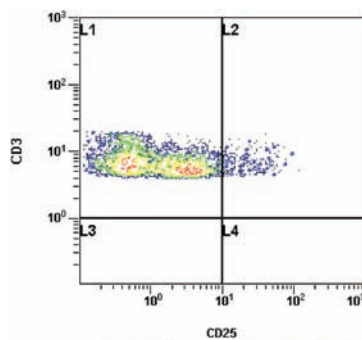


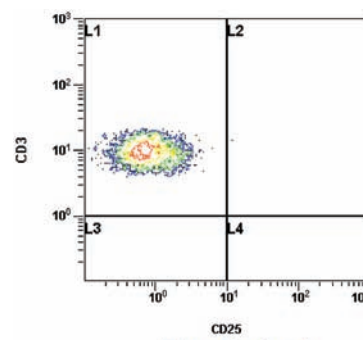
# Paterson Institute for Cancer Research

## SCIENTIFIC REPORT 2006





**Leukapheresis  
Product**



**Depleted  
Product**

Leukapheresis blood product is depleted of CD25<sup>+</sup> lymphocytes prior to infusion back into the patient. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cells are thought to be instrumental in allowing a growing cancer to evade immunological attack and the rationale behind this clinical trial is that their removal may unmask an anti-tumour immune response in patients with cancer (See Biological Immune and Gene Therapy Group report on page 36).

### **Illustration Credits**

Many illustrations in this report were taken by Jenny Varley and Paul Cliff

Cancer Research UK

# Paterson Institute for Cancer Research

SCIENTIFIC REPORT 2006



Paterson  
Institute for Cancer Research



The University of Manchester



Cancer Research UK Paterson Institute for Cancer Research Scientific Report 2006

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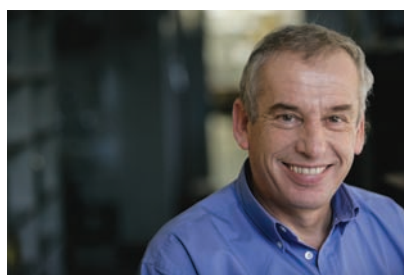
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# Director's Introduction

2006 has been a very important year in the history of the Paterson Institute. It became an Institute of The University of Manchester and at the heart of the newly developed Manchester Cancer Research Centre.



Nic Jones, Director of the Paterson Institute

Hospital. The Trust provided significant financial support to the Institute as well as laboratory facilities to house our research efforts. However, on the 1st January of this year the Institute transferred to become an Institute of The University of Manchester retaining its current level of operational autonomy. The major reason for such a move was to initiate the development of The Manchester Cancer Research Centre (MCRC) which integrates all the cancer research efforts of the University and to provide a comprehensive approach to cancer research in very close partnership with the Christie NHS Trust and Cancer Research UK. The goals and aspirations of the Centre are described in the next section and are focused on ensuring that advances in the understanding of the molecular and cellular basis of cancer is translated into new approaches to therapy and meaningful diagnostics. Through the development of this Centre the Paterson can not only continue to support basic research but also facilitate translational and clinical research and thereby achieve its full potential as a Cancer Research UK core funded Institute. In addition, not only are our interactions with the rest of the University research activities enhanced, but our interaction within the Christie NHS Trust is also strengthened through the MCRC-Christie partnership and the increased investment in research that this partnership will bring.

A major ambition of the MCRC is to significantly increase the volume of cancer research on the Paterson/Christie site which will necessitate major investment in new laboratory facilities. A new research building is being planned but in the meantime it is important that existing facilities are renovated and developed to maximise their use for supporting our research efforts. This year saw the completion of a significant phase in such development with the opening of the TRF1 (Translational Research Facilities 1) laboratories. These laboratories provide first class facilities for many of our translational research activities – some that exist already and some for new activities such as developments in molecular pathology. One floor of the new facility is devoted to activities in pharmacology supporting the major phase 1 trial activities in the Christie NHS Trust. This includes the Clinical and Experimental Pharmacology Group headed by Caroline Dive and Malcolm Ranson (see pages 22-23) and the Translational Angiogenesis Group headed by Gordon Jayson (pages 44-45). The refurbishment of this space has allowed us to optimise the use of these facilities, such as the provision of a Good Clinical Laboratory Practice (GCLP) laboratory that facilitates our ability to assess clinical samples from early phase trial patients under the very strict regulations that are now required. The provision of the GCLP laboratory was aided by a very generous donation from the PACCAR Foundation. We are very grateful for this support and the support we receive from numerous other private donations (see pages 78-79).



The newly refurbished basement labs within TRF1

In addition to the academic interactions discussed above, it is vital that we have strong interaction with industry especially in the translational and clinical research areas. This year has seen the development of fruitful interactions with AstraZeneca one of the biggest pharmaceutical companies in the world. An

alliance between the MCRC and AstraZeneca has resulted in joint initiatives in biomarker research and in training which is of considerable benefit to the translational research of the Institute. A novel training programme for clinical pharmacologists has been initiated which we hope will help to alleviate the shortage of qualified and experienced researchers in this important area.

This year has also seen the completion and implementation of a new Pay and Grading system for all Institute staff. This has been developed with Cancer Research UK and will operate across all their core funded Institutes. Although it involved considerable effort, overhauling our previous system was much needed and has resulted in a fair and transparent system that allows us to reward achievements and success and hopefully will prove motivating to all Institute staff.



Our career structure for Group Leaders has two main categories: Junior Group Leaders who are at an early stage of their independent career development and Senior Group Leaders who have well established, internationally competitive research programmes. It is vitally important that the balance between Junior and Senior Group Leaders reflects the opportunities the Institute has in developing scientific careers but equally important that we ensure that only Junior Group Leaders with outstanding records and a high reputation in their respective fields are promoted to a senior position. This was certainly the conclusion that an international appointments panel reached when they considered the promotion of Karim Labib who was made a Senior Group Leader in August. Karim has made outstanding contributions to our understanding of the process of DNA replication, a process that is fundamentally important for the stable inheritance of the genome and is known to be defective in cancer. It is a very conserved process and Karim's studies using budding yeast as a convenient model system have provided direct insights into this process in mammalian cells. Not surprisingly given the importance of replication and its correct timing in the cell cycle, it is a process that is very highly regulated. Karim's research has led to the identification of a new and essential complex called GINS which interacts with additional proteins to form a 'Replication Progression Complex' (see pages 14-15). This work is at the forefront of his field and his promotion and consequent expansion of his research group is richly deserved.

The coming year will provide many new and exciting challenges and opportunities. Up to three additional Group Leaders will be recruited to enhance our research activities and strengthen our research focus in tumour cell biology, tumour microenvironment and stem cell biology. We will initiate new programmes in the development of advanced mouse models of cancer for pre-clinical research and therapeutic assessment and in the development of drug discovery. This latter activity is a major new initiative undertaken with Cancer Research UK aimed at the development of small molecule drugs against new potential targets some of which will emerge from our basic science programmes. This initiative will strongly complement the research that we currently do and plan for the future. The first step in this initiative will be to recruit a Senior Drug Discovery Scientist to lead the development. We will invest further in our research services by enhancing our capabilities in mass spectrometry based proteomics and advanced imaging. Finally we will continue to play a central role in the development of the MCRC to ensure that the promise and potential of the Centre will be realised.



# Manchester Cancer Research Centre

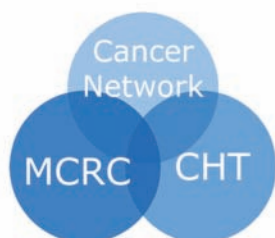
The Manchester Cancer Research Centre (MCRC) has been established to integrate the cancer research efforts within the University of Manchester in partnership with the Christie Hospital NHS Trust and Cancer Research UK. Its goal is to become a world-leading centre for basic, translational and clinical cancer research by 2015 with the major aims of:

- Supporting fundamental scientific research to better understand the processes that drive the initiation, progression and maintenance of cancer in different tissues and through which new targets for therapeutic intervention are identified and validated.
- Support translational and clinical research to develop and test new promising therapies as well as advances in diagnosis and prognosis.
- Support extensive clinical and non-clinical cancer research training and development.

In order to achieve these aims the Centre has developed a strategic plan for future development and for the focus of its cancer research efforts, is coordinating the recruitment of world-class basic and clinical researchers, is developing laboratory-based and clinically-based research infrastructure and developing a matrix of basic and clinical research programmes which will provide a framework for matching strengths in basic research with interests and strengths in translational and clinical research. The Joint Research Strategy Group of the MCRC and the Christie Trust is where the main objectives are developed and steps for delivery identified.

The opportunities and strengths of the MCRC come from the expertise and ambitions of the partners involved. The University of Manchester is the biggest single-site University in the country with the stated ambition to be among the top 25 Universities in the world by 2015. It has significant existing activities in cancer research within the Faculties of Medical and Human Sciences and Life Sciences augmented by the recent incorporation of the Paterson Institute. The Paterson Institute is a leading cancer research institute core-funded by Cancer Research UK, supporting programmes in basic and translational research. The Christie Hospital NHS Trust is a specialist tertiary centre for cancer treatment and one of the major centres in the UK for clinical trials and radiotherapy research involving cancer patients. It is the largest single-site cancer treatment centre in Europe treating approximately 12,000 new patients per year and supporting a network population of 3.2 million across Greater Manchester and Cheshire. Cancer Research UK is the world's leading cancer charity supporting the research activities of over 3000 scientists, doctors and nurses working across the UK.

## MCRC/CHT Joint Research Strategy Group



- Strategic Research Planning
- Prioritise recruitment & investment
- Develop new activities

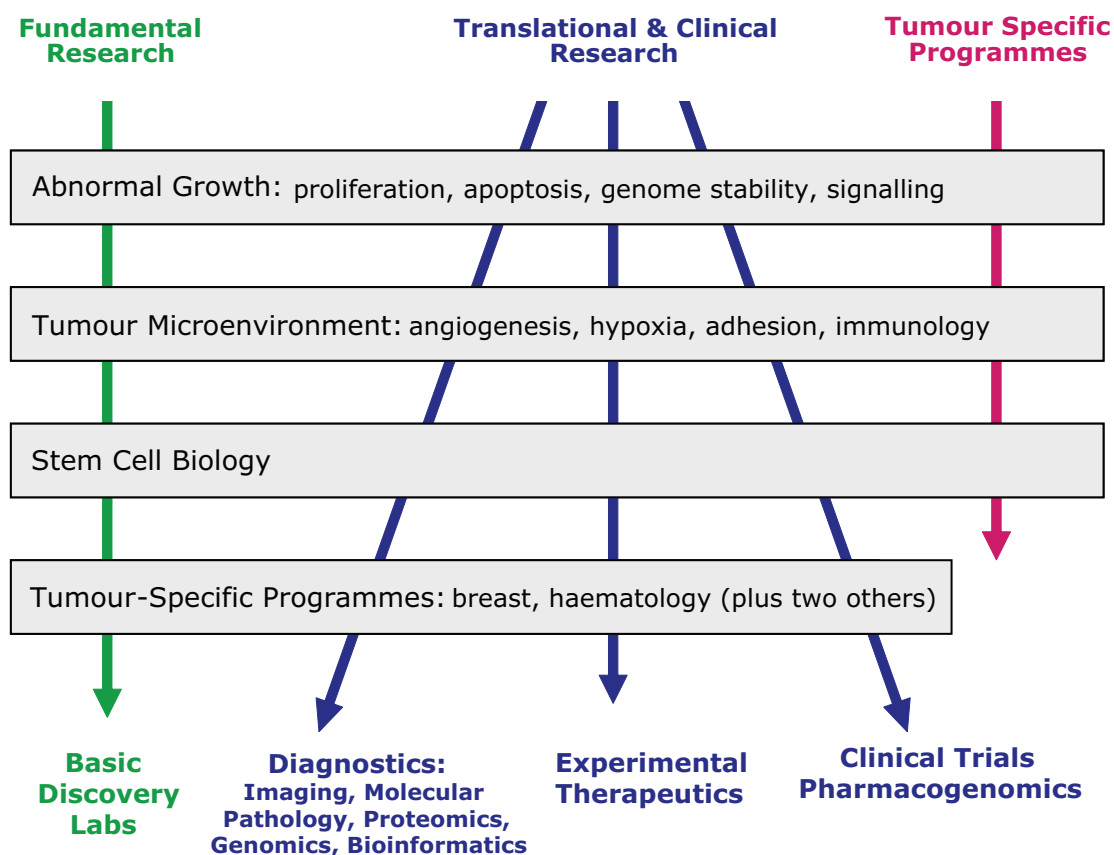
Manchester Cancer Research Centre - Creating a world leader in the fight against cancer

Over the next 5-10 years significant investment in people, technology and infrastructure across the whole basic to clinical research spectrum will take place. Further investment in basic research will be made to understand in more depth the events critical for the initiation, progression and maintenance of tumours, the interactions between the tumour cells and their immediate cellular environment and the mechanistic basis of drug resistance. This will be enhanced by continued provision of state-of-the-art technologies and new laboratory facilities. At the heart of translational research in the MCRC is experimental therapeutics and biomarker discovery. Early phase clinical trial activity is already a significant strength at the Christie



# MANCHESTER CANCER RESEARCH CENTRE

Hospital site with dedicated patient facilities and expertise associated with clinical and experimental pharmacology. The MCRC will significantly increase first-into-man trials of mechanism based therapies particularly focussing on targets that match its biological strengths and interests. In addition it will expand cell-based therapies that aim to harness the immune system to recognise and kill tumour cells. This drug development and biomarker discovery focus is being augmented by further development of molecular imaging and clinical proteomics as well as the development of a new programme in drug discovery. To fully capitalise on the large patient population associated with the Christie and the associated cancer network, the Centre will increase and strengthen its activities in clinical and population-based research by facilitating increased recruitment into clinical trials, developing pharmacogenomic research to identify and validate biomarkers with diagnostic and prognostic potential using major patient cohort studies and sample collections and by developing tumour-specific research programmes.



The MCRC is still in its infancy and many challenges lie ahead. However, the opportunities to develop a world-class comprehensive centre are great. In its first year significant progress has already been made. Most notable is its success in attracting over £3M to expand and develop the phase I clinical trial unit which will double its current capacity and in so doing, will become the biggest phase 1 unit in the world. It is anticipated that this expansion will take place over the next year. Recruitment of basic and clinical scientists and development of the plans for a new laboratory build will be the focus of the coming year.



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**GRADUATE STUDENTS**  
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Laura Edwards (with Georges Lacaud)  
Danny Bitton

## Bioinformatics Group

<http://www.paterson.man.ac.uk/groups/bioinf.jsp>

The majority of the work in the Bioinformatics Group involves interpreting high-throughput gene expression data, most of it arising from the Institute's Affymetrix system. The group is strongly interdisciplinary by nature (the team's backgrounds bring together a mix of computer science, mathematics, and biology), and the aim is to maintain a friendly and collaborative environment that brings the informatics and biology together as much as possible.

### Microarray gene expression data

Over the last year, a number of biological collaborations have begun to come to fruition; Claire Wilson (a previous postdoc in the group) spent a significant amount of time working on a microarray project investigating the effects of oestrogen on gene expression in epithelium and stroma of normal human breast tissue in collaboration with Andy Sims and the Breast Biology group, headed by Rob Clarke. Claire has also worked with Tony Whetton's Stem Cell and Leukemia Proteomics Laboratory (SCALPL) at the University of Manchester, on work that revealed post-translational control as a regulatory factor in primary hematopoietic stem cells. Her contribution was towards the underlying bioinformatics necessary to bring the quantitative proteomics data alongside Affymetrix gene expression data. Other projects, including one looking at Formalin Fixed Paraffin Embedded Tissue (FFPET) in collaboration with Stuart Pepper and the Molecular Biology Core Facility (MBCF; page 54), Kim Linton, Tony Freemont at the University of Manchester and John Radford at the Christie Hospital are also generating promising results, and we have continued to work extensively with other

groups in the Institute, most notably through Graeme Smethurst and Laura Edwards, who have joint PhD projects with Peter Stern (page 24) and Georges Lacaud (page 28).

### Detailed annotation of microarray data

The more computational side of our research is also yielding exciting data. We have continued our work with BioConductor and R, and have contributed the package *PLIER* to the BioConductor project, which provides access to Affymetrix' *PLIER* algorithm for expression summarization. Affymetrix microarrays use 25-mer oligonucleotide probes to target individual transcripts of interest. Typically, 11 probes are used to target each transcript, and these are grouped in software to form a probeset, and their combined data summarized to generate an estimated concentration for each transcript. Michal Okoniewski has been interested in the specificity of the probeset to transcript relationship, and, along with Tim Yates, built a database of probe-probeset-transcript mappings by searching, *in silico*, the target probe sequences against a database of well annotated mRNA sequences. Michal was able to use this database to identify 'multiply-targeted' probes that were able to bind to multiple transcripts, possibly from different genes, and then to show that this was a significant effect that could lead to apparent, but spurious, relationships between genes. This work is also interesting because these events are most likely to happen when sequences are similar. Since sequence similarity is often used to infer functional relationships, we might expect the effect to be most frequently observed between functionally related genes. This is important to consider, when, for example, using correlation based techniques to predict functional associations by clustering gene expression data.

## Analysis of Exon arrays

Recently, Affymetrix released a new generation of microarrays that aim to interrogate every known and predicted exon in the human genome. The ability to investigate transcription at such fine levels of detail is clearly exciting, but makes some significant demands on data analysis, not least, because their interpretation requires access to a comprehensive description of the fine-grained structure of every gene of interest. Michal and Tim have been applying their expertise in databases and their understanding of probe-set-transcript-gene relationships to these arrays for some time now, and have developed a set of software tools to support their analysis. Tim's database, X:MAP, can be found online at <http://xmap.picr.man.ac.uk>. We are now starting to use their software to explore microarrays at the exon level, and have a number of collaborative exon array projects underway.

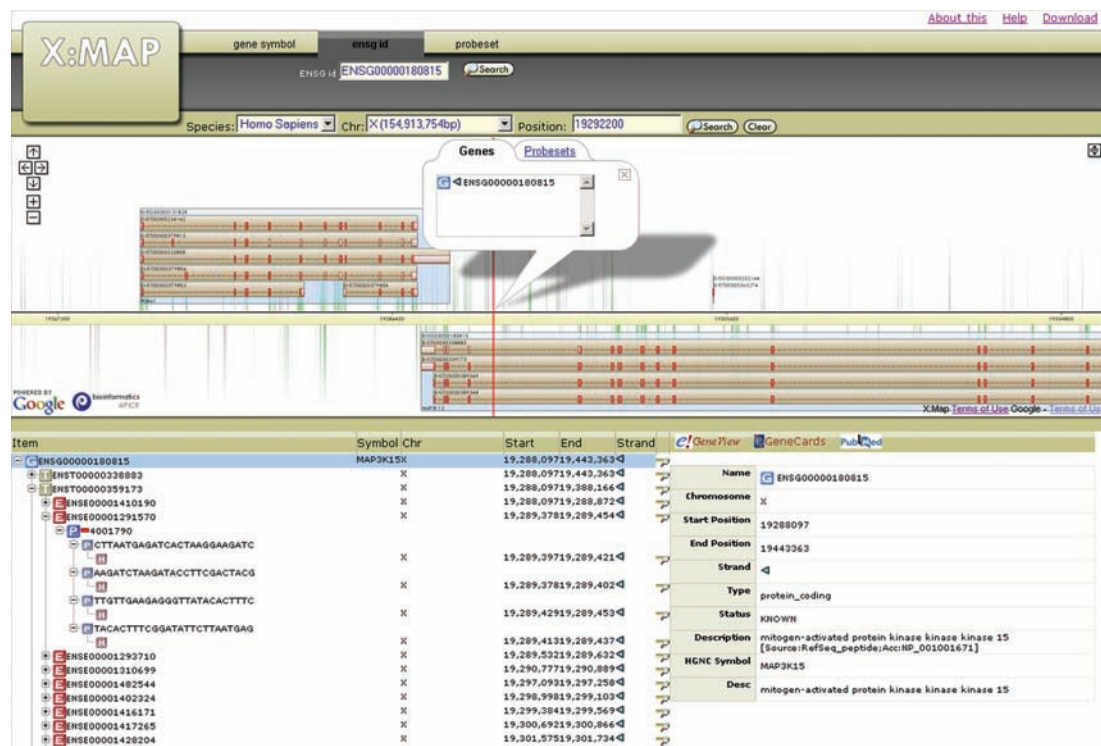
Underpinning this work was a validation study done in collaboration with the MCBF and Cancer Research UK Affymetrix service (page 51), to consider how well these new arrays perform in comparison to the previous generation of expression arrays from Affymetrix. We found that with the right data analysis high-correspondence could be found, and

this has given us enough confidence to conduct some new projects to these arrays.

## Development of novel statistics for gene expression analysis

Meanwhile, Carla Möller Levet has been working with Catharine West from the University of Manchester's Academic Department of Radiation Oncology (ADRO), looking at clinical microarray data. Carla has also been developing novel analysis techniques, and this has led to the development of a promising new method for finding correlations within gene expression, important because correlation based techniques are a fundamental set of tools for gene expression analysis.

Finally, in the last few months, Danny Bitton has joined us as a PhD student investigating novel transcriptional and translational events in microarray and proteomics data, and Páll Jónsson has just arrived as a postdoc to bring knowledge of protein interaction networks. Over the next year we aim to begin to bring all of these different strands together, and to continue to apply them to our datasets.



X:MAP, an annotation database representing the relationship between Affymetrix Exon I.OST array probesets and gene structure.



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## Carcinogenesis Group

<http://www.paterson.man.ac.uk/groups/carcino.jsp>

Many types of cancer treatment exploit the cell killing effects of therapeutic agents that can generate a variety of types of damage in DNA. But the cell killing effects can be prevented by a variety of cellular DNA repair pathways that operate to remove this 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 the ensuing DNA repair processes that follow exposure to certain types of alkylating agents, one example of which is the CR-UK drug, Temozolomide. Other projects include studies of the relationship between single nucleotide polymorphisms in the *O*<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) gene and cancer risk, and the characterisation, in fission yeast, of a close relative of MGMT, which appears to be part of a novel DNA alkylation damage repair pathway.

### Background

Chemotherapeutic alkylating agents generate varying amounts of a dozen different types of lesions in DNA and there is increased understanding of the mechanism/s by which some of these lesions result in cell killing. Thus, methylating agents such as Dacarbazine and Temozolomide produce *O*<sup>6</sup>-methylguanine in DNA and this kills cells *via* the action of the post replication mismatch repair (MMR) system. These agents also generate 3-methyladenine, which kills cells by blocking DNA replication.

Repair pathways that probably evolved to deal with low levels of endogenously produced damage, and which may be important in preventing the carcinogenic effects of such damage, reduce both the ther-

apeutic efficiency and also the toxic side effects of alkylating agents. Thus there is increasing interest in attenuating the expression of such pathways in tumours, and enhancing them in normal tissues, in order to increase the differential killing effects and thus to improve clinical outcome. These repair processes, especially that repairing damage at the *O*<sup>6</sup>-position of guanine, i.e. MGMT, and more recently alkylpurine-DNA glycosylase (APG) which instigates the base excision repair system that processes 3-methyladenine, have therefore become targets for modulation. MGMT removes alkyl groups from the *O*<sup>6</sup>-position of guanine by stoichiometric transfer to a cysteine residue in its active site, a process that results in its irreversible inactivation. Because of this, inactivation of MGMT results in increased cellular sensitivity until more protein is synthesised.

### Development of the MGMT inactivating drug, Lomeguatrib

Over the last decade, in collaboration with Prof Brian McMurry and the late Dr Stanley McElhinney (and their group at the Chemistry Department, Trinity College, Dublin), and with the support of CR-UK Drug Development and Formulation Units and also Cancer Research Technology, we have developed a drug that is a “pseudosubstrate” for MGMT. This drug is PaTrin-2, officially called Lomeguatrib (Figure), and it effectively inactivates MGMT and sensitises human tumour xenografts to the killing effect of Temozolomide. The first-time-into-man Phase I clinical trial of this drug started here at Christie Hospital in 2000, and established a dose combination of Lomeguatrib and Temozolomide for use in Phase II trials. These trials, carried out under the auspices of KuDOS Pharmaceuticals (who were recently purchased by Astra-Zeneca), to whom Lomeguatrib is licensed, are ongoing in several centres in the UK, USA and Australia. The dose and schedule of the two drugs is currently being optimised. In addition, another



trial, sponsored by CR-UK is addressing the effectiveness of Lomeguatrib in inactivating MGMT in a number of tumour types in order to establish the doses required for complete inactivation. These trials have required us to develop and validate to Good Clinical Laboratory Practice standards, quantitative assays for both functionally active and total MGMT protein. Brain, prostate, breast and colorectal tumours are being examined in dose escalation studies. Once the effective dose is established, Phase II trials will be undertaken. We are also investigating the role of MGMT promoter methylation in regulating MGMT expression and influencing response.

Aspects of these clinical trials and also of our cancer chemotherapy molecular epidemiology studies have involved the determination of the major DNA methylation product, 7-methylguanine in DNA by means of a sensitive immunoslot-blot assay. The levels of this product in lymphocyte DNA of temozolomide-treated patients varies very considerably and recent results suggest that this may be a consequence of the activity of APG (see above).

## MGMT polymorphism studies

Our previous single nucleotide polymorphisms (SNP) studies that demonstrated allelic expression imbalance (AEI) of the two MGMT alleles in subjects that are heterozygous for an expressed polymorphism have been confirmed and extended. A human cell line that contains an expressed polymorphism has been identified and this may allow us to establish a possible mechanism of MGMT AEI that could then be explored in human samples.

## Preclinical studies with MGMT inactivating agents

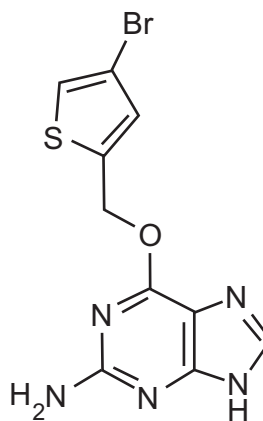
We are also involved in preclinical studies aimed at establishing clinical strategies that will reduce the dose-limiting bone marrow toxicity of agents like Temozolomide, work that we had been undertaking in collaboration with the late, and greatly missed, Lez Fairbairn and his group. We have shown that the P(140)K mutant of MGMT is highly resistant to inactivation by Lomeguatrib and that a human haemopoietic cell line (K562) transduced with a retroviral vector encoding MGMT(P140K) is highly resistant to the cytotoxic effects of Temozolomide in combination with Lomeguatrib. This is therefore a suitable combination for chemoprotective gene therapy. We have also shown in collaborations with Prof Chris Baum (Hamburg and Cincinnati) and Dr Samy Chinnasamy (Milwaukee) that self-inactivating gammaretroviral and lentiviral vectors with comparable internal expression cassettes had similar MGMT expression properties. These vectors are of interest because of the safety concerns associated with the use of the standard

retroviral vectors.

It has been reported previously that over-expression of MGMT might be disadvantageous to haematopoietic cells. To allow regulated functionality of MGMT, a 4-hydroxytamoxifen (4-OH-T) inducible MGMT(P140K) was constructed by fusing a mutated version of the mouse oestrogen receptor (ERT) to the C-terminus of MGMT(P140K). Following transduction with a retrovirus containing the MGMT(P140K)-ERT fusion, the human cell line K562, which does not express endogenous MGMT, expressed high levels of MGMT activity. Growth analysis showed that the negative effect of MGMT(P140K) overexpression in the absence of drug selection was reduced by the ERT-fusion. Further evaluation is in hand.

## Alkyltransferase-like (ATL) proteins: a novel DNA repair pathway

The *alkyltransferase-like* (ATL) proteins contain primary sequence motifs resembling those found in MGMT but in the putative active site of some ATL proteins a tryptophan residue replaces the cysteine found in the alkyltransferases. In collaboration with David Williams (Sheffield) we have shown using gel shift assays that the ATL from *E.coli* binds to short single- or double-stranded oligonucleotides containing a number of O<sup>6</sup>-alkyl-substituted guanines including Benzyl, hydroxyethyl and 4-bromophenyl (i.e. Lomeguatrib embedded into an oligonucleotide, which is the most potent MGMT inactivating agent so far described). Inactivation of the ATL gene (that we have christened *Atl1*) in *S. pombe* sensitises them to the toxic effects of a wide range of O<sup>6</sup>-alkylating agents, demonstrating that, in wild-type cells, the Atl1 protein confers resistance to such agents. The mechanism of this repair pathway is currently being investigated.



Chemical structure of the MGMT inactivating drug, Lomeguatrib (O<sup>6</sup>-(4-bromophenyl)guanine; previously called PaTrin-2, and having the trade mark, Patrin™)



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Frederick van Deursen

## Cell Cycle Group

<http://www.paterson.man.ac.uk/groups/cellcycle.jsp>

Our group studies chromosome replication and cytokinesis in eukaryotic cells. During 2006 we identified a key role for the four-protein GINS complex at DNA replication forks.

We showed that GINS allows the MCM helicase to interact with the Cdc45 protein that is essential for the progression of DNA replication forks. In contrast, we found that the Sld3 protein is required to establish but not maintain MCM-Cdc45-GINS complexes. Sld3 is recruited to origins before GINS but is subsequently displaced during the initiation process, and does not normally travel with DNA replication forks. In parallel with this work, we identified a novel factor that we call "Ingressin" or Inn1, which is required for cytokinesis. We found that Inn1 associates with the actomyosin ring at the end of mitosis and is required for contraction of the ring to be coupled to ingression of the plasma membrane.

### **Regulation of the establishment and progression of DNA replication forks**

In a systematic study of the essential budding yeast proteins of previously unknown function we identified four factors required for chromosome replication (Kanemaki, Sanchez-Diaz *et al.*, (2003), *Nature* 423, 720-724). Together with Hiro Araki's group in Japan we showed that these proteins interact to form a complex called GINS. By purifying GINS from yeast cell extracts, Aga Gambus found that GINS associates with the MCM helicase at nascent DNA replication forks and allows large "Replisome Progression Complexes" (RPCs) to form around MCM (Gambus *et al.*, 2006). It appears that RPCs exist uniquely at DNA replication forks as they are only found during S phase, on chromatin, require prior recruitment of the MCM helicase to origins during G1 phase, and are preserved if the progression of replication forks is blocked artificially. GINS is required for the normal progression of DNA replication forks and Aga identified one

important reason for this, namely that GINS maintains the interaction of MCM with Cdc45 that is also essential for fork progression.

Hiro Araki's lab previously identified Sld3 as a DNA replication protein that appeared to behave in a very similar manner to Cdc45. Both proteins are recruited to early origins during G1 phase in budding yeast, in a mutually dependent manner, but Sld3 and Cdc45 are only recruited to late origins during S phase around the time that these origins are activated. Sld3 and Cdc45 can apparently interact with each other, and Sld3 is required to establish the interaction between Cdc45 and MCM during initiation. More recently, Araki's lab showed that Sld3 could also interact with GINS in 2-hybrid screens, and Sld3 is required for loading of GINS at origins during the establishment of DNA replication forks.

We were struck, therefore, by the fact that Sld3 is not a component of Replisome Progression Complexes. This observation led us to suspect that Sld3 may only be required during initiation to mediate events such as the recruitment of Cdc45 and GINS, but might then be excluded from the nascent replisome, in contrast to Cdc45 and GINS that are required together with MCM for progression of forks. To address this possibility, Masato Kanemaki used a sensitive and quantitative chromatin immunoprecipitation (ChIP) assay based on real-time PCR to follow the association of replication proteins with DNA replication forks (Kanemaki and Labib, 2006). He found that Sld3 is indeed displaced from origins during initiation without becoming incorporated into the replisome, in contrast to Cdc45 and GINS that move away with DNA replication forks, presumably as part of RPCs. He then used degron technology to generate a novel and extremely tight new allele of SLD3. This required the addition of the degron cassette to a version of SLD3 that also contained a mutation that contributed to the resultant temperature-sensitive phenotype. Masato used this new allele, called

sld3-7td, to show that Sld3 is not required for the completion of chromosome replication after the establishment of DNA replication forks, in agreement with our finding that Sld3 is not part of RPCs and does not move with DNA replication forks (Kanemaki and Labib, 2006).

## Ingressin links contraction of the actomyosin ring to ingression of the plasma membrane during cytokinesis

In our systematic screen for new cell cycle proteins Alberto Sanchez-Diaz identified a protein that we call “Ingressin” or Inn1, which is essential for cell division but dispensable for the continuation of the nuclear cycle of replication and mitosis. In collaboration with Terry Allen’s group we have used electron microscopy to show that cells lacking Inn1 are truly defective in cytokinesis.

Alberto fused Inn1 to Green Fluorescent Protein (GFP) and found that it associates at the end of mitosis with the actomyosin ring that marks the site where cell division will subsequently occur. Contraction of the actomyosin ring is coupled by an unknown mechanism to ingression and subse-

quent abscission of the plasma membrane, thus producing two cytoplasms from one. Alberto has shown that the localisation of Inn1 requires other essential components of the ring, and Inn1 remains associated with the ring during subsequent contraction.

He then used the degron strain to show that Inn1 is specifically required for ingression of the plasma membrane. The Inn1 protein contains a predicted lipid-binding domain, and a purified recombinant form of Inn1 is able to bind to inositol phospholipids *in vitro*. These data suggest that Inn1 may link the actomyosin ring to the plasma membrane during cytokinesis.

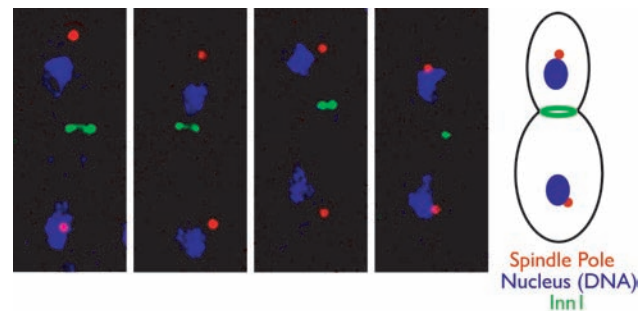


Figure 2  
Inn1-GFP associates with the contractile actomyosin ring at the end of mitosis. The picture shows cells that express Inn1 fused to Green Fluorescent Protein, as well as a fusion of Red Fluorescent Protein to a Spindle Pole Body component. Cells were fixed and stained with a DNA binding dye.

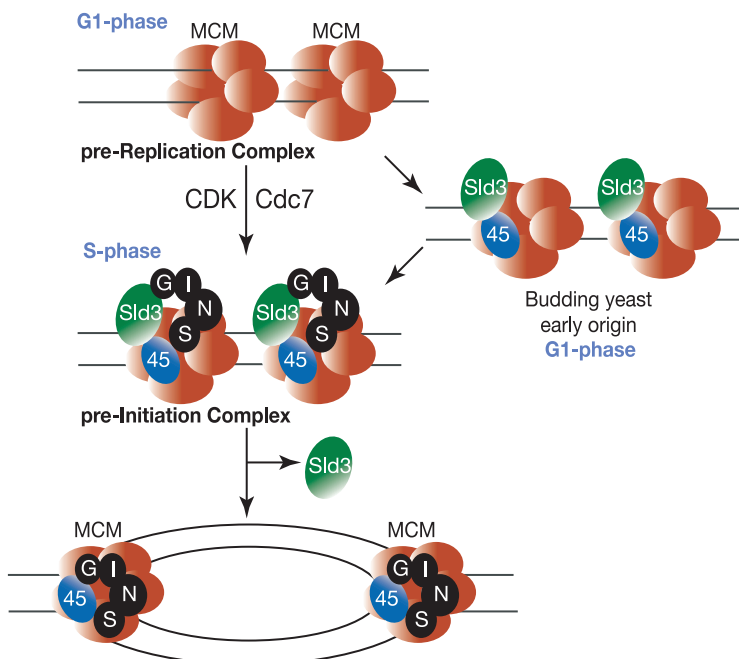


Figure 1  
A model for the role of Sld3 during the initiation of chromosome replication. See text for details.





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## Cell Division Group

<http://www.paterson.man.ac.uk/groups/celldiv.jsp>

Microtubule behaviour underpins many aspects of cancer biology. Alteration in the function of these filaments within the mitotic spindle can affect chromosome transmission to alter the balance of tumour suppressor and tumour promoter genes and so precipitate carcinogenic changes in genome composition. Microtubules also play an integral part in the migration of these cancer cells during their invasion of secondary sites to form metastases and again in the generation of the vasculature to provide the blood supply that is essential for the survival of these secondary tumours. Thus, drugs that disrupt microtubule function, such as taxol, have proved highly effective chemotherapeutic agents. Further, understanding microtubule biology will extend therapeutic options by identifying novel ways to target the microtubule cytoskeleton and define better ways to exploit existing drugs. As the structure, composition and function of the microtubule cytoskeleton is highly conserved, we study the simple and highly malleable, unicellular yeasts to learn lessons that can then be applied to tumour biology.

### Microtubule growth

Microtubules form by the polymerisation of tubulin heterodimers. They are polar structures. Subunits add onto one end much faster than the other. Consequently this end is known as the plus end and the opposing end the minus end. Free tubulin subunits only associate with microtubules when it has bound GTP. The incorporation of GTP tubulin into the polymeric microtubule lattice then stimulates the hydrolysis of the GTP to GDP. This hydrolysis induces a conformational switch in the tubulin molecule. Thus, the microtubule polymerises as a flat sheet of GTP tubulin that curls up upon GTP hydrolysis to form a tube of GDP tubulin. If there are no GTP associated molecules at the end of the microtubule, the structural change

induced by GTP hydrolysis within the lattice induces the subunits to peel away from the microtubule lattice leading to the rapid depolymerization of the microtubule. Thus, if at any point in the life of a microtubule, the hydrolysis rate exceeds the polymerisation rate, the microtubule will lose its protective cap of GTP tubulin and depolymerise. As the loss of a GTP cap is a random event, microtubules are constantly switching from growth to shrinkage independently of one another. The result is a dynamic microtubule cytoskeleton that is constantly probing the 3-dimensional space. This enables it to rapidly establish contacts that are critical in cell migration or chromosome segregation.

### The +TIP class of microtubule associated proteins

While the inherent GTPase activity of tubulin generates a dynamic cytoskeleton, it is the activity of a number of molecules that associate with microtubules that modulate microtubule behaviour and it is the control of these microtubule associated proteins (MAPs) that underpins the activity of the microtubule cytoskeleton. There are several types of MAP: motor proteins that migrate along microtubule lattice, structural MAPs that associate along the length of the microtubule and the +TIPs that associate specifically with the +ends of microtubules. Because it is the dynamic +end of the microtubule that mediates many microtubule functions, there is considerable interest in understanding how these +TIPs function. The +TIP CLASP was identified by virtue of its association with the founder +TIP, CLIP170, leading to the proposal that the association with CLIP170 enables CLASP to modulate microtubule dynamics. Subsequent analyses demonstrated that a number of other +TIPs also associate with one another, and the proposal that CLASP influences microtubule function through its association with a second +TIP, EB1.

# CELL DIVISION

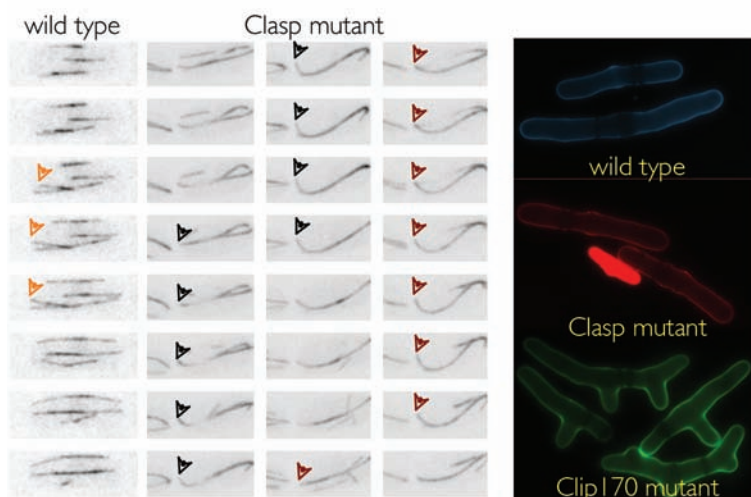
## Fission yeast CLASP

We have been studying fission yeast CLASP. Like its higher eukaryotic counterparts, fission yeast CLASP regulates microtubule behaviour at the cell cortex. When microtubules arrive at the ends of these highly polarised cells they establish an end-on association with the cortex, continue to polymerise for a while at this attached end before finally depolymerising so that their end ultimately shrinks away from the cell tip. The polymerisation rate of microtubules that have established end-on associations at cell tips is slower than before contact is made. We found that this reduction in polymerisation rate depended upon CLASP function. Other aspects of cortical function were also altered by ablating CLASP function and many microtubules failed to make an end-on attachment but continued elongating to curl around cell tips. Unexpectedly we failed to find strong evidence for any interplay between fission yeast CLASP and its CLIP170 or EB1 homologues. Instead, we found a strong link between CLASP and the multi-subunit motor protein dynein. Because dynein moves towards the minus end of microtubules it either transports car-

goes to minus ends or pulls the plus ends towards a structure to which the dynein is anchored. We found striking similarities in the consequences of losing the function of CLASP and dynein.

## Lessons from yeast

The ability to manipulate genes at will in a simple organism whose primary purpose is to divide is enabling us to explore the finer points of microtubule biology. 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 microtubule biology.



Left: Consecutive images of a wild type and a clasp mutant cell. Arrows indicate the ends of dark microtubules that elongate to cell tips where they pause before de-polymerising. Right: Placing mutations in a background that produces highly elongated cells shows that *clasp* plays no role in morphogenesis.



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## Cell Regulation Group

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Cells commonly respond to extracellular signals by modulating the activity of specific transcription factors and subsequently the expression of many target genes. We are particularly interested in the response to cytotoxic and genotoxic stress which results in the mobilisation of a battery of protective and repair mechanisms or the induction of apoptosis. Failure to respond appropriately can result in cellular damage and thereby drive tumourigenesis.

The AP-1 transcription factor plays a key role in the response of cells to extracellular signals. In mammalian cells it is regulated by a plethora of physiological and pathological stimuli including mitogens, hormones, genotoxic agents, stress signals, viral infections and cytokines. Not surprisingly therefore, it has been linked to many cellular events including cell proliferation, differentiation as well as apoptosis. AP-1 plays important roles in tissue stress responses such as inflammation and ischemia and is implicated in the onset and progression of tumours. The factor and its regulation is complex since it is not a single entity but rather a mixture of dimeric complexes composed of members of the Jun, Fos, ATF and MAF protein families. Different dimeric combinations can recognise slightly different sequence elements and be regulated by distinct signalling pathways. A well characterised signalling cascade involves the activation of the mitogen-activated protein (MAP) kinases ERK and stress-induced MAP kinases JNK and p38 which directly phosphorylate and modulate the activity of various members of the AP-1 complex. Over the last few years considerable progress has been made in elucidating the function of individual AP-1 proteins through the characterisation of genetically modified mice and cells that derive from them.

Homologues of AP-1 family proteins are found in all eukaryotic organisms and their involvement in

stress responses is highly conserved. In fission yeast the major transcriptional responses to stress conditions are coordinated by the transcription factors Atf1 and Pap1, which are related to mammalian ATF and Jun proteins respectively. In addition the activity of Atf1 is regulated by the Sty1 kinase, a homologue of the mammalian p38 kinase. Thus fission yeast serves as a useful model for understanding the role and regulation of AP-1 proteins in mediating stress responses.

### Functional Characterisation of ATF2

ATF2 is a member of the AP-1 family and can bind to DNA either as a homodimer or as a heterodimer with other AP-1 family members, most prominently c-Jun. ATF2 is activated by the p38 or JNK kinases through phosphorylation of two N-terminal threonine residues T69 and T71. Many reports, mostly using *in vitro* systems, have implicated ATF2 in numerous growth and developmental programs and in response pathways after stimulation with geno- and cytotoxic stresses.

To better understand the biological importance of ATF2 we have generated a number of genetically modified mice where ATF2 activity is compromised. The germline deletion of the DNA binding domain in ATF2 leads to postnatal lethality due to deficiencies in the control of breathing. A similar defect is uncovered by specifically deleting ATF2 in neuronal cells by Cre/loxP mediated conditional gene inactivation. Therefore, we could show that ATF2 has essential roles in the central nervous system, and at least one essential function is the establishment or correct placement of respiratory centres in the hindbrain.

The essential role of ATF2 in embryonic development is uncovered by simultaneously deleting ATF2 and its closest homologue ATF7 in the germline. The resulting double homozygous embryos display

severe deficiencies in the developing liver due to severely enhanced apoptosis. During large periods of embryogenesis, the embryonic liver consists of developing hepatocytes as well as haematopoietic precursor cells and in the absence of functional ATF2 and ATF7, both cell types showed increased apoptosis. This increase is driven by high levels of activated p38 kinase and is reversed by the addition of a specific p38 inhibitor. The accumulation of active p38 is due to a decrease in a number of dual specificity phosphatases, including DUSP1/MKPI, which serve to negatively regulate the activity of MAP kinases. ATF2 binds directly to the promoter regions of these phosphatases and regulates their transcription, thereby establishing a negative feedback loop. We also generated mice containing an allele of ATF2 where the phospho-acceptor threonine residues (T69 and T71) are altered to alanines (ATF2-AA) and thus rendering ATF2 inactive for response to upstream MAP kinases. On an ATF7 mutant background, these mice demonstrate the same embryonic phenotypes as the deletion in the DNA binding domain. Furthermore, since the ATF2/7 mutations phenocopy the germline mutation of JNK and p38 activating kinases MKK4, and MKK7, it is likely that during liver development ATF2/7 are essential effectors of MAP kinase signaling.

Cells derived from ATF2/7 mutant embryos have been established in culture. Compared to wild-type control cells ATF2/7 mutant cells are characterised by saturation at a higher cell density suggesting a defect in contact inhibition of growth. The cells are also sensitive to specific pro-apoptotic signals such as TNF $\alpha$ . When immortalised and expressing oncogenic Ras, ATF2 deficient cells generate significantly larger tumours following injection into immunodeficient mice suggesting a negative role for ATF2 in tumour growth. In this context, the importance of regulation of MAP kinase activity through the transcriptional activity on MAP kinase phosphatase genes is currently being established.

ATF2/7 knockout cells, as well as other tissue specific knockout models, are being used to further address the role of ATF2 in tumourigenesis or the response of tumour cells to therapeutic approaches.

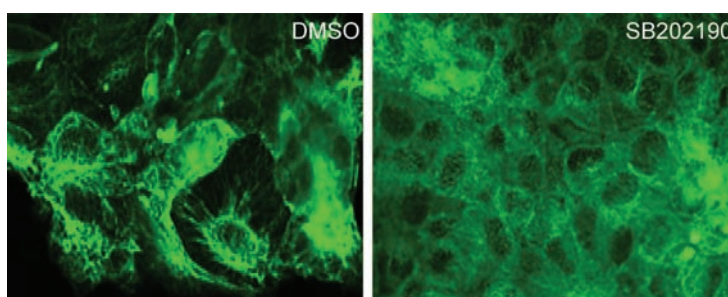
## Stress Response in Fission Yeast

We use fission yeast as a model system for studying stress responses since there appears to be remarkable conservation involving similar signalling pathways and the mobilisation of closely related transcription factors. All cells sense and react to changes in their environment. Single-celled organ-

isms, in particular, must contend with fluctuations in nutrients, pH, temperature and external osmolarity, as well as exposure to UV irradiation and a range of potentially toxic environmental compounds. Appropriate responses to these environmental stresses must be induced for cell survival and proliferation.

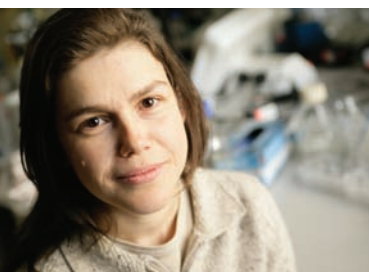
Both the Sty1 kinase and the Atf1 transcription factor are crucial for fission yeast to respond normally to a range of different stress conditions by orchestrating the expression of a common set of environmental stress response genes encoding numerous defence and repair proteins. We are investigating how Sty1 modulates Atf1 activity. Atf1 is directly phosphorylated by Sty1 and we have shown that this regulates Atf1 stability. Our results suggest that Atf1 phosphorylation by Sty1 is not required for activation of gene expression *per se*. However, Atf1-dependent transcription absolutely requires Sty1 which suggests that the MAPK plays some other role in the activation of gene expression. We are currently investigating the mechanism whereby Sty1 activates expression and find that the kinase gets recruited to Atf1 dependent promoters upon stress.

We have gone on to identify an E3 ubiquitin ligase involved in Atf1 turnover and are currently characterising the mechanisms by which phosphorylation interferes with this turnover. Furthermore, this ligase itself seems to be subject to stress dependent regulation. In addition, we have used mass-spectrometry approaches to identify a number of novel protein interactions with Atf1. The functional importance of these interactions is being characterised and should lead to a more detailed understanding of how this factor is regulated.



Chemical inhibition of p38 kinase blocks growth arrest in ATF2/7 mutant embryonic hepatocytes.





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## Cell Signalling Group

<http://www.paterson.man.ac.uk/groups/cellsig.jsp>

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 essential for Ras-mediated *in vitro* transformation. Recently, data has emerged to directly implicate Rho proteins in tumour initiation and progression *in vivo*. Our group's focus is on identifying signalling events downstream of Rho proteins that modulate tumour susceptibility and disease progression.

Similarly to Ras, 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. In the active state, Rho proteins bind and stimulate effector molecules that in turn govern cell morphology, adhesion, motility, as well as cell cycle progression and cell survival. Of relevance to cancer, Rho proteins are transforming in *in vitro* assays, particularly when expressed in combination with Ras effectors, and they are required for Ras-induced transformation. 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. Interestingly, Tiam1 preferentially associates with activated GTP-bound Ras through a Ras-binding domain (RBD). Activated Ras and Tiam1 synergize to induce formation of Rac-GTP (Lambert *et al.*, Nature Cell Biol 2002; 4: 621). Further, Tiam1-deficient cells are resistant to Ras-induced cellular

transformation (Malliri *et al.*, Nature 2002; 417: 867).

### Tiam1/Rac signalling and tumorigenesis *in vivo*

Mice deficient for Tiam1 are resistant to the formation of skin tumours induced by application of a two-stage chemical carcinogenesis protocol (Malliri *et al.*, Nature 2002; 417: 867). This protocol entails tumour initiation in epidermal keratinocytes by treatment with the carcinogen 7,12-dimethyl-benzanthracene, which induces oncogenic activation of the *c-Ha-Ras* gene. Subsequent repeated treatments with the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) result in the outgrowth and progression of initiated cells. 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 skin of Tiam1-deficient mice.

Tiam1 is also a potent modifier of intestinal tumorigenesis (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 its activation. However, few genes targeted by this pathway have been demonstrated to affect tumour development *in vivo*. Tiam1 is a Wnt-responsive gene. It is expressed in the proliferative compartments (crypts) of the adult mammalian intestine where the Wnt pathway is normally active. It is also up-regulated in adenomas from patients with either sporadic colorectal polyps or familial adenomatous polyposis (FAP), as well as in adenomatous polyps in Min (multiple intestinal neoplasia) mice. In each instance, the Wnt pathway is hyperactivated due to a mutation in the *apc* tumour suppressor gene. Further, 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 tumorigenesis *in vivo* demon-

strate 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 the context of oncogenesis, activation of this signaling module promotes tumour initiation and growth. Moreover, this role is specific to Tiam1 since its loss cannot be compensated for by other Rho GEFs.

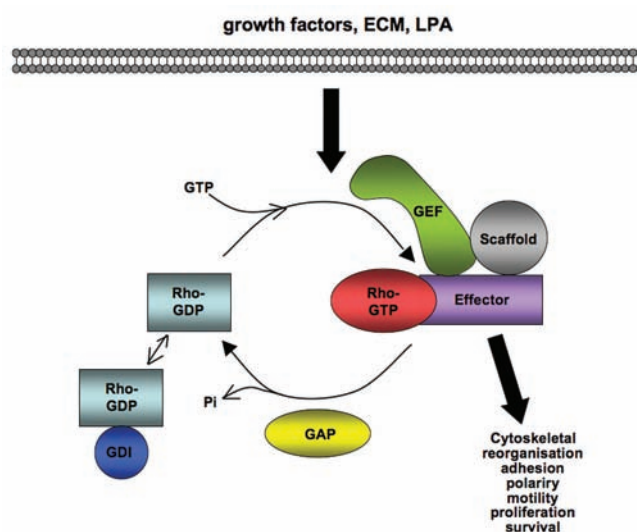
## Tiam1/Rac signalling and tumour invasion

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. Paradoxically, 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 antagonize Ras during tumour invasion. Similarly in intestinal tumours, lack of Tiam1 increased the invasiveness of malignant cells (Malliri *et al.*, J Biol Chem 2006; 281: 543).

One probable mechanism by which Tiam1/Rac antagonizes 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 and the accompanying induction of an epithelioid phenotype in a number of 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). Intriguingly, Asef, another Rac specific exchange factor, promotes intestinal tumour cell migration and invasiveness in *in vitro* models through down-regulating cadherin-mediated adhesion (Kawasaki *et al.*, Nature Cell Biol 2003; 5: 211).

The diverse and even opposing roles of Rho GEFs in certain processes clearly indicate that Rho GEFs

do more than simply activate Rho molecules, and 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. Tiam1 contributes to the signalling specificity downstream of Rac via associating with IB2/JIP2, a scaffold that promotes Rac activation of p38 kinase cascade over JNK MAP kinase cascade (Buchsbau *et al.*, Mol Cell Biol 2002; 22: 4073). Tiam1 can also influence Rac signalling specificity through its interaction with spinophilin, a scaffold that binds to p70 S6K, another kinase regulated by Rac. Spinophilin binding suppresses the ability of Tiam1 to activate Pak1, a different Rac effector (Buchsbau *et al.*, J Biol Chem 2003; 278: 18833). In our lab, we are using biochemical approaches to identify Rac and Rac GEF interacting proteins involved in different aspects of transformation including malignant progression (acquisition of invasiveness). We are also investigating the potential role of the Tiam1 homologue, Stef, in tumourigenesis and the impact of down-regulating more than one GEF simultaneously in different aspects of the transformed phenotype.



The Rho GTPase cycle. Rho-like GTPases cycle between an active GTP-bound and an inactive GDP-bound form. This is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Guanine nucleotide dissociation inhibitors (GDIs) inhibit nucleotide dissociation and control cycling of Rho GTPases between membrane and cytosol. Signals like growth factors, extracellular matrix (ECM) or lysophosphatidic acid (LPA) are able to activate Rho-like GTPases. Active GTPases interact with effector molecules to elicit various cellular responses. Additionally GEFs could work as scaffold proteins by either binding directly to Rac effectors or other scaffold proteins that bind to effectors.

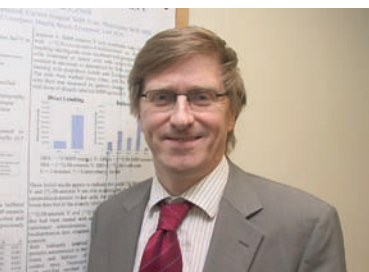


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# Clinical and Experimental Pharmacology Group

<http://www.paterson.man.ac.uk/groups/cep.jsp>

CEP incorporates teams in pre-clinical drug target validation and biomarker discovery. Within the recently refurbished, specialised PACCAR Good Clinical Laboratory Practice (GCLP) laboratory CEP also develop, validate and implement pharmacokinetic (PK) and pharmacodynamic (PD) assays for Phase I trials at the Christie Hospital's Derek Crowther Unit (DCU). Our research focuses on novel agents targeted to apoptosis pathway components (e.g. IAP and Bcl-2 inhibitors) and, in collaboration with Gordon Jayson, we are validating a panel of biomarkers for trials of anti-angiogenic drugs (e.g. GSAO). Technology development initiatives in CEP include generation of preclinical models with inducible expression of wild type or mutant drug targets, optimisation of serum proteomics in a new clinical proteomics facility, the isolation of circulating tumour cells, the use of Q Dots to enhance immuno-histochemical (IHC) analyses and implementation of multiplexed bead arrays.

## Clinical Trial Facilities at the Christie Hospital's DCU

Our translational research is associated directly with clinical trials in DCU. During 2005/2006, DCU had 118 trials involving 635 patients. The Christie Hospital NHS Trust, became an Experimental Cancer Medicines Centre (ECMC) led by Malcolm Ranson in 2006 and recent award of a Clinical Research Infrastructure bid will fund a doubling of DCU capacity making it one of the largest units of its kind worldwide. The CR-UK Phase I trial of Aegera Therapeutics' AEG35156 (anti-sense XIAP) showed that it is well tolerated up to 125mg/m<sup>2</sup> x 7 days every 3 weeks with dose limiting toxicity of asymptomatic, reversible increase in liver enzymes. There was preliminary evidence of single agent anti-tumour activity in refractory breast cancer and in refractory non-Hodgkin's lymphoma. The Phase I Trial of Allos Therapeutics' bio-reductive alkylating agent RH-1 concluded this year. Clinical PK/PD data are now being integrated and modelled in collaboration with Prof Leon Aarons, UoM.



### The Critical Importance of Biomarkers in Early Clinical Trials

In an era of mechanism-based therapies there is clear imperative to determine the right dose and schedule for the right patient and thus increased requirement for pharmacodynamic (PD) biomarkers. These biomarkers allow proof of concept demonstrations of target modulation in a patient; they can facilitate rational choice of correct dose and schedule, and, if implemented to the appropriate quality standard they can contribute to clinical decision making. Ultimately biomarkers can help explain or predict clinical outcomes and decrease costs of drug development. CEP has therefore put increased emphasis on development and validation of PD biomarkers and on biomarker discovery strategies.

### Pharmacodynamic Assay Development

CEP has focussed on IHC, flow cytometric and ELISA-based PD biomarkers. With a Bcl-2 small molecule inhibitor phase I trial due to be initiated in DCU in 2007, we worked up a series of IHC methods to detect Bcl-2 family proteins and apoptosis. We have progressed and validated markers of tumour vasculature and vascular density assessment within biopsies. In collaboration with AstraZeneca and Richard Byers (UoM), CEP is also evaluating the utility of Q-dot technology for multi-analyte detection in IHC. Multiple serological ELISA-based biomarkers have been evaluated for clinical use including three surrogate markers of cell death and a panel of biomarkers of angiogenesis. CEP is currently developing methods to utilise cytometric bead arrays to measure multiple analytes in small volume clinical samples. Protocol development is ongoing to implement 4 colour flow cytometric analysis of apoptosis in serial samples from lymphoma patients (see Figure).

### Development of a GCLP Quality System

To use biomarkers for clinical decision making, studies must be performed to GCLP standard. With expansion of the QA team and relocation to the TRF, the QA system now covers facilities compliance, equipment qualification, resource logs and archiving. CEP continues to innovate in QA/lab management with introduction of *pro forma* lab books and fast track validation, establishing the lab/clinic (CEP/DCU) QA interface and development of custom designed electronic resources.

### Pre-clinical validation of novel agents against paediatric tumours.

RH-1 and a novel HDAC inhibitor demonstrated effectiveness against paediatric tumour cell lines *in vitro*; RH-1 also showed efficacy *in vivo*. We validated two agents that target XIAP which is expressed

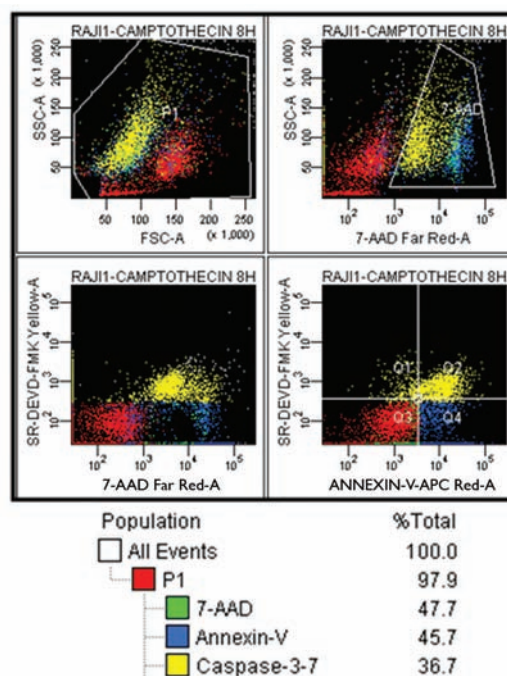
across our paediatric cell line panel. Repression of XIAP by shRNAi or antisense oligonucleotide can sensitise to cytotoxic agents. A novel XIAP small molecule inhibitor is effective against these cell lines and synergizes with conventional cytotoxic agents in a number of them.

### Biomarker Discovery

In a joint venture with Tony Whetton's group (UoM) and in collaboration with Beyond Genomics Medicine Inc, we are optimising a workflow for large-scale proteomic screening of human plasma. With a pilot clinical study and process validation underway, and recruitment of a bioinformatician (in collaboration with Crispin Miller, PICR), we anticipate our first clinical proteomics data sets early in 2007.

### Biomarkers Club and the AstraZeneca Serological Biomarker Alliance

AZMU Biomarkers Club, a forum to discuss biomarkers of efficacy and toxicity with colleagues at AstraZeneca met on the topics of apoptosis, invasion and hypoxia. The recruitment of three staff and purchase of an ELISA Robotic station in 2006 activated the AZ/CEP Serological Biomarker Alliance to investigate surrogate markers of tumour cell death in AstraZeneca-driven clinical trials.



Four-colour Flow Cytometric Apoptosis Assay

Cell death in lymphoma cells from patients treated with AEG 35156 XIAP antisense was assessed using CD20 positive staining to discriminate lymphoma cells, Annexin V for early apoptosis and cleaved caspase 3,7 for late apoptosis with membrane permeable (necrotic) cells staining positively for 7-AAD.



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## Immunology Group

<http://www.paterson.man.ac.uk/groups/immuno.jsp>

A consistent theme of the Immunology Group's research has been the investigation of shared properties of developmental tissues and tumours or cancer cells with a view to identifying new targets/markers of value in diagnosis, prognosis or therapy. One particular success has been the 5T4 oncotrophoblast molecule where several different types of immunotherapies have progressed through preclinical and early clinical studies and are now entering phase III studies. Our clinical studies of 5T4 and HPV related therapies are now reported in the Biological Immune and Gene Therapy section. In the past year we have investigated the early differentiation of embryonic stem cells of both mouse and human to further study the cellular role of 5T4 molecules. In addition we have exploited the early upregulation of surface 5T4 expression of embryonic stem (ES) cells committed to differentiation rather than self renewal, to identify additional gene expression changes which may be important in both development and cancer.

### 5T4 expression in ES cells

The 5T4 oncofoetal antigen is a cell surface glycoprotein that is transiently expressed during mouse ES cell differentiation and correlates with decreased pluripotency of such cells. We have now shown that the 5T4 antigen is a transient marker of human ES cell differentiation and that 5T4 phenotype, colony seeding density and culture conditions significantly influence the maintenance of pluripotent hES cells and their differentiation to neural lineages (Exp Cell Res. 2006; 312: 1713-26). In collaboration with Chris Ward, we have shown that mouse and human ES cell differentiation is also associated with loss of cell surface E-cadherin and up-regulation

of N-cadherin proteins. The E- to N-cadherin switch during ES cell differentiation correlates with up-regulation of Snail and Slug proteins, both of which show nuclear localisation in OCT-4 negative cells. ES cell differentiation is also associated with up-regulation of transcripts encoding matrix metalloproteinases (MMP)-2 and -9 and increased gelatinase activity. Although E-cadherin is down-regulated during ES cell differentiation it is not required for maintenance of undifferentiated ES cells. Furthermore, E-cadherin, N-cadherin and 5T4 proteins are independently regulated during ES cell differentiation and are not required for induction of epithelial-mesenchymal transition (EMT)-associated transcript expression. Abrogation of E-cadherin mediated cell-cell contact in undifferentiated ES cells resulted in a reversible mesenchymal phenotype in the absence of EMT-associated transcript expression. The loss of cell surface E-cadherin in undifferentiated ES cells results in translocation of the 5T4 antigen from the cytoplasm to the cell surface in an energy-dependent manner. We conclude that E-cadherin protein is likely to function in ES cells to stabilise cortical actin cytoskeletal arrangement and this can prevent cell surface localisation of the 5T4 antigen, a newly identified component of the EMT process. Interestingly, a study (with Renehan, O'Dwyer *et al.* Dept. Surgery) of pseudomyxoma peritonei (PMP), a rare neoplasm of mainly appendiceal origin, demonstrated a specific pattern of adhesion-related protein expressions of increased N-cadherin, reduced E-cadherin, and increased vimentin ( $P=0.004$ ), a phenotype suggesting a possible epithelial-mesenchymal transition state. The similarities between PMP and colorectal adenocarcinoma, also reveal a specific cadherin phenotype that may characterise the distinct non-metastasising behaviour of PMP (Brit. J. Cancer 2006; 95: 1258-1264).

5T4 has been used as a marker to define and separate populations of pluripotent mouse ES cells (5T4 negative) and early differentiating cells (5T4 positive) for comparison by microarray analysis. Many established transcriptional changes that occur during differentiation were identified, including the down-regulation of the transcription factors Krüppel like factor 4 and oestrogen receptor related  $\beta$ , very recently suggested to have roles in ES cell self-renewal. In addition, a number of previously unreported transcriptional changes were identified. One of these was the significant down-regulation of CD26 during differentiation. CD26 is a multifunctional cell surface molecule which inhibits SDF-1 chemokine induced cell migration. In various cancers loss of CD26 expression is associated with increased metastatic potential and SDF-1 responsiveness (Figure). The inverse correlation between 5T4 and CD26 expression during mouse ES cell differentiation, and the known roles of these molecules in cell migration/motility, suggests that molecular events are common to both ES cell differentiation and tumour metastasis. Understanding the co-ordination of these events may provide new strategies for cancer treatment.

In our ongoing studies into the 5T4 oncofoetal antigen we have backcrossed 5T4 KO heterozygote mice against the 129 and C57BL/6 backgrounds allowing us to assess the consequences of the KO phenotype. The null 5T4 C57BL/6 animals are viable but adult animals show some structural disorganisation within the brain and exhibit a high frequency of hydrocephaly. Importantly, the 5T4 null mice have been used to generate useful monoclonal antibodies and cytotoxic T cell responses against the mouse 5T4 target. This will allow the detailed analysis of the consequences of generation of autologous antigen specific immunity in animals as a result of vaccination (Cancer Immunol Immunother. 2006; 56 : 165-180), adoptive transfer of natural or targeted T cells or their combination use (e.g. J Immunology 2006; 177: 4288-98), all of which are potential goals for clinical trials in man.

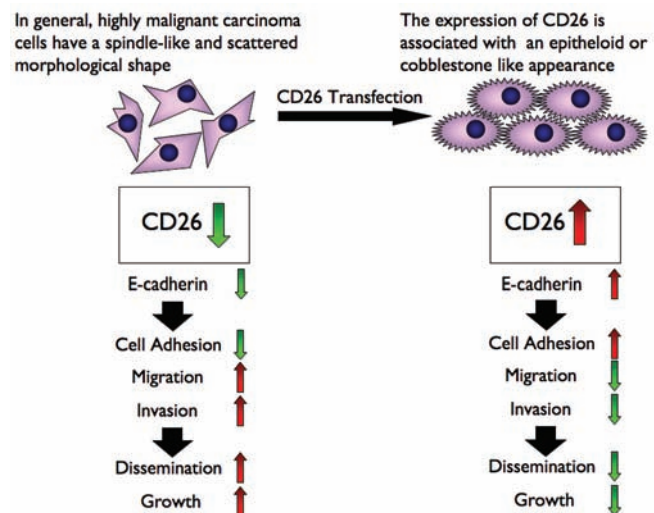
## 5T4 immunotherapy

We have shown that human CD8-T cell repertoire versus 5T4 antigen exists and can be detected in the absence of CD4 T cells (either helper or regulatory) and HLA-A2 epitopes have been identified (Int J Cancer. 2006; 119: 1638-47). Significant 5T4 responses of CD4 T cell enriched populations are most apparent with depletion of the CD25<sup>+</sup> T cells and some MHC Class II restricted epitopes have been identified. These observations imply that the availability of the 5T4 repertoire of both CD4 and CD8 T cells may be optimally accessed in the absence of T regulatory activity. In many different types of cancer there are significantly increased

numbers of CD4<sup>+</sup>CD25<sup>high</sup> regulatory cells which express the FOXP3 marker. We are examining the modulation of repertoire of recognition of 5T4 in patients in trials aimed at reducing T regulatory activity in CD25<sup>+</sup> depletion in renal cell carcinoma and in upper gastrointestinal cancer patients receiving anti-CTLA-4 therapy.

## HPV studies

High risk HPV infection is a necessary cause of cervical cancer. An ideal vaccine would have both prophylactic and therapeutic effects. Such a vaccine would have immediate benefit in contrast to the inevitable delay before the clinical impact of prophylactic vaccines. A fusion protein comprising HPV16 L2, E6 and E7 is a candidate combination preventive and therapeutic HPV vaccine. In collaboration with Richard Roden at Johns Hopkins University we have analysed sera from previous clinical trials of the TA-CIN vaccine for L1 and L2-specific and neutralising serum antibody titers. In particular vaccination three times at monthly intervals in a phase I randomised double-blind placebo controlled dose escalation trial in 40 healthy volunteers, and a phase II trial of TA-CIN at the maximum dose in 29 women with high grade anogenital intraepithelial neoplasia (AGIN). Vaccination of healthy volunteers induced L2-specific serum antibodies that were detected one month after the final vaccination and neutralized both HPV16 and HPV18 *in vitro*. There was also an antigen-specific proliferative response of the vaccinated healthy volunteers that showed a significant trend with increasing vaccine dose and correlated with the L2-specific antibody response. However, the AGIN patients responded less effectively to vaccination at the highest dose than the healthy patients with respect to the induction of HPV16 L2-specific and neutralising antibodies and proliferative responses. We are exploring the means to increase such cross neutralising antibody titres with different adjuvants.



Publications listed on page 60





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# Radiochemical Targeting and Imaging Group

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Research activity over the last year focussed on i) developing multi-modality imaging with radio-quantum dots, allowing both radionuclide and fluorescent signals to be detected using the same probe and ii) investigation of  $^{18}\text{F}$ -Fluorothymidine ( $^{18}\text{F}$ -FLT) as a PET probe for cell proliferation. In the quantum dot project, a  $^{109}\text{CdSe}(\text{ZnS})$  probe capable of detecting a combined fluorescence and radionuclide signal was developed and evaluated in *in vitro* and *in vivo* studies. Radio-quantum probes were synthesized at a tracer level, hence overcoming a major toxicity issue associated with conventional fluorescence only quantum dots. The bio-distribution and autoradiography profile of intravenously administered probe showed accumulation in reticulo-endothelial system (RES) tissues, particularly the liver and spleen, followed by metabolism and significant clearance over 24 hours. To demonstrate biological targeting, the radio-quantum dot was conjugated to the monoclonal antibody rituximab and found to bind at the surface of lymphoma cells expressing the anti-CD20 protein target. In the second project we have investigated the uptake kinetics of  $^{18}\text{F}$ -FLT and enzyme activity in tumour cell lines and related the nucleoside kinase activity to the concentration of radiotracer in tumour samples. Nucleoside kinase activity (pmoles per  $\mu\text{g}$  protein) of tissues from H460 tumour-bearing mice were higher for  $^3\text{H}$ -thymidine and  $^{18}\text{F}$ -FLT than for radioiodinated IUdR and FIAU. The higher *in vivo* stability of  $^{18}\text{F}$ -FLT compared to the iodinated analogues enabled better cell incorporation and its improved overall kinetics showed better reflection of S-phase enzyme activity than in the case of the iodinated nucleosides.

## **Radiotracer quantum dots**

We have established a model system, based on  $^{109}\text{CdSe}$ , for the development and validation of the

radio-quantum dot approach to multi-modality imaging. We have synthesised radiotracer quantum dots which emit both gamma-ray and fluorescence signal. Such probes were found to be non-toxic in various cell lines and were significantly (40-60% in 30 minutes) incorporated in lung and colorectal tumour cells. Whole body autoradiography of  $^{109}\text{CdSe}/\text{ZnS}$  quantum dots (630nm) showed accumulation mainly in the RES system followed by metabolism and clearance through the liver and kidneys (figure 1). In tumour-bearing mice, the uptake of  $^{109}\text{CdSe}/\text{ZnS}$  quantum dots at 24 hours was 10-fold higher than at 1 hour. Uptake is mainly driven by endocytosis with greater retention in tumour cells than in normal organs including non-RES tissues. To demonstrate non-toxicity, stability and biological compatibility, the radio-quantum dots were conjugated to the anti-CD20 monoclonal antibody rituximab and the binding of the probe was demonstrated in lymphoma xenografts expressing the CD20 protein (figure 2).

## **Nucleoside-based PET probes**

The development of  $^{18}\text{F}$ -FLT as a probe to measure cell proliferation has continued over the last year. In terms of thymidine kinase activity, in both normal and tumour bearing animals, FLT showed higher level of the kinase in proliferative tissues (bone marrow, spleen, intestine) as well as in NSCLC xenografts. Despite high thymidine phosphorylase activity of  $^{124}\text{I}$ -IUdR, measured as the number of cleaved iodouracil molecules, the higher stability of  $^{18}\text{F}$ -FLT resulted in higher and more specific uptake of the PET signal in both tumours and the proliferative cells of the bone marrow, spleen and intestine. These studies, along with our previous observation, of the  $^{124}\text{I}$ -IUdR kinetic profiles in animal and human studies indicate that despite the higher DNA incorporation of  $^{124}\text{I}$ -IUdR, it has lower specific signal sensitivity compared to  $^{18}\text{F}$ -FLT due to the extensive metabolism of the radioiodinated pyrimidine analogue. This observation was substantiated in our pilot clinical study which showed a biodistribution pattern main-

ly of radiolabelled metabolites in the case of the [ $^{124}\text{I}$ ]-IuDR compared to [ $^{18}\text{F}$ ]-FLT.

## Imaging pharmacokinetics of superantigen targeted therapy

Clinical trials of superantigen targeted therapy directed against the tumour associated antigen 5T4 have shown clinical efficacy in lung cancer (unpublished) and in renal cancer (Shaw *et al* in press). In the latter study, a sub-set of patients was assessed for clinical effect using FDG-PET, which gave a reliable, early indication of disease control which correlated with long term survival. In order to

study the pharmacokinetics of such therapy, we have, in collaboration with Immunology and Medical Oncology, designed, optimised and validated a clinical labelling strategy to label the drug ANYARA with the positron emitting isotope  $^{124}\text{I}$ . Proof of principle of drug targeting was demonstrated in renal cancer patients and the radiolabelled drug uptake was found to be antigen-specific as validated by immuno-histochemistry on biopsy samples.

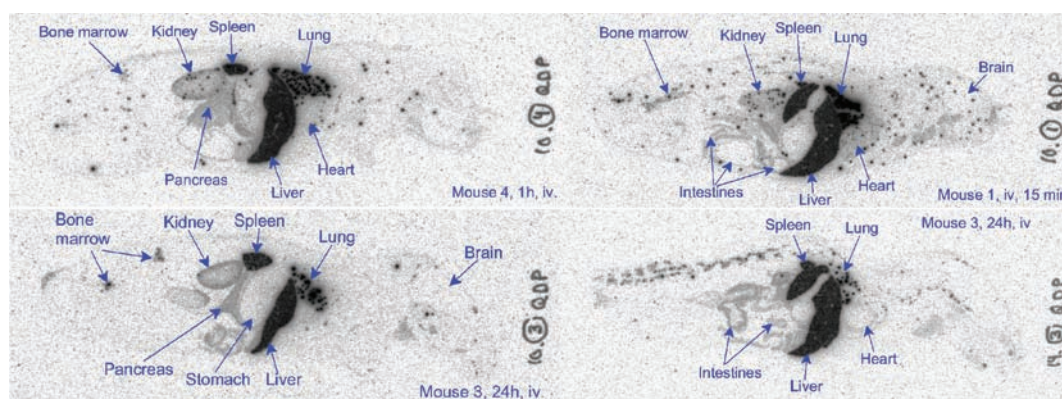


Figure 1. Whole body autoradiographs of  $^{109}\text{CdSe/ZnS}$  radio-quantum dots following intravenous administration of the probes into mice.

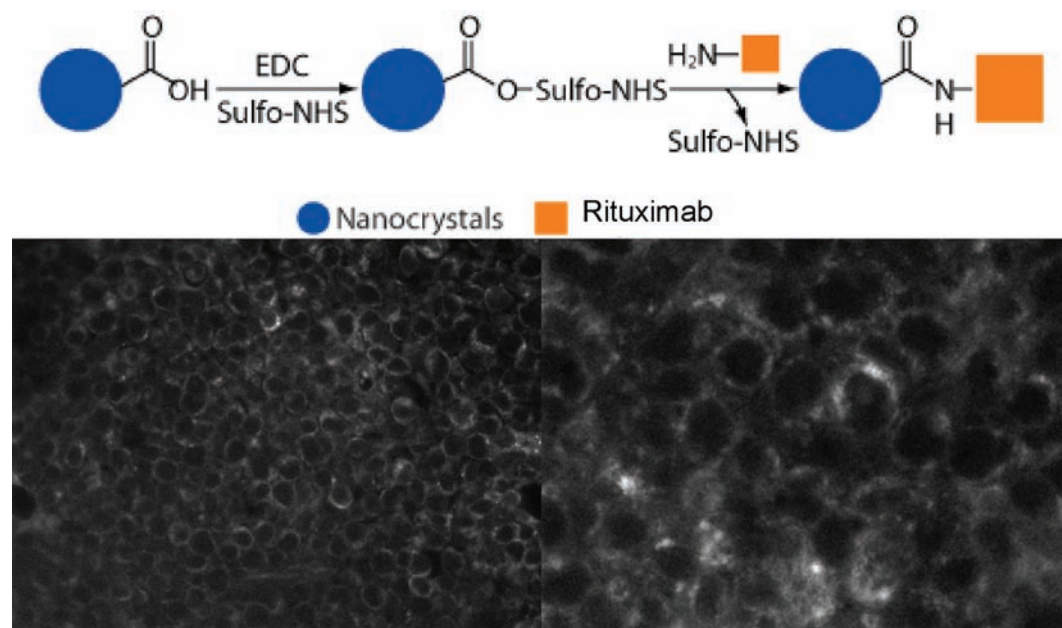
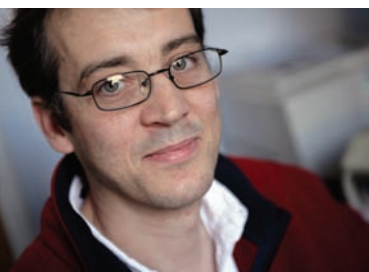


Figure 2. Conjugation of  $^{109}\text{CdSe/ZnS}$  radio-quantum dots to the anti-CD20 monoclonal antibody rituximab (top) and binding of the conjugated antibody to the surface of lymphoma cells expressing the CD20 protein (bottom).



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## Stem Cell Biology Group

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The AML1/Runx1 transcription factor is a frequent target of gene rearrangements and mutations in human acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). Consistent with its initial implication in leukemias, Runx1 has been shown to be critical for normal haematopoietic development. The MOZ gene is involved in three independent myeloid chromosomal translocations fusing MOZ to the partner genes CBP, P300 or TIF2.

Using the *in vitro* differentiation system based on mouse embryonic stem (ES) cells and mouse models, our goals are to further define the role of *Runx1* and *MOZ* in early haematopoietic development and how alterations of their function leads to leukemogenesis.

### Early haematopoietic development and haemangioblast

The earliest site of blood cells development in the mouse embryo is the yolk sac where blood islands, derived from mesodermal cells, develop at approximately day 7.5 of gestation. The yolk sac blood islands consist of two lineages, a population of primitive erythroid cells surrounded by a layer of angioblasts that eventually form the developing vasculature. The parallel development of these lineages in close association provided the basis for the hypothesis that they arise from a common precursor, a cell called the haemangioblast.

The differentiation of embryonic stem (ES) cells in culture offers a powerful alternative approach to study the development of lineages that are established very early in embryonic life. Using this model system, a precursor was identified that generates blast colonies containing precursors of endothelial and haematopoietic lineages. The blast colony-forming cells (BL-CFC) that generate these colonies represent a transient population that appears in the embryoid bodies (EBs) prior to the emergence of any other haematopoietic lineage precursors. The characteristics of the BL-CFC suggest that it represents the *in vitro* equivalent of the haemangioblast and as such the earliest stage of haematopoietic development described to date.

The existence of the haemangioblast has been demonstrated recently *in vivo* in early embryos.

### Development of blast colonies and characterization of the onset of blood cell development.

We have initiated a series of studies to determine the series of cellular events leading to the generation of a blast colony from a BL-CFC. For this, we have followed the maturation of the precursor in time lapse experiments and identified different developmental stages. In collaboration with Terry Allen (Structural Cell Biology Group) we are currently further investigating by electron microscopy these early events of generation of blood cells.

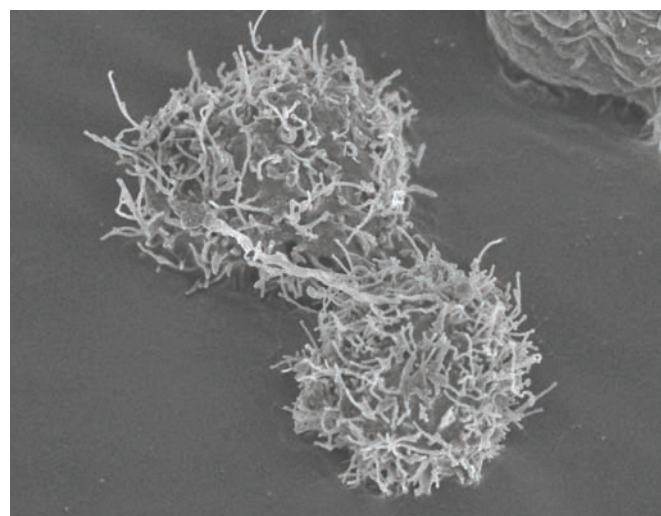


Figure 1: Electron microscopy of early haematopoietic cells generated from ES cells.

### A critical function of Runx1 in Haemangioblast development.

To investigate the role of *Runx1* at the earliest stage of haematopoietic commitment, we have analysed its expression pattern and function during ES/EB differentiation and in early yolk sac development. Expression analyses indicated that *Runx1* is expressed in yolk sac mesodermal cells prior to the establishment of the blood islands and within the BL-CFC in EBs. Analysis of early EBs revealed a profound defect in the potential of the *Runx1*<sup>-/-</sup> ES cells to generate blast colonies. Altogether these results provide evidence that *Runx1* does function



at the haemangioblast stage of development and position *Runx1* as a master regulator of the onset of blood development.

#### Downstream transcriptional targets of Runx1 at the onset of blood development

Our results have indicated that *Runx1* is required for definitive haematopoietic development at the level of the BL-CFC and is therefore likely to regulate a specific set of genes at this time of development. To identify these genes, we have 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 have then validated the differential expression of candidates on samples generated from the ES/EB system. We have 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 these genes at the onset of haematopoietic development.

#### Runx1 DNA binding partners

We have created *Runx1* variants containing a N- or C- tag which can be biotinylated *in vivo* by a biotin ligase enzyme. We have validated that both constructs are still able to rescue the haematopoietic defects in *Runx1* deficient cells. We have expressed both constructs in human Jurkat T cells and purified proteins associated with Runx1 using streptavidin beads. Peptide sequencing and mass spectrometry indicated the association of Runx1 with several components of the nucleosome remodeling and histone deacetylase (NuRD) complex, one of the major transcriptional co-repressor complexes in mammalian cells. Future experiments will investigate using this experimental approach the nature of the proteins associated with Runx1 at the onset of haematopoietic development.

#### Runx1 isoforms

Previous studies have shown that *Runx1* is expressed as multiple naturally occurring spliced isoforms that generate proteins with distinct activities on target promoters. One intriguing hypothesis is that the different isoforms of this transcription factor could fulfill distinct functions at the different stages of the establishment of the haematopoietic system. We have determined that expressions of some of these isoforms are differentially regulated during early haematopoietic development. We have generated ES cells containing reporter genes knock-in in the different isoforms or knock-out altering

the specific expression of these isoforms. We are currently investigating the respective expression of these isoforms, the biological potential of the cells expressing them and the respective function of each isoform.

#### A critical function of MOZ in haematopoietic development.

MOZ is found translocated in cases of AML with either CREB binding protein (CBP), the nuclear receptor TIF2 or the P300 transcriptional co-activator. All these genes encode enzymes containing a histone acetyl transferase domain (HAT) suggesting that aberrant modification of histones or other factors could provide the first step in the route to oncogenicity. To evaluate the role of the HAT activity of MOZ, we have created a mouse model with a point mutation inactivating the HAT activity of MOZ. Analysis of these mice has revealed a profound defect in haematopoiesis. The numbers of haematopoietic foetal liver precursors is dramatically affected by the mutation. Haematopoietic stem cells carrying the mutation present a strong defect in competitive repopulation assays. These *in vivo* results were substantiated with ES cells mutated for the HAT activity of MOZ. Much less primitive and definitive haematopoietic precursors were generated with these mutated cells. These studies with embryonic stem cells allowed us to further pinpoint the defect in absence of the HAT activity of MOZ to the balance between normal proliferation and differentiation of haematopoietic precursors. We are currently trying to identify the critical genes whose expression is epigenetically regulated by the HAT activity of MOZ.

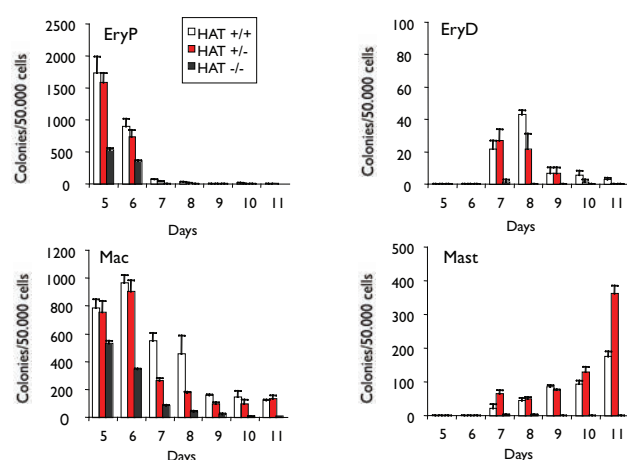


Figure 2: Analysis of primitive and definitive haematopoietic potential of HAT<sup>+/+</sup>, HAT<sup>+/-</sup> and HAT<sup>-/-</sup> MOZ ES cells. Primitive erythroid (EryP), definitive erythroid (EryD), macrophage (Mac) and mast cell (Mast) colonies generated by HAT<sup>+/+</sup>, HAT<sup>+/-</sup> and HAT<sup>-/-</sup>.





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# Stem Cell and Haematopoiesis Group

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The differentiation of embryonic stem (ES) cells offers a powerful approach to study mechanisms implicated in cell fate decision. A major hurdle however is to promote the directed and efficient differentiation of ES cells toward a specific lineage. To overcome that challenge, we have established a serum-free culture system allowing the selective and efficient differentiation of ES cells toward haematopoietic progenitors. We have defined in serum-free media the minimal growth factors that are required to control each step of the differentiation process. BMP4 (Bone morphogenetic protein 4) promotes the very efficient formation of mesoderm, FGF (Fibroblast growth factor) and Activin A induce the differentiation of these mesodermal precursors to the haemangioblast fate, and VEGF (Vascular endothelial growth factor) is required for the production of fully committed haematopoietic progenitors. This step-wise control of differentiation is extremely efficient and provides a perfect system for understanding the molecular machineries involved in blood progenitor commitment.

## ***In vitro* differentiation of ES cells as a model system to study lineage specification:**

Upon differentiation, mouse ES cells can give rise to primitive and definitive haematopoietic precursors, an *in vitro* process that was shown to accurately recapitulate the *in vivo* development of yolk sac haematopoiesis. This progressive differentiation can be monitored by measurement of gene expression and quantitative analysis of biological potential. As they lose their self-renewal and pluripotent characteristics, ES cells form epiblast-like cells which differentiate further to give rise to mesodermal precursors. The first blood precursor, the haemangioblast, derives from the mesoderm and gives rise to primitive and definitive haematopoiesis, smooth muscle and endothelium. This carefully orchestrated process can be reproduced *in vitro* upon the differentiation of ES cells in well-defined

conditions, fetal calf serum been one of the key components of the culture conditions. Although the inherent characteristics of ES cell lines are likely to be important for an efficient differentiation, the batch of serum used appears critical in obtaining the most effective differentiation. This fact illustrates the complexity of serum composition and the many parameters that may modulate positively or negatively the differentiation process. To dissect and understand the molecular mechanisms of cell fate specification, refined culture conditions are needed to allow the specific and efficient differentiation of ES cells toward haematopoiesis.

## **Selective and efficient differentiation of ES cells toward the formation of blood progenitors:**

To define the minimal requirement for the generation of blood progenitors from mouse ES cells in absence of serum, we assessed several serum-free media and soluble factors previously shown to induce some degree of mesoderm or haematopoiesis specification. To monitor mesodermal differentiation, we used an ES line (GFP-Bry) carrying the cDNA for green fluorescence protein targeted to the Brachyury locus. We have previously shown using this ES cell line that GFP expression recapitulated effectively Brachyury expression and could be used to track mesoderm formation *in vitro* and *in vivo*. Using the *in vitro* differentiation of GFP-Bry ES line, we have studied the requirement for each step of the differentiation (figure 1): ES cells to epiblast-like cells (step 1), epiblast-like cells to mesodermal precursors (step 2), mesodermal precursors to haemangioblast (step 3) and haemangioblast to committed blood precursors (step 4).

Altogether, our data demonstrate that commitment to the haematopoietic lineage can be driven efficiently by only four factors in the absence of serum and that each step of this differentiation can be controlled by only one or two factors (Figure 1). The removal of leukemia inhibitor factor (LIF) and feeder cells which together keep ES cell undifferentiated, is sufficient to trigger the progression from

# STEM CELL AND HAEMATOPOIESIS

ES cell to epiblast-like cell stage (step 1). These data suggest that no added factors are necessary for the transition from ES cells to epiblast-like cells. The transition from epiblast-like cells to mesodermal precursors (step 2) requires the addition of BMP4 in a dose dependent manner. The specification of haemangioblast from the mesodermal precursors (step 3) is induced very efficiently and rapidly upon stimulation with FGF and Activin A. The last step of differentiation (step 4), allowing the formation of committed blood precursors from haemangioblast is triggered upon the addition of VEGF to the culture already stimulated by the sequential addition of BMP4, Activin A and FGF. The very high fraction of haematopoietic cells within the EBs underscores the efficiency with which this combination of factors specifically promotes haematopoietic commitment at the expense of other lineages.

## Molecular analysis of haemangioblast precursor specification:

A robust increase in the number of haemangioblast precursors can be detected within a few hours of stimulation with Activin A and FGF (Figure 2). This very rapid response suggest that Activin A/FGF might promote the specification of mesodermal precursors to the haematopoietic program rather than inducing the proliferation of a few haemangioblast already present in the EBs. To dissect the molecular mechanisms implicated in haemangioblast commitment, we performed a screening for genes implicated in this process via Affymetrix microarray analysis. Three independent samples for each time point were harvested: (T0) prior to stimulation with Activin A and FGF and 6 hours later

either without (T6) or with the addition of Activin A/FGF (T6+). Secondary replating assay was performed to assess the number of haemangioblast precursors in each population (Figure 2A). Analysis of the microarray data revealed that 33 genes were differentially regulated with a fold change greater than 2 and with a p value lower than 0.001 (Figure 2B). We are now in the process of investigating the role of these selected genes in the specification of haemangioblast precursors.

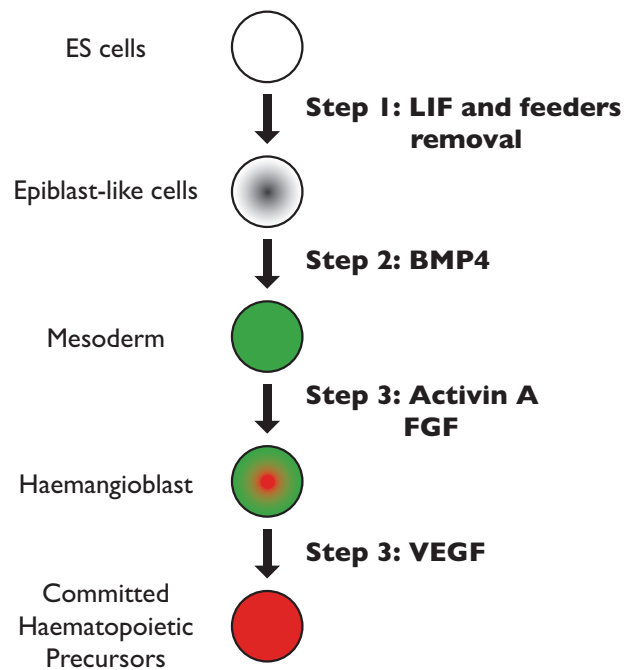


Figure 1: Schematic representation of ES cell differentiation to the haematopoietic fate.

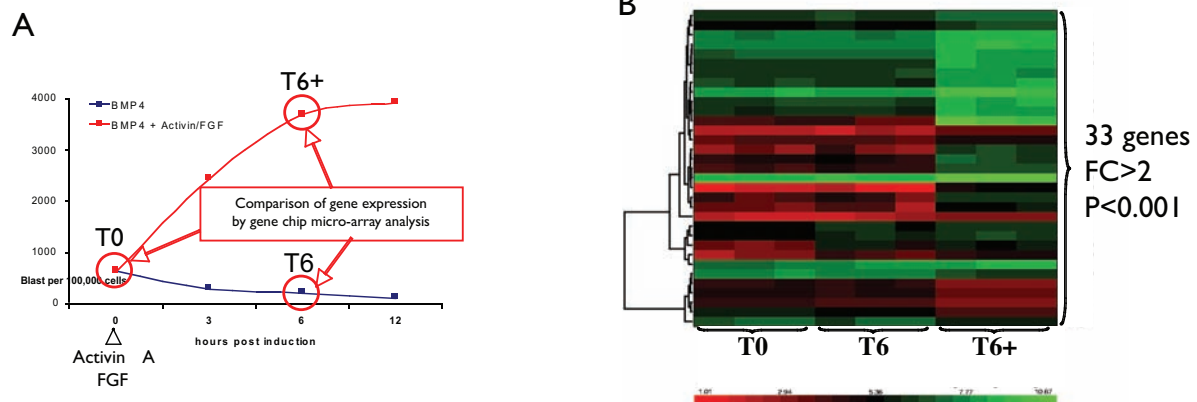


Figure 2: (A) Kinetic of blast colony formation upon stimulation with Activin A and FGF. (B) Heat map representation of transcripts found to be differentially expressed upon Activin A and FGF induction.



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# Structural Cell Biology Group

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Higher eukaryotes are characterised by the separation of nucleus and cytoplasm with a nuclear membrane, which undergoes two major dynamic events during the cell cycle. At mitosis the entire structure is dismantled and reformed, and during interphase the nuclear surface area and pore complex populations increase by a factor of two. Our experimental endpoint is direct, *in situ*, 3D macromolecular visualisation of specific proteins and multiprotein complexes. These studies incorporate GFP labelling for live cell imaging coupled with immunogold localization at the EM level. We are also investigating the roles of condensin and cohesin in the processes of chromatin condensation and chromosome separation. Collaborations within the Institute currently involve projects on apoptosis, yeast mitosis and haematopoietic stem cell interactions.

## Nucleoporin interactions during nuclear pore formation.

New nuclear pore complexes (NPC) are inserted directly into the nuclear envelope (NE) throughout interphase. At mitotic NE reformation however, the nucleoporin (nup)107/160 complex associates directly with the chromatin surface before NE membrane reformation. This complex locates to kinetochores during prophase, but also over the chromosome arms from metaphase onwards. Labelling for other structural nucleoporins by mAb 414 is absent from the chromosomal surface until anaphase, and appears to be concentrated around the spindle poles. Thus although NPC formation is initiated by binding of the nup107/160 complex to chromosome surfaces at late metaphase, it may not be completed until the chromosomes have migrated towards the spindle poles for recruitment of the remaining NPC structural elements.

## The structural visualisation of cohesin and condensin in complex with condensing chromatin.

Cohesin is a multisubunit complex that mediates sister-chromatid cohesion. Cohesin complexes link replicated DNA duplexes by forming huge ring structures which 'embrace' both duplexes until cleavage is brought about during anaphase by the cysteine protease separase. We are studying cohesin loading onto DNA duplexes in S-phase, investigating both the possible *de novo* assembly of cohesin on chromatin and the transient opening of the cohesin ring to allow entry of DNA molecules. Cohesin may also mediate the repair of double-strand DNA breaks. We are using immunogold and fluoronanogold labeling by light microscopy (LM) and Field Emission In Lens Scanning Electron Microscopy (FEISEM) to investigate the predicted ring structure of cohesion.

Condensins are structurally related to cohesin, possessing two SMC (Structural Maintenance of Chromosomes) core subunits, but differ in action to produce condensation of replicated chromatin. Condensins I and II consist of the hSMC2 and hSMC4 core subunits in combination with three non-SMC regulatory subunits, forming distinct complexes which interact with chromatin at specific stages during condensation. Our data support a model in which the condensins function as molecular hinges, contacting two points on a chromatin fibre in their open conformations and bringing them into closer proximity upon its closure.

## Maintenance and regulation of structural organization at the nuclear surface

Our studies into the function of the human orthologue of the integral membrane nucleoporin gp210 have involved down-regulation of protein expression using the pSUPER vector system. FEISEM studies have revealed that a reduction in gp210 expression results in a loss of spatial organisation where individual NPCs are no longer maintained at



a uniform distance from each other. Ultra-structural visualisation indicates that the nuclear lamina interacts with the “spoke-ring” component of NPCs. This is a large annular structure, thought to be at least partly composed of gp210 oligomers, that encircles the channel through which molecules are transported and is embedded within the lumen of the NE. Therefore it is likely that this structure contributes to the positional regulation of NPCs within the plane of the NE. FACS analysis of HeLa cells transfected with our pSUPER gp210 knock-down constructs shows that a significant proportion of these cells contain activated caspase-3. This strongly suggests that gp210 is an essential protein without which cells initiate apoptosis.

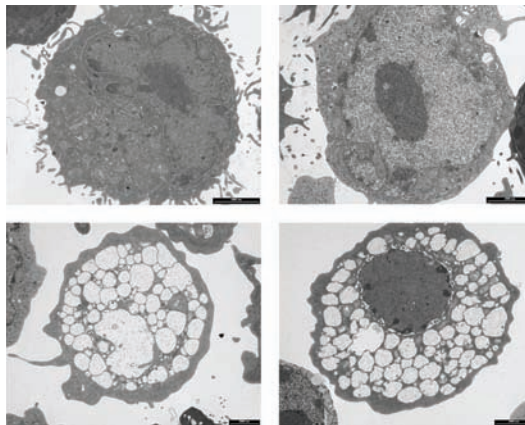


Fig 1 Increased c-SRC expression in HCT 116 colorectal cancer cells results in intense cytoplasmic vacuolation and nuclear disorganization of detached cells, which undergo cell death in a characteristic manner, but without classic apoptotic morphology. (top micrographs from attached cells, lower micrographs from detached cells).

## Ultrastructural aspects of cell death

Accidental (necrosis) versus Programmed (apoptosis) cell death is now too limited an option to describe the spectrum of cellular demise. Necrosis is best used for features that appear after the cell has died, and Oncosis, (Greek –swelling), describes accidental death. Apoptosis is the mechanism for reducing cell numbers in tissue, characterized by nuclear alteration of chromatin condensation and fragmentation, blebbed membranes, loss of endoplasmic reticulum and mitochondrial integrity, followed by phagocytosis by adjacent cells. Of the various pathways leading to an apoptotic endpoint, ‘anoikis’ (Greek - state of homelessness) is triggered by ‘loss of cell anchorage’ The ability to overcome detachment-induced apoptosis is a crucial step in the oncogenic transformation pathway. In a recent collaborative study involving an induced

increase in c-SRC expression levels in a colorectal cancer cell line (Welman *et al* Neoplasia 2006, 8, 905), ultrastructural observations confirmed the induction of a cell death pathway in detached cells characterised by nuclear alteration and intense cytoplasmic vacuolization, which was associated with the accumulation of cells in the G2 phase (Figure 1).

In collaboration with the Stem Cell Biology group, we have initiated investigations into the cellular interactions during the early stages of blast colony forming cell differentiation (see page 28). Figure 2 shows images visualizing cell interactions in haematopoietic differentiation *in vitro*. Cultures have been observed by LM time lapse (Fig 2:2), then fixed *in situ* for both SEM and TEM with specific areas relocated for direct comparison by vertical or ‘en face’ sectioning by TEM, (Fig 2:3) or whole cell interactions by SEM (Fig 2:1).

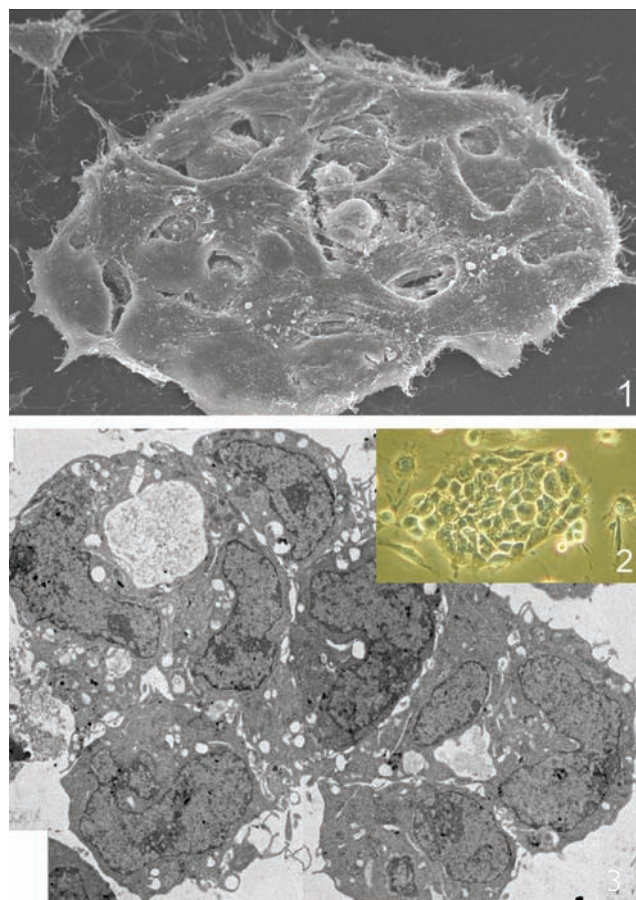


Fig 2 Haematopoietic cell association in culture, visualized by SEM (1), LM (2), and sections cut parallel to the growing surface for TEM (3).



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# Cancer Studies Academic Radiation Oncology: Translational Radiobiology Group

The Translational Radiobiology Group, part of Academic Radiation Oncology (ADRO) in the Christie Hospital (head Prof Pat Price) has laboratory space within the Paterson Institute. The group explores and develops methods for the individualisation of radiotherapy. With the advent of high-throughput techniques, the group is interested in characterising molecular profiles that reflect relevant biological phenotypes and predict tumour and normal tissue response to radiation.

## Development of a hypoxia transcriptome in head and neck cancer

The group has a long interest in evaluating methods for assessing tumour hypoxia and a prospective study was established in patients with head and neck cancer to investigate the potential of RNA microarrays (Priy Silva). This work involves collaboration with Prof Adrian Harris at the Weatherall Institute of Molecular Medicine in Oxford, Dr Francesca Buffa at the Gray Cancer Institute, Dr Crispin Miller (Bioinformatics Group), Dr Nick Slevin (Clinical Oncology), Mr Jarrod Homer (Surgery) and Prof Phil Sloan (Pathology, Manchester Royal Infirmary). Tumour (n=59) and normal (n=11) tissue samples were arrayed and a signature representing 99 up-regulated genes was derived by clustering around the tumour expression of 10 well-validated hypoxia-associated genes (eg VEGF, CA9). The median RNA expression of all 99 genes was used to investigate the prognostic ability of the signature (HS-up). In multivariate analysis in an independent head and neck cancer dataset including an intrinsic classifier and established clinico-pathological prognostic variables, HS-up was a significant and independent prognostic factor outperforming the intrinsic classifier. HS-up also had prognostic information in a published breast cancer

dataset, again independent of established variables and a trained intrinsic signature. During the past year work was established to validate the signature in terms of investigating the hypoxia induction of novel genes identified in the work (Sara Bhana).

## Development of a homogeneous database of head and neck patients

Validation work for the hypoxia signature will include examining the prognostic significance of novel genes. A homogeneous database of head and neck cancer cases was established, therefore, comprising patients with oropharyngeal cancers (tonsil/tongue base) who received radiotherapy for their primary tumour; 133 patients were identified and tumour blocks obtained from 79 (Priy Silva, Helen Valentine). Features associated with poor locoregional control were low Hb level ( $p=0.035$ ) and advancing disease stage ( $p=0.007$ ). HIF-1 $\alpha$  expression was a more significant adverse prognostic factor in tonsil (HR=23.1, 95% CI 3.04-176.7) than tongue base (HR=2.86, 95% CI 1.14-7.19) tumours ( $p=0.03$ , interaction test). High tumour HIF-1 $\alpha$  expression associated with low Hb levels ( $p=0.03$ ). In multivariate analysis, the only independent prognostic factors for locoregional control were increasing Hb level (HR=0.84, 95% CI 0.72-0.98,  $p=0.03$ ), tongue base cancer (HR=2.03, 95% CI 1.04-3.96,  $p=0.04$ ) and high tumour HIF-1 $\alpha$  expression (HR=6.60, 95% CI 2.92-14.86,  $p<0.001$ ). The work showed differences in radiotherapy outcome within a homogeneous subsite of head and neck cancers related to molecular marker expression.

## Assessment of hypoxia-associated markers in oesophagogastric cancer

Evidence for the presence and prognostic significance of hypoxia is lacking in oesophagogastric cancer. The latter, along with the need to develop

# CANCER STUDIES ACADEMIC RADIATION ONCOLOGY: TRANSLATIONAL RADIOBIOLOGY GROUP

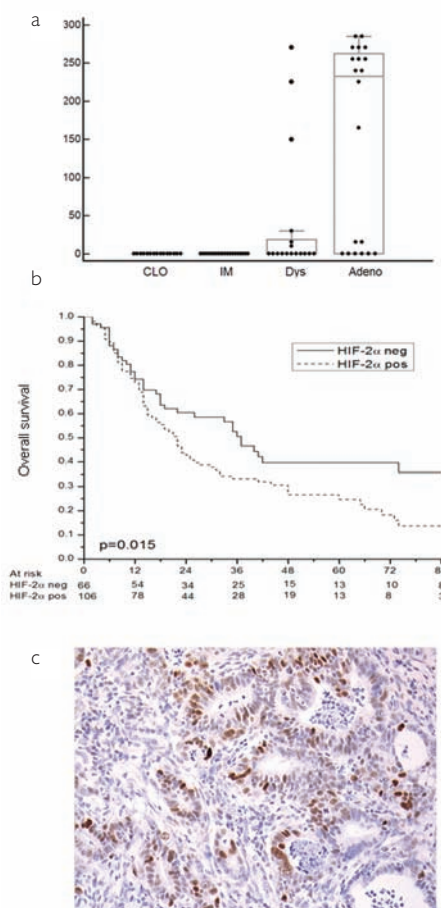
approaches for selecting patients likely to benefit from adjuvant radiotherapy, led to the initiation of a project looking at the role of hypoxia in the disease. This work (Ewen Griffiths, Helen Valentine) involves collaboration with Mr Ian Welch and Dr Sue Pritchard (Wythenshaw Hospital). Hypoxia-associated markers were studied in oesophageal and gastric carcinogenesis sequences, and 177 surgically-treated oesophagogastric cancers. Marker expression increased with progression along the carcinogenesis sequences. HIF-2 $\alpha$  was expressed late in the Barrett's sequence, and was only seen in dysplasia and cancer samples. HIF-1 $\alpha$  and HIF-2 $\alpha$  were expressed in 53% (2% >30% staining) and 63% (44% >30% staining) of tumours, respectively. HIF-1 $\alpha$  expression at the invasive tumour edge was associated with a median survival of 18 *vs* 33 months for negative tumours ( $p=0.019$ ). High HIF-2 $\alpha$  was an adverse prognostic factor ( $p=0.015$ ). Neither protein provided independent prognostic information in multivariate analysis. The late expression of HIF-2 $\alpha$  in the carcinogenesis models and its high expression in tumours (Figure) suggest it is worth further study as a marker of disease progression in patients with Barrett's dysplasia and as a cancer therapeutic target.

## Radiogenomics: assessment of polymorphisms for predicting the effects of radiotherapy (RAPPER)

Individual radiosensitivity is considered to be an inherited complex trait dependent on interactions between multiple genes/gene products. RAPPER is designed to explore associations between single nucleotide polymorphisms (SNPs) in candidate genes and radiotherapy toxicity. It is a multi-centre study with a planned recruitment of 2200 patients and is powered to detect common alleles conferring a moderate risk of late morbidity and rarer ones with larger effects. Breast, prostate or gynaecological cancer patients are being recruited to identify polymorphisms affecting radioresponse across tumour types. The project involves collaboration with Drs Neil Burnet and Alison Dunning (Cambridge), Prof Soeren Bentzen (Wisconsin) and numerous clinical oncologists locally and nationally. The day-to-day administration of RAPPER is carried out by Rebecca Elliott. The work began in July 2005 and sample accrual is on target with blood collected from 1075 patients. Radiation toxicity is being scored using LENT-SOMA. Rapid developments in high-throughput genotyping may allow study of 120 genes using SNP-tags to cover all common variation in each gene. Selection of the candidate list will be finalised in ~2 years but will focus on cell cycle checkpoint control, DNA damage response and cytokine pathways.

## VORTEX-BIOBANK

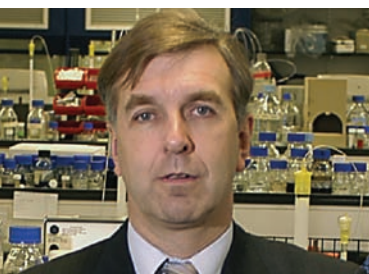
Funding was obtained from the CR-UK for VORTEX-BIOBANK. VORTEX is a national randomised trial currently in set-up. Patients with soft tissue sarcoma of the extremity who undergo post-operative radiotherapy: 66 Gy in 33 fractions once daily for 5 days a week over 6.5 weeks will be randomised to one of two different radiation volumes and the primary outcome measures are limb functionality and time to local recurrence. The target accrual is 400 patients based on non-inferiority of local control but improved limb function in patients with reduced irradiation volumes. Joely Irlam-Jones will manage the prospective sample collection for the trial. The translational hypothesis underlying the work is that a pre-treatment tumour molecular profile will define patients with a high risk of treatment failure following surgery and post-operative radiotherapy, and that the profile could be used in a subsequent study to identify patients who might benefit from adjuvant systemic therapy. The secondary hypothesis is that there is an association between SNPs in relevant candidate genes and individual patient variability in normal tissue radiation toxicity. It is envisaged that samples for microarray analysis will be collected at surgery from at least half of the patients. Paraffin blocks for tissue microarrays and blood samples for future genotyping should be collected from the majority of patients.



Box and whisker plot of HIF-2 $\alpha$  expression in the Barrett's (15 columnar-lined metaplasia, 20 intestinal metaplasia, 17 dysplasia, 20 adenocarcinoma) carcinogenesis sequence (a). Tumour HIF-2 $\alpha$  expression vs overall survival in 177 oesophagogastric cancer patients (b). Tumour section showing high HIF-2 $\alpha$  expression (c).

Publications listed  
on page 62





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## Cancer Studies: Biological, Immune and Gene Therapy Group

In order to translate laboratory discoveries into effective clinical therapies, partnerships involving scientific and clinical researchers as well as the commercial sector are key. It is from this platform that the Biological, Immune and Gene Therapy Group has been established. The focus of the group is on development of novel immunotherapies with a particular interest in cellular based therapies, genetic vaccines and antibodies. This report details the group's current clinical trial activity and highlights the translational interface that exists from target discovery through to later stage Phase II and III trials. In recent years the group has spearheaded the development of two novel agents from pre-clinical development through Phase I and on to Phase III trials. The group is also involved with other trials and is the largest recruiter to trials in the Christie Clinical Trials Unit (Derek Crowther Unit).

#### Clinical trials of TroVax – 5T4 vaccine

With Oxford Biomedica we have been involved in the development of 5T4 vaccines from pre-clinical and through all phases of clinical trials. The main Phase I trial (Clin Can Res. 2006; 12: 3416-24) of TroVax in colorectal cancer showed safety and immunological efficacy as well as encouraging correlates of survival with immune responses. Subsequent Phase II studies combining TroVax with chemotherapy in colorectal cancer and with interferon in renal cancer have been successfully completed and have led to the development of Phase III studies in each of these diseases. An international Phase III study of TroVax in combination with standard therapy for renal cancer has been initiated by Oxford Biomedica with Manchester as the lead UK site. For colorectal cancer we are developing a national study with the

NCRN colorectal group combining TroVax with chemotherapy and Bevacizumab.

A mechanistic study sponsored by CR-UK investigated the effect of TroVax in patients undergoing resection of colorectal cancer liver metastases. The objectives in this trial included assessment of systemic immune responses before and after surgery as well as local tumour immunity and clinical outcomes. Twenty patients undergoing resection of liver metastases by David Sherlock at North Manchester General Hospital received 2 vaccinations pre-operatively, 2 post-operatively and 2 further boosting vaccinations if immune responses were detected. Immunological assays indicate that 15 of the 20 patients have T-cell or T-cell plus antibody responses to 5T4 or subunit peptide within 14 weeks. Of the 16 protocol-compliant patients 13 have demonstrated responses. Currently we are investigating the levels of regulatory T-cells (T<sub>reg</sub>) in peripheral blood and local immune factors by immunohistochemistry and isolated tumour infiltrating lymphocytes.

#### 5T4 superantigen therapy

Targeting of superantigen to a tumour-associated antigen can direct T-cell mediated cytotoxicity. Building on previous successful Phase II studies with a first generation agent (Shaw *et al* in press) we have now undertaken a Phase I trial (with Active Biotech) with a re-engineered molecule. This shows improved tolerability and encouraging clinical results. A PET mechanistic study has also been completed (with Peter Julian / David Hastings / Jamal Zweit) and this shows tumour localization of the fusion protein in all patients tested. An International Phase II/III study in renal cancer is due to commence shortly with Manchester as the lead centre.



# CANCER STUDIES: BIOLOGICAL, IMMUNE AND GENE THERAPY GROUP

## Adoptive transfer of T<sub>reg</sub> depleted autologous T-cells

With the Urology Group (Noel Clarke/Vijay Ramani) we have demonstrated that CD4+CD25<sup>high</sup> T<sub>regs</sub> are present in increased numbers in the peripheral blood of patients with advanced cancer compared to normal controls. T<sub>regs</sub> suppress proliferation and cytokine release from effector cells and, in animal models, depletion of CD25<sup>+</sup> T-cells can induce tumour responses. We therefore initiated a study to examine the effects of T<sub>reg</sub> depletion on renal cancer. Six patients with advanced renal cancer were recruited and underwent leukapheresis followed by conditioning chemotherapy with cyclophosphamide and fludarabine. The autologous leukapheresis product was depleted of CD25<sup>+</sup> cells by magnetic selection using the CliniMACS<sup>®</sup> system then reinfused into the patient. T<sub>reg</sub> depletion of the leukapheresis product was efficient and there was no toxicity of the infused cellular product. Transient reductions in T<sub>regs</sub> in the peripheral blood of patients were noted with evidence of increased T-cell responses to the tumour-associated antigen 5T4 in at least one patient. This was associated with mixed/minor responses in two patients. We are now looking to combine this treatment with vaccines or with engineered T-cells.

## Anti-CTLA-4 monoclonal in advanced oesophago-gastric cancer

CTLA4 is a transmembrane protein which is a crucial inhibitor of T-cell activation. Anti-CTLA4 antibody blockade has been shown to have therapeutic effects in melanoma. We are therefore exploring the effects of an anti-CTLA4 monoclonal antibody (Ticilimumab, Pfizer) in a Phase II study in advanced oesophago-gastric cancer. As well as clinical efficacy we are examining pharmacodynamic effects on lymphocyte phenotype (eg. CD25, CTLA4, and FoxP3), and for the development of active immune responses against 5T4. The trial opened in September, and has recruited eleven patients to date. Toxicity has been acceptable, and the first patients are due formal re-staging of their disease by the New Year.

## Sequential therapy of imiquimod and photodynamic therapy for vulval intraepithelial neoplasia (VIN)

The aim of this Phase II study (with Henry Kitchener) was to demonstrate the tolerability of Imiquimod and photodynamic therapy (PDT) used sequentially and to assess lesion and immunological response in women with VIN. The hypothesis was

that VIN lesions pre-treated with an immune response modifier would respond more favorably to PDT. Treatment consisted of Imiquimod and PDT using methylated ALA as a photosensitiser. Follow up at 12 months is available for 10 women and response rates are maintained at this time point with 3 complete responses and 4 partial responses. Pharmacodynamic analysis showed that Imiquimod significantly increased CD8 T-cells within VIN lesions and that non-response to Imiquimod was associated with an increase in T<sub>regs</sub> within VIN lesions.

## Trials with engineered T-cells

Trials of engineered T-cells (see Cell and Gene Therapy Group) are awaiting final approval by the MHRA following recent changes in the regulations governing first into man trials of immunotherapy. For our CR-UK sponsored trial targeting CEA the GMP virus has been produced and production runs of gene modified T-cells carried out to GMP (with Eric Austin at Manchester Blood Centre). Likewise assay validation for immunological and molecular monitoring of the trial has been completed and ethical approvals are in place. The trial targeting CD19 is at a similar point although delays in production of GMP virus mean the trial could not start before March 2007.

## Other large scale trials

We are also involved in trials of agents targeting other biological pathways in renal and oesophago-gastric cancer. In renal cancer a number of major trials have been completed and these are changing the way we treat renal cancer. Building on successful recruitment to trials of anti-angiogenic drugs we are investigating how these interact with the immune system as a means of rationally developing combination trials. Following our presentation at ESMO of interesting results from a large Phase III trial with Lapatinib (Glaxo) in renal cancers over-expressing EGFR we are also planning to look at combination studies.

## Summary

The group, through interactions with laboratory groups in Manchester and elsewhere and with the pharmaceutical industry, has a vibrant trials portfolio focusing on a variety of ways to manipulate the immune system.



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## Children's Cancer Group

The Children's Cancer Group (CCG), formed in 2000, was previously located within the laboratories of the Institute of Cancer, Queen Mary University of London Charterhouse campus. In October of 2006, the group relocated to the Paterson Institute for Cancer Research at the University of Manchester. The focus of our research lies in improving the outcome of children with acute lymphoblastic leukaemia (ALL). On the clinical front, this involves designing and running of novel international clinical trials for those with high risk disease. In the laboratory we are exploring biological explanations for the variations in response to therapy.

### Clinical Trials:

#### ALL R3

This trial is for children with ALL who relapse after initial therapy. The trial recruits patients from all 21 paediatric centres in UK and Ireland as well as from centres in Netherlands, Australia and New Zealand. An indigenously designed web-based remote entry database with decision support tools has been made to run the trial. Minimal residual disease estimation after 5 weeks of therapy is used to risk stratify children. Those who have high levels of disease at this stage are offered allogeneic stem cell transplantation. The trial opened in the UK in 2003 and to date has recruited 178 patients. The overall survival of patients is 70% at 3-years. A number of clinical and cytogenetic markers of poor prognosis have been identified. An interim analysis is being performed to better inform us of the progress of the trial and help us design the next trial when this one closes in 2010.

#### EsPhALL

This trial is randomising the use of imatinib in children with Philadelphia positive (Ph+) ALL. All children with this disease in the UK are eligible for

allogeneic transplantation after completing 5 blocks of treatment. We have obtained funding from the Leukaemia Research Fund to monitor the speed of response to therapy, identify key mutations and unique gene expression patterns in these patients. Our preliminary studies suggest that mutations in the ABL kinase domain are present in some children with Ph+ ALL, but this does not necessarily predict for a poor response to therapy. On the other hand mutations in cell cycle kinase genes appear to do so. This trial will continue to 2010.

### BIOV-111

This is a phase II trial of the drug Clofarabine in resistant and relapsed ALL. It is currently recruiting in 10 countries throughout Europe and will close early next year. At the moment the response rate is 28% and some refractory patients have been transplanted in remission. This is highly promising for a single agent in this population. Both pharmacokinetics and molecular pharmacology studies are incorporated into this trial.

### Laboratory Investigations:

#### Targets of ETV6

The ETS-related transcription factor *ETV6* is frequently found disrupted in many human malignancies, in particularly in childhood ALL, where 25% of patients have ETV6-RUNX1, t(12;21) translocation. ETV6 binds to a specific DNA sequence consisting of GGAA/T core motif within the promoters of its target gene and as a result, repress its transcription. To date, there are few known target genes of ETV6, namely BCL<sub>XL</sub>, MMP3 and Fli1. We have used a Gene-ACE technique to convert ETV6 into an activator and used microarray analysis to identify putative direct targets of ETV6. Promoter and chromatin immunoprecipitation assays identify two new target genes of ETV6, namely the cytokines CCL3 and CSF2.

## Chromosome 21 and childhood ALL

We have identified and characterised a new cytogenetic sub-type of childhood ALL. This is characterised by a intrachromosomal amplification of chromosome 21 (iAMP21). A common region of amplification (CRA) has been identified between 33.192 and 39.796Mb and a common region of deletion (CRD) between 43.7 and 47Mb in 100% and 70% of iAMP21 patients, respectively. Supervised gene expression analysis showed a distinct signature for eight patients with iAMP21, with 10% of overexpressed genes located within the CRA. The mean expression of these genes was significantly higher in iAMP21 when compared to other ALL samples. Although genomic copy number correlated with overall gene expression levels within areas of loss or gain, there was considerable individual variation. A unique subset of differentially expressed genes, outside the CRA and CRD, were identified when gene expression signatures of iAMP21 were compared to ALL samples with ETV6-RUNX1 fusion or high hyperdiploidy with additional chromosomes 21. From this analysis, AEP was shown to be overexpressed in patients with iAMP21. Genomic and expression data has further characterized this ALL subtype, demonstrating high levels of 21q instability in these patients.

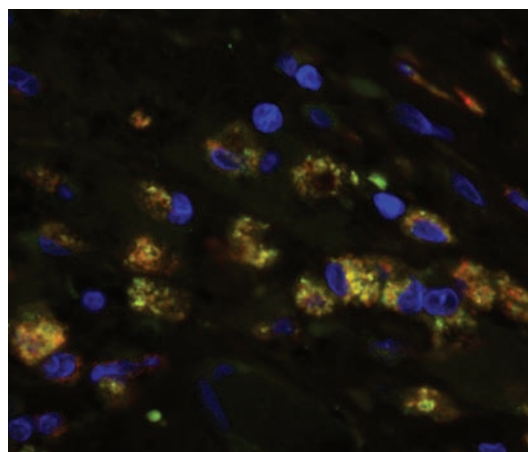
## AEP over expression and high risk ALL

We have now further demonstrated that AEP is overexpressed in high risk ALL and those with extramedullary relapse. Western analysis identifies high levels of active AEP in blast cells obtained from these patients. Leukaemic cell lines over-expressing AEP show increased migration and invasiveness *in vitro* assays and produce extramedullary

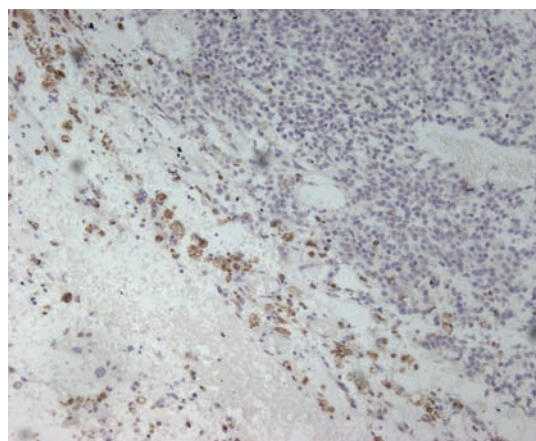
tumours in NOD-SCID mice. This behaviour is inhibited by AEP specific inhibitors. AEP also cleaves the drug Asparaginase and we believe this not only activates the drug but may increase its MHC Class II processing resulting in the production of inactivating antibodies.

## Plans for 2007

The group is in the process of reorganisation and will add a new senior post doctoral fellow and clinical senior lecturer in 2007. The clinical trials will move towards further integration with the laboratory with the development of a national cell bank in Manchester for relapsed and refractory ALL. We plan to use the new chromosome 21 tiling arrays to further investigate the differences in the transcriptome of chromosome 21 in childhood ALL. The ETV6 targets will be further examined using a ChIP on chip approach and we will investigate the possibility of collaboration between ETV6 and RUNX1 on the CSF2 and CCL3 promoter. The role of AEP in resistant disease is being investigated using a number of different tools.



The AEP expressing cells are tumour associated macrophages. Blue = DAPI; Red = antiCD68; Green = AEP



HE section of glioblastoma showing cells highly positive for AEP expression (brown) at the edge of the tumour.



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## Medical Oncology: Breast Biology Group

Normal tissues are maintained by the self-renewal capacity of a rare population of stem cells, which divide asymmetricaly both to replace themselves and to generate progenitors. After limited cell division, progenitor cells produce the non-dividing differentiated cells specific for each tissue. An emerging concept is that in leukemia as well as in neural and epithelial cancers, including breast cancer, only a minority of cells, i.e. the “cancer stem cells”, have the capacity to initiate tumours; the others are committed to differentiation pathways and senescence. Thus, characterising the cancer stem cell and understanding the molecular basis for dysregulated self-renewal will be crucial for identification of targets for effective therapeutic intervention.

### Stem cell self-renewal pathways

Identification of stem cell self-renewal pathways is emerging as important for cancer prevention, and for the future therapy of treatment-resistant cancer stem cells that have the capacity to initiate tumours and recurrence. Although some cell-specific markers and signal transduction pathways are similar in normal and cancer stem cells, the tight regulation of self-renewal that is operative in the normal stem cell may well be disrupted in cancer. Thus, understanding the regulation of self-renewal will be crucial for improving therapeutic intervention by targeting the cancer stem cell that is predicted to be inherently chemo- and endocrine resistant and to initiate tumour recurrence.

Our current aim is to exploit culture and *in vivo* techniques to address the regulation of normal and cancer stem cells in the breast. We have already established methods to isolate human mammary epithelial stem cells from normal tissue, using

Hoechst dye-efflux to obtain the mammary cell ‘side population’. More recently, we have exploited the expression of ALDH1 in stem cells by using the commercial substrate Aldefluor to enrich for stem cell populations. Both these methods can be coupled with the use of mammary stem cell surface markers. To identify the factors that regulate breast stem cell phenotype, we are collecting material from consenting patients undergoing surgery for either breast reduction, removal of a benign or primary tumour, ductal carcinoma *in situ* or pleural effusions (from advanced breast cancer patients).

One current focus is the signalling pathways regulating stem cell self-renewal of which Notch, Wnt, ErbB, Prl and ovarian hormones are of particular interest. We have shown the aberrant activation of the Notch receptor signaling pathway in breast cancer (see Stylianou *et al.*, 2006). We have extended these studies of Notch signaling and have data demonstrating its importance in regulation of breast cancer stem-like cells, using methods for non-adherent mammosphere suspension culture (see figure), analogous to neurosphere culture that enriches for brain stem cells. Our recent results indicate that in this culture system self-renewing stem cells can be enriched and passaged, and the contribution of the above signalling pathways can be assessed. Their importance in an *in vivo* model of tumourigenesis (the gold standard stem cell assay) is also being actively investigated.

### Gene expression and functional genomics

Gene expression arrays and functional genomics methods are being employed to identify novel pathways that participate in stem cell regulation. For the functional genomics studies, we are collaborating with Rene Bernards’ group at the NKI, Amsterdam to use a retroviral short hairpin (sh) RNA library that targets approximately 8,000 genes (Nature,



# MEDICAL ONCOLOGY: BREAST BIOLOGY GROUP

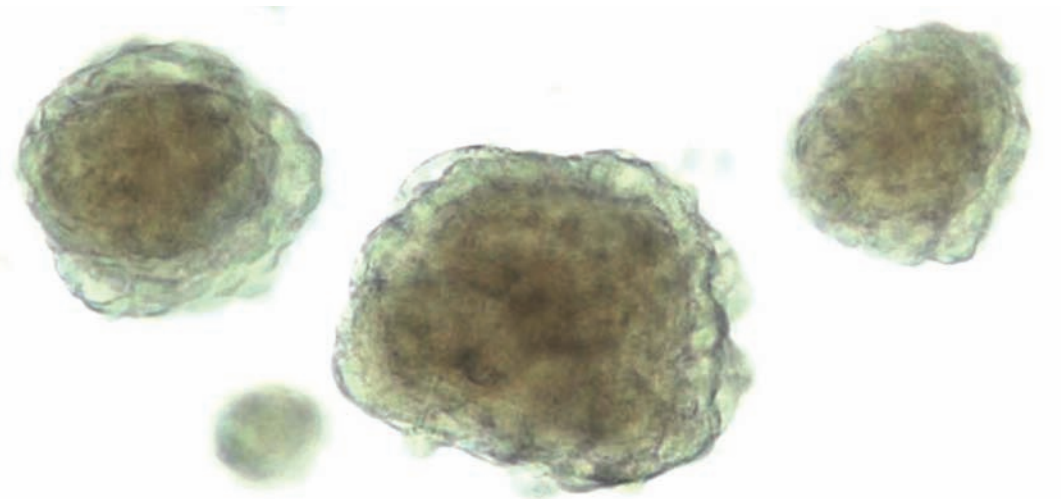
2004, 428: 431-7). We use the library to screen for genes that function in stem cell self-renewal by using several rounds of mammosphere culture to enrich for integrated shRNA sequences that increase production of non-adherent sphere colonies. DNA must be collected at each passage and 'bar-coded' by amplification, labelling and hybridisation to custom arrays of the shRNA sequences. The enriched sequences light up on the array and the most important ones will be increased by each passage of mammospheres. We are currently testing the candidate shRNA sequences in our normal and cancer stem cell assays.

## Gene expression and risk of breast cancer

Another area of focus is gene expression and risk of breast cancer where we compare tissues from women at high or normal risk of breast cancer, for example, parous versus nulliparous women or those with a family history of breast cancer versus population controls. To inform these investigations, we have developed an *in vivo* model where nulliparous human breast tissue is exposed to pregnancy levels of ovarian hormones and we can observe significant changes in genes previously identified in other model species. If these genes can be modulated in

women, then it may be possible to reduce the increasing incidence of breast cancer. We therefore collaborate with Professors Howell and Evans at the South Manchester Family History Clinic to examine the effects of preventative strategies to see whether relevant gene expression is altered following a clinical intervention such as a calorie restricted diet.

It is hoped that the results of these investigations should lead to an increased understanding of the biology of the normal human breast which, in turn, could lead to the development of new strategies or new targets for breast cancer prevention and therapy.



The photomicrograph shows a bright field image of mammospheres grown from dissociated normal human breast epithelial cells obtained from a reduction mammoplasty specimen. The mammospheres are colonies grown in suspension from single stem-like cells. They are analogous to neurospheres that have been demonstrated to enrich for brain stem cells. Magnification x400



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## Medical Oncology: Cell and Gene Therapy Group

The primary focus of the Cell and Gene Therapy group is upon the development of engineered T cells to eradicate cancer. Pre-clinical studies generated over the past five years have formed the basis of two clinical trials which are presently at the final regulatory hurdle (see Biological, Immune and Gene Therapy Group report). Consequently, the focus of our work has moved towards the use of model systems to investigate the mechanisms of activity of engineered T cell therapies with the aim of improving the overall therapeutic approach. A key recent development is the ATTACK programme which is a Framework 6 EU funded project (Co-ordinator Professor Hawkins) which brings together 14 laboratories to work on the pre-clinical development of engineered T cell strategies. Importantly, local collaborative work with various groups within the PICR (including Immunology and Carcinogenesis) is exploiting these model systems in order to discover how combinations of therapeutic approaches may synergise thereby producing more potent therapies.

### Engineered T cells

The core activity of the research group is the genetic engineering of T cells. In essence, this involves the stable introduction of genes encoding chimeric immune receptors (CIR) into primary T cells. CIRs consist of scFv (derived from antibodies) fused to the signalling domain of important T cell receptors (e.g. CD3 $\zeta$ ). The group has spent some considerable time optimising methods for the gene modification of primary human and mouse T cells with the result that we have protocols which use retroviral vectors to reliably and efficiently introduce the

CIR gene into T cells. T cells engrafted the CIR then target tumour cells through the scFv part of the CIR resulting in the specific destruction of the tumour cell. There are two principal targets used in the group – carcino-embryonic antigen (CEA – gastro-intestinal cancers and others) and CD19 (B cell lymphoma in collaboration with Prof. J. Radford, Medical Oncology) and these are the targets of the two clinical trials currently held up in regulation (see clinical trial section). Human T cells engrafted with anti-CEA.CD3 $\zeta$  or anti-CD19.CD3 $\zeta$  receptors kill their respective antigen-expressing tumour cell lines *in vitro* and also can effectively challenge the growth of these tumour lines in immuno-compromised animal models.

While immuno-compromised animal models provide a setting to investigate the functionality of human T cells, these models lack the power to fully explore the implications of adoptive cell therapy in the context of a normal, functioning host immune system. To this end, we have developed immuno-competent models for both CEA and CD19 and mouse T cells harbouring the respective receptors are indeed active in challenging the growth of tumours. However, in order to achieve high levels of T cell engraftment, lymphodepleting chemotherapy is required which transiently reduces the number of white cells in the circulation thereby allowing the therapeutic T cells to survive long enough to carry out their anti-tumour function. Optimising the combination of chemotherapy and engineered T cell infusions in these model systems is the key to the future translation of the therapy. However, one safety concern is the possibility of auto-immunity driven by the engineered T cells targeting tissues expressing normal or physiological levels of target antigen. To investigate this, we are using mice transgenic for CEA (CEATg) which

# MEDICAL ONCOLOGY: CELL & GENE THERAPY GROUP

express CEA protein to levels similar to that observed in man. Initial experiments have proven encouraging where CEATg animals given chemotherapy and CEA specific T cells remained tumour-free with no evidence of auto-immunity while cohorts treated with the same regime but with non-specific control T cells developed tumour.

The concept of combining therapies is being explored in a broader context. Together with the Immunology group, we have recently demonstrated that mouse T cells engineered with specificity for the 5T4 tumour-associated antigen synergise with 5T4 vaccination and dendritic cell therapy to significantly improve therapy against established tumours (Jiang *et al.*, 2006). We are currently seeking to improve the 5T4 specific chimeric receptor in order to investigate the mechanisms underlying this observation. In a similar vein, in a further collaboration with the Immunology and Carcinogenesis Groups, we are exploring whether DNA damaging chemotherapy may potentiate immune therapies in our model systems. Furthermore, improving the power of the receptor itself is being undertaken with more powerful signalling domains being incorporated which may further boost the activity of the engineered T cell.

## Chimeric immune receptors in other immune cell types.

T cells are potent immune effector cells. However, many other immune cells can play an important role in eradicating tumour and exploiting these cells through chimeric receptor targeting has been one avenue of research. Our group was the first to successfully show that monocyte/macrophages can be transduced to express a chimeric receptor and to effectively combat the growth of tumour cells *in vivo* (Biglari *et al.* 2006). Current studies are working upon the expression of chimeric receptors in Natural Killer cells (collaboration with Immunology) and also seeking to exploit gene-modified haematopoietic stem cells as a source to re-populate recipient animals with multiple lineages of immune cells harbouring tumour specific chimeric receptors.

## Homing of T cells to tumour.

As an aside to our studies investigating engineered T cell function in patients with advanced CEA<sup>+</sup> tumours, we have identified the presence of an unusual chemokine within colorectal tumours which have metastasised to the liver. Chemokines play an important role in controlling the migration of cells around the body and we have identified that the chemokine Eotaxin-2 is present to very high levels in colorectal metastases and is also present

in primary colorectal tumours (Cheadle *et al.*, submitted). Eotaxin-2 is more commonly found playing a role in allergy and at present it is not clear what the biological significance of the presence of this chemokine is in colorectal tumours. However, the presence of specific chemokines in tumours does provide a potential tag with which to attempt to target engineered T cells to specifically migrate to tumour and our future work will involve attempts to manipulate T cells to respond to the presence of these tumour derived chemokines.

## Summary

The translational research carried out by our group has formed the basis for two clinical trials which are due to commence in 2007. Further development to generate future trial proposals will be dependent upon model systems being currently used in the laboratory to investigate the potential and also to assess the potential risks of gene and immune therapies.



Photo of ATTACK / CRUK sponsored Symposium in Manchester December 2006. The meeting featured the global leaders in the field.

Publications listed  
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**GROUP LEADER**  
**Gordon Jayson**

**POSTDOCTORAL  
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Alison Backen  
Claire Cole  
Steen Hansen  
Will Stimpson

**CLINICAL FELLOWS**  
Andrew Clamp  
Sin Lau  
Claire Mitchell  
Daniela Rosa

## Medical Oncology: Translational Angiogenesis Group

We have established a Translational Angiogenesis Group in collaboration with Professor Caroline Dive with the aim of defining biomarkers for anti-angiogenic agents. We will run the translational research programme for two MRC/NCRN international randomised trials of anti-angiogenic agents in ovarian cancer. The laboratory programme has identified critical FGFs in ovarian cancer and developed heparin oligosaccharide synthesis.

### Ovarian Cancer Laboratory Programme

The obligate dependency of the Fibroblast Growth Factors (FGF) on heparan sulphate (HS) for their biological activity led us to examine this axis in ovarian cancer. In previous work we had shown that stromal syndecan 1 was of prognostic significance in the disease and that syndecan 3, conventionally found on neuronal tissue, was aberrantly expressed in the tumour endothelium. Using a unique molecular probe we showed that the heparan sulphate on the tumour vascular endothelium has the capacity to activate FGF2. While the stroma bound this probe only moderately, the tumour cells were largely negative.

We investigated why the tumour cells do not express growth factor activating heparan sulphate and showed, using *in situ* hybridisation, that the tumour cells strongly express 6-O-sulphotransferase, an enzyme implicated in the synthesis of biologically active heparan sulphate. In addition we showed that the core proteins for proteoglycans were present on the surface of ovarian cancer cells. These data implied that the tumour cells make heparan sulphate but that active heparan sulphate is not present on the cell surface. By adding exoge-

nous FGF2 we were able to probe for non-activating sequences of HS but this was also reduced on the tumour cell surface suggesting that there is a global rather than sequence-specific reduction in cell surface HS. Both heparanase and H-sulf1 were expressed by the tumour cells but only heparanase could account for the findings. Thus these data suggest that heparanase is a key mediator of heparan sulphate depolymerisation at the tumour cell surface, augmenting its known role in invasion and angiogenesis.

In an extensive study of FGFs in ovarian cancer we have identified a switch in the receptor type upon transformation. This is associated with the response of the tumour cells to the appropriate ligand FGFs and enhanced chemosensitivity in inhibition studies. Thus our data (manuscript in preparation) suggest that FGF2 is relevant to angiogenesis while other FGFs play a role in transformation and the malignant phenotype, highlighting the potential of FGF inhibitors in the disease.

### Therapeutic Programme

We had previously shown that heparin octasaccharides inhibit angiogenesis *in vivo* and over the last year have developed the organic chemistry to make synthetic heparin octasaccharides. This work is ongoing and will provide uniquely pure reagents to test structure-function relationships in the next year.

### Clinical Trial Programme

We have completed phase I trials of anti-angiogenic agents that include a pure anti- $\alpha_v$  integrin antibody and a pure anti-VEGFR2 antibody. These trials have yielded unique insights into the possible roles that the drugs might have. In the integrin trial we showed that the antibody was very well tolerated

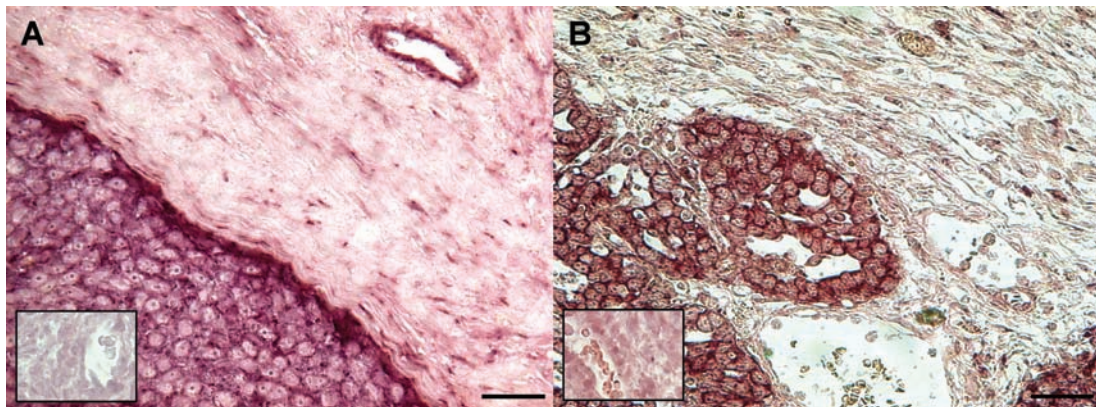


# MEDICAL ONCOLOGY: TRANSLATIONAL ANGIOGENESIS GROUP

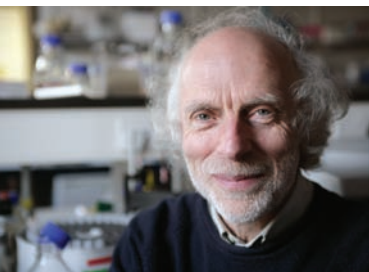
and that one patient with angiosarcoma underwent rapid improvement of her disease with single agent therapy. In the anti-VEGFR2 trial we demonstrated growth retardation using serial imaging strategies (collaborators: Jackson, Parker, Univ. Manchester) but did not see changes in vascular permeability, thereby challenging the dogma that all active VEGF inhibitors reduce vascular permeability as assessed by dynamic contrast enhanced Magnetic Resonance Imaging.

In collaboration with Prof. Sean Kehoe (Oxford) we have gained approval from CTAAC to expand the preliminary randomised study of neoadjuvant chemotherapy for the treatment of ovarian cancer. The CHORUS trial will now be completed over the

next 2 years and will test the acceptability of deferring surgery in ovarian cancer through the use of pre-operative chemotherapy.



**A.** HS6ST1 ISH shows RNA to be present in ovary tumour and endothelial cells, but absent in stroma and normal ovary (inset).  
**B.** HS6ST2 ISH shows RNA to be present in ovary tumour cells, but absent in endothelial cells, stroma and normal ovary (inset). Each scale bar represents 400mm.



**GROUP LEADER**  
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**GRADUATE STUDENTS**  
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Annie Wat  
Eun –Ang Raber (joint with Salford University)

**PLACEMENT STUDENT**  
Nicole Yan

**RESEARCH COLLABORATORS**  
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Stem Cell Biology Group

Professor Gordon Jayson and Colleagues  
Translational Angiogenesis Group

Dr Catherine Merry,  
Materials Science, University of Manchester

## Medical Oncology: Proteoglycan Group

Heparan sulphate (HS) is a near-universal cell surface co-receptor for many growth factors and cytokines. Its mode of action is still unclear, although specific structural motifs in the HS polysaccharide chain are known to be essential for binding growth factors and enabling their efficient engagement with tyrosine kinase receptors. We are investigating the molecular design of HS in different cell types, including embryonal stem (ES) cells, and examining how its unique domain structure and conformational flexibility drive the assembly of ligand-receptor signalling complexes on the plasma membrane.

### Glycosaminoglycan recognition sites in complex modular proteins

#### Hepatocyte Growth Factor/Scatter Factor

Hepatocyte growth factor/scatter factor (HGF/SF) is comprised of an N-terminal domain (N), four kringle domains (K1 to K4) and a serine proteinase homology domain (SP). It contains both Met receptor and glycosaminoglycan (GAG) co-receptor-binding sites. The alternatively transcribed NK1 fragment of HGF/SF retains the major GAG-binding site that recognises two structurally-distinct GAGs, HS/heparin and dermatan sulphate. We have recently investigated the independent GAG-binding properties of the six individual N, K and SP domains (provided by Dr Ermanno Gherardi, MRC Centre, Cambridge), using gel mobility shift and HPLC size exclusion chromatographic assays. Interestingly, GAG-binding sites were revealed in three distinct domains, with relative affinities of N>K1>>SP. All three sites bound heparin but only the N domain bound dermatan sulphate with high affinity. NMR titration data (in collaboration with Dušan Uhrín, University of Edinburgh), also indicate a substantial overlap of

the highest affinity heparin- and dermatan sulphate-binding sites within NK1. Together, these data suggest that the N domain provides a single high affinity site for both GAGs, but that long GAG chains, especially HS/heparin, may be able to engage in additional, weaker, cooperative interactions with other HGF/SF modules.

#### Factor H Complement Regulatory Protein

In collaboration with Dušan Uhrín, we have investigated the GAG-binding properties of factor H, an inhibitor of complement amplification on self-surfaces. Factor H is comprised of 20 tandem complement control protein modules. Our gel mobility shift assay demonstrated binding of a fully-sulphated heparin tetrasaccharide to the C-terminal 19-20 module pair. NMR chemical shift mapping allowed the GAG-binding site to be delineated within the newly-derived 3D solution structure of this module pair (Herbert *et al.* J. Biol. Chem. 2006; 281:16512). Strikingly, missense mutations associated with atypical haemolytic uraemic syndrome congregate in this GAG-binding site and may thus disrupt the GAG self-recognition properties of factor H. Similarly, we have also shown that the Tyr-to-His mutation at residue 402 in module 7, that is known to predispose to acute macular degeneration, causes a reduction in affinity of the isolated module 7 for heparin oligosaccharides. This is consistent with the positioning of this residue on the edge of a separate GAG-binding site.

#### Studies on mouse embryonic stem cells

#### Characterisation of ES cells with mutations in HS endosulphatases

The Sulfs are a family of extracellular enzymes which act to modify mature HS chains, removing 6-O-sulphate groups from specific sites. The loss of these groups significantly alters the biological func-

# MEDICAL ONCOLOGY: PROTEOGLYCAN GROUP

tion of HS by influencing the ability of the chains to interact with a variety of growth factors and morphogens, such as BMP and FGF. Although their activities are tightly controlled during development, Sulfs are mis-regulated in various tumours including bladder and breast cancer. Working with collaborators in Germany (Prof. Dierks, Bielefeld) we analysed the structure and function of HS extracted from mice mutants of one or both members of the Sulf family. Surprisingly, we found that there was an element of co-operativity in the functions of these enzymes, a feature not observed in previous studies. In addition, we demonstrated that the pattern of HS sulphation observed in these *in vivo* models was different to that predicted from *in vitro* experiments, with considerably more 6-O-sulphate groups being targeted by the enzymes than previously thought. We have developed a non-invasive assay for detecting Sulf activity, and we hope to use this method to define the extent of Sulf loss/gain in and around tumour sites *in vivo*.

## HS and the regulation of ES cell biology

ES cells are a useful model system to study the inter-relationships between HS structure and function. We can manipulate the levels of HS biosynthetic enzymes and core proteins using either RNAi, or by deriving new ES cell lines from existing mutant mice. As pluripotent ES cells differentiate and commit to a defined lineage (e.g. neural, haematopoietic), they change the sulphation patterns of their HS chains. The expression of lineage-specific HS may influence the ability of differentiating cells to respond to the range of HS-dependent factors that direct ES cell differentiation. ES cells deficient for the iduronate-2-O sulphotransferase enzyme (Hs2st) appear to be restricted in their ability to differentiate to specific neural lineages. Various members of the FGF family are critical in this pathway and we are investigating how loss of 2-O-sulphation influences their activity. There is growing interest in the role of extracellular matrix components in directing ES cell behaviour, and we have been able to conduct experiments demonstrating that specific HS oligosaccharides can be used to influence neural and mesodermal differentiation pathways. We are also working with

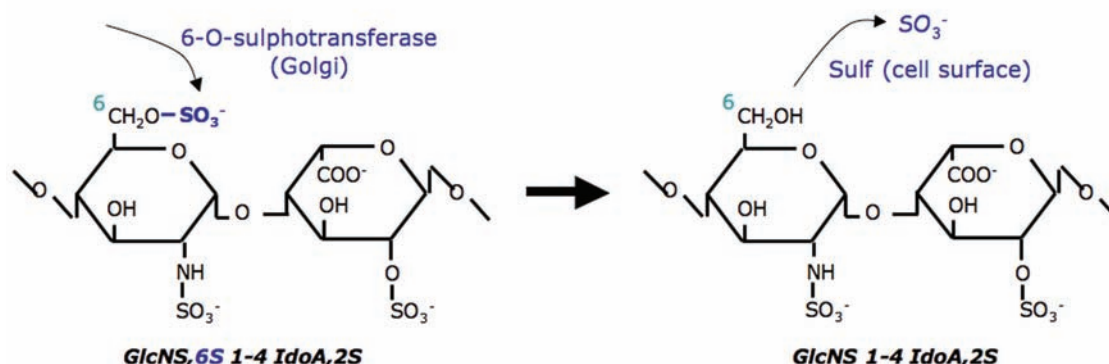
the Manchester Stem Cell Centre on a variety of projects.

## Observations on the interaction of HS with FGF1 using an enzyme protection-based approach

The fibroblast growth factors (in particular FGF1 and FGF2) are an important area of research because of the roles they play in tumour growth and angiogenesis. At the cell surface, 2:2 complexes of FGF and FGFR (FGF-receptor) are responsible for signal transduction; however, these complexes cannot form in the absence of HS or heparin a chemical analogue of the sulphated domains (S-domains) of HS. We have demonstrated that specific heparin saccharides drive the assembly of 2:1 FGF1: saccharide complexes by a co-operative binding mechanism that correlates with the capacity of these saccharides to induce FGF1-mediated cell proliferation. We have now begun to analyse FGF1 interactions with HS using a protection assay in which **preformed** HS-FGF1 complexes are incubated with heparinase enzymes to degrade unprotected HS. This showed that FGF1 conferred almost complete protection on the heparinase I cleavage sites in HS (i.e. the S-domains). This was surprising since only a minority of S-domains bind FGF1 following their excision from HS. Another surprising observation was that FGF1 also protected transition zones (T-zones) of HS from degradation. T-zones are positioned at the interface of the S-domains and the non-sulphated sections of HS. They have an intermediate level of sulphation and were not expected to interact with FGF1. Our findings indicate that FGF1 binding to T-zones and S-domains may be facilitated by co-operative conformational effects resulting from their close apposition in the HS chain. It is unclear whether FGF1 is dimerized at any of these binding regions in HS. In collaboration with Professor Tom Blundell and colleagues (University of Cambridge), we are now exploring the interaction of FGF-receptors with HS-FGF1 complexes with a view to identifying the active sites in HS for assembly of signalling complexes.

Sulf enzymes "edit" the sulphation pattern of HS

Sulphation at C6 of N-sulphated disaccharides in HS is dynamically regulated by an interplay of sulphotransferase and endosulphatase (Sulf) enzymes. The extracellular Sulfs specifically remove 6-O-sulphate groups and in so doing regulate HS-mediated cell growth.



Publications listed on page 67





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Jamie Honeychurch  
Lijun Mu

**GRADUATE STUDENT**  
Andrei Ivanov

**CLINICAL RADIOIMMUNOTHERAPY GROUP**

**SENIOR CLINICAL SCIENTIST**  
Maureen Zivanovic

**RADIOPHARMACIST**  
Anna McNicholas

**SENIOR PHYSICIST**  
Mathew Guy

**CLINICAL TRIALS ADMINISTRATOR**  
Emma Burke

**RESEARCH NURSES**  
Sue Neelson  
Tracy Gardener

## Cancer Studies: Targeted Therapy Group

The research aims of the Targeted Therapy Group are to increase our understanding of the interactions between monoclonal antibodies (mAb) and irradiation in the treatment of cancer and to develop novel clinical trial protocols using radionuclide targeted therapies. There are two research domains which inter-relate to form a cohesive programme of preclinical work under the title of radioimmunotherapy (RIT) of cancer, which include optimisation of RIT and irradiation and immunoregulation. In the first project, the roles of mAb as both vectors to deliver radiotherapy to tumours and in direct cancer cell killing are further explored. The specific aims are to further understand the mechanisms of action of RIT and define the relative contributions of targeted radiation and mAb effector mechanisms to the clearance of tumour. In the second project, mAb are used to augment T-cell responses to tumour by blocking or stimulating co-receptors in the immune system. This project aims to further define the factors which are important in combining irradiation and immunoregulatory mAb.

### Preclinical Group

The last year has been very successful in creating a cohesive preclinical laboratory and clinical translational groups. In February 2006 Dr Jamie Honeychurch relocated from Southampton and two new post-doctoral fellows Dr James Hainsworth and Dr Lijun Mu were appointed later in 2006.

Over the last years, we have substantially increased our understanding of the relative contributions of antibody effector mechanisms and targeted radiation to the eradication of tumour by using well defined syngeneic animal models of B-cell lymphoma. We have demonstrated for the first time in a variety of different syngeneic models of lymphoma that successful RIT leading to long-term tumour protection consists of both targeted irradiation

and mAb effector mechanisms. Our results confirm that RIT is much more than targeted radiation and provide a scientific rationale to support the use of selecting combinations of mAb in RIT rather than using a single mAb. We have shown that a radiation dose response exists for RIT in B-cell lymphoma, when the targeted dose of radiation is augmented in the presence of signalling mAb. We have demonstrated for the first time *in vivo* that this type of combination mAb approach is effective in RIT and are attempting to translate these preclinical findings to clinical studies. Our recent work has focused on the importance of the micro-distribution of radiolabelled mAb to the delivery of targeted radiotherapy to tumour. We have found important therapeutic differences between targeting different tumour antigens and their ability to deliver tumouricidal doses of radiation at the microscopic level.

Another line of investigation has been the intracellular signalling pathways underlying the combined treatment of anti-CD20 antibodies and irradiation in non-Hodgkin lymphoma cells. We have observed a synergistic cytotoxic effect when the anti-CD20 mAb B1 was combined with irradiation. The additive effect seen with B1 mAb and radiation was not however observed with another anti-CD20 mAb, Rituximab. This synergistic effect was accompanied by activation of ERK/MAPK pathway and could be reversed with MEK inhibitors and siRNA approaches.

Our other preclinical laboratory interests involve exploiting the therapeutic potential of combining immuno-modulatory mAb such as anti-CD40 with irradiation or cytotoxic chemotherapy. We have been able to demonstrate that irradiation and anti-CD40 mAb can act in concert to eradicate lymphoma and induce long-term survival under conditions whereby either treatment alone is ineffective. When anti-CD40 mAb is given in combination with EBRT a clear radiation dose-dependent increase in survival is seen with long-term CD8 T-cell dependent protection. We are hopeful that this type of



# CANCER STUDIES: TARGETED THERAPY GROUP

combination approach using tumour cytoreduction with standard anti-cancer approaches such as cytotoxic chemotherapy and irradiation followed by host immunostimulation may provide an excellent therapeutic opportunity for future clinical testing. Our current research is focused on improving our understanding of the fate of irradiated tumour cells *in vivo* following combination therapy, and determining which type of cell death provides the greatest stimuli to the host immune system and which are more “immunologically silent”. To address these issues we are currently developing a number of systems for manipulating individual antigen presenting cell (APC) populations *in vivo*, including transgenic models, specific depleting agents, and mechanisms for altering the recognition of dying tumour cells by phagocytes. Using fluorescently labelled tumour cells, we have been able to demonstrate that following EBRT lymphoma cells are rapidly cleared *in vivo* by macrophages in a radiation dose-dependent manner. These studies are currently being extended to assess the role of macrophages and other APC such as dendritic cells and B-cells in priming immune responses following combination therapy. To increase our understanding of how the mode, site and quantity of cell death can affect immunogenicity we are also characterising and comparing the efficacy of cells treated *ex vivo* with chemotherapy or radiotherapy in prophylactic and therapeutic immunisation protocols.

## Clinical Translational research

A major focus of the targeted therapy group is to work alongside and to interact with the clinical trial programme. The clinical RIT group has made tremendous progress this year in building sufficient infrastructure to enable the delivery of high quality novel clinical research over the coming years. We were delighted to welcome Maureen Zivanovic, a senior clinical scientist who relocated from Southampton, Anna McNicholas a radiopharmacist and to have secured the services of Mathew Guy to lead the clinical radionuclide dosimetry. A major project over the coming year and beyond will be the building of a new radiopharmacy following the recent Christie Trust approval.

One of the major successes from the laboratory programme which we have translated from the clinic to the laboratory has been the use of fractionated RIT as well as the development of a novel anti-Idiotypic against Rituximab that can be used to measure serum Rituximab levels in patients. We have completed a Phase I/II dose escalation study of fractionated <sup>131</sup>I Rituximab and are now embarking on a multicentre investigator led study using fractionated RIT. This phase II study (FIZZ) in follicular lymphoma will be the first study to be performed with fractionated 90Y Ibritumomab tiux-

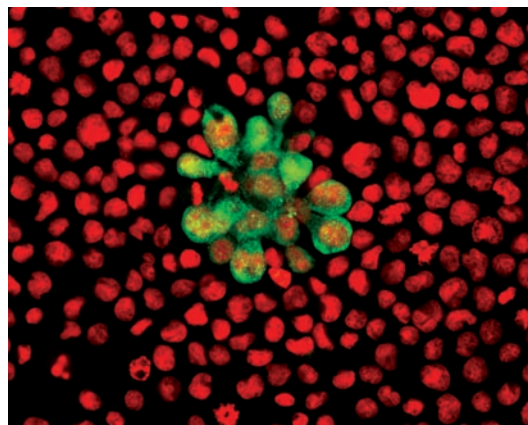
etan (Zevalin) in previously untreated follicular lymphoma and will be led from the Christie and involve collaborations with investigators in France and Italy.

The Phase I/II study in diffuse large B cell lymphoma is the first attempt to integrate RIT into a combination chemotherapy schedule. It is being led from the Christie along with 5 other UK sites. More recently the CR-UK and an industrial partnership have funded a further novel trial approach using abbreviated chemotherapy followed by RIT in relapsed follicular lymphoma. This study (SCRIFT) will again be led from the Christie but will run through the NCRI lymphoma group.

Another novel clinical trial developed in Manchester and launched this year is the GemBex study in Cutaneous T cell lymphoma. This is first multi-institutional national study ever to have been attempted in this rare cancer and is again funded by the CR-UK. An important element of this Phase II clinical trial using Gemcitabine and Bexarotene in addition to the clinical response rates, will be the skin assessment but a detailed quality of life assessment. Finally we plan to develop the anti-Idiotypic against Rituximab in clinical trials to measure serum Rituximab levels in national clinical trials. This will be done via a collaboration with the Clinical Experimental Group in the Paterson Institute, led by Caroline Dive.

## Collaborators

Sue Owens Nuclear Medicine; John Radford Medical Oncology; Richard Cowan Clinical Oncology (all Christie Hospital) and members of Manchester Lymphoma Group; Richard Myers Pathology, MRI; Martin Glennie, Peter Johnson, Mark Cragg; Cancer Sciences Division, Southampton University; Franck Morschauser, Lille, France; Giovanni Martenelli, Milan, Italy



ERK protein activated in cells undergoing homotypical adhesion following treatment with the anti-CD20 antibody B1. Immunofluorescence of active ERK (Alexa fluor 488, green) and DNA (Propidium Iodide, red)

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HEAD OF RESEARCH SERVICES  
**Jenny Varley**

## Research Services

<http://www.paterson.man.ac.uk/facilities/scifacs.jsp>

There have been a number of significant developments over the past year in the Research Services. A review of the requirements of the Institute for Electron Microscopy has resulted in the formation of a Transmission EM service unit which will roll out in January 2007, and which will be run by Stephen Murray who currently works for Terry Allen. The proteomics service component of the Molecular Biology Core Facility has been boosted tremendously by the appointment of Duncan Smith who is working closely with Yvonne Connolly to provide an excellent proteomics service. We have continued to invest in equipment, including computers and software, to make sure that the services remain at the forefront. This year major purchases have included a new Qiagen robot for the MBCF and a new time lapse microscopy system. The facilities work closely together to provide a range of integrated activities such as cell sorting or laser capture microdissection for microarray work, and genotyping transgenic pups.

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### Advanced Imaging Facility

Steve Bagley

<http://www.paterson.man.ac.uk/facilities/advmimg.jsp>

One of the principal challenges in the study of biological processes is the temporal localisation of events hence the facility has concerned itself over the last five years with the visualisation of some-time fleeting relationships between proteins and a study of their function. Working in conjunction with other facilities within the institute, the analysis of structure, function and relationship assists in building up a snapshot of biological activity.

Over the previous year the institute has purchased another time lapse microscope which has been designed for the analysis of biological activity where the environment (temperature, nutrition, vibration) around the microscope is neutral to the

cell and the very act of imaging the cell has as low photo-toxic effect as possible. This microscope system enables the researcher to record not only in a manner that does not affect the biological activity of the cell but also allows large numbers of cells to be examined thus producing statistically viable results. The requirement for specific processing tools to achieve numerical characterisation of particle and cell movement has increased which has led to the purchase of another 64-bit workstation and the building of software which utilises mathematical modelling software.

Currently usage of the microscopes is up to 80% which includes overnight and weekend usage, hence the amount of useful data being generated increases exponentially year on year. As a result of this amount of data, together with that generated by other facilities and groups in the Institute, IT department is looking at a storage and archival solution due to the terabytes of data being generated.

Challenges over the coming year include high throughput imaging and three dimensional histological imaging which would allow the automatical-ly imaging and creation of whole tissue visualisation. An important avenue of study would also be the localisation of specific protein interactions with tissue array data. The requirement for high resolution, low light, and low impact imaging also increases as it becomes a technique adopted by several research groups within the institute consequently there is a constant drive for the update of the current equipment and software to allow the systems to become more photon efficient and hence impact less upon biological activity.

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### Biological Resources

<http://www.paterson.man.ac.uk/facilities/bru.jsp>

The Biological Resources unit is a modern transgenic facility that continues to support the scientific research programmes at the Paterson whilst ensuring that the highest quality of welfare and

health status of the animals is maintained at all times. This year has seen the introduction of additional animal caging as more genetically altered strains are developed and maintained.

The use of animals in medical research does still remain a controversial issue but where ever possible alternative methods such as tissue culture are used but there is still a need for some research involving animals to allow the understanding of cancer that will lead to the development of better treatments for patients. All animal work at the Paterson Institute that involves the use of rodents is covered by both Project and Personal licences that are issued by the Home Office and reviewed by a local ethical committee.

Since 2005 we have brought in several new genetically altered strains of mice through our Quarantine suite. These new models are vital to ensure we remain at the forefront of scientific research. Once the animals have been re-derived they are then integrated into the BRU where they are housed in individually ventilated cages (IVCs). These cages prevent the spread of potential disease from one cage to another and each cage has an individual Hepa filtered air supply that gives approximately 72 air changes per hour and a fixed exhaust system. In addition to protecting the mice from disease these cages also help to protect the staff from exposure to allergens from the mice and bedding. Exposure of staff to Laboratory Animal Allergens (LAA) is a current hot topic with the Health and Safety Executive (HSE). Long term exposure to LAA can induce asthma and skin or eye allergies in staff handling the animals. The use of IVC caging dramatically reduces this risk.

All the cages are provided with environmental enrichment, in the form of nesting material, a variety of mouse houses, wooden chew blocks or play tunnels. The addition of these items increases socialisation and environmental stimulation for the mice and reduces aggression amongst some strains of males.

We undertake routine health screening from our colonies to ensure that the mice are free from a list of specific pathogens (SPF) and any new strains brought into the unit are health screened before introduction into the facility.

Two thirds of the space in the unit has been given over to the development and breeding of genetically altered mice, which are important in providing

mouse models of human disease or understanding the function of a particular gene in the living organism. The transgenic service has continued to develop with pronuclear and ES cell injections, and over the last year we have successfully developed several novel strains for groups within the Institute. The routine archiving of mouse lines continues through cryo-preservation and sperm freezing and re-derivation of embryos.

The Molecular Biology Core facility provides a full genotyping service of the genetically altered mice produced on site for the users which allows us to efficiently identify the required genotypes.

The remaining third of the unit is dedicated to the care and housing of experimental mice, and a dedicated staff of highly skilled technicians can undertake a range of procedural studies involving xenograft models and associated therapies. There is close liaison between the BRU staff and the scientific groups within the Institute and the University of Manchester.



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### Cancer Research UK GeneChip Microarray Service.

Head: Stuart Pepper

<http://bioinf.picr.man.ac.uk/mbcf/index.jsp>

The last year has seen numerous developments to the GeneChip service. Since joining with CR-UK Research Services and introducing full cross charging we have expanded in size and increased the range of services. Notably this year we have started offering a SNP array service in conjunction with Mike Churchman, early in the year we undertook training on the Affymetrix 50K SNP array, however such is the pace of change in microarrays that before the end of the year we have already moved on to arrays with 250K SNPs, and early in 2007 we will be offering half a million SNPs on one array.

During this year we have also added two extra posts to the team. Firstly a bioinformatician has been recruited to add in house support for the service. Having a bioinformatician as part of the team is proving to be very valuable for day to day troubleshooting and protocol comparison work, this post also provides a string link between the microarray service team and Crispin Miller's Bioinformatics group.

Last year we had started working with Epistem who were providing access to technology for amplification of small samples. Although we have stopped short of offering single cell profiling as a routine service, we have now supported several projects based on either LCM samples or small numbers of FACS sorted cells. This work has been helped by improvements in sensitivity of the Agilent Bioanalyzer Picochips, such that we can now perform QC on less than 1ng of total RNA.

The major development for this year has been the introduction of new arrays known as Exon arrays. These arrays are significant step forwards in expression profiling as they allow detection of splice variants. Traditional arrays have detect transcripts at one location, however the Exon arrays have a stunning 1.4 million probe sets, such that each exon of a transcript is analysed independently, the arrays also include a large number of probe sets targeted against introns sequences as controls. These arrays use completely different chemistry to previous Affymetrix expression arrays, and require a very different approach to data analysis. Support from the PICR Bioinformatics Group has been particularly important to enable work with these arrays, they have put a significant effort into writing novel software aimed at allowing streamlined analysis of such large data sets. Our two in-house pilot studies have been presented at the European Affymetrix Core Lab Directors Meeting and have generated a lot of interest; we are now offering these arrays as a standard service and already have four projects at various stages of processing.

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### Central Services

Head: Martin Chadwick

<http://www.paterson.man.ac.uk/facilities/censerv.jsp>

A modern and efficient central services facility provides a vital role in supporting the research carried out in the Institute. The roles carried out by the group include the preparation of media and sterile services, central stores, porters. The services pro-

vided can often go un-noticed by staff as it runs smoothly and efficiently, which is a sign that the group works well.

During the last year we have had three new members of staff including a Central Services Assistant Manager in Maurice Cowell who is learning the job inside out so that he can cope with any problem in Martin's absence. We have also had Catherine and Andrew join the team to replace the retirements of Ken, Colin and Gordon. The service has not only expanded in personnel but also the services the group provide, in the last year we have taken over the lunch time cover of reception as well as providing a delivery service on the institute freezer programs. The planned integration of the service is continuing with the new employees and some of the old able to transfer between departments to cover which ever may be understaffed. This integration is set to continue with a few more ideas to provide an even more efficient and effective service.

The barcode system is now fully operational and helping in the department with recharges stock monitoring. In the last twelve months we have also introduced an online intranet ordering system not only for our stores but also for our six onsite fridge/freezer programs providing the media and enzymes from Bioline, Invitrogen, Promega, Qiagen, Roche, and Sigma.

Stores continues to grow at a rapid rate, there are over 200 different products available in stores for the end users, and with over 1500 individual products being delivered by the porters a month.

The media and plate pouring service is proving more popular than ever now with four members of staff having to work hard to keep up with increasing demand in an attempt to provide a next day delivery service, the service still continues to grow each month. All the lab aides are assigned labs which they tend to spend a few hours a week in helping with basic technical duties along side the lab staff. Along side the Media Services the Sterile Services provide an important role ensuring the labs are fully stocked with sterile glassware, simple buffers and boxes of tips. All lab aides are responsible for ensuring the smooth running of the labs they are assigned.

The porters have been running smoothly and still provide an essential service and remove all rubbish from the Institute as well as moving equipment. They also not only deliver the normal deliveries but also the goods ordered from the 'intranet ordering



system' for both stores and freezers to the labs during the day. We have also managed to switch supplier of our dry ice contact with no disruption to the institute and save over £500 a week.

The Porters now recycle as much of the Institute's waste as possible, existing contracts mean we are able to recycle the plastic inserts from tip trays, as well as all the cardboard and waste paper that leaves the Institute.



### Flow Cytometry Unit

Head: Mike Hughes

<http://www.paterson.man.ac.uk/facilities/facs.jsp>

The Flow Cytometry service unit has two cell sorters, the FACSVantage and the FACSria that can sort up to 22,500 cells a second. They are operated by two full time staff. There are also two multi-user cell analysis machines, the FACScan and the FACSCalibur. All staff wishing to operate these machines are given an initial training course and late this year a more advanced course on compensation and red laser alignment has been offered. The service is undergoing a review so that we can accommodate an expected increase in demand. A new database has been produced for monitoring usage and performance of the service.

There have been several new projects this year on the cytometers. The Targeted Therapy Group has focused on the immunogenicity of apoptotic cells. Tumour cells were treated with different chemical drugs to induce apoptosis. These cells were used *in vivo* to see whether they can enhance the immune response or not. Doxorubicin, one of the drugs used, is red in colour with a broad spectrum, making it impossible to use viability dyes that could be excited with a 488nm laser. DAPI was therefore used to measure the exact number of apoptotic cells and the FACSVantage with the UV laser used to excite the DAPI.

The Medical Oncology group has started using the FACSria as part of the framework 6 ATTACK project to optimize *in vitro* culture conditions for chimeric immune receptor expressing T cells. In order to identify potentially useful cytokine combinations for culture, they want to look for differences in receptor expression patterns between patient groups with different cancers and to follow receptor expression during culture. To do this they have developed a 5-colour (FITC, PE, PECy5, PECy7, Pacific Blue) panel of antibodies that can be run on the FACSria that enables them to study expression of a number of surface molecules (CD3, CD4, CD8, CD25, IL-7Ralpha, IL-15Ralpha, IL-18Ralpha, IL-21Ralpha and FOXP3) in just 4 tubes, thus minimizing the number of cells required and maximizing the information gained. This panel may also be extended in the future to make use of the Aria's capacity to run more colours in a single sample.

During this year we have been working with the Molecular Biology Core Facility to develop protocols that allow microarray profiling of small numbers of FACS analysed cells. By working together the services aim to provide users with a seamless service from initial cell sort through to microarray data. Various options for cell collection have been tested for their compatibility with downstream RNA extraction and microarray analysis. Although this work is technically challenging it has already been applied to projects with some success and now provides an excellent option for detailed expression analysis of small cell populations.

### Histology

Head: Garry Ashton

<http://www.paterson.man.ac.uk/facilities/histology.jsp>

The unit specialises in the histological preparation and analysis of a wide range of samples including mouse model tissues, whole mouse embryo preps, cell preps, zebra fish and human biopsies. Requests from groups new to histology, as well as increases in workload from our existing customers, have resulted in a very busy year for the unit. All our key services have once again seen heavy demand.

Once again the unit has worked closely with Cell Regulation to help characterise the developmental phenotypes arising from functional deletion of the transcription factor ATF2. Protocols have been optimised to detect proliferation, differentiation and apoptosis markers in the ATF2 embryonic

knockout mice. In addition, research into the 3D reconstruction of tissues from optimally prepared sections has been undertaken in collaboration with the Advanced Imaging Facility.

Immunohistochemistry was used to show that CEA+ colorectal hepatic metastases produce and secrete large quantities of the chemokine Eotaxin-2 compared to adjacent normal liver. Primary bowel tumours were also shown to produce Eotaxin-2 that was elevated compared to non-cancerous bowel. These data might be of use in the targeting of T-cell immunotherapies to sites of tumour or in the development of other targeted therapies.

Adoptive transfer of mouse T-cells genetically engineered to express a chimeric receptor consisting of an anti CD19 scFv linked to the CD3zeta signalling molecule leads to the regression of established B-cell lymphomas *in vivo*. Immunohistochemical techniques are currently being employed to study the T-cell infiltrate in these tumours before they are destroyed by the transferred T-cells.

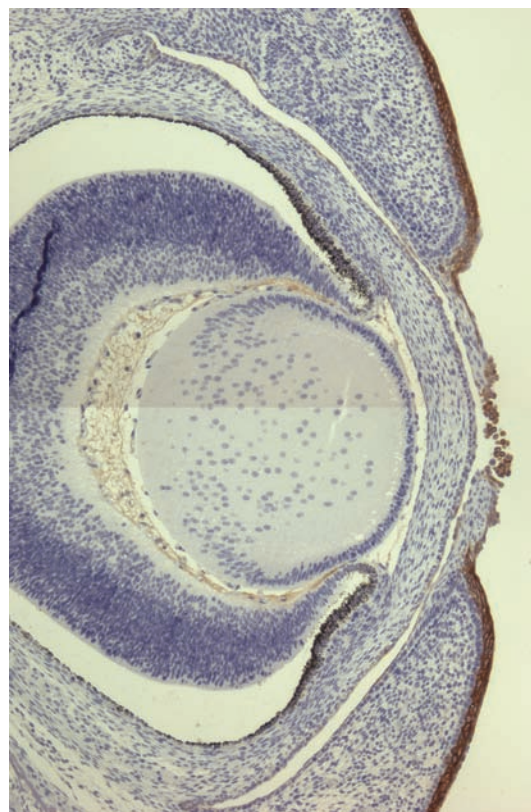
With the Immunology group, laser capture microdissection has been used to isolate regions of tumour and normal tissue to study HLA expression in patients undergoing surgical resection of colorectal carcinoma metastases to the liver. The isolated RNA has been converted to cDNA and used to assess the transcription from different HLA class I gene loci between the normal and tumour areas using real time PCR. These data will then be compared to immunohistochemical analysis of HLA class I expression using locus- and allele-specific antibodies. This work will identify evidence and mechanism of HLA down-regulation of primary importance to escape from tumour immunity.

Recently and in conjunction with the Cell Signalling group, and again using LCM, the expression of Tiam1, STEF and pRex1 homologues in zebra fish is being determined in wild type, benign and malignant melanophores.

Tissue microarray construction has been expanded over the last year. Several hundred samples have now been incorporated into new TMA blocks, allowing for high throughput and standardisation of methodology, both of which are essential in translational research. Improvements in template design and post construction processes have resulted in increased numbers of samples per array.

Finally, as well as a new request form and sample tracking system being introduced, the unit hopes to

automate some of the antibody optimisation and troubleshooting services by purchasing an automated staining robot over the next few months. Linked with this, two new epitope retrieval systems will also be introduced, allowing for improvements in standardisation.



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### Molecular Biology Core Facility

Head: Stuart Pepper.

<http://www.paterson.man.ac.uk/facilities/mbcf.jsp>

The Paterson Institute Molecular Biology Core Facility has now been in existence for 6 years. In that time it has grown from a team of two people offering DNA sequence and plasmid isolation to a team of nine people offering a full range of state of the art techniques for analyzing DNA, RNA and protein. This year a total of three extra posts joined the team, two of which are supporting the CR-UK wide GeneChip Microarray service which is detailed separately on these pages.

Duncan Smith has joined the team and brings with him a strong background in chromatography and MS protocol development and implementation. Over this year phosphorylation mapping has become a standard service and data have now been generated for approximately ten projects. These

## RESEARCH SERVICES

services have had a significant impact on the work of groups in the Institute, allowing research programs to include phosphorylation mapping as a matter of routine. As the year draws to a close the facility is developing strategies that will enable quantitative phosphorylation mapping. Techniques for analyzing global phosphorylation patterns are also being evaluated, and we would hope that next year both quantitative and global phosphorylation mapping will be offered to research groups within PICR.

In 2005 we had started using an EpMotion robot to set up real time reactions in 384 well format so that large quantitative PCR projects could be undertaken more efficiently. Over the last year this system has been used to complete several large projects which have been written into publications. We are currently testing a new qPCR system from Beckman which may well offer another significant jump in throughput for qPCR; pilot studies should be completed early in 2007.

The core services of DNA sequencing and small scale plasmid isolation have remained stable this year; our new Qiagen 8000 robot has performed reliably since install in May, and the 3100 sequence system has run as reliably as ever – the 3100 has now been running every day for 6 years and has delivered over 80 000 sequences for the Institute.

Looking ahead to next year there will be more challenges – particularly the growing interest in micro RNA analysis will require more development within the facility.

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### Kostoris Library

Head: Steve Glover

<http://www.christie.nhs.uk/profinfo/departments/library/default.htm>

The Kostoris Library provides a service to staff and students at Christie Hospital NHS Trust and the Paterson Institute for Cancer Research. Sited near Clinical Oncology on the 2<sup>nd</sup> floor of the Nathan House courtyard, the library holds textbooks, PhD theses and journals.

Staff of the Paterson Institute can access the online journals of the University of Manchester from their desktop PCs within the PICR. The Kostoris Library staff work in close liaison with the health sciences and electronic resources teams at the uni-

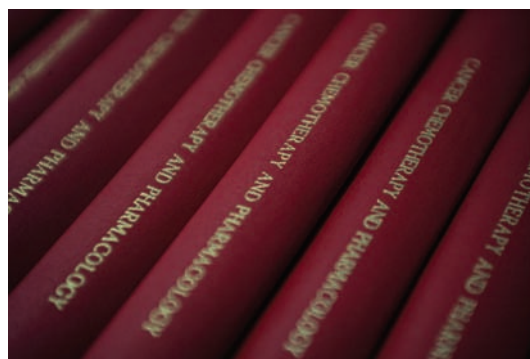
versity and provide an onsite point of contact for access to resources. Access to university resources is primarily based on IP address but certain resources require usernames or access via Athens.

In addition to a liaison role with the university the Kostoris Library provides an onsite document supply service. Documents not available from the Internet, or through subscriptions or in print copy in the library are sourced from other research institutes, hospitals, or the British Library Document Supply Centre. In 2006 the library supplied 101 articles to PICR staff.

The library also provides a centralised alerting service using publishers' table of contents (etocs). In 2006 the library supplied over 20,000 etoc alerts to Christie and PICR staff for over 154 different journal titles. PICR and Christie staff downloaded over 53,000 full text PDFs from subscribed journals. The American Association of Cancer Research journal *Cancer Research* was the most heavily downloaded title with a figure of 4059.

The library provides a database surveillance service and will set up monthly queries against PubMed, ISI Web of Science, EMBASE, or BIOSIS. The results can be sent to PICR staff via email on a monthly or weekly basis. The library carried out 730 set queries for PICR staff in 2006.

The library aims to support the information needs of research staff and has developed its services to give access to published research as soon as it is available. This includes delivering information to the desktop and alerting users to newly published articles within their fields of interest. Integrating online services with a responsive enquiry service and efficient document supply system enhances the core oncology and related sciences of the reference collection available onsite.





# Publications

## BIOINFORMATICS GROUP (page 10)

Crispin Miller

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Okoniewski, M.J. and Miller, C.J. (2006) Hybridization interactions between probesets in short oligo microarrays lead to spurious correlations. *BMC Bioinformatics*, **7**, 276.

Unwin, R.D., Smith, D.L., Blinco, D., Wilson, C.L., Miller, C.J., Evans, C.A., Jaworska, E., Baldwin, S.A., Barnes, K., Pierce, A., Spooncer, E. and Whetton, A.D. (2006) Quantitative proteomics reveals post-translational control as a regulatory factor in primary hematopoietic stem cells. *Blood*, **107**, 4687-4694.

Wilson, C.L., Sims, A.H., Howell, A., Miller, C.J. and Clarke, R.B. (2006) Effects of oestrogen on gene expression in epithelium and stroma of normal human breast tissue. *Endocr Relat Cancer*, **13**, 617-628.

### Other Publications

Pepper, S.D., Hey, Y., Newton, G., Okoniewski, M. and Miller, C. (2006) A core lab case study – exon array challenges and opportunities. *The Core – Affymetrix Core Lab Communication Bulletin*. September.  
([http://www.affymetrix.com/userForum/technology/arraysContents/CoreLabCaseStudy\\_Pepper.uf](http://www.affymetrix.com/userForum/technology/arraysContents/CoreLabCaseStudy_Pepper.uf))



## CARCINOGENESIS GROUP (page 12)

Geoff Margison

### Refereed Research Papers

Chinnasamy, D., Milsom, M.D., Shaffer, J., Neuenfeldt, J., Shaaban, A.F., Margison, G.P., Fairbairn, L.J. and Chinnasamy, N. (2006) Multicistronic lentiviral vectors containing the FMDV 2A cleavage factor demonstrate robust expression of encoded genes at limiting MOI. *Virology*, **3**, 14.

Harrison, K.L., Crosbie, P.A., Agius, R.M., Barber, P.V., Carus, M., Margison, G.P. and Povey, A.C. (2006) No association between N7-methyldeoxyguanosine and 8-oxodeoxyguanosine levels in human lymphocyte DNA. *Mutat Res*, **600**, 125-130.

Hornillo-Araujo, A.R., Burrell, A.J., Aiertza, M.K., Shibata, T., Hammond, D.M., Edmont, D., Adams, H., Margison, G.P. and Williams, D.M. (2006) The syntheses and properties of tricyclic pyrrolo[2,3-d]pyrimidine analogues of S<sup>6</sup>-methylthioguanine and O<sup>6</sup>-methylguanine. *Org Biomol Chem*, **4**, 1723-1729.

Horsman, M.R., Bohm, L., Margison, G.P., Milas, L., Rosier, J.F., Safrany, G., Selzer, E., Verheij, M. and Hendry, J.H. (2006) Tumor radiosensitizers—current status of development of various approaches: report of an International Atomic Energy Agency meeting. *Int J Radiat Oncol Biol Phys*, **64**, 551-561.

Lees, N.P., Harrison, K.L., Hall, C.N., Margison, G.P. and Povey, A.C. (2006) Human colorectal mucosal O<sup>6</sup>-alkylguanine DNA-alkyltransferase activity and DNA-N7-methylguanine levels in colorectal adenoma cases and matched referents. *Gut*, epub Aug 4.

Margison, G.P., Povey, A.C. and Santibanez-Koref, M.F. (2006) Genetic variation at the human MGMT locus and its biological consequences. *Curr Pharmacogenomics*, **4**, 133-144.

Pearson, S.J., Wharton, S., Watson, A.J., Begum, G., Butt, A., Glynn, N., Williams, D.M., Shibata, T., Santibanez-Koref, M.F. and Margison, G.P. (2006)



A novel DNA damage recognition protein in *Schizosaccharomyces pombe*. *Nucleic Acids Res*, **34**, 2347-2354.

Povey, A.C., O'Donnell, P., Barber, P., Watson, M., Margison, G.P. and Santibanez-Koref, M.F. (2006) Smoking is associated with a decrease of O<sup>6</sup>-alkylguanine-DNA alkyltransferase activity in bronchial epithelial cells. *Int J Cancer*, **119**, 463-466.

Ranson, M., Middleton, M.R., Bridgewater, J., Lee, S.M., Dawson, M., Jowle, D., Halbert, G., Waller, S., McGrath, H., Gumbrell, L., McElhinney, R.S., Donnelly, D., McMurry, T.B. and Margison, G.P. (2006) Lomeguatrib, a potent inhibitor of O<sup>6</sup>-alkylguanine-DNA-alkyltransferase: phase I safety, pharmacodynamic, and pharmacokinetic trial and evaluation in combination with temozolomide in patients with advanced solid tumors. *Clin Cancer Res*, **12**, 1577-1584.

Saad, A.A., O'Connor, P.J., Mostafa, M.H., Metwalli, N.E., Cooper, D.P., Margison, G.P. and Povey, A.C. (2006) Bladder tumor contains higher N7-methylguanine levels in DNA than adjacent normal bladder epithelium. *Cancer Epidemiol Biomarkers Prev*, **15**, 740-743.

Schambach, A., Bohne, J., Chandra, S., Will, E., Margison, G.P., Williams, D.A. and Baum, C. (2006) Equal potency of gammaretroviral and lentiviral SIN vectors for expression of O<sup>6</sup>-methylguanine-DNA methyltransferase in hematopoietic cells. *Mol Ther*, **13**, 391-400.

Schambach, A., Schiedlmeier, B., Kuhlcke, K., Verstegen, M., Margison, G.P., Li, Z., Kamino, K., Bohne, J., Alexandrov, A., Hermann, F.G., von Laer, D. and Baum, C. (2006) Towards hematopoietic stem cell-mediated protection against infection with human immunodeficiency virus. *Gene Ther*, **13**, 1037-1047.

Shibata, T., Glynn, N., McMurry, T.B., McElhinney, R.S., Margison, G.P. and Williams, D.M. (2006) Novel synthesis of O<sup>6</sup>-alkylguanine containing oligodeoxyribonucleotides as substrates for the human DNA repair protein, O<sup>6</sup>-methylguanine DNA methyltransferase (MGMT). *Nucleic Acids Res*, **34**, 1884-1891.

Southgate, T.D., Garside, E., Margison, G.P. and Fairbairn, L.J. (2006) Dual agent chemoprotection by retroviral co-expression of either MDR1 or MRP1 with the P<sub>140</sub>K mutant of O<sup>6</sup>-methylguanine-

DNA-methyl transferase. *J Gene Med*, **8**, 972-979.

White, S.C., Lorigan, P., Margison, G.P., Margison, J.M., Martin, F., Thatcher, N., Anderson, H. and Ranson, M. (2006) Phase II study of SPI-77 (sterically stabilised liposomal cisplatin) in advanced non-small-cell lung cancer. *Br J Cancer*, **95**, 822-888.

Woolford, L.B., Southgate, T.D., Margison, G.P., Milsom, M.D. and Fairbairn, L.J. (2006) The P<sub>140</sub>K mutant of human O<sup>6</sup>-methylguanine-DNA-methyltransferase (MGMT) confers resistance in vitro and in vivo to temozolomide in combination with the novel MGMT inactivator O<sup>6</sup>-(4-bromophenyl)guanine. *J Gene Med*, **8**, 29-34.

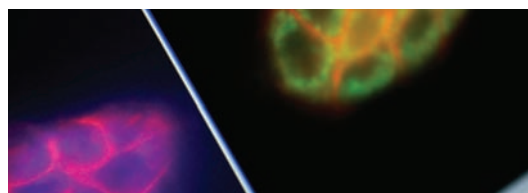
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O<sup>6</sup>-Substituted guanine derivatives, a process for their preparation and their use in treating tumour cells. McMurry, T.B.H., McElhinney, R.S., McCormick, J.E., Elder, R.H., Kelly, J., Margison, G.P., Rafferty, J.A., Watson, A.J. and Willington,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

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

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Ionizing radiation or diathermy-switched gene therapy vectors and their use in antitumour therapy. Margison, G.P. Marples B., Scott, S.D. and Hendry, J.H. through CR Technology Ltd.



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## CELL CYCLE GROUP (page 14)

Karim Labib

### Refereed Research Papers

Gambus, A., Jones, R.C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R.D. and Labib, K. (2006) GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat Cell Biol*, **8**, 358-366.

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## CELL DIVISION GROUP (page 16)

Iain Hagan

### Refereed Research Papers

Grallert, A., Beuter, C., Craven, R.A., Bagley, S., Wilks, D., Fleig, U. and Hagan, I.M. (2006) S. pombe CLASP needs dynein, not EB1 or CLIP170, to induce microtubule instability and slows polymerization rates at cell tips in a dynein-dependent manner. *Genes Dev*, **20**, 2421-2436.

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Hagan, I.M. and Palazzo, R.E. (2006) Warming up at the poles. *EMBO Rep*, **7**, 364-371.

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## CELL REGULATION GROUP (page 18)

Nic Jones

### Refereed Research Papers

Sinclair, J., Weeks, M., Butt, A., Worthington, J.L., Akpan, A., Jones, N., Waterfield, M., Allanand, D. and Timms, J.F. (2006) Proteomic response of *Schizosaccharomyces pombe* to static and oscillating extremely low-frequency electromagnetic fields. *Proteomics*, **6**, 4755-4764.

Weeks, M.E., Sinclair, J., Butt, A., Chung, Y.L., Worthington, J.L., Wilkinson, C.R., Griffiths, J., Jones, N., Waterfield, M.D. and Timms, J.F. (2006) A parallel proteomic and metabolomic analysis of the hydrogen peroxide- and Sty1p-dependent stress response in *Schizosaccharomyces pombe*. *Proteomics*, **6**, 2772-2796.

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Angeliki Malliri

### Refereed Research Papers

Malliri, A., Rygiel, T.P., van der Kammen, R.A., Song, J.Y., Engers, R., Hurlstone, A.F., Clevers, H. and Collard, J.G. (2006) The RAC activator Tiam1 is a Wnt-responsive gene that modifies intestinal tumour development. *J Biol Chem*, **281**, 543-548.

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Caroline Dive and Malcolm Ranson

### Refereed Research Papers

Blackhall, F., Ranson, M. and Thatcher, N. (2006) Where next for gefitinib in patients with lung cancer? *Lancet Oncol*, **7**, 499-507.

Booton, R., Ward, T., Heighway, J., Taylor, P., Power, F., Ashcroft, L., Morris, J. and Thatcher, N. (2006) Xeroderma pigmentosum group D haplotype predicts for response, survival, and toxicity after platinum-based chemotherapy in advanced nonsmall cell lung cancer. *Cancer*, **106**, 2421-2427.

Cummings, J. and Dive, C. (2006) A chromatographic screening method to identify agents that reverse intrinsic drug resistance in colon cancer cells. *LC GC Europe*, **16**, 363-370.

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Cummings, J., Ranson, M., Lacasse, E., Ganganagari, J.R., St-Jean, M., Jayson, G., Durkin, J. and Dive, C. (2006) Method validation and preliminary qualification of pharmacodynamic biomarkers employed to evaluate the clinical efficacy of an antisense compound (AEG35156) targeted to the X-linked inhibitor of apoptosis protein XIAP. *Br J Cancer*, **95**, 42-48.

Hussein, D., Estlin, E.J., Dive, C. and Makin, G.W. (2006) Chronic hypoxia promotes hypoxia-inducible factor-1alpha-dependent resistance to etoposide and vincristine in neuroblastoma cells. *Mol Cancer Ther*, **5**, 2241-2250.

Isa, A.Y., Ward, T.H., West, C.M., Slevin, N.J. and Homer, J.J. (2006) Hypoxia in head and neck cancer. *Br J Radiol*, **79**, 791-798.

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Southgate, T.D., Sheard, V., Milsom, M.D., Ward, T.H., Mairs, R.J., Boyd, M. and Fairbairn, L.J. (2006) Radioprotective gene therapy through retroviral expression of manganese superoxide dismutase. *J Gene Med*, **8**, 557-565.

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Welman, A., Cawthorne, C., Ponce-Perez, L., Barraclough, J., Danson, S., Murray, S., Cummings, J., Allen, T.D. and Dive, C. (2006) Increases in c-Src expression level and activity do not promote the growth of human colorectal carcinoma cells in vitro and in vivo. *Neoplasia*, **8**, 905-916.

White, S.C., Lorigan, P., Margison, G.P., Margison, J.M., Martin, F., Thatcher, N., Anderson, H. and Ranson, M. (2006) Phase II study of SPI-77 (sterically stabilised liposomal cisplatin) in advanced non-small-cell lung cancer. *Br J Cancer*, **95**, 822-888.

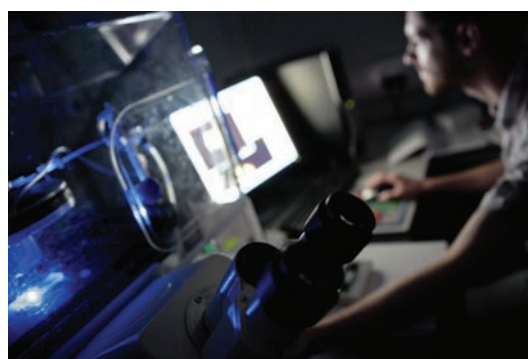
Williams, H., Julyan, P., Ranson, M., Zweit, J., Gillies, J. and Hastings, D. (2006) Does (124)Iodo-deoxyuridine measure cell proliferation in NSCLC? Initial investigations with PET imaging and radiometabolite analysis. *Eur J Nucl Med Mol Imaging*, epub Nov 16.

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Peter Stern

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Ali, S., Mulryan, K., Taher, T. and Stern, P.L. (2006) Immunotherapy success in prophylaxis cannot predict therapy: prime-boost vaccination against the 5T4 oncofoetal antigen. *Cancer Immunol Immunother*, **56**, 165-180.

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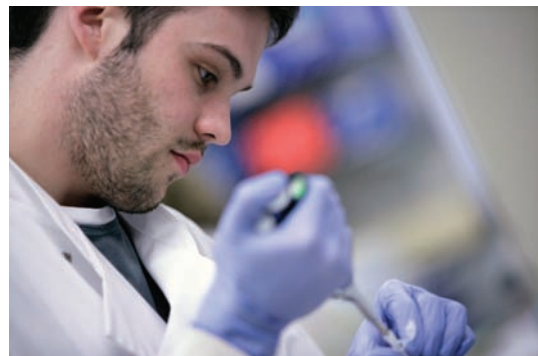
Smyth, L.J., Elkord, E., Taher, T.E., Jiang, H.R., Burt, D.J., Clayton, A., van Veelen, P.A., de Ru, A., Ossendorp, F., Melief, C.J., Drijfhout, J.W., Dermime, S., Hawkins, R.E. and Stern, P.L. (2006) CD8 T-cell recognition of human 5T4 oncofetal antigen. *Int J Cancer*, **119**, 1638-1647.

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Robson, L., McKenna, P., McCanny, T., Ledingham, K.W.D., Gillies, J.M. and Zweit, J. (2006) High-power laser production of PET isotopes. In: *Lecture notes in physics. Lasers and nuclei*. Vol **694** pp 191-203. Springer Berlin/Heidelberg

## RADIOCHEMICAL TARGETING AND IMAGING (page 26)

Jamal Zweit

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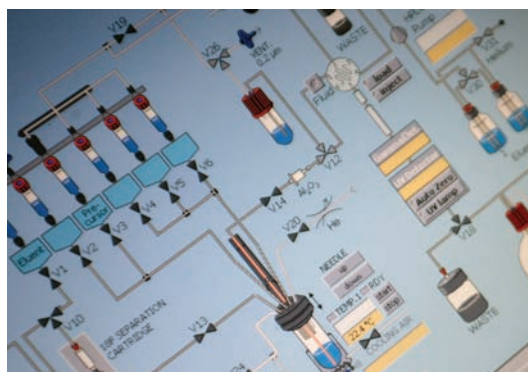
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## STEM CELL BIOLOGY GROUP (page 28)

Georges Lacaud

#### Refereed Research Papers

Chan, W.Y., Follows, G.A., Lacaud, G., Pimanda, J.E., Landry, J.R., Kinston, S., Knezevic, K., Piltz, S., Donaldson, I.J., Gambardella, L., Sablitzky, F., Green, A.R., Kouskoff, V. and Gottgens, B. (2006) The paralogous haemopoietic regulators Lyl1 and SCL are co-regulated by Ets and GATA factors yet Lyl1 cannot rescue the early SCL<sup>-/-</sup> phenotype. *Blood*, epub Oct 19.



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## STEM CELL AND HAEMATOPOIESIS GROUP (page 30)

Valerie Kouskoff

### Refereed Research Papers

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## STRUCTURAL CELL BIOLOGY GROUP (page 32)

Terry Allen

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Catharine West

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Kabuubi, P., Lancaster, J.A., Davidson, S.E., Hunter, R.D., Kobylecki, C., Stratford, I.J. and West, C.M. (2006) No relationship between thymidine phosphorylase (TP, PD-ECGF) expression and hypoxia in carcinoma of the cervix. *Br J Cancer*, **94**, 115-120.

Nordsmark, M., Loncaster, J., Aquino-Parsons, C., Chou, S.C., GebSKI, V., West, C., Lindegaard, J.C., Havsteen, H., Davidson, S.E., Hunter, R., Raleigh, J.A. and Overgaard, J. (2006) The prognostic value of pimonidazole and tumour pO<sub>2</sub> in human cervix carcinomas after radiation therapy: a prospective international multi-center study. *Radiother Oncol*, **80**, 123-131.

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Robert Hawkins and Peter Stern

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Board, R.E., Thistlethwaite, F.C. and Hawkins, R.E. (2006) Anti-angiogenic therapy in the treatment of advanced renal cell cancer. *Cancer Treat Rev*, epub Oct 26.

Chong, G., Bhatnagar, A., Cunningham, D., Cosgriff, T.M., Harper, P.G., Steward, W., Bridgewater, J., Moore, M., Cassidy, J., Coleman, R., Coxon, F., Redfern, C.H., Jones, J.J., Hawkins, R., Northfelt, D., Sreedharan, S., Valone, F. and Carmichael, J. (2006) Phase III trial of 5-fluorouracil and leucovorin plus either 3H1 anti-idio-

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## CANCER STUDIES – CHILDREN'S CANCER GROUP (page 38)

Vaskar Saha

### Refereed Research Papers

Strefford, J.C., van Delft, F.W., Robinson, H.M., Worley, H., Yiannikouris, O., Selzer, R., Richmond, T., Hann, I., Bellotti, T., Raghavan, M., Young, B.D., Saha, V. and Harrison, C.J. (2006) Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. *Proc Natl Acad Sci U S A*, **103**, 8167-8172.

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Shankar, A. and Saha, V. (2006) Non Hodgkin's lymphomas of childhood. In: *Lymphomas 2<sup>nd</sup> Edition*. Eds Canellos, G.P., Lister, T.A. and Young, B.D. W.B. Saunders

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## CANCER STUDIES – MEDICAL ONCOLOGY: BREAST BIOLOGY GROUP (page 40)

Rob Clarke

### Refereed Research Papers

Bramley, M., Clarke, R.B., Howell, A., Evans, D.G., Armer, T., Baildam, A.D. and Anderson, E. (2006) Effects of oestrogens and anti-oestrogens on normal breast tissue from women bearing BRCA1 and BRCA2 mutations. *Br J Cancer*, **94**, 1021-1028.

Flasza, M., Nguyen Huu, N.S., Mazaleyrat, S., Clemence, S., Villemant, C., Clarke, R. and Baron, M. (2006) Regulation of the nuclear localization of the human Nedd4-related WWP1 protein by Notch. *Mol Membr Biol*, **23**, 269-276.

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Robert Hawkins

#### Refereed Research Papers

Biglari, A., Southgate, T.D., Fairbairn, L.J. and Gilham, D.E. (2006) Human monocytes expressing a CEA-specific chimeric CD64 receptor specifically target CEA-expressing tumour cells in vitro and in vivo. *Gene Ther*, **13**, 602-610.

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Chong, G., Bhatnagar, A., Cunningham, D., Cosgriff, T.M., Harper, P.G., Steward, W., Bridgewater, J., Moore, M., Cassidy, J., Coleman, R., Coxon, F., Redfern, C.H., Jones, J.J., Hawkins, R., Northfelt, D., Sreedharan, S., Valone, F. and Carmichael, J. (2006) Phase III trial of 5-fluorouracil and leucovorin plus either 3H1 anti-idiotypic monoclonal antibody or placebo in patients with advanced colorectal cancer. *Ann Oncol*, **17**, 437-442.

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121974) and gemcitabine compared with gemcitabine alone in advanced unresectable pancreatic cancer. *BMC Cancer*, **6**, 285, epub Dec 11.

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## CANCER STUDIES – MEDICAL ONCOLOGY: TRANSLATIONAL ANGIOGENESIS GROUP (page 46)

Gordon Jayson

### Refereed Research Papers

Board, R.E., Bruijns, C.T., Pronk, A.E., Ryder, W.D., Wilkinson, P.M., Welch, R., Shanks, J.H., Connolly, G., Slade, R.J., Reynolds, K., Kitchener, H.C. and Jayson, G.C. (2006) Stage- and CA125-related survival in patients with epithelial ovarian cancer treated at a cancer center. *Int J Gynecol Cancer*, **16 Suppl 1**, 18-24.

Buonaccorsi, G.A., Roberts, C., Cheung, S., Watson, Y., O'Connor, J.P., Davies, K., Jackson, A., Jayson, G.C. and Parker, G.J. (2006) Comparison of the performance of tracer kinetic model-driven registration for dynamic contrast enhanced MRI using different models of contrast enhancement. *Acad Radiol*, **13**, 1112-1123.

Clamp, A., Blackhall, F.H., Henrioud, A., Jayson, G.C., Javaherian, K., Esko, J., Gallagher, J.T. and Merry, C.L. (2006) The morphogenic properties of oligomeric endostatin are dependent on cell surface heparan sulfate. *J Biol Chem*, **281**, 14813-14822.

Clamp, A.R., Maenpaa, J., Cruickshank, D., Ledermann, J., Wilkinson, P.M., Welch, R., Chan, S., Vasey, P., Sorbe, B., Hindley, A. and Jayson, G.C. (2006) SCOTROC 2B: feasibility of carboplatin followed by docetaxel or docetaxel-irinotecan as first-line therapy for ovarian cancer. *Br J Cancer*, **94**, 55-61.



Cummings, J., Ranson, M., Lacasse, E., Ganganagari, J.R., St-Jean, M., Jayson, G., Durkin, J. and Dive, C. (2006) Method validation and preliminary qualification of pharmacodynamic biomarkers employed to evaluate the clinical efficacy of an antisense compound (AEG35156) targeted to the X-linked inhibitor of apoptosis protein XIAP. *Br J Cancer*, **95**, 42-48.

Duff, S.E., Jeziorska, M., Rosa, D.D., Kumar, S., Haboubi, N., Sherlock, D., O'Dwyer, S.T. and Jayson, G.C. (2006) Vascular endothelial growth factors and receptors in colorectal cancer: implications for anti-angiogenic therapy. *Eur J Cancer*, **42**, 112-117.

Jayson, G. and Harris, J. (2006) How participants in cancer trials are chosen: ethics and conflicting interests. *Nat Rev Cancer*, **6**, 330-336.

Parker, G.J., Roberts, C., Macdonald, A., Buonaccorsi, G.A., Cheung, S., Buckley, D.L., Jackson, A., Watson, Y., Davies, K. and Jayson, G.C. (2006) Experimentally-derived functional form for a population-averaged high-temporal-resolution arterial input function for dynamic contrast-enhanced MRI. *Magn Reson Med*, **56**, 993-1000.

Rosa, D.D., Clamp, A., Mullamitha, S., Ton, N.C., Lau, S., Byrd, L., Clayton, R., Slade, R.J., Kitchener, H.C., Shanks, J.H., Wilson, G., McVey, R., Hasan, J., Swindell, R. and Jayson, G.C. (2006) The interval from surgery to chemotherapy in the treatment of advanced epithelial ovarian carcinoma. *Eur J Surg Oncol*, **32**, 588-591.

Rosa, D.D., Harris, J. and Jayson, G.C. (2006) The best guess approach to phase I trial design. *J Clin Oncol*, **24**, 206-208.

Whitworth, M.K., Sheen, A., Rosa, D.D., Duff, S.E., Ryder, D., Burumdayal, A., Wiener, K., Hawkins, R.E., Saunders, M., Valle, J.W., Sherlock, D. and Jayson, G.C. (2006) Impact of laparotomy and liver resection on the peritoneal concentrations of fibroblast growth factor 2, vascular endothelial growth factor and hepatocyte growth factor. *J Cancer Res Clin Oncol*, **132**, 41-44.

#### Other Publications

Baka, S., Clamp, A.R. and Jayson, G.C. (2006) A review of the latest clinical compounds to inhibit VEGF in pathological angiogenesis. *Expert Opin Ther Targets*, **10**, 867-876.

Thistlethwaite, F. and Jayson, G.J. (2006) Anti-angiogenic therapy in colorectal cancer: an update. *Gastrointestinal Cancer Abstracts*, **17**, 2-6.

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## CANCER STUDIES – MEDICAL ONCOLOGY: PROTEOGLYCAN GROUP (page 46)

John Gallagher

#### Refereed Research Papers

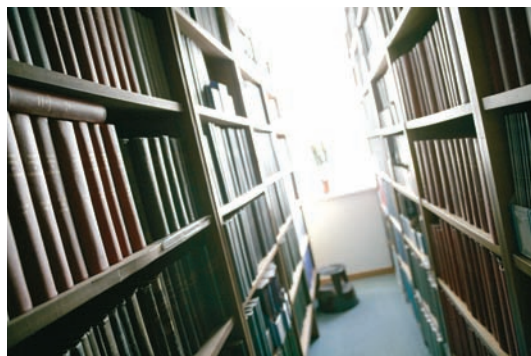
Clamp, A., Blackhall, F.H., Henrioud, A., Jayson, G.C., Javaherian, K., Esko, J., Gallagher, J.T. and Merry, C.L. (2006) The morphogenic properties of oligomeric endostatin are dependent on cell surface heparan sulfate. *J Biol Chem*, **281**, 14813-14822.

Harmer, N.J., Robinson, C.J., Adam, L.E., Ilag, L.L., Robinson, C.V., Gallagher, J.T. and Blundell, T.L. (2006) Multimers of the fibroblast growth factor (FGF)-FGF receptor-saccharide complex are formed on long oligomers of heparin. *Biochem J*, **393**, 741-748.

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Lamanna, W.C., Baldwin, R.J., Padvá, M., Kalus, I., Ten Dam, G., van Kuppevelt, T.H., Gallagher, J.T., von Figura, K., Dierks, T. and Merry, C.L. (2006) Heparan Sulphate 6-O-endosulphatases - Discrete in vivo activities and functional cooperativity. *Biochem J*, **400**, 63-73.





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Ozensoy, O., Kockar, F., Arslan, O., Isik, S., Supuran, C.T. and Lyon, M. (2006) An evaluation of cytosolic erythrocyte carbonic anhydrase and catalase in carcinoma patients: an elevation of carbonic anhydrase activity. *Clin Biochem*, **39**, 804-809.

Robinson, C.J., Mulloy, B., Gallagher, J.T. and Stringer, S.E. (2006) VEGF165 binding sites within heparan sulfate encompass two highly sulfated domains and can be liberated by K5 lyase. *J Biol Chem*, **281**, 1731-1740.

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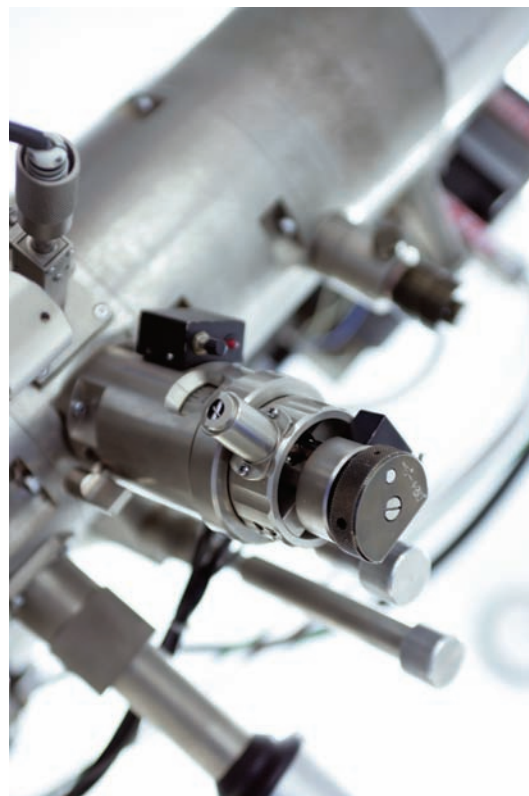
Uchimura, K., Morimoto-Tomita, M., Bistrup, A., Li, J., Lyon, M., Gallagher, J., Werb, Z. and Rosen, S.D. (2006) HSulf-2, an extracellular endoglycosaminase-6-sulfatase, selectively mobilizes heparin-bound growth factors and chemokines: effects on VEGF, FGF-1, and SDF-1. *BMC Biochem*, **7**, 2.

#### Other Publications

Gallagher, J.T. (2006) Multiprotein signalling complexes: regional assembly on heparan sulphate. *Biochem Soc Trans*, **34**, 438-441.

Lyon, M. (2006) The co-factor role of glycosaminoglycans in the activity of hepatocyte growth factor/scatter factor and the possibilities for therapeutic inhibition. Chapter 15 In: *New Developments in Therapeutic Glycomics*. Eds Delehedde, M. and Lortat-Jacob, H. Research Signpost, Trivandrum India

Sarrazin, S., Adam, E., Lyon, M., Depontieu, F., Motte, V., Landolfi, C., Lortat-Jacob, H., Bechard, D., Lassalle, P. and Delehedde, M. (2006) Endocan or endothelial cell specific molecule-1 (ESM-1): A potential novel endothelial cell marker and a new target for cancer therapy. *Biochim Biophys Acta*, **1765**, 25-37.





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## CANCER STUDIES – TARGETED THERAPY GROUP (page 48)

Tim Illidge

### Refereed Research Papers

Kalejs, M., Ivanov, A., Plakhins, G., Cragg, M.S., Emzinsh, D., Illidge, T.M. and Erenpreisa, J. (2006) Upregulation of meiosis-specific genes in lymphoma cell lines following genotoxic insult and induction of mitotic catastrophe. *BMC Cancer*, **6**, 6.

Weigert, O., Illidge, T., Hiddemann, W. and Dreyling, M. (2006) Recommendations for the use of Yttrium-90 ibritumomab tiuxetan in malignant lymphoma. *Cancer*, **107**, 686-695.

## ADDITIONAL PUBLICATIONS

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Araki, Y., Lau, C.K., Maekawa, H., Jaspersen, S.L., Giddings, T.H., Jr., Schiebel, E. and Winey, M. (2006) The *Saccharomyces cerevisiae* spindle pole body (SPB) component Nbp1p is required for SPB membrane insertion and interacts with the integral membrane proteins Ndc1p and Mps2p. *Mol Biol Cell*, **17**, 1959-1970.

Brown, M.D., Hart, C.A., Gazi, E., Bagley, S. and Clarke, N.W. (2006) Promotion of prostatic metastatic migration towards human bone marrow stroma by Omega 6 and its inhibition by Omega 3 PUFAs. *Br J Cancer*, **94**, 842-853.

Lalloo, F., Varley, J., Moran, A., Ellis, D., O'Dair, L., Pharoah, P., Antoniou, A., Hartley, R., Shenton, A., Seal, S., Bulman, B., Howell, A. and Evans, D.G. (2006) BRCA1, BRCA2 and TP53 mutations in very early-onset breast cancer with associated risks to relatives. *Eur J Cancer*, **42**, 1143-1150.

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of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin Cancer Res*, **12**, 1157-1167.

# Seminar Series 2006

2005 saw a lot of disruption to the seminar programme of the Institute due to refurbishment of the lecture theatre. This was all completed by 2006, so we could return to full strength and hosted some excellent speakers. In addition to the external speaker programme, there are regular Gene Therapy seminars and also seminars in the Christie Hospital. Last, but definitely not least, the postdocs continue to run an internal seminar series which is extremely well attended by staff in the Institute.

***Peter Angel***

German Cancer Research Centre, Heidelberg, Germany.

***Frances Balkwill***

Centre for Translational Oncology, Institute of Cancer, London.

***Stephen Bell***

MRC Cancer Cell Unit, MRC Research Centre, Cambridge.

***Dominique Bonnet***

Cancer Research UK London Research Institute.

***Allan Bradley***

The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge.

***John Couchman***

Division of Biomedical Sciences, Imperial College London.

***John Diffley***

Cancer Research UK, Clare Hall.

***Amanda Fisher***

MRC Clinical Sciences Centre, Imperial College Faculty of Medicine, London.

***Holger Gerhardt***

Cancer Research UK London Research Institute.

***Ermanno Gherardi***

MRC Research Centre, Cambridge.

***Eyal Gottlieb***

Cancer Research UK Beatson Institute for Cancer Research, Glasgow, Scotland.

***Paul Harkin***

Queen's University, Belfast, Northern Ireland.

***Christine Harrison***

Leukaemia Research Fund Cytogenetics Group, University of Southampton.

***Ron Hay***

Centre for Interdisciplinary Research, University of Dundee, Scotland.

***Marja Jäättelä***

Apoptosis Department, Institute of Cancer Biology, Copenhagen, Denmark.

***Stephen Keyse***

Stress Response Laboratory, Biomedical Research Centre, Dundee.



**Michael Knop**

EMBL, Heidelberg, Germany.

**Walter Kolch**

Cancer Research UK, Beatson Institute for Cancer Research, Glasgow, Scotland.

**Willy Krek**

Institute of Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland.

**Johan Kreuger**

Uppsala University, Sweden.

**Dieter Marmé**

Tumor Biology Center, Freiburg, Germany.

**Eric Miska**

The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge.

**Inke Näthke**

Cell & Developmental Biology, University of Dundee, Scotland.

**Daniel Peeper**

Division of Molecular Genetics, Netherlands Cancer Institute, Amsterdam, The Netherlands.

**Francesc Posas**

Universitat Pompeu, Fabra, Barcelona, Spain.

**Kai Rothkamm**

Medical Sciences Division, University of Oxford, Oxford.

**Erik Sahai**

Cancer Research UK, London Research Institute.

**Owen Sansom**

Cancer Research UK, Beatson Institute for Cancer Research, Glasgow, Scotland.

**Viesturs Simanis**

Cell Cycle Control Laboratory, ISREC, Switzerland.

**Helle Ulrich**

Cancer Research UK, Clare Hall Laboratories.

**Maarten Van Lohuizen**

The Netherlands Cancer Institute, Amsterdam, The Netherlands.

**Laura van't Veer**

The Netherlands Cancer Institute, Amsterdam, The Netherlands.

**Fiona Watt**

Cancer Research UK, London Research Institute.

**Michael White**

University of Liverpool, Liverpool.

**Gareth Williams**

Department of Pathology, University College London

**Wolfgang Zachariae**

Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

**Pascale Zimmermann**

Molecular Genetics Section, K.U. Leuven, Belgium.



## **POSTGRADUATE TUTOR**

Graham J Cowling

## **EDUCATION COORDINATOR**

Julie Edwards

## **EDUCATION COMMITTEE 2006**

Iain Hagan – Chairman  
Fiona Blackhall  
Noel Clarke  
Graham Cowling  
Caroline Dive  
Dave Gilham  
Tim Illidge  
Gordon Jayson  
Valerie Kouskoff  
Karim Labib  
Catherine Merry  
Crispin Miller  
Mark Saunders  
Jenny Varley

## **STUDENT REPRESENTATIVES 2006**

Hiroko Morohashi  
Helen Spencer  
Claire Rooney  
Katalin Boros  
Martin Brandenburg

# Postgraduate Education

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

Developing first rate cancer research and treatment in future decades depends on today's quality training of our clinical and science graduates. The Paterson Institute continues to act as the centre for postgraduate study in many aspects of molecular, cellular and translational cancer research by providing the major elements required of a training programme for postgraduate research students and clinical research fellows studying for MPhil, MD and PhD degrees by research. As the major centre for research and teaching in the Division of Cancer Studies, School of Medicine, University of Manchester, our active research environment serves all our students equally and they can draw on the wide expertise of our scientists and clinicians as well as gaining benefit from our modern laboratories and first-rate service units.

### **The Paterson PhD Programme**

In 2006 we welcomed eight new Paterson four-year PhD students from the around the world, who were selected from over 30 interviewed candidates who were chosen from 250 applications received by the Institute during the year. The School of Medicine also awarded two strategic PhD studentships in Medical Oncology and a further four new clinical fellows who started work in the Institute. Two of these were the prestigious AstraZeneca Clinical Fellowships who will spend half their degree working in Professor Caroline Dive's laboratory and the rest working at AstraZeneca leading the clinical trials of new cancer drugs. The contributions of all our postgraduates to the scientific work of the Institute are described elsewhere in this report. The four-year PhD students from previous years contin-

ue to master a remarkable range of skills and are achieving a strong basis for their final thesis research. Several have already published parts of their work. We say goodbye to Graeme Smethurst at the end of 2006 who is our first four-year PhD student and wish him all success as he begins a new career in investment with a city bank. Our programme is still evolving and, in 2007, we are offering more of these flexible PhD training programmes. Successful UK, European and International candidates can opt to work on one main project or three 10 week options in their first year.

### **Research Degree Structure**

Postgraduate students entering the Institute to study for the degree of PhD are under the direct guidance of an appointed Supervisor(s) and are also allocated an Advisor, with whom they meet regularly to review and record progress, set new targets and identify any assistance required. All postgraduates are also required to participate in regular research meetings within their research group and to attend an organised series of seminars by national and international speakers, which runs throughout the year in the Paterson Institute, Christie Hospital and Manchester Universities. To further enhance our training programme, the monthly series of cancer biology master-classes has been continued, allowing Lead Researchers in the Paterson and Christie Hospital to explain the "what", the "how" and the "why" of their area of research. These relaxed evening sessions are proving extremely popular with all of our postgraduate students and many of the postdoctoral fellows take part in the scientific discussions. Students also participate in a structured Faculty graduate training programme of short



# POSTGRADUATE EDUCATION

courses and workshops depending on their research needs. This includes sessions on statistics and data handling (project dependent), bioinformatics (optional), pharmacology (optional), safety (compulsory), innovation (compulsory), written and oral presentations (compulsory), careers, animal usage (compulsory if using animal models) and ethics (compulsory if using human tissue). The Faculty Training sessions has continued to allow students to develop generic research and personal skills.

In the Division of Cancer Studies all new postgraduate research projects are assessed for quality. Based on Research Council principles, the Education Committee, with help from internal and external assessors ensure that the work will form the basis of a stimulating and intellectually challenging postgraduate programme. This process often takes place before the candidate is chosen. The Education Committee, a body made up of senior scientists, postdoctoral fellows and student representatives, continue to assess student progress throughout their 3-4 years of study and ensure, along with their Supervisors and Advisors, that the student achieves their degree goal. Assessment is through written reports at 3 months (literature review), 1 year and 2 years and short talks to the Institute at similar times (6, 12, 24 months and as

part of the final *viva voce*). All students have the opportunity to give written feedback on any aspect of their degree programme. Students can ask advice of any member of the Education Committee or use their student representatives. All Students are automatically members of the Cancer Studies Postgraduate Association, an independent student body at the Institute and Christie Hospital.

When our students are not working, our student representatives have been busy organising a programme of activities outside of the laboratory. This begins in September when all our students join the Institute for the annual Colloquium. These three days of intensive science also includes two nights of relaxation that allow new students to find their feet for hard work and play. All students are invited to annual student socials to meet other new postgraduates and postdoctoral fellows and to finish the year, the Simon students social takes the form of ice skating followed by a meal.



Paterson students 2006



**Director of Operations**  
Pippa McNichol

**DEPUTY**  
Margaret Lowe



# Operations Services

<http://www.paterson.man.ac.uk/facilities/adminfacjs.jp>

Operations provide the foundations upon which the Paterson Institute runs. This encompasses Administration and Reception, the Director's office, Estates, Finance and Purchasing, Health & Safety, HR, IT and the MCRC team.

2006 has been a very interesting and busy year for the Operations teams. On 1 January 2006 we legally transferred the Paterson Institute from the Christie Hospital NHS Trust to The University of Manchester. This meant that all the HR, Payroll and Finance systems changed and so the staff had to adapt very quickly to these new systems. I am delighted to report that the changeover was so smooth that the majority of Paterson staff were unaware of it. I would like to thank all the operations staff for their perseverance and patience during the transfer.

As well as managing the Paterson transfer, Operations staff were also responsible for implementing the new CR-UK contribution-based national Pay & Grading scheme. Following a consultation period with the union and staff, this was agreed and implemented in September. Ricky Van Deursen (Cell Cycle) suggested that staff from within the Paterson should design the performance review forms that will be used with the new system. To this end a small cross-section of staff have been meeting and the new forms will be ready for distribution early in the New Year. Towers Perrin, the consultants working on the project, will be running training sessions for all managers, staff and the performance review panel in January and February 2007 ready for the first cycle of reviews, scheduled for March/April next year.

The MCRC team were recruited within the year to provide support in establishing the MCRC.

---

## Admin & Reception Services

Manager: Julie Hallett  
Sharon Barnes (temp), Trevor Haughton, Shirley Leonard, Carl Oluwole

There were a number of staffing changes in the Administration Department during 2006. Julie Hallett returned from maternity leave and Sharon Barnes, who had been providing additional cover, left. Sharon made a valuable contribution to the team, assisting Shirley Leonard, who was 'acting up' into the Manager's position. Shirley adapted to this role extremely well and enjoyed the challenge that this opportunity provided.

Trevor Haughton, Front of House Security/Receptionist, also decided it was time to move on to pastures new. Carl Oluwole took over the reins and settled in well.

Despite these changes the team continued to provide a comprehensive secretarial / administration / reception service to the Institute. The needs of the business continue to evolve and the diverse nature of the work being given is proving to be a very challenging experience for the team.

---

## Director's Office

Director's PA: Elaine Mercer

Throughout 2006, the Director's office has continued to organise the Paterson Seminar series with a full and exciting programme of eminent speakers visiting the Institute. This has served to foster collaboration and encourage positive interaction within the wider scientific community, covering a diverse range of fields. The 2007 series has now been planned and guest speakers invited, with a full programme for the start of the year.

Following the merger with The University and the development of the MCRC, this office has under-

# OPERATIONS SERVICES

taken a great deal of work with regard to the planning and organisation of meetings involving both internal and external personnel. Another major focus has been on the recruitment of potential new Group Leaders with emphasis on the development of research programmes.

The newsletter has continued to be a great success with regular meetings of the Newsletter Editorial Board taking place. Copies have been sent out to a wider audience over the past year, with much positive feedback from the recipients. Members of the public who have made regular donations to the Institute and those that have shown an interest in fund-raising, have all appreciated being able to read about the work and the staff of the Institute.

This office has continued to support the Director and his management team, in an administrative capacity, through an extremely busy and eventful year.

---

## Estates

Manager: Steve Alcock

Graham Hooley, John Lord, Dennis O'Shea, Tony Woollam,

The Estates function has a new look to it this year, since John Lord left and was replaced by Graham Hooley and Tony Woollam. The additional post was created in recognition of the expanding workload and the opening of TRF1. Graham and Tony have made quite an impact since joining in June, with response times to outstanding work and fault reports improving considerably.

Several capital schemes have been undertaken throughout the year including laboratory refurbishments and a replacement lift for the central stairwell. Dennis O'Shea managed the TRF scheme which was handed over in summer 2006, so many thanks to Dennis for all his hard work.

An Estates user group was established this year to give users an opportunity to comment upon the services they receive. The team has been pro-active throughout the year which has improved the overall service to the Institute.

---

## Finance & Purchasing

Manager: Margaret Lowe

Yana Anderton, Catherine Bentley, Liz Fletcher, David Jenkins, Denise Owen, Debbie Suthern

Once the Paterson Institute transferred to The University of Manchester, life within the Finance Department became a series of challenges. Contacts within the various departments within The University were established and obstacles were overcome. The University introduced a new Finance System (Oracle) that went live on 1st June; this proved to be an additional challenge for the team as they had just become acclimatised to the previous system. As a result of its transfer to The University, the Paterson now has two different financial years that it needs to work within – April to March for CR-UK (and the Christie Hospital NHS Trust) and August-July for The University.

The team would like to think that the majority of the Institute have been unaware of the problems they have faced. They have all given immense support to provide an efficient service to keep the groups and service units functioning.

During the year the Paterson instigated a European tender process for the new IT storage and archiving system (overseen by The University's Procurement Manager) which will be implemented during 2007.





CR-UK have introduced a new budget planning process and so the team have been working to ensure that the Paterson complies with this.

In 2007 it is planned to introduce Internet Procurement throughout the Institute. Several key members of staff within the groups and service units have already received training and courses are on-going for any further staff wishing to attend.

---

## Health & Safety

Manager: Colin Gleeson

Wide-ranging health and safety advice and guidance is provided to staff at all levels within the Institute. The main issues for staff are around biological and chemical hazards. To address these concerns a number of initiatives have been developed and implemented in the last twelve months.

Firstly, an Institute wide Bio-safety survey was designed and implemented which surveyed the Institute's laboratories. This focused on laboratory environment and estate-related issues and on laboratory waste disposal procedures. As a result of this survey, estates-related and waste disposal procedural deficiencies have subsequently been rectified or are programmed to be so.

Secondly, an Institute-wide review of genetic modification (GM) risk assessments was undertaken to ascertain their sufficiency and suitability in light of the regulations. Some improvements in the level of detail of these risk assessments have been made, to better reflect the hazard and risk potential posed by the work.

It is pleasing to note that the Paterson Institute's GM risk assessment format and its administrative procedures for GM risk assessment review and its GM committee's operations are looked on favourably by the University; and are held up as a model of good practice for the main University campus and other campuses.

Thirdly, the training presentations for working with biological agents and hazardous chemicals have been further developed to address frequently occurring themes and concerns posed by staff, over the last year. Adapting these and other presentations to address concerns raised by staff will be continued next year.

More generally, departmental health and safety

inspections are run on a rolling annual programme. This helps to spread any necessary minor remedial works more evenly throughout the year. Statutory performance-testing of the Institute's hundred fume cupboards and microbiological safety cabinets, (including those in the Kinnaird Road labs), have been carried out. Finally our relationship with The University's Health & Safety Services team continues to develop, especially via contributions to the Biological and GM Hazard Advisory Group.

---

## HR

Manager: Anna Pearson  
Laura Humes

The HR department has continued to provide professional advice to managers and staff on all employment-related matters such as policy guidance, legislation and best practice.

Throughout 2006, the team has undertaken several large tasks including the co-ordination of HR issues relating to the merger with The University of Manchester, the administration of the new CR-UK Pay and Grading system, the evaluation and improvement of the induction process and the transfer of all staff data onto The University's payroll software, Resourcelink.

A Joint Negotiation Committee with three management representatives and three union members has been created to ensure effective partnership working in the development of HR policies and other change processes.

The HR Manager is currently developing the HR strategy based on high standards of employment practice to ensure it is aligned with the needs of the Institute. This will almost certainly produce an exciting and varied workload for the team.

---

## IT

Manager: Malik Pervez  
Brian Poole, Steve Royle, Zhi Cheng Wang, Matt Young

The IT department has continued to maintain high levels of support to users in terms of response times to support calls and extremely low downtimes ensuring that research and development is supported at the highest level.



## OPERATIONS SERVICES

The key challenge facing the Institute is the ever increasing demand for higher levels of storage capacity. In response to this plans are being put in place to develop a new storage solution that will quadruple the available storage capacity. The new storage solution is scheduled for implementation in early 2007 and can be easily added to, which should provide adequate storage for subsequent years. To meet legal and legislative requirements an archive solution is also being developed in conjunction with the storage strategy. The new solution will initially provide 50 Terabytes (Tb) of storage and archiving, with the potential to increase this to 200 Tb in the future.

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### Web & Graphic Design

Mark Wadsworth

To enhance the image of the Institute there are plans to redevelop and modernise the Institute's website. A small committee has been working on this and the revamped site will go live in Spring 2007. The internal intranet will also be redesigned and this will be ready by Summer 2007.

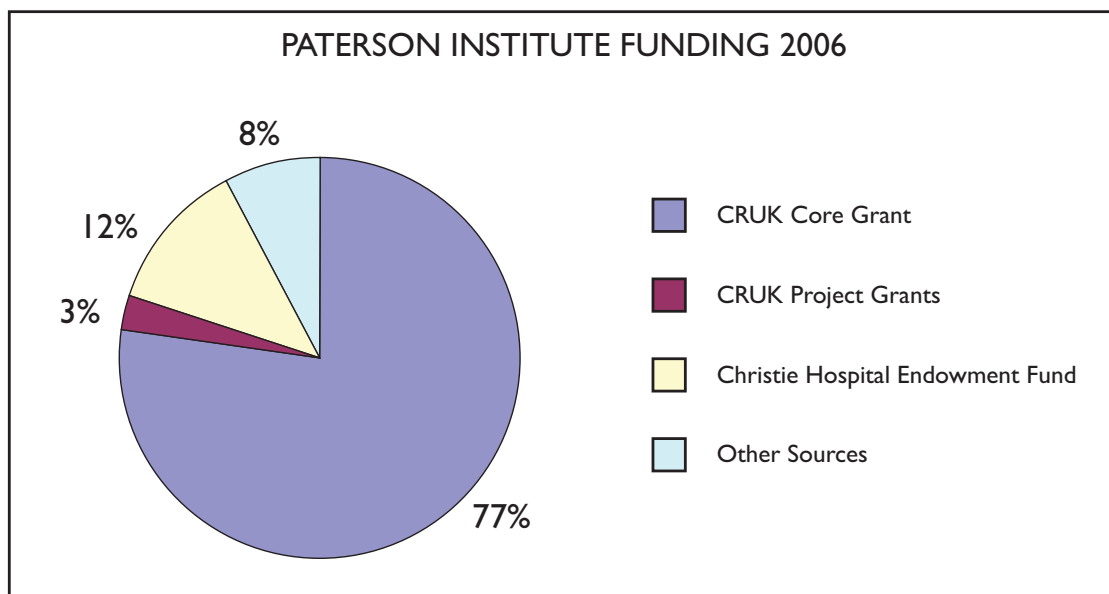
The design of the Paterson newsletter, annual report, stationery and other promotional material has now been brought in-house, giving greater flexibility and allowing us to increase our profile through a variety of different media and events.



Operations team

## Acknowledgement for Funding of the Paterson Institute

The major source of funding (77%) of the Paterson Institute is through a core grant from Cancer Research UK (CR-UK). This is divided between the various scientific groups and service units within the Institute to enable them to carry out their research. In addition to this a further 3% of funding has been received from CR-UK for Project Grant Work and Studentships.



The infrastructure of the Institute is funded by the Christie Hospital Endowment Fund and together with specific project grants accounts for just over 12% of the total income.

The final 8% 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:

- National Translational Cancer Research Network (NTRAC)
- European Commission
- Medical Research Council
- Astra Zeneca
- Wellcome Trust
- Active Biotech
- Trillium Therapeutics

We are immensely grateful to all our sponsors.

### Donations to the Institute in 2006

#### Legacies

- In memory of Mrs Catherine Ord
- In memory of Mrs Mary Hyde

## Donations

- The PACCAR foundation (through CR-UK)
- Mr Neil Raghib
- Oldham SNU Church
- Mrs E Barton
- In memory of Jeremy Daintree: Mr & Mrs S Rubin; Mr & Mrs B L Singer; Mr & Mrs A Miller; Mr & Mrs V Harris; Mr & Mrs M Moss; Mr & Mrs M Collins; Mr & Mrs H I Silverberg; Mr & Mrs P Showman; Mr & Mrs B Rothmer; Mr & Mrs Goldenfield; Ms M Milton & Mr M Press; Mrs G Daintree; Mrs B Kay; Mrs A Nicholson; Mrs A Halton; Mrs J Reuben.
- Mr G T Mercer in memory of Mr H Roberts
- Mrs P Johnson
- In memory of Rhoda Luscott-Evans (formerly Rhoda Langmead-Smith): Mr & Mrs R Evans; Mrs E Haworth; Mrs B Teale and Friends at Kidderminster Health Centre; Mrs A Henderson; Mr M Luscott-Evans; Jane Johns and Friends.
- ServiceMaster Contract Services
- In memory of Audrey Beardmore: Mr J Beardmore, Mrw H Roberts; Mrs M K Sutcliffe and members of the Alkrington Townwomen's Guild; Mrs Neta Smart.
- Ms Eileen Hamilton
- Mrs H M Emerson in memory of Mr G Emerson
- Mrs M Collier in memory of Mr Bill Collier
- The Ladies Committee of Prosperity Lodge No 5206, Accrington
- Mr M Gannon
- Mr V Timson in memory of Mrs Eileen Bottom
- Women's Trust Fund



Stuart Heys (left) formally opened the new PACCAR laboratory in October. The opening was attended by senior members of the University, the Christie Hospital NHS trust and Paterson Institute. Also shown (left to right) are Nic Jones, David Newbigging (Chairman of CR-UK), Caroline Dive and Alex Markham (Chief Executive of CR-UK)

# Career Opportunities

The Paterson Institute is located alongside the Christie Hospital, 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. Recently the Manchester Cancer Research Centre has been created with partners including the Paterson Institute, the Christie Hospital NHS Trust, The University of Manchester and Cancer Research UK (<http://www.mcrc.manchester.ac.uk/>). This is an extremely exciting development which will enhance 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, 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 72). 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 compliment 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. Links to sources of potential funding for fellowships are provided on <http://www.pater-son.man.ac.uk/djs?jid=222>.

In addition to postgraduate and postdoctoral opportunities, the Institute is still seeking to recruit outstanding candidates (clinical and non-clinical) 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/vacancies>), but suitably qualified and enthusiastic individuals should contact the Institute at any time to enquire about career possibilities.



# How to Find 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

