Budding yeast cells lacking the Inn1 protein are unable to complete cytokinesis. These cells express a fusion of a green fluorescent protein to a marker of the plasma membrane, and have red fluorescent proteins attached to components of the spindle poles and actomyosin ring (Sanchez-Diaz et al., Nature Cell Biology 2008; 10: 395).

Additional images:

Front cover image supplied by Helen Rushton, Simon Woodcock and Angeliki Malliri (Cell Signalling Group). The image is of a mitotic spindle in fixed MDCK (Madin-Darby canine kidney) epithelial cells, which have been stained with an anti-beta tubulin antibody (green), DAPI (blue) and an anti-centromere antibody (CREST, red) which recognises the kinetochores of the chromosomes. The image was taken on the spinning disk confocal microscope using a 150 x lens.

Rear cover image supplied by Andrei Ivanov and Tim Illidge (Targeted Therapy Group). Visualisation of tubulin (green) and quadripolar mitosis (DNA stained with DAPI), Burkitt’s lymphoma Namalwa cell after 10 Gy irradiation.

ISSN 1740-4525
Copyright 2008 © Cancer Research UK
## Contents

4  Director’s Introduction

**Researchers’ pages – Paterson Institute for Cancer Research**

<table>
<thead>
<tr>
<th>Page</th>
<th>Name</th>
<th>Research Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Crispin Miller</td>
<td>Applied Computational Biology and Bioinformatics</td>
</tr>
<tr>
<td>10</td>
<td>Geoff Margison</td>
<td>Carcinogenesis</td>
</tr>
<tr>
<td>12</td>
<td>Karim Labib</td>
<td>Cell Cycle</td>
</tr>
<tr>
<td>14</td>
<td>Iain Hagan</td>
<td>Cell Division</td>
</tr>
<tr>
<td>16</td>
<td>Nic Jones</td>
<td>Cell Regulation</td>
</tr>
<tr>
<td>18</td>
<td>Angeliki Malliri</td>
<td>Cell Signalling</td>
</tr>
<tr>
<td>20</td>
<td>Caroline Dive &amp;</td>
<td>Clinical and Experimental Pharmacology</td>
</tr>
<tr>
<td></td>
<td>Malcolm Ranson</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Peter Stern</td>
<td>Immunology</td>
</tr>
<tr>
<td>24</td>
<td>Nullin Divecha</td>
<td>Inositolide Laboratory</td>
</tr>
<tr>
<td>26</td>
<td>Tim Somervaille</td>
<td>Leukaemia Biology</td>
</tr>
<tr>
<td>28</td>
<td>Georges Lacaud</td>
<td>Stem Cell Biology</td>
</tr>
<tr>
<td>30</td>
<td>Valerie Kouskoff</td>
<td>Stem Cell and Haematopoiesis</td>
</tr>
<tr>
<td>32</td>
<td>Akira Orimo</td>
<td>Stromal-Tumour Interaction</td>
</tr>
</tbody>
</table>

**Researchers’ pages – The University of Manchester School of Cancer and Imaging Sciences**

<table>
<thead>
<tr>
<th>Page</th>
<th>Name</th>
<th>Research Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Catharine West</td>
<td>Academic Radiation Oncology; Translational Radiobiology Group</td>
</tr>
<tr>
<td>36</td>
<td>Robert Hawkins &amp;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peter Stern</td>
<td>Biological, Immune and Gene Therapy</td>
</tr>
<tr>
<td>38</td>
<td>Vaskar Saha</td>
<td>Children’s Cancer Group</td>
</tr>
<tr>
<td>40</td>
<td>Tim Illidge</td>
<td>Targeted Therapy</td>
</tr>
<tr>
<td>42</td>
<td>Robert Hawkins</td>
<td>Medical Oncology; Cell Therapy</td>
</tr>
<tr>
<td>44</td>
<td>John Gallagher</td>
<td>Medical Oncology; Glyco-Oncology</td>
</tr>
<tr>
<td>46</td>
<td>Gordon Jayson</td>
<td>Medical Oncology; Translational Angiogenesis</td>
</tr>
</tbody>
</table>
48 Research Services

48 Steve Bagley  Advanced Imaging Facility
49 Biological Resources Unit
51 Stuart Pepper  Cancer Research UK GeneChip Microarray Service
51 Morgan Blaylock  Flow Cytometry Facility
52 Garry Ashton  Histology
53 Steve Glover  Kostoris Library
54 Mark Craven  Laboratory Services
54 Maurice Cowell  Logistics
54 Stuart Pepper  Molecular Biology Core Facility
54 Duncan Smith  MBCF Biological Mass Spectrometry Facility

56 Publications
66 Seminars
68 Postgraduate Education
70 Operations Services
73 Local Engagement and Development
74 Acknowledgement of Funding
75 Career opportunities
76 How to find us
Welcome to the 2008 Paterson Institute annual Scientific Report. As always it has been a busy year scientifically with further recruitment and consolidation of the partnership represented by the Manchester Cancer Research Centre.

Recruitment of new scientific leaders is essential for maintaining research excellence and building up areas of research strength. It is particularly important for a research Institute such as the Paterson Institute to recruit young group leaders and to provide the support and research environment that will allow their research development to flourish. Building on a very busy recruitment schedule last year, we have recruited another Junior Group Leader, Ivan Ahel, who comes to us on the back of a very successful and productive postdoctoral period in Steve West’s laboratory at our sister Institute, the London Research Institute. Ivan’s research interests have focused on DNA repair mechanisms and in particular on novel repair proteins that contain a poly (ADP-ribose)-binding zinc finger motif (PBZ). He will continue this work when he joins us on 1st January 2009, with an emphasis on how the interaction of PBZ-containing proteins with other proteins that are modified by poly (ADP-ribosyl)-ation orchestrates the generation of complexes that function in ensuring an efficient cellular response to DNA damage. Another recruit to the Institute is Donald Ogilvie who will head the new and exciting development of a Drug Discovery Centre. This new initiative is a key component of Cancer Research UK’s strategic plan to increase its capability and activity in the development of small molecule drugs. Donald comes to us after a very distinguished career with the pharmaceutical giant AstraZeneca where he coordinated the development of a number of new drugs some of which are currently being tested in phase III clinical trials. He will join the Institute on the 1st February 2009 and will begin the challenging task of building a drug discovery team with major medicinal chemistry expertise and identifying promising targets that will be the initial focus of the centre. It is an important development for us since it provides a scientific bridge between our activities and strengths in basic cancer biology and our strengths in translational research associated with early clinical trials and development of new biomarkers.

In contrast to these exciting new comings, sadly we saw the going of John Gallagher, head of the Glyco-Oncology group, who is retiring after an association with the Institute of over 30 years. John is a world expert in glyco-biology particularly with respect to heparan sulphates found on the surface of all cells.
where they function as important co-receptors for a number of growth factors including the fibroblast growth factors and hepatocyte growth factor. John’s work has highlighted the complexity of different sulphation patterns seen with the heparan sulphates and how these patterns affect such key biological processes such as stem cell differentiation and malignant transformation. John shone through with his enthusiasm, dedication and above all his modesty. He will be sorely missed.

During this last year, the Clinical and Experimental Pharmacology (CEP) group in the Institute was site-visited by an international panel of experts. These site visits provide a stringent assessment of the international standing of the research programme in question by examining the progress and impact of the research over the last five years and a view on the potential and importance of the proposed research programme for the next five years. This was a particularly important review for the Institute since CEP is a relatively new group which is at the heart of the Institute’s translational research efforts and which has grown rapidly over the last few years. It was therefore very pleasing and reassuring that the group received a very positive review and whose research was judged to be at the international forefront. CEP works very closely with the early phase clinical trials unit (The Derek Crowther Unit, DCU) and has as its mission the discovery and validation of biomarkers that provide indications on the efficacy and toxicity of new molecules being tested in clinical trials. This type of research is crucially important for modern drug development and lies at the heart of the need to identify early in the development programme, drugs that are likely to be effective and patients are likely to respond to – in other words, the implementation of personalised medicine. If the results of such research are used to inform clinical decision making, then it has to be conducted under very strict regulatory guidelines. The CEP has established itself as one of the leading centres internationally with this capability. CEP is lead by Caroline Dive working closely with the head of the DCU, Malcolm Ranson. They were both very deservedly congratulated by the visiting party for developing such an exciting and important research programme which exemplified the power of a strong and effective clinical-laboratory interface. Over the next few years further development and investment in this research area will take place, the most significant development being the expansion of the clinical trials facility as part of the £35M development by The Christie NHS Foundation Trust. This will result in a 3-fold increase in the capacity of the DCU, creating one of the largest early clinical trials units in the world.
There were a number of research highlights during the year. The Stem Cell Biology group led by Georges Lacaud characterised in detail the developmental steps leading to the generation of haematopoietic cells, which despite receiving a lot of attention from many groups over a number of years, remained controversial with two conflicting theories predominating. One theory hypothesised that haematopoietic cells arise from a mesodermal progenitor called the haemangioblast whilst the other suggested that such cells arise from a specialised endothelial cell that has haematopoietic potential, the haemogenic endothelium. Studies from Georges’s laboratory have beautifully reconciled these opposing views showing that both theories are merged into a single developmental process where the haemangioblast gives rise to a haemogenic endothelium intermediate which then further differentiates to generate haematopoietic cells. Furthermore, they identified specific transcription factors that regulate different stages of this differentiation pathway. This important work will be published early in 2009 in the journal Nature. The Cell Signalling Group led by Angeliki Malliri has been studying a regulator (Tiam 1) of the Rac GTPase which, amongst many of its roles, functions to control cell-cell adhesion. Previous work from the group had shown that deletions of Tiam1 confer resistance to the formation of both skin and intestinal tumours in appropriate mouse models. They have now discovered that Tiam1 is phosphorylated and regulated by the oncoprotein Src, a tyrosine-kinase implicated in malignant transformation. This phosphorylation occurs preferentially at cell-cell contacts resulting in Tiam1 degradation and disruption of cell adhesion. This regulation of Tiam1 is likely to be important for Src-mediated cancer cell invasion and metastasis. Interestingly, in a range of different cancers, they found a correlation between Tiam1 phosphorylation and Src-activity consistent with this regulatory mechanism operating in tumour malignancies. This work will be published early in the New Year in the journal Molecular Cell. The Cell Cycle laboratory headed by Karim Labib has been using yeast as a model system to investigate the regulation of DNA replication and cell division. During the last year they reported in Nature Cell Biology the discovery of a novel factor called Inn1 which is essential right at the end of the cell cycle for cytokinesis, the process whereby the eukaryotic cell divides into two. This has to be regulated in time to ensure that it does not occur before nuclear division, and in space to ensure that the division plane lies between the two nuclei formed during mitosis. The characterisation of Inn1 has provided new insight into a relatively poorly understood yet vital process. Powerful technologies are now available to quantitate the cellular levels of RNA transcripts through microarray analysis and proteins through quantitative mass spectrometry proteomics. The Applied Computational Biology and Bioinformatics laboratory led by Crispin Miller has been developing new tools to integrate microarray and proteomics data. A new and powerful approach was recently refined and published in BMC Bioinformatics. These are just a few selected examples of the research progress that has been made over the last year.

The Manchester Cancer Research Centre (MCRC) which is now in its 3rd year continues to make good progress in integrating cancer research efforts across Manchester and realising the benefits and opportunities that the partnership between The University of Manchester, The Christie NHS Foundation Trust and Cancer Research UK can provide. It is galvanising researchers to work together to develop areas of research strength, to develop research infrastructure, to co-ordinate research training and to ensure close alignment of basic and clinical research. Significant advances have been made in a number of areas: early phase clinical trials and biomarker research as
described above; centralised tumour biobanking involving five NHS Trusts across the Manchester conurbation; development of radiation-related research; development of molecular pathology through the recruitment of a new Chair (Goran Landberg); consolidation and expansion of the formal alliance with AstraZeneca which supports research and training across a number of research areas. The MCRC is very much in line with the major initiative of Cancer Research UK to develop a number of centres across the Country where research activities are closely linked with patient care and public engagement. The MCRC already fulfils much of what is envisaged in such a centre.

In December the results of the national Research Assessment Exercise (RAE) were announced. This exercise provides a comprehensive assessment of the quality of research in UK universities. It was therefore tremendous news that cancer research in Manchester was officially ranked as the best in the UK. This was a great endorsement of the research taking place in the Paterson, The University of Manchester and The Christie NHS Foundation Trust and a major boost to the MCRC partnership.

As always the coming year will provide many new challenges and opportunities for the Institute. The 5-year Institute Review will take place in June 2009. These reviews are obviously important for Cancer Research UK since they judge the overall success and strategy of the Institute. They can also be valuable to the Institute and it was discussions at the last review that precipitated talks that lead to the development of the MCRC. The development of the Drug Discovery Centre will also be high on the agenda. The big challenge will be to continue to develop the Institute and support all our current research activities in a particularly difficult economic climate.

Nic Jones
Director
Bioinformatics and Computational Biology exist to help us make sense of the complex datasets generated by research in the biosciences. In the Applied Computational Biology and Bioinformatics Group, we are interested in the development and application of software tools and analytical strategies for the analysis of cancer related datasets. We collaborate closely with other groups working on both the clinical and the molecular biology aspects of cancer research.

Genome annotation

Until recently, the genome was viewed as primarily involved in defining proteins, via a set of protein coding genes separated by large tracts of ‘junk’ DNA. Over the last few years, this perspective has changed markedly. We now know that as much as 90% of the human genome can, in some circumstances, be transcribed, and that much of this transcription leads to RNA that, although never translated into protein, is functional in its own right. In addition, the process of alternative splicing, by which a cell can systematically include or exclude parts of a gene’s sequence in order to yield a set of distinct gene products from a single locus, has also been shown to be prevalent. Both non-coding RNA and alternative splicing have been implicated in many cellular processes and pathways, and both are known to be involved in a variety of human diseases, including cancer. We are interested in developing software tools and databases (e.g. X:Map; xmap.picr.man.ac.uk) to help describe and interpret these regions within the genome, in order to better exploit the data generated by new technologies such as next generation sequencing, SNP, exon and tiling arrays, and quantitative proteomics.

Affymetrix exon arrays

DNA microarrays consist of an inert substrate, such as a glass slide, onto which groups of single stranded DNA molecules are fixed at known locations. Each group, or ‘spot’, contains millions of identical molecules, which are designed so that their sequence is complementary to that of a particular transcript or region of interest. When labelled, fragmented, DNA or RNA is applied to the surface of the array it will hybridize when it encounters a spot with the appropriate complementary sequence, bringing its (typically fluorescent) label with it. The hybridized array can then be imaged and analysed using a computer to measure the relative amount of binding at each spot, thus providing an estimate of the amount of material in the original sample. Microarrays make it possible to generate global measurements of gene expression for many thousands of transcripts in parallel, or to look for Single Nucleotide Polymorphisms (SNPs) or changes in DNA copy number. Unlike most microarrays, for which there is generally a one-one mapping between probe(set) and gene, Affymetrix exon arrays aim to include a separate probeset for every known and predicted exon in the entire genome. This allows differential expression to be considered at the exon level, important given the prevalence of alternative splicing. We are collaborating with a number of fundamental and clinical research groups to use these tools available to other researchers, and they are used internationally. Current research is focused on developing novel approaches for interpreting exon array data, and in particular, on generating the meaningful summaries that are required in order to reduce the data from the arrays’ 1.4
million probesets to a more manageable size suitable for human interpretation.

**Quantitative Proteomics**

Tandem mass spectrometry (MS/MS) is a technique that allows proteins to be identified by fragmenting them into peptides that are then fragmented further and analysed using a mass spectrometer. This results in a set of characteristic ion masses that can then be used to identify each peptide (and thus their originating protein) via a database search. A number of techniques such as iTRAQ allow protein identification to be coupled with quantitation in order to measure the relative abundance of multiple proteins between samples. Typically, this is done by averaging the signal from a protein’s individual peptides, to generate a protein level consensus value. The path from DNA to RNA to protein is a complex one, involving many stages of regulation and control. We are interested in integrating quantitative protein and mRNA level data in order to help explore the space between transcription and translation. In Bitton et al. (2008), we showed for the first time that it was feasible to integrate transcription data generated using Affymetrix exon arrays with quantitative proteomics data from iTRAQ. The increased resolution of exon arrays makes it possible to do this at the level of individual peptides, which are then mapped back to their originating exons via the genome. When this was done, we found very high correlation between the protein and transcript data in a steady state cell line system, chosen to reduce biological variability in order to provide a suitable evaluation dataset. This demonstrates both the feasibility of the approach and the quality of the data generated by both platforms. Most recently we have started to combine these data with copy number information generated using Affymetrix SNP arrays.

**Analysis of archival material**

An issue with using microarrays for expression profiling arises because of the need for high quality RNA samples for analysis. Many archival samples have been stored as Formalin-Fixed Paraffin-Embedded Tissue (FFPET), in which the RNA has been chemically modified and/or degraded, making them challenging to analyse by microarray. A collaboration with the Department of Medical Oncology at the Christie Hospital and the Cancer Research UK Affymetrix service, explored the possibility of using this material on Affymetrix GeneChip Arrays. We found that with appropriate attention to Quality Control, it was possible to generate clinically relevant reliable data from these samples. (Linton et al. 2008).

Publications listed on page 56
The treatment of cancer often involves the use of drugs that kill cells by damaging their DNA. That some tumours do not respond to such treatments can be attributed to the presence of repair processes which can remove potentially lethal damage from DNA. Understanding how DNA damage leads to cell death, and how these repair systems process the damage, may provide opportunities to improve the effectiveness of existing cancer therapies, and develop new agents. Our main focus is on DNA damage and repair following exposure to certain types of alkylating agents, for example the CR-UK drug, Temozolomide. Clinical trials of a drug that was designed to ablate one of the repair processes that is critical in Temozolomide resistance are now essentially completed. In fission yeast, we found a different mechanism for dealing with toxic DNA damage, and our efforts are now directed towards the characterisation of this novel repair pathway.

**Background**

Alkylating agents are a family of chemicals that are used in the treatment of several types of cancer including brain tumours (glioma) and skin tumours (melanoma) but there is considerable room for improvement in their effectiveness. These agents generate a dozen different types of damage in DNA and there is increased understanding of the mechanisms by which some of these result in cell killing. Thus agents such as Dacarbazine and Temozolomide generate $O^6$-methylguanine in DNA and this kills cells via the action of the post replication mismatch repair system. Cell killing by this pathway can be prevented by the prior action of the damage reversal protein, $O^6$-alkylguanine-DNA alkyltransferase (MGMT). This protein most probably evolved to protect organisms against the toxic effects of endogenously or environmentally generated lesions in DNA. However, it can also reduce the effectiveness of these chemotherapeutics and hence there is increasing interest in ablating the activity of MGMT in tumours in order to improve clinical outcome. One of the strategies we have pursued has involved the development and use of Lomeguatrib, a drug that has now completed phase II clinical trials in combination with Temozolomide. MGMT removes alkyl groups from the $O^6$-position of guanine by stoichiometric transfer to a cysteine residue in its active site, a process that results in its irreversible inactivation. We recently discovered, using computer-based amino acid sequence analysis, several proteins that have extensive homology to MGMT, but without this critical cysteine residue. These proteins are present in a number of organisms, including E. coli and the fission yeast, S. pombe. We have named this family the alkyltransferase-like (ATL) proteins and we are currently intensively investigating their modus operandi.

**Clinical trials of Lomeguatrib**

Lomeguatrib ($O^6$-[4-bromothenyl]guanine, previously called PaTrin-2) is one of the products of a very fruitful collaboration with Prof Brian McMurry and the late Dr Stanley McElhinney (and their group at the Chemistry
Department, Trinity College, Dublin). It is a very potent inactivator of MGMT and it effectively sensitises human cells and tumour xenografts to the killing effect of Temozolomide and other agents of that type. With the support of CR-UK Drug Development and Formulation Units and also Cancer Research Technology, Phase I clinical trials of this drug started here at Christie Hospital in 2000 in the hands of Drs Malcolm Ranson and Mark Middleton (now at the Churchill Hospital, Oxford). These trials established a dose combination of Lomeguatrib and Temozolomide for use in Phase II trials, which have been carried out under the auspices of KuDOS pharmaceuticals, to whom Lomeguatrib was licensed. The clinical trials required us to develop and validate to Good Clinical Laboratory Practice standards, quantitative assays for both functionally active and total MGMT protein. Malignant melanoma patients were recruited from several centres in the UK and Australia. They were treated with either Temozolomide alone or a Lomeguatrib-Temozolomide combination. In addition, patients displaying disease progression on Temozolomide (alone) therapy were treated with the Lomeguatrib-Temozolomide combination to assess if this would reverse Temozolomide resistance. The outcome of the trial was disappointing: neither the response rates nor survival were improved in the Lomeguatrib-Temozolomide combination treated patients in comparison with Temozolomide alone, including the group progressing on the latter. However, laboratory analyses of tumour biopsy samples collected during these studies showed that MGMT activity was recovering in tumour tissue very soon after treatment. Therefore a period of treatment with Lomeguatrib alone was included at the end of the combination treatment, and subsequently extended, in attempts to maintain suppression of MGMT activity. This also produced no survival benefit in the Lomeguatrib-Temozolomide combination provide any clinical benefit in colorectal cancer patients. The reasons for this outcome have yet to be established and are the subject of much speculation.

**Alkyltransferase-like (ATL) proteins**

We previously reported the isolation of the ATL-encoding genes from *E. coli* and *S. pombe* and we named the latter, *Atl1*. We have shown that, in vitro, the *Atl1* protein binds to methylated DNA and by doing so, can inhibit the action of MGMT. In collaboration with David Williams (University of Sheffield) we initially showed using gel shift assays that a purified fusion protein of maltose binding protein and the ATL from *E. coli* binds to short single- or double-stranded oligonucleotides containing a number of *O*-alkyl-substituted guanines including methyl, benzyl, hydroxyethyl and 4-bromothenyl (i.e Lomeguatrib embedded into an oligonucleotide, which we have shown to be the most potent MGMT inactivating agent so far described). However, whilst binding to these potentially toxic lesions can be demonstrated, there is no evidence of an MGMT-like alkyl transfer, nor of glycosylase or endonuclease activity, so the protein seems likely to be a specific DNA damage sensing protein that signals to other DNA repair networks. We have now extended these studies using native *Atl1* protein in enzyme-linked immunosorbent assays and surface plasmon resonance methods to quantify these molecular interactions.

To establish if the *Atl1* protein has any role in the sensitivity of *S. pombe* to alkylating agents, the gene was inactivated by gene disruption. The resulting deletants had similar growth characteristics to the wild type strain, but had substantially increased sensitivity to the growth inhibitory effects of N-methyl-N-nitro-N-nitrosoguanidine, a methylating agent that generates the same lesions in DNA as Temozolomide. *Atl1* also protects against a number of related alkylating agents, but there was no detectable effect on sensitivity to the toxic effects of other classes of DNA damaging agents. *Atl1* is therefore an important protein in the protection of *S. pombe* against the toxic effects of these alkylating agents. Exactly how it achieves such protection has yet to be established, but, crossing the *atl1* deletant with deletants in established DNA repair pathways (epistasis analysis) has suggested that *Atl1* is part of, or signals to, the nucleotide excision repair pathway and not the base excision or recombination repair pathways (see Figure). Further studies of this type along with an analysis of the proteins that interact with *Atl1* to achieve repair of the damage may confirm this.

**Publications listed on page 56**

Effects of the methylating agent N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) on growth in liquid culture of WT, *Atl1*, *Rad13* and *Atl1/rad13* double deletant *S. pombe* strains. *Rad13* is part of the nucleotide excision repair system and the data indicate that *Atl1* and *Rad13* are both on this pathway.
In a systematic screen for new cell cycle proteins we identified a previously uncharacterised protein that is required for cell division. We modified the chromosomal locus of each of the essential budding yeast genes so that the encoded protein was fused to the heat-inducible degron, allowing us to analyse the effects of rapid depletion (Kanemaki et al., Nature 2003; 423: 720). In this way we found that inactivation of the budding yeast protein Ynl152w blocked cytokinesis, and we named the protein Inn1 because it is required for ingressio of the plasma membrane (Sanchez-Diaz et al., 2008).

Using a strain in which Inn1 was fused to Green Fluorescent Protein (GFP) we found that Inn1 is recruited at the end of mitosis to the site of the contractile actomyosin ring (Figure 1). Recruitment of Inn1 was dependent upon essential components of the actomyosin ring, and we found that Inn1 co-purified from cell extracts with other previously described components of the actomyosin ring such as the SH3-domain protein Hof1 and the IQGAP-domain protein Iqg1 (Sanchez-Diaz et al., 2008).

In animal cells and in yeasts, the actomyosin ring defines the site at which cell division will occur. At the end of mitosis the ring becomes activated and then contracts into the cytoplasm, and this is coupled by an unknown mechanism to ingressio of the plasma membrane. We found that the actomyosin ring can still form in the absence of Inn1, and contraction is still initiated in all cells at the end of mitosis. In contrast, however, ingressio of the plasma membrane does not occur in the absence of Inn1 (Figure 2). The Inn1 protein is thus required in some way for contraction of the actomyosin ring to be coupled to ingressio of the plasma membrane at the end of mitosis.

The first 134 amino acids of the Inn1 protein are predicted to form a C2-domain, which is a class of membrane targeting domain that was first described in Protein Kinase C. The C2 domain comprises eight β-strands that form a sandwich of two β-sheets. From one side of the sandwich, two or three loops protrude and these usually interact with the targets of the C2 domain, which can be the head groups of membrane lipids, or other proteins. We found that mutation of
conserved positively charged residues in Loop1 of the C2 domain of Inn1 blocks membrane ingression and cytokinesis, but does not prevent recruitment of the Inn1 protein to the cleavage site. This indicates that the C2 domain is essential for ingression of the plasma membrane during cytokinesis.

Removal of the C2-domain blocks cytokinesis but does not prevent the regulated targeting of the rest of the protein to the cleavage site. In contrast, the remainder of Inn1 after the C2-domain is required for localisation at the cleavage site, and contains multiple ‘PXXP’ motifs that are likely to be binding sites for SH3 proteins. These findings suggested a model for the action of the Inn1 protein, whereby the majority of the protein serves to target Inn1 to the cleavage site so that the C2-domain can then fulfill some essential role during cytokinesis. To test this model, we generated a diploid strain lacking one copy of the INN1 gene and in which we had fused artificially the C2-domain to the Hof1 component of the actomyosin ring. Upon sporulation of this diploid we found that haploid cells that lacked the INN1 gene and that expressed wild type HOF1 were inviable and formed chains of undivided cells, indicating a failure of cytokinesis. In contrast, however; expression of the C2-Hof1 fusion protein was able to suppress the defects normally associated with absence of Inn1, and restore ingression of the plasma membrane during cytokinesis, so that inn1Δ C2-HOF1 cells grew as well as wild type cells (Sanchez-Diaz et al., 2008).

These findings indicate that the recruitment of the C2-domain of Inn1 to the cleavage site is a key requirement for ingression of the plasma membrane during cytokinesis in budding yeast. We are currently trying to understand how Inn1 is recruited to the cleavage site, how recruitment is regulated during the cell cycle, and how the C2-domain promotes ingression of the plasma membrane.

Publications listed on page 57

![Figure 1](image1.png)

**Figure 1.** Inn1 is recruited to the actomyosin ring at the end of mitosis. The figure shows timelapse analysis of the contracting actomyosin ring in a cell that expresses Inn1-GFP and that also has the red fluorescent protein Tomato fused to the budding yeast type II myosin, Myo1.

![Figure 2](image2.png)

**Figure 2.** Inn1 is required for ingression of the plasma membrane during cytokinesis in budding yeast. The picture shows timelapse images of cells that have just completed mitosis in the presence (a) or absence (b) of Inn1. The cells express GFP fused to the Ras2 protein that is associated with the plasma membrane, and the cells also express Myo1-Tomato. The cell in panel (b) has the heat inducible degron fused to Inn1 (inn1-td) and also expresses the red fluorescent protein eqFP fused to the spindle pole body component Spc42.
MAP kinase cascades lie at the heart of most stress response pathways. They comprise three kinases that are activated in sequence: a MAP kinase kinase kinase (MAPKKK) stimulates a MAP kinase kinase (MAPKK) to activate the MAPK (figure 1). In all organisms these cascades are activated by a variety of stimuli to promote the phosphorylation of a number of targets to modify cell metabolism and physiology in order to cope with the environmental changes. As major changes in cell physiology are needed to adapt to the new environment, transcription factors are prime targets. The activation of these transcription factors in turn promotes the transcription of a cohort of genes to adapt to the new environment. For example, cells produce a number of reducing agents in response to heightened levels of oxidative stress.

Stress responses, cell division and growth control
In addition to changing cell constitution to tolerate stress, stress response pathways can arrest growth and division until the adaptation is complete. Presumably this ensures fidelity of division and minimises the long-term impact of stress, should either process be attempted whilst any damage remains un-repaired. Once the adaptive response has dealt with the novel environment, specific signalling events are required to re-initiate growth and division. These recovery pathways often rely upon the same stress response pathways that instigated the arrest in the first place. Quite how they do this is largely unclear at present. A broad goal of our research effort is therefore to understand how stress responses exploit cell cycle regulators and growth controls to regulate the timing and execution of cell division and growth following stress. We employ fission yeast as a model system for these studies as its cell cycle controls and stress response pathways mirror those of humans.
Stress responses in fission yeast

Two MAP kinase cascades maintain homeostasis in fission yeast. The so-called “stress response pathway” (SRP) incorporates the MAPK Sty1/Spc1. Like its mammalian JNK/p38 counterparts, the SRP responds to a broad range of stresses, including oxidative, osmotic, heavy metal ion and heat stresses. The “cell integrity pathway” (CIP) utilizes the MAP kinase, Pmk1, and is required to maintain cell integrity in response to osmotic shock and hydrostatic pressure as well as regulating cytokinesis. There is interplay between these two pathways as SRP signaling both promotes and attenuates CIP signaling depending upon the circumstances.

Specific stress recovery pathways

Phosphorylation of the conserved cell cycle regulator polo kinase on serine 402 restores both growth and division of cells after they have been arrested in a mild heat shock induced stress response (Petersen and Hagan, Nature 2005; 435: 507). Curiously, serine 402 phosphorylation is not stimulated by other stresses such as osmotic stress, even though these stresses also arrest cell growth. We therefore studied the impact of osmotic stress upon the cell growth machinery over the last year.

Growth in fission yeast

Cell growth in fission yeast mirrors the interplay between the actin and microtubule cytoskeletons that underlies growth, migration, and signaling in human cells. Cell extension is promoted as a consequence of polarisation of the actin cytoskeleton by the same GTPase cascades that promote the polarisation of the actin cytoskeleton to drive migration of human cells. In yeast, the polarisation of the actin cytoskeleton directs the secretion of cell wall material to generate the robust polysaccharide wall that acts as an “exoskeleton” to define the rod shape of wild type fission yeast cells (figure 2). This cell wall contains a range of polysaccharides including 1→3 β-D-glucan that is stained by the fluorescent probe calcofluor. The selection of the site at which the actin becomes polarised to generate growth of the cell tip is mediated by microtubules. Therefore, perturbation of the microtubule cytoskeleton, or the microtubule associated factors that communicate the inputs from microtubules to the actin cytoskeleton, cause cells to branch.

SRP signalling regulates growth recovery at multiple levels

We found that osmotic shock induced the deposition of novel calcofluor staining structures at cell tips that persisted after cells resumed growth (arrows in figure 3). We used these landmarks to monitor the timing with which tip growth resumed after the stress as foci were displaced from the tip by tip growth following recovery (figure 3 stage 1>2>3). This established that the resumption of growth was independent of CIP signaling as it was promoted by SRP stimulated transcription. The transcriptional nature of this recovery pathway was underscored by its insensitivity to the mutation of serine 402 of polo kinase to block the post-translational SRP response that is so critical in recovery from heat shock. The recovered tips of cells lacking the SRP MAPK Sty1 failed to re-establish polarised growth, as they ballooned out in a random fashion rather than pursuing the polarised growth that produces the linear extension of cells with an intact SRP (figure 2). This indicated that SRP signalling is required to both re-initiate growth and re-establish polarity. SRP signalling may also regulate a further aspect of recovery as cells lacking the SRP associated microtubule polarity factor Wsh3 were able to re-initiate growth on cue, however they could not select the right site at which to do so and became bent and branched following recovery (figure 2). Having identified these SRP signalling functions in growth control we can study the molecular basis of these controls to identify how stress responses modulate cell structure.

Lessons from yeast

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

Publications listed on page 57

Figure 2. Calcofluor staining of unstressed wild type cells and two mutant strains one hour after the imposition of osmotic stress when growth patterns clearly deviate from the linear extension seen following recovery of cells in which the wild type sty1Δ and wsh3Δ genes are present (figure 3).

Figure 3. Calcofluor staining of a mixture of cdc1 0v50 cells, 30 (cells marked 1), 60 (cell marked 2) and 90 minutes (cells marked 3), after osmotic stress at 36°C. Bright foci of calcofluor staining appear at the tip 30 minutes after imposition of stress (arrows). The dots persist as new growth initiates driving these landmarks progressively back towards the cell equator (cells 1>2>3). The cell cycle mutation is simply used to block division for the duration of the experiment and so highlight the apparent “movement” of the foci.
The MAP (mitogen activated protein) kinase signalling pathways are central to the ability of the cell to respond to various stress conditions and in so doing protect the cell from potential damage that could arise. The highly conserved pathways are essential for a wide variety of biological activities which in mammalian cells range from cell proliferation and differentiation to regulation of apoptosis; their deregulation has been associated with numerous disease conditions such as inflammation and cancer.

Previous efforts in our laboratory have focused on two mammalian MAP kinase targets, the transcription factors ATF2 and ATF7, which are both regulated by phosphorylation mediated by the stress activated MAPKs p38 and JNK. ATF2 and ATF7 are both members of the AP-1 family which controls the transcription of an extensive repertoire of genes including cell cycle and apoptotic regulators, numerous cytokines and growth factors. We have analysed the biological role of ATF2 and ATF7 using the genetically amenable mouse model system. Germ line mutations uncovered new insights into their role in development: simultaneous deletion of both genes leads to embryonic lethality as a result of massive apoptosis in the embryonic liver involving both developing hepatocytes and haematopoietic cells. Characterisation of cultured cells derived from mutant embryos revealed a role in limiting cell proliferation resulting in increased cell growth at high density. Interestingly, upon oncogenic transformation with K-Ras, cells lacking ATF2 and ATF7 showed a profound increase in tumour growth upon grafting into appropriate recipient mice. In both the embryonic phenotype and the cell growth phenotype the role of the ATF factors in regulating MAPK signalling is critical. A number of dual specificity phosphatases, including MKP1, constitute major downstream targets and in the mutant background their transcription is decreased resulting in loss of essential negative feedback regulation of MAP kinase pathways.

The scope for in vivo analysis using mouse knockouts is limited by the early lethality of the mutations. To circumvent this difficulty we have developed a number of tissue-specific ATF2 knockouts. These models have revealed roles for ATF2 in specific tissues and disease conditions: a brain specific deletion of ATF2 leads to defects in hindbrain development and cerebellum functions resulting in death soon after birth due to a respiratory defect that resembles meconium aspiration syndrome; deletion of ATF2 specifically in endothelial cells leads to defects in the microvasculature of the gut upon postnatal intestinal growth. These results emphasise the importance of ATF2 in the development and homeostasis of mammalian tissue.

Conditional knockout models have also provided additional insights into a potential role for these factors in tumourigenesis. A tumour suppressor role has been revealed in a skin tumourigenesis model using a mutant mouse where ATF2 is specifically deleted in keratinocytes. Upon tumour initiation and promotion, the mutant animals demonstrate a significantly earlier onset of papillomas as well as greater numbers. The potential role of ATF2 in the progression of papillomas to malignant carcinomas is currently being assessed. Likewise, irradiation of mice with ATF2 specifically deleted in T-cells results in earlier onset of T-cell lymphomas further supporting a tumour suppressor role.

In contrast, in other tumour contexts there is indirect evidence for ATF2 being pro-tumourigenic. In order to assess this possibility directly, appropriate tumour models...
are being developed and characterised. For example, we are characterising a B-cell specific ATF2 knockout model and analysing the effect of ATF2 deletion on lymphoma initiation and development following B-cell specific expression of the c-myc oncogene. Similarly, the role of ATF2 in hepatocellular carcinoma (HCC) is being examined using two different approaches: one involves the chemical induction of HCC in control and liver specific ATF2 knockout mice which will determine whether the absence of functional ATF2 (and ATF7) leads to significant changes in onset, frequency and burden of HCC; the second utilises the recently developed orthotopic transplantation of hepatocyte precursors (hepatoblasts) to produce chimaeric models of liver tumours.

Homologues of the AP-1 family are found in all eukaryotic organisms and their involvement in stress response is highly conserved. Model organisms could provide useful models for understanding the role and regulation of AP-1. Fission yeast is a particularly pertinent model system – stress responses are coordinated through the Sty1 signalling pathway which is analogous to the mammalian p38 pathway. Furthermore, many of the changes in the transcriptional profile of cells following stress is orchestrated through the Atf1 and Pap1 transcription factors which are related to mammalian ATF2 and cJun respectively.

The transcriptional changes following stress are extensive and complex: a common set of genes are activated in response to all stresses accompanied by the activation of genes that are specific to the particular stress being imposed. Therefore each stress has its own transcriptional pattern resulting in the up-regulation of the appropriate defence and repair mechanisms. The complexity of the response is best illustrated by characterising the events following exposure to oxidative stress: different patterns of transcriptional activation are seen dependent upon the exact nature of the oxidant as well as the dose.

Given the central role that Atf1 plays in the stress response, we have characterised in detail its regulation and its interaction with the Sty1 kinase. Using ChIP assays we demonstrated that Atf1 is essential to target and tether the Sty1 kinase to stress-response gene promoters. Both the targeting and transcriptional activation is dependent upon Sty1 kinase activity. Surprisingly however, the key kinase target is not Atf1 itself since Sty1 is found at promoters in the presence of Atf1 protein that can no longer be phosphorylated (Atf1-11M mutant). We hypothesise that Sty1 phosphorylates and regulates another factor(s) found at the promoter; possibly a component of chromatin remodelling complexes or the polymerase complex itself. A number of approaches are being taken to identify Sty1 targets.

Phosphorylation of Atf1 by Sty1 regulates its stability; phosphorylation results in an increase in the half-life of Atf1 and its accumulation to significantly higher levels in the cell following stress. This increase in levels of Atf1 is critical for a robust transcriptional response upon exposure to H2O2 and for the ability of cells to mount an adaptive response providing resistance to acute levels of osmotic stress. The key regulatory step in Atf1 degradation is the interaction of the transcription factor with the E3 ligase, SCFfbl. This interaction results in the ubiquitination of Atf1 and its destruction via the proteasome. The substrate specificity of the SCFfbl complex is determined by the F-box protein, Fbh1. Crucially, the interaction between Atf1 and Fbh1 is sensitive to Atf1 hyper-phosphorylation; accordingly the interaction is lost upon stress and Atf1 is stabilised. Consistent with these findings, disruption of the interaction between Fbh1 and the rest of the SCF E3 ligase complex, results in an increase in Atf1-11M protein and a rescue of the phenotypes displayed by the atf1-11M mutant. Interestingly, Fbh1 levels appear to be regulated by Atf1 suggesting that a complex interplay exists between the F-box protein and its target which serve to finely tune the abundance of the Atf1 transcription factor upon stress.

Publications listed on page 57
Rho proteins, such as Rac1, RhoA and Cdc42, are guanine nucleotide binding proteins that cycle between an inactive GDP-bound state and an active GTP-bound state. The activity of Rho proteins is controlled by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs activate small GTPases by promoting the exchange of GDP for GTP, whereas GAPs enhance the intrinsic rate of hydrolysis of bound GTP for GDP, leading to inactivation. Tiam1 (for T-lymphoma invasion and metastasis protein) belongs to the GEF family of proteins and selectively activates Rac in response to growth factors and cell-substrate interactions. Precisely how these upstream events engage the Tiam1-Rac signalling module is unclear. One possible mechanism is suggested by the observed association of Tiam1 with the second messenger Ras through a Ras-binding domain (RBD). Activated Ras and Tiam1 then cooperate to activate Rac (Lambert et al., Nature Cell Biol 2002; 4: 621). Significantly, Tiam1-deficient cells are resistant to Ras-induced cellular transformation (Malliri et al., Nature 2002; 417: 867), implying that this interaction is important for tumourigenesis.

Tiam1/Rac signalling and tumourigenesis in vivo
Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by topical application of chemical carcinogens and consequent oncogenic activation of the c-Ha-Ras gene (Malliri et al., Nature 2002; 417: 867). Tiam1-deficient tumours were not only fewer but also smaller than wild-type tumours and this correlated with increased apoptosis and reduced proliferation in carcinogen-exposed Tiam1-deficient mice. Tiam1 also acts as a potent modifier of intestinal tumourigenesis (Malliri et al., J Biol Chem 2006; 281: 543). The majority of intestinal tumours are caused by mutations in the canonical Wnt signaling pathway, leading to aberrant expression of Wnt-responsive genes. Tiam1 is a Wnt-responsive gene, and is expressed in crypts of the adult mammalian intestine as well as in adenomas from patients and Min (multiple intestinal neoplasia) mice. In each of these settings, the Wnt pathway is activated. Furthermore, by comparing tumour development in Min mice expressing or lacking Tiam1, it was found that Tiam1 deficiency significantly reduces the formation as well as growth of polyps in vivo (Malliri et al., J Biol Chem 2006; 281: 543).

These two studies on tumourigenesis in vivo demonstrated that two independent oncogenic signalling pathways of major clinical significance (Ras and Wnt) recruit the Tiam1-Rac signalling pathway by specific, albeit distinct mechanisms. In 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 has emerged to directly implicate Rho proteins in tumour initiation and progression in vivo. Our group investigates how the activities of certain regulators of the Rho protein Rac are controlled. We are also identifying signalling events downstream of Rac that modulate tumour susceptibility and disease progression.
the context of oncogenesis, activation of this signalling module promotes tumour initiation and growth. Moreover, this role is specific to Tiam1 since its loss cannot be compensated for by other Rho GEFs.

Tiam1/Rac signalling and the regulation of cell-cell adhesion

The skin carcinogenesis model revealed an additional role for Tiam1 in tumourigenesis. The few skin tumours arising in Tiam1-deficient mice progressed more frequently to malignancy than those in wild-type mice, suggesting that Tiam1 deficiency promotes malignant conversion (Malliri et al., Nature 2002; 417: 867). Analysis of Tiam1 expression in skin tumours of wild-type mice revealed that benign papillomas maintained high levels of Tiam1 expression, whereas expression was reduced in squamous cell carcinomas and was completely lost in highly invasive spindle cell carcinomas. The increased Ras signalling associated with advanced skin malignancies (resulting from amplification of the mutated Ras allele) seems to be responsible for the reduction or loss of Tiam1 expression in the later stages of tumour progression, as demonstrated in vitro for Ras-transformed MDCK cells (Zondag et al., J Cell Biol 2000; 149: 775). Thus, while Tiam1/Rac co-operate with Ras in establishing tumours, they antagonist Ras during tumour invasion.

One probable mechanism by which Tiam1 and Rac suppress malignant progression is through their ability to stimulate cell-cell adhesion. In vitro studies have shown that over-expression of activated Rac or Tiam1 can promote the formation of adherens junctions (AJs) and the accompanying induction of an epithelial-like phenotype in a number of mesenchymal cell lines (Malliri & Collard, Curr. Opin. Cell Biol 2003; 15: 583). Moreover, using both RNA interference and cells derived from Tiam1-deficient mice, it has been shown that endogenous Tiam1 is required for both the formation as well as the maintenance of cadherin-based adhesions (Malliri et al., J Biol Chem 2004; 279: 30092). The oncoprotein Src, a non-receptor tyrosine kinase implicated in malignant progression, potently induces epithelial–mesenchymal transition (EMT) by targeting AJs for disassembly. We recently showed that direct phosphorylation of Tiam1 by Src is required for 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-Sos complex, ERK becomes activated and triggers the localised degradation of Tiam1 at AJs through, in turn, activating calpain proteases. Significantly, we demonstrated that in human lung, colon, and head and neck cancers phosphorylation of Y384 of Tiam1 positively correlated with Src activity, while total levels of Tiam1 were inversely correlated with Src activity, consistent with the above-mentioned post-translational regulatory mechanism operating in malignancies. Abrogating Tiam1 phosphorylation and degradation 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. These data establish a new paradigm for regulating local concentrations of Rho-GEFs, as well as linking Tiam1-Rac signalling with a further oncprotein.

It is increasingly apparent that Rho GEFs do more than simply activate Rho molecules; several studies now point to their role in influencing the choice of biological response elicited by a given Rho protein. GEFs have been shown to bind to effectors directly or to scaffold proteins that complex with components of effector pathways. Thus Tiam1 interacts with IB2/JIP2, a scaffold that promotes Rac activation of p38 kinase cascade over JNK MAP kinase cascade (Buchsbaum et al., Mol Cell Biol 2002; 22: 4073), and also with spinophilin, a scaffold that promotes Rac activation of p70 S6K over Pak1, a different Rac effector (Buchsbaum et al., J Biol Chem 2003; 278: 18833). In our lab, we are using biochemical approaches to identify Rac and Ras GEF interacting proteins involved in different aspects of transformation including malignant progression (acquisition of invasiveness).

Publications listed on page 57

Model for the regulation of Tiam1 by Src activation. Src directly phosphorylates Tiam1, preferentially at sites of cell-cell adhesions. Phosphorylated Tiam1 recruits the Grb2-Sos complex which, via MEK, increases activation of the ERK associated with Tiam1, and hence the local activation of calpain proteases at cell-cell adhesions. Calpain mediated proteolysis of Tiam1 results in its inactivation, reducing the activity of Rac that is necessary to maintain cadherin adhesions. The weakening of cell-cell adhesions in this way by Src would allow increased migration of cells.
The development of molecularly targeted anticancer drugs mandates a parallel development of biomarkers in order to give the right patient the right dose and schedule of treatment. CEP develops and validates pharmacokinetic (PK), pharmacodynamic (PD) and predictive biomarker assays and implements biomarker qualification within clinical trials at the Christie Hospital’s Derek Crowther Unit (DCU) focusing on novel agents targeted to apoptosis and angiogenesis. In 2008, biomarker research included development of a robust plasma proteomics workflow for biomarker discovery, enumeration and characterisation of circulating tumour cells, detection of oncogene mutations in circulating free DNA and multiplexing circulating and tissue biomarker assays.

Disease orientated translational research focus on lung, colorectal and paediatric cancers and pre-clinical studies includes drug target validation, drug combination optimisation and the impact of hypoxia on drug efficacy.

Clinical Trials at the Christie Hospital’s DCU
Research in CEP is predicated on those novel agents entering clinical trial at the DCU. DCU typically supports c100-120 trials with c6400 patient visits p.a. In order to meet research and service requirements, proposals for a new £35M Cancer Treatment Centre were developed in 2008. The new Cancer Treatment Centre will provide comprehensive facilities for clinical trials, experimental treatment, and service chemotherapy and is due to open in 2010. The expansion of the DCU within the treatment centre will make it one of the largest early clinical trials centres worldwide and integral to this development are enhanced laboratory facilities for translational research to strengthen further the CEP-DCU axis. One example of the CEP DCU axis in action is the biomarker enhanced CR-UK Phase I trial of AEG35156 (Aegera Therapeutics, antisense XIAP). We reported this first in class, first in man clinical trial in the Journal of Clinical Oncology (Dean et al., 2008). CEP has also developed a suite of biomarkers to accompany ongoing early clinical trials of the BH-3 mimetic class of apoptosis promoting drugs that target interactions between Bcl-2 family proteins.
Biomarker Research in CEP in 2008 is exemplified by the following highlights: i) application of cytokeratin 18 (CK18) based circulating biomarkers of cell death as pharmacodynamic biomarkers; ii) demonstration of the potential of circulating tumour cells (CTCs) as pharmacodynamic and predictive biomarkers; iii) the potential predictive utility of oncogene mutation detection in circulating free DNA and iv) the development of multiplexed circulating biomarkers of angiogenesis.

(i) CK18 based circulating biomarkers of cell death
In collaboration with Abbott Laboratories, we demonstrated using a small cell lung cancer (SCLC) xenograft model that the BH-3 mimetic ABT 737 provoked tumour cell apoptosis that was reported by the appearance of full length and caspase cleaved CK18 in the blood stream as assessed by M65 and M30 ELISAs respectively. These circulating biomarkers were elevated rapidly after drug administration before detectable tumour shrinkage and informed on appropriate blood sampling times for the ongoing Phase I clinical trial where biomarker analysis is underway.

(ii) Circulating Tumour Cells
We evaluated CTCs as a useful biomarker for trials of BH-3 mimetics in SCLC. In preparation for upcoming trials, CTC number was evaluated in patients on standard chemotherapy using the Veridex CellSearch system; CTC numbers decreased post drug treatment in accordance with patient response in cycle 1 of therapy. The levels of drug targets in CTCs may serve to predict which patients will respond to BH-3 mimetics thus assays were developed to identify amplification of bcl-2 (a potential marker of drug sensitivity, measured using FIISH, see figure) and upregulation of Mcl-1 (a potential biomarker of drug resistance for the BH-3 mimetic ABT 263, by immunohistochemistry). Efforts are underway using the Metagenex ISET system that separates CTCs from white blood cells on the basis to size exclusion to isolate RNA from purified CTCs of sufficient quantity and quality to undertake transcript profiling with the goal to discover signatures of drug resistance and sensitivity and identify novel drug targets.

(iii) Oncogene Mutations in Circulating Free (cf) DNA
A number of tumour specific mutations e.g. within EGFR, B-Raf and K-Ras, can predict response to certain novel therapeutics and progression free survival. Mutation detection is traditionally performed on archival tissue samples which may no longer reflect current tumour biology, and can be difficult to obtain, especially within the context of clinical trials. Reliable detection of tumour specific mutations within cfDNA provides an alternative that is minimally invasive, can be performed on serial samples and potentially provides a ‘real time’ assessment of the tumour mutation status to guide clinical decision making. We have optimised methods for extracting circulating DNA and Standard Operating Procedures were produced for blood processing and for DNA extraction (Board et al., 2008b). Derelegation of the PI-3K pathway in cancer is prevalent most notably with loss of the tumour suppressor PTEN or activating mutations in the PIK3CA gene that encodes the catalytic p110α subunit of PI-3K. The PI-3K signalling pathway is considered to be a tractable drug target in cancer treatment, however, the precise impact of PIK3CA mutations on drug responsiveness remains incompletely defined. In order to begin to address the clinical utility of PIK3CA mutation detection as a circulating biomarker and within our CR-UK/AstraZeneca Clinical Pharmacology Fellowship Scheme and with experts at DxS diagnostics, we participated in the generation of a novel assay based on Amplification Refractory Mutation System (ARMS) allele-specific PCR and Scorpion primers (DxS Diagnostics) to detect the 4 most common mutations in the PIK3CA gene. The resultant high throughput, multiplexed ARMS assays of these PIK3CA mutations are more sensitive than sequencing (Board et al., 2008a). Our first and ongoing clinical study in metastatic breast cancer suggests that there is a high concordance rate between plasma and tumour PIK3CA incidence. The assay will be applied in 2009 to trials of PI-3K and mTOR inhibitors to assess its potential to predict drug responses.

(iv) Multiplexing circulating biomarkers of angiogenesis
Molecular therapies targeting tumour vasculature have recently achieved clinical recognition through randomised trials of conventional therapy with or without Vascular Endothelial Growth Factor (VEGF). However, prospective identification of patients who will benefit from VEGF inhibitors has not yet been achieved and remains a significant obstacle to the approval of this type of drug on the NHS. There are plentiful potential circulating pharmacodynamic and/or predictive biomarkers of angiogenesis but it is not yet clear how to select an optimal biomarker panel for trial implementation. CEP working with Gordon Jayson’s Translational Angiogenesis team has used the Endogen Searchlight platform to develop and validate multiplexed ELISAs that can now be used in upcoming trials of anti-angiogenic therapies using minimal blood volumes. Of critical importance, and ongoing is the integration of these circulating biomarkers with imaging biomarkers to obtain a comprehensive picture of patient drug responses where RECIST criteria may not be sufficiently informative.

Publications listed on page 57
The Immunology Group has a major goal of understanding and exploiting the function and/or expression of ST4 oncofoetal molecules in the context of cancer associated changes in motility, and adhesion contributing to metastasis. Successful translational studies have led to ongoing Phase 3 clinical trials of ST4 directed immunotherapies. This report details studies of both animal model and human immune responses to ST4 antigen aimed at improving both vaccine and antibody delivered therapies for cancer. In addition progress on the development of immunotherapies for HPV associated cancers are reported.

**Immune regulation and escape in TroVax vaccinated patients**
We have recently reported the results of a phase II trial in which two TroVax (MVA-ST4) vaccinations were given to patients both pre- and post-surgical resection of liver metastases secondary to colorectal cancer (CRC). ST4-specific cellular responses were assessed at entry and two weeks after each vaccination by proliferation of fresh lymphocytes and ELISA for antibody responses; 18 of 19 CRC patients mounted a ST4-specific cellular and/or humoral response (see also Biological, Immune and Gene Therapy Report). We have now completed a comparison of individual and between patient responses over the course of the treatments using cryopreserved peripheral blood mononuclear cells (PBMC) samples from baseline until after the fourth vaccination at 14 weeks using a proliferation assay with ST4-Fc fusion protein, overlapping 32-mer ST4 peptides, MVA-LacZ and MVA-ST4 infected autologous monocytes. Significant responses to 32-mer peptide pools were seen in 8 of 19 CRC patients by time of surgery and 13 of 19 who received 2 more vaccinations by week 14. We also assessed the levels of systemic T regulatory cells, plasma cytokine levels, phenotype of tumour infiltrating lymphocytes including T regulatory cells and tumour MHC class I loss of expression. Approximately half of the patients showed phenotypes consistent with relative immune suppression and/or escape highlighting the complexity of positive and negative factors challenging any simple correlation with clinical outcome.

**Mouse models of ST4 immunotherapy**
We have shown that transient depletion of T regulatory cells (Tregs) can enhance ST4 immunity in renal cell carcinoma patients (Thistlethwaite et al., 2008), suggesting that Treg modulation might improve the efficacy of the ST4 vaccine (TroVax). To investigate the role of Tregs in ST4 immunity we utilised ST4 knockout (KO) C57BL6 mice to map the ST4 T cell repertoire. By immunising wild type (WT) and ST4 KO mice with a single dose of a replication defective adenovirus vaccine encoding murine ST4 (Ad-mST4) and re-stimulating these primed cells *in vitro* with overlapping 32-mers, we identified mST4 derived epitopes recognized by CD4 and CD8 T cells using interferon gamma (IFNγ) ELISPOT. ST4 KO mice generated strong and high avidity ST4-specific CD4 and CD8 T cell responses. In WT mice, ST4-specific CD4 T cells were either absent or anergized and the CD8 T cell response weaker and lower avidity. The latter might result from a lack of help and/or active suppression by Tregs. To look for evidence of a natural ST4 specific Treg cell population, CD4+CD25+ T cells were isolated from naïve
WT and ST4 KO animals and their suppressive activity on ST4 primed KO effector cells assessed by IFN-γ ELISPOT. Our data demonstrate that WT CD4+CD25+ T cells have a stronger specific suppressive activity than ST4 KO CD4+CD25+ T cells (p < 0.01). Ongoing studies are evaluating methods to selectively modulate ST4 specific Tregs as a means to improve efficacy of ST4 vaccines in the clinic.

**Immunotherapy of HPV associated VIN**

The introduction, in autumn 2008, of UK wide human papillomavirus (HPV) vaccination programmes for prevention of cervical cancer represents a magnificent dividend for the national and international HPV research community. UK based scientists and clinicians are continuing to make important contributions to the challenges of implementation and education (Garland *et al.*, 2008). In addition, we have continued to develop therapeutic approaches for treatment of HPV associated neoplasia including high grade vulval intraepithelial neoplasia (VIN). The chronic nature of this condition might be explained by a balance between the consequences of the virus infection and immune control (see figure). To improve the management of VIN, we undertook 2 phase II studies using a topical immunomodulator cream – imiquimod followed by either photodynamic therapy (PDT) or therapeutic HPV vaccination with TA-CIN, a fusion protein of HPV16L2E6E7. The initial use of imiquimod in this study was designed to create a more favorable local environment characterized by increased T-cell infiltration for the subsequent PDT especially, because the response to the latter has been associated with increased CD8 T-cell infiltration. In addition, other previous work had shown that patients with lesions containing a higher density of T cell infiltration were more likely to respond clinically following HPV vaccination. This investigation has used immunohistochemistry to investigate the levels and types of VIN infiltrating immune cells including Tregs, as the latter have been shown to be associated with poor clinical responses in various cancers. The imiquimod treatment is characterised by increased local infiltration of CD8 and CD4 T cells but in non-responders (failure to clear VIN on vulvoscopy or histology) this is accompanied by increased Tregs whereas in the responders (clearance of VIN on vulvoscopy and histology) these are at significantly lower density. In the imiquimod/PDT trial, responders had significantly increased pre-existing lympho-proliferative responses to the HPV 16 compared to non-responders but there was no stimulation of HPV immunity with treatment. Following vaccination in the imiquimod/vaccine trial, patients showing clinical responses had significantly increased lympho-proliferation to the vaccine compared to the non-responders. Imiquimod followed by PDT or TA-CIN both show promise as non surgical therapies for VIN. As a correlation between pre-existing HPV response, response to vaccination and clinical response was noted, any therapeutic anti-HPV treatment could be very valuable. Non-responders showed higher level of T-regulatory cells in situ consistent with their negative prognostic value in some cancers. The therapeutic impact of treatment may depend on the differential immune response of responders and non-responders.

Publications listed on page 58
Phosphoinositides are a family of lipid second messengers interlinked by the activities of an extensive and highly regulated network of kinases and phosphatases that modulate phosphoinositide levels in response to environmental changes. Alterations in phosphoinositide levels can regulate many different cancer-relevant cellular pathways including survival, proliferation, migration, cell substratum interactions and transcription. PtdIns(4,5)P\textsubscript{2} is at the heart of phosphoinositide signalling, being the substrate for both phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PIC). The PI3K /PTEN pathway is deregulated in tumours promoting cell survival and proliferation through the activation of PKB and flux through the PIC pathway is upregulated in human tumours. Furthermore PtdIns(4,5)P\textsubscript{2} is itself a regulator of cytoskeletal dynamics, cell survival pathways and cell polarity (see Panbianco et al., 2008). Cells monitor PtdIns(4,5)P\textsubscript{2} levels and a decrease in their levels can lead to the induction of apoptosis. As PtdIns(4,5)P\textsubscript{2} levels are sensitive to extracellular matrix signalling, the induction of apoptosis in response to decreases in PtdIns(4,5)P\textsubscript{2} may constitute a metastasis suppressive mechanism. It is likely that tumour cells must by-pass this process to become metastatic. Interestingly a number of oncogenes that suppress cell death in response to loss of extracellular matrix signalling also suppress the decrease in PtdIns(4,5)P\textsubscript{2} and are consequently able to maintain growth factor signalling.

Understanding how PtdIns(4,5)P\textsubscript{2} levels are regulated and how changes in PtdIns(4,5)P\textsubscript{2} are transduced in to regulating cellular function.

PtdIns(4,5)P\textsubscript{2} is present in the plasma membrane where its levels can be regulated in response to receptor activation. PtdIns(4,5)P\textsubscript{2} is also synthesised within the nucleus where its levels can be regulated distinctly from the plasma membrane pool. Understanding how these two pools of lipids are controlled and which cellular pathways they regulate represents a major goal of the laboratory. PtdIns(4,5)P\textsubscript{2} can be synthesised by the action of two distinct but related kinases (collectively termed PIP kinases). PIP5Ks phosphorylate PtdIns4P on the 5' position, while PIP4Ks phosphorylate PtdIns5P on the 4' position. It is likely that PIP5Ks are the major producers of PtdIns(4,5)P\textsubscript{2} while the role of PIP4K may be to regulate cellular PtdIns5P levels and/or a distinct minor pool of PtdIns(4,5)P\textsubscript{2}. PIP5Ks are upregulated in nearly all cancer cell lines tested and overexpression of PIP5K can induce dramatic changes in cell morphology, increase migratory capacity and attenuate apoptosis in response to cellular stressors. PIP4Ks on the other hand appear to regulate the levels of cellular PtdIns5P which can impinge on the activity of the tumour suppressor protein p53 and a number of other transcriptional regulators that are implicated in the development of cancer and on PKB activation.
One approach to understanding how PIP kinases are regulated is to elucidate how they may be post-translationally modified and what proteins they interact with. Post-translational modifications such as phosphorylation, acetylation, methylation or ubiquitination can induce changes in enzymatic activity, localisation, interaction partners and stability. Using mass spectrometry we have identified 12 different sites of phosphorylation on PIP5K and 3 sites of phosphorylation and 8 sites of acetylation on PIP4Kβ. We have generated modification-specific antibodies and are using these to elucidate how and when the sites of modification are regulated and what the impact of modification is on the subcellular localisation and activity of the enzymes. Using methods such as mass spectrometry and yeast two hybrid we are investigating what proteins interact with PIP kinases. For example the small molecular weight G protein Rac interacts with and regulates the localisation of PIP5K. We have identified an allele of PIP5Kα that has attenuated ability to interact with Rac and demonstrated that this mutant protein no longer localises to the membrane. In vivo PIP5K activity modulates focal adhesion stability required during neuronal retraction, an important process in guiding neurones to their targets. This role of PIP5K is blocked if its interaction with Rac is attenuated. We have constructed a targeting vector to knock in this mutation to study the role of the interaction between PIP5K and Rac in vivo. We have also identified a number of proteins that interact with PIP4Kβ. We are investigating whether these interacting proteins are regulated by or regulate the activity or the localisation of PIP4Kβ.

To define the array of pathways that are regulated by PtdIns(4,5)P2 and PtdIns5P we are identifying lipid interacting proteins as these likely represent downstream lipid regulated proteins. In collaboration with Clive D. Santos (PROBE proteomic platform, Dept of Biomedicine University of Bergen) isolated nuclei have been treated with reagents that tightly interact with phosphoinositides and therefore are able to dissociate endogenous proteins that are bound to nuclear phosphoinositides. The released proteins have been analysed by mass spectrometry and include transcriptional regulators and chromatin remodelling complexes. Candidate proteins are being tested for their phosphoinositide binding characteristics.

**PIPSK as a target for drug development.**

In collaboration with CRT (Cancer Research Technology) we have screened and identified small molecular weight compounds that are able to inhibit PIP5K activity. Chemical modification and structure-function studies have identified important chemical moieties on these small molecules which have led to the development of more potent PIP5K inhibitors. The rationale behind PIP5K as a cancer relevant target is shown in the figure. PtdIns(4,5)P2 is the substrate for PI-3-kinase which regulates the oncogenic activity of PKB. We hypothesised that depletion of PtdIns(4,5)P2, using an inhibitor to PIP5K, may compromise the receptor mediated synthesis of PtdIns(3,4,5)P3 and may therefore attenuate the activation of PKB. The inset shows that treatment with one of our PIP5K inhibitors attenuates both insulin (ins) and H2O2-induced PKB activation. Future experiments will concentrate on driving this concept forward to define which types of cancer cells may be sensitive to inhibition of PIP5K.

*Publications listed on page 59*
The cancer stem cell (CSC) model posits that many human malignancies consist of two functionally distinct cell types: (i) CSCs, which are self-renewing cells with the capacity to initiate, sustain and expand the disease, and (ii) non-self-renewing progeny cells, derived from CSCs through differentiation, which may make up the bulk of the tumour and account for disease symptomatology. In order for malignancies to be cured, it may be necessary and sufficient to exclusively eliminate CSCs. Consequently, there is considerable interest in further understanding the biologic and molecular properties of these cells, by comparison with both their non-self-renewing downstream progeny and their normal adult stem cell counterparts.

CSCs (also called tumour-propagating or tumour-initiating cells) were first formally described in human acute myeloid leukaemia (AML) as rare cells that share an immunophenotype with normal hematopoietic stem cells (HSCs). This paradigm has recently been substantially revised based on two significant observations. First, in murine models of human leukaemia induced by the MLL-AF9 oncogene, self-renewing leukaemia stem cells (LSCs) may account for up to a quarter of cells within the leukaemia clone and exhibit mature myeloid immunophenotypes (Somervaille and Cleary, Cancer Cell 2006; 10: 257). Secondly, protocols that enhance engraftment of human leukaemia cells in xenogeneic transplant assays demonstrate the presence of LSCs in leukaemia cell sub-populations previously considered to be devoid of them.

Since LSCs may be more numerous and mature than originally proposed, the nature and generality of the hierarchical organization of malignancies has recently been questioned. However, consistent with the CSC model, only a subset of AML cells have clonogenic potential in in vitro assays, and human AML blast cells undergo differentiation in vivo to mature granulocytes, as may murine LSCs initiated by MLL-AF9 (Somervaille and Cleary, Cancer Cell 2006; 10: 257).

To further elucidate the hierarchical disposition of AML, a major goal is to identify transcriptional programs, genes and pathways that specifically correlate with and promote the retention of LSCs within the self-renewing compartment of leukaemias. It is not known whether such LSC maintenance programs are synonymous with programs responsible for leukaemia initiation, for example Hoxa/Meis in MLL leukaemogenesis. It is also not clear whether they share features with transcriptional programs expressed in adult or embryonic stem cells (ESCs), or whether there is a relationship with genes and pathways implicated in the function of AML stem cells such as NFκB, phosphatidylinositide-3-kinase, CTNNB1, Bmi1, Pten and Junb.

In work performed at the Paterson Institute in the past year by members of the Leukaemia Biology group, and also previously by Tim Somervaille at Stanford University in the United States, we investigated the genetic determinants that maintain LSC frequencies and leukaemia cell hierarchies using a mouse model that faithfully recapitulates many of the pathologic features of AML induced by chromosomal...
translocations of the MLL gene, which occur in about 5-10% of human AMLs. Confirming recent speculation that CSC frequency may differ between distinct tumour types, LSC frequency in AML was found to vary substantially according to the initiating MLL oncogene. This feature, and the observation that LSC frequency varies within the leukaemia cell hierarchy, was used to derive a transcriptional program for LSC hierarchical maintenance. The program indicates that MLL LSCs are maintained in a self-renewing state by co-option of a transcriptional program that shares features with ESCs and is transiently expressed in normal myeloid precursors rather than HSCs or mature neutrophils. Furthermore, the shared transcriptional features of LSCs, ESCs, normal mid-myeloid lineage cells, and a diverse set of poor prognosis human malignancies supports the broader conclusion that CSCs may be aberrantly self-renewing downstream progenitor cells whose frequency in human malignant disease correlates with and dictates prognosis.

The transcription/chromatin regulatory factors Myb, Hmgb3 and Cbx5 are critical components of the LSC hierarchical maintenance program and suffice for Hoxa/Meis-independent immortalization of myeloid progenitors when co-expressed, establishing the cooperative and essential role of an ESC-like LSC maintenance program ancillary to the leukaemia initiating MLL/Hox/Meis program. This work is currently in press in Cell Stem Cell and will be published early in 2009.

**Publications listed on page 60**

---

**Figure 1.** Murine acute myeloid leukaemia initiated by MLL-AF1p.

**Figure 2.** In vitro semi-solid culture of murine acute myeloid leukaemia cells generates colonies of cells. The single cells that initiate these colonies have leukaemic stem cell potential.
The transcription factor AML1/RUNX1 is a frequent target of gene rearrangements and mutations in human acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). Consistent with its implication in leukemias, RUNX1 has also been shown to be critical for haematopoietic development. The MOZ gene is involved in three independent myeloid chromosomal translocations fusing MOZ to the partner genes CBP, P300 or TIF2. Our group studies the function of MOZ and RUNXI in haematopoietic development and maintenance with the aim to better understand how alterations of these functions lead to leukemogenesis. We use complementary approaches such as in vitro differentiation of mouse embryonic stem (ES) cells and in vivo mouse models.

RUNXI and leukemia.

Human acute leukemias are characterized by the presence of recurrent chromosomal abnormalities, which frequently result in the formation of chimeric transcription factors. The core binding factors AML1/RUNXI and CBFβ are the most frequent targets of these genetic alterations. The t(8;21) translocation resulting in AML1-ETO fusion and the inv(16) generating the SHMMC-CBFβ fusion accounts together for more than 20% of all the AML cases. Animal models have indicated that the full length AML-ETO, expressed either upon viral transfer or as a transgene, is not able to induce alone leukemia in mice. However an alternatively spliced form of AML1-ETO has recently been shown to cause following retroviral transfer a rapid development of leukemia in mice. Based on this new finding, we are developing an animal model in which the expression of this form of AML1-ETO is inducible. This new tool will allow us to study the molecular events leading upon expression of AML-ETO to the development of leukemia.

Early haematopoietic development

The earliest site of blood cells 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 instead associates the first haematopoietic cells to a phenotypically differentiated endothelial cell with haematopoietic potential, i.e. a haemogenic endothelium. Support for the haemangioblast concept was initially provided by the identification during embryonic stem (ES) cells differentiation of a clonal precursor, the blast colony-forming cell (BL-CFC), which gives rise after 4 days to blast colonies with both endothelial and haematopoietic potential. Although recent studies have now provided evidence for the presence of this bipotential precursor in vivo, the precise mechanism of generation of haematopoietic cells from the haemangioblast still remains completely unknown.

A new model of haematopoietic development

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

**Transcriptional targets of RUNX1/AML1**

Our initial studies revealed a profound defect in the potential of the RUNX1-/- ES cells to generate blast colonies. More recently we demonstrated that RUNX1 is critical for the generation of definitive haematopoietic from haemogenic endothelium during the formation of blast colonies. RUNX1 is likely to regulate the expression of an important set of genes at this stage of development. To identify these genes, we compared the patterns of gene expression of haemangioblast-enriched-cell-populations or haemangioblast-derived-cell-populations from either RUNX1 deficient or RUNX1 competent ES cells. We further validated the differential expression of candidates on samples generated from the ES/EB system and further documented the regulation by RUNX1 of the transcription of several of these genes by promoter assays or chromatin immunoprecipitation. We are currently evaluating the specific function of some of these genes at the onset of haematopoietic development and testing their potential to rescue haematopoietic development in absence of RUNX1. We have selected previously uncharacterized transcriptional target genes of RUNX1 and have initiated a series of experiments, such as conditional knock-out and knock-in, to determine the pattern of expression and function of these new genes.

**Expression and Function of RUNX1/AML1 isoforms**

RUNX1/AML1 is expressed as multiple naturally occurring spliced isoforms that generate proteins with distinct activities on target promoters. We have generated ES cells containing a reporter gene knock-in in the different isoforms and produced knock-outs altering the specific expression of these isoforms. We have demonstrated that the expression of these isoforms is differentially regulated during early haematopoietic development both in vitro and in vivo and that their expression defines specific stages of haematopoietic development. We are investigating the biological potential of cells expressing the respective isoforms and the function of each isoform.

**RUNX1 and chromatin remodelling**

We have examined in collaboration with the group of Constanze Bonifer (Institute for Molecular Medicine, University of Leeds) the molecular mechanisms leading to the expression of the transcription factor PU.1 and its target Colony-Stimulating-Factor 1 Receptor gene (CSF1R) at the onset of haematopoietic development. Our results indicate that chromatin remodeling at the PU.1 locus is initiated by transient binding of RUNX1 at an earlier developmental stage than expected. Subsequent CSFR-1 expression requires prior PU.1 expression and RUNX1 binding. Once a stable transcriptional haematopoietic circuit has been established, RUNX1 is in contrast dispensable. These results indicate stage specific functions and requirements for RUNX1 in the development and maintenance of the haematopoietic program.

**Function of the HAT activity of MOZ**

The MOZ gene is involved in leukemia in three independent myeloid chromosomal translocations fusing MOZ to the partner genes CBP, P300 or TIF2. All these genes encode enzymes containing a histone acetyltransferase domain (HAT) suggesting that aberrant modification of histones or other factors could provide the first step in the route to oncogenicity. We specifically addressed the role of the HAT activity of MOZ during haematopoiesis by generating a mouse strain that carries a single amino acid change in the HAT domain of MOZ. Analysis of these mice has revealed a profound defect in haematopoiesis. The numbers of haematopoietic stem cells and their potential is dramatically affected in homozygous mice. These in vivo results were confirmed with ES cells mutated for the HAT activity of MOZ as again less haematopoietic precursors are generated with the mutated ES cells. Altogether these results demonstrate the critical role of MOZ driven acetylation in the balance between proliferation and differentiation during haematopoiesis. We are currently investigating the precise molecular and cellular mechanisms affected in absence of the HAT activity of MOZ.

**Publications listed on page 60**
The emergence of haematopoietic cells during embryonic life occurs soon after gastrulation. A tight coordination of proliferation, differentiation and migration during the generation of these first blood cells ensures the proper survival and growth of the developing embryo. Understanding the molecular mechanisms that control the formation of these blood precursors from the mesodermal germ layer is the major focus of our laboratory. Several lines of evidence suggest that during adult life leukemogenesis can result from the re-initiation of an embryonic program or from the inappropriate expression of genes controlling critical steps of this embryonic program. A clear understanding of the molecular mechanisms orchestrating the onset of haematopoietic specification should help us to better define the basis of deregulated proliferation and differentiation observed in haematological malignancies.

**A model system to study haematopoietic specification.**

The in vitro differentiation of Embryonic Stem (ES) cells offers a powerful approach to advance our understanding of many developmental processes. Murine ES cells can be induced to differentiate and generate primitive and definitive haematopoietic precursors, an in vitro process that accurately recapitulates the in vivo development of yolk sac haematopoiesis. This progressive differentiation, leading to the formation of fully mature blood cells, can be monitored by the measurement of gene expression via real-time polymerase chain reaction (PCR), the analysis of cell surface expression by multi-colour flow-cytometry and the quantitative analysis of biological potential using clonogenic replating assays. The first known progenitor specified to the blood program is the haemangioblast. This precursor is characterized by its expression of Flk1, the VEGF (vascular endothelial growth factor) receptor 2, and its ability to generate both primitive and definitive haematopoietic precursors as well as endothelium and smooth muscle lineages. The hemangioblast, in presence of VEGF, give rise to fully committed blood precursors, characterized by their expression of CD41, the alpha2b integrin chain. Using microarray expression profiling of subpopulations at various stages of differentiation, we have identified novel cell surface markers and transcription factors implicated in the specification of blood precursors from the mesodermal germ layer.

**Multi-parameter analysis of blood specification.**

In our effort to define novel cell surface markers expressed during the specification of the haematopoietic lineages, we identified CD40 and Icam2 as two molecules substantially up-regulated upon haemangioblast commitment. Both genes code for proteins expressed on the cell surface of adult leukocytes and are actively implicated in immunological responses. To define a possible function for these two molecules at the onset of blood formation, we first investigated their pattern of expression during the differentiation of ES cells to blood progenitors. Interestingly, we observed a progressive and sequential up-regulation of these two cell surface molecules during the maturation of mesodermal precursors to fully committed blood cells.

Figure 1 illustrates the dramatic shift in CD40 and Icam2 profile observed between day 3 and 4 of ES cell differentiation. Detailed studies allowed us to establish that low levels of CD40 expression specifically define the Flk1⁺ haemangioblast subpopulation. As these precursors differentiated to generate blood-restricted precursors Icam2 expression is switched on while CD40 expression becomes significantly higher. In multi-parameter analysis, the integration of CD40 and Icam2 expression pattern into previously defined pathway of blood differentiation allowed us to further refine and identify discrete steps during the specification of mesoderm into fully restricted blood cells.
Control of blood precursor differentiation by Sox genes:
The microarray profiling analyses also led us to investigate the possible function of Sox transcription factors in the overall orchestration of blood cell development. Sox genes belong to the HMG (High Mobility Group) superfamily; they are highly conserved throughout evolution and implicated in the regulation of many developmental processes. Sox genes are subdivided into 7 groups according to their respective degree of homology in both their HMG boxes and trans-activation domains. Sox7, 17 and 18 are all members of the F group and became the focus of our investigation. Our expression analysis revealed the sharp up-regulation of Sox7 and Sox18 expression at the onset of haematopoietic development. However, upon further differentiation to generate fully committed blood cells and endothelium, the expression of both Sox7 and Sox18 was down-regulated. To address the significance of this transient expression, we assessed the effect of sustained Sox7 or Sox18 expression during haematopoietic differentiation using a doxycycline inducible ES cell system. Enforced expression of either gene using a progenitor replating assay resulted in the generation of colonies blast-like in appearance while the number of mature myeloid or erythroid colonies was strongly decreased. Further analysis revealed that enforced expression of either gene promoted a dramatic increase in cell proliferation coupled with an arrest in differentiation toward mature blood cells. Doxycycline removal led to the down-regulation of Sox7/18 expression, resulting in a progressive reduction of cell proliferation and promoting the maturation toward all myeloid and erythroid lineages. Insight into the molecular program sustained or initiated by Sox7 and Sox18 revealed the activation of the canonical Wnt pathway (figure2). We are now further dissecting this molecular program promoting the self-renewal of early haematopoietic precursors. Interestingly, the outcome of the mis-regulation of these two Sox genes is highly reminiscent of the leukemogenesis process in which immature blood progenitors loose their differentiation potential while acquiring an uncontrolled capacity to proliferate.

Publications listed on page 60
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. During 2008, we studied 1) how tumour-associated stroma becomes altered and co-evolves with tumour cells during the course of tumour progression 2) how the stroma facilitates progression of tumour 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 biologically complex stroma composed of various types of mesenchymal cells as well as extracellular matrix (ECM), both of which create the complexity of the tumour microenvironment. The significant contribution of stroma to the development of a wide variety of tumours has been supported by extensive clinical evidence; this contribution is highlighted by the higher incidence of tumour formation in tissues exhibiting a chronically inflamed stroma as well as those undergoing various types of wound healing, in which the stroma plays a central role. Use of mouse models of tumorigenesis also reveals that stromal cells, notably inflammatory cells, vascular cells, and fibroblasts, actively support tumour growth.

Large 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 of tumour stroma and the stroma present in sites of wound healing, both containing large numbers of myofibroblasts, raises the following questions: are myofibroblasts present in tumour biologically equivalent to those observed in wound healing or, alternatively, do tumour-associated myofibroblasts acquire “cancer-specific alterations” that distinguish them from those present in wounds? Such questions still remained to be investigated.

Stromal fibroblasts, termed carcinoma-associated fibroblasts (CAFs), were extracted from human carcinomas and these cells include collectively both fibroblastic and myofibroblastic cell populations. CAFs are known to substantially promote growth of nearby carcinoma cells co-injected into immunodeficient mice. This striking tumour-promoting property was indeed observed in CAFs extracted from various different types of human carcinomas. Independent of those, we demonstrated that fibroblasts present in the invasive human mammary carcinoma mass are biologically very different from their counterparts located outside tumour masses and from mammary stromal fibroblasts prepared from reduction mammoplasties in several important functional respects: (i) CAFs extracted from invasive human breast carcinomas
CAFs stimulate tumour angiogenesis

Sections from MCF-7-ras human breast tumours containing various fibroblasts were stained by anti-CD31 antibody (d, e, and f) or by Masson’s trichrome (a, b, and c).

Scale bar, 100 μm. (Orimo et al., Cell 2005; 121:335).

are more competent than normal fibroblasts in enhancing tumour growth by comingled breast cancer cells.

(ii) CAFs include larger populations of myofibroblasts, which exhibit high levels of α-SMA expression and increased collagen contractility.

(iii) When comingled with a line of human breast cancer cells, CAFs give rise to highly vascularized tumours in contrast to the poorly vascularized tumours generated by admixed normal stromal fibroblasts (see figure).

(iv) CAFs release increased levels of SDF-1 (stromal cell-endothelial progenitor cells (EPCs)) into a tumour mass, thereby boosting tumour angiogenesis.

In addition, the SDF-1 secreted from CAFs enhances tumour growth by direct paracrine stimulation via the CXCR4 receptor displayed by human breast carcinoma cells, thereby revealing a second role for stromal SDF-1 in promoting tumour progression in vivo.

(v) Both the tumour-enhancing and myofibroblastic properties of CAFs are stably retained by these cells in the absence of ongoing contact with breast carcinoma cells.

Evolution of tumour stromal fibroblasts in tumours

CAFs retain their myofibroblastic properties and tumour-promoting phenotypes, even after they have been passaged for ten population doublings (PDs) in vitro without ongoing contact with carcinoma cells. Accordingly, even though the CAFs appear to have initially acquired a myofibroblastic phenotype under the influence of carcinoma cells, once it is acquired, they display this trait in the absence of further signalling from the carcinoma cells. Unanswered by these observations are (i) how do CAFs acquire and maintain their activated, tumour-enhancing phenotypes? (ii) might CAFs harbour genetic and/or epigenetic alterations that act to confer their unique phenotypes?

Some reports indicate that stromal regions microdissected from human breast cancers exhibit a high frequency of genetic alterations, such as chromosomal regions of loss of heterozygosity (LOH) and somatic mutations. A recent report also suggests that stromal fibroblasts that have undergone p53 loss are clonally selected during tumour progression, yielding a highly proliferative stroma. However, another report indicates that myofibroblasts isolated from human mammary breast carcinomas exhibit no detectable genetic alterations, as gauged by array CGH and SNP array analyses; this suggests that any stably maintained phenotype may depend on epigenetic modifications of the genome, such as DNA methylation.

Alternatively, the stabilization of their phenotype may depend on some type of positive-feedback signalling of the sort created by autocrine signalling loops.

We note that our CAFs show no detectable aneuploidy as determined by karyotype analysis, no anchorage-independent growth in culture, and no tumorigenicity in vivo. Moreover, some of the CAFs begin to senesce after 15 PDs in culture, similar to the behaviour of normal human stromal fibroblasts.

We speculate roles of the epigenetic alteration(s) in regulating behaviour of CAFs as the following:

1) the alterations acquired during the course of tumour progression must be a rare event that provokes stable reprogramming in the resulting fibroblasts in tumours. Once established, they help initiate and maintain CAFs phenotypes in a stable fashion.

2) they can be, however, not eligible to transform these fibroblasts into tumourigenic cells.

3) only particular fractions of CAFs are present as bona fide cells that acquired the stable alterations in tumours, since CAFs are basically composed of heterogenous cell populations by constantly recruiting into tumour-associated stroma locally preexisting mesenchymal cells and/or bone marrow-derived hematopoietic cells. Just after the recruitment, the latter would be converted into tumour-supporting CAFs in the absence of acquisition of the stable alterations.

4) these CAFs thus depend largely on ongoing signaling from nearby carcinoma cells in supporting the carcinoma growth, while stably altered CAFs depend on the alterations that regulate them in a cell-autonomous fashion. This thought together with the description above reflects the notion that the stable alterations may not encourage the resulting fibroblasts to clonally expand.

5) out-growth of the CAFs would be also under the control by carcinoma cells; this schema enables the latter to orchestrate virtually all mesenchymal cells in tumours, facilitating to develop eventually full-blown tumour.

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

Each year about 650,000 people are diagnosed with cancer of the head and neck worldwide and 350,000 people will die from the disease. Over the last 20 years, the overall 5-year survival rate has remained at ~50% despite significant advances in surgery and oncology practice. There is a need to increase understanding of biological factors associated with a poor prognosis and to improve the individualisation of treatment in order to increase survival. There is considerable evidence that high levels of tumour hypoxia are associated with a poor prognosis in patients with head and neck cancer. Various approaches are being studied to assess the level of tumour oxygenation, one of which involves measuring the level of tumour expression of hypoxia-related proteins. Carbonic anhydrase 9 (Ca9) is a transmembrane glycoprotein which is expressed in some types of normal tissue, such as duodenal, jejunal, hepatic and pancreatic tissue. The protein is widely expressed in some tumours including head and neck squamous cell carcinoma. It is a hypoxia-inducible protein and tumour expression has been linked with oxygenation status. We were the first group to associate tumour expression with a poor prognosis following radiotherapy in cervix tumours. Subsequent work by the group has also shown a relationship between high expression in a homogeneous group of oropharyngeal cancers and poor outcome following radiotherapy. Work over the past year investigated expression of Ca9 in a large series of larynx cancers (Catriona Douglas & Helen Valentine in collaboration with Mr Jarrod Homer and Dr Nick Slevin). From a database of 423 patients who underwent potentially curative radiotherapy, scores for tumour CA9 expression were available for 310, a considerably larger number of patients than any series reported in the literature. High (≥10%; n=109) vs low (<10%; n=201) expression was associated with a worse locoregional recurrence-free (P=0.032; figure 1) and cancer specific (P=0.040) survival on univariate analysis. Associations of high expression with a poor prognosis were retained on multivariate analysis for both local control (HR=2.16, 95% CI=1.07–4.34; p=0.031) and cancer specific survival (HR=2.53; 95% CI=1.01–6.34; p=0.048). This is an important finding clinically because surgery is an option for treating the disease and is associated with a similar outcome as radiotherapy. Radiotherapy is generally preferred because it results in better preservation of organ function, i.e., voice, but if local recurrence occurs following treatment a total laryngectomy is usually carried out.
Development of a hypoxia-associated gene signature in head and neck cancer

With our collaborators in Oxford (Prof Adrian Harris, Dr Francesca Buffa) and Applied Computational Biology & Bioinformatics (Dr Crispin Miller) we have continued work on our hypoxia-associated gene expression signature (Guy Betts & Carla Möller-Levet). Over the past year the signature has been streamlined and reduced from 99 to 26 genes, and a comparison made of Exon and Affymetrix U133plus2 GeneChip data. With the recent award of an MRC Biomarker grant, the group is looking forward to validating and qualifying the signature using a PCR approach in multiple clinical datasets.

RAPPER and VORTEX-BIOBANK

The Translational Radiobiology group co-ordinates the biobanking associated with several national radiotherapy trials. RAPPER (Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy) is collecting samples from a number of national trials. The CR-UK funded project aims to explore associations between genetic variation expressed as single nucleotide polymorphisms and radiation toxicity. This year we exceeded our planned recruitment of 2,200 patients with breast, prostate, gynaecological or rectal cancer (figure 2). Day-to-day administration is carried out by Rebecca Elliott and Kathryn Fellows recruited patients at the Christie Hospital. Collaborators include Drs Neil Burnet & Alison Dunning (Cambridge), Prof Søren Bentzen (Wisconsin) and numerous clinical oncologists locally and nationally. Sample collection will continue from national radiotherapy trials for a second validation phase of genotyping. The first sample for VORTEX-BIOBANK (Joely Irlam-Jones) was received in 2007 and we have now banked 36 matched fresh tumour and normal tissue samples and 32 blood samples.

Publications listed on page 60
Chimeric immune receptor (CIR) T-cell trials

Tumour antigen specific CIRs enable T cells to target and specifically destroy tumour cells in a MHC-independent manner. Two first-in-man phase I clinical trials of CIR T-cell trials are now open and recruiting patients at The Christie. In both trials patients receive conditioning chemotherapy prior to an autologous infusion of CIR T cells and the patients subsequently receive intravenous IL2 to support the in vivo survival and expansion of the CIR T-cells. The first trial targets CEA in patients with solid tumours that have shown to express CEA. Recruitment to the first cohort of patients in this trial is now complete and well underway for the second cohort. More recently a second trial targeting CD19 in NHL has opened and a number of patients have been recruited to the first cohort. In both trials, prior to use for treatment, the autologous expanded CIR T cells are extensively phenotyped by flow cytometry to allow for correlation with any subsequent engraftment. A validated assay based on quantitative PCR allows the detection of the level of transduced cells in each patient. The first results indicate that following transfusion of the expanded CIR T cells, their levels initially drop but a transient return can be seen following the end of IL-2 treatment; one of three patients showed persistence of transduced T cells beyond 6-weeks post infusion. Ongoing secondary and scientific assays include molecular monitoring of clonal populations with T cell spectratyping and the assessment of retroviral integration via LAM-PCR.

An MVA-based vaccine targeting the oncofoetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases

We investigated the use of a therapeutic vaccine, TroVax in patients undergoing surgical resection of colorectal cancer liver metastases. Systemic immunity generated by vaccination before and after resection of metastases was measured in addition to assessing safety and analyzing the function and phenotype of tumour-associated lymphocytes. Twenty patients were scheduled to receive two TroVax vaccinations at 2-week intervals pre-operatively and two post-operatively; if immune responses were detected two further vaccinations were offered. Blood was taken at trial entry and two weeks after each vaccination; tumour biopsies were collected at...
surgery. ST4-specific cellular responses were assessed by lymphocyte proliferation and ELISPOT, with antibody responses by ELISA. Immunohistochemistry characterized the phenotype of tumour infiltrating lymphocytes. Seventeen of 19 colorectal cancer patients showed ST4 expression in the liver metastases or surrounding stroma and 18 mounted a ST4-specific cellular and/or humoral response. In patients who received at least four vaccinations and potentially curative surgery (n=15), those with above median ST4-specific proliferative responses or T cell infiltration into the resected tumour showed significantly longer survival compared to those with below median responses (figure). Seven of 8 patients that had pre-existing proliferative responses to ST4 were longer term survivors; these patients showed significantly higher proliferative responses following vaccination than those who subsequently died. These data suggest that the magnitude of ST4 proliferative responses and the density of CD3 cells in colorectal cancer liver metastases are associated with longer survival. These observations warrant more studies to identify the precise underlying mechanisms.

**Immune regulation and cancer therapy**

The immune system can recognise cancer, but immune driven cure is rare. Cytotoxic T lymphocyte-associated antigen 4 (CTLA4), a crucial inhibitor of T cell activation, has a role in regulating host immune responses against cancer. It is expressed by natural regulatory T cells (Treg) and by other T cells on activation. CTLA4 blockade can induce immune driven cure in some animal models, and has shown promising results in phase I and II clinical trials in melanoma patients. Second-line chemotherapy in advanced oesophageal and gastric cancers has poor response rates which are rarely sustained and high levels of toxicity; new treatment strategies are needed. Thus, 18 adult patients with metastatic oesophageal or gastric adenocarcinomas received CP-675,206 (Pfizer), a human monoclonal antibody against CTLA4 in a Phase II clinical trial. Twelve received a single cycle, five two cycles, and one patient remains on treatment after five cycles. Four patients had stable disease on CT scan; two had clinically beneficial tumour shrinkage; one has an ongoing response with marked reduction of tumour markers. Changes to lymphocyte phenotype (measured by flow cytometry) and lymphocyte proliferative responses to ST4 tumour antigen with CTLA4 blockade were assessed. By day 15, parallel rises in expression of FoxP3 (Treg marker), CTLA4 and another immunomodulatory target, PD1, were observed. By day 60 most of these rises have returned to near baseline levels, but increased CTLA4 expression in CD4+CD25low cells was sustained. These complex patterns of expression are being further explored functionally. Importantly, enhanced proliferative T cell responses to specific ST4 peptide pools were seen in 7 of 13 patients. This is consistent with anti-CTLA4 releasing potentially useful anti-tumour immunity. Responses to other tumour antigens such as CEA are in progress. Analysis of the cellular mechanisms of anti-tumour effect of anti-CTLA-4 monoclonal antibodies has provided conflicting results in cancer patients. We are currently investigating whether the antitumor effect of CTLA-4 blockade is due to increased T cell activation or inhibition of Treg activity.

**ST4 antibody targeted superantigen therapy**

We are leading a Phase III trial of targeted superantigen therapy based on our previous clinical/translational studies. Extended immune analyses are addressing several questions. What is the level and activity of superantigen specific T cell in the treated patients? This involves direct detection of the patients’ drug reactive T cells by flow cytometry and superantigen antibody dependent cell-mediated cytotoxicity of tumour cells. Is there any bystander effect on ST4 specific immunity? Such ST4 specific immunity is monitored using ST4 peptide lymphocyte proliferation or by specific ELISA for antibodies. Ultimately the goal is to discover whether the active immunity correlates with clinical outcome!

**Publications listed on page 61**

Kaplan-Meier overall survival curves for patients stratified according to below or above median anti-ST4 immune responses and CD3+ T cell infiltration into the tumor. The curves show ST4 antibody responses (A), ST4 proliferative responses (B), combined ST4 proliferative and antibody responses (C), CD3+ T cell infiltration into the tumor (D), and combined ST4 proliferative responses, ST4 antibody responses and CD3+ T cell infiltration (E) (Elkord et al., 2008b).
Clinical Trials
In 2008, Carly Leighton left and Catriona Parker joined the team as Clinical Trials Manager. Our flagship clinical trial, ALLR3, is for children with relapsed ALL. In that, we had asked a randomised question of the efficacy of Mitoxantrone (test drug) over Idarubicin. The randomisation was stopped in January of this year, earlier than planned, as the test drug Mitoxantrone proved to be superior to the standard drug (figure 1). Curiously, the differences in outcome do not correlate to the kinetics of disease clearance, as both drugs show the same minimal residual disease patterns. Moreover, the efficacy of Mitoxantrone appears to be related to a low relapse rate post transplant. This suggests that Mitoxantrone may be more effective in eradicating cellular niches where ALL cells may escape therapeutic annihilation (see below). This is the first ever trial in relapsed childhood ALL to provide an answer to a randomised question. Overall the trial has produced some of the best results ever achieved in this cohort of children. Last year we also completed the first Pan-European phase II trial in childhood ALL with the drug Clofarabine. As the drug showed a beneficial effect, the high risk arm of ALLR3 is now being amended to incorporate Clofarabine. This will open in early 2009 and form the basis of a Pan-European trial EuReALL in 2011 at which time we will close ALLR3. We also coordinate in the UK, on behalf of a European interstudy group, a clinical trial of Imatinib in children with Philadelphia positive ALL in the UK. This trial is also expected to run till 2011.
Laboratory

Naina Patel with the help of Shekhar Krishnan has identified a novel mechanism of resistance to the key anti-leukaemic drug Asparaginase. The lysosomal cysteine proteases CTSB and AEP both cleave and inactivate the drug. Naina has identified the cleavage sequence and shown that mutation of the first cleavage site is sufficient to protect the drug. Shekhar has shown that active AEP appears to be localised in a distinct lysosomal compartment found at the periphery of the cell (figure 2). Working with Paul Bates’ group at the London Research Institute, Naina is continuing to modify Asparaginase to make it less degradable, less toxic and more effective. This work has led to a successful grant application from the Leukaemia Research Fund to correlate the expression of these proteases with clinical response to therapy. This is the first biomarker study to be carried out in children with ALL in the UK and will be one of the largest of its kind ever carried out. This work is being carried out by Ashish Masurekar and Jizhong Liu and is co-ordinated by Catriona Parker.

Our work has uncovered the presence of large lysosomes in ALL cells. There is evidence from other cancers that lysosomal proteases participate in mechanisms that promote cell survival. These include processes such as cell migration invasion and autophagy. Mark Holland has identified ALL cell lines that invade across matrigel and endothelial barriers. He has taken a global proteomic approach to investigate the changes in the plasma membrane proteome of the invading cells. With the help of Professor Anthony Whetton and Duncan Smith, using SILAC and iTRAQ, Mark has identified an actin-related signature of invasion. He is now working with Peter Stern’s group to develop an animal model to validate this observation. Seema Alexander has developed lentiviral strategies to investigate the relationship of lysosomal proteases, such as AEP, in the invasion process. Her initial experiments suggest that though AEP expressing cell lines are invasive, AEP per se is not responsible for this phenomenon. Clare Dempsey is working with a leukaemia-associated fusion transcript known to be associated with endosomal trafficking. She has transduced cell lines and haematopoietic stem cells with a lentivirus expressing CALM-AF10 and will now examine its leukaemogenic potential and its effect on cargo trafficking. As endocytosis and signalling are dependent on the microenvironment, Jizhong Liu is investigating the interactions between normal and malignant haematopoietic cells and mesenchymal tissue. He has established a 3D bone marrow culture system to study these interactions. His investigations suggest that mesenchymal cells protect haematopoietic stem cells (HSCs) from toxic stimuli. It is likely that a subpopulation of ALL cells is able to mimic HSCs and find refuge in mesenchymal niches. As discussed earlier, this may be the explanation of the differential effect of Mitoxantrone in ALLR3. We hope that the 3D model that Jizhong has developed will help us answer this question.

Overall, as a translational research group, we continue to conduct hypothesis-driven laboratory research, based on observations made on patients undergoing clinical trials. Finally, we are pleased to report that both Frederik van Delft and Shai Senderovich successfully defended their doctoral dissertations in 2008.

Publications listed on page 62
The 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. This will be achieved by the specific objectives which are i) to investigate how the recognition of radiotherapy (RT) induced tumour cell death by different antigen presenting cells in the tumour microenvironment can impact on the ensuing immune response; ii) to investigate the role of myeloid derived suppressor cells (MDSC) in tumour regrowth after RT and to develop strategies to enhance RT tumour control by modifying host immune response after RT and iii) to translate our experimental research findings into developing early phase clinical trials.

Mechanisms of action of radioimmunotherapy (RIT)

Our recent work has focused on the molecular mechanisms of action of RIT induced tumour cell death in vitro. We have investigated the downstream signalling events in a variety of human B cell lymphomas after treatment with RT and anti-CD20 mAb using a number of clinically relevant anti-CD20 mAb. Anti-CD20 mAb can be broadly sub-divided into either type I (eg rituximab) or type II (eg tositumomab). Rituximab and other Type I anti-CD20 mAb engage complement effectively and cause target cell lysis. In contrast type II mAb such as tositumomab (B1) are generally potent at inducing cell death in target cells. We observed increased tumour cell death with tositumomab and RT, which was not seen with rituximab and RT. This increased tumour cytotoxicity was reversed with the MEK inhibitors (U0126, PD98059) as well as siRNA targeting MEK1 or MEK2. Furthermore the addition of U0126 reversed the loss of clonogenic survival triggered by combining tositumomab with RT. Phosphorylated ERK1/2 (pERK) was found to accumulate in the nucleus following tositumomab and the nuclear accumulation of pERK was greatly enhanced in combination with RT. In contrast rituximab caused early ERK phosphorylation but this was not sustained and remained within the cytoplasm suggesting that this might underlie the lack of additive tumour cell death. In summary our data indicate that activation and nuclear accumulation of pERK appear to be required to produce the synergistic effect produced by combining tositumomab and RT (Ivanov et al., 2008).

Recently, in collaboration with Dr Mark Cragg’s group in Southampton we have investigated a new form of mAb induced cell death in B-cell lymphomas. Using both lymphoma cell lines and primary chronic lymphocytic leukemia (CLL) cells, we have demonstrated for the first time the importance of lysosome-mediated cell death for antibody therapy elicited by clinically relevant mAb directed against two different target antigens namely CD20 and HLA DR (figure). By virtue of a detailed and kinetic approach we have determined that death is preceded by homotypic adhesion with both adhesion and death being dependent upon actin redistribution. Malignant B cells, undergoing homotypic adhesion, actively communicate via ~ 5 nm wide temporary inter-cytoplasmic bridges. The formation of these channels is accompanied by the mutual exchange of plasma membrane components and importantly the extent of plasma membrane swapping correlates with the extent of cell death induced by both anti-CD20 and anti-HLA DR mAb. To our knowledge there are no known precedents for these phenomena recorded for these cell types. The fact that similar findings have been
recorded after treatment with functionally different mAb direct to CD20 and HLA DR antigens suggests that the phenomena observed may be the general mechanism of lymphoma cell killing by activating antibodies. The latter is potentially of great interest as it provides a potential means to bypass the often dysregulated apoptotic death pathways of tumour cells allowing for effective tumour cell killing in the presence of apoptotic inhibition. Interestingly both of the mAbs studied evoke lysosomal non-apoptotic cell death pathway and this is likely to go some way to explain their efficacy in vivo. This experimental work has been selected for an oral presentation at ASH 2008 and has been submitted to the Journal of Clinical Investigation after successful pre-submission enquiry.

**Immune response to RT induced dying tumour cells**

The work in this research area has focused on enhancing the therapeutic potential of RT by investigating combining RT with immunotherapeutic approaches. Our recent work has focused on understanding the nature of the host immune response to RT induced tumour cell death

Over the last few years we have successfully developed a number of powerful tools that will in the future programme facilitate the exploration of the possible roles of subsets of professional antigen presenting cells (APC) in the immune response to tumour cell death induced by RT. We have focused our attentions on two types of APC, namely macrophages (MΦ) and Dendritic cells (DC). Recently we have demonstrated that by manipulating MΦ within the tumour microenvironment we can induce protective anti-tumour CD8 T-cell responses with anti-CD40 against irradiated lymphoma cells that are in themselves poorly immunogenic. In these studies we have shown the potential importance of MΦ in cellular vaccination and have demonstrated that depletion of MΦ using clodronate-encapsulated liposomes considerably enhances primary vaccination efficacy in the presence of adjuvant anti-CD40 mAb (Honeychurch et al., submitted).

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 (Honeychurch et al., 2008).

**Clinical Translational applications of the laboratory research programme**

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

**Anti-idiotype against Rituximab (serum rituximab assay)**

The serum rituximab ELISA assay that we previously developed has now been validated to GCLP in collaboration with the Clinical and Experimental Pharmacology Group (CEP). A robust, reliable and reproducible ELISA which can accurately determine serum rituximab concentrations has been established as a national reference laboratory resource. The first study to use the validated serum rituximab assay is the NCRI Phase III study which compares the policy of “Watch and Wait” versus rituximab (Grace Hampson is funded by a 2008 CR-UK TRICC grant awarded to Illidge/Dive). We plan to apply this assay to more national clinical studies in the forthcoming year.

**Early Phase Clinical Trials of Radioimmunotherapy**

The clinical RIT group has made considerable progress over the last few years in leading early phase clinical trial design both nationally and internationally, with a substantial portfolio of early phase clinical trials. The highlight of the year was the publication in Blood of the Phase II dose escalation RIT study. This study was the first of its kind to investigate the effect that induction therapy (4 weekly infusions of 375 mg/m² rituximab) has on the subsequent efficacy and toxicity of anti-CD20 RIT in relapsed indolent B cell Lymphoma (Illidge et al., 2008). Induction therapy with rituximab was found to significantly increase the effective half-life of 131I-rituximab and higher serum levels of rituximab at week 6, after rituximab induction therapy, correlated with an increased effective half-life of the radioimmunoconjugates

An important observation we made from this study was that induction therapy with multiple doses of rituximab did not appear to compromise the clinical efficacy or increase toxicity of subsequent 131I-rituximab RIT. The overall response rate (ORR) was 94%, with complete response (CR) rate 50%. The median time to progression was 20 months, significantly longer than for the last qualifying chemotherapy with ongoing durable remission of more than 60 months. Fractionation of 131I-rituximab allowed cumulative whole body doses of over 120 cGy, around 60% greater than those previously achieved with a single administration of a murine radioimmunoconjugate, to be delivered without significant hematological toxicity.

**Publications listed on page 62**
The Chimeric Immune Receptor: Molecular Interactions with the T cell Receptor

The gene that is introduced into the patient’s T cells encodes for a protein called a Chimeric Immune Receptor. This receptor consists of an extracellular domain that can bind to target proteins present on the cell surface of the target cell and is fused to the transmembrane and cytoplasmic domain of the CD3ζ receptor; which is one of the components of the T cell receptor (TCR) complex. Natural CD3ζ proteins bind to the TCR through electrostatic interactions between charged amino-acids present within their transmembrane domains. We have shown that our chimeric receptor is also incorporated within the TCR complex present on the surface of the gene-modified T cell by biochemical and by Fluorescence Resonance Energy Transfer (FRET, Figure 1).

Mutations in the charged amino-acids reduce the ability of the chimeric receptor to interact with the TCR; consequently, this significantly reduces the sensitivity of the chimeric receptor function suggesting that the formation of the TCR-chimeric receptor complex is critical for optimal anti-tumour activity of the T cell. However, important questions arise from this observation. Does the chimeric receptor interfere with the activity of the normal T cell? Could there be safety issues associated with the long-term formation of the TCR/chimeric receptor complex? To explore these questions, we have developed two model systems (based upon our clinical trials) to investigate the potency of chimeric receptors and to assess the long-term safety of gene-modified T cell therapy.

Genetic Modification of Mouse T-cells

In order to test chimeric receptor technology in suitable tumour models, we have had to develop and improve protocols to allow high levels of gene transfer into primary mouse T cells. For reasons which are not entirely understood, mouse T cells tend to be more refractory to the viral gene transfer systems which work efficiently in human T cells. We have optimised retroviral gene transfer into primary mouse T cells and have established culture conditions which permit the expansion of these T cells to high numbers ex vivo for use in adoptive transfer experiments. We have passed on our methods to collaborators who have confirmed the robustness of the protocol (Prof. Zelig Eshhar, Weizmann Institute, Israel). Using these improved protocols, we are currently examining how the culture condition and duration of culture impacts upon T cell persistence and anti-tumour activity in vivo.

Models of gastrointestinal cancer and B-cell lymphoma.

Our first trial (sponsored by Cancer Research UK) involves generating T cells with specificity for CEA through the expression of a chimeric immune receptor called MFEz. The second (sponsored by the Kay Kendall Leukaemia Fund) involves targeting B cell lymphoma using T cells armed with a chimeric receptor specific for the CD19 protein (CD19z). In
order to obtain regulatory approval for these trials, we have generated model systems for both CEA and CD19 which have demonstrated the efficacy of human T cells targeting the relevant tumour types in immuno-compromised mouse models.

In order to further understand and to improve the approach, we are using mouse T cells armed with chimeric receptors to target mouse tumours expressing the relevant protein target. In an initial set of experiments, we showed that T cells injected close to the site of tumour proved more much effective at challenging the growth of CEA+ tumours than T cells given systemically. This suggests that getting the T cells to the site of the tumour is critical for the therapy to succeed. However, immuno-compromised mice (i.e. mice lacking a functional immune system) bearing established CEA+ tumours can be effectively treated using gene-modified T cells. These observations suggest that the presence of competing immune cells may inhibit the functionality of the gene-modified T cells. Consequently, conditioning of the patient (i.e. transiently depleting the immune system) prior to T cell infusion may be important in driving an effective anti-tumour response.

Indeed, in our CEA model system, mice receiving either chemotherapy or radiotherapy prior to T cell infusion showed significantly reduced levels of tumour growth compared to animals treated with T cells alone (Figure 2). In the B cell lymphoma model, the combination of human CD19z T cells with chemotherapy effectively treated the Raji B cell lymphoma in vivo (Cheadle et al., 2008) while the combination of chemotherapy and mouse CD19z T cells was optimal in eradicating a long term (13 day) established mouse B-cell lymphoma in immune competent mice (Cheadle et al., in press). Importantly, these studies demonstrate that achieving high levels of circulating gene-modified T cells is an important factor to predict for anti-tumour response. These studies support the central aim of both the CEA and CD19 clinical trials which is to determine which therapeutic conditions can achieve high levels of circulating gene-modified T cells in the patient.

Tumour Infiltrating lymphocytes

Aside from the genetic modification approach, we are actively investigating the isolation of antigen specific T cells from the tumours of patients. This approach has been pioneered by Dr Steven Rosenberg (NCI, Washington DC) in malignant melanoma with some spectacular clinical responses. In collaboration with Mr Gary Ross (Dept. of Surgery) and Dr Paul Lorigan (Medical Oncology), we have tested biopsy samples from ten melanoma patients with approximately 120 TIL cultures being initiated. Of these, approximately 50% generated sufficient T cells for testing with only one patient of the ten failing to generate any TIL cultures. Importantly, of the first three patients tested, two have generated TILs that are able to respond against autologous tumour cells. We are further developing this protocol in order to move towards clinical testing of melanoma TILs in Manchester within the next two years.

Summary

The focus of the Cell Therapy group over the last twelve months has been upon the development of model systems to test adoptive T cell therapies. These models are now advancing our knowledge of gene modified T cell biology which will translate into improved future clinical protocols.

Publications listed on page 63

Figure 1. FRET Analysis of TCR-CIR Interactions – Jurkat T cells expressing “TCR-interacting” MFE23.CD3ζ and “TCR non-interacting” MFE23.htm.CD3ζ were stained with Alexa-647 conjugated anti-TCR antibodies and Alexa-555 conjugated CEA protein. Cells were imaged using an Axiovert-Time lapse microscope incorporating Metamorph software. FRET signal is observed in cells expressing the MFE23.CD3ζ CIR but not the MFE23.htm.CD3ζ CIR.

Figure 2. Improved tumour free survival of mice involves pre-conditioning of the mice prior to infusion of tumour specific engineered T cells.
Cancer cells are characterised by progressive growth and the capacity to invade and colonise distant sites. Heparan sulphate (HS) is a cell surface co-receptor for many of the growth and migration factors involved in the dissemination and vascularisation of human tumours. Our research has shown that patterns of sulphation along the HS chain act as binding sites for growth factors and enable their efficient engagement with tyrosine kinase receptors. Using novel analytical methods we have investigated the molecular design and function of HS in different cell types, including embryonic stem (ES) cells. Our recent findings indicate that the unique domain structure and conformational flexibility of HS drives the assembly of ligand-receptor signalling complexes on the plasma membrane.

**Hepatocyte Growth Factor/Scatter Factor (HGF/SF): a single, primary co-receptor binding site with dual glycosaminoglycan specificities**

HGF/SF is a potent mitogen and migration factor for epithelial and endothelial cells. It acts via a dual receptor system of the MET tyrosine kinase receptor and the glycosaminoglycan (GAG) chains of proteoglycan co-receptors. Excessive HGF/SF-MET signalling occurs in many carcinomas, sarcomas and also multiple myeloma, where the degree of elevation correlates with tumour invasiveness, metastasis and poor disease prognosis. There is a strong case for HGF/SF-MET as a target in anti-cancer therapy. Our aim has been to elucidate how GAGs are recognised by HGF/SF, and how they act as co-receptors, to exploit this as a potential route to the development of inhibitors of co-receptor function and thus HGF/SF action. This is complicated by the unusually high affinity of HGF/SF for both heparan sulphate (HS)/heparin and dermatan sulphate (DS), two GAG types which differ significantly in structure. Recently we have completed a two-pronged experimental approach, whereby we have tested the binding and activating properties of two different groups of molecules: (i) a wide array of natural and modified GAGs/sulfated polysaccharides (Catlow et al., 2008), and (ii) a panel of minimal-binding tetrasaccharide sequences of variable but well-defined sulfation patterns.

These studies revealed that binding required a minimum of two sulphate groups within a tetrasaccharide, in combination with the presence of iduronate residues, but surprisingly is independent of sulphate positioning. Indeed, using a newly-developed technique, combining our previous gel mobility shift assay (GMSA) (Lyon et al., J Biol Chem 2004; 279: 43560) with reverse-phase-HPLC, we have now been able to show conclusively that all heparin tetrasaccharide isomers, that contain two sulphates at either N-, 2-O- or 6-O-positions (see figure) bind equally well, and identically to a DS tetrasaccharide that is 4-O-sulphated (Deakin et al., in press). Thus the ability to bind either HS/heparin or DS appears to arise from a non-specific ability to accommodate a variety of sulphate configurations in iduronate-containing GAGs. Absolute affinities correlate with the overall level of sulphation, suggesting a major role for electrostatic interactions in complex formation.

In collaboration with Dr Dusan Uhrín (University of Edinburgh), we were able to show that the NMR chemical shifts induced in the truncated NK1 variant of HGF/SF by titration with either heparin or DS oligosaccharides were very similar, strongly indicating that both these GAGs bind to a single site of relatively low specificity (Deakin et al.). These
results will hopefully impact upon the future development of HGF/SF inhibitors based upon mimics of GAG co-receptors which we described in our 2007 Report (Raiber et al., Bioorg Med Chem Lett 2007; 17: 6321).

**Heparan sulphate in the regulation of embryonic stem cell (ES) cell differentiation**

ES cells are a valuable model to study the role of HS during development. ES cell differentiation is accompanied by dramatic alterations in HS sulphation that enable each cell/organ to respond characteristically to extrinsic signals in the form of peptide growth factors and morphogens. In previous reports we described the alterations in HS that occurred during neural differentiation. To highlight the essential requirement for HS for progression along the neural lineage we have characterised the differentiation potential of HS-deficient Ext1-/- ES cells. These cells were found to be defective in both neural and mesodermal/haematopoietic differentiation (see below), mimicking the block in development seen in HS-deficient mouse embryos. However, remarkably differentiation could be restored by the addition of soluble HS or heparin oligosaccharides to the growth media. These important findings open the way for investigating whether different HS species can dictate cell fate decisions in ES cells.

We have also characterised the dynamic modifications in HS sulphation motifs during mesoderm formation and blood formation (Baldwin et al., 2008). This paper highlighted the expression of a specific HS epitope (detected by antibody HS4C3) that is selectively and transiently expressed during formation of the haemangioblast, a cell expressing Flk1 (VEGF receptor) which is capable of forming both blood and endothelial progeny. Functional assays revealed that cells positive for HS4C3 and Flk1 had a dramatically increased ability to form blood cells compared to those expressing Flk1 alone. The development of these cells is dependent upon the HS-binding growth factor VEGF, suggesting this HS epitope may be important for correct binding of VEGF. In vivo studies showed remarkable correlation with in vitro findings, with expression of the HS4C3 epitope restricted to newly formed mesodermal tissues during gastrulation. We believe this is the first time a defined HS epitope has been implicated in a specific developmental pathway and that this additionally provides a novel enrichment technique for the isolation of haemangioblasts from mixed differentiated ES cell cultures. This work was done in collaboration with Valerie Kouskoff, Georges Lacaud and Catherine Merry.

**Co-operative dimerisation of Fibroblast Growth Factor (FGF) on Heparan Sulphate drives the assembly of FGF/FGF-Receptor signalling complexes**

Heparan sulphate is a mandatory co-receptor for the fibroblast growth factors (FGFs). Heparin saccharides of defined length (8-to-10 sugars) and sulphated at the N-, 2- and 6- positions (see figure) were used as chemical analogues of the sulphated regions of HS. At very low concentrations, these saccharides elicit a potent FGF2 mitogenic response which correlates with their ability to dimerise FGF2 in a co-operative manner. These results, together with parallel studies on acidic FGF (FGF1), have revealed that co-operative ligand dimerisation by heparin and HS is likely to be the main driving force in assembly of FGF signalling complexes on the cell surface. The predicted stoichiometry of these complexes from size exclusion chromatography (SEC) is 2:2:1 FGF:FGFR-HS where FGFR is the FGF-receptor.

Investigations of these complexes by multi-angle light scattering (MALLS) available at the Biomolecular Analysis Facility (University of Manchester) has yielded data that strongly support the stoichiometries predicted from SEC. A particularly interesting observation was that saccharide concentration had no effect on the stoichiometry of FGF-heparin complexes. This is what we would expect from our published model of co-operative ligand dimerisation. (Robinson et al., J Biol Chem 2005; 280: 42274). Further studies by our collaborators Professor Tom Blundell and Alan Brown in Cambridge on the thermodynamic properties of monomeric and dimeric FGF-heparin complexes using isothermal titration calorimetry (ITC) have largely confirmed the co-operative binding model. Overall these studies highlight an unexpected structural plasticity of HS and heparin that enables a monomeric ligand like FGF to induce a conformational change that creates a highly favourable proximal site for a second FGF. This HS-bound dimeric FGF efficiently recruits two receptors that then deliver signals across the cell membrane.
Medical Oncology: Translational Angiogenesis Group

Group Leader  Gordon Jayson

Senior Fellows
Egle Avizienyte

Postdoctoral Fellows
Marek Barath
Claire Cole
Steen Hansen

Clinical Fellows
Gireesh Kumaran
Claire Mitchell
Nishanth Murukesh

Scientific Officers
Alison Backen
Karen Brookes
Graham Rushton

Postgraduate Student
Karl Broburg

Rotation Student
Kelvin Wilkinson

Over the last year the group has progressed in two directions; the GCLP validation and implementation of imaging and blood borne biomarkers for anti-angiogenic agents and the second approach has been to develop research platforms that will assess the contribution of heparan sulfate proteoglycans in human epithelial ovarian cancer. The translational proteoglycans programme has emerged from the basic science studies that our group has performed over the last few years within Professor John Gallagher’s laboratory.

Biomarkers for Anti-Angiogenic therapy (Collaborators: Dive, Jackson, Parker)

Over the last few years the group has identified and evaluated a series of imaging based biomarkers for anti-angiogenic therapy. Within the last year we have reported a phase I trial of a novel anti-VEGFR2 di-Fab compound (Ton et al., 2007 Clin Cancer Res; 13: 713), which differed from other broader spectrum VEGF inhibitors in that it did not impact on $K_{trans}$, the endothelial permeability-surface area product. On the other hand, biopsy of hemangiomata that were induced by the compound confirmed that the compound was present in areas of non-phosphorylated receptor, that is, that the biopsy data were compatible with the proposed mechanism of action. By changing the traditional phase I drug design to expand the number of patients at each dose level we were able to increase the statistical power of the study and, through measurements of tumour volumetrics, were able to detect a dose-level vs volumetric response. This is important as the traditional method of reporting radiological evaluation of tumour response, RECIST, failed to detect this relationship. Thus our findings highlight tumour volumetric measurements as a potential novel biomarker for mechanism based therapeutics.

In a second programme we have developed and validated to the standards of GCLP multiplex and singleplex assays of sixteen angiogenic proteins. Traditional assays are based on singleplex ELISAs that are costly and consume significant amounts of plasma. By multiplexing nine proteins, for the first time, we have impacted significantly on the cost, time and amount of plasma needed for these assays. The protocols have been submitted for publication and will be used in forthcoming evaluations of plasma samples in ICON7, a randomised trial of carboplatin and paclitaxel with and without bevacizumab in the first line treatment of ovarian cancer: Additional samples that will be evaluated will be obtained from randomised trials of anti-angiogenic agents in pancreatic and colorectal cancer. Finally, within the next twelve months we will apply the technology within an in-house study that unites the single and multiplex studies developed in the last year; with our longstanding imaging programme.

Recognising that the data obtained from imaging would be significantly augmented through the inclusion of measurements of circulating anti-angiogenic biomarkers we have nearly completed a study were we have compared dynamic MR and dynamic CT measurements of patients undergoing treatment for ovarian cancer. This trial will be finished within the next few months and the data are revealing novel insights into the relationship between imaging and circulating biomarkers. In a second combination
We are currently analysing the distribution of sulfated HS in ovarian serous cancers and normal ovarian tissues. Our preliminary data show that N-sulfated HS is localized specifically to the ovarian tumour vascular network, but not to tumour tissue, while sulfated HS staining in normal ovaries is predominantly detected in the perivascular and stromal areas (see figure). We have a large panel of characterised antibodies that specifically recognise sulfation (collaboration with Dr. G ten Dam, Nijmegen Center for Molecular Life Sciences). Using these antibodies we were able to show that HS sulfated at different positions of hexosamine is mostly confined to the vascular endothelial cells in ovarian tumours. In addition, the antibody recognising 6-O sulfated HS species highlighted perivascular region of tumour vasculature. HS sulfation specific antibodies stained normal ovarian tissue predominantly at stroma and basal side of the vessels.

Our programme has hitherto focused on FGF2 and we aimed to define the cytokines involved in ovarian cancer angiogenesis in greater detail. We are currently screening a panel of ovarian cancer cell lines for the expression of angiogenic factors using angiogenesis antibody arrays and multiplex ELISAs. We have already discovered high levels of FGF2 and IL-8 in a subset of ovarian cancer cell lines. Using human umbilical vein endothelial cells (HUVECs) we have established in vitro assays to evaluate endothelial cell ability to form tubules either on Matrigel or in fibrin or collagen gels.

We are translating our basic science HS programme towards the clinic in two ways: it is clear that certain moieties in the HS chain are responsible for growth factor activation and using retroviral expression of RNAi for particular HS synthetic enzymes our aim is to identify critical enzymes that generate the moieties essential for growth factor activation. Lastly, we have developed a technique that analyses the composition of HS in very small amounts of biological samples. This technique will be used to start to analyse samples provided both from the surgical and non-surgical oncological environments.

Publications listed on page 64

Relationship between sulfated HS and endothelium. Normal ovarian tissue was fluorescently stained with antibodies that recognise the endothelium (von Willebrand factor, vW) and sulfated heparan sulfate (H). The data show that the perivascular layer contains sulfated heparan sulfate.
Early in 2008 Steve Murray left the Institute and this precipitated a review of the requirements for a TEM service. Given the costs of maintaining, housing and staffing the facility together with very limited and intermittent requirements for TEM, the decision was made to transfer the equipment to the University, with access available to Institute Groups as required.

Some much-needed refurbishments were completed at the beginning of the year, giving better facilities for the Molecular Biology Core Facility, the Flow Cytometry Facility and for the Mass Spectrometers. We also increased the space for the Histology Facility because of both an increased workload and the inclusion of the MCRC Biobank within their remit.

To maintain cutting edge services we need to constantly upgrade or replace equipment, and this year has been no exception. Details of each new development are given in the appropriate section below, but installation of a new Nipkow spinning disk confocal microscope, a Zeiss Mirax whole slide scanning system, a Nano Acquity UPLC system and LTQ-Orbitrap XL, an XY clone laser, an LSRII and a BD InFlux cytometer, and imminently a new laser capture microdissection system have all taken place in the last twelve months.

Advanced Imaging Facility
Head: Steve Bagley

Over the year we have considerably extended the imaging capabilities of the Institute with the installation and development of new equipment. The requirement for complex imaging techniques has increased considerably to embrace a range of applications. Extensive examination of cellular morphology, quantification of protein levels, quantification of turnover rates and interactions between multiple low light signals are now all covered in the portfolio of supported technologies.

A Nipkow spinning disk confocal microscope was introduced early in the year. This technology is particularly good for imaging multiple fluorescent proteins in a 3-D volume. The scanning technology it employs enables the user to record absolute localization in multiple channels in 3-D and so generate a detailed map of spatial interactions. The extended sensitivity of this system and high-end optics permit the visualization of very faint signals that would be below the threshold of a conventional wide-field microscope. This enhanced sensitivity also reduces the levels of illumination required for imaging, which in turn reduces photo-bleaching and thus greatly extends the periods over which a sample can be imaged. The system also supports the visualization of multiple XY positions so that thousands of cells can be imaged during a single investigation, considerably reducing the amount of microscope time required to gain statistical valid datasets. The system is currently being employed for studies ranging from yeast cell division to the dynamics of the cytoskeleton during the migration and adhesion of mammalian cells.

A whole slide imaging system has transformed histological imaging within the Institute. Over 8.5 TB of data have been generated by nine research groups over the 10 months up to October 2008. The system scans both tissue sections and...
tissue micro-arrays (TMA) at x20 magnification; fields of view are ‘stitched’ together by software to produce a data set that describes the whole slide. At full resolution (1:1) a monitor of five meters in width would be required to display a whole 4x1.5cm TMA slide. Once loaded, the system can process 300 slides over a weekend without further intervention. A second positive attribute of this system is that all of the data are captured in a standardized format, reducing the variations that plague manual processing of such large datasets.

We have worked with a number of users to develop and apply new hardware and software tools. Over the past year the following technologies have been developed: imaging of whole tissue culture flasks via image montage techniques; time lapse investigations have been modified to incorporate complex journals to extend the functionality of the equipment; cell and particle tracking has been improved; a 488nm FRAP system has been modified to reduce the spot size of the laser at the sample to approach the refraction limit of the system. This latter system lets users study protein turnover at specific locations within a cell, such as focal adhesions. FRET (436/535nm and 555/647nm) has been utilized extensively to quantify the co-localization of proteins such as the study of the function of chimeric immune receptors on a molecular basis and their interaction with endogenous T cell receptors. The sensitivity of the low-light microscope has been improved so that 5T4 oncofoetal antigen in embryonic stem cells can be visualized to elucidate expression, localization and function. A minor improvement to the way the facility runs that has made a huge impact upon the users has been migration of the software controlling microscope hardware and image capture to a common software interface. This has enabled users to move between instruments in response to particular imaging requirements with far greater ease than was possible in previous years. The provision of such a common interface also extends the range of novel software tools that can be applied to our range of microscopes.

In the coming year the deltavision system and mammalian time lapse system are to be upgraded to improve image capture speeds and reduce the induction of photo-stress. In conjunction with the IT department the temporary storage of data and longer term archiving are being reorganized. Finally a deep UV photo-bleaching/induction system will be introduced to enable the study of DNA damage and stress-responses.

**Biological Resources Unit**

2008 has seen a considerable increase in the number of transgenic lines produced in house at the Paterson and to date we have over 120 mouse lines that are actively producing progeny for experimental use. All animal work is carried out in accordance with the Animal Scientific Procedures Act 1986 which ensures that high quality scientific results are obtained whilst considering the cost/benefit. The Ethical Review Process (ERP) continues to provide a vital role advising the Certificate Holder regarding Project licence applications and standards of animal care and welfare; they also develop initiatives leading to the widest possible application of the 3Rs so that procedures are refined to
minimise suffering, numbers of animals used are reduced and animal use is replaced wherever possible.

Transgenic Services

This area of the facility continues to have a high throughput and in the last year we have microinjected over 30 clones using both embryonic stem cell and pronuclear injection techniques. There has also been major investment with the purchase of the XY clone laser which will assist with a number of procedures to ensure that a high quality service is provided. Some of the techniques that are being developed are as follows:

- Zonae ablation, this allows a more effective delivery of embryonic stem cells into tetraploid blastocysts
- Intra Cellular Sperm Injection (ICSI) - the laser allows sperm to be injected into unfertilised oocytes which will allow us to utilise sperm more readily in comparison to cryopreserving embryos. This will lead to not only a reduction in numbers but also a refinement

As well as producing novel genetically altered animals there have been a number of strains obtained from UK and worldwide establishments to facilitate the research:

- CD169-DTR - a conditional cre that knocks out CD169 positive macrophages on administering Diphtheria toxin, this will be a useful tool to look at immune responses in tumour cells
- CKO runx 1 - a conditional knockout to be used to study the role of RUNX1 in adult haematopoiesis
- Emu-Myc - expression of the mouse myc transgene is restricted to the B-cell lineage and these animals have the potential to develop spontaneous lymphomas
- K-ras - mice carrying the K-ras mutation have extensive tumour burden by approximately 300 days of age which is predominantly seen in the lung
- Lysosome-cre - expresses cre recombinase from the endogenous Lyzs locus and when crossed with a relevant LoxP line it leads to the deletion of monocytes, mature macrophages and granulocytes
- Mx-cre and Tie2-cre - to be used to delete genes that maybe implicated in haematopoiesis

These lines have been re-derived into the facility through the quarantine area using an established embryo transfer method. We have continued to archive our transgenic lines at F5 following a standard freezing protocol for mouse embryos and have now begun to develop in house the reactivation of frozen sperm. The banking of these tissues for protection against genetic drift and potential breakdown in health status remains a priority to ensure high quality biomedical tools.

Experimental Services

Technical support has continued throughout 2008 for a number of established and newer research groups facilitating the scientific goals of the Institute. Licenced technicians have carried out routine health and welfare monitoring including tumour measurements, tissue sampling and post mortems. Predominantly, procedural techniques have focussed around a number of xenograft models as well as partial and total body
irradiations with a variety of strains used including genetically altered, immuno-competent and immuno-compromised. There has also been the opportunity to learn and develop a number of unfamiliar techniques such as:

- Intra-femoral injection of bone marrow cells post ablation
- Intra-splenic/intra-hepatic injection for orthotopic transplantation
- IVIS imaging - a non invasive procedure to track tumour growth

Equipment Purchase

There has been considerable investment undertaken with the purchasing of the following equipment,

- Three replacement cage changing stations for carrying out routine husbandry tasks and animal manipulation
- XY Clone Laser Ablator to facilitate a number of transgenic procedures
- Controlled Rate Freezer for Cryopreservation

Cancer Research UK GeneChip Microarray Service

Head: Stuart Pepper

The microarray facility at the Paterson maintains an ongoing program of development to ensure that array services offered to CR-UK funded scientists are state of the art. Over the last couple of years we have seen a growing number of publications featuring data sets we have generated; in this report three publications are described which show the range of work that the facility undertakes.

In April our work with paraffin embedded archival material lead to a joint publication with Dr Kim Linton (Christie Hospital) examining the expression profiles of sarcoma samples (Linton et al., Br J Cancer 2008; 98: 1403). There have been very few publications so far on these challenging samples and even fewer with significant validation at both RNA and protein level. Since this paper came out we have continued to develop expression profiling of archival samples and have just submitted a follow up publication examining the use of Affymetrix Exon arrays for work with FFPE material.

For the last couple of years we have been encouraging the use of Exon arrays as they give a more complete picture of transcription than 3’ expression arrays. Exon arrays have much higher content than conventional arrays with each transcript being interrogated by multiple probe sets along the full length rather than just at the 3’ end. Several publications describing the use of Exon arrays have already come from this facility. In October we contributed to a joint paper with Alvin Lee and Charles Swanton looking at the accuracy of gene level summaries generated by Exon arrays and concluding that these gave excellent comparability to qPCR results (Lee et al., Cell Cycle 2008; 7: 3947).

Over several years of operation the facility has collected various comparison data sets; when testing new arrays or labelling protocols our usual approach is to run replicates of two cell lines, MCF7 and MCF10A, and compare the data to previous data sets. Last year Andy Sims (Breast Biology Group) was looking at ways to combine and analyse data from different projects and our in house data sets proved to be of great value for testing possible algorithms. This work was written up and published in September this year (Sims et al., BMC Med Genomics 2008; 1: 42).

Finally, this year is seeing a major change in how the facility will be managed. As part of a major restructuring within Cancer Research UK the service will be leaving the Research Services division to be managed directly by the Paterson Institute. As the year draws to a close we will be saying a fond farewell to our many colleagues in Research Services as we look ahead to the exciting challenges of 2009.

Flow Cytometry Facility

Head: Morgan Blaylock

The 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 to users.

The facility currently has three bench top cytometers including the newly purchased BD LSRII which is a 17-colour instrument. We also have three BD sorters ranging from the Vantage SE which is capable of basic 2-way sorting, the BD FACSArra which offers complex 9-colour 4-way sorts to our most recent acquisition the BD InFlux which offers 14-colour 4-way sorting capabilities with added bio-safety.

The LSRII was purchased to assist with our increasing workload and allow us to move into the field of polychromatic flow, the new system allows us to detect up to 18 colours from four different laser sources with a much higher cellular throughput. In addition this system is fully digital providing accurate logarithmic conversions and a full compensation matrix which allows automatic compensation of every fluorochrome in an antibody cocktail.

The BD InFlux was initially purchased as a replacement for the FACS Vantage SE which is over 15 years old, however the facility has seen an increase of over 70% in the level of
sorting conducted in the Institute so the new purchase will provide an additional 35 sorting hours a week to the facility. The InFlux is fully configurable allowing us to move and add lasers to the system thereby expanding the number of colours which we can use for sorting by six allowing more complex sorts to be conducted thereby helping to define smaller cellular subsets. We have recently purchased a new 592nm laser for this system which will provide a dedicated laser for cells transfected with mCherry, mPlum and mStrawberry, additionally we can now tandem sort these fruity proteins with GFP. As part of our constant drive to improve the facility we conducted an internal review on the type of sorts being conducted in the facility and there is a trend towards sorting more hazardous material. The InFlux offers increased bio-safety over our current stock in that it is enclosed in a HEPA hood system with constant vacuum applied to the sort chamber to provide containment of the sample while providing a more sterile sorting environment. In addition, we have implemented a new sample risk form to be included with all sorts.

We have also reviewed the data analysis tool available to the user and have purchased a site licence for FlowJo which is the industry standard, a single copy of which has been offered to each lab.

We have been involved in the continuation and development of a number of projects this year, one of the most interesting has been with the Leukaemia Biology group who are studying the developmental control of human haematopoietic stem cells and their progenitors. They are mainly using human bone marrow to isolate different populations of human haematopoietic cells for this study. They have been using flow cytometry to immuno-phenotype and sort cells, producing purified populations highly enriched for previously defined cellular potentials. By applying microarray and proteomic analysis to these cells they are expecting to generate a detailed gene and protein expression map in human haematopoiesis.

In the New Year we will be continuing the changes in the facility, we will take possession of a new laboratory area which will house the bench top analysers (except the FACScan) and will hopefully include a small analysis suite. The current FACS lab will be reassigned as a dedicated FACS sorting suite which will minimise the traffic through the room providing a more stable environment for the sorters.

**Histology**

**Head:** Garry Ashton

This year has seen several changes to the unit. To allow the unit to continually evolve whilst also offering a comprehensive and flexible service, we have recruited a new scientific officer. Once again we have seen heavy demand in all of our key services.

In early 2009 we plan to replace our existing Arcturus PixCell II laser capture microdissection system. This will allow the unit to continue with the high quality research currently undertaken whilst also focussing on the development of the laser capture microdissection service. Several improved systems have been evaluated, all of which allow the downstream analysis of DNA, RNA, proteins and living cells. Our evaluation involved the capture and extraction from benign and malignant melanocytes in the zebrafish melanoma model, endothelial cells from ovarian tumours based on immuno-phenotype and normal breast tissue.

In 2008 the Histology laboratory was extended to incorporate the MCRC Biobank. The lab now performs all the central processing and storage of samples collected from the
five collaborating NHS Trusts. The aim of the Biobank is to facilitate translational research by allowing researchers access to high quality samples from targeted cancer disease groups. Standard operating procedures have been compiled that ensure that sample collection and processing is performed in a uniform manner and is of the highest standard. Initial quality control checks on the samples have been very encouraging. The Biobank has recently been expanded to include the collection of haematological malignancies.

Tissue microarrays allow for high throughput and standardisation of methodology, whilst also preserving rare samples. In response to the anticipated future demand from new and existing groups and the MCRB Biobank, we are currently evaluating a new high throughput automated platform, whilst still retaining the manual platform for more specialised construction. The new automated platform will also allow more accurate donor core acquisition using an H & E reference slide as guidance. Cell pellets are also being used to make disease specific tissue microarrays. Disease areas include paediatric (Ewing's sarcoma, neuroblastoma), lung (small- and non small-cell), breast, ovarian, colorectal and prostate cancers. These specific tissue microarrays will enable rapid screening of cell lines for immunohistochemical markers of interest and help identify suitable controls for use alongside patient tissue samples in clinical trials.

Immunohistochemistry and the optimisation of novel antibodies comprises a large proportion of our work. Multiple chromogenic and fluorescent labelling studies have been performed and alternative sample handling and fixation regimes have been explored.

Immunohistochemical analyses of tissues derived from mouse knockout experiments have continued. Specifically tissue specific Cre lines restricted in their expression to haematopoietic and vascular tissues were tested. To differentiate tissue types we used myeloid specific markers (e.g. F4/80 for macrophages) and vascular markers (e.g. CD31, VE-Cadherin) in the presence of Cre-dependent reporter genes (e.g GFP, lacZ). In cases where tissue specific gene mutations produced inflammatory phenotypes we evaluated inflammatory markers (e.g. P-IKK) leukocyte infiltration markers (e.g. CD45, F4/80) and apoptotic markers (e.g. cleaved Caspase 3).

Immunohistochemistry has also been used to identify genetically modified T cells in studies looking at their ability to eradicate tumours. Using mouse models, tagged T cells were identified at the site of tumour and within the spleen and lymph nodes of treated animals. Importantly, this process is being used to identify and enumerate T cells in other tissues that could be potentially involved with tissue toxicity, such as CEA-specific T cells residing within the mucosal tissue of the gastro-intestinal tract expressing the normal tissue level of the target antigen.

Similarly the role played by IGF-I signalling in colorectal cancer initiation and progression is being investigated, by immunohistochemically determining and correlating the distribution of the IGF-I receptor (IGF-IR) and markers of cell lineage, proliferation and receptor activity. Staining will also be correlated with serum levels of IGF-I related proteins and BMI to determine the role that obesity plays in the disease.

In collaboration with the Translational Radiobiology Group, in situ hybridisation is being used to examine the role of viral oncogenes in head and neck squamous cell carcinoma (HNSCC). Epstein Barr-Virus (EBV) is an important etiological factor in nasopharyngeal carcinoma but has only recently been implicated in the development of oropharyngeal carcinomas. Similarly, high risk human papilloma virus (HPV 16) has long been associated with the development of cervical cancer but recent studies have shown that it may also play a role in a distinct subset of HNSCCs. Investigation of these viruses in a large series of patients is now being undertaken.

Kostoris Library
Head: Steve Glover

The Kostoris Library provides a service to staff and students at The Christie NHS Foundation Trust and the Paterson Institute for Cancer Research. The library works closely with the John Rylands University Library service at The University of Manchester to provide staff with desktop access to the world’s primary research in life sciences. In addition to over 5000 online journals the library provides access to databases including PubMed, Medline, BIOSIS, ISI Web of Knowledge, EMBASE, and Scopus. The library also offers a personalised database surveillance service in which regular search queries are run on a monthly basis to keep staff up-to-date with the latest published research. The library also provides an electronic table of contents service with links to full text delivered to the desktop.

This year the library has launched an Institutional Repository of Research Publications that currently holds over 2000 articles going back to 2001. The repository contains peer-reviewed papers published by the staff of the Paterson Institute and The Christie NHS Foundation Trust. Newly published records are added on a monthly basis whilst retrospective deposits are due to go back as far as 1950. The database is hosted on the Christie website and is accessible to be searched on the Internet. Most records are linked to the publishers’ websites using Digital Object Identifiers (DOIs) which will provide persistent links via a resolving service. Whilst the repository currently holds journal articles, reviews and editorials it is hoped that conference abstracts and posters may be added in the future.
The Kostoris Library achieved a stage 3 award when it underwent external accreditation in 2008. This was the third consecutive time since 2000 that the library has achieved this award which is the highest level of accreditation for NHS libraries. The Health Care Libraries Unit (HCLU) carried out the accreditation visit in May on behalf of the NHS North West (NHS NW) Strategic Health Authority.

Laboratory Services
Head: Mark Craven

During 2008 Laboratory Services became a stand-alone service and a new head of the service was appointed. Since then the provision of media services within the Institute has been reviewed. The media service has adapted to a doubling in the requirements for liquid media over the year and continues to adapt to meet this challenge. Currently over 1000 litres of media per month are supplied throughout the Institute. Alongside a general upgrading of facilities within the department, we have created a clean room for the production of microbiological plates. This allows us better control and flexibility to meet the demands from our customers within the Institute.

Laboratory Services continue to provide daily sterile glassware and plastics deliveries and the removal of used glassware for washing and autoclaving. Laboratory aides are assigned to each research group and they can undertake a range of technical and laboratory management duties.

Logistics
Head: Maurice Cowell

The Logistics department provides a comprehensive and efficient portering service for the Institute. The main duties are the receipt, checking, booking in and distribution of goods ordered by numerous personnel in the Institute, accurately and efficiently. The porters are responsible for the collection and removal of waste, be it general rubbish, clinical or GM waste. They are also responsible for the collection of liquid nitrogen containers from laboratories, transporting them to the loading bay for refilling and returning to the labs.

Ordering and distribution of the Central Stores stock via the intranet email is also the duty of the Logistics department, ensuring adequate stock levels are maintained at all times. In addition Logistics maintain the Institute freezers (Sigma, Invitrogen, Roche, Promega and Qiagen), having responsibility for the ordering, distribution and stock levels. The Institute’s 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, for example, by tracing and confirming delivery of goods with suppliers, dealing with missing, damaged or incorrect items, managing the moving of heavy equipment or furniture, and setting up various meeting rooms for numerous events.

Molecular Biology Core Facility
Head: Stuart Pepper

The Molecular Biology Core Facility comprises three separate teams; two posts provide a range of core services described here, two further teams provide comprehensive microarray and proteomics services detailed separately in this section.

One of the major tasks for the core team is provision of a genotyping service for biological samples. This service processes around 400 samples per week and aims to return data within 10 days. For many samples multiple PCR reactions are needed meaning that typically we run around 2000 PCR reactions per week. High throughput for this service is achieved by keeping all work in 96-well format, including the final analysis on agarose gels.

Demand for the DNA sequencing service has increased this year however since the upgrade of our 3100 to a 3130xl we have been able to increase the number of samples run each day to keep up with demand. Alongside the DNA sequencing we have maintained our miniprep service; scientists on site are able to have plasmid DNA prepared each morning in time for inclusion in sequencing runs later in the same day. This offers excellent turnaround for projects requiring sequence checking of clones.

The core facility also provides support for expression profiling by quantitative PCR. An AB7900 in conjunction with an Eppendorf epMotion allows qPCR to be carried out in 384 well formats, facilitating large projects to be processed efficiently. For projects looking at large numbers of genes we also have a Beckman GeXP system. This system allows a high level of multiplexing of PCR reactions allowing for a greater throughput than traditional real time PCR approaches.

MBCF Biological Mass Spectrometry Facility
Head: Duncan Smith

The remit of the biological mass spectrometry facility is to support all the protein and peptide mass spectrometry requirements of the Institute’s research groups. This support covers a whole spectrum of activities ranging from provision of routine services, implementation of new technologies onsite, through to dedicated collaborations designed to
develop novel applications enabling cutting-edge cancer research. The major development activities in the facility during 2008 have been driven from the installation of our new LCMS platform in April. The Nano Acquity UPLC system in conjunction with the LTQ-Orbitrap XL have revolutionised both our routine services and development progress since their installation. Our routine protein identification service has benefited from a sensitivity enhancement of over two orders of magnitude! In addition, our phosphorylation mapping service now utilises linear ion trap multi-stage activation. This LTQ-enabled technology was implemented and optimised in collaboration with the Cell Division group. The technology has facilitated the definition of sites of phosphorylation previously invisible with our last generation instrumentation. Moreover, multi-stage activation enables us to perform protein identification and phosphorylation mapping services simultaneously. These significant enhancements have boosted demand for these routine services with over 3200 samples processed in between May and November 08! In order to support this level of demand, further facility investment has lead to the purchase and installation of a robotic sample handling system.

The nUPLC-LTQ-Orbitrap platform also promises many potential advantages compared to our last generation instrumentation in quantitative proteomics applications. We have wasted no time in transferring existing SILAC (in collaboration with the Children’s Cancer Group) and iTRAQ (in collaboration with the Molecular Pathology Group) global protein profiling approaches onto the new platform with significant enhancements in both speed and sensitivity of analysis. These enhancements significantly improve the quality and penetrance of resulting datasets. These complex mixture applications have also benefitted from the successful development and implementation of a new multi-dimensional LC approach where we utilise reverse phase chemistries at high pH (first dimension) and low pH (second dimension). Implementation of these highly successful complex mixture applications has driven multiple informatic developments fundamental to exploiting this new quality and depth of biological information. Fruitful collaboration with the Applied Computational Biology and Bioinformatics Group has been instrumental in developing and evolving this dynamic informatic pipeline.

There are numerous active developments targeted at enhancing the research of groups in the Institute. These include the definition of sites of ubiquitination and sumoylation (in collaboration with the Cell Signalling Group), ADP Ribosylation (in collaboration with the DNA Damage Response Group) and acetylation. Moreover, we are actively developing an Orbitrap enabled quantitative phospho site mapping protocol (in collaboration with the Cell Division Group) in addition to a global phosphoproteome profiling approach (in collaboration with the Cell Regulation Group). Our research and development portfolio is extremely vibrant and will deliver a massive enhancement to routine service portfolio moving into 2009.
Research Publications

Crispin Miller  (page 8)
Applied Computational Biology and Bioinformatics Group

Refereed Research Papers


Other Publication


Geoff Margison  (page 10)
Carcinogenesis Group

Refereed Research Papers


Other Publication


Active Patents


Other Publications


Other Publication


**Other Publications**


**Peter Stern**

*Immunology Group*

**Refereed Research Papers**


Other Publications


Active Patents


Nullin Divecha (page 24)
Inositide Laboratory

Refereed Research Papers


Other Publications

Tim Somervaille  (page 26)
Leukaemia Biology Group

Refereed Research Papers


Georges Lacaud  (page 28)
Stem Cell Biology Group

Refereed Research Papers


Valerie Kouskoff  (page 30)
Stem Cell and Haematopoiesis Group

Refereed Research Papers


Catharine West  (page 34)
Academic Radiation Oncology: Translational Biology Group

Refereed Research Papers


Robert Hawkins and Peter Stern (page 36)

Biological, Immune and Gene Therapy

Refereed Research Papers


Other Publications


Vaskar Saha (page 38)  
Children’s Cancer Group  

Active Patent  

Tim Illidge (page 40)  
Targeted Therapy Group  

Refereed Research Papers  


Other Publications  


Robert Hawkins (page 42)

Medical Oncology: Cell Therapy Group

Refereed Research Papers


Other Publications


John Gallagher (page 44)

Medical Oncology: Glyco-Oncology Group

Refereed Research Papers

Gordon Jayson (page 46)
Medical Oncology: Translational Angiogenesis Group

Refereed Research Papers


Clamp, A.R., Schoffski, P, Valle, J.W, Wilson, R.H., Marreaud, S.,


Other Publications


Additional Publications


Seminar Series 2008

We had another exceptionally good seminar series again in the Institute, with speakers from around the world. This series is complemented by seminars within the Christie Hospital, a Gene Therapy seminar series with a mixture of local, national and international speakers and many seminars at The University of Manchester. Finally the postdoctoral lunchtime seminar series was once again very popular.

Peter Adams
CR-UK Beatson Institute, Glasgow

Kurt Ballmer-Hofer
Paul Scherrer Institute, Switzerland

Jiri Bartek
Danish Cancer Centre, Copenhagen, Denmark

Clare Bennett
Royal Free Hospital, London

Constanze Bonifer
Institute of Molecular Medicine, Leeds

Hugh Brady
Great Ormond Street Hospital, London

Cathrin Brisken
Swiss Institute for Cancer Research, Epalinges sur Lausanne, Switzerland

Keith Burridge
University of North Carolina, USA

Susan Chan
University of Strasbourg, France

Mark Cragg
University of Southampton

Michaela Frye
Wellcome Trust Centre for Stem Cell Research, Cambridge

Simone Fulda
University Children's Hospital, Ulm, Germany

Michelle Garrett
Institute for Cancer Research, Haddow Laboratories, Sutton

Susan Gasser
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

David Gillespie
CR-UK Beatson Institute, Glasgow

Angela Glading
University of Pittsburgh, Pittsburgh, USA

Nadia Harbeck
University of Munich, Germany
Doug Higgs  
Weatherall Institute of Molecular Medicine, Oxford

Wolfgang Huber  
EBI, Wellcome Trust Genome Campus, Cambridge

Phil Jones  
Addenbrooke’s Hospital, Oxford

Jos Jonkers  
National Cancer Institute, Amsterdam, The Netherlands

Michael Lisanti  
Kimmel Cancer Centre, Philadelphia, USA

Richard Marais  
Institute for Cancer Research, Chester Beatty Laboratories, London

Masanori Mishima  
Wellcome Trust/Gurdon Institute, Cambridge

Eduardo Moreno  
Spanish National Cancer Centre, Madrid

Duncan Odom  
CR-UK Cambridge Research Institute, Cambridge

Avtar Roopra  
University of Wisconsin, Madison, USA

Owen Sansom  
CR-UK Beatson Institute, Glasgow

Matthew Smalley  
Breakthrough Breast Cancer Research Centre, London

Arnoud Sonnenberg  
National Cancer Institute, Amsterdam, The Netherlands

Christina Steulten  
Johns Hopkins Medical School, Baltimore, USA

Jesper Svejstrup  
London Research Institute, Clare Hall Laboratories

Kay-Uwe Wagner  
University of Nebraska Medical Centre, Omaha, USA

Alan Warren  
MRC Laboratory of Molecular Biology, Cambridge

Gareth Williams  
Royal Free and University College Medical School, London

Mark Winey  
University of Colorado, Boulder, USA

Brad Wouters  
Ontario Cancer Institute, Toronto, Canada
World-class research is backed up with access to world-class facilities, and we aim to match these with a challenging but rewarding graduate training programme that helps students reach their full potential. Recruitment is highly competitive, and generally involves two days of interviews with group leaders and members of the Education Committee. The goal is to find exceptional students with research interests that closely match those of their prospective supervisor. This year we brought recruitment forward to the last week of November when we spent four days interviewing students for the 2009 intake. Overall the interview process was very successful and we expect to recruit again at a similar time next year.

The Paterson Institute’s major role within the Manchester Cancer Research Centre (MCRC) provides an opportunity to design projects that sit at the interface between fundamental and translational research, through collaborations with other groups at the Christie Hospital and The University of Manchester. The Clinical Fellowship scheme introduced in 2007, run in collaboration with AstraZeneca, continues to be successful and has developed further over the past year. 2008 has seen a number of changes in postgraduate education, including the appointment of a new Postgraduate Tutor for Cancer, Dr Crispin Miller and the establishment of an education sub-committee (Crispin Miller, Iain Hagan, Jenny Varley, Julie Edwards) to help streamline the administration process.

The Paterson Graduate Programme
Paterson PhDs are four years long, structured with a set of key milestones. These include talks, literature reports and project planning meetings, and provide formal points at which progress (of both the student and the project) can be assessed, and research goals developed and discussed. Graduate training is monitored by the Education Committee, which features Group Leaders, senior clinicians and scientists, and student representatives. Each project is peer reviewed in advance and considered in terms of its potential scientific contribution and training opportunity. Every student is assigned an Advisor (similar to a personal tutor on an undergraduate programme), whose role is to provide impartial advice and support while further support can also be provided by the Postgraduate Tutor and a Student Welfare Group.

The Paterson runs an external seminar series, featuring talks from many of the key players in cancer research, and students are also expected to attend postdoctoral seminars and to present their work at lab meetings within the Institute. This year also featured the introduction of a student seminar series, conceived and organised by our graduate students as an additional forum in which to discuss their work.

Ultimately though, it is the direct research experience that is most important, and this is perhaps best measured by the number and quality of publications featuring work by our Graduate Students.
Education Committee 2008
Iain Hagan – Chair
Fiona Blackhall
Richard Cowan
Caroline Dive
Julie Edwards
David Gilham
Tim Illidge
Gordon Jayson
Valerie Kouskoff
Karim Labib
Crispin Miller
Vaskar Saha
Tim Somervaille
Jenny Varley
Catharine West

Student representative 2008
Dorota Feret (until September)
Natalie Reeves (until September)
Monique Melis (from September)
Andrzej Rutkowski (from September)
Our relationship with the union (Unite) has strengthened throughout 2008 as new policies were negotiated and implemented. The formal Joint Negotiating Committee (JNC) met quarterly and regular meetings have been held with the union to ensure that we work in true partnership.

Management training is important in any organisation and courses were run for Scientific Officers, Postdoctoral Fellows and a ‘Management Masterclass’ was held for senior managers and Group Leaders. One result of the Scientific Officers’ training was that a Scientific Officer Working Group has now been formed to provide a platform for Scientific Officers to share information, working experiences and expertise, with each other and with other staff members. One of their objectives is to present Scientific Officers as a coherent, professional body that acts as an interface between Scientific Officers and the rest of the Institute. This has already proved invaluable, when the Director of Operations was able to attend one of their meetings to discuss cost savings for 2009/10. As CR-UK’s funding to the Institute will be cut next year, a cost saving strategy will need to be formulated.

The Paterson Newsletter has gone from strength to strength and favourable feedback continues to be received from its readership.

The Paterson’s website is currently being overhauled and the new fresher, modern-looking website will go live in the New Year. Work is also being undertaken on the internal intranet and this will be relaunched in the New Year too. An off-the-shelf intranet system is being tested at the moment which will provide much needed microscope booking services, a multi-room booking facility and an annual leave system which is designed to allow staff to manage their own leave and book it online.

The Estates department oversaw the installation of our second Translational Research Facility, which was handed over on time and on budget.

Social activities grew within the Paterson with the formation of the Wine Tasting, Games Console and Film clubs. The Fantasy Football competition has been a great success with 29 teams battling it out for the Paterson Trophy. The Five-a-side Football and Badminton clubs continue to flourish. The Christmas party was held off-site for the first time this year, and was enjoyed by all the staff and students who attended. As social activities help with the cohesion of the Institute, a summer BBQ is planned (weather permitting) for next year.

Staff and students from the Paterson continued to assist CR-UK with their fundraising efforts by volunteering to help at local Races for Life and the Tatton Park 10K race.
Administration and Reception Services
The administration department has seen a number of changes during the year with the streamlining of processes and procedures to ensure a time efficient and cost effective structure. The catering function has increased and new contracts have been negotiated, which have resulted in cost savings. The Operations Manual has been significantly updated to ensure that all practices are now current and fully documented. The administration department continues to oversee the Reception services and now provides lunchtime cover for the Receptionist.

Some of the highlights over the last year include the opportunity to provide the Assistant Director (Research) with full support in the organisation of this year’s annual Colloquium in September, which was very successful. In addition, the department was able to provide administrative support to the Clinical and Experimental Pharmacology group on their recent quinquennial review, the outcome of which was very positive.

Director’s Office
The Paterson’s Seminar Series had an impressive list of external speakers during 2008. The series has proved to be informative, varied and exceptionally popular within the Institute.

One of the main administrative duties of this office has been to support the Director in the search and appointment of senior staff.

Estates
June 2008 saw the completion of the Institute’s second Translational Research Facility. This £1.8m project has provided much-needed additional accommodation of 800m² for research staff.

A number of minor projects have also been completed which have improved the environment and services for the Flow Cytometry Department, Molecular Biology Core Facility and Purchasing.

The Estates team has been pro-active throughout 2008 and has identified plant and building fabric that requires attention or replacement over the coming years. They have also helped to reduce the amount of backlog maintenance within the Institute.

There are some interesting projects that will be undertaken next year, including the replacement of the existing emergency generators and an increase in capacity.

The team endeavors to provide a quality service to reduce the amount of disruption to research and the scientists. The Estates team are working alongside The University of Manchester’s Estates team to achieve compliance with legislation, such as the control of Legionella in buildings.

Finance and Purchasing
The department continues to strive to provide a comprehensive purchasing, travel and finance service on a daily basis to the Research Groups and Service Units within the Institute. New groups have been incorporated into the system and all necessary training has been arranged for new staff.

The relationship with the Faculty of Medical and Human Sciences continues to develop. This year has seen the devolvement of Research Administration to school level. This has resulted in additional training courses for the various University research systems and has also enabled the department to network with the Research Business Managers of the different research schools within the Faculty. The University will be implementing a new grant-costing tool in the New Year which should prove very useful as the level of Contract Research Agreements and Grant Applications continue to increase.

Health and Safety
A review of equipment maintenance covered by statutory requirements was undertaken. This included local exhaust ventilation (e.g. microbiological safety cabinets and fume cupboards), pressurised vessels, all portable electrical equipment (approximately 8,000 items), fire extinguishers and alarm systems. This resulted in some changes to the service providers, with subsequent cost savings and improved levels of service. Also, the installation of a new access control system is underway, which will further enhance security within the Institute, and build on the improvements made last year.

Evaluation of online health and safety training was undertaken, using staff in the Administration and Logistics departments. Whilst this exercise proved useful and met with some approval amongst staff, it is evident that there is still a place for a more formalised presentation style of training. Accordingly, more bespoke health and safety training will be devised, such as the induction session, biological agents, hazardous chemicals and risk assessment and activities such as office safety may be rolled out as online training.

The annual health and safety inspection programme was undertaken and reports produced for each department. The inspection programme identified relatively minor non-compliance issues, which were department specific. These have been, or will be, addressed in due course. Health and Safety advice and guidance was given to staff on a daily basis,
covering a wide range of issues, but predominately related to laboratory activities.

Visits from the enforcement authorities - the Environment Agency and the Biological Agents unit of the Health and Safety Executive (HSE) - occurred early in the year. Both visits were successful in that little or no remedial action was required.

**HR**

Over the past year the HR Department has continued to deliver high quality, cost effective and professional services that make a real difference to the Institute. This year the department has recruited to 35 vacancies with highly skilled individuals that will enhance the expertise within the workforce enabling the Institute to continue to develop world-class science and research.

There has been a focus on improving the induction process to enable new recruits to settle into the Institute smoothly and effectively. We have concentrated on providing all the vital information that new employees will need and are able to assimilate, without being overwhelmed or diverting them from the essential process of integration into the Institute.

This year the HR department introduced the new on-line Contribution Review appraisal system. The main objective of the Contribution Review Process is to review performance, potential and to identify any training and career planning needs for the workforce to enable staff to continue to develop to their full potential.

Joint partnership working with the unions has continued throughout the year which has resulted in the agreement of several revised policies including Maternity Leave, Paternity Leave and the Adoption Leave.

The significant challenges of the future will increasingly require the department to lead change and improvement programmes, adopting a transformational approach in all we do. Therefore, the HR Department is currently in the process of undergoing a transformation that will enable it to provide a proactive HR service delivered through systems and processes that are aligned to the Institute’s goals and objectives.

**IT**

2008 saw the completion of an extensive project to upgrade the storage capacity of the Institute’s IT systems. However, during the year a colossal surge in demand for data storage occurred as a result of improvements in analytical systems producing even more complex images and increased amounts of data. To cope with the extra demand further storage upgrades are required and steps have been put in place to resolve this including the development of a clear storage and archive strategy that will enable the Institute to manage any future storage expansion needs.

As a result of the increased reliance on high quality data and the expansion in complex data storage solutions, the department has refreshed and tested the disaster recovery strategy to ensure resilience. In addition, the development of the Translation Research Facility stage 2 (TRF2) has resulted in the extension of the network infrastructure.
The ambitious five year Science Strategy unveiled by Cancer Research UK at the end of 2008 includes a small but significant strand of activity called Local Engagement and Development (LEAD). It is a feature of a much broader priority to establish up to 20 ‘Centres of excellence’ across the UK, linking research activities with patient care, public engagement and prevention initiatives.

The aim of LEAD is to highlight Cancer Research UK’s impact on cancer by engaging supporters with its research at a local level. It has never been more important for the Charity to demonstrate its impact to donors. As the economic downturn deepens and people start to review their personal spending, LEAD will help the Charity make a powerful case for support.

Last year, the Paterson Institute took part in Project Local, the original pilot for LEAD and James Dunphy, who heads up the initiative in Manchester, has since demonstrated the benefits of having a LEAD manager based on site.

The Institute’s researchers have been extremely supportive of LEAD, attending a wide variety of fundraising events and hosting a regular programme of lab tours. Over the last year around 100 representatives have come into contact with over 13,000 supporters, of which as many as 500 have had the opportunity to talk directly to a researcher. Ahmet Acar (Stromal-Tumour Interaction Group) who has met supporters on a number of occasions said: “It was great attending the events, seeing so many people support the Charity made me feel even more motivated about my research”.

The number of lab tours has been increased and they are particularly popular with supporters. Tess Doughty, a Race for Life participant enthused: “It was a real honour to have been invited to the Paterson Institute and to be able to see directly how the money we’ve raised is being used to help fund vital research into beating cancer.”

In the years ahead, LEAD will help achieve a number of Cancer Research UK’s 2020 goals. Through building local partnerships it will be possible to enhance the impact of the Charity’s cancer prevention agenda by helping people understand how they can reduce their risk of getting the disease. In Manchester this work has already begun. Local relationships have been developed with key bodies such as the Greater Manchester and Cheshire Cancer Network, providing relevant information on prevention to the people that need it the most.

As Cancer Research UK’s Centres Initiative unfolds in cities across the country, each location will appoint a Local Engagement and Development manager. The managers will learn from the successes in Manchester and develop locally relevant partnerships to ensure even more people make the connection between Cancer Research UK funded research and the impact it has on people’s lives.
Acknowledgement of Funding for the Paterson Institute

The total funding of the Paterson Institute for 2008 was £13.3M. The major source of this funding (83%) was through a core grant from Cancer Research UK (CR-UK). The actual value of this award in 2008 was £11.01M. This is divided between the various scientific groups and service units within the Institute to enable them to carry out their research.

After the merger with the University in January 2006 it was agreed that the infrastructure of the Institute would continue to be funded by the Christie Hospital Endowment Fund until 31 December 2008 and this accounts for 7% (£875K) of the total income.

From January 2009 the infrastructure will be funded by HEFCE generated income (4%).

The final 6% 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
- Abbott Laboratories
- European Commission
- ECMC
- BBSRC

The Institute has also been successful in obtaining two grants from the Leukaemia Research Fund which will be activated in 2009.

We are immensely grateful to all our sponsors.

Donations to the Institute in 2008
- Mrs E Lloyd in memory of Mr Cyril Taylor
- Mrs V Jones in memory of Mr Cyril Taylor
- Mrs E Lloyd in memory of Joanne Wood
- Mrs B Armstrong in memory of Mrs Doreen Kempster
- Mrs M Emerson in memory of Mr Gordon Emerson
- Mrs D Caine
- Mrs E Hamilton
- Mr A Wilkinson and Class 9AWi St Bede’s RC High Blackburn
- Mrs P Johnson and family
- Oldham SNU Church
- K Bennett
- Clifford Schofield
- Mrs G M Hunter
- Mr and Mrs E Molyneux
- Mr Michael Gannon
- Mr and Mrs J E Slowley
- Mrs J Hitchen
- Mrs J Harewood
- Mrs K Lord
- Dr S J Lintott
- Mrs N Black
- Mr P Thornton
- Mrs P Johnson
- Mrs M Collier

We are particularly indebted for the continuing support of the PACCAR Foundation, and for a very generous donation from Sir John Zochonis.
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 was created nearly three years ago with partners including the Paterson Institute, The Christie NHS Foundation Trust, The University of Manchester and Cancer Research UK.

This is an extremely exciting development which is enhancing all aspects of cancer research, education and treatment. The Institute offers excellent laboratory facilities and outstanding core facilities, including molecular services, a microarray platform, proteomics, flow cytometry, histology, the production of knock-in/knock-out animal models, real-time PCR and advanced imaging. Details of all groups and facilities are given throughout this report, and can guide interested parties to the appropriate contacts.

Opportunities exist at a number of levels in the Institute. We have a well-established programme of degrees by research which is described in the section on Postgraduate Education (page 68). 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 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/), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.
Finding us

The Paterson is well placed for both national and international travel, with Manchester Airport only around a 30 minute drive away. The region is very well-served by the motorway network and the West Coast mainline rail service.