmain, A., Bruder, G., Chaplin, D.J., Double, J.A., Everitt, J., Farningham, D.A., Glennie, M.J., Kelland, L.R., Robinson, V., Stratford, I.J., Tozer, G.M., Watson, S., Wedge, S.R. and Eccles, S.A. (2010)
Guidelines for the welfare and use of animals in cancer research. *Br J Cancer*, **102**, 1555-1577.

External Seminar Speakers 2010

2010 produced another set of excellent speakers for our external seminar series. As in 2009 the Paterson seminars were complemented by seminars in the Breakthrough Breast Cancer Research Unit series which produced an outstanding range of speakers covering a wide range of topics. The Institute also has weekly postdoctoral seminars which continue to be very well attended.

Christoph Ballestrem
Wellcome Trust Centre for Cell-Matrix Research, The University of Manchester

Chris Boshoff
University College London Cancer Institute

Michael Boutros
German Cancer Research Centre, Heidelberg, Germany

Keith Caldecott
Sussex Centre for Genome Damage and Stability, University of Sussex, Brighton

Jason Carroll
Cambridge Research Institute

Matthew Collin
Cambridge Research Institute

Simon Cook
The Babraham Institute, Cambridge

Adrian Harris
University of Oxford, Weatherall Institute of Molecular Medicine, Oxford

Nick Hastie
MRC Human Genetics Unit , Edinburgh

Kristian Helin
Biotech Research & Innovation Centre (BRIC), University of Copenhagen, Denmark

Penny Jeggo
Sussex Centre for Genome Damage and Stability, University of Sussex, Brighton

Peter Laslo
Leeds Institute of Molecular Medicine, University of Leeds

Alison Lloyd
University College London

Ultan McDermott
Wellcome Trust Sanger Institute, Cambridge

Claus Nerlov
MRC Centre for Regenerative Medicine, University of Edinburgh

Jim Norman
The Beatson Institute, Glasgow

Klaus Pantel
University Medical Center Hamburg-Eppendorf, Germany

Philippe Pasero
Institute of Human Genetics, Montpellier, France

Matthias Peter
Swiss Federal Institute of Technology, Zurich

Jordan Raff
Sir William Dunn School of Pathology, University of Oxford

Peter Ratcliffe
Nuffield Department of Clinical Medicine, University of Oxford

Joel Richter
King's College London

Pablo Rodriguez-Viciana
University College London

Peter Ten Dyke
Queen Mary, University of London

Bart Van Haesenbrook
Queen Mary, University of London (QMUL)

Ashok Venkitaraman
MRC Research Centre, Cambridge

Juan Jose Ventura
Wellcome Trust Centre for Stem Cell Research, Cambridge

John York
Duke University Medical Center, Durham USA

Philip Zegerman
Gurdon Institute, Cambridge University

Breakthrough Breast Cancer Research Unit Seminar Series 2010

Gabriela Dontu
Department of Academic Oncology, Guy's Hospital

Mitch Dowsett
Royal Marsden Hospital

Barry Gusterson
Head of Pathology & Gene Regulation and Forensic Medicine & Science, University of Glasgow

Lars Holmberg
Division of Cancer Studies, King's College London School of Medicine

Rudolf Kaaks
Division of Cancer Epidemiology, German Cancer Research Centre

Yibin Kang
Department of Molecular Pathology, Princeton University

Lucio Miele
Ergon Professor of Medicine and Pharmacology Director, University of Mississippi Cancer Institute, University of Mississippi Medical Center

Salvatore Pece
University of Milan, Italy

John Stingl
CRUK Cambridge Research Institute

Postgraduate Education

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

Postgraduate Education Manager

Julie Edwards

Postgraduate Tutor

Crispin Miller

A well-supported graduate programme is of fundamental importance to a research institute such as the Paterson, both to train the researchers of tomorrow, and for the valuable contribution made by our students to the labs they are working in. In 2010, we welcomed another ten graduate students from around the world to join our PhD programme, working in fields as diverse as yeast genetics, stem cells and clinical research. This year ten PhD students and three Clinical Fellows were awarded their PhDs.

The Paterson Graduate Programme

We aim for each student to receive high quality 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 course of their studies, 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 monitored, but also to help develop the presentation skills that are so fundamental to the majority of careers in science and elsewhere. Graduate training is monitored by an Education Committee, which features Group Leaders, senior clinicians and scientists, and student representatives (see below). Each student is 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 expected to attend all of these external seminars. The speakers are internationally renowned scientists and we consider it essential that our students are exposed to outstanding work from the leaders in different disciplines, which will give them a broad understanding of many aspects of cancer research and basic biology. In addition we hold a series of weekly postdoctoral research seminars which the students attend, and they have the opportunity to present their own work in lab meetings within the institute.

PhD studentships

All our CR-UK funded studentships are of four years duration, and consist of an approved research project in one of our research groups. Some students have joint supervisors in different groups, fostering collaborations and giving the students exposure to different disciplines. Recruitment is highly competitive, with hundreds of applications competing for around ten places each year. Interviews are typically conducted over a two-day period in early January.

All our students benefit from access to advanced state-of-the-art facilities including advanced imaging, biological mass spectrometry, microarrays, flow cytometry, histology and next generation sequencing. 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-base 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. Clinical training includes one research per 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 2010

Jenny Varley (chair)
Richard Cowan
Julie Edwards
David Gilham
Ian Hampson
Valerie Kouskoff
Karim Labib
Crispin Miller
Donald Ogilvie
Vaskar Saha
Tim Somervaille
Catharine West
Caroline Wilkinson

Student Representatives

Emily Holmes (from November)
Tim Maculins
Andrjez Rutkowski (until November)

Operations

Director of Operations
Pippa McNichol

2010 has been a year of consolidation. After the shock of the financial situation and subsequent decrease in the Institute's budget from CR-UK in 2009, staff across the Institute rose admirably to the challenge of keeping costs down and driving hard deals with all of the Institute's suppliers. As a consequence at the end of the third quarter, the deficit has decreased to £96K.

At the beginning of the year the heavy snow and bad weather caused the Institute to shut down for two days, which had never happened before. Consequently, when it was re-opened the Major Incident Plan was updated to encompass all the lessons learned. The IT department worked hard to introduce video-conferencing facilities in time for the annual budget session with CR-UK.

The HR department worked miracles to ensure that CR-UK's new pay and grading system for non-scientific and managerial staff was implemented in a timely manner.

Two new staff joined the Operations team during 2010: Becky Allen as the Administration Services Co-ordinator and Muhammad Raja as the Finance Assistant. Both these posts were vacant posts rather than new posts.

CR-UK organised a large new fundraising event in Manchester in April – Shine – a midnight walking marathon. Paterson staff undertook demonstrations of science at the start of the walk at Manchester Central and showed the walkers how to extract DNA from strawberries. The Paterson Institute was the six mile mark and so another group of staff volunteered to provide the walkers with drinks and encouragement. The atmosphere was brilliant and the event was a huge success for CR-UK.

The Paterson Football team hosted a return match for the Beatson Football team (our sister Institute) in October. The event was turned into a Family Fun Day for Paterson staff and £1167 was raised for Cancer Research UK. The Paterson team had lost the last two matches to the Beatson so there was a lot at stake. It is very gratifying that the Paterson team won the match 4-2. All their training had at last paid off.

The long awaited new intranet for staff – PICRboo was launched in December.

Just before Christmas it was announced that the Director, Professor Nic Jones would be stepping down as Director of the Institute to take up the very prestigious position of Chief Scientist for CR-UK. The news has left the Institute a little shell-shocked but absolutely delighted for Nic. An interim Senior Management team is being established to run the Institute until a replacement director can be found, so 2011 will be a year of great challenges.

Admin and Reception Services
Manager: Amy Weatheritt

Over the year the admin department has evolved and is now providing a productive and effective administrative service to the Institute. The appointment of a permanent Security Coordinator and Receptionist and Administration Services Coordinator has enabled the department to ensure a high quality service and provide support across the groups within the Institute. The department has assisted with many different events over the year, from fundraising to conferences and has expanded its service to provide professional assistance in the arranging of events internally and externally. The department was responsible for helping to organise the 2010 colloquium which went well and looks forward to arranging the 2011 colloquium at its new location in Lancaster.

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

The 2010 Paterson seminar series was a great success with over 40 speakers visiting the Institute including two distinguished seminar talks taking place in November. The 2011 seminar series is currently being arranged and it is hoped this will be just as productive. The seminar series is vital for the Institute, particularly for the students and postdoctoral fellows in helping them to become exposed to world-class research. It brings together and connects researchers and provides an opportunity for interaction for both the speaker and the staff at the Institute. A list of speakers for 2010 can be found on pages 76/77.

Recruitment has been a high priority for the office which has worked closely with HR. It was good to welcome John Brognard who was appointed as a Junior Group Leader of the Signalling Networks in Cancer Group to the Institute. Recruitment is ongoing to fill another Group Leader position. Furthermore the organisation of Angeliki Malliri's tenure, viva examinations and high profile meetings have also kept the department busy.

It has also been a busy yet exciting year for the MCRC, and the Director's Office has been supporting the operations and administration of the Centre. As the new build progresses the Director's Office will be assisting the Director of Operations throughout the building process.

The Director's Office will continue to provide a supportive role to the Institute and its Group Leaders and aid the MCRC through what is expected to be an extremely busy but exciting period over the next year.

Estates
Manager: Steve Alcock

2010 has been a challenging time for the Estates team. There has been a considerable amount of capital work carried out in the Institute. The schemes include: reconfiguring an additional GCP lab for CEP; office remodelling for two group leaders and a write up area for their staff; refurbishing the area vacated by Nic Jones into a two person office and write up area. The Building Management System has recently been upgraded to Schnyder's latest platform which will improve the controls and legislative record keeping capabilities. Refurbishment of the photocopying room was recently undertaken to accommodate the institute scanner and printers, plus many other small schemes to improve facilities for the scientific groups.

A popular area of concern is sustainability, and reducing the Institute's carbon footprint as much as possible is one of the Institute's goals. Whenever a new scheme is required, energy-saving devices are utilised and more efficient heating/cooling and ventilation systems are installed. One recent scheme involved the removal of eight ageing individual DX air conditioning units, replaced with a single VRF (variable refrigerant flow) system which will move heat taken from the labs (via the refrigerant gas) and redistribute it in the office areas that the system serves. The VRF heating/cooling

system is very efficient and has lowered energy consumption and improved the working environment of the areas it serves.

The Estates team have been pro-active throughout the year with positive responses from the Estates user group. The Estates team members have attended relevant courses to improve their skills and keep their knowledge up to date with current working practices and changing legislation.

Finance and Purchasing
Manager: Margaret Lowe

The Finance Department is responsible for providing a comprehensive purchasing, travel and finance service on a daily basis to the Research Groups and Service Units within the Paterson Institute. The procurement team continues to work with the groups to identify savings in consumable spending. In addition to this they advise on capital purchases, working with the researchers to ensure legal legislation is adhered to and that the best possible price is obtained for the equipment.

In the current economic climate is it crucial that the management information provided is current and accurate at all times to assist Group Leaders in managing their budgets and for the Directors to have a full overview of the Institute finances.

Group Leaders are also assisted with costing up grant applications and receive full post-award administration support.

Health and Safety
Manager: Colin Gleeson

An Institute-wide Risk Management survey was devised and completed. The survey listed, in a checklist format, safety-related tasks and duties which are essential to the safe running of a laboratory or department. The purpose of the survey was to ensure that individual laboratories and departments had named individuals to carry out the safety-related tasks and duties and to indicate whether the selected persons are trained and competent to carry out their allotted tasks. Analysis of the survey results is continuing but it has already identified some training needs for key tasks, for certain individuals and/or laboratories. The survey will also help ensure that there is no loss of management control when key staff leave the Institute as their safety-related tasks are recorded and can be passed on to others.

Health and safety training has been provided at the monthly Induction along with more detailed information in training sessions on work with biological agents, hazardous chemicals, radiation safety and risk assessment. Also a successful risk assessment workshop was held, whereby individuals discussed and developed risk assessments of actual techniques employed in the laboratory. Feedback form the workshop was positive and this format will certainly be repeated next year.

Statutory required performance testing of fume cupboards, safety cabinets and other local exhaust ventilation equipment was completed by a cost effective and time efficient one-hit testing schedule by the chosen contractor. Other statutory required inspections, such as examination of liquid nitrogen pressure vessels, have also been completed as have equipment maintenance regimes. The rolling programme of formal safety inspections of laboratories and other areas has also been carried out and action-point reports fed back to those concerned with follow up to check actions are completed.

The health and safety pages of the local intranet have been updated and this will continue in to next year.

Human Resources
Manager: Rachel Powell

Over the past year the HR Department has continued to successfully deliver a high quality professional service to the Institute. The focus has been on developing a more customer-focused approach to enhance and support the aims and objectives of the Institute.

The department has continued its drive for efficiency by further improving the recruitment and selection process and evaluating where vacancies are advertised to ensure that that they are cost effective and providing value for money. This year 31 highly skilled scientists and support staff have been recruited to compliment and further enhance the work of the Institute.

It has been a busy and challenging 12 months with the department heavily involved in the transfer of the non scientific staff onto a new pay and grading structure. This was successfully achieved through effective communication and consultation with staff.

Joint partnership working with the unions has continued throughout the year which has resulted in the agreement of several new and revised policies, including the Sickness Absence policy, Secondment policy and the launch of a new Mentoring Framework. The aim of the Mentoring Framework is to allow for the transfer of skills and experience within a supportive environment. This is available when an individual is taking on a new role or would like to increase their knowledge in a specific area.

The main focus for the next twelve months is the successful implementation of the Absence Management system and the online probationary process system.

Information Technology
Manager: Malik Pervez

With continuous demand for superior, faster, cutting edge technology to support the research programme, the IT department has stepped up to the challenge of delivering an excellent IT infrastructure.

During 2010, the introduction of a new desktop operating system and upgrading back-end systems have provided the response time, stability and security required to support pioneering research. The constant need for additional data storage has provided a further challenge and the implementation of an online archive system has alleviated the need to purchase additional storage. The need to process large data sets commands enormous computing power. The task for the IT department this year was to balance the computing power demanded by researchers whilst at the same time reducing the carbon footprint of the solution required. This meant detailed planning and design which resulted in the successful implementation of a new computer cluster without increasing our carbon footprint. Information is the lifeblood of any organisation. Sharing information effectively and quickly requires organisations to have systems in place that are responsive and easy to access. To maximise the flow of information a new Intranet was developed that provided functionality and interactions that were lacking in previous systems. This was launched in December 2010. 2010 has provided all organisations with a challenging financial climate which has led to the need to reduce costs. The introduction of specific technologies such as video conferencing has been pursued to maximise scientists' time whilst reducing travelling costs for routine meetings.

In a year that has presented many challenges for IT, the team has yet again succeeded in delivering very good core systems, new, innovative solutions that are cost effective and environmentally friendly as well as maximising value for money.

Cancer Research Technology
Manager: Martyn Bottomley

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

At CRT we bridge the fundamental gap between cutting edge academic research and industrial development of cancer therapeutics and diagnostics. We achieve this by working closely with prestigious international research institutes, such as the Paterson Institute for Cancer Research, 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. Our 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).

Our relationship with the Institute reflects the specific requirements of the scientist, the Paterson Institute, 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 Institute dedicated to working closely with the staff at the PICR. We are here to offer access to oncology focused expertise in technology evaluations, patent applications and management, funding for development, commercialization, drug discovery, market intelligence, and project management. CRT also works closely with the recently formed Drug Discovery Laboratories to facilitate the development of small molecules drug therapies to satisfy the unmet clinical needs of cancer patients. CRT is currently actively managing a broad portfolio of development programmes and robust licensing opportunities originating from the Paterson that continue to attract commercial partners (ranging from enabling technologies through to drugs in late stage Clinical Trials). We look forward to building on our successes and continuing to work closely with the Institute to advance discoveries to beat cancer.

Cancer Research UK's Local Engagement and Development

LEAD Manager
James Dunphy

2010 has proved to be another busy year of local engagement and development activity in Manchester. Researchers have been involved in fifty engagement events, with 30,000 Cancer Research UK supporters being reached, and a further 500 having the opportunity to visit the Institute. In addition to the monthly lab tour programme and open day, an extra event was held to commemorate the opening of the new Drug Discovery laboratories. This was attended by fifty supporters who had been involved in helping to promote and organise Cancer Research UK's Race for Life events across the northwest.

Researchers have continued to support their local fundraisers with inspirational speeches conducted at key Race for Life, Run 10k, Relay for Life and Committee events throughout the year. In addition to this the Institute has once again actively fund-raised for the charity with the Keswick to Barrow and Relay for Life teams raising £3000. A fun day was also organised as part of the Paterson Football Club versus Beatson Football Club game and £1200 was raised from on the day activities and sponsorship.

An important element of local engagement activity in Manchester involves making connections with local schools and colleges to inspire and inform their students about the potential to become cancer researchers of the future. This years School's day was the biggest yet with thirteen local schools sending 66 students to the Institute to gain a practical insight into the work undertaken here. The feedback was excellent:

"Being in the labs and being able to do real techniques confirmed that I definitely do want to work in a lab in the future".

The Institute was heavily involved in the promotion and on the night activity for Shine, CR-UK's inaugural night-time walking marathon. A team of researchers hosted an interactive area at the start, another team manned the official pit stop at the Institute and a further ten representatives took part in the walk. This pilot event was a resounding success with 7500 walkers taking part and raising over £1.8 million for Cancer Research UK. The importance of the Institute involvment in this event was highlighted by a participant:

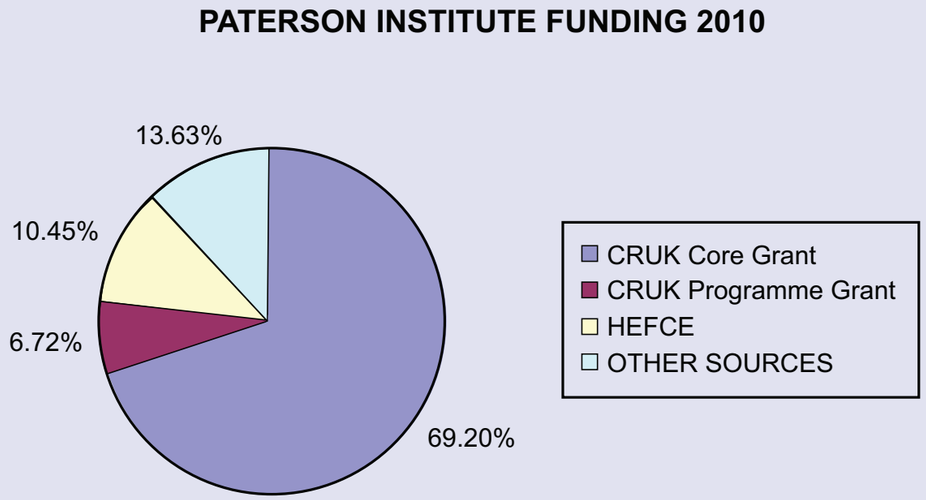
"I was a participant in SHINE on Saturday night and just wanted to thank the staff of the Institute for turning out and supporting us. The guys that I met at Central were very patient and explained what the money is used for brilliantly. Then when we got to the Institute it was lovely to be cheered on. It was so nice to actually meet the people who spend their work lives trying to help people with this nasty disease. I would be grateful if you could pass on my thanks to all and tell them how much their time was appreciated on Saturday and how much their work is appreciated every day"

Shine will be returning to Manchester in 2011 and the success of the activity undertaken in Manchester will be replicated at the new Glasgow and London events.

Paterson scientists outside the Institute during the Shine night time walk

Acknowledgement for Funding of the Paterson Institute

The total funding of the Paterson Institute for 2010 was £15.48M. The major source of this funding (69.2%) was through a core grant from Cancer Research UK (CRUK). The actual value of this award in 2010 was £10.7m. 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 CRUK awarded us £1m to run the Drug Discovery unit (6.72%).



The infrastructure of the Paterson Institute is funded by HEFCE generated income at a cost of £1.6m (10.45%)

The final 13.63% 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 & Lymphoma Research Fund
- Novartis
- DXS
- Chugai
- Abbott Laboratories
- GeminX

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 five 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, next generation sequencing 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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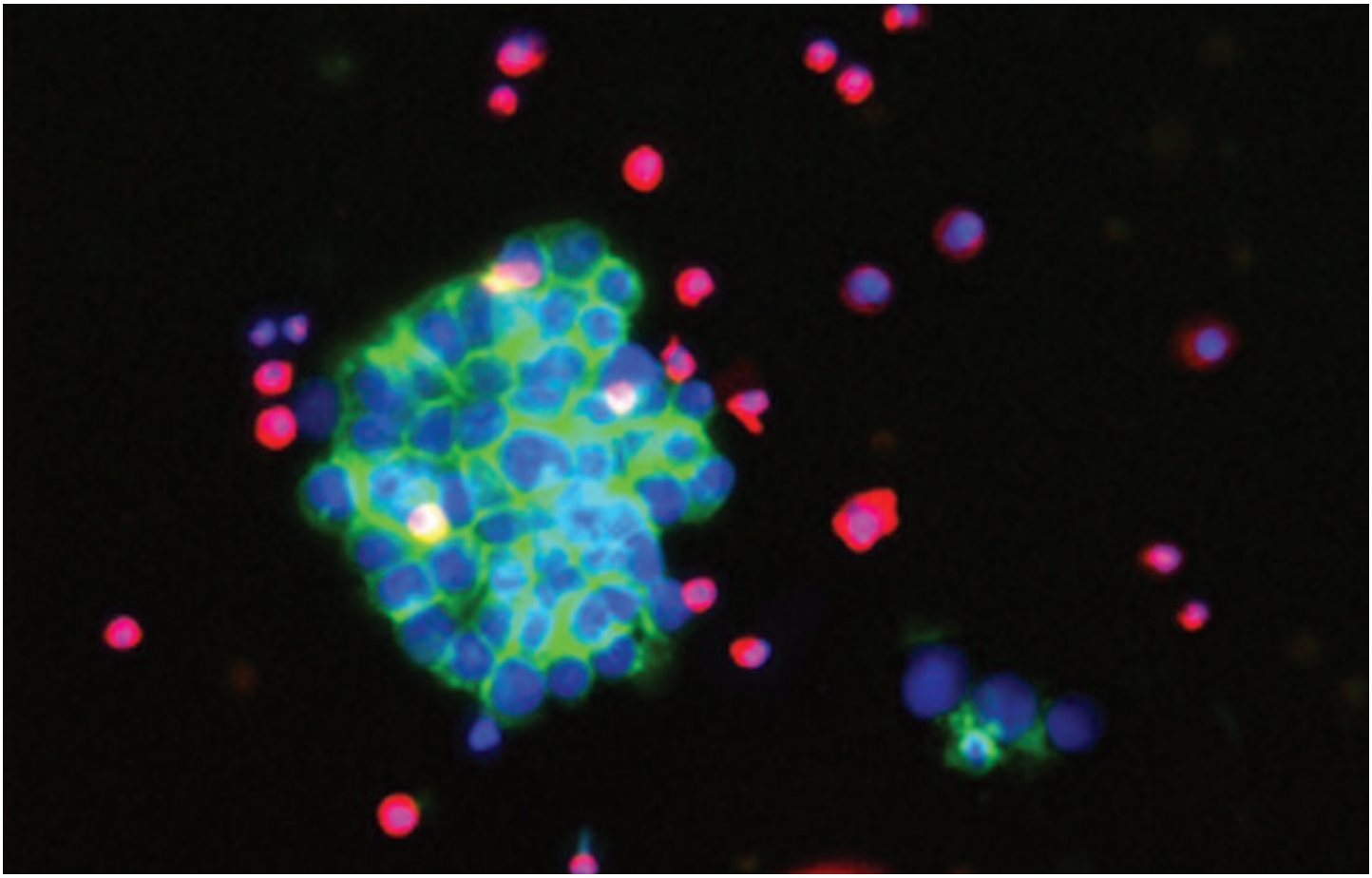

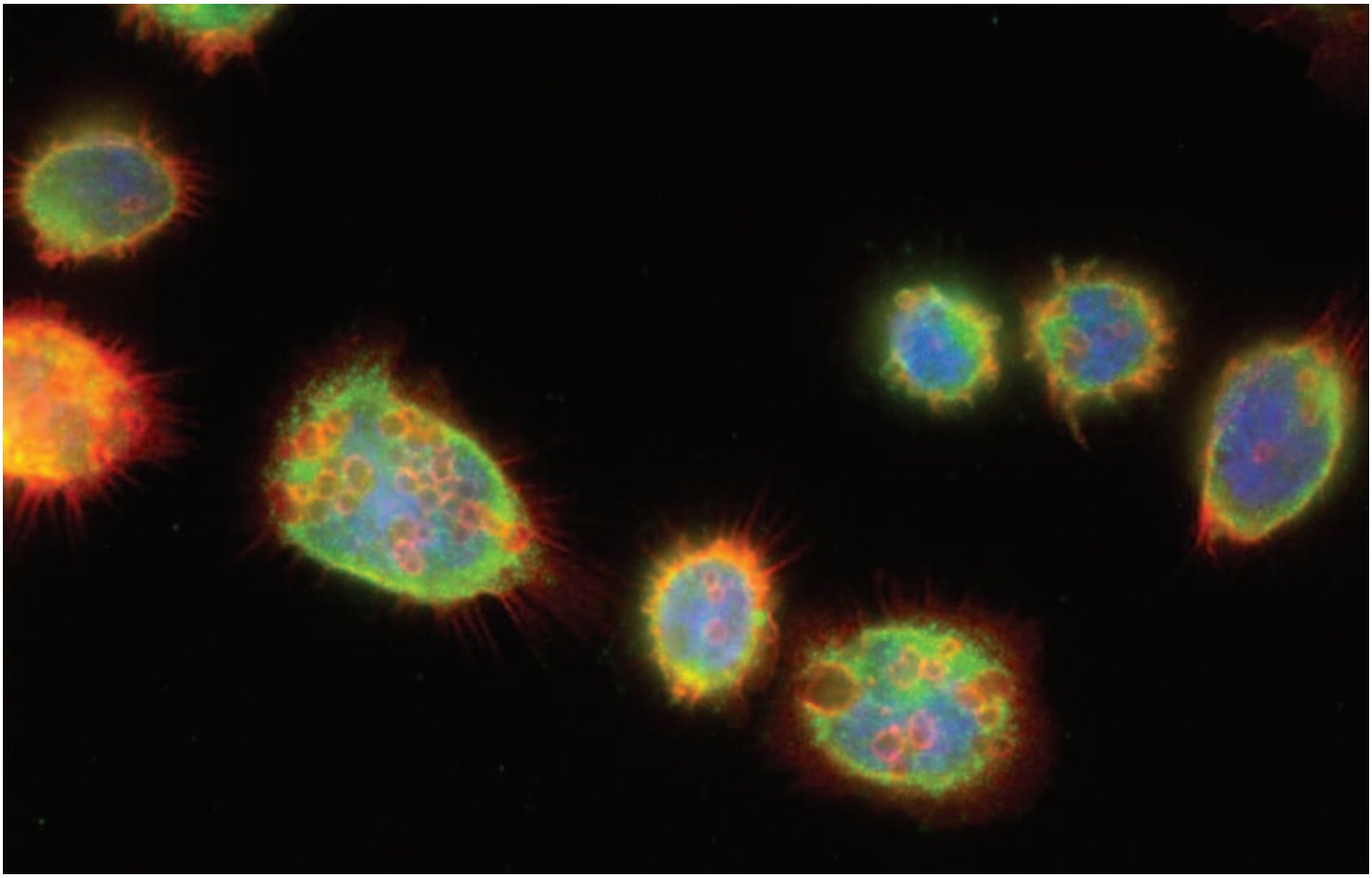

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