Mouse back skin arrector pili muscles (APMs) that are inserted to the region of the hair follicle stem cells (bulge). Image provided by Hironobu Fujiwara (Watt laboratory).

Displastic small intestinal villi stained with WGA488 and DAPI. Image provided by André Neves (Brindle laboratory) in collaboration with the Winton laboratory.
Multiphoton image of a zebrafish eye, imaged on the LaVision TriMScan system. Green: second harmonic generation (SHG) signal; Blue: DAPI nuclear counterstain. Image provided by Lorraine Berry (Light Microscopy).
## Contents

<table>
<thead>
<tr>
<th>Director’s Introduction</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research Highlights</td>
<td>7</td>
</tr>
</tbody>
</table>

### Research Groups

- Shankar Balasubramanian 10
- James Brenton 12
- Kevin Brindle 14
- Carlos Caldas 16
- Jason Carroll 18
- Fanni Gergely 20
- John Griffiths 22
- Duncan Jodrell 24
- Florian Markowitz 26
- Gillian Murphy 28
- Adele Murrell 30
- Masashi Narita 32
- David Neal 34
- Duncan Odom 36
- David Ponder 38
- Nitzan Rosenfeld 40
- John Stingl 42
- Simon Tavaré 44
- David Tuveson 46
- Doug Winton 50

### Core Facilities

- Matthew Eldridge 54
- Bioinformatics 55
- Allen Hazlehurst 55
- Biological Resources Unit 56
- Bob Geraghty 56
- Biorepository and Cell Services 57
- Jane Gray 57
- Equipment Park 58
- Richard Grenfell 58
- Flow Cytometry 59
- James Hadfield 59
- Genomics 60
- Will Howat 60
- Histopathology and In Situ Hybridisation 61
- Stefanie Reichelt 61
- Light Microscopy 62
- Donna Smith 62
- Pharmacokinetics and Pharmacodynamics 63
- Kevin Brindle and John Griffiths 63
- Pre-clinical Imaging 64
- Clive D’Santos 64
- Proteomics 66

- Research Publications 66
- External Funding 76
- Seminars and Conferences 78
- Cambridge Cancer Centre 80
- Outreach and Fundraising 81
- Academic Administration 82
- Institute Administration 84
- Theses 87
- Contact Details 88
The Cancer Research UK Cambridge Research Institute
The Institute’s location on the Cambridge Biomedical Campus facilitates collaborations with Addenbrooke’s Hospital and other Institutes and University of Cambridge Departments on the site.
This year, our fourth, the first two of our original Junior Group Leaders achieved tenure. We completed recruitment (pending the release of the third floor), welcoming two distinguished new Senior Group Leaders: Shankar Balasubramanian, and Doug Fearon, who is joining in early 2011. We plan to launch a new pilot core resource, the Biomarkers Laboratory, to support our translational research. Our important interactions with the wider Cambridge scientific community and public continue to thrive, with a very successful event to mark the formal launch of the Cambridge Cancer Centre, a vigorous programme of symposia and meetings, and formal proposals invited by the University for recognition of cancer as a ‘Strategic Initiative’.

The first of our outstanding group of Junior Group Leaders to achieve tenure were Jason Carroll and Duncan Odom. Jason works on molecular and genomic aspects of oestrogen receptor mediated transcription in breast cancer cells, extending his previous work at the Dana Farber Cancer Institute in Boston. Exciting recent data from the lab implicate Pax-2 in mechanisms of tamoxifen resistance. Jason won the British Association for Cancer Research Frank Rose Award for a young investigator in translational research in 2009, and this year an EMBO Young Investigator Award. Duncan uses a combination of genomics, systems and evolutionary approaches to understand the mechanisms underlying transcriptional regulation. In a key recent publication, he used a mouse carrying a human chromosome to show that transcription factor binding is almost exclusively determined by DNA sequence. Duncan won a major ERC Start Grant in 2008, and an EMBO Young Investigator Award in 2009. Both Jason and Duncan epitomise the combination of the highest quality science with an active interest in clinical application, which is what the CRI is about.

Shankar Balasubramanian is the Herchel Smith Professor of Medicinal Chemistry in the University, a post that is held jointly between the Department of Chemistry and the Clinical School. We are fortunate that Shankar was keen to make the CRI his base on the Biomedical Campus. He is an outstanding chemist who has a feel for biology, with current interests in nucleic acid chemistry and target discovery. He has a strong record in entrepreneurship and practical innovation as scientific founder of Solexa, whose DNA sequencing technology was recently acquired by Illumina. This year (as well as inclusion in The Times’ list of most influential scientists in the UK), he won the BBSRC ‘Innovator of the Year’ Award for Solexa DNA sequencing.

Doug Fearon, a distinguished immunologist, comes to the CRI to continue his very exciting work on a new approach to immunology-based treatment of tumours by elimination of a cell type in the tumour stroma that has a strong immunosuppressive effect. His primary collaboration will be with Dave Tuveson in the mouse pancreatic model; but other links are forming fast. As well as an exciting and timely project, Doug will bring needed additional expertise to the CRI in the areas of immunology and tumour microenvironment.

The Cambridge Cancer Centre was officially launched at the CRI on 3rd February. One motivation for setting up the CRI as a new Institute in Cambridge was the potential for interaction with the science of the University and associated Institutes, biotech, and of course Addenbrooke’s Hospital. The Cancer Centre was first proposed in 2002, and has been active since 2006. Its new formal identity will further strengthen our mechanisms for joint planning between the partners. The launch day was a sell-out with fundraisers and the general public, who were able to visit the laboratories and hear about our work; so popular that we ran a second day in November to celebrate our Experimental Cancer Medicine Centre, including lectures and tours, which was again a huge success. The Cancer Centre Annual Scientific Symposium in June attracted some 260 attendees from across Cambridge to hear talks spanning from physics through chemistry and genomics to brain tumour biology; the keynote speaker was Mariano Barbacid, Director of the Spanish National Cancer Centre. The postdoc day in November was similarly well attended, with a keynote by Mel Greaves from the Institute of Cancer Research. Two excellent ‘Models and Mechanisms’ meetings for postdoc
and student speakers interspersed with ‘outside experts’ were organised jointly between the CRI, the Sanger Institute, Biochemistry and the MRC Cancer Cell Unit. Finally, we held another International Symposium in our ‘Unanswered Questions’ series on tumour monitoring. An exceptional group of international leaders came as speakers and Chairs, the Chairs giving short talks at the end of each session to pose the questions for a 45-minute structured discussion: a format which has worked well. The next symposium, in November 2011, will be on ‘Unanswered Questions in Transcription’: outstanding speakers are already signed up.

Congratulations, finally, to the several members of the CRI who achieved distinction this year. Florian Karreth (PhD student, Tuveson lab) won one of the five ‘Future Leaders in Cancer Research’ Awards given in annual world-wide competition by the American Association for Cancer Research, and presented his work at the winners’ symposium. This is the third successive year that a PhD student from either the CRI or our Oncology Department has won one of these awards, which we are told is unique. Tiago Rodrigues (postdoc, Brindle lab) won the meeting prize for his poster at the Marie Curie Fellows’ meeting in Turin. Ferdia Gallagher (formerly Brindle lab and AACR Future Leader 2009), now a Cancer Research UK Clinician Scientist, jointly won the Cancer Research UK Pontecorvo Prize for a thesis submitted by a Cancer Research UK-funded student. Matt Eldridge (Head, Bioinformatics Core Facility) received a 2010 caBIG® Building Capabilities Award for his work on a microarray data management system (caBIG® is a bioinformatics grid initiative developed by the United States National Cancer Institute).

Jason Carroll’s EMBO Young Investigator Award was mentioned earlier; Masashi Narita won a Human Frontier Science Programme Research Award for a collaborative project with Japanese colleagues. John Griffiths was awarded the prestigious 2010 European Magnetic Resonance Award by the European Magnetic Resonance Forum. Carlos Caldas was elected to the Fellowship of the European Academy of Cancer Sciences, and Bruce Ponder won the 2010 Donald Ware Waddell Award from the Arizona Cancer Centre. David Tuveson was awarded a personal Honorary Professorship by the University of Cambridge, with the title of ‘Professor of Pancreatic Cancer Medicine’.

This coming year, in April, we have the first Institute external five-year review. We look forward to reflecting on our progress and on our priorities for the future.

Professor Sir Bruce Ponder

High-grade serous (HGS) carcinoma is the most common subtype of ovarian cancer, with tumours arising from the ovary, Fallopian tube or peritoneum. These cancers are collectively referred to as high-grade pelvic serous carcinoma (HGPSC). In this paper, the authors found that mutations in the TP53 gene were present in 96.7% of the HGPSC tumour DNA samples that they examined. This is the highest known rate of TP53 mutations of any solid tumour. In the samples which had no TP53 mutations, and had been confirmed as HGPSC, they found evidence of dysfunction in the p53 protein. Overall, p53 dysfunction rate approached 100% in confirmed HGPSCs. This shows that mutations in the TP53 gene are a key step in the development of aggressive ovarian cancer, and presents a target for the development of new treatments.


Integrins are receptors that mediate cell attachments to other cells or the extracellular matrix, and play key roles in cell signalling. In normal epidermis, integrin expression is restricted to the basal layer, which contains dividing stem cells. In many skin tumours, however, integrins are also expressed in the non-dividing, differentiated suprabasal layers, resulting in upregulation of Erk mitogen-activated protein kinase (MAPK) signalling. Using a mouse model that expresses an activated MAPK kinase 1 (MEKI) transgene in the suprabasal layers, the authors found that wounding induces benign skin tumours. Non-dividing cells that express MEKI stimulate adjacent cells to divide and become incorporated into the tumour. Tumour formation is associated with an inflammatory response – IL1α is expressed at high levels in the tumours. Blocking the inflammatory response and depleting levels of inflammatory cells such as γδ T cells and macrophages reduces tumour formation. These results show that differentiated, non-dividing epidermal cells can trigger tumour formation through the activation of inflammation.


Resistance to chemotherapy in ovarian cancer is poorly understood. The disease can often be hard to treat with treatment often failing after an initial good response. The aim of this study was to determine whether this resistance to treatment is caused by the chemotherapy drugs themselves (analogous to antibiotic resistance) or if the resistant cells are already present in the tumour and are lying dormant before treatment begins. To find out, the researchers investigated the genetic evolution in high-grade serous (HGS) ovarian cancers by analysing cell line series derived from three cases of HGS carcinoma before and after platinum resistance had developed. They found that the chemotherapy resistant cells grow as part of the tumour, such that when patients have treatment, the susceptible cells are destroyed, leaving the resistant cells behind to regrow the tumour. The development of treatments tailored to these resistant cells would improve overall treatment success.


This paper shows that a single gene, FOXA1, controls the oestrogen-fuelled growth of breast cancer cells. Estrogen receptor-a (ER) is the key feature of most breast cancers and binding of ER to DNA correlates with expression of FOXA1. The authors found that FOXA1 is necessary for ER to bind to chromatin – in breast cancer cells lacking FOXA1, they found that almost none of the genes normally switched on by ER were activated. This means that ER was left floating around the cell, unable to bind to DNA and trigger cell growth and division. FOXA1 also plays an essential role in tamoxifen response, since tamoxifen also uses FOXA1 to bind to DNA and prevent ER from binding instead. The implications are that developing drugs to target FOXA1 could help women with ER-positive breast cancers who have developed resistance to standard hormone treatments like tamoxifen.
The lining of the intestine, the epithelium, has a capacity for rapid self-renewal and regeneration. This is typical of tissues where growth is supported by stem cells – a small number of stem cells within a tissue produce all of the different cell types in the tissue as and when they are needed. The pattern of stem cell turnover in the intestinal epithelium wasn’t clear, however. It was previously thought that stem cells in the gut replace each other according to a predetermined system, where the fate of each daughter cell is already decided. This would mean that there is a hierarchy where a small number of different stem cells produce the many different types of cells in the gut that make up the gut epithelium. Instead, the authors found that there is one overall stem cell population, with no hierarchy. Stem cells replace their neighbours as they are lost, which means that each stem cell has an equal chance of producing other stem cells and therefore all cell types in the gut. It is likely that normal stem cells replace themselves in a way that is more akin to cancer stem cells than previously thought. Cancer stem cells are more resistant to treatment, so understanding stem cell behaviour in the gut could ultimately have implications for improving treatments.


McIntyre DJ, Howe FA, Ladroue C, Lofts F, Stubbs M, Griffiths JR. Can localised 19F magnetic resonance spectroscopy pharmacokinetics of 5FU in colorectal metastases predict clinical response? Cancer Chemother Pharmacol 2010; Sep 7 [Epub ahead of print]

19F magnetic resonance spectroscopy can be used in vivo to measure the half-life and metabolism of 5-fluorouracil (5FU), a widely used colorectal cancer treatment. Previous studies have reported that longer 5FU half-life in tumours is associated with better survival. This paper investigated 5FU pharmacokinetics in 32 patients undergoing treatment for colorectal cancer, 15 of whom had liver metastases. Using a 1.5T MRI scanner the authors measured 5FU half-life and looked for the presence of fluoronucleotides (the products of 5FU which kill cancer cells) in each patient. Unlike previous studies, they found no association between 5FU half-life, tumour burden and survival. They also had a counterintuitive finding in the group with liver metastases, showing that high levels of fluoronucleotides were associated with poorer survival. This may simply be because patients with higher tumour burdens have higher levels of fluoronucleotides, since fluoronucleotide levels are higher in tumour tissue than in normal liver. While the current technique allows for straightforward detection of fluoronucleotides, which are present at very low concentrations throughout both tumour and normal liver tissue, the authors recommend that more sensitive scanning techniques are needed to resolve tumour from normal liver tissue.


Transcription factors (TFs) are proteins that direct gene expression by binding to specific DNA regulatory regions upstream or downstream of the target gene. It has been widely assumed these TF binding sites would be highly conserved through evolution, just like their target genes. To explore the evolution of gene regulation further, the authors compared the binding of the TFs CEBPA and HNF4A in the genomes of five vertebrate species – human, dog, mouse, opossum and chicken – which last shared a common ancestor around 300 million years ago. In all five species, CEBPA and HNF4A are regulators of liver-specific genes. Comparing the maps of where these TFs bind in each species, they found that in most cases neither the location nor the sequence of the TF binding sites was conserved. Despite this, these TFs still manage to regulate the largely conserved gene expression and function of liver tissue. These differences in binding locations and regulatory sequences help us to understand the evolution of gene regulation and therefore gene mis-regulation, which plays a key role in the development of cancer.


The microseminoprotein-beta (MSMB) protein regulates apoptosis, and previous studies have shown that a single nucleotide polymorphism in the promoter region of the MSMB gene is linked to an increased risk of developing prostate cancer. It is thought this is due to the risk allele reducing promoter activity, reducing MSMB levels in benign tissue and leading to increased prostate cancer risk. In this study, the authors found that MSMB levels in prostate tissue and urine were much lower in men with prostate cancer. They also found that men with the risk allele (but no prostate cancer) also had lower levels of urinary MSMB, and that the level of urinary MSMB was better than urinary PSA levels at differentiating men with prostate cancer at all Gleason grades. These results have the potential to lead to more sophisticated tests, using the levels of MSMB in tissue and urine as a biomarker of prostate cancer risk, diagnosis and disease monitoring, possibly in conjunction with the PSA test.
The CRI’s laboratories undertake research in four main areas:

1. Basic research into the cellular and molecular biology of cancer.
2. Research in molecular imaging, genomics, bioinformatics and biomolecular modelling.
3. Research focussed on specific cancer sites, which form a bridge between laboratory and clinic.
4. Clinical investigations including experimental medicine based clinical studies, conducted jointly with the University of Cambridge and National Health Service (NHS) clinical departments.
Recent advances in the understanding of nucleic acid function have shown that non-coding sequences have key roles in regulating many cellular processes, from transcription and translation to cell division and genome stability.

Rather than having typical Watson-Crick base pairs that form a double helix, many non-coding sequences in DNA and RNA display non-standard structural features. For example, guanine-rich sequences can adopt stable four-stranded structures called G-quadruplexes. We hypothesise that the formation of such structures in vivo is critical to biological function and medicine. We aim to elucidate the role of such structures in cancer and in normal cells. By the application of small chemical molecules that selectively target such non-canonical structural elements, we further aim to develop novel approaches that could be used in the treatment of cancer.

While nucleic acids generally adopt a well-known double helical structure through guanine-cytosine and adenine-thymine base pairing, some sequences can take on alternative structures. In guanine (G)-rich regions, G bases can adopt stable intramolecular arrangements mediated by Hoogsteen hydrogen bonding to form several stacked G-tetrads. Within the human genome, we have shown that potential G-quadruplex forming sequences, with the consensus $G_{1-6}N_{1-7}G_{1-6}N_{1-7}G_{1-6}N_{1-7}G_{1-6}$, are common (Figure 1). These sequences show particular concentrations near or in the promoters and first introns of many genes, including oncogenes such as MYC, KIT and RAS. The accumulated evidence for G-quadruplex structure and function is based largely on data from biophysical, structural and in vitro studies. We are therefore investigating the existence of G-quadruplex nucleic acids in living systems, and are seeking robust evidence of their biological function and their validity as drug targets.

DNA G-quadruplexes

DNA G-quadruplexes are implicated in a range of biological processes from the control of cell division to the regulation of gene transcription. We are investigating several aspects of DNA G-quadruplex biology. First, we wish to prove the existence and survey the extent of G-quadruplex formation in living cells, and how this might be regulated in cancer phenotypes. To do this we are using a variety of probes to stabilise G-quadruplexes in cells. We have therefore chemically synthesised a number of small molecules with high affinity for G-quadruplex over duplex DNA. We are also using G-quadruplex-binding proteins as probes and are exploiting natural binding proteins, such as nucleolin, or synthetic proteins such as specific G-quadruplex-binding antibodies and zinc-finger proteins that we have generated. By isolating genomic DNA bound to these probes, we can use chromatin immunoprecipitation together with next-generation sequencing (ChIP-seq) technologies to determine the sites of G-quadruplex formation genome-wide.

We are also exploring how G-quadruplexes in promoters influence gene transcription and/or DNA replication in cancer cells. Many groups including ours have intensely studied the biophysical and structural characteristics of G-quadruplexes found in the promoters of human oncogenes. Chemical biological studies on cancer cells in culture have also shown that G-quadruplex-binding small molecules can suppress oncogene expression. While such studies are highly suggestive of G-quadruplex formation at promoters during transcription,
DNA damage and uncapping of telomeres is induced by a G-quadruplex-binding small molecule. In treated cells (b), POT1 (green spots) is lost from telomeres compared to untreated cells (a). The compound induces DNA damage as measured by gamma-H2AX foci (red spots in c). This suggests that a DNA damage response is stimulated by loss of POT1 from telomeres. Double staining, under conditions of partial POT1 loss shows that DNA damage occurs at telomeres (see Rodriguez et al., J Am Chem Soc 2008; 130: 15758).

RNA G-quadruplexes
There is much evidence that G-quadruplexes are widely present in RNA and that they may be associated with several key aspects of RNA biology. For example, G-quadruplexes in the 3′-UTR of insulin-like growth factor II mRNA play a role in post-transcriptional endonucleolytic cleavage. Furthermore, we have recently shown that a conserved RNA G-quadruplex motif in the 5′-UTR of the human NRAS proto-oncogene can modulate translation (Figure 3). We have also shown that small molecule ligands can target such RNA G-quadruplexes and thus influence translation. As RNA helicases with G-quadruplex resolving activity have recently been identified, this suggests that RNA quadruplexes exist normally in vivo. Our bioinformatics analysis highlights that large numbers of human RNA transcripts contain a potential G-quadruplex forming region. This raises an important question: how widespread are G-quadruplexes in RNA transcripts and what is their functional relevance? To address this we are therefore using a combination of genome-wide ChIP-seq and chemical biology approaches to identify and map the existence of G-quadruplex structures within the transcriptome.

Non-coding RNA function
More generally, we are exploring other non-coding structural elements formed by RNA molecules. Recently, much evidence has pointed to a role for micro-RNAs and long non-coding (inc) RNAs in epigenetic regulation and cancer. For example, IncRNAs are thought to associate with chromatin-modifying complexes to delineate active versus silent chromatin domains. In breast tumours, the IncRNA HOTAIR shows high expression, with increased levels correlating with greater tumour spread and cell death. HOTAIR is thought to interact with the Polycomb repressive complex 2 (PRC2) to remodel chromatin to a more embryonic phenotype. In cell culture models, over-expression of HOTAIR promotes a metastatic phenotype while repression inhibits cancer invasiveness. In collaboration with Adele Murrell’s laboratory, we are defining which domains in HOTAIR are required for HOTAIR-PRC2 interaction. We are also designing small molecule ligands that disrupt HOTAIR binding to PRC2 with a view to reversing metastasis.
Our laboratory focuses on discovering improved treatments for epithelial ovarian cancer using laboratory and clinical studies. Ovarian cancer has a high healthcare burden because of low cure rates and frequent recurrent disease that causes significant symptoms for patients. This is despite the fact that ovarian cancer is initially sensitive to systemic treatments and most patients are free of disease after completing initial surgery and chemotherapy. The fundamental problem that we are addressing is to understand how ovarian cancer cells escape initial treatment and the molecular mechanisms by which they acquire resistance to further therapy. Using genomic and functional studies we are identifying new biomarkers and treatment targets for testing in clinical trials.

Genomic studies of chemotherapy response in vivo
To identify genetic alterations that are selected for during the acquisition of drug resistance we are carrying out prospective clinical studies that collect cancer samples before and during neoadjuvant treatment. Our initial studies have focused on the drugs carboplatin and paclitaxel as these are the most important therapies in ovarian cancer. By using expression analysis and bioinformatics methods that have been developed to model the acquisition of resistance, we have identified clinically relevant biomarkers that overlap with independently identified genes from RNA interference screens (Swanton et al., Cancer Cell 2007; 11: 498).

Our studies depend upon having homogeneous patient cohorts with similar clinical characteristics. However, response to treatment in tumour masses can be heterogeneous and mixed response frequently occurs at different anatomical sites. For example, primary ovarian masses may respond better than peritoneal metastases. This differential response may be a result of variable blood supply and hypoxia that limits delivery and efficacy of chemotherapy.

We have confirmed these observations using functional magnetic resonance imaging for perfusion (Saia et al., Eur Radiol 2010; 20: 491) and diffusion and are now using imaging data to target the collection of tissues from responding and non-responding areas. This will allow us to calibrate genomic profiles much more precisely and to better identify the molecular determinants of resistance. High throughput sequencing with Illumina technologies is being used to quantitate expression and genomic changes and to identify novel fusion transcripts and mutations (Figure 1).

Differential sensitivity to paclitaxel as compared to carboplatin may depend on cellular pathways involved in maintaining chromosomal stability (CIN). To ask whether this may be clinically relevant we have tested surrogate expression markers of CIN in samples from a prospective neoadjuvant study and have shown that high measures of CIN predict resistance to paclitaxel and increased sensitivity to carboplatin (Swanton et al., PNAS 2009; 106: 8671) (Figure 2). Thus, measuring CIN pre-treatment may optimise choice of treatment for patients.

The key oncogenic and tumour suppressor genes for high-grade ovarian serous carcinoma have not been identified as this type of tumour has high rates of genomic instability, where many of the described alterations may be passenger mutations. Numerous studies have tested the association between TP53 mutations in ovarian...
cancer and prognosis but these have been consistently confounded by limitations in study design, methodology and/or heterogeneity in the sample cohort. To identify the true prevalence of TP53 mutations in high-grade pelvic serous carcinoma, we sequenced exons 2–11 and intron-exon boundaries in tumour DNA from 145 patients with invasive serous carcinoma of the ovary, fallopian tube and primary peritoneal cancer. Surprisingly, pathogenic TP53 mutations were identified in 97% (n = 119/123) of HGS cases (Ahmed et al., J Pathol 2010; 221: 49). This is the first comprehensive mapping of TP53 mutation rate in a homogeneous group of high-grade pelvic carcinoma patients and shows that mutant TP53 is a driver mutation in the pathogenesis of HGS cancers.

Mechanisms of taxane resistance and the role of extracellular matrix
Taxanes, such as paclitaxel, interfere with the dynamic growth of microtubules by directly binding to them, leading to mitotic arrest and apoptosis. Paclitaxel is widely used to treat ovarian and breast cancers but drug resistance limits its clinical usefulness to only half of patients who receive it.

Alterations in the ratio of tubulin isoforms or mutations in tubulin can alter microtubule stability and sensitivity to taxane drugs. By studying cell line models of taxane resistance along with clinical samples we have recently shown that loss of the ECM protein, transforming growth factor beta induced (TGFBI), was sufficient to induce paclitaxel resistance in cells and ovarian cancer tissues (Ahmed et al., Cancer Cell 2007; 12: 514). We have also shown that TGFBI induces microtubule stabilisation that is dependent upon integrin-mediated FAK and RHO signalling pathways. Extracellular matrix proteins have been implicated in the acquisition of drug resistance in ovarian cancer although the mechanism by which this is achieved is unclear. Loss of TGFBI induces resistance by altering microtubules which are the direct pharmacodynamic target of paclitaxel. This work shows that the effects of ECM proteins on drug resistance may be very specific to particular cytotoxic treatments. As 30% of ovarian cancers do not express TGFBI, it may be an important biomarker for paclitaxel response.

Current projects are characterising how TGFBI interacts with integrins and other cell surface receptors and how this may be modulated therapeutically. It is now clear that TGFBI exerts its effects specifically through beta-3 integrins but is also co-regulated, and interacts with, other ECM proteins implicated in drug resistance. To identify the downstream pathways from FAK and RHO that alter microtubule stability, we have generated knock-out somatic cell lines using homologous recombination. These knock-out models have provided a powerful system to identify microtubule associated proteins responsible for effects on paclitaxel resistance. As TGFBI has complex roles in organising interactions between cells and ECM, we have studied its function in early development in Xenopus to identify how it may affect cell migration. Both loss and gain of function experiments have shown that TGFBI is required for somite development in Xenopus.

Publications listed on page 66
The primary aim of our laboratory is to develop clinically applicable imaging methods that can be used to detect early tumour responses to treatment. These could be used in early stage clinical trials of new drugs to get an indication of efficacy and subsequently, in the clinic, to guide therapy in individual patients.

Patients with similar tumour types can show markedly different responses to the same therapy. The development of new treatments would benefit, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment for a specific patient (Brindle, *Nat Rev Cancer* 2008; 8: 1).

A targeted imaging agent for detecting cell death

The agent is based on the 14 kDa C2A domain of the protein synaptogamin, which binds to the phosphatidylserine (PS) exposed by dying cells. Initially we labelled the molecule with tags that could be detected using magnetic resonance imaging (MRI) and have subsequently labelled it with radioactive metals for radionuclide imaging. We made a site-directed mutant (C2Am) in which a serine residue distant from the active site was replaced with a cysteine residue (S78C) which a serine residue distant from the active site was replaced with a cysteine residue (S78C) which a serine residue distant from the active site was replaced with a cysteine residue (S78C). The mutant has a similar affinity for PS as the wild-type protein—using sulphydryl-selective reagents, we have produced homogeneous preparations that have been labelled at this single site with a DOTA chelate. A patent application has been filed on this mutant. This year, using the 111In-loaded chelate and following installation of a single photon computed tomography (SPECT) instrument in the CRI, we have demonstrated that we can detect cell death in a drug treated tumour using SPECT (Figure 1). Targeted radionuclide-labelled imaging agents based on Annexin V, which like C2Am binds to PS, have been trialled in the clinic for the detection of cell death in tumours post treatment. However, there were problems with their biodistribution. Initial studies suggest that C2Am has better specificity for detecting cell death than Annexin V, in that it shows lower binding to viable cells (Alam et al., *Bioconjug Chem* 2010; 21: 884).

Imaging metabolism with hyperpolarised 13C-labelled cell substrates

MRI gives excellent images of soft tissues, such as tumours. The technique works by mapping, in 3D, the distribution and MR properties of tissue water protons, which are very abundant (60 – 70 M in tissues). However, we have known since the 1970s that we can also use MR to detect metabolites in vivo. The problem is that these molecules are present at 10,000× lower concentration than the protons in tissue water. This makes them hard to detect and almost impossible to image, except at very low resolution. We have been collaborating with GE Healthcare in the development of a technique, termed ‘hyperpolarisation’, that increases sensitivity in the MRI experiment by more than 10,000×. With this technique we inject a hyperpolarised 13C-labelled molecule and now have sufficient sensitivity to image its distribution in the body and the distribution of the metabolites produced from it. We have shown that we can detect early treatment response in lymphoma tumours by monitoring decreased tumour utilisation of one cell metabolite, pyruvate, and then detect subsequent cell death by watching the increased metabolism of another cell metabolite, fumarate (Gallagher et al., *Proc Natl Acad Sci USA* 2009; 106: 19801). This year we consolidated this work by demonstrating that the technique will work with other tumour types such as breast (Witney et al., *Br J Cancer* 2010; 103: 1400) and glioma (Day et al., *Magn Reson Med* 2010; 18 Nov EPub), and with other types of drugs, notably combretastatin A4 phosphate, which is an anti-vascular drug (Bohndiek et al., *Mol Cancer Ther* 2010; 9: 3278). In the latter case we showed that measurements of hyperpolarised pyruvate and fumarate metabolism could provide a more sustained and sensitive indicator of response to a vascular disrupting agent than dynamic contrast agent enhanced (DCE)- or diffusion weighted (DW)-MRI respectively, which...
Figure 1
Imaging cell death in EL-4 murine lymphoma tumours using 111In-labelled C2Am and SPECT. Representative images from untreated (A and C) and drug-treated (B and D) animals at 4 and 24 hours after injection of 111In-C2Am-malDOTA. The locations of the tumour (T), kidneys (K) and bladder (B) are indicated. The arrows indicate the presence of radioactivity in the tumour of the treated mouse.

are used currently in the clinic to detect the action of anti-vascular and anti-angiogenic drugs.

Imaging tumour cell glycosylation
Aberrant glycosylation is a hallmark of cancer. We are currently developing a novel molecular imaging platform for the non-invasive assessment of tumour glycosylation, in which sugar analogues are incorporated metabolically by tumour cells in vivo and subsequently detected by a highly selective chemical reaction with a reporter probe that has been labelled with an imaging agent. This year we demonstrated that this technique can be used to image tumour glycans in vivo, using both fluorescence and radionuclide (SPECT) imaging. This methodology could provide new insights into tumour cell proliferation, response to therapy, and perhaps more importantly, metastasis. The technique also has the potential for subsequent translation into a clinical setting, using nuclear imaging techniques.

Genetically engineered models of disease
We are developing a platform technology that enables rapid bioluminescent imaging of autochthonous Cre/loxP-dependent tumour models in vivo. We have designed lentiviral and transposon-based vectors to couple tumour induction with the establishment of expression of the luciferase reporter transgene. By simply varying the route of vector administration, we will be able to selectively induce tumour formation in various organ types. Bioluminescent imaging will allow individual animals to be identified very early during tumour development and then selected for analysis with lower throughput but clinically applicable imaging modalities, such as CT, MRI and PET.

Future directions
With the arrival of microPET and SPECT scanners in the CRI we will continue the development of radiolabelled C2Am with a view to translating the most promising derivative to the clinic. We will apply the glycan imaging approach to animal models of early dysplasia to determine whether the technique can give prognostic information on tumour progression. We will extend an image analysis method that we have developed previously in animal models of disease to the clinic, in particular to determine whether it can detect early treatment response in glioma. We will introduce new hyperpolarized 13C-labelled cell substrates for imaging metabolism and evaluate these for detecting treatment response in new animal models of disease.

Publications listed on page 67
Individualised therapy for breast cancer patients will require a detailed molecular characterisation of the tumour, as well as of the germline polymorphisms that modulate, for example, pharmacokinetics of drugs, as well as other aspects of individual host responses. The challenge is the great heterogeneity of breast cancer. Our laboratory is identifying improved biomarkers with potential clinical utility, and characterising the initiating events (mutations) that transform cells within the breast epithelial hierarchy and give rise to cancer.

Translational breast cancer genomics: applications of molecular profiling in prognosis, prediction and novel therapeutics

The detailed description of the complex molecular taxonomy of breast cancer is the first step towards robustly identifying markers that have true clinical utility.

We have now characterised the genomic and transcriptomic landscapes of 2000 breast cancers using high-resolution SNP arrays (Affymetrix SNP6.0), gene expression arrays (Illumina) and focused mutation analysis (TP53). This has revealed a more complex taxonomy than that gleaned from expression analysis alone. By implementing joint copy number/expression-based clustering we have identified at least 10 subtypes of breast cancer with markedly different clinical outcomes. Most of these subtypes robustly capture the copy number-driven events that dominate the landscape (ERBB2, Cyclin D, 1q, 8p12, 8q24, 20q13). Moreover the high resolution of the arrays used and the large number of tumours profiled allow precise mapping of copy number events, as illustrated with the ERBB2 amplicon (Figure 1). We have also identified subtypes with lower genomic instability where expression is driven by copy number-independent events. These tumours are being prioritised for epigenetic and mutational analysis to help identify the driver events. We are also, in collaboration with Sam Aparicio in Vancouver, sequencing the exomes and transcriptomes of triple-negative (ER−/PR−/Her2−) breast cancers, which are revealing significant heterogeneity in this important subtype. We continue to contribute to the International Cancer Genome Consortium (ICGC) effort to characterise breast cancer genomes to single nucleotide resolution by deep whole-genome sequencing. Importantly, the availability of 2000 breast cancers with a minimum of five years of clinical follow up constitutes a unique and valuable validation platform for the clinical significance of cancer genes identified by ICGC.

To date more than 8,500 paraffin-embedded samples from a population-based cohort and from four randomised clinical trials have been included in tissue microarrays (TMA). We have started to deploy this unique resource of clinically annotated tumours to robustly identify and validate prognostic and predictive markers. We have now shown that BCL2 is an independent indicator of favourable prognosis for all types of early-stage breast cancer and its addition to currently used prognostic models improves survival prediction. We have contributed the largest TMA to the study showing that in women with early breast cancer receiving adjuvant chemotherapy, the most powerful predictor of benefit from anthracyclines is Ch17CEP duplication, and not Her2 amplification as previously proposed. We have been major contributors to the study showing that six subtypes of breast cancer defined by expression of five markers show distinct behaviours with important differences in short term and long term prognosis. This study has definitively shown that triple-negative breast cancers are not a homogeneous entity and this has major implications to the design of clinical trials with agents that target defective homologous recombination.
Visiting Workers ctd
Stephen Sammut
Ina Schulte
Martin Sæt
Hans Kristian Moen Vollan
Jamie Weaver

We have followed our published observations on the importance of immunity in breast cancer outcomes by showing that immune response pathway modules antagonise or synergise to delineate novel prognostic subtypes.

Our efforts in pharmacogenetics, the study of germline genetic variation and its influence in drug disposition, have progressed. Using a large population-based cohort of breast cancer patients treated with adjuvant tamoxifen we have shown absence of association with survival in frequent variants of CYP2D6, including CYP2D6*4, questioning the validity of the reported association between CYP2D6 genotype and treatment response in breast cancer.

Collaborators: Sam Aparicio (University of British Columbia), Simon Tavaré (CRI), Paul Pharoah (Strangeways Research Laboratory), Helena Earl (Department of Oncology and Addenbrooke’s Hospital), Gordon Wishart and Elena Provenzano (Addenbrooke’s Hospital), Paul Edwards (Hutchison/MRC Research Centre), and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC)

Functional breast cancer genomics: characterising tumour initiating/cancer stem cells in breast cancer subtypes
We have continued our work to identify and characterise which cells initiate different types of breast cancer and what is the nature of the pathways that are disrupted. We are particularly interested in the role of miRNAs, TGFβ, a new oncogene on 8p12 (ZNF703), and NOTCH signalling in the regulation of normal and malignant breast stem cells. We are testing whether disruption of these pathways alters the self renewal of tumour initiating cells, which could lead to novel therapeutic approaches to eradicate tumours.

ZNF703 is a new breast cancer oncogene, and amplification occurs almost exclusively in more aggressive ER-positive tumours. We have shown that ZNF703 over-expression in epithelial progenitors in normal human mammary epithelium favours luminal differentiation and attenuates basal differentiation. This might explain why ZNF703 amplification/over-expression predominates in Luminal B breast cancers.

Collaborators: John Stingl, Jason Carroll and Bruce Ponder (CRI), Eric Misra (Cancer Research UK/Wellcome Trust Gurdon Institute) and Shankar Balasubramanian (Department of Chemistry and CRI)

Publications listed on page 67
Oestrogen receptor is the defining feature of luminal breast cancers, where it functions as a transcription factor to induce cell cycle progression. ER is also the target of most endocrine therapies, including tamoxifen and aromatase inhibitors, which are effective treatments. However, some women can develop resistance to these drugs and in many cases, ER simply gets switched back on again, despite the presence of the drug. Therefore, understanding how ER functions is an important issue and one that has not been completely resolved. ER transcriptional activity requires a number of co-factors and co-operating transcription factors that possess enzymatic activity to alter chromatin structure, the outcome of which determines transcriptional activity. It is currently known that a number of ER co-factors can either assist in transcription (including SRC-1 and AIB-1) or are involved in gene repression by tamoxifen (including N-CoR and SMRT).

Recently, using chromatin immunoprecipitation (ChIP) combined with high-throughput sequencing (ChIP-seq), we mapped all ER binding sites in a breast cancer cell model after oestrogen treatment. This unbiased identification of the genomic contact points of ER revealed a number of surprising features about ER biology. These included the observation that ER rarely regulates genes from promoter regions, but instead utilises distal enhancers. We also identified the role of a ‘pioneer factor’ called FoxA1, which is critical for ER to function. Our lab is interested in extending these findings to fully define the cis- and trans-elements that contribute to ER activity in breast cancer cells, with particular emphasis on the pioneer factors that stabilise ER-DNA interactions.

Characterisation of the role of pioneer factors in ER biology
We are interested in identifying and characterising the role of the pioneer factor FoxA1 in regulating ER activity. We have found that FoxA1 is required for all ER-DNA interactions and for ER to promote cell growth. In the absence of FoxA1, ER function is blocked and cells do not proliferate (Figure 1). We also find that for tamoxifen to successfully block ER function, FoxA1 is also required. This unexpected finding is possibly due to the fact that tamoxifen-ER actively represses gene transcription, which still requires functional ER-chromatin interactions. We find that FoxA1 is essential for cell growth in models of drug resistance, suggesting that FoxA1 may constitute an attractive drug target for endocrine resistant breast cancer. We can show that FoxA1 expression in non-breast cancer cells is sufficient to enable ER to interact with the DNA and to switch on gene transcription. These findings implicate FoxA1 as the sole mediator of ER function and response to hormonal stimuli, in both drug sensitive and drug resistant breast cancer.

Co-operative interactions between ER and RARα
We have observed that a number of estrogen-ER regulated genes are subsequently utilised by the ER complex itself in order for effective transcription to take place, resulting in feed-forward loops. We recently found that RARα, another nuclear receptor, is an estrogen-ER target gene that can feedback to the ER complex. RARα has been shown to be required for oestrogen-mediated gene transcription and proliferation. We have shown, on a genomic scale, that ER and RARα co-occupy similar regions of the genome, which occur in a co-operative manner. This was achieved by establishing re-ChIP (double...
Figure 1
ER binding is dependent on the pioneer factor FoxA1. (A) Examples of ER-DNA binding events, determined by ChIP-sequencing experiments in the presence (siControl) or absence (siFoxA1) of FoxA1. (B) A model representing the role of FoxA1 in mediating ER interactions with chromatinised DNA.

ChIP sequencing for the first time. RARα was subsequently shown to be required for oestrogen-ER to recruit co-factors, providing a bridge between ER and some of the essential co-factors. This investigation links two nuclear receptors together in a co-operative role and may help explain why women with breast cancer can, in some cases, respond to retinoic acids.

Genomic analysis of ER function in primary breast cancer
All ER genomic studies to date have been limited to a single breast cancer cell line, yet they have revealed extraordinary features about ER biology. We have now been able to extend genomic transcription factor mapping experiments into frozen primary breast cancer samples, by performing ER ChIP-sequencing in luminal breast cancer material. The data confirm that ER ChIP-seq can be performed in primary breast cancer samples and that the ER binding events accurately represent the binding sites in the cell lines. However, there are significant numbers of ER binding events that are acquired in tumours with a poor clinical outcome and even more ER binding events acquired in metastatic material that originated from an ER positive breast cancer. These ER binding events, associated with poor prognosis tumours and metastatic samples, occur at genes that predict a poor clinical outcome in patient cohorts. Furthermore, the alterations in ER binding profiles in tumours with poor prognosis and in metastatic samples provide insight into specific genes that may be influencing drug response and risk of metastasis.

Publications listed on page 68
Emerging evidence, however, suggests that the centrosome also acts as a communication hub that spatially concentrates diverse signalling pathways. While centrosome number, structure and function are carefully regulated within healthy cells, tumours display a multitude of centrosomal abnormalities. How such anomalies contribute to tumourigenesis is an important and as yet unresolved question.

The regulation of centrosome number and function underlies bipolar mitotic spindle formation and genetic integrity. In normal cells, the centrosome is usually composed of a pair of barrel-shaped structures, the centrioles, which are embedded in an electron-dense amorphous matrix, the pericentriolar material. The latter provides the site for microtubule nucleation and therefore strongly influences microtubule numbers and organisation throughout the cell cycle. Malignancies exhibit a wide variety of centrosome abnormalities that range from numerical and structural to functional and positional aberrations. It is not yet understood how these abnormalities arise in cancer and how they contribute to tumourigenesis. We have two basic goals in the laboratory. First, we want to address what the consequences are of deregulation of centrosomal proteins on mitotic spindle formation and genome stability. Second, we want to study the molecular mechanisms and signals that govern centrosome behaviour in response to environmental cues. These goals aim to provide insight into basic biological processes whose deregulation is implicated in the development of cancer.

CDK5RAP2: linking centrosomes with mitotic spindle poles

To investigate the function of specific centrosomal proteins during cell division we have turned to the chicken B-cell line, DT40. These vertebrate cells have an exceptionally high ratio of targeted versus random integration of transfected DNA constructs, and are therefore genetically manipulable. DT40 cells have provided us with particularly exciting results regarding the function of the highly conserved centrosomal protein, CDK5RAP2. In search of a mitotic role for CDK5RAP2, we have deleted two evolutionarily highly conserved regions of the cdk5rap2 gene in DT40 cells. Both cdk5rap2 mutants exhibited poor cell viability and a decrease in the clonogenic potential of individual cells. However, in these mutants the most striking phenotype became
Figure 2
CEP63 is required for centrosome duplication. Top panels show a wild-type DT40 cell in which two centrosomes are visible each within a pole of a bipolar mitotic spindle. In contrast, bottom panels show a cep63-mutant DT40 cell in which only one centrosome is present (yellow arrow) and therefore the mitotic spindle is monopolar. The centrosome is marked with an antibody that recognises CDK5RAP2, whereas microtubules are stained with anti-α-tubulin antibodies. DNA is blue in merged image. Scale bar is 5μm.

apparent only during mitosis. As soon as cells began to assemble a bipolar mitotic spindle, the centrosomes detached from the mitotic spindle poles (Figure 1). This phenotype gradually worsened as mitosis progressed and, by anaphase, the centrosomes detached from the majority of the mitotic spindle poles. We therefore propose that CDK5RAP2 serves as a platform for microtubule anchoring within the mitotic centrosome, thereby connecting centrosomes and spindle poles (Barr et al., J Cell Biol 2010; 189: 23). During the course of these studies we identified at least two centrosome components that depended on CDK5RAP2 for their centrosomal localisation, however the molecular mechanism by which CDK5RAP2 links centrosomes and mitotic spindle poles is not fully understood and is the subject of our current investigations. Importantly, mitotic spindle positioning relies on astral microtubules, polymers that emanate from the centrosomes and interact with the cell cortex. Centrosome detachment from mitotic spindle poles, however, leads to mitotic spindles without astral microtubules. We therefore predict that cdk5rap2 mutant cells will be defective for spindle positioning. This is an exciting possibility, since the ability of a cell to position its mitotic spindle is central to asymmetric cell division, a process that generates daughter cells with different fates. Normal homeostasis of many of our tissues is dependent on controlled asymmetric cell division, and deregulation of this process occurs in malignancies.

CDK5RAP2 and DNA damage signalling
In order to maintain their genome stability, cells respond to DNA damage by activating a complex signalling cascade that triggers cell cycle checkpoints, repair of damaged lesions or apoptosis in cases of irreparable damage. Intriguingly, at least three centrosomal components have been reported to be required for cell cycle arrest in G2 in response to DNA damaging agents. We have now shown that CDK5RAP2 also belongs to this group of proteins, since following irradiation cdk5rap2 mutant cells exhibit a reduced capacity to maintain G2 arrest. Thus, in addition to its role during mitosis, CDK5RAP2 is also an integral part of a DNA damage-sensing signalling network at the centrosome. It is unclear how CDK5RAP2 promotes an effective G2 checkpoint, but our working hypothesis is that its role in the process involves the recruitment and/or maintenance of Chk1 at the centrosome. Indeed, levels of Chk1 kinase are reduced in the centrosomes of cdk5rap2 mutant cells. Chk1 kinase is central to the DNA damage checkpoint in cells, and its centrosomal accumulation prevents premature mitotic entry not only in the presence of DNA damage but also during unperturbed cell cycles. We now want to understand the molecular mechanism by which CDK5RAP2 regulates Chk1 levels at the centrosome and address the broader role of the centrosome in DNA damage sensing.

CEP63 controls centrosome duplication
Centrosomes duplicate once and only once per cell cycle. Centrosome duplication is under strict control in cells, like DNA replication, since abnormal centrosome number can lead to tumour formation, mitotic catastrophe and aneuploidy. Despite its importance, control of centrosome duplication is still poorly understood. Our most recent work reveals a new regulator of this process, the core centrosomal protein, CEP63. We generated cells that are deficient in CEP63 and found that these cells contained too few centrosomes. They grew more slowly than control cells and displayed monopolar instead of bipolar spindle formations indicative of abortive centrosome duplication cycles (Figure 2). We are currently investigating where CEP63 fits into the molecular network that controls centrosome number in cells.

Publications listed on page 68
Magnetic resonance imaging and spectroscopy (MRI and MRS) have many uses in cancer research. Our group uses these methods, both in the laboratory and in patients, to study basic cancer biology, to improve non-invasive methods for tumour diagnosis and grading, to personalise therapy to individual patients, and to develop biomarkers for monitoring the action of anticancer drugs.

**Tumour cell biology**

Normal cells adapt to hypoxia by the HIF-1 pathway, which is upregulated in many cancers and accelerates their growth; it also upregulates their glycolytic pathway. Monika Golinska’s PhD project (in collaboration with Adrian Harris, University of Oxford), is on Hepa c4 tumours, which cannot activate HIF-1 or upregulate glycolytic enzyme expression but still perform glycolysis normally. Interestingly, Monika has found that they upregulate glycolysis allosterically by maintaining low ATP and high AMP, which doubles the activity of phosphofructokinase-1, the main regulatory enzyme of glycolysis. The high AMP in c4 tumours also upregulates AMP-dependent protein kinase, which tends to slow their growth. These results suggest a mechanism whereby tumours might resist the anti-HIF drugs that are under development, and that monitoring tumour glycolysis by FDG-PET would not necessarily indicate whether they are working in a patient.

Also in collaboration with Adrian Harris, Shen-Han Lee, a PhD student, is studying the role of the enzyme carbonic anhydrase IX (which converts CO₂ to bicarbonate and which is overexpressed in many cancers) on tumour extracellular pH (pHₑₓ). Using ISUCA, a pH sensitive MRS probe, Shen-Han has shown that tumours that overexpress carbonic anhydrase IX tend to have lower pHₑₓ. He is now looking at the role of this mechanism in tumour growth and metastasis.

**Metabolomics**

Madhu Basetti leads several metabolomic projects, including a study of cellular senescence in collaboration with the Narita laboratory. Senescence is a fail-safe mechanism to prevent malignant transformation of cells when their genome is under stress. In this project Madhu (in collaboration with the Tavare laboratory) has implemented a novel method of metabolite-metabolite correlation analysis, which has demonstrated numerous unexpected metabolic interactions, many of which are altered by the induction of senescence. Tonci Sustic, a new joint PhD student with Masashi Narita, will be working on the metabolomics of senescence and autophagy.

Another metabolomics project is being performed by Sara Dietz, a joint PhD student with Colin Watts (Department of Neurosurgery), on the characterisation of the metaboome in stem-like cells derived from human glioblastoma multiforme tissue, and in cell lines produced by inducing differentiation in these cells. Sara has observed marked metabolic differences between the cell types.

**Preclinical MRI and MRS**

Preclinical MRI and MRS studies are led by Dominick McIntyre, together with Davina Honess. Leanne Bell’s PhD project is on the Tuveson laboratory’s KPC pancreatic tumour model that, like human pancreatic tumours, responds poorly to gemcitabine, the current standard of care for this cancer. In previous studies with the Tuveson laboratory (Olive et al., Science 2009; 324: 1457) we demonstrated that the vascular insufficiency in KPC tumours, which is probably due to their dense collagenous stroma, contributes to their chemoresistance. Leanne has continued DCE-MRI studies of the vasculature and developed magnetisation transfer MRI methods to monitor the tumour matrix in the KPC mouse model and ectopic KPC allografts. She will now be
collaborating with the Tuveson laboratory on studies using novel anticancer drugs designed to break down the collagenous matrix of KPC tumours and enhance the action of gemcitabine, using magnetisation transfer as a biomarker of matrix breakdown.

Nicola Ainsworth, a clinical research fellow (jointly supervised by Jonathan Gillard, Department of Radiology, and in a collaboration with Susan Harden, Department of Oncology), is studying cerebral metastasis of small cell lung cancer (SCLC). About half of patients with SCLC develop cerebral metastases, but since we cannot predict which half, the current practice is to give all of them prophylactic cranial irradiation, a therapy with significant long-term side effects. Nicola’s project is aimed at developing MR methods for detecting these metastases much earlier, so that patients who would not benefit from prophylactic cerebral irradiation can be spared this therapy. Nicola has developed a mouse model of brain metastasis and she is developing various MRI methods, including magnetisation transfer, which could be used in patients. She is also recruiting SCLC patients, nine so far, into a study (CLUB-01) in which they are imaged before and after prophylactic cranial radiotherapy.

We are continuing our joint programme with Martin Leach, Ian Judson and Paul Workman (Institute of Cancer Research, Sutton) on monitoring the actions of novel anticancer drugs by MRS and MRI, in order to develop non-invasive biomarkers for use in drug trials or in the clinic.

Clinical MRI and MRS
Mary McLean leads our work on tumours in patients. We are collaborating with James Brenton (CRI) and Evis Sala (Department of Radiology) in an MRI and MRS study (OVO3) on the response to chemotherapy of cancer of the ovary. Three papers have been published.

We are continuing our participation in an NCI-funded international collaboration, which has developed a method for individualising therapy for patients with non-Hodgkin’s lymphomas by using MRS to predict response; 16 studies have been performed.

Another collaboration with Evis Sala, Vincent Gnanaprasam (Department of Surgery) and David Neal (CRI) is concerned with the use of DWI, DCE-MRI and MRS for prediction and early detection of prostate cancer response to androgen deprivation in advanced prostate cancer. We have so far performed 47 examinations on 25 patients. One paper is in press and another in preparation.

In a collaborative study with the Jodrell laboratory we are developing methods for quantification of the oral anticancer drug capecitabine and its metabolites in breast tumours and in the liver. Five patients have so far been studied and both capecitabine and its metabolite fluoro-β-alanine have been detected; preclinical studies are also planned.

Sidhartha Nagala is taking a PhD under the supervision of Jonathan Gillard (Department of Radiology) on the use of MRS and DWI for the diagnosis of cancer in follicular thyroid nodules and parotid lumps. Thirty eight examinations have been performed so far. Tumour biopsies from these examinations will be studied by HRMAS 1H MRS to correlate metabolomics data with the clinical findings. Accurate pre-operative diagnosis, which is difficult for these lesions, will enhance surgical planning as well as reducing unnecessary operations.

Publications listed on page 68
Pre-clinical model systems are used to inform clinical trial design for novel agents and combination strategies. The PDDG is closely linked with the CRI pharmacokinetics (PK) and pharmacodynamics (PD) core facility and the Early Phase Clinical Trials Team (EPCTT) based in the Cambridge Cancer Trials Centre, at Addenbrooke’s Hospital.

The model systems used are generally pre-clinical models of pancreatic cancer. These include cell line models, human tumour xenografts and, through collaboration with David Tuveson (CRI), the KPC GEM (genetically engineered mouse) model. We developed a novel LC-MS assay for use in that model to assess the PK and activation of gemcitabine in pancreatic tumour tissue. This assay is suitable for clinical use and collaborative clinical studies are being planned. We will also modify the method to quantify gemcitabine metabolites incorporated into tumour tissue DNA. In view of the poor drug delivery to pancreatic cancer tissue, a joint PhD student (with the Tuveson laboratory) is investigating the biology of the hypovascular phenotype in KPC tumours, in order to manipulate tumour/vasculature/stromal interactions, to improve drug efficacy.

Our work on the PK and PD of the fluoropyrimidine capecitabine continues. The metabolism and delivery of active capecitabine metabolites to tumour tissue has been identified in pancreatic cancer allografts and the KPC autochthonous mouse model. Capecitabine inhibited tumour growth in the allograft model and efficacy in the KPC model is currently being tested. Combination strategies, using capecitabine, are being evaluated in in vitro models, prior to commencing in vivo studies.

In collaboration with John Griffiths (CRI), a study in patients is assessing the feasibility of non-invasive magnetic resonance fluorine spectroscopy, to assess the metabolism and accumulation of capecitabine and its metabolites in normal liver and breast tumour tissue. Clinical studies are in progress to assess the PK of capecitabine in specific sub-groups of patients following surgery: gastrectomy in gastric cancer patients and Whipple’s procedure in pancreatic cancer patients. Each of these studies incorporates a pharmacogenetic assessment of patients’ capecitabine metabolising capacity, which will be linked to the PK data. In addition, laboratory studies are underway to characterise the impact of specific genetic polymorphisms in fluoropyrimidine metabolising enzymes.

In the last year, the EPCTT has completed a Phase II trial, assessing a novel vaccine approach for colorectal cancer. Nine studies are currently recruiting patients. In addition to our capecitabine studies, the following studies are enrolling patients: three phase I trials of novel drug combinations (in patients with breast, CNS and pancreatic cancers), a phase I trial of a novel growth factor receptor inhibitor, a phase I trial of an immunotherapeutic in patients with melanoma, and a biomarker study of an anti-angiogenic therapy for renal cancer. This latter study includes both PET and MR based PD studies, highlighting our close collaboration with researchers developing these emerging technologies. Six further protocols (four phase I) are in development, for initiation in 2011. We are collaborating with Tim Eisen (Department of Oncology), hosting a Hales Clinical Fellow, who is identifying functional defects in the VHL tumour suppressor protein caused by mutations detected in renal cancer, and linking these to patient outcome.

Aurora kinases (AK) have been identified previously as potential targets for anticancer therapeutics. The aurora family of serine/threonine protein kinases plays a critical role in cell division, with key roles in...
the mitotic spindle checkpoint. Deregulation of AK expression or function is believed to provoke genetic instability and AK-A has been identified as a cancer susceptibility gene. Elevated expression levels of AK are detected in a high proportion of melanoma, colon, breast, ovarian, gastric and pancreatic tumours and our collaborators have shown over-expression of AK-A in urothelial tumours (Veerakumarasivam et al., Cell Cycle 2008; 7: 3525).

In the lab, we have been comparing a number of AK inhibitors with different potency/specificity and are now proceeding to test rational combinations of similar agents in vitro, in selected cancer cell lines. Ashok Venkitaraman (Hutchison/MRC Research Centre) has shown that AK-A over-expression (at levels found in tumour cells) overrides the spindle assembly checkpoint signal, inducing resistance to taxanes in vitro (Anand et al., Cancer Cell 2003; 3: 51). We are investigating the interaction surfaces of AK inhibitors and paclitaxel combinations in pancreatic and urothelial cell lines and have preliminary data that confirm that these interactions are complicated, with regions of synergy and regions of antagonism. It will be critical to understand these in order to optimise the dose scheduling for clinical trials. Assessments of cell sensitivity are being linked to measurements of cytokinetics and gene expression, developing assays that can be used as potential PD markers. Effective drug combinations will be tested in appropriate xenograft and/or transgenic models of cancer.

In general, it is assumed that combinations of agents have similar effects on normal and tumour cells, but this is not always the case. The optimal combination would show synergy in cancer cells and antagonism in normal cells, reducing the toxic side effects that often limit dosing. We are investigating which of the available normal diploid cell types are most relevant for these comparisons. This project also incorporates mathematical modelling approaches, with a model of the cell cycle and spindle assembly checkpoint being used to predict the effects of the drugs. The mathematical models are also being linked with PKPD modelling approaches, to allow the prediction of the efficacy of certain drugs/combinations/dosing schedules in vivo (a collaboration with Bob Jackson, Pharmacometrics). We will extend our pre-clinical findings into clinical trials, incorporating the biomarkers we identify.

We also have collaborations with Steve Ley and Rebecca Myers (Department of Chemistry) and Fanni Gergely (CRI). We are co-supervising a Cancer Research UK Medicinal Chemistry Programme PhD student, who is synthesising and evaluating selective allosteric inhibitors of the kinesin motor protein HSET, a promising new anticancer target. We have also established a mouse xenograft model of disseminated ovarian cancer, in which tumour growth is monitored by bioluminescent detection of the luciferase-expressing tumour cells in the abdomen, to support a collaboration with Gillian Murphy (CRI), investigating TNF-α converting enzymes (TACE) as therapeutic targets.

Publications listed on page 69
Natural perturbations: Somatic copy number alterations
Natural perturbations like copy number alterations and SNPs can promote cancer development. The development of cancer is a complex process involving the accumulation of multiple mutations and genomic aberrations. A consequence of these alterations is the deregulation of cell signalling pathways central to the control of cell growth, cell fate and other important cellular functions. With our partners at the CRI we aim to characterise disruptions of signalling pathways in tumours and to identify genomic alterations that drive tumour evolution.

Dr Yinyin Yuan (in collaboration with Carlos Caldas’ lab) works on statistical methods to quantify the impact of copy-number alterations on gene expression in tumours. The methods she has developed identify differential regulation between breast cancer subtypes by comparing regulatory hotspots (Yuan et al., BIBM 2010; B405). Yinyin also works on image analysis methods for quantitative cellularity scoring of tumour samples, which can greatly increase the signal-to-noise ratio in copy-number profiles (Figure 1; manuscript in preparation).

Dr Roland Schwarz develops statistical methods for sequence analysis based on finite-state transducers. This framework allows for very accurate phylogenetic tree reconstruction (Schwarz et al., PLoS ONE 2010; 5: e15788). We use these methods in collaboration with James Brenton’s lab to infer tumour evolution and its main drivers in ovarian cancer within individual patients.

Dr Mauro Castro, who started in May 2010, develops methods for integrating functional information into diagnostic signatures. While signatures based on individual genes are highly redundant and hard to interpret mechanistically, our assumption is that integrating known pathways and networks in the analysis will lead to easier to interpret signatures.

Experimental perturbations: RNA interference
Experimental perturbations like RNAi are key approaches at the forefront of functional genomics. A goal that is becoming more and more prominent in both experimental as well as in computational research is to leverage gene perturbation screens to the identification of molecular interactions, cellular pathways and regulatory mechanisms. Research focus is shifting from understanding the phenotypes of single proteins to understanding how proteins fulfill their function, what other proteins they interact with and where they act in a pathway. Novel ideas on how to use perturbation screens to uncover cellular wiring diagrams can lead to a better understanding of how cellular networks are deregulated in diseases like cancer.

In our group we work on several projects to analyse gene perturbation screens in terms of pathways and cellular networks. For example, we develop methodology for network analysis of high-throughput RNAi screens (Markowetz, PLoS Comp Biol 2010; 6: e1000655; Wang et al., Bioinformatics 2011; Epub 22 Jan). A particular focus of the lab is on nested effects models (NEMs), a statistical approach that is specifically tailored to reconstruct features of pathways from perturbation effects in downstream reporters (Markowetz et al., Bioinformatics 2007; 23: i305). Based on NEMs, we are developing an integrated experimental and computational approach to identify new branches in the NFκB pathway, a key pathway involved in the immune response as well as in cancer, working in collaboration with the group of Thomas Meyer (Max-Planck Institute for Infection Biology, Berlin).

The theory of NEMs has so far been mainly limited to static snapshots of perturbation effects. In a major conceptual improvement,
Figure 1
Automated quantitative cellularity scoring improves detection of copy number alterations. In a stained tumour image (step 1) individual nuclei are classified into different cell types (step 2). Tumour cells cluster together and classification performance is improved by spatial smoothing (step 3). The percentage of tumour cells among all cells yields a quantitative cellularity score, which greatly improves the signal to noise ratio in DNA copy number profiles (step 4).

Xin Wang, a PhD student in the group, has combined hidden Markov models with NEMs to reconstruct rewiring events in pathway topologies from time-series data derived after silencing pathway components. Xin applies his methods to gene expression time-series in mouse embryonic stem cells to infer changes in pathway activity in the early stages of differentiation.

In a related project we are interested in the coordinated interplay between epigenetic, transcriptional, and translational mechanisms that are required for the molecular regulation of stem cell fate. We are approaching this question in collaboration with the group of Ihor Lemischka (Mount Sinai School of Medicine, New York) by undertaking a dynamic systems level study of cell fate changes in murine embryonic stem cells. Global changes in histone acetylation, chromatin-bound RNA polymerase II, mRNA, and nuclear protein levels were measured over five days after down-regulation of Nanog, a key pluripotency regulator (Lu et al., Nature 2009; 462: 358). This data set provides a rich resource that allows us to untangle the complexity of the multilayer regulatory mechanism responsible for stem cell fate. We anchored our analyses on changes in nuclear protein expression and found that many lacked concordant changes in mRNA expression, pointing to important roles for translational and post-translational regulation of ESC fate (Lu et al., Nature 2009; 462: 358). Recently, we complemented these analyses with an in-depth study of the relationship between histone acetylation and gene expression in the same data set (Markowetz et al., PLoS Comp Biol 2010; 6: e1001034).

In the future, our lab plans to strengthen its ties with our experimental collaborators in order to approach pivotal questions in biology and medicine by computationally guided experimentation. Biological and clinical questions motivate the development of novel statistical algorithms, which guide the next round of experiments.

Publications listed on page 69
Understanding the roles of proteases in tumour biology

The successful development of tumours is determined by the tissue environment in which the ‘host’ participates in the induction, selection and expansion of the neoplastic cells. Malignant tumour cells recruit vasculature and stroma through the production of stimulatory factors. The locally activated host environment (both cells and extracellular matrix) in turn modifies the proliferative and invasive behaviour of tumour cells. The nature and function of the activating factors involved and the subsequent effectors are important areas of basic biological research in the field of cancer studies. Extracellular proteases are major effectors of both cell-cell and cell-extracellular matrix (ECM) interactions, modifying ECM integrity, growth factor availability and the function of cell surface signalling systems, with consequent effects on cellular differentiation, proliferation and apoptosis. Early data from screens of cancer tissues have shown that different patterns of protease elevation occur and that the relationship of expression to tumour progression and the contribution of individual cell types – tumour cells, fibroblasts, endothelial cells and inflammatory cells – requires detailed dissection. A major aim of the drive to understand protease biology within a specific tumour environment relates to the need to assess potential targets within the interface between tumour cells and the ‘host’ cells that may be appropriate for therapeutic intervention. It is anticipated that the understanding and the manipulation of protease function will give clear leads as to the critical stages in the breakdown of the normal tissue-cell ‘society’ that occurs in neoplasia.

Within this remit our research is focussed on cell surface associated forms of the zinc-dependent proteases, notably the aminopeptidase N (CD13), the membrane type matrix metalloproteinase-1 (MMP14) and members of the disintegrin-type metalloproteinases (ADAMs), as well as the tissue inhibitors of metalloproteinases, TIMPs. We aim to elucidate how these metalloproteases and inhibitors function in the regulation of extrinsic effectors at the cellular and molecular level, as well as proceeding to complex tumour models addressing tumour-stromal interactions. The fundamental data accrued will drive the development of novel reagents for disease therapy and diagnosis.

Membrane associated metalloproteases

The aminopeptidase CD13 is a homodimeric 140-150 kDa type II transmembrane
Research Groups   |   29

**Figure 1**
The ADAM17 metalloprotease (red) activates multiple pathologically significant substrates. ADAM17 solubilises ErbB growth factors (blue), pro-inflammatory cytokines (orange) and can also cleave signalling receptors (green).

metallo-ectopeptidase that preferentially removes neutral or basic amino acids from the unblocked N-termini of bioactive peptides or proteins. It also participates in cell migration and invasion of extracellular matrix and is important in both angiogenesis and tumour progression. We are studying its role as a mediator of membrane remodelling in both tumour cells and endothelial cells and some aspects of its regulation, notably in relation to cellular trafficking.

The membrane type 1 matrix metalloprotease MT1 MMP plays a major role in tumourigenesis, including tumour cell migration, aspects of stromal cell function and angiogenesis. As a potentially key target for therapeutic approaches to cancer we are addressing its involvement in intracellular signalling with a focus on the role of its different domains in important interactions. We have elucidated novel roles for the MT1 MMP cytoplasmic domain in its regulation during cellular trafficking and have identified several intracellular and extracellular interaction partners. We are evaluating their significance and roles in (among others) VEGF receptor regulation, collagen degradation and cell migration. Future studies will look at the effects of MT1 MMP on gene transcription. The characterisation of scFv antibodies to MT MMPs that we have isolated is being used to address the question of the importance of exosite interactions in the proteolytic capacity of MT1 MMP.

The disintegrin metalloproteinases are also regulators of cellular signalling and we are studying ADAM10 and 17 in this respect. Biochemical studies have focussed on the structure-function relationships of ADAM17 and projects on the role of different ADAM domains in the proteolytic ‘shedding’ of cell surface proteins are in progress (Figure 1). We are particularly interested in the generation of soluble EGF receptor ligands which are key drivers of a number of different tumour types.

The significance of ADAM activity in cell models is being investigated using shRNA and siRNA techniques and novel antibody tools that we have recently developed. ADAM regulation by G-protein coupled receptors and redox mechanisms are being investigated. The role of ADAM10 and ADAM17 in the development of ovarian, and gut cancers are being evaluated using gene ablation or over-expression studies in animal models.

**TIMPs**
Protein engineering of members of the tissue inhibitor of metalloproteinase (TIMP) family has given us insights into their enzyme target specificity and how to modify them for the development of potential therapeutics. We are able to engineer forms of these inhibitors that distinguish to some extent between the MMPs and ADAM proteinases, and between individual ADAMS. With this toolbox of TIMP mutants their efficacy in cell models of proteolysis is being evaluated. With our collaborators we have prepared adeno-viral and lentiviral delivery systems for studies in cell and animal models of cancer. Alongside siRNA techniques the TIMPs are good tools to assess the role of individual proteinases in specific proteolytic events.

**3D in vitro models**
In order to carry out molecular studies and inhibitor screens on the complexity of cells within tumour tissue we have set up several more complex 3D models (tumour cells, endothelial cells and fibroblasts in collagen gels) for the evaluation of metalloproteinase function and eventual therapeutic abrogation. Such models are being used to study the roles of fibroblast MT1 MMP, CD13, ADAM10 and ADAM17 in angiogenesis and tumour growth. We are also using a model of small cell lung cancer to elucidate novel determinants of chemoresistance.

**Publications on page 69**
Epigenetic refers to mitotically stable changes in gene expression that cannot be attributed to DNA sequence changes. DNA methylation and post translational histone modifications, together with chromatin structure, underpin the epigenetic organization of the genome. Our laboratory works towards understanding the epigenetic mechanisms whereby genomic imprinting is lost in cancer.

Genomic imprinting is a process of marking the gametic origin of some genes and results in the expression of one parental allele and the reciprocal silencing of its homologue. Aberrant imprinting of the insulin-like growth factor 2 (IGF2) gene locus is part of the aetiology of congenital growth disorders such as Beckwith Wiedemann syndrome (BWS, OMIM#130650), as well as various human cancers including Wilms’ tumour, rhabdomyosarcoma, hepatoblastoma, colorectal and breast carcinomas (Cooper et al., Eur J Hum Genet 2009; 13: 1025; Murrell, ScientificWorldJournal 2006; 6: 1888).

Distinct epigenetic mechanisms are involved in the establishment and maintenance of the imprint of IGF2 and the neighbouring H19 gene which transcribes a long non-coding RNA. Establishment involves erasure of prior imprints by demethylation which is followed by sex specific de novo methylation and further chromatin modifications. Maintenance of imprinting in somatic cells requires semi-conservative maintenance of methylation and maintenance of polycomb/thithorax memory systems. Loss of imprinting (LOI) refers to either biallelic silencing or biallelic expression of imprinted genes and can potentially occur due to aberrant establishment of the imprint during germline development; an inability of the cell to recognise the imprint; or failure to maintain imprinting. BWS is a complex heterogeneous disease involving a number of genes within a cluster of imprinted genes on chromosome 11p15. BWS children with biallelic expression of IGF2 together with silencing of H19 have a predisposition to Wilms’ tumour. Biallelic IGF2 expression is a feature of many tumours but has also been reported to be constitutively present in some individuals without an inherited growth disorder (Sandovici et al., Hum Mol. Genet. 2005; 14: 2135). The latter group of individuals with so called polymorphic imprinting are potentially at risk for developing adult onset cancers. Early embryonic environment factors in mice have been shown to have an effect on gene expression after birth and recent reports suggest that in humans assisted reproduction technologies may also increase the risk of imprinting disorders and cancer (Paolini-Giacobino, Fertil Steril 2007; 88: 761). These observations suggest that LOI in cancer may be constitutive.

Imprinting of IGF2 and H19 are regulated by an insulator element upstream of H19. The zinc finger protein CTCF binds to the insulator and mediates its function, such that when CTCF is bound on the unmethylated maternal allele, the maternal copy of IGF2 cannot access enhancers downstream of the H19 gene (Bell and Felsenfeld, Nature 2000; 405: 482). Methylation at the insulator sequence on the paternal allele prevents CTCF binding, inactivates the insulator function and enables the IGF2 gene access to the enhancers. We have previously shown that in mice chromatin looping is mediated by CTCF binding at the insulator (Murrell et al., Nat. Genet. 2004; 36: 889), and hypothesised that DNA methylation of the insulator sequence would result in differential loops on the maternal and paternal allele. In mouse models with the insulator sequence deleted or mutated on the maternal allele, the maternal IGF2 allele becomes methylated and looks more like the paternal allele (Lopes et al., Hum Mol Genet 2003; 12: 295).
Our initial studies in humans have indicated that:
1. Methylation at the insulator is associated in cis with methylation upstream of the IGF2 gene (the DMR0 region) (Murrell et al., PLoS ONE 2008; 3: e1849).
2. The epimutations that lead to loss of imprinting in congenital disease are different from those found in cancer. Indeed, even when BWS individuals with constitutive loss of imprinting develop Wilms’ tumours, the methylation patterns associated with the tumours are different from the methylation patterns associated with the syndrome (Murrell et al., PLoS ONE 2008; 3: e1849).
3. In breast and colorectal cancers, DNA methylation and expression profiles are disconnected suggesting that imprinting can be lost without epigenetic switching to a paternal identity (Ito et al., Hum Mol Genet 2008; 17: 2633).
4. In colorectal and breast cancers, hypomethylation of the IGF2 DMR0 is frequently associated with cancer independently of IGF2 expression state. DMR0 hypomethylation is actually more prevalent in cancer than loss of imprinting, and could potentially even be indicative of cancer (Ito et al., Hum Mol Genet 2008; 17: 2633).
5. IGF2 DMR0 hypomethylation when found in cancer is not constitutionally present prior to cancer (Ito et al., Hum Mol Genet 2008; 17: 2633).

In order to understand how the human IGF2-H19 locus maintains its methylation profile in somatic cells and to identify active and silent chromatin domains at the locus, we characterised the chromatin conformation (3C) state by looking at interactions between CTCF binding sites across the locus. Recently genome-wide studies have shown that cohesin complexes co-localise onto the same DNA sequences as CTCF (Wendt et al., Nature 2008; 451: 796). We therefore speculated that cohesin may have a function in holding chromatin loops together by connecting two DNA molecules in cis, in an analogous manner to its role in holding two sister chromatids together (Figure 1). We found that CTCF sites upstream, within and downstream of the locus bound CTCF and cohesin on both alleles and that the H19 insulator sequence was the only CTCF site that bound CTCF and cohesin on one allele only. It was further found that when cohesin was depleted by RNAi, that IGF2 expression was upregulated and biallelically expressed, but that methylation at the insulator sequence was not changed and that H19 expression levels were unaffected. Our 3C experiments in which cells were first synchronised in G1 or G2 phases showed that CTCF sites interact with one another to divide the locus into looping domains with CTCF forming the base of these domains. Cohesin depletion resulted in significant reduction of the interaction frequencies between CTCF binding sites suggesting that cohesin is required for stabilisation of chromatin loops. These results also indicate that in somatic cells, changes in chromatin conformation can result in loss of imprinting independent of DNA methylation changes (Nativio et al., PLoS Genet 2009; 5: e1000739). We are now testing various cancer cell lines with and without methylation defects and loss of imprinting to see if aberrant looping between CTCF binding sites occurs in cancer.

Further work is concentrating on isolating proteins that bind to the DMRs in imprinted genes and to create mouse models and cell based assays in which we can screen for additional proteins that have trans effects on imprinted gene expression.

**Publications listed on page 70**
Cellular senescence is a state of stable cell cycle arrest with active metabolism. Similar to apoptosis, senescence can be a failsafe program against a variety of cellular insults. In contrast to apoptosis, in which cytotoxic signals converge to a common mechanism, senescence is typically a delayed stress response involving multiple effector mechanisms. These effector mechanisms include epigenetic regulation, the DNA damage response, and the senescence-associated secretion phenotype. The relative contribution of these effectors varies depending on the trigger and cell type, and it is possible that the combination and balance of these effectors determines the quality of the senescence phenotype. Thus, to understand the senescence program, it is important to identify new effector mechanisms and examine how they associate with each other, and also to identify which effector mechanisms could be potential targets for cancer therapy.

Identification of senescence-associated chromatin factors
To further understand the molecular basis underlying the irreversibility of senescence arrest, we have been using a biochemical approach to analyse the alteration of the cell’s chromatin protein profile during senescence. Using SDS-PAGE to visualize the protein composition of each chromatin preparation, we have identified HMGA proteins as new components of senescence (Narita et al., Cell 2006; 126: 503). Considering the identification of HMGA proteins in this system as a proof of concept, we are currently taking a more systematic approach for a thorough analysis of the chromatin protein profile in senescent cells. So far, we have several candidate proteins that specifically associate with chromatin to form senescent cells. Now we are in the process of verifying and analysing them in the context of senescence.

Genome-wide analysis of heterochromatin components in SAHFs
Certain types of cells undergo distinct alterations in chromatin structure during senescence, called senescence-associated heterochromatin foci (SAHF). SAHF have been widely used as a marker of senescence, and more importantly, several new components of senescence machinery have been successfully identified using SAHF as a readout. Thus, it is important to understand SAHF structure in more detail and how SAHF are actually formed. Interestingly, we have shown that SAHF are indistinguishable from the inactive X (Xi) chromosome, one of the best studied heterochromatin models, and other groups recently suggested that each individual SAHF might represent each chromosome territory. In contrast to Xi, SAHF formation can be dynamically regulated in normal human diploid fibroblasts (HDFs), thus providing a unique tool to study not only senescence, but also chromatin biology. To further characterise SAHF in detail, we are currently investigating a dynamic redistribution of the specific histone modifications and their adaptor proteins using confocal microscopy, with a new panel of highly specific monoclonal antibodies against histone marks (in collaboration with Dr. Hiroshi Kimura, Osaka University, Japan). In addition,
we are currently analysing the genome-wide redistribution of these chromatin components by chromatin-IP coupled with deep sequencing (ChIP-seq).

Figure 1
Concept of stress-responsive gene regulation.

From mRNA to proteins
Oncogene induced senescence (OIS) is a very dynamic process where cells typically undergo an initial burst of cell proliferation (‘mitotic phase’), followed by the induction of pro-senescent factors, including p16 and HMGA2 (‘transition phase’). Eventually, the senescent phenotype dominates (‘senescence phase’). During the transition phase, oncogenic and pro-senescent activities work against each other; and senescence usually prevails in normal cells. How cells can achieve such a drastic phenotype remodelling is unclear. A new area of interest in my group is in another layer of gene expression control, namely protein metabolism, during the senescence and transition phases. We reason that global epigenetic alteration should be coupled with efficient protein turnover as a part of the execution of epigenetic ‘blue prints’, in such an emergent context (Figure 1). Consistent with this idea, we have identified that autophagy, a bulk protein degradation program, facilitates synthesis of IL6/8, which are pro-senescent secretory proteins (Young et al., Genes Dev. 2009; 23: 798). This new functional link between senescence and autophagy will be further characterised in the future.

Identification of senescence-associated p53 function
A tumour suppressive transcription factor, p53, plays a critical role in many stress-responsive phenotypes, including DNA damage checkpoints, apoptosis, and senescence. Although ample data have supported a role for p53 in senescence, the precise mechanism is not clear. To address this issue, we are currently using HDFs, where we can induce different phenotypes depending on environmental stimuli or other conditions (Figure 2). By adding either retroviral- or lentiviral-mediated stable RNAi to HDFs, we are comparing the impact of p53 knockdown on the gene expression profile in each condition, which represents a phenotype-specific p53 function. We have finished the array experiments, and are now attempting to build a comprehensive picture of p53’s functions. So far, in a primary analysis, we have identified several genes whose products are upregulated in a p53-dependent manner during senescence, but not in the other stress responsive contexts (e.g. apoptosis). We are currently undertaking functional verification of one of the genes.

Publications listed on page 70
We are a translational research group with a focus on castration independent prostate cancer, and have strong clinical and surgical links.

Our clinical practice informs and underpins the research. We have the largest NHS practice in robotic prostatectomy; biorepositories from this, and from ProtecT (the largest ever surgical randomised controlled trial in prostate cancer) have led to important collaborative research with Doug Easton at the Strangeways Research Laboratory and Ros Eeles at the Institute of Cancer Research (ICR) (Al Olama et al., Nat Genet 2009; 41: 1058; Eeles et al., Nat Genet 2009; 41: 1116). Another collaborative project with Colin Cooper at the ICR (CancerMAP) is now completed and undergoing analysis for expression profiling stratified by TMPRSS2-ERG status. A further collaborative application is under consideration for in-depth sequencing of prostate cancers.

Ian Mills has left to become a Group Leader at the Centre for Molecular Medicine in Oslo and Charlie Massie has left to take up a new post-doctoral position. We have been joined by Lee Fryer; Mohammad Asim and Gökmen Altay. Frank O’Brien has joined the clinical academic department to establish joint research with Colin Cooper at the ICR (CancerMAP). Ian Mills, Mohammad Asim and Gökmen Altay. Frank O’Brien has joined the clinical academic department to establish joint research with Tim Eisen (Department of Oncology).

Main discoveries
We have now completed ChIP sequencing for the androgen receptor coupled with ChIP for Pol II. This research has led to a new understanding about how the AR binds to the genome and discoveries about how this influences major metabolic signalling pathways. Two papers have been completed and a third is being written on our ChIP sequencing studies on human tissue.

We have also taken forward one of the SNP risk alleles we reported last year in a paper in Nature Genetics to begin functional studies (Whitaker et al., PLoS ONE 2010a; 5: e13363; Whitaker et al., Prostate 2010b; 70: 333) and have completed mechanistic studies on a biomarker reported last year (Thirkettle et al., Clin Cancer Res 2009a; 15: 3003; Thirkettle et al., Oncogene 2009b; 28: 3663).

We have comprehensively mapped AR binding sites in two models of prostate cancer using ChIP-seq and mapped transcriptionally active targets using ChIP-seq for phosphorylated RNA polymerase II, combined with expression profiling. This approach identified thousands of novel targets, defined distinct characteristics of transcriptionally active AR binding sites and identified signalling pathways directly regulated by the AR. Amongst these, we identified calcium/calmodulin kinase kinase 2 (CAMKK2) as over-expressed in castrate resistant prostate cancer and as being functionally important for proliferation. Our data provide new direct links between the AR and signalling pathways and offer the potential for novel therapeutic interventions. We are now expanding these studies into human material and have discovered several novel binding sites that appear to be functional.
2. Studies on MSMB (PSP94)

Our recent collaborative genome-wide association studies have shown an association of a SNP two base pairs upstream of the 5’ UTR of the microseminoprotein-beta (MSMB) gene with an increased risk of developing the prostate cancer (Eeles et al., Nat Genet 2009; 41: 1116). MSMB expression is high in normal and benign prostate tissue and lowered or lost in prostate cancer, suggesting that it might be a useful tissue biomarker for prostate cancer diagnosis (Figures 1 and 2). Members of the cysteine-rich secretory protein family and laminin receptors have been shown to bind MSMB at the cell surface and in serum, thereby regulating apoptosis. Both full length MSMB and a short peptide comprised of amino acids 31-45 have been tested for potential therapeutic benefit in mouse models and humans. Our recent data also show links between the risk allele in normal prostate and levels of expression (Figure 3). MSMB has potential as a biomarker of prostate cancer development, progression and recurrence and potentially as a target for therapeutic intervention (Whitaker et al., PLoS ONE 2010a; 5: e13363; Whitaker et al., Prostate 2010b; 70: 333).


A neuroendocrine profile is associated with castration-independence. We have found that a pro-neural expression signature — including over-expression of Ascl1, HES6, and neurotensin — is associated with advanced and castration-independent prostate cancer, and that transduction of androgen sensitive prostate cancer cells with Hes6 delivers a more aggressive phenotype. We are now studying the interaction between HES6 and c-myc and are testing the role that HES6 plays in castration independent cell growth in vivo.


ARRB1 plays a role in cancer progression and some tumours show elevated levels in the nucleus where it may regulate gene expression via epigenetic mechanisms. We aim to determine the potential role played by ARRB1 in prostate cancer and to identify novel biomarkers and therapeutic targets. Prostate cancer displays elevated levels of ARRB1 that correlate with stage and aggressiveness, it is also present in the nucleus in high-grade cancer. We have identified several genes whose expression is differentially regulated by ARRB1 and they are involved in processes such as the cell cycle, cell motility and metabolism. Using ChIP-sequencing, we have identified several binding sites for endogenous ARRB1, which reside mainly in enhancers or proximal promoters and include genes involved in the unfolded protein response and autophagy. A paper has been submitted.

Publications listed on page 70
The control and evolution of cellular gene expression

The proteins that control DNA, known as transcription factors, bind to it in a combinatorial manner in yeast and bacteria, and my early work showed that this combinatorial binding occurs in mammalian tissues as well. Master regulators in primary human hepatocytes form a highly interconnected core circuitry that frequently bind promoter regions in clusters, particularly at highly regulated and transcribed genes (Odom et al., Mol Syst Biol 2006; 2: 2006.0017) (Figure 1). More surprisingly, we have recently found that transcriptional regulation can vary much more rapidly and widely than previously appreciated among homologous tissues from many mammals (Schmidt et al., Science 2010; 328: 1036; Odom et al., Nat Genet 2007; 39: 730). The experiments in our laboratory allowed the identification of specific genetic architectures that appear to preserve a small handful of transcription factor binding events across large evolutionary timescales (>300 million years) (Schmidt et al., Science 2010; 328: 1036).

In asking why rapid variation occurs among most transcription factor binding events, we realised that a number of causative factors could contribute. These possible causes may be the result of variability of genetic sequences, the types and number of marks left in the histone proteins that package DNA (commonly thought of as an epigenetic code), or even diet or environmental differences between different species. In order to isolate a single one of these variables, we used a previously created mouse model of Down’s syndrome that carries a virtually complete copy of a human chromosome (O’Doherty et al., Science 2005; 309: 2033). By exploiting this aneuploid mouse strain, a unique and powerful genetic tool designed for an entirely different purpose, my laboratory was able to determine that genetic sequence dominates all others in directing transcription (Wilson et al., Science 2008; 322: 434).

The origin, regulation, and evolution of non-coding RNAs

We have been using similar comparative functional genomics approaches to look at the regions of the genome that are transcribed, but which do not code for proteins. These regions are known as non-coding RNAs, and range from well-characterised species like tRNAs and rRNAs to newer categories of regulatory nucleic acids like microRNAs, piRNAs, and endogenously expressed RNAi. In addition, we are using the unbiased maps of transcriptional regulation we have generated to date to investigate the regulation of these molecules (Figure 2).
Figure 1
Core human hepatocyte regulatory circuitry. The black ovals represent transcription factors that are required for the creation and maintenance of liver specific transcription. The red arrows represent the autoregulatory loops at the apex of core regulatory circuitry in human liver. The blue arrows represent the regulator to regulator connections that exist in vivo in human liver.

Figure 2
RNA polymerase III regulation of tRNA loci in six mammals.

Few tRNAs are bound by pol III in all placental mammals.
To date our work has been following up the results of the genome-wide association studies in breast cancer that we and our colleagues initiated. The chance of an individual developing breast cancer is roughly two-fold greater if that individual has a close relative with breast cancer. Twin studies indicate that this risk is largely genetic. The genes that confer this risk have been sought either by genetic linkage mapping in multiple-case families, or by genome-wide association studies (GWAS). The former have identified rare but higher risk alleles such as those of BRCA1 and BRCA2, while GWAS have identified common variants that each carry only a small risk of cancer. BRCA1 and 2 explain about 15–20% of the estimated total genetic risk of breast cancer, and loci identified through GWAS a further 10%.

One question is how to find the genes that account for the ‘missing’ 70% or so of heritability. Larger and more powerful GWAS will find some, while genome resequencing will identify an unknown contribution from rare genetic variants. Pending these studies, we are exploring other approaches in breast and in lung cancer.

In breast cancer, Ana-Teresa Maia is making a catalogue of genes in which there are common variants that cause different levels of expression of the two alleles in a heterozygous individual. Since many of the genetic variants so far identified in GWAS studies in general appear to have their effects through altered gene regulation, the subset of genes that show differential allelic expression (DAE) should be enriched for genes involved in susceptibility. If correct, this information would allow prioritisation of genes for further study from the very large numbers of loci that show borderline levels of significance in existing GWAS. We are now testing this hypothesis.

The common genetic variants identified through GWAS individually have very small effects. They can be thought of as causing small perturbations of regulatory gene networks within the cell – the combined effect of many variants produces a greater perturbation that leads to disease. A regulatory variant in the fibroblast growth factor receptor 2 (FGFR2) gene is the common variant with the greatest effect on breast cancer susceptibility. We are using a systems biology approach to understand the function of this predisposing gene. To this end we have treated the well-studied oestrogen dependent cell line MCF-7 with oestrogen and FGF10, an activator of the FGFR signalling pathway. Microarray analysis has identified downstream target genes that are differentially regulated (Figure 1). We will try to identify the network of genes that are coordinately up or down regulated after FGFR and oestrogen receptor signalling in a time-dependent manner. Highly connected genes, forming a hub in the network, are likely to be master regulators and might themselves act as predisposing genes. These hubs may also be promising therapeutic targets that could be modulated in order to correct a de-regulated network.

We have also continued our work to identify the functional variants within the risk loci identified by GWAS for breast cancer. We have focussed this work on the TNRC9 gene (Udler et al., *Hum Mol Genet* 2010; 19: 2507) and 8q24 region (Meyer et al., submitted). The latter is a gene-poor region that contains multiple susceptibility loci for prostate, breast, colon and bladder cancers. By studying chromatin conformation and protein-DNA interactions both in vitro and in vivo, we have identified a functional variant for one of the 8q24 prostate cancer predisposition loci. The protective allele is able to bind YY1 and mediates transcriptional repression. Chromatin conformation capture (3C) suggests that this region is able to interact...
Figure 1
Differentially expressed genes in MCF-7 cells after activation of the FGFR and oestrogen signalling pathways. The following comparisons were carried out – red circle: untreated versus oestrogen (E2) treated; blue circle: oestrogen treated versus oestrogen plus FGF10 (E2.FGF10); green circle: oestrogen treated versus FGF10 and FGFR inhibitor (E2.FGF10.PD).

with both the MYC and PVT1 oncogenes, located more than 600kb downstream of the risk interval. Furthermore, we find that increased PVT1 expression correlates with the presence of the risk allele in normal prostate samples.

In lung cancer, we propose to explore a different hypothesis to identify the ‘missing genetic heritability’ which in this cancer is possibly even greater than in breast cancer. Cigarette smoking is the major cause of lung cancer, but only 15% of heavy smokers develop cancer. We hypothesise that this is not chance, but that the susceptible 15% differ; because of genetic variation, in their response to cigarette smoke injury to the airway cells. If so, analysis of relevant phenotypes in those normal cells (or possibly in a surrogate such as blood lymphocytes) may provide an integrated readout of the effects of genetics and smoke exposure, that determines the likelihood that cancer will develop. We will start by analysing: (1) Gene expression patterns in airway epithelium of smokers who do, and do not, have lung cancer. We will examine both nasal and bronchial epithelium to see if nasal epithelium can provide an accessible surrogate tissue; and (2) H2AX phosphorylation as a measure of DNA damage response in irradiated cultured lymphocytes. If the results are promising, we have access to a large prospective study for confirmation.

Publications listed on page 72
Rational clinical decisions on the management and treatment of cancer rely on accurate diagnostic information. Molecular analysis of tumour samples has been used to predict prognosis or response to treatment, but should be complemented by non-invasive methods for monitoring disease progression or dynamics. Circulating DNA in plasma and serum include tumour-specific sequences that are a promising source of diagnostic information.

The mechanisms through which tumour DNA reaches blood circulation are unclear, although fragmentation patterns of DNA in the plasma of cancer patients suggest it may originate from cell death. Overall levels of circulating DNA are higher in cancer patients compared with healthy controls, but these differences are not consistent enough for robust diagnostic tools. The maturation of genomic technologies allows circulating tumour-specific DNA to be used as personalised biomarkers (Figure 1).

Circulating tumour DNA (ctDNA) can be measured by tying together genomic and molecular techniques. First, tumour-specific somatic alterations must be identified on a case-by-case basis. Second, sequence-specific molecular assays must be designed that can precisely detect and measure tumour-specific sequences in the background of circulating genomic DNA. Finally, these assays must be applied to body fluid samples such as blood plasma that have been carefully collected and processed to extract circulating DNA.

Circulating tumour DNA may be useful for identifying the presence of cancer mutations, for detecting systemic or residual tumour burden, or for non-invasive monitoring of tumour changes. Preliminary studies suggest that ctDNA compares favourably to imaging or to currently used protein markers. Our goal is to translate this potential into diagnostic applications, by integrating new quantification methods and computational insights with clinical research.

**Measurement and noise in molecular biology**

Quantitative measurements in molecular biology are challenging; objects of study are highly sensitive biochemical systems and repeated sampling is limited since living organisms are highly variable and dynamic. Reliability depends on our ability to take into account biological variation, measurement noise and biases.

In earlier studies (at the Weizmann Institute of Science), time-lapse microscopy and fluorescent reporter fusions were used to study gene regulation circuits. These studies demonstrate one approach to overcoming biological variation, by performing measurements in individual living cells.

Medical diagnostics poses different challenges. Clinical samples are often limited and heterogeneous, and can vary in collection conditions or contain a mixture of tumour and other material. Molecular quantification methods introduce additional noise and bias. We need to understand these effects and consider their impact on the design of diagnostic tests.

We are studying collection and processing protocols for peripheral blood samples, to optimise these for measurement of ctDNA and adapt them for simplified clinical use. We quantify DNA using parallel or ‘digital’ PCR, arguably the most accurate method for quantification of nucleic acid sequences. Template molecules are distributed into multiple independent reactions, reducing background interference. Quantification is obtained through counting of positive amplifications, and does not rely on calibration standards or curves.
Figure 1
Workflow for studies on circulating tumour-specific DNA. DNA obtained from a patient's tumour or biopsy sample is used to identify tumour-specific genomic alterations. Assays are designed to specifically measure these tumour-specific DNA sequences. Assays are validated using tumour DNA as positive control and DNA from other subjects (and normal) as negative controls. The assays are used to measure ctDNA levels in blood samples from the same patient. These data are compared to clinical information to study ctDNA dynamics and diagnostic potential.

Diagnostic algorithms
A major challenge in designing diagnostic tests is in defining categories that are clinically informative and can also be robustly identified. Tumours can be classified, for example, as positive or negative for hormone receptors, indicating the suitability of hormonal treatment. To be effective, diagnostic algorithms need to take into account both measurement limitations and clinical considerations.

In previous projects (at Rosetta Genomics Ltd.), microRNA expression levels were used to classify tumour histological types and sites of origin. Classification was based on strong biomarkers and intuitive, ‘logical’ decision criteria. The robust design of these algorithms enabled their rapid translation into clinical tests. This practical approach to molecular classification is likely to be effective in translating other types of diagnostic assays into clinical practice.

We use state of the art genomic tools to identify somatic changes in the DNA from a tumour or biopsy sample, and design tumour-specific molecular assays. The complex analysis of tumour material shifts the burden of proof and makes the measurement of ctDNA in blood samples direct and unequivocal. We believe that these personalised biomarkers will prove to be highly informative and clinically effective.

Non-invasive diagnostics using ctDNA
The study of ctDNA requires carefully collected samples from clinical studies that include tumour or biopsy material and matched collections of blood samples. It is possible to accurately measure ctDNA in these samples using the methods that we are developing and utilising. These data must then be compared to clinical follow-up data to identify associations and potential diagnostic roles of ctDNA (Figure 1).

We work in close collaboration with clinical groups (such as the Brenton, Caldas and Neal laboratories at the CRI) to study the dynamics and utility of ctDNA in epithelial cell cancers, with the aim of developing findings into robust diagnostic assays. In 2010, we recruited the lab’s first postdoctoral scientists, integrated technologies, and produced our first proofs of concept. These show early evidence for the informative content of ctDNA as a personalised biomarker for cancer monitoring, as well as its analytical robustness.

Publications listed on page 73
Mammary Stem Cell Biology
www.cambridgecancer.org.uk/johnstingl

My laboratory is interested in identifying and characterising the cells that make up the normal mammary epithelium, and how these cells relate to those present in different types of human breast tumours. We are particularly interested in studying mammary stem and progenitor cells since we hypothesise that these cells are the initial targets for malignant transformation and that they may function as cancer stem cells that propagate tumour growth. The laboratory also has an interest in characterising the cellular hierarchies present in human serous ovarian tumours and in normal and malignant human prostate tumours.

My laboratory currently has six main research themes:

1. Characterisation of normal mammary epithelial stem and progenitor cells
Mammary stem and progenitor cells are perceived to be the cell of origin of breast tumours since only these cells have the replicative capacity that allow the multiple mutations required for tumour progression to accumulate. My previous research demonstrated that functionally distinct mammary cells can be purified and detected via the use of flow cytometry and functional assays (Figure 1). We have recently identified two novel types of progenitor cells within the mammary epithelium and are currently determining their properties and how they relate to breast tumours.

2. Determining the cell of origin in breast cancer
Breast cancer is a heterogeneous disease with approximately five molecular subtypes and 18 histological subtypes identified. Our laboratory is interested in elucidating the mechanisms that account for this heterogeneity. One possible mechanism is that different types of breast cancers initiate in, and are propagated by, different types of mammary cells. To test this directly, we are conducting experiments in which we are reverse engineering human breast tumours onto different cellular backgrounds. We will also be examining the influence of a variety of tumour suppressor genes on mammary stem and progenitor cell function to determine if loss of common tumour suppressor genes can impart some properties of stem cells to committed progenitor cells. The tumour suppressor genes that we are focusing on are those associated with basal-like breast tumours, which are very aggressive types of breast cancer.

3. Identification of the molecular mechanisms that regulate stem cell self-renewal
Self-renewal is perceived to be a defining property of stem cells. Cellular pathways that regulate stem cell self-renewal are considered to be good targets for therapeutic intervention since tumours should eventually exhaust their proliferative capacity in the absence of these pathways. Since we have developed strategies that enable us to isolate stem cells to high purities, our approach to identify these pathways is to compare the gene expression patterns of mammary stem cells isolated from different developmental states (e.g. states of stem cell expansion vs. non-expansion).

4. Characterisation of human breast tumour stem cells (collaborative project with Carlos Caldas, CRI)
Previous reports in the literature have demonstrated that human breast tumours contain a cancer stem cell component and that these cells can be prospectively isolated on the basis of the expression of certain cell surface
A flow cytometry dot plot demonstrating the distribution of epithelial cell adhesion molecule (EpCAM) and alpha 6 integrin (CD49f) among freshly dissociated human mammary epithelial cells. The differentiated luminal (NCL), luminal progenitor (LP) and basal cell populations are indicated. The LP population generates colonies of pure luminal cells, whereas the basal cell population is enriched in bipotent progenitors, which generate mixed lineage colonies in vitro. Re-plating of these mixed colonies demonstrates that they are precursors to myoepithelial-restricted progenitors. Scale bars are 1mm.

It is not known if these properties are universal for all breast cancer stem cells or if different types of breast tumours have stem cells with unique properties. To address this issue, we have initiated experiments in which the breast cancer stem cell component of human breast tumours is identified and characterised using flow cytometry in combination with functional assays. We will be able to understand the cellular context of these cancer stem cells by using the markers that we have previously determined to characterise different subsets of normal mammary epithelial cells. Long-term experiments include tracking the evolution of these cancer stem cells over time and examining their functional heterogeneity, both of which have large implications for response to therapy.

5. Characterisation of human ovarian cancer stem cells (collaborative project with James Brenton, CRI)
Serous ovarian cancer is an aggressive disease that initially responds to chemotherapy, but approximately 70% of patients will relapse and become resistant to therapy. It is our hypothesis that this resistance is mediated by the emergence of a subpopulation of ovarian cancer stem cells. We are currently evaluating the proliferative potential of phenotypically distinct subsets of ovarian tumour cells in order to identify the putative cancer stem cells. Future research includes tracking experiments to follow the fate of individual clones during chemotherapy and gene expression profiling of these cells.

6. Characterisation of the normal and malignant prostate epithelial cell hierarchy (collaborative project with David Neal, CRI)
We are applying our expertise in the characterisation of the mammary epithelial cell hierarchy to the human prostate epithelial cell hierarchy. We are particularly interested in characterising progenitor cells within the human prostate and their developmental relationships.

Publications listed on page 73
Computational Biology and Statistics
www.cambridgecancer.org.uk/simontavare

Our work has continued its focus on five main areas:
Statistical methods for microarray data, analysis of Solexa second-generation sequencing data, evolutionary approaches to cancer, statistical genetics, and analysis of genomics data.

We have continued our development of statistical methods for the analysis of Illumina BeadArray technologies. Illumina’s technology uses randomly assembled arrays of beads, each of which has a probe for a specific genomic feature. Illumina’s software can report full bead-level data, access to which allows for more detailed quality assessment and more flexible statistical analyses. In collaboration with Mark Dunning (Bioinformatics Core), we have redesigned our open-source Bioconductor software, beadarray, to provide a statistical environment for such analyses.

beadarray 2.0 is a complete re-write of the original beadarray package with the aim of providing a more flexible interface to analyse all kinds of Illumina BeadArray data. Rather than being specific to expression data, users can now import methylation or genotyping data and easily adapt the standard visualisation and summarisation options provided in the package to their particular data type. The new package structure also facilitates the development and implementation of new methods for expression data.

Another aspect of our research develops evolutionary approaches to cancer. Realistic agent-based models are proving useful in capturing the complex interactions, spanning multiple space and time scales, that drive cancer growth. We have developed cellular Potts models to describe the dynamics of colon crypts and colorectal tumours. Statistical inference for agent-based models is a challenging problem that has hampered the adoption of agent-based models for complex systems. To this end, we have developed the first approximate Bayesian computation methods for such models, an approach that is likely to revolutionise agent-based modelling. We are using molecular markers such as DNA methylation to infer aspects of the stem cell dynamics in crypts, and to study heterogeneity in colorectal tumours.

Measuring the methylation status of single molecules continues to be a focus of our experimental work. We have developed a cheap, automated, emulsion PCR-based method for identifying CpG methylation in hundreds of thousands of molecules at a small number of CpG islands, together with the computational tools needed to analyse such data. We are using this method to generate data for the cancer modelling described above. This technique has a number of other uses, such as the quantification of allele-specific expression, the identification of rare variants, and the validation of findings from second-generation sequencing experiments.

We continue to collaborate on several projects that involve the statistical analysis of resequencing data. We have continued development of our BayesPeak package for the analysis of ChIP-seq experiments, this is now freely available as part of Bioconductor. In collaboration with Illumina we have developed statistical methods for identifying copy number aberrations from second-generation sequencing data obtained from matched tumour-normal pairs. Data-mining and data visualisation remain an important focus of our work. To this end, we have developed custom databases to mine network interactions in genomic data. These tools incorporate information from diverse data types, and have been used in collaborations with the Caldas and Narita labs at the CRI, and the Fearon lab at the University.

We have continued our work with the Caldas laboratory on the statistical analysis of the METABRIC project that has assayed germline and somatic copy number variants, and their impact on expression variation in some 2,500 breast tumours and matched normal samples, using high-density microarrays (Figure 1).
Trans-acting aberration hotspots are evident in breast cancer and modulate concerted molecular pathways. From top to bottom: Manhattan plot illustrating the genomic location of cis and trans expression associated CNAs, where the directionality of the association is indicated by shading – cis-positive (red), cis-negative (pink); trans-positive (blue), trans-negative (light blue). A matrix of predictor-expression associations with Bayes’ factor greater than five are plotted, illustrating a strong off diagonal pattern at several loci including regions on chromosomes 1q, 7p, 8, 11q, 14q, 16, 17q, and 20q. The frequency of mRNAs associated with a particular predictor further illuminates these trans-acting aberration ‘hotspots’.

Following up on our initial findings, we are performing targeted resequencing of specific patient sub-populations to survey the mutational spectrum of putative cancer genes. We are also generating transcriptomic and paired-end sequence data on a subset of these tumours to study alternative splicing and structural variation. Additionally, we are pursuing integrative bioinformatic and statistical methods to further delineate mechanisms of differential pathway disruption within the novel subgroups. In collaboration with the Bioinformatics core facility we have developed analysis pipelines for production runs on Illumina expression arrays. We have also developed novel methods for detecting aberrations in tumour samples that exploit cross-sample information, and for identifying interacting SNPs in genome-wide association studies.

Dr Benilton Carvalho joined the lab as a postdoc. Benilton completed his PhD in Biostatistics with Rafael Irizarry at Johns Hopkins University, where he developed CRLMM, a genotyping algorithm for SNP arrays. He is now working on statistical methods for the next generation of Affymetrix and Illumina SNP chips, and for second-generation sequencing data. Daniel Andrews completed Part III in Mathematics in Cambridge, and joined us as a research student. Daniel is working on methods for inferring cellularity from sequencing data.

Sergii Ivakhno and Doug Speed completed their PhDs. Sergii is now in the bioinformatics group at Illumina, and Doug is a postdoc in the Balding laboratory at University College London. Dr Nuno Barbosa-Morais obtained a Marie Curie Fellowship to work in Ben Blencowe’s lab at the University of Toronto, and Dr Christina Curtis has taken up her position as an Assistant Professor in Preventive Medicine in the Keck School of Medicine at the University of Southern California.

Publications listed on page 73.
Oncogenes initiate and sustain carcinogenesis, and therefore a detailed understanding of oncogene function is pertinent in the design of effective strategies to treat, diagnose and prevent cancer.

We have chosen to model malignant progression in patients and genetically engineered laboratory mice, focusing upon pancreatic cancer and more recently melanoma as prototypical diseases with poor prognoses despite the identification of the predominant oncogene in each disease. Our approach entails the characterisation of molecular and cellular events following driver oncogene expression, the identification of cell intrinsic and extrinsic pathways in tumour evolution, and the evaluation of therapeutic and diagnostic strategies in these model systems. These preclinical results have prompted the initiation of a number of clinical trials that our laboratory directs.

Cell autonomous and non-cell autonomous events in carcinogenesis

Both cell autonomous and non-cell autonomous events shape the evolution of carcinogenesis through internal signalling events and homotypic and heterotypic cellular interactions. Whereas such events are difficult to characterise using human specimens, they can be readily pursued with our murine cancer models through a variety of biochemical, cellular and genetic approaches.

We have undertaken both unbiased and targeted approaches to identify important mediators of KrasG12D transformation. Indeed, a global proteomic and transcriptional evaluation of primary cells following oncogenic Kras expression has revealed alterations in Reactive Oxygen Species (ROS) metabolism as an important facet of cellular immortalisation in primary cells. Although ROS have previously been proposed to promote cellular transformation, we instead find that endogenous ROS is deleterious to cellular immortalisation and that KrasG12D expressing cells enact a cellular programme to rapidly neutralise ROS and other cellular toxins. The regulation of altered ROS metabolism in KrasG12D expressing cells is due to signalling by the MAP kinase cascade, and thus represents a possible therapeutic target in early neoplasms. Other common oncogenes such as BrafV600E and c-Myc also activate the ROS metabolism pathway to lower ROS levels, suggesting that this is a general pathway of relevance during the initiation of cancer. In addition to the MAPK pathway, we are seeking alternative methods to inhibit this ROS metabolism pathway as a means to prevent the onset of cancer or treat established cancers.
Figure 1
The effect of normal cells on pancreatic preneoplasm development. (A) and (B) Kras<sup>G12D</sup> was expressed in a complete manner in LSL-Kras<sup>G12D</sup>; p48-cre mice and a mosaic manner in LSL-Kras<sup>G12D</sup>; pdx-cre mice as shown by gfp staining when crossed to a cre reporter strain. (C) The presence of normal cells results in delayed preneoplasm development with time. (D) A cartoon illustrating where neighbour suppression is postulated to operate.

Our targeted approaches include the conditional ablation of several pathways that may cooperate with oncogenic Kras to promote tumourigenesis. For example, to determine whether specific branches of the Raf signalling pathway are required to sustain tumourigenesis, we have concomitantly deleted CRAF or BRAF while expressing Kras<sup>G12D</sup> in lung epithelial cells in vivo. We find that CRAF, but not BRAF, is required to promote early lung cancer. Current work is focused upon elucidating the exact molecular pathways downstream of CRAF, besides MAPK, that are required to promote Kras<sup>G12D</sup> oncogenesis.

We also use both classical and forward genetic approaches to uncover novel genes and pathways that facilitate pancreatic cancer initiation and metastasis. This entails high throughput sequencing of spontaneous and transposon-stimulated tumours, and the validation of potential candidates in cell culture and in vivo. We recently identified a major new pathway important in pancreatic cancer genesis, and are currently establishing its clinical relevance.

Cell biological approaches afford the opportunity to explore the non-cell autonomous interactions in developing tumours and thereby identify features that may both suppress and promote tumourigenesis. Our initial efforts have focused on the identification of the cell types that comprise the immune, stromal and vascular constituents of the tumour microenvironment. We recently reported the presence of immature myeloid cells in pancreatic preneoplasms and invasive tumours. Such cells might stimulate tumourigenesis as they have been implicated as potential suppressors of the acquired immune response. The role of inflammation in the genesis of pancreatic cancer is also being investigated. To do this, we are analysing the importance of the immune system and stromal cells in the generation of the desmoplastic stroma common in preinvasive and advanced pancreatic cancer; and we are investigating cell autonomous paracrine and autocrine factors that could stimulate the microenvironment. Work done in collaboration with the laboratory of Douglas Fearon (Department of Medicine) has revealed that the cancer fibroblasts in our pancreatic tumours are immune suppressive. We are currently evaluating this finding.

Pancreatic Cancer Medicine advances in the preclinical and clinical settings
We have established with our mouse model of pancreatic ductal adenocarcinoma that the intratumoural vasculature in such tumours is sparse and highly compressed, correlating with poor tissue perfusion and therapeutic delivery. These observations have been extended to human tumours, and we have developed various strategies to correct this defect by targeting the tumour stroma. We have initiated several clinical trials to confirm our findings, including a pre-surgical trial that uses a Hedgehog pathway inhibitor for two weeks prior to surgery. Likewise, our work has directly stimulated the opening of three other clinical trials that our laboratory either clinically directs or supports.

Publications listed on page 74
The epidermis consists of a multilayered epithelium, the interfollicular epidermis, and associated hair follicles, sweat glands and sebaceous glands. All of the different lineages within the epidermis are maintained through proliferation of stem cells and differentiation of their progeny (Watt and Jensen, EMBO Mol Med 2009; 1: 260). By investigating how stem cell renewal and differentiation are controlled in normal tissue, we hope to identify new approaches to preventing and controlling tumours of the epidermis and other stratified squamous epithelia (Watt and Driskell, Phil Trans Roy Soc B 2010; 365: 155).

Stem cell renewal and lineage selection
One of our ongoing interests is in how stem cell behaviour is regulated by extrinsic signals from the local microenvironment, or niche. Two different and complementary approaches can be taken to investigate this: observing stem cells in vivo and recreating the niche in vitro. Stem cell behaviour in vivo is a composite response to all niche signals, whereas in vitro it is possible to parse out the response to individual signals.

For our in vitro studies we have developed, in collaboration with Wilhelm Huck (Department of Chemistry, University of Cambridge and Radboud University, Nijmegen), micro-patterned extracellular matrix (ECM)-coated glass substrates that selectively capture single human epidermal stem cells. The substrates are amenable to microscopic analysis of living cells, allowing us to perform FRET and image cytoskeletal dynamics. In addition, we can perform single cell gene expression profiling of cells on these substrates (Gautrot et al., Biomaterials 2010; 31: 5030; Connelly et al., Nat Cell Biol 2010; 12: 711). We found that when spreading is restricted on small circular islands, cells exit the stem cell compartment and differentiate. When cells can spread on large circular islands, however; they do not differentiate (Connelly et al., Nat Cell Biol 2010; 12: 711). We also found that differentiation does not depend on ECM composition or density. Instead, the state of assembly of the actin cytoskeleton regulates differentiation by controlling serum response factor (SRF) transcriptional activity. Differentiation requires SRF and its co-factor MAL, and is also dependent on the presence of growth factors. SRF target genes, the API transcription factors FOS and JUNB, are required for differentiation. c-Fos mediates serum responsiveness, while Jun-B is regulated by actin and MAL. We are now investigating how stem cells respond to differences in substrate stiffness and topology, and whether environmental responsiveness is altered in cells from squamous cell carcinomas.

Complementing the in vitro studies, we have continued to investigate the stem cell compartment in vivo, using genetically modified mice. One of the key pathways that regulates epidermal stem cells is the Wnt pathway. We have compared the responses of different stem cell populations to activation of Wnt signalling (Baker et al., Dev Biol 2010; 343: 40). We have found that activation of β-catenin in the stem cells of the hair follicle bulge stimulates proliferation, but not the formation of additional hair follicles. In contrast, when we target cells at the base of the sebaceous gland they readily form ectopic follicles. In contrast, when we target cells at the base of the sebaceous gland they readily form ectopic follicles. We are currently investigating whether this reflects intrinsic differences between the cells, or differences in their local microenvironment.

Stem cells and tumour formation
Interactions between epidermal cells and stromal cells profoundly influence normal differentiation and tumour formation, and we are using a variety of approaches to study these
interactions (Jensen et al., Nat Protoc 2010; 5: 898). In a recent study we found that activation of Notch signalling in the epidermis leads to profound changes in the underlying dermis, with the accumulation of cells that express markers of the neural crest, melanocytes, smooth muscle and peripheral nerve (Ambler and Watt, Development 2010; 137: 3569). These effects are dependent on epidermal upregulation of the Notch ligand Jagged 1. Gene expression profiling reveals that epidermal Notch activation results in upregulation of a number of growth factors and cytokines, including TNFα, whose expression is dependent on epidermal Jagged 1 expression. Since the Notch pathway is activated in a large number of different tumour types, the effects that we have observed may provide a general mechanism for how Notch signalling alters the tumour stroma.

Another example of how aberrant activation of a signalling pathway in one cell population can have a profound impact on neighbouring cells comes from our ongoing studies of the consequences of aberrant integrin expression. Integrin expression is normally confined to the basal epidermal layer, but in many squamous cell carcinomas expression extends to the suprabasal cell layers. Suprabasal integrin expression results in upregulation of Erk mitogen-activated protein kinase (MAPK) signalling and we have modelled this by expressing an activated MAPK kinase 1 (MEK1) transgene in the suprabasal, non-dividing, differentiated epidermal cell layers (InvEE transgenics). We have found that wounding induces benign skin tumours in InvEE mice (Arwert et al., Proc Natl Acad Sci USA 2010; 107: 19903). Differentiating, non-dividing cells that express MEK1 stimulate adjacent cells to divide and become incorporated into the tumour (Figure 1). Tumour formation is associated with epidermal expression of IL1α and can be inhibited by blocking inflammation using dexamethasone. Depletion of γδ T cells and macrophages also reduces tumour formation. Our results are quite unexpected, because they show that differentiated epidermal cells can trigger tumorigenesis without re-acquiring the ability to divide.

Publications listed on page 74
Renewing tissues and many cancers are maintained by a small number of long-lived stem cells and this explains interest in trying to define their distinguishing properties. Most models of stem cell organisation take account of their longevity and the fact that they self-renew, and also assume that they are stable populations carrying unique identifying characteristics. For decades the assays used to test different cell populations for their ‘stemness’ have appeared consistent with such deterministic models. These assays commonly challenge the ability of cells, separated into discrete populations based on the expression of cell surface antigens, to undergo growth when cultured or engrafted. Cells that are able to support long-term growth are taken as being synonymous with stem cells, and it is assumed that the differential expression of transcription factors underpins the fate of stem cell populations.

However, this interpretation of stem cell organisation now seems too simplistic. For example: cell fate is likely determined by small changes in the expression of regulatory transcription factors in the context of transcriptional networks; the cell surface signatures of stem cells may not be as stable over time as previously thought; the success of stem cell engraftment may be partly determined by properties of the recipient rather than the transplanted cells (Chang et al., Nature 2008; 453: 544; Quintana et al., Nature 2008; 456: 593). Stem cell biology may be driven by stochastic switching between different states in response to variations in the balance of signals coming from complex transcriptional networks. In accordance with this view we have recently demonstrated, by following the dynamics of clonal growth in situ, that intestinal stem cell turnover is a constant and rapid stochastic process that follows a pattern of neutral drift (Lopez-Garcia et al., Science 2010; 330: 822).

Given the above our approach is pragmatic: to identify novel ways of assaying stem cells in situ with respect to the functional endpoints that are integral to their biology.

**What is the multi-potentiality of stem-like cells in intestinal cancers?**

Our long-term objective is to determine the repertoire of differentiation options available to cancer stem cells, how this differs from normal stem cells, and thereby identify unique opportunities for therapies. To measure potentiality we are exploiting the known differences between cell types in the timing of DNA replication during the cell cycle. Genes associated with maintaining pluripotency are replicated early in S-phase, while those associated with neural lineages are replicated late in S-phase (Azuara et al., Nat Cell Biol 2006; 8: 532). The pattern of replication timing for key transcription factors has been described as a barcode of potentiality, indicative of the accessibility of the chromatin for subsequent expression.

We are attempting to devise such a barcode for intestinal stem cells to identify changes in potentiality during carcinogenesis. S-phase cells can be isolated and sorted by DNA content into four fractions. Immunoprecipitation for BrdU allows newly synthesised DNA to be analysed. To date we have shown reproducible differences in replication timing between different loci. For example, the neural transcription factor Mash1 is replicated late, while the transcription factor Ngn3, expressed in the intestine, is replicated early. Currently, the amount of material obtained on pull-down
is restrictive. We aim to increase genomic coverage by amplification to generate a comprehensive characterisation of replication timing. The effect of deleting the APC tumour suppressor gene on replication timing patterns is also being determined — deleting this gene also results in dramatic changes in cell type (loss of secretory cell lineages) and differentiation.

Role of quiescent stem cells
Label retaining cells, identified by their ability to sequester and retain label, have long been thought to be synonymous with quiescent stem cells. Using inducible expression of nuclear-localised fluorescent protein (Histone H2B-YFP) we have identified a population of crypt-base cells that appear to divide either very slowly or to be quiescent. Conventional views of stem cell organisation would place these cells as potential long-lived cells acting at the apex of a proliferative hierarchy. However, such an interpretation is not compatible with the dynamics that we have documented: rapid stem cell turnover with neutral drift. We aim to characterise these cells and define both their normal fate and whether they can be tumour initiating in a cancer setting. To this end we are currently performing detailed transcriptional analysis of purified label-retaining cells.

Cancer models and tumour progression
At a molecular level the development of intestinal cancers is well characterised, with the most common genetic changes incorporated into a paradigm of progression for colorectal cancers in which loss of APC is a central early event (as described by Bert Vogelstein’s lab at Johns Hopkins University). Despite this it has been shown that many other gene specific mutations can also be associated with the disease (Sjoblom et al., Science 2006; 314: 268). APC has been deleted in animal models by a variety of strategies that usually lead to the development of benign adenomas. Introduction of additional mutational events in candidate genes has only been partly successful in creating the full carcinomatous (cancer-like) disease. Our ability to induce deletion of APC in the intestinal epithelium lends itself to investigating the nature of other gene mutations that might interact with APC and contribute to the formation of malignant disease. Therefore as an alternative unbiased approach to identifying such genes we are using our Cre models to mobilise a Sleeping-Beauty activated transposable element in mice predisposed to intestinal tumorigenesis by virtue of APC deficiency (Collier et al., Nature 2005; 436: 272). Cloning and sequencing of the insertion sites in tumours allows affected genes to be identified and associated with tumour pathology. Currently we have identified around 900 such genes (Figure 1). Their validation will be a priority for the coming year.

Publications listed on page 74
The CRI’s Core Facilities provide state-of-the-art services and equipment to support the cutting-edge research of the Institute, as well as working towards applying new technologies to cancer research. Each facility has a team of scientific staff who provide scientific support, advice, and training for all CRI researchers and students in the use of their facility’s particular speciality, as well as keeping fully up-to-date on developing technologies.

Four colour tissue analysis on the Compucyte iCys Research Imaging Cytometer of an ear papilloma of an aggregate chimeric mouse. Yellow: Ki67, showing proliferating cells; Green: eGFP; Red: Histag showing cells with activated MEK1; Blue: DAPI nuclear counterstain. In this system, post-mitotic, activated MEK1-expressing cells can recruit undifferentiated normal cells into a tumour and form its proliferative compartment (Arwert et al., PNAS 2010; 107: 19903). Image courtesy of Heather Zecchini (Light Microscopy).
The Bioinformatics Core offers a statistics and bioinformatics analysis service to CRI research scientists and develops software and analysis pipelines to support high-throughput technologies such as next generation sequencing and microarrays.

Analysis of datasets generated by high-throughput sequencing and microarrays has continued to be a major focus for the Bioinformatics Core. In the past year, our team has supported a large number of research projects by consulting with scientists, providing input into experimental design, analysing the raw data generated and helping with interpretation of the results. Illumina BeadChip expression arrays and ChIP-seq projects run on the Illumina Genome Analyzer and HiSeq sequencers remain the most popular applications requiring support from the Core, but we have also contributed to projects involving copy number profiling using SNP arrays, exon arrays, amplicon resequencing for SNP analysis, chromosome conformation capture, and resequencing to explore structural variation in cancer genomes.

Throughout the year, we have been running weekly experimental design sessions, jointly with the Genomics Core for genomics projects, and recently have extended the scope of these to cover other technologies. We also run a weekly statistics clinic providing help with the statistical aspects of a wide range of experiments and research questions.

We have continued to develop our analysis pipeline for Illumina BeadChip expression arrays and have tailored our training course in microarray analysis to better reflect the typical interaction of CRI scientists with the Core, placing emphasis on experimental design issues and interpretation of the output from the pipeline. We are also developing an analysis pipeline for ChIP-seq experiments incorporating multiple peak callers to give a high confidence consensus set of locations for DNA-bound proteins or epigenetic marks.

In the coming year, we anticipate increasing demand for downstream bioinformatics support for proteomics data. Working closely with the Proteomics Core, we have assisted with the analysis and visualization of SILAC pulse-chased mass spectrometry data to investigate protein turnover rates; these are time-course experiments in which incorporation rates for labelled peptides are quantified and anomalous turnover patterns identified.

A further aspect of the Core’s work involves maintenance of local installations of key bioinformatics databases and applications, including Galaxy, a web application that enables researchers to carry out their own analyses and the Core to deploy analysis and visualization tools developed in-house.

Finally, in 2010 the Core ran training courses on functional and network analysis, motif searching, and microarray analysis, and a seminar series on next generation sequencing.

Publications listed on page 75
The Biological Resources Unit at the CRI facilitates the work of research groups that use mouse models to understand the genetic causes of cancer, by providing technical advice and expertise in animal care and use.

Cancer is a complex disease which requires scientific advancements in a variety of biomedical research disciplines in order to be better understood, diagnosed and treated. Relevant research results can be obtained with the combined use of computers, in vitro experiments, studies done on patients and in human populations, and in vivo mouse models.

Each year in the UK, more than a quarter of a million people are diagnosed with cancer. Doctors and researchers estimate that about one in three of us will get some form of cancer at some point in our lives. The transgenic mice at the CRI develop cancers that very closely resemble cognate human cancers in terms of both genetic make-up and behaviour. These mice are used to develop new methods for early diagnosis, and to understand the determinants of response or resistance to new treatments.

In order to best assist the ground-breaking science within the CRI, the BRU provides a wide range of skills and services. Our dedicated animal husbandry and services staff are both highly skilled in the care and welfare of animals and have vast experience in a full range of licences and technical procedures. For example, our newly refurbished surgical suite, trained staff and surgery workshops help Institute staff to further develop and refine their techniques (Figure 1).

Regular health screens are carried out on our colony and all animals are housed within IVC cages, helping to maintain our clean health status. Animals are only accepted from designated suppliers or colonies with proven known health status to ensure the colony is not compromised. Any animals that arrive from a non-supplier are housed within our isolation suite until their health status can be verified and they can be released into the general colony (Figure 2).

Our isolation and containment area has been developed to allow animals with certain named pathogens to enter the CRI in order to take advantage of some of the facilities we are able to offer, for example the Xenogen scanning machine, CAT2 area, PET/SPECT scanners and the MRI suite. We can also import and export whole animals and tissues from both collaborators and suppliers located nationally and internationally.

We are able to offer advice and guidance in the development and application of all licences required to enable in vivo science to take place within the CRI. We administer the Animal Ethics Committee for the CRI on behalf of the Certificate Holder and, as a result, are able to offer advice on the CRI’s attitude towards ethical issues and bring concerns and administrative items relating to licences to the attention of the committee.
Biorespository and Cell Services

Our service allows simple access to storage, tracking and risk management of tissue samples, cell lines and any other biological samples, including human tissue samples, in accordance with current legislation. We also provide up-to-date expertise, training and troubleshooting in all aspects of cell and tissue culture, to maintain a consistently high standard throughout the Institute. The Biorepository is used extensively by most research groups at the CRI.

Cell culture

We provide basic cell culture training for all scientists at the CRI, as well as a regular and comprehensive mycoplasma testing service, a batch testing service for serum and other cell culture media components, and quality controlled bulk culture of research cell lines, including mouse embryonic fibroblasts (MEFs). We also offer a routine human cell line authentication service using multiplex PCR and short tandem repeat (STR) profiling. Regular cell line authentication is important both to confirm integrity of data and as a requirement for publication in many leading journals. Biorepository staff also keep fully up-to-date with the latest cell and tissue culture based technology, methods and equipment, by attending relevant conferences, meetings, seminars and courses.

The Human Tissue Act

Biorespository staff advise on, monitor and control the import, use, storage and disposal of human tissue samples for research, to ensure full compliance with the Human Tissue Act and the Human Tissue Authority (HTA) Codes of Practice, a statutory requirement for all research involving human tissue samples. We advise on how to request human tissue samples from the Addenbrooke’s Hospital Tissue Bank and other sources, and how to obtain Local Research Ethics Committee approval for new research projects involving the use of human tissues.

2010 developments

We have expanded and developed to provide an ever improving service to a growing Institute. New services include:

• Introduction of a routine (monthly) human cell line authentication service using STR profiling.
• Introduction of a second Essen BioScience IncuCyte™ instrument. This is a compact, automated live cell imaging platform designed to provide kinetic, non-invasive live cell imaging. The instrument is located in a 5% CO₂ incubator and acquires high-definition phase contrast images of live cells in microplates, cell-culture dishes and cell-culture flasks. Custom image processing software calculates a variety of image metrics, such as cell proliferation assays and growth curves, wound closure assays, optimisation of cell based assays and the optimisation of cell culture media components. The instrument has proved to be extremely useful and popular and is heavily used by many of the research groups at the CRI.

Future developments

We have identified a number of new services and initiatives that will further facilitate and enhance research at the CRI when implemented. These include:

• Introducing and optimising a phage display antibody library. A human single fold scFv library will enable us to very rapidly derive monoclonal antibodies to almost any target molecule requested.
• We plan to install a third Essen BioScience IncuCyte™ instrument. Our two existing instruments are used at full capacity and are producing high quality data. A third instrument would give us the option of running screens in containment level 2 or low oxygen conditions.
The Equipment Park provides CRI scientists with access to a range of state-of-the-art equipment and specialised technologies.

We offer technical/scientific advice, troubleshooting support and appropriate training for all the facility’s equipment. We also routinely test the capabilities of our equipment, optimise current techniques and horizon scan to maximise the quality of data generated and to provide the best possible advice to CRI scientists. This year we have focused on equipment used for Western blotting and quantification of low volume DNA, RNA and protein samples, as these are important techniques routinely carried out by the Institute’s researchers.

Protein Gel Electrophoresis
We provide access to a wide range of gel electrophoresis equipment for analysis of protein samples. We have the capability for both 1- and 2-dimensional separation of proteins including 2D Fluorescence Difference in-Gel Electrophoresis (2D-DiGE). Together with our range of digital camera and scanner imaging systems, we can digitise images which improves accuracy of quantification, saves time and reduces costs. This year we have undertaken a collaborative project with the Proteomics Core Facility to understand how 2D-DiGE compares with a mass spectrometry technique, iTRAQ. The aim is to improve the range of proteomic techniques on offer.

Biosensor
The Biacore T100 measures molecular interactions in real-time. It provides label-free measurements of the affinity and kinetics of interactions, as well as the thermodynamic properties underlying association and dissociation rates. This instrument has proved pivotal in a number of research studies this year, carried out with the Murphy and Brindle laboratories, investigating the kinetics of protein-protein and protein-phospholipid interactions.

Plate readers and spectrophotometers
The Equipment Park provides access to two high specification plate readers: the Tecan Infinite M200 is used extensively by most research groups at the CRI for absorbance, fluorescence and/or luminescence assay work, and we also house a BioTek Clarity, a dedicated luminescence plate reader. A third UV-visible cuvette spectrophotometer, the Cecil Super Aquarius 9500, is particularly suited to quantification of low-concentration samples. We will soon be introducing a new high specification plate reader, BMG PHERAstar FS, which will allow users to perform new assays including time-resolved fluorescence, fluorescence polarisation and Alphascreen.

Imaging Systems
Four imaging systems are available that produce digital images from a wide range of different samples. The Typhoon Trio produces images of radioactive, visible fluorescent or chemiluminescent samples while the Li-Cor Odyssey images fluorescence specifically in the infrared region. Both systems are used routinely for Western blotting and cell-based assays. The ImageScanner III is a high-resolution flatbed scanner for imaging non-fluorescent samples. We also have a high resolution camera system, Syngene Dyversity, capable of capturing both fluorescent and chemiluminescent images. Dedicated analysis software packages can accurately quantify protein/DNA bands or spots captured by any of these imaging systems.

Molecular Biology Applications
The Equipment Park houses an 8-channel NanoDrop as well as a Qubit for quantification of small volume nucleic acid (and protein) samples and has the capability for both standard and real-time PCR. We also have a pulsed-field gel electrophoresis system, CHEF III, for separation of large DNA molecules and an E-Gel iBase for fast separation of DNA and RNA.
The Flow Cytometry core facility provides state-of-the-art flow cytometric instrumentation, technical expertise, training, and software analysis in a collaborative environment. Our mission is to develop cytometric technologies that will best assist CRI researchers in finding answers for the treatment, prevention, and understanding of cancer.

**Services**

Our lab offers a full range of educational and cytometric services that includes immunophenotyping, cell cycle analysis, translocation and co-localisation of cell activation markers, chromatin density, and apoptotic and necrotic analysis. In addition we are capable of performing cell sorting for researchers so that they can isolate cell populations needed for further studies.

Users are offered an array of educational programs in the theory, anatomy, applications and science of flow cytometry. Additional workshops are offered on data analysis using all of our software programs and on practical applications of current protocols in cytometry. We also collaborate with other scientists in the Cambridge Cancer Centre on our specialised equipment.

**Equipment**

**FACS Aria SORP (BD Biosciences)** – The Aria is a high-speed sorter. It is equipped with five lasers: a UV, 407nm, 445nm, 488nm, and 633nm. Our optical configuration allows us to see three UV, six violet, three indigo, six blue and three red parameters.

**LSR II (BD Biosciences)** – The LSR II is an analytical bench top flow cytometer. It is comprised of four lasers: a UV, a violet (407nm), a blue (488 nm) and a red (633 nm). Our optical configurations allow users to see two UV, six violet, seven blue and three red fluorescent parameters.

**FACS Caliburs (BD Biosciences)** – These flow cytometers are routinely used for phenotyping (to look at antigen, cytokine, or GFP expression), cell cycle analysis, and apoptosis studies. They are equipped with 488nm and 635nm lasers that allow users six parameter analysis.

**ImageStream (Amnis)** – The powerful combination of quantitative image analysis and flow cytometry in a single platform creates exceptional new experimental capabilities. 405nm, 488nm and 635nm lasers for four colour/six parameter analysis as well as EDF capability for FISH analysis are available.

**Influx Cell Sorter (BD Biosciences)** – This high speed cell sorter is contained within a biosafety cabinet to enable the isolation of cell populations from human tissue. It has four lasers at 405nm, 488nm, 561nm, 640nm and is equipped with 12 fluorescence detectors.

**RoboSep (Stem Cell Technologies)** – This magnetic bead separator unit has customisable programs allowing positive or negative selection of virtually any cell type from any species. Up to four samples can be processed simultaneously.

**Vi-CELL (Beckman Coulter)** – The Vi-CELL automates the widely accepted trypan blue cell exclusion method, with video imaging of the flow-through cell, to obtain results in minutes. The software conforms to key regulatory requirements with its electronic signature capability, audit trail, secure user sign on and user level permissions for clinical or preclinical studies.
The Genomics core facility allows researchers at the CRI to access state-of-the-art DNA and RNA analysis resources. The tools in Genomics help researchers to understand the cancer genome and unravel the genetic causes of cancer.

DNA Sanger sequencing is a workhorse of scientific research used by all groups at the CRI. DNA sequencing has recently been revolutionised by next-generation technologies, and at the CRI we are now able to sequence a human genome in around five days and perform unbiased genome-wide experiments to see what the underlying sequence differences are in cancer genomes. We have installed the Illumina HiSeq 2000 DNA sequencing technology (Figure 1). The CRI has invested significant time and resources in both the Genomics and Bioinformatics core facilities to become a centre of excellence in this technology. Having the capability to access these new systems puts the CRI at the forefront of genomic research.

Real-time PCR is commonly used for lower throughput gene expression, SNP genotyping, allelic expression and copy number analysis. We have two AB7900 systems and have recently installed a new system from Fluidigm. This allows us to run very high throughput projects and also to amplify regions of the genome for targeted resequencing using the Illumina sequencers.

Other technologies in the facility include a Pyrosequencer to look at methylation of DNA, Agilent Bioanalyser capillary electrophoresis instruments to quality control RNA and DNA samples before genomic analysis, and Qiagen robotics for nucleic acid extraction.

An important part of the Genomics core facility is the staff. The technologies used in Genomics are complicated and the staff in the facility undertake projects for the Institute’s research groups as well as training individuals to use the equipment in the facility.

Publications listed on page 75
The Histopathology/ISH core facility at the Cambridge Research Institute offers a variety of histological techniques, immunohistochemistry, in situ hybridisation, laser capture microdissection as well as automatic slide digitisation and analysis to CRI scientists.

**Histology**

The facility processes, embeds and sections human and animal tissues or cell lines into frozen or paraffin formats and stains these with the standard haematoxylin and eosin (H&E) or special stains, as needed by the researcher to complement their work. During the past year, the facility has worked up further special stains, the Herovici stain for new deposition of collagen and the Leder stain for mast cells (Figure 1), which can be added to the list of special stains being run by the facility.

**Immunohistochemistry (IHC)**

A third Bondmax was purchased last year for antibody work-up and has contributed to an increase in the number of available antibodies for routine staining (170), and to a 51% increase in the number of slides stained by the facility this year to 20,000. We are currently undertaking a project in collaboration with the Caldas laboratory to assist in the profiling of 150 antibodies across 3,000 breast cancers in tissue microarrays. Dual fluorescence has also been worked up on the system (Figure 2).

**In situ hybridisation (ISH)**

Our gold standard ISH protocol utilises S\textsuperscript{35}-labelled riboprobes and we offer fluorescence ISH (FISH) for the Y chromosome and HER-2 CISH as part of the routine service. In collaboration with the Caldas laboratory, we have worked up a micro-RNA ISH protocol that is working well for high-medium abundance targets. Our aim in the next year is to apply this in a reconditioned Bondmax system that has been purchased for this purpose.

**Digitisation and analysis**

The Zeiss Mirax system, purchased last year, has increased the facility’s capacity for digitising brightfield and fluorescence slides, raising our scanning output by 39% to 30,000 slides. We have continued to assist users in maximising the use of the analyses that can be carried out (Bolton et al., *Cancer Epidemiol Biomarkers Prev* 2010; 19: 992) and have been involved in a major collaboration with the Breast Cancer Association Consortium (Sherman et al., *Cancer Epidemiol Biomarkers Prev* 2010; 19: 966). The numbers of slides being captured for image analysis has steadily increased and we hope to grow this further.

**Publications listed on page 75**
The Light Microscopy Facility provides the CRI with state-of-the-art light microscopy and develops new imaging modes.

The facility specialises in the following areas: advanced live-cell imaging using wide-field and spinning disc imaging systems; high-quality confocal scanning light microscopy; non-linear imaging techniques such as multi-photon, second harmonic, fluorescence life-time imaging (FLIM); live imaging at high resolution; quantitative high throughput image acquisition and analysis (e.g. iCys imaging cytometer).

We are also constantly monitoring new developments in imaging techniques, and are actively engaged in developing new applications for cancer research with new instrumentation design. Our further scientific aim is to obtain cancer-relevant comparative imaging results in collaboration with colleagues within the CRI, with newly developed imaging modalities, such as FLIM, non-linear imaging and Raman microscopy, which is John Harris’ expertise. He worked on laser development and new imaging instrumentation at the Centre of Biophotonics, Strathclyde, before joining the CRI.

The LaVision TriMScope system has now been equipped with an additional optical parametric oscillator, which provides fs-pulsed MP-excitation ranging from 690nm to 1600nm. The TriMScope is a very sensitive and rapid multi-photon scanning system, which scans the specimen with 64 beams simultaneously. The TriMScope is used mainly for live applications, which Lorraine Berry has developed with CRI research groups.

The CompuCyte iCys research imaging cytometer has become a very popular research tool in the CRI, providing quantitative high-throughput image analysis. Heather Zecchini works on this with CRI researchers. Current applications include ligand uptake in prostate cancer, apoptosis, death receptor involvement in human smooth muscle cells, tumour vasculature and drug distribution and ligand uptake and DNA damage in cancer cells.

Research and Development
Current projects include the following: (1) Second harmonic imaging based on a scattered signal, e.g. to demonstrate the formation of vessels from endothelial cells as well as the extracellular matrix in tumours, and cell behaviour in collagen matrices. (2) CARS imaging is being combined with fluorescence imaging in cancer drug up-take studies in collaboration with Sumeet Mahajan at the Cambridge Centre for the Physics of Medicine. (3) Large-area high-efficiency macro imaging using the prototype Mesolens, developed by Brad Amos (MRC Laboratory for Molecular Biology) is being explored by Stefanie Reichelt with the aim of recording subcellular detail throughout 6mm-long mouse embryos and has already been used (in collaboration with Scott Lyons, CRI) to follow individual bioluminescence-labelled tumour cells.

(1) 3D volume measurement of the extracellular matrix collagen-vessel relationship in a mouse tumour. Collagen (SHG, blue), vessels (FITC, green), Lectin (mStrawberry, red) (images: L Berry, samples: W English, Murphy lab).

Multi-photon images of mouse tissues: Ear (2), Kidney (3) expressing a GFP-nuclear marker and Td-tomato membrane marker (images: L Berry, samples: N Berry, MRC-LMB).

(4) Multi-photon image of mouse epidermis (whole mount), two sebaceous glands associated with the hair follicle (image: J Harris, samples: G Donati, Watt lab).

*joined in 2010 †left in 2010
Pharmacokinetics (PK) is the study of what the body does to drugs. It is the mathematical study and description of the absorption, distribution, metabolism and excretion processes used by the body when a drug is administered. In order to obtain good PK data, bioanalysis forms an integral part of the science and to facilitate this we have two liquid chromatography-mass spectrometry systems (LC-MS/MS) within the facility (Figure 1). These state-of-the-art systems enable us to detect very low levels of drugs in a variety of biological matrices such as blood, plasma, tumour and cell cultures. During 2010 we developed and validated several bioanalytical methods to support both research and clinical studies. This included a validated assay to simultaneously measure capecitabine and three of its metabolites in a single sample of human plasma. Other assays include those for gemcitabine, paclitaxel, retinoic acid and azido sugars.

During 2010 we have evaluated the use of dried blood spots (DBS) as a sampling technique for bioanalysis. Initial results are very promising and we will be developing this further in 2011. This leading edge bioanalytical technology uses small volumes of blood for sampling (typically around 30 µL). This can have a positive impact on the refinement and reduction of in vivo studies with the potential to obtain high quality PK data from efficacy studies. Michael Williams presented a poster at the European Bioanalytical Forum in Barcelona of our findings on using this technique to stabilise the degradation of pro-drugs in whole blood.

Pharmacodynamics (PD) is the study of what the drug does to the body (i.e. its effect). By relating PD effects to PK parameters, the PK/PD relationship can be determined. To this end, a variety of PD assays (e.g. biomarker assays) were established to support several clinical trials. We are looking to expand our portfolio for 2011 including evaluating the use of DBS in this field.

As we are working with clinical samples the facility will be compliant to the MHRA guidelines entitled ‘GCP in the Clinical Laboratory’.

In addition to the analysis of PK samples we can also offer advice on the design of PK and efficacy studies.
Optical
IVIS 200 and IVIS lumina imaging systems (Caliper Lifesciences) are available for whole-animal in vivo photonic imaging. These can perform sensitive and relatively high-throughput in vivo bioluminescence imaging. Typical scans take less than one minute and up to five subjects can be imaged at a time. Fluorescence imaging can also be performed in vivo, although this approach is less sensitive.

MRI
The unit has two Varian MRI systems; a 9.4T with higher sensitivity, and a 7T whose smaller susceptibility effects make it more suitable for techniques such as echo-planar imaging. Both perform 1H MRI and multi-nuclear MRS, assisted by integrated monitoring, gating heating and anaesthesia. We have produced DCE-MRI data for vascular characterisation of tumour models, notably autochthonous pancreatic tumours (Tuveson laboratory). We have implemented improved 1H MRS methods that minimise chemical shift artefacts and we are developing quantitative MT-MRI and motion-insensitive DW-MRI methods for abdominal tumours, which are subject to respiratory and cardiac motion. The unit is currently being refitted to support in vivo hyperpolarised 13C spectroscopy, using a Hypersense system.

Radiotracer methods
A NanoPET/SPECT/CT (Mediso/Bioscan/Philips) system for multimodality radionuclide imaging was installed in 2010. This offers the greatest sensitivity of any in vivo imaging modality and can provide non-invasive assessment of pharmacological (target tissue exposure, target engagement and functional activity) and biological processes (blood flow, perfusion and metabolism). These scanners can resolve at nanolitre resolution (~0.4mm for SPECT and ~1mm for PET) and so are ideal for small animal imaging.

Static and dynamic imaging can be implemented, with or without respiratory/cardiac gating. We can label biologically active molecules with positron emitters and perform kinetic modelling. A PET/CT/SPECT/MRI compatible animal bed system delivers gaseous anaesthesia, maintains body temperature and allows precise co-registration across imaging modalities. Simultaneous dual isotope studies are possible using nanoSPECT and we are investigating multimodality imaging approaches for the integrated molecular imaging of cancer.

Installation of a 68Ga generator will take place in 2011. We are establishing a laboratory for radiolabelling with 68Ga and SPECT radionuclides in collaboration with the radiopharmacy and PET/CT facility at Addenbrooke’s Hospital.

Molecular imaging probes currently available for PET include [18F]FDG, [18F]FLT, [18F]FMISO. Preliminary collaborative projects with CRI laboratories will focus on novel molecular marker development and use of PET and SPECT to measure early response of tumours to therapy. The Brindle laboratory are investigating the C2A domain of synaptotagmin, labelled with 111In for SPECT and 64Cu for PET, as a novel probe for detection of tumour cell apoptosis post treatment and the use of [11C]acetate for early detection of malignant transformation. The Tuveson laboratory plan to compare the sensitivity of FDG and FLT to measure pancreatic tumour responses to novel therapeutic protocols.

Metabolomics
The facility is based on a Bruker 600MHz NMR instrument. Ongoing collaborative projects include studies on cellular senescence (Narita laboratory), correlations of epigenetic markers with metabolomics (Murrell laboratory), monitoring response to treatment (Neal laboratory) and 19F NMR of anticancer drug metabolites (Tuveson laboratory). Metabolite correlation methods are being developed, with the Tavaré laboratory, to help interpret the biochemical data.

Pre-clinical imaging is a collaborative facility that manages a wide range of imaging machines for the CRI.
The Proteomics core facility focuses on the systematic study of proteins, particularly their structures, interactions and expression levels. The facility is equipped with state-of-the-art instrumentation for CRI researchers requiring access to proteomics technology and expertise.

Proteomics applications can help obtain a better understanding of the processes that contribute to the development of cancer. The facility has been designed to apply the latest proteomic methods to key areas of cancer research: the definition and comparison of protein profiles in normal and pathological samples, the identification of disease mechanisms at a molecular (protein) level, the identification of temporal patterns of expression, and to help define the function of uncharacterised proteins.

The Proteomics core facility provides help in designing experimental strategies and implements and validates previously developed proteomic workflows to profile proteins from diverse biological samples. We also aim to modify or develop entirely new methods and assays when warranted. In addition, we have bioinformatics support for data management and analysis as well as software development.

The facility has already been well equipped with state-of-the-art analytical instrumentation for proteomic studies, including the latest orbitrap mass spectrometer, the LTQ Velos Orbitrap (Thermo), which has been configured to a Dionex Ultimate 3000 RSLC nanoHPLC system (Figure 1). In addition an Agilent 6520 QToF with ChIP cube technology was installed in 2010. The mass spectrometers are supported by off-line chromatography platforms: two Dionex Ultimate 3000 capHPLC systems for multidimensional chromatography at the protein and peptide level. These are supported by 1D and 2D gel electrophoresis systems as well as a GE Healthcare Typhoon Trio+ imager available in the equipment park run by Jane Gray. Data analysis is supported by an array of bioinformatics and statistical analysis tools.

Specific methods and areas of interest include:

- Protein profiling of complex biological samples, e.g. serum, tissue, cell extracts
  - Profiling by nanoLC/MS
  - Multidimensional protein/peptide fractionation by capLC and/or gelLC.

- Targeted protein identification by nanoLC/MS/MS
  - Coomassie and silver stained gel bands of purified proteins
  - In solution digestion of purified proteins.

- Identification of protein and peptide modifications
  - Phosphorylation sites
  - Protein modifications such as acetylation and methylation
  - Coomassie stain only, purified proteins.

- Relative quantitation by nanoLC/MS/MS
  - SILAC - stable isotope labeling of amino acids in cell culture
  - ITraq - an isobaric peptide tagging system.
Laser scanning cytometer mosaic image (x60 objective) of a pancreatic tumour section labelled with the following to show areas of hypoxia within the tumour and surrounding tissue: Blue – DAPI nuclear counterstain; Green – hypoxyprobe, indicating hypoxic areas; Red – lectin-647, showing blood vessels. Image taken by Heather Zecchini (Light Microscopy), sample provided by Michael Jacobetz (Tuveson laboratory).
Shankar Balasubramanian (page 10)
Chemical Biology of Nucleic Acids Laboratory
Primary research papers
Fegan A, Shirude PS, Ying L, Balasubramanian S. Ensemble and single molecule FRET analysis of the structure and unfolding kinetics of the c-kit promoter quadruplexes. Chem Commun (Camb) 2010; 46: 946-8

James Brenton (page 12)
Functional Genomics of Ovarian Cancer Laboratory
Primary Research Papers
magnetic resonance imaging: feasibility in metastatic ovarian cancer at 3 Tesla. *Eur Radiol* 2010; 20: 491-6
Shearman JW, Myers RM, Beale TM, Brenton JD, Ley SV. Total syntheses of the bromotyrosine-derived natural products ianthelline, 5-bromoverogamine and jBIR-44. *Tetrahedron Lett* 2010; 51: 4812-4

Kevin Brindle (page 14)

Molecular Imaging of Cancer Laboratory

Primary research papers


Other publications


Witney TH, Brindle KM. Imaging tumour cell metabolism using hyperpolarized 13C magnetic resonance spectroscopy. *Biochim Soc Trans* 2010; 38: 1220-4

Carlos Caldas (page 16)

Breast Cancer Functional Genomics Laboratory

Primary research papers


Other publications


Swanton C, Caldas C. From genomic landscapes to personalized cancer management—is there a roadmap? Ann NY Acad Sci 2010; 1210: 34-44


Jason Carroll (page 18)
Nuclear Receptor Transcription Laboratory
Primary research papers


Other publications


Fanni Gergely (page 20)
Centrosomes, Microtubules and Cancer Laboratory
Primary research papers


Nicholas AK, Khurshid M, Desir J, Carvalho OP, Cox JJ, et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. Nat Genet 2010; 42: 1010-4

John Griffiths (page 22)
Magnetic Resonance Imaging and Spectroscopy (MRI and MRS) Laboratory
Primary research papers


Other publications
Griffiths J. Editorial. NMR Biomed 2010; 23: 1
Duncan Jodrell (page 24)
Pharmacology and Drug Development Group
Primary research papers
Florian Markowetz (page 26)
Computational Biology Laboratory
Primary research papers
Other publications
Gillian Murphy (page 28)
Proteases and the Tumour Microenvironment Laboratory
Primary research papers
Grossman M, Tworowski D, Dym O, Lee MH,
Roghi C, Jones L, Gratian M, English WR, Murphy G. Golgi reassembly stacking protein 55 interacts with membrane-type (MT) 1-matrix metalloproteinase (MMP) and furin and plays a role in the activation of the MTI-MMP zymogen. FEBS J 2010; 277: 3158-75
Other publications
Lawson MH, Cummings NM, Russell R, Morjaria JB, et al. Tissue banking of diagnostic lung cancer biopsies for extraction of high quality RNA.

Adèle Murrell (page 30)
Epigenetics and Imprinting Laboratory
Primary research papers
Other publications
Huddleston J, Woodfine K, Murrell A. Role of antisense transcripts at the DIRAS3 locus. Genetics Res 2010; 92: 76

Masashi Narita (page 32)
Mechanisms of Cellular Senescence Laboratory
Primary research papers
Other publications
Narita M. Quality and quantity control of proteins in senescence. Aging (Albany NY) 2010; 2: 311-4

David Neal (page 34)
Prostate Research Laboratory
Primary research papers
Gudmundsson J, Besenbacher S, Sulem P,


Yoshimatsu M, Toyokawa G, Hayami S, Unoki M, Tsunoda T, et al. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. *Int J Cancer* 2010; Epub 5 Apr.

Yoshimatsu M, Toyokawa G, Hayami S, Unoki M, Tsunoda T, et al. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. *Int J Cancer* 2010; Epub 5 Apr.

Other publications


Neal DE. PSA testing for prostate cancer improves survival-but can we do better? *Lancet Oncol* 2010; 11: 702-3.

Parry M, Elliott G, Abo R, Camp NJ, Neal DE,
Sharma N, Papadopoulos A, Shah NC, Neal DE. First 500 cases of robotic-assisted laparoscopic prostatectomy from a single UK centre: Learning curves of two surgeons. BJU Int 2010; 106: 4
Sharma N, Scott HE, Mills IG, Massie CE, Neal DE. GA-binding protein alpha: an ETS family member with an important role in prostate cancer. BJU Int 2010; 106: 53
Whitaker HC, Neal DE. RAS pathways in prostate cancer - mediators of hormone resistance? Curr Cancer Drug Targets 2010; Epub 19 Aug

Duncan Odom (page 36)
Regulatory Systems Biology Laboratory
Primary research papers
Other publications

Bruce Ponder (page 38)
Genetic Susceptibility to Cancer Laboratory
Primary research papers
Hamamoto R, Cho HS, Suzuki T, Dohmae N, Hayami S, et al. Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. Cancer Res 2010; Epub 7 Dec
Yoshimatsu M, Toyokawa G, Hayami S, Unoki M, Tsunoda T, et al. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. Int J Cancer 2010; Epub 5 Apr
Other publications
Ponder B. Polycyclic aromatic hydrocarbon exposure in oesophageal tissue and risk of
oesophageal squamous cell carcinoma in north-eastern Iran. Arch Iran Med 2010; 13: 457-8
Ponder BAJ. Genome-wide approaches to risk: Lessons from breast and prostate. J Thorac Oncol 2010; 5: S216

Nitzan Rosenfeld (page 40)
Molecular and Computational Diagnostics Laboratory
Primary research papers

John Stingl (page 42)
Mammary Stem Cell Biology Laboratory
Primary research papers
Other publications
John Stingl (page 42)
Mammary Stem Cell Biology Laboratory
Primary research papers
Other publications

Simon Tavare (page 44)
Statistics and Computational Biology Laboratory
Primary research papers
Other publications
University Press.

David Tuveson (page 46)
Tumour Modelling and Experimental Medicine Laboratory
Primary research papers

Fiona Watt (page 48)
Epithelial Cell Biology Laboratory
Primary research papers
Ambler CA, Watt FM. Adult epidermal Notch activity induces dermal accumulation of T cells and neural crest derivatives through upregulation of Jagged 1. Development 2010; 137: 3569-79
Jensen KB, Driskell RR, Watt FM. Assaying proliferation and differentiation capacity of stem cells using disaggregated adult mouse epidermis. Nat Protoc 2010; 5: 898-911
Other publications

Douglas Winton (page 50)
Cancer and Intestinal Stem Cells Laboratory
Primary research papers
McCutchion SC, Jones K, Cumming SA, Kemp R,

Bioinformatics (page 54)
Matthew Eldridge

Primary research papers

Genomics (page 59)
James Hadfield

Primary research papers

Histopathology and In Situ Hybridisation (page 60)
Will Howat

Primary research papers
Chowdhury F, Howat WJ, Phillips GJ, Lackie PM. Interactions between endothelial cells and epithelial cells in a combined cell model of airway mucosa: effects on tight junction permeability. Exp Lung Res 2010; 36: 11-1
External Funding
The Cambridge Research Institute gratefully acknowledges those organisations that have provided support during the period of this report to the individuals and laboratories listed.

Academy of Medical Sciences
Christine Parkinson (Brenton laboratory)

Addenbrooke’s Charitable Trust
Briit Basu (Murphy laboratory)
Tom Booth (Brindle laboratory)
Christine Parkinson (Brenton laboratory)

Association for International Cancer Research
Kathryn Woodfine (Murrell laboratory)

Biotechnology and Biological Sciences Research Council (BBSRC)
Hélène Bon (Neal laboratory)
Holly Canuto (Brindle laboratory)
Audrey Fu (Tavaré laboratory)

BBSRC/CASE
Tim Witney (Brindle laboratory)

Boehringer Ingelheim Fonds
Florian Karreth (Tuveson laboratory)

Breast Cancer Campaign
Kelly Holmes (Carroll laboratory)
Michael Prater (Stingl laboratory)

Breast Cancer Research Foundation
Ana-Teresa Maia (Ponder laboratory)

Brian Cross Memorial Trust
Nicola Ainsworth (Griffiths laboratory)

British Heart Foundation
Anne Leclerq (Murphy laboratory)

Caja Madrid Foundation
Pedro Perez Mancera (Tuveson laboratory)

Cambridge Cancer Research Fund
Neal laboratory

Cambridge Commonwealth Trust and Overseas Research Studentship
Charlotte Ng (Brenton laboratory)

Cambridge Overseas Trust, TNK/BP Kapitza Cambridge Scholarship
Sergii Ivakhno (Tavaré laboratory)

Caring for Carcinoid Foundation
Tuveson laboratory

Commonwealth Scholarship and Fellowships Plan
Sarah-Jane Dawson (Caldas laboratory)
Caryn Ross-Innes (Carroll and Odom laboratories)

Deutscher Akademischer Austausch Dienst (DAAD)
Albrecht Neese (Tuveson laboratory)
Alexander Kuznetsov (Watt Laboratory)

EC FP VII (Health)
Caldas laboratory
Watt laboratory

EC Marie Curie Initial Training Network
Joana Borlido (Neal laboratory)
Esther Hoste (Watt laboratory)
Bianca Schmitt (Odom laboratory)
Christine Weber (Watt laboratory)

EC Marie Curie International Re-integration Grant
Klara Stefflova (Odom laboratory)

EC Marie Curie Intra-European Fellowship
Juan Boren (Brindle laboratory)
Alejandra Bruna (Caldas laboratory)
Christine Feig (Tuveson laboratory)
Klaas Mulder (Watt laboratory)
Tiago Rodrigues (Brindle laboratory)

Engineering and Physical Sciences Research Council (EPSRC)
Doug Speed (Tavaré laboratory)
Brenton laboratory

EU Seventh Framework Programme (FP7)
Helen Ross-Adams (Neal laboratory)

EUREKA EU
Light Microscopy (with the Universities of Utrecht and Heidelberg, the MRC Laboratory of Molecular Biology and Nikon Europe)

European Molecular Biology Organisation (EMBO)
Carroll laboratory
Odom laboratory
European Research Council (ERC)
Carroll laboratory
Odom laboratory

European Science Foundation (ESF)
Markewetz laboratory

Experimental Cancer Medicine Centre
Bin Liu (Caldas laboratory)

Federation of European Biochemical Sciences
Sara Cipolat (Watt laboratory)

Foundation for Science and Technology (Portugal)
Pedro Correa de Sampaio (Murphy laboratory)
Jose Sandoval (Caldas laboratory)

Gates Foundation
Nathan Benaich (Watt laboratory)
Timothy Humpton (Tuveson laboratory)
Thornton Thompson (Winton laboratory)

Girton College
Sandra Fulton (Brindle laboratory)

GlaxoSmithKline
Brindle laboratory
Jodrell laboratory
Tuveson laboratory

Hales Clinical Fellowship
Carles Escriu (Gergely laboratory)
Lucy Gossage (Jodrell laboratory)

Human Frontier Science Program (HFSP)
Narita laboratory

Institut National du Cancer/DoH
Anthea Messent (Murphy laboratory)

Irish Research Council for Science, Engineering and Technology
Aisling Redmond (Carroll laboratory)

Italian Association for Cancer Research
Daniele Perna (Tuveson laboratory)

Japan Foundation for the Promotion of Science
Ken Natsuga (Watt Laboratory)

KWF Dutch Royal Fellowship
Wilbert Zwart (Carroll laboratory)

Medical Research Council (MRC)
Tom Booth (Brindle laboratory)
Jonathan Cairns (Tavaré laboratory)
Valerie Curry (Murphy laboratory)
Helen Gillingham (Murphy laboratory)
Stephen Goldie (Watt laboratory)
Elizabeth Shedden (Murphy laboratory)
Caldas laboratory
Neal laboratory

Merck & Co., Inc.
Griffiths laboratory
Tuveson laboratory

Microsoft Research
Brenton laboratory

National Institute for Health Research
Alastair Lamb (Neal laboratory)
Christine Parkinson (Brenton laboratory)
Naomi Sharma (Neal laboratory)
Maxine Tran (Neal laboratory)
Bruce Ponder (Ponder laboratory)
Neal laboratory

National Institutes of Health, USA (NIH)
Leanne Bell (Griffiths laboratory)
John Connelly (Watt laboratory)
Ryan Fiehler (Watt Laboratory)
Charlotte Hodgkin (Griffiths laboratory)
Griffiths laboratory
Tuveson laboratory

Nuffield Foundation
Gergely laboratory

Pancreatic Cancer UK
Shivan Sivakumar (Tuveson laboratory)

Prostate Cancer Charity
Neal laboratory

Prostate UK
Ajoeb Baridi (Stingl and Neal laboratories)
Neal laboratory

Royal Society University Research Fellow
Fanni Gergely

Schultheiss-Reiser Foundation
Sven Quist (Watt laboratory)

Science and Technology Facilities Council
Brenton laboratory

Swiss National Science Foundation
Claudia Kutter (Odom laboratory)

The Leukemia and Lymphoma Society, USA
Piotr Dzien (Brindle laboratory)

Uehara Memorial Foundation
Hiro Fujiwara (Watt laboratory)
Mahito Sadaie (Narita laboratory)

University of Cambridge
Alexandra Jauhiainen (Tavaré laboratory)

Yousef Jameel Scholarship
Sui Seng Tee (Brindle laboratory)
Seminars and Conferences

CRI Seminars in Cancer
The following speakers gave talks as part of the CRI Seminars in Cancer series:

January
Douglas Fearon, Wellcome Trust Immunology Unit, Cambridge
A tumoral stromal cell that mediates immune suppression

Katarina Wolf, Department of Cell Biology, Nijmegen Centre for Molecular Life Sciences
Physical limits of cell migration

February
Axel Behrens, Cancer Research UK London Research Institute, Lincoln’s Inn Fields Laboratories
JNK signalling in stem cells and cancer

April
Sara Zanivan, Department of Proteomics and Signal Transduction, Max-Planck Institute for Biochemistry
In vivo quantitative proteomics to study skin cancer progression

May
Michael Boutros, Division of Signalling and Functional Genomics, German Cancer Research Centre
Mapping synthetic genetic interaction networks by high-throughput RNAi

June
Stephen Baylin, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore
View of the cancer epigenome from biological and translational perspectives

September
Eran Segal, Weizmann Institute of Science, Israel
Transcriptional Lego: Predictable control of gene expression by manipulating promoter building blocks

October
Mitch Dowsett, Institute of Cancer Research, Royal Marsden Hospital
Biological lessons from presurgical studies of breast cancer

November
Michael Karin, University of California, San Diego
Inflammation, metabolism, ageing and cancer: Dangerous Liaisons

Other CRI Seminar series
A range of seminar series run within the Institute and in the University of Cambridge Department of Oncology, covering different aspects of cancer research:

Lectures in Cancer Biology
This lecture series is compulsory for all first year graduate students and acts as an introductory course on oncology, covering all aspects of cancer from basic cell biology to clinical applications (weekly).

Cambridge Oncology Seminars
These seminars are the main departmental oncology seminars and cover basic, clinical and translational aspects of oncology, and feature internal and external speakers (weekly).

Institute Lunchtime Seminars
This series is an opportunity for graduate students, postdoctoral scientists and other scientific staff to present their research to the Institute (weekly).

Group Leader Chalk Talks
For group leaders only, this is an opportunity for them to meet and discuss ideas and present research (weekly).

Computational Biology and Genomics Seminar Series
In this series speakers discuss computational approaches to cancer research and genomics (fortnightly).

Conferences
Cambridge Cancer Centre Annual Symposium
25 June

Attended by over 250 delegates, the meeting featured a series of diverse talks from invited speakers and keynote lecture from Mariano Barbacid, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid. See page 80 for more information.
CRI Annual Symposium
19–20 March

The third CRI symposium on ‘Unanswered Questions in Tumour Monitoring’ took place on 19 and 20 March 2010 at the CRI. The scientific organising committee of Kevin Brindle, Rebecca Fitzgerald (CRI Adjunct Faculty), John Griffiths and Florian Markowetz put together a programme that was designed to facilitate discussion and tackle unanswered questions in each of the four sessions:

1. The translation barrier; problems and solutions in moving from animal models to clinical trials.
   Chair: Harvey Herschman, University of California, Los Angeles.
   Speakers: Sanjiv Gambhir, Stanford University; Antoni Ribas, University of California, Los Angeles; Wolfgang Weber, University Medical Center Freiburg; Vasilis Ntziachristos, German Research Centre for Environmental Health; Nicholas Hughes, Stanford University (selected talk from submitted abstracts).

2. The potential clinical use of MRS in oncology.
   Chair: Sarah Nelson, UCSF Helen Diller Family Comprehensive Cancer Center.
   Speakers: Patrick Bolan, University of Minnesota; Ferdia Gallagher, Cambridge Research Institute; Ingrid Gribbestad, Norwegian University of Science and Technology; Arend Heerschap, Radboud University Nijmegen Medical Center; Rui Simões, Universitat Autònoma de Barcelona (selected talk from submitted abstracts).

3. Detecting genomic biomarkers from high-dimensional clinical data.
   Chair: James Brenton, Cambridge Research Institute.
   Speakers: Nicholas Dracopoli, Johnson & Johnson; Joseph Lucas, Duke Institute for Genome Sciences & Policy; Jill Mesirov, Broad Institute; Rainer Spang, University of Regensburg; Christina Curtis, Cambridge Research Institute (selected talk from submitted abstracts).

4. Opportunities of preneoplasia for a pre-emptive strike.
   Chair: Rebecca Fitzgerald, MRC Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge.
   Speakers: Carlo Maley, The Wistar Institute; Sanford Markowitz, Case Western Reserve University; Nicholas Wright, Barts and the London School of Medicine and Dentistry and the Cancer Research UK London Research Institute; Senthil Muthuswamy, Cold Spring Harbor Laboratory and Ontario Cancer Institute; Frank McCaughan, MRC Laboratory of Molecular Biology (selected talk from submitted abstracts).

Each session was followed by a 45 minute panel discussion, led by the chair; which led to many lively debates and fruitful discussions. Delegates also had the opportunity to present posters. The Symposium concluded with a dinner held in Jesus College.

CRI Retreat
23–24 September

This year’s retreat was held at the Institute and staff enjoyed two days of talks and collaborative discussions. For the first time we had two sessions of talks for non-scientists, where PhD students and postdocs gave short talks on their work to an audience of the CRI’s non-research staff. These were very popular and well attended, and will be held again in the future.

There were four poster sessions, with a prize awarded for each session. Toni Hurtado (Carroll lab) won session 1, Tamir Chandra (Narita lab) won session 2, James Jones (Fearon lab) won session 3, and Stewart MacArthur; Sarah Vowler and Suraj Menon (Bioinformatics core) won session 4.

The team building exercises – building a hat on the theme of ‘a day at the zoo’ with provided materials and writing a story using a set of words pulled out of a hat – led to some very imaginative and alternative creations from staff.
The CCC was re-launched in February by Cancer Research UK and in its new form – it is a partnership between the Charity, the University of Cambridge, Cambridge University Hospitals NHS Foundation Trust and the Medical Research Council. The vision of the CCC is to build strong links across disciplines from the laboratory to the clinic.

The annual symposium took place in June and was attended by around 260 participants from University departments, institutes, biotech companies and journal editorial offices in Cambridge. The keynote lecture on ‘Targeting Ras oncogenes in cancer’ was given by Mariano Barbacid from the CNIO, Madrid. The invited talks were given by Cambridge-based researchers to highlight current research and interdisciplinary collaborations, the speakers were: David Tuveson (CRI), Colin Watts (Cambridge Centre for Brain Repair), James Brenton (CRI), David Adams (Wellcome Trust Sanger Institute), Jason Carroll (CRI) and Shankar Balasubramanian (Department of Chemistry and CRI). Awardees of CCC pump-priming project grants in 2008 and 2009 also presented the results of their projects, the speakers were: Athene Donald (Cavendish Laboratory), Ashok Venkitaraman (MRC Cancer Cell Unit) and Laura Itzhaki (MRC Cancer Cell Unit).

The second meeting for postdoctoral staff took place in October and was very well attended by around 100 postdoctoral staff and final year PhD students from University departments and research institutes across Cambridge. The day consisted of talks, a poster session, a series of workshops, a speed collaboration activity and the keynote lecture. The keynote lecture on ‘Natural history of childhood acute lymphoblastic leukaemia’ was given by Mel Greaves from the Institute of Cancer Research in Sutton.

The first review of the CCC by its Scientific Advisory Board (SAB) took place in September. The SAB of the CCC are George Demetri (Dana-Farber Cancer Institute), Susan Horwitz (Albert Einstein College of Medicine), Patrick Johnston (Queen’s University, Belfast), Peter Jones (University of Southern California Norris Comprehensive Cancer Center), William Kaelin (Dana-Farber Cancer Institute), Gilbert Lenoir (Institut Gustave-Roussy), Keith Peters (GlaxoSmithKline), John Potter (formerly at the Fred Hutchinson Cancer Research Center), Brian Ross (University of Michigan), Alan Smith (Genzyme), Terry Speed (University of California, Berkeley) and Zena Werb (UCSF Helen Diller Family Comprehensive Cancer Center). The review consisted of brief presentations under the theme ‘personalised cancer medicine’ by members of the CCC followed by a discussion session.

This year Cancer Research UK has set up a network of 16 cancer centres across the UK, which have a vision similar to the Cambridge Cancer Centre (CCC).
Outreach and Fundraising

CRI staff have had a busy year participating in outreach and fundraising activities.

![Prof Fiona Watt speaking to the participants at Race for Life.](image)

The Cambridge Science Festival in March attracted around 35,000 visitors over the two week period. The theme of this year’s Festival was the diversity of science and we had three displays at the Festival on the biology of cancer, cancer care: from laboratory to bedside, and cancer and art.

This year we again hosted the East Regional Final of the Debating Matters competition, which is a national competition organised by the Institute of Ideas for sixth-form students to debate current issues in science and other subjects.

CRI staff regularly give talks at local primary and secondary schools, as well as to groups of Cancer Research UK fundraisers and supporters who visit the Institute. A number of staff were also involved with the new Healthcare Science Programme run by Addenbrooke’s Hospital. The programme gives students an insight into medical and research professions available on the Cambridge Biomedical Campus.

At the official launch of the Cambridge Cancer Centre in February we held an evening of talks, a poster session and laboratory tours for high-profile members of the Cambridge academic community, colleagues, and Cancer Research UK fundraisers and supporters. We held a second open day on the translational and clinical trials research undertaken at the Cambridge Experimental Cancer Medicine Centre, for the public, patients, sixth-form students, nurses, researchers and doctors. The day consisted of five sessions of talks by a researcher, nurse and patient, along with a poster session and a number of demonstrations.

Around 100 CRI staff ran or volunteered at the Cancer Research UK races in Cambridge. Prof Fiona Watt and Dr Hayley Whitaker (Neal laboratory) gave short speeches at the start of the races thanking all the participants for their support and fundraising efforts. The Race for Life took place in Cambridge on Sunday 4 July and saw 8,000 women running or walking through the city centre. A CRI scientist, Esther Rodriguez (Brindle laboratory), sprinted into 1st place in 18 minutes 5 seconds and she was also the first woman to finish the Run 10k race in Cambridge on Sunday 17 October.

CRI staff are active fundraisers for the Charity. A team from CRI took part in the first Cancer Research UK Shine event – a night-time walking marathon – and raised around £2500. A number of CRI staff also took part in the Cambridge Dragon Boat Festival in September, along with 37 teams from companies and Institutes in Cambridge. This was the first year we had a team entered in the event and they did well, but did not bring home the trophy!

![CRI Staff participating at the Cambridge Dragon Boat Festival.](image)
The graduate student body in the Cambridge Research Institute is composed of PhD students, MPhil students and clinical research training fellows. Details of the graduate training programme are co-determined by Cancer Research UK, the Institute, the University Department with which a student is affiliated, and the University of Cambridge. The entire staff of the Institute are committed to making it a great place to work and study and all provide support at all levels to our students.

The Cambridge Experience
There are currently 52 graduate students at the CRI which corresponds to approximately one third of our entire research population. Our graduate students are fully integrated into their research groups where they are expected to make valuable contributions to the success of their groups. Fifteen students commenced study in October 2010 with a further four starting in January 2011, of these, four are clinical research training fellows, and one is a MPhil student. Our student body is highly international – out of 19 new starters, three are from the UK, nine from the EU and the remaining seven from further afield.

Support and Mentoring
Each student has a supervisor who is a group leader and is also assigned a second supervisor who acts as a mentor and provides support. In addition, Ann Kaminski (the Head of Scientific Administration) acts as the first point of contact for any student with a query or difficulty that is not directly related to their scientific work. All student matters in the Institute are overseen by the Studentships and Fellowships Committee, chaired by Fiona Watt. This committee has the well-being of our students at heart, while ensuring that they are fulfilling the requirements of the University of Cambridge for obtaining their degree.

The Graduate Programme
Soon after their arrival, all of our new graduate students join the University graduate intake to attend the compulsory introductory safety courses organised by the University, followed by an Institute specific safety course and induction. All students and group leaders are invited to attend a reception in the Institute, where the students were welcomed this year by the Institute’s director of operations, John Wells, and other members of the administration team. This is their first opportunity to meet some of the staff who will help them over the years to come.
All first year graduate students are required to attend a series of around 30 lectures in cancer biology, which are organised by the Department of Oncology. These lectures are given by specialists in their fields and the aim is to provide the students with a comprehensive overview of cancer biology, ranging from basic cell biology through to cancer diagnosis and treatment. This excellent and unique resource is available to all members of the University and is widely attended. The students are also briefed by the core facilities managers to learn of the services available to them. They also attend courses specific to the demands of their projects.

After two months in the Institute all first year students give 15 minute talks to all members of the Institute to explain the nature of the projects. In accordance with University regulations, all graduate students studying Biological Sciences in Cambridge are not at first registered for PhD studies and must qualify for registration by successfully completing a first year report followed by a viva. Two examiners assess a student’s report and then write a report on their progress over the past year.

Our second and third year PhD students give research talks as part of the Institute Lunchtime Seminar series, attended by all Institute staff. In addition, students complete a written report towards the end of their second year which summarises their work to date and also forms the basis for discussions regarding further work. Our graduate students all follow the three year graduate programme supported by the University of Cambridge; a further year is available if necessary to complete their thesis, which must be submitted within four years.

Like their colleagues in London, our students are encouraged to attend numerous courses planned to hone their transferable skills. These courses range from advice on how to make scientific posters to the Cancer Research UK-organised Graduate Students Public Engagement with Science and Technology (GRADPEST) course.

The Graduate Society
The graduate students have organised themselves into a very active society which organises monthly journal clubs and a wide variety of social events including movie nights, punting and the occasional wine-tasting. The students also arrange meetings with visiting speakers and have a Christmas dinner with an invited speaker — this year’s speaker was Professor Sir Tim Hunt from the Cancer Research UK London Research Institute. The society has also introduced a highly effective mentoring scheme in which all first year students have two mentors located in different parts of the building. This provides new students with recognisable friendly faces in other labs and also helps them to settle in much quicker.

Our second and third year PhD students give research talks as part of the Institute Lunchtime Seminar series, attended by all Institute staff. In addition, students complete a written report towards the end of their second year which summarises their work to date and also forms the basis for discussions regarding further work. Our graduate students all follow the three year graduate programme supported by the University of Cambridge; a further year is available if necessary to complete their thesis, which must be submitted within four years.

Like their colleagues in London, our students are encouraged to attend numerous courses planned to hone their transferable skills. These courses range from advice on how to make scientific posters to the Cancer Research UK-organised Graduate Students Public Engagement with Science and Technology (GRADPEST) course.

The Graduate Society
The graduate students have organised themselves into a very active society which organises monthly journal clubs and a wide variety of social events including movie nights, punting and the occasional wine-tasting. The students also arrange meetings with visiting speakers and have a Christmas dinner with an invited speaker — this year’s speaker was Professor Sir Tim Hunt from the Cancer Research UK London Research Institute. The society has also introduced a highly effective mentoring scheme in which all first year students have two mentors located in different parts of the building. This provides new students with recognisable friendly faces in other labs and also helps them to settle in much quicker.
Institute Administration

The administration team facilitates the smooth running of the Institute by providing infrastructure and support to the Director.

The team provides administrative support to group leaders and supports research activities through management of the laboratories and core facilities. The team also coordinates graduate student administration and laboratory finance.

In addition to laboratory management each of the group leaders has administrative support provided by one of the dedicated research administrators.

Scientific Administration
Graduate student and summer student administration is overseen by Ann Kaminski (page 82). The team organises quinquennial reviews and mid-term reviews for the research groups, and reviews for the core facilities.

With the formal opening of the Cancer Research UK Cambridge Cancer Centre (CCC) on 3 February, Katrien Van Look has started in a new post as Cambridge Cancer Centre and Outreach Coordinator. Katrien supports administration of the CCC, organises CCC symposia and meetings, co-ordinates grants and budgeting processes, manages external communications, and co-ordinates CCC and CRI outreach activities. More details on the CCC’s activities are available on page 80, outreach on page 81.

Julie Bailey has taken up the new role of Scientific Communications Administrator, and is primarily responsible for developing the CRI symposium and events such as the retreat. The scientific administration team is also responsible for the running of symposia, seminars, chalk talks and committees that take place in the Institute, including providing full audio visual cover. We produce the Institute’s publications including the annual report, the CRI newsletter, leaflets and posters for fundraising and outreach activities, write for and edit the intranet, and provide content for the internet.

The team is also responsible for internal and external communications, coordination with the Cancer Research UK press office and is involved in the organisation of fundraising visits. We have also been heavily involved in the co-ordination of volunteering efforts at Cancer Research UK fundraising events this year. CRI staff took part in the Cambridge Race for Life and Run 10k, for more details see page 81.

Human Resources
Human Resources (HR) work in partnership with the Institute to provide support and guidance in the areas of recruitment, personal and team development, pay and grading, employment law and staff wellbeing. Frequent interactions between the HR Managers of the five Cancer Research UK core funded Institutes allow the Institutes to remain aligned in their approaches to HR. The Institute has a mixed economy of staff from Cancer Research UK and the University of Cambridge so collaboration is essential to provide a seamless employment experience, allowing the Institute to focus on research.

This year has seen the Institute continue to move from start-up phase to one of growth and maturity. Therefore HR has focused its efforts on development by devising a programme for postdoctoral research fellows that will be launched in 2011. This programme will provide a portfolio of development opportunities to support scientists on their journey to academic independence. With the implementation of the Equality Bill on 1 October 2010, fostering equal opportunity is high on the agenda for the Institute. In collaboration with the University of Cambridge, a lunchtime seminar was held for all members of the Institute to increase awareness and understanding of the responsibility of both the organisation and the individual to continue to make the Institute an environment conducive to high quality research.
As we move into 2011, HR will continue to work with the Institute to foster collaborations with the wider biomedical community, in order to provide an appropriate employment infrastructure that supports a highly skilled and motivated workforce.

Finance

The finance team:
- Help budget holders efficiently manage their budgets.
- Provide financial analysis to CRI management to inform decision-making.
- Assist with the budget and business planning process for the CRI.
- Assist with the acquisition and management of grants.
- Provide a link between the Cancer Research UK finance department and the CRI.
- Help with ad hoc queries and concerns.

Laboratory Management

The Laboratory Management team are part of the Institute administration and continue to provide a vital role in underpinning the Institute’s ever-evolving research activities. Once again this has been a challenging year as the research groups and core facilities have continued to grow, with Ferdia Gallagher and Athena Matakidou joining the CRI as Clinician Scientists, and Shankar Balasubramanian as a group leader. Doug Fearon is joining us in early 2011. In addition, the Imaging Facility has expanded to include PET/SPECT and CT.

We have also continued to develop and support an efficient, highly-serviceable range of centralised shared facilities on the research floors, with increased usage of containment level 2 facilities for research involving human tissue samples. The laboratory support team continue to maintain and extend a cost-effective and efficient centralised system of ordering and stock control for the most widely used lab consumables, and coordinate repairs to the diverse equipment stock.

The team also play a key role in liaising with other CRI and Cancer Research UK departments including Health and Safety, IT, Property Services, Procurement and Finance, and other Cancer Research UK Institutes. The team has also developed close links with Addenbrooke’s Hospital and similar Institutes on the Cambridge Biomedical Campus as well as in the wider Cambridge area. The team also continue to be heavily involved in supporting Fundraising and Supporter Marketing by providing laboratory tours and talks.

Glasswash and Media

The Glasswash and Media service is part of the Laboratory Management team and plays an essential role in supporting the Institute’s research. As well as providing a high quality centralised glass-washing and sterile supplies service, the team produces a range of basic solutions and liquid/solid media that are replenished in the laboratories on a daily basis. The team can also supply more complex solutions and media to order. They also manage the consignment stock of Invitrogen products, which includes tissue culture reagents and the core consumable stocks of tissue culture plastics and other necessary laboratory sundries.

Health and Safety

Maintaining a good standard of health and safety is important in everyday work life. It is vital in helping us to protect employees and visitors from harm. Thus, improving the work environment, employee morale and productivity enables the Institute to continue to undertake cutting-edge scientific research into the causes and treatment of cancer.

In 2010 we recruited a new Health and Safety Advisor: In conjunction with other support staff we developed new safety procedures for contractors working at the Institute. We reviewed and updated general safety guidance, developed a safety scorecard, and conducted a series of safety inspections with a view to improving overall safety performance. We assisted the scientists with the preparation of their risk assessments, and we worked closely with the Pre-Clinical Imaging core facility to finalise the safety arrangements for our new PET/SPECT system.

In 2011 we will be undertaking a review of the emergency arrangements with Institute management, and will continue to assist the scientists in the review of their risk assessments. We will also encourage managers to continue to promote good safety performance within their departments through toolbox talks and safety presentations. We will organise and deliver safety training to meet the needs of the Institute, go on with our inspection program, and develop new safety policies such as the lone working policy.

Our overall aim is to encourage the scientists to take more ownership for health and safety, and thereby create a positive safety culture. We will work with the scientists to develop pragmatic safety solutions that safeguard their welfare, and also ensure regulatory compliance.

Procurement

The CRI procurement team is supported by the Purchasing Operations Team, which is based in London. Procurement helps
departments accomplish their objectives by undertaking a range of activities that achieve value for money. We do this through good procurement practice and increased efficiency whilst mitigating operational, commercial and compliance risk to Cancer Research UK.

- We are working with key suppliers to set up central discounted price agreements, reducing the cost of consumable items.
- We are working with laboratory management teams in London and Cambridge to set up central service contracts.
- We are working across directorates on a number of proposals to implement new ways of buying goods and services into the organisation, enabling further efficiencies through increased automation of buying processes.
- We are playing our part in helping Cancer Research UK meet its environmental targets. We are integrating sustainability into our procurement process and are working with our suppliers to minimise waste and packaging and to support recycling schemes.

**Property Services**
The Property Services team provide three main services: maintenance, facilities and security. The main aim of the whole team is to ensure the smooth, safe and efficient running of Institute operations, in order to support the CRI’s research efforts.

We ensure that the building is clean, well maintained, and secure for staff and visitors. The team provides or administers the following services: electrical maintenance, cleaning, car parking, environmental and climate control systems, post room, recycling, catering, mechanical engineering, security, decorating and repairs, furniture, energy management and carbon reduction, waste removal and disposal, reprographics and photocopying, catering and hospitality, stationery. The team liaises with other Institutes on the Cambridge Biomedical Campus to ensure that the CRI is kept up to date with developments on the site, and contributes to the site’s environmental goals and initiatives.

The CRI hosts a large number of seminars and events for staff and external visitors. Larger events can last for up to three days with 200 visitors. The Facilities team manage the infrastructure and the catering requirements for these meetings. This includes providing partnering, changing the CRI’s communal areas into exhibition spaces through provision of the correct furniture such as poster boards, and helping to ensure that events run smoothly.

Property Services adopt a Total Facilities Management strategy, working towards integrating the provision of all of these services to the Institute. We are ambitious and progressive and we work alongside the end-users of these services to deliver and maintain a world-class facility, whilst reducing our environmental and economic impact wherever possible.

**IT and Scientific Computing**
From April 2010, provision of information technology and scientific computing facilities and expertise at the Cambridge Research Institute has been the responsibility of the new IT and Scientific Computing Department. The new department has nine staff with a wide range of technical and programming skills.

The Institute’s scientists and laboratory equipment use a variety of Mac, Windows PC and Linux desktops and laptops to support their work, with Voice Over IP phone systems and 1Gb network connectivity to labs and offices. All users have access to backed-up networked file systems to store and share their data.

The Institute’s core facilities continue to produce high volumes of data, with the Genomics Illumina sequencers still the major producers; however Light Microscopy and Histopathology are currently also producing several terabytes of data each month. We have upgraded the existing systems and now have more than 250TB of backed-up live storage. With the Institute producing nearly this amount of data each year, we have purchased a disk-based archival system with an initial capacity of 240TB, to hold last year’s data, and the ability to grow year on year to accommodate future growth.

With the increasing focus on data analysis and the development of systems biology research programmes, the Institute’s computing cluster has also been expanded, with 512 cores, over 1TB of memory and a 48TB parallel file system. Further expansion is already scheduled.

During 2010 we delivered: expansions to storage and cluster computing facilities, the new archive, new database servers, a LIMS for tracking of transgenic samples, a front desk visitor registration system and a database for tracking Cancer Research UK and University staff working in the building. The team also took on full responsibility for local management of desktops and software for Institute staff.
The following CRI students submitted theses in 2010:

**PhD**
- **Jean Abraham**, Caldas laboratory
  Pharmacogenetics of breast cancer treatment
- **Israt Alam**, Brindle laboratory
  Imaging tumour cell death using the C2A domain of synaptotagmin-I
- **Esther Arwert**, Watt laboratory
  The contribution of the inflammatory microenvironment to wound-induced epidermal tumour formation
- **Alexis Barr**, Gergely laboratory
  Characterising the function of CDK5RAP2 in the vertebrate centrosome
- **Meredith Caldwell**, Tuveson laboratory
  Cellular fitness in response to oncogenic Kras
- **Gina deNicola**, Tuveson laboratory
  K-Ras oncogene-induced ROS detoxification promotes tumorigenesis
- **Aarthi Gopinathan**, Tuveson laboratory
  Utilizing genetically engineered mouse models of pancreatic cancer: evaluating the role of cathepsin B and the efficacy of farnesyl thiosalicylic acid
- **Daniel Holland**, Caldas laboratory
  ZNF703 is a luminal breast cancer oncogene
- **Jo Huddleston**, Murrell laboratory
  Mechanisms regulating the imprinted tumour suppressor gene DIRAS3 in normal development and cancer
- **Mahesh Iddawela**, Caldas laboratory
  Genome wide copy number and gene expression profiling using archived tissue for molecular marker studies in breast cancer
- **Mike Jacobetz**, Tuveson laboratory
  Optimizing drug delivery in pancreatic ductal adenocarcinoma
- **Florian Karreth**, Tuveson laboratory
  Modulation of oncogenic transformation by RAF proteins
- **Shane Kelly**, Winton laboratory
  Proteomic analysis of APC deficient mouse intestinal epithelium
- **Malcolm Lawson**, Murphy laboratory
  Determinants of chemoresistance in small cell lung cancer
- **Doug Speed**, Tavaré laboratory
  Exploring nonlinear regression methods, with application to association studies
- **Sofie Willems**, Murphy laboratory
  ADAMs as EGFR ligand sheddases in prostate cancer
- **Tim Witney**, Brindle laboratory
  Detection of tumour treatment response using hyperpolarised carbon-13 magnetic resonance spectroscopy

**MSc**
- **Gemma Sharp**, Caldas laboratory
  Mammary stem and progenitor cells and the origin of breast cancer

**MPhil**
- **Sarah Dombernowsky**, Murphy laboratory
  Regulation of ADAM17 – biochemical and cell based studies
- **Julia Frede**, Murphy laboratory
  The role of aminopeptidase N (CD13) in cell migration
- **Monika Pütz**, Gergely laboratory
  Characterization of a centrosomal protein CEP135 in vertebrate cells
- **Thornton Thompson**, Winton laboratory
  DNA replication timing, differentiation and cancer in human colon cell lines
- **Amary Wagner**, Tuveson laboratory
  Identifying mutations that cooperate with BRafV600E in melanoma development using forward genetics
An electronic copy of this report is available on our website.

By road: from M11 junction 11, follow the signs to Addenbrooke’s Biomedical Campus, or follow the signs to Trumpington Park and Ride and take the Addenbrooke’s Shuttle Bus.

By rail: take the train to Cambridge, then take the Stagecoach Citi 1 or Citi 7 bus to Addenbrooke’s.

By air: the nearest airport to the CRI is London Stansted Airport.

Cancer Research UK
Registered charity number in England and Wales: 1089464; in Scotland: SC041666
Registered as a company limited by guarantee in England and Wales number: 4325234
Registered address: Angel Building, 407 St. John Street, London EC1V 4AD

Telephone: +44 20 7242 0200
www.cancerresearchuk.org
Mouse back skin arrector pili muscles (APMs) that are inserted to the region of the hair follicle stem cells (bulge). Image provided by Hironobu Fujiwara (Watt laboratory).

Displastic small intestinal villi stained with WGA488 and DAPI. Image provided by André Neves (Brindle laboratory) in collaboration with the Winton laboratory.