LUDWIG INSTITUTE FOR CANCER RESEARCH

ANNUAL RESEARCH HIGHLIGHTS REPORT 2006

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Ludwig Institute for Cancer Research 2006 Annual Research Highlights Report

In the 35 years since the Ludwig Institute for Cancer Research was established by Daniel K. Ludwig, the world of cancer research, diagnosis and therapy has changed remarkably.

In 1971, technologies today deemed critical to cancer research did not exist. DNA sequencing would not be invented for another five years, and the first human gene would not be cloned for another seven. The first oncogene, *src*, had been discovered the previous year, yet the discovery of the first tumor-suppressor gene, *p53*, would not occur for another eight years. We did not know the basis of cancer heredity. The etiological role of infectious agents in human cancer was beginning to be understood, but the association between human papillomavirus (HPV) infection in cervical cancer was more than 20 years away. Finally, the bluntest of weapons—chemotherapy and radiotherapy—constituted the cancer therapy arsenal.

Today, scientists routinely utilize genomic, transcriptomic and proteomic technologies to analyze the sequence and expression of thousands of genes and proteins in a single experiment. Some would argue that investigators have all but completed the identification and comprehensive study of oncogenes and tumor suppressor genes and the proteins they encode. Medicine is now using so-called 'biomarkers' to diagnose cancer earlier, and to predict familial and other cancer risks. A vaccine against HPV infection, which is predicted to prevent 70% of cervical cancers, was this year approved by regulatory bodies around the world. Equally importantly, therapies that specifically target proteins promoting cancer onset, growth and spread are revolutionizing our therapy arsenal. 'Personalized medicine'—therapy tailored to the genetic basis of the tumor and/or patient—is no longer in the realm of science fiction.

The Ludwig Institute for Cancer Research (LICR) has played a central role in many crucial developments in cancer research. The Institute is a pioneer of the field of cancer immunology and immunotherapies, and is known for research findings in cell and biological processes critical to cancer onset and spread, growth factor signaling, cytokine signaling, DNA repair mechanisms, and mitosis. The Institute is also a major contributor of transcriptomic and 'immunomic' data to publicly-available databases. LICR has not only tackled multiple facets of basic cancer research but has also made a commitment to the entire discovery continuum, from the laboratory to the clinic. This commitment began with the 'Ludwig Breast Cancer Study Group' (now the 'International Breast Cancer Study Group'), which was established in 1976 and conducted some of the earliest Phase III clinical trials for adjuvant breast cancer therapies. Today, LICR—having cloned the first cancer antigen—is conducting clinical trials of cancer vaccines as a continuing contribution to the development of this new therapeutic modality.

The Institute remains dedicated to making meaningful contributions to cancer research and to translating its laboratory findings into applications for human benefit. This report offers some highlights of the progress that LICR made to these ends in 2006.

BRANCHES / CENTER

The Institute's nine Branches and the Melbourne Center (see p35) are each physically and functionally associated with a university or research institute and/or a non-profit hospital. This arrangement guarantees an environment conducive to collaborative cancer research and provides access to local institutional resources and expertise in both the laboratory and the clinic.

Brussels Branch

Belgium — *Catholic University of Louvain*
*Saint-Luc's University Clinic**

Lausanne Branch

Switzerland — *Swiss Institute for Experimental Cancer Research (ISREC)*
University of Lausanne (UNIL)
Federal Polytechnic School of Lausanne (EPFL)
*Central Hospital of the Vaudois University (CHUV)**

London Branch

UK — *University College London*

Melbourne Branch

Australia — *University of Melbourne*
Melbourne Health

* Clinical trials sites

Melbourne Center

Australia — *Austin Health**

New York Branch

USA — *Memorial Sloan-Kettering Cancer Center**

San Diego Branch

USA — *University of California, San Diego*

São Paulo Branch

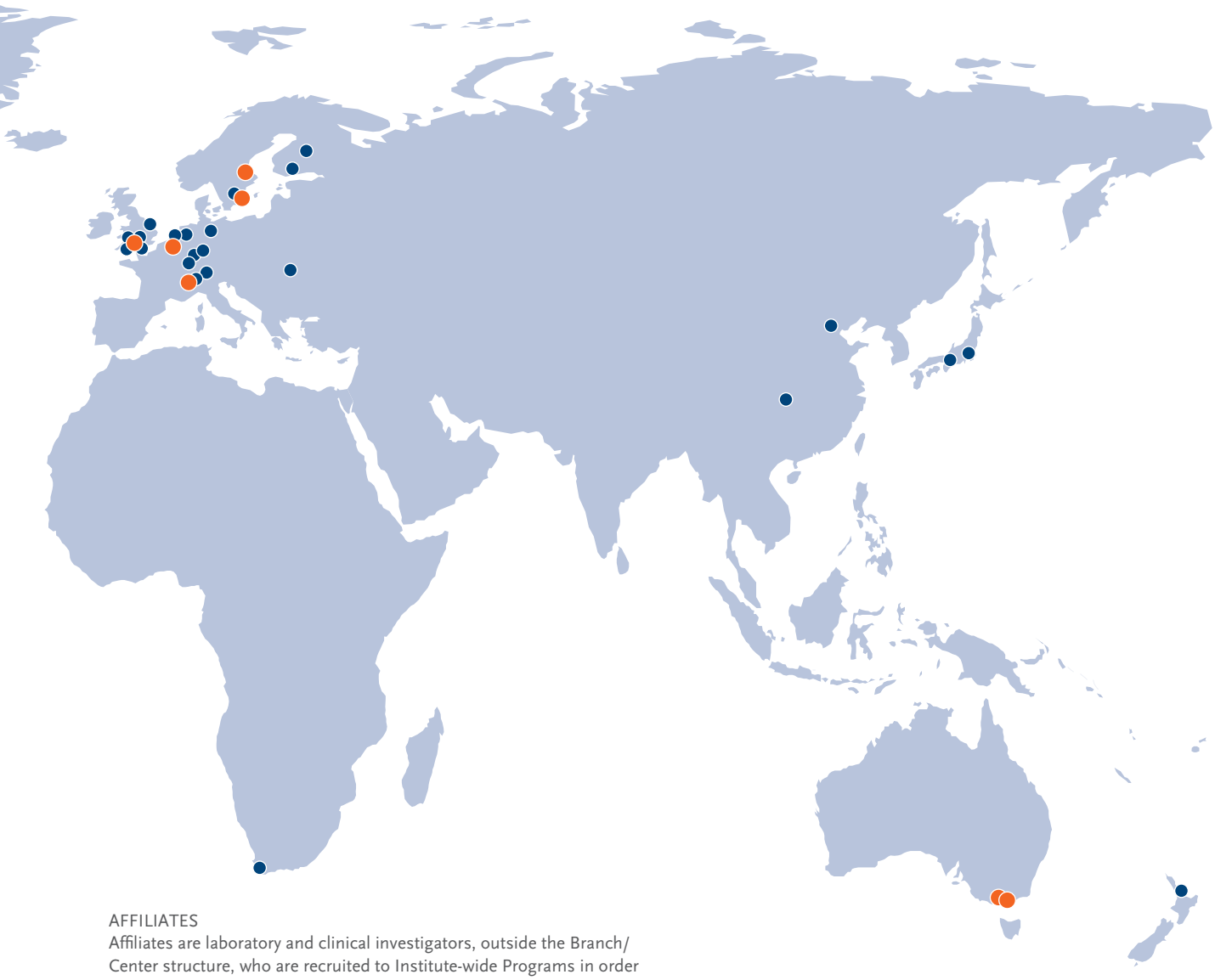
Brazil — *Hospital Alamão Oswaldo Cruz*

Stockholm Branch

Sweden — *Karolinska Institute*

Uppsala Branch

Sweden — *Uppsala University*



AFFILIATES

Affiliates are laboratory and clinical investigators, outside the Branch/Center structure, who are recruited to Institute-wide Programs in order to extend the knowledge and expertise required to achieve Program goals. LICR Affiliates are located in the following cities:

Auckland, New Zealand
- *University of Auckland**

Beijing, China
- *Peking University*

Belo Horizonte, Brazil
- *Federal University of Minas Gerais*

Buffalo, NY, USA
- *Roswell Park Cancer Institute**

Cape Town, South Africa
- *University of the Western Cape*

Frankfurt, Germany
- *Krankenhaus Nordwest**

Heidelberg, Germany
- *German Cancer Research Center (DKFZ); Heidelberg University Clinic*

Helsinki, Finland
- *University of Helsinki*

Hamburg, Germany
- *University Hospital Hamburg-Eppendorf*

Homburg, Germany
- *University of Saarland Medical School**

Houston, TX, USA
- *M.D. Anderson Cancer Center*

Ithaca, NY, USA
- *Cornell University*

Konstanz, Germany
- *University of Konstanz*

Kuopio, Finland
- *University of Kuopio*

Kyiv, Ukraine
- *Institute of Molecular Biology and Genetics*

Leiden, The Netherlands
- *Leiden University Medical Center*

London, UK
- *Institute of Neurology*
- *Imperial College*
- *St Mary's Hospital*
- *University College London*

Mie, Japan
- *Mie University School of Medicine*

New Haven, CT, USA
- *Yale University*

New York, NY, USA
- *Columbia-Presbyterian Medical Center**
- *New York University Cancer Institute**
- *Weill Medical College of Cornell University**

Nijmegen, The Netherlands
- *University Hospital Nijmegen**

Oxford, UK
- *John Radcliffe Hospital**

Petropolis, Brazil
- *National Laboratory of Scientific Computation*

Philadelphia, PA, USA
- *Wistar Institute*
- *Hospital of University of Philadelphia**

Pittsburgh, PA, USA
- *University of Pittsburgh Cancer Institute*

Ribeirao Preto, SP, Brazil
- *School of Medicine of Ribeirao Preto, São Paulo State University*

São José do Rio Preto, SP, Brazil
- *School of Medicine of São José do Rio Preto, São Paulo State University*

São Paulo, SP, Brazil
- *São Paulo University*

St Louis, MO, USA
- *Washington University School of Medicine*

Stockholm, Sweden
- *Karolinska Institute*

Tokyo, Japan
- *The Cancer Institute, University of Tokyo*

Xi'an, China
- *Fourth Military Medical University*

Zürich, Switzerland
- *Zürich University Hospital**

* Clinical trials sites

Ludwig Cancer

In 1971, Daniel K. Ludwig established the global Ludwig Institute for Cancer Research. Upon Mr. Ludwig's death in 1992, a Trust was created to manage a second gift, the Virginia & D.K. Ludwig Fund for Cancer Research, for the benefit of cancer research at six distinguished US institutions chosen by Mr. Ludwig: Harvard Medical School, The Johns Hopkins School of Medicine, Massachusetts Institute of Technology, Memorial Sloan-Kettering Cancer Center, Stanford University School of Medicine, and The University of Chicago Pritzker School of Medicine.

In 2006, the Trustees of the Fund—Lloyd J. Old, M.D. (Chair), R. Palmer Baker, Jr., John D. Gordan, III, George Jenkins, Edward A. McDermott, Jr., and Sir Derek Roberts—distributed $120 million cash and company stock valued at $196 million in what is believed to be the largest single gift to cancer research by a US foundation. This distribution established a Ludwig Center at each of the six beneficiary institutions. Together the Ludwig Centers and LICR comprise "Ludwig Cancer." The objectives of Ludwig Cancer are to:

Change the paradigm of research support

Ludwig believed that cancer research should not be hindered by the changing priorities of government funding. His gifts to cancer research ensure reliable and continuing resources, enabling laboratory and clinical investigators to tackle long-term research objectives and rapidly incorporate new knowledge.

Harness the exponential power of collaboration

With the emerging understanding of the extraordinary complexity of cancer the Trustees believe that every effort should be made to forge and facilitate strong research alliances. The Ludwig Centers and the Ludwig Institute each have strengths and capabilities that can be leveraged to form a powerful network—Ludwig Cancer.

Make human benefit our unyielding yardstick

The Trustees believe that the importance of any fundamental insight into cancer gleaned from laboratory research cannot be truly measured until its impact on the alleviation of human suffering is known. Ludwig Cancer will seek to broadly explore the clinical potential of its discoveries.

Provide scientific vision

Leadership is indispensable to the success of this endeavor. Ludwig Cancer engages some of the most eminent scientists in cancer research today, and empowers them to make even greater contributions to altering the course of this disease.

"Success in any complex enterprise consists in bringing the best minds to bear on each problem, in providing the best resources possible, and in putting each concept into practice whenever and wherever the opportunities are most favorable. I believe firmly in the value of applying these principles in grappling with tasks as momentous as finding ways to relieve the human suffering caused by cancer."

—Daniel K. Ludwig, December 17, 1974









From top left
Andrew J.G. Simpson, Ph.D.; George D. Demetri, M.D.; Kenneth Kinzler, Ph.D.; Bert Vogelstein, M.D.; James P. Allison, Ph.D.; Robert A. Weinberg, Ph.D.; Irving Weissman, M.D.; Geoffrey L.Greene, Ph.D.; Ralph R. Weichselbaum, M.D.

Ludwig Center at Dana-Farber/HMS

George D. Demetri, M.D. led one of the first pivotal clinical trials to demonstrate that therapies targeting a specific mutant protein can extend cancer patient survival. The Dana-Farber Cancer Institute/Harvard Medical School team is now designing and implementing scientific studies that expand knowledge gained in early drug development trials.

Ludwig Center at Memorial Sloan-Kettering

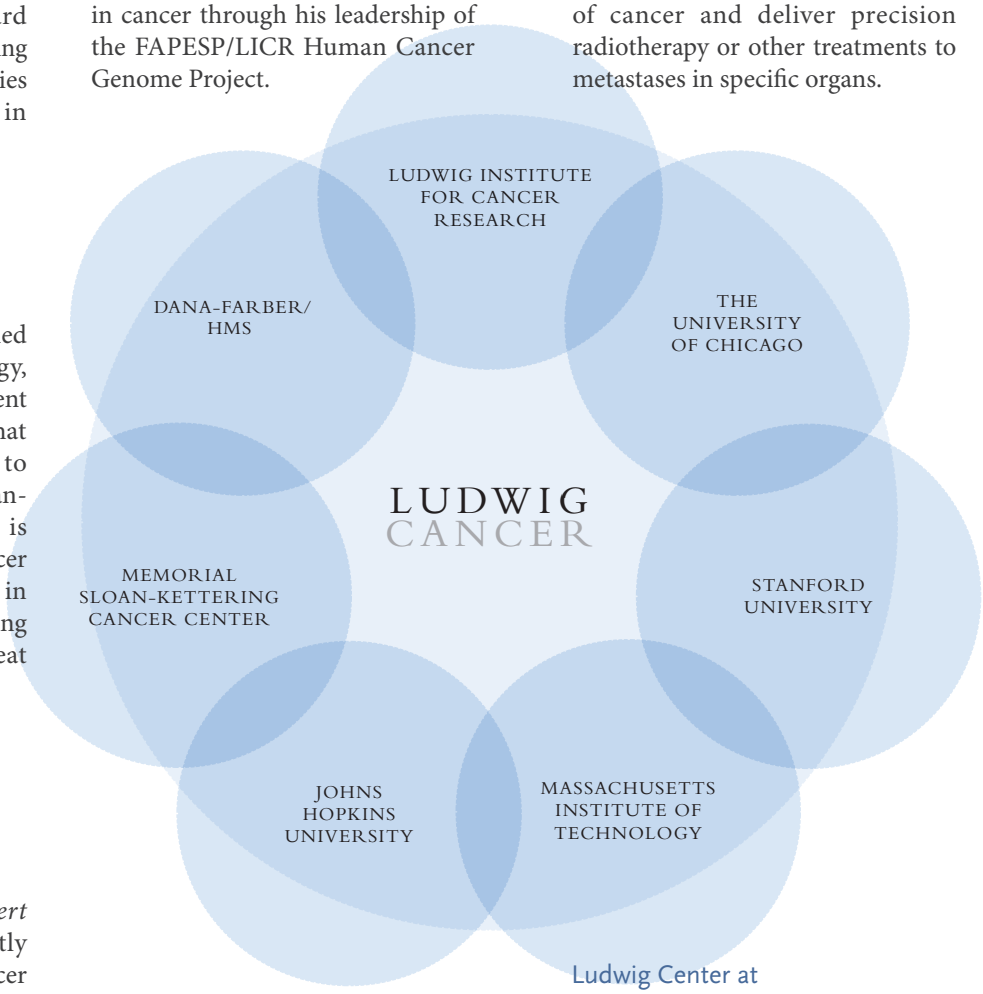
James P. Allison, Ph.D. is a renowned leader in the field of immunology, and particularly in the development of novel therapeutic concepts that harness the immune system to fight cancer. The Memorial Sloan-Kettering Cancer Center team is evaluating several innovative cancer immunological approaches, now in clinical trials, to identify promising new ways to diagnose and treat people with cancer.

Ludwig Center at Johns Hopkins

Kenneth Kinzler, Ph.D. and *Bert Vogelstein, M.D.* are consistently ranked in the top-most cited cancer researchers in the world, and have just published the most comprehensive study identifying gene mutations in breast and colon cancers. The Johns Hopkins University Kimmel Cancer Center team will continue to focus on the genetic underpinnings of cancer and how to use these discoveries to improve cancer patient care.

Ludwig Institute for Cancer Research

Andrew J.G. Simpson, Ph.D., as LICR Scientific Director will coordinate collaborations with the Ludwig Centers. Dr. Simpson made a significant contribution to the identification of the complete set of human genes and their expression in cancer through his leadership of the FAPESP/LICR Human Cancer Genome Project.



Ludwig Center at MIT

Robert A. Weinberg, Ph.D. is an internationally recognized authority on the genetic basis of human cancer, his group having isolated the first human cancer-causing gene and the first known tumor suppressor gene. The MIT team will focus particularly on the understanding of metastasis, the spread of cancer.

Ludwig Center at The University of Chicago

Geoffrey L.Greene, Ph.D., a leader in the study of steroid hormones and their specific receptors, and *Ralph R. Weichselbaum, M.D.,* a pioneer in radiation targeted gene therapy, have formed a team to find novel ways to direct radiolabeled hormones to their receptors to detect the spread of cancer and deliver precision radiotherapy or other treatments to metastases in specific organs.

Ludwig Center at Stanford University

Irving Weissman, M.D. and his colleagues were the first to identify and isolate normal adult (or tissue-specific) stem cells, and have since identified the malignant counterparts of these cells in leukemia and solid tumors such as breast cancer. The Stanford University team is working to isolate cancer stem cells in all human cancers and develop new therapies that target these cells to eliminate the disease at its source.

Metastasis

Metastasis is the process by which cancer cells migrate to distant sites in the body and initiate new tumors. The metastatic cancer cell disrupts normal cell adhesion and migration so that it can detach from the primary tumor, enter nearby blood or lymph vessels, then exit the vasculature and attach to a new, distant site. As the direct cause of death in the majority of cancer patients, the development of therapies to prevent metastasis is an area of profound interest.

CAR and Tight Junctions

The coxsackie and adenovirus receptor (CAR) is a transmembrane protein associated with intercellular 'tight junctions' that control adhesion between both endothelial and epithelial cells. Metastatic cancer cells must break these tight junctions when detaching from the surrounding tumor cells and/or when passing between the endothelial cells that form the blood or lymph vessels. A team from the Stockholm Branch this year showed that CAR is associated with epithelial cell tight junctions but not with endothelial cells of the vasculature *in vivo*. As the receptor for adenoviruses, the presence of CAR marks cells as targets for adenovirus-based gene therapies. In a second study, the team found that CAR is expressed in human male germ cells and interacts with a protein involved in spermatid differentiation. This suggests CAR may have a hitherto unsuspected role in male fertility. The LICR team also found that CAR expressed in spermatozoa would likely be inaccessible to adenovirus-based vectors, meaning that the risk of cancer gene therapies targeting normal male germ cells is low.

Raschperger E, Thyberg J, Pettersson S, Philipson L, Fuxe J, Pettersson RF. 'The coxsackie- and adenovirus receptor (CAR) is an in vivo marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis.' Experimental Cell Research 2006 312(9):1566-80.

Mirza M, Hreinsson J, Strand ML, Hovatta O, Soder O, Philipson L, Pettersson RF, Sollerbrant K. 'Coxsackievirus and adenovirus receptor (CAR) is expressed in male germ cells and forms a complex with the differentiation factor JAM-C in mouse testis. Experimental Cell Research 2006 312(6):817-30.

Novel Occludin Regulator

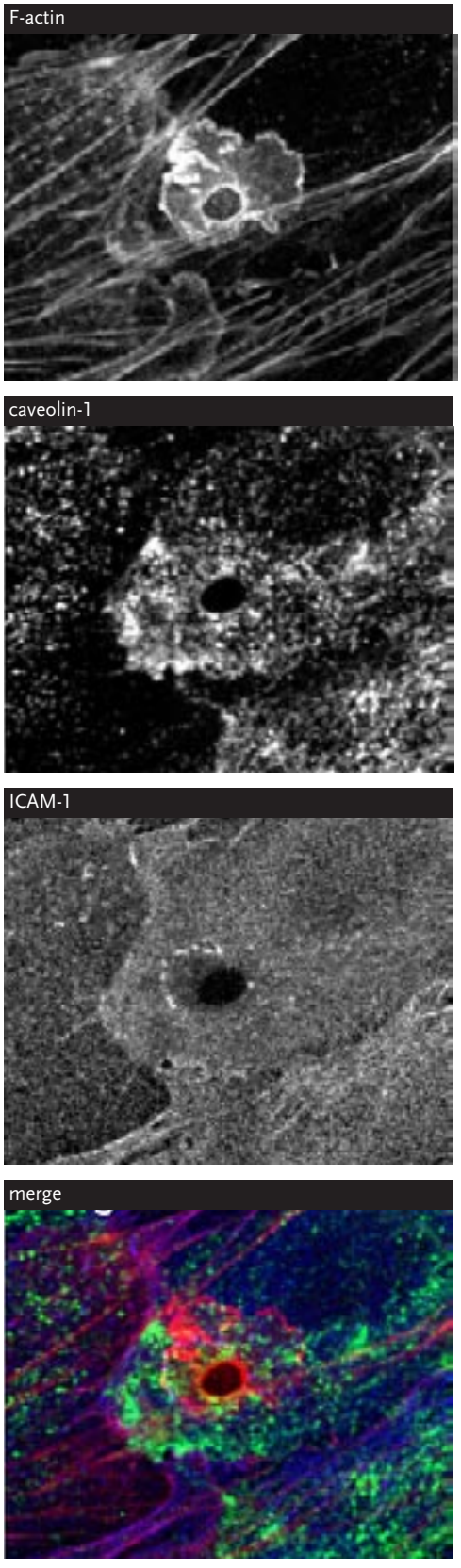
Occludin, a major component of tight junctions, is regulated by phosphorylation (addition of a phosphate group) and dephosphorylation (removal of a phosphate group). Targeting the regulation of tight junction permeability via occludin has been suggested as a therapeutic approach to prevent metastasis. Investigators at the London Branch have now discovered that occludin is phosphorylated by a kinase, CKI epsilon, that is involved in a variety of cell processes, including membrane transport, cell division and DNA repair. The finding that CKI epsilon is a novel occludin kinase suggests ideas for new therapeutic approaches that may prevent cancer cell detachment from the primary tumor plus reduce the blood and lymph vessel permeability that allows cancer cells to enter the bloodstream.

McKenzie JA, Riento K, Ridley AJ. Casein kinase I epsilon associates with and phosphorylates the tight junction protein occludin. FEBS Letters 2006 580(9):2388-94.

The Rac Family in Metastasis

The cell's 'cytoskeleton' determines cell 'morphology' (shape) and is remodeled dynamically during migration. The Rac family of proteins—Rac1, Rac2 and Rac3—is integral to the transduction of signals from growth factor and other cell surface receptors that drive cytoskeleton remodeling, but the individual roles of the family members are not known. A team from the London Branch has been analyzing the role of each Rac protein by engineering 'macrophages' (highly migratory cells of the immune system) such that they lack Rac1, Rac2, or both Rac1 and Rac2. The team has previously found that macrophages lacking Rac1 have altered cell shape and reduced cytoskeleton remodeling, but normal migration speed. Their latest studies have shown that Rac1 and Rac2 do not appear to be required for macrophage migration or 'chemotaxis' (movement toward a chemical stimulus), but each has distinct roles in cell morphology, migration and invasion. Importantly, macrophages lacking Rac1 lost their capacity for tissue invasion, suggesting this protein as a potential target to inhibit or prevent metastasis.

Wheeler AP, Wells CM, Smith SD, Vega FM, Henderson RB, Tybulewicz VL, Ridley AJ. Rac1 and Rac2 regulate macrophage morphology but are not essential for migration. Journal of Cell Science 2006 119(Pt 13):2749-57.



Transcellular Migration

Cancer cells must pass through or around the endothelial cells of the blood and lymph vasculature to metastasize. A team from the London Branch and Affiliates in New Haven (USA) analyzed the mechanisms of leukocyte transendothelial migration from capillaries into surrounding tissues as a model for how metastatic cancer cells pass in and out of the vasculature. One of the adhesion receptors to which leukocytes bind, ICAM-1, is internalized into the cell following translocation to cell membrane areas rich in the structural proteins F-actin and caveolin-1. Remarkably, the LICR team showed that the leukocytes bound to ICAM-1 and then crossed through the cell itself—without breaking the junctions between cells—via passages formed by F-actin and caveolin-1. The team also specifically decreased the levels of the caveolin-1 protein and showed that this reduced lymphocyte transcellular migration.

Millan J, Hewlett L, Glyn M, Toomre D, Clark P, Ridley AJ. 'Lymphocyte transcellular migration occurs through recruitment of endothelial ICAM-1 to caveola- and F-actin-rich domains.' Nature Cell Biology 8(2):113-23, 2006.

These images, from the London Branch, show individual proteins F-actin, caveolin-1 and ICAM-1 as they form a transcellular passage (bottom image).

Cell Differentiation

Differentiation, the process by which cells mature and become specialized, is driven by gene expression networks that determine the cell's size, 'morphology' (shape) and function. Typically, cancer cells are relatively undifferentiated, which might enable aberrant behavior, such as uncontrolled proliferation and the capacity to invade and metastasize (spread around the body). Most normal adult cells are terminally (irreversibly) differentiated. They are all derived originally from embryonic stem (ES) cells—the primitive embryonic cells that become mature, differentiated cells—which are used as models for studying differentiation. One important outcome of studying differentiation in ES cells is the building of understanding and tools that might one day benefit humankind through replacement cell therapies for non-oncology indications. In the future, cardiomyocytes induced from ES cells might treat cardiac diseases, and stem cell transfer might treat neurological conditions such as Parkinson's disease.

Cardiogenesis and Notch Signaling

The Notch pathway—mediated by four Notch receptors and three ligands—plays a key role in the differentiation of several cell lineages during development. Upon activation, the Notch receptor is cleaved and the intracellular domain translocates to the nucleus, where it interacts with a transcription factor to express target genes. Notch is known to play a substantial role in 'cardiogenesis' (heart formation) during embryonic development: mice lacking Notch die, in part, from heart defects. A team from the Lausanne Branch studied the ability of the Notch pathway to control cardiogenesis following the differentiation of ES cells into cardiomyocytes, the cell type that constitutes the heart muscle. The team found that Notch signaling inhibits the differentiation of ES cells into cardiomyocytes, and that blocking cardiogenesis is specifically mediated only by Notch1 receptor signaling. Analysis of proteins that mark cell lineage indicated that Notch signaling inhibits the ES cell from committing to the precursor of the cardiomyocyte cell type, the mesodermal lineage. In fact, it appears that Notch signaling may be a vital part of the biochemical switch that commits ES cells to become neurons rather than cardiomyocytes during embryonic development.

Nemir M, Croquelois A, Pedrazzini T, Radtke F. Induction of cardiogenesis in embryonic stem cells via downregulation of Notch1 signaling. Circulation Research 2006 98(12):1471-8.

Epithelial-Mesenchymal Transition in Cancer

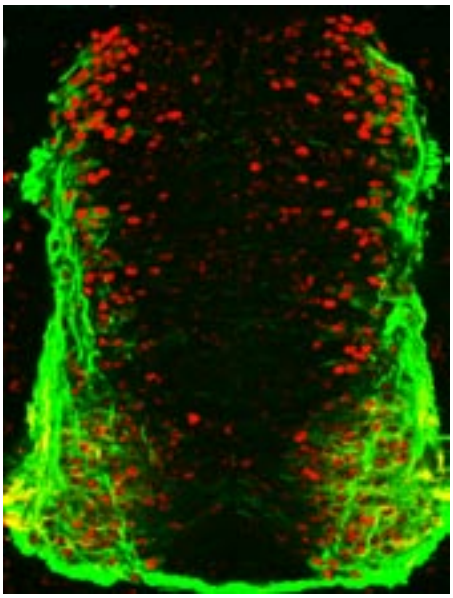
Epithelial-mesenchymal transition (EMT) is the process by which a fully differentiated epithelial cell dedifferentiates to become a more primitive mesenchymal cell. EMT occurs naturally during embryogenesis, but is reactivated inappropriately during carcinogenesis. The molecular mechanisms that control EMT remain largely unexplored. A team from the Uppsala Branch is studying EMT initiated by transforming growth factor (TGF) β (see pp36-37) signaling pathways, specifically those transduced by the Smad proteins. Gene expression profiling indicated that the transcription factor HMGA2 is induced during Smad-mediated EMT. The team found that the addition of ectopic HMGA2 caused irreversible EMT, and that HMGA2 controls the expression of known regulators of EMT. These findings delineate a pathway that links TGFβ signaling to the control of tumor invasiveness and metastasis.

Thuault S, Val court U, Petersen M, Manfioletti G, Heldin CH, Moustakas A. Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition. Journal of Cell Biology 2006 174(2):175-83.

Neurogenesis and Sox Signaling

Neuronal stem cells are frequently used to study how stem cells are maintained in an immature state and the mechanisms by which they are then induced to exit the cell cycle (see pp10-12) and differentiate into specialized neuronal cell types. A team from the Stockholm Branch has found that the Sox4 and Sox11 proteins, part of a family of HMG-box transcription factors, operate as transcriptional activators downstream of the proneural bHLH transcription factor proteins, in neuronal stem cells, to initiate the neurogenic program. The study also revealed that Sox4 and Sox11 are the targets of a transcriptional repressor protein, REST/NRSF, that has been shown to block neuronal gene expression. Taken together, these findings show that Sox4 and Sox11 mechanistically separate the gene expression necessary for neuronal differentiation and cell cycle exit during neuronal maturation.

Bergsland M, Werme M, Malewicz M, Perlmann T, Muhr J. The establishment of neuronal properties is controlled by Sox4 and Sox11. Genes & Development 2006 20(24):3475-86.



A section through the embryonic chick spinal cord labeled with antibodies against a neuronal protein (green) and the transcription factor Sox11 (red) which mechanistically separates neuronal differentiation and cell cycle exit.

Differentiation of Dopamine Cells

Scientists at the Stockholm Branch are analyzing the developmental pathways of neurons to understand the transcription networks that drive stem cell differentiation into dopamine cells. Dopamine cells, particularly those of a 'mid-brain identity,' are clinically important since they degenerate in Parkinson's disease. The team identified two transcription factors, Lmx1a and Msx1, necessary for the specification of 'midbrain' dopamine cells. Lmx1a is required to trigger dopamine cell differentiation, which it does partly by inducing the expression of Msx1. This was confirmed by the finding that 'midbrain' dopamine neurons could be induced by artificially expressing Lmx1a in ES cells. The Stockholm Branch team also found that a nuclear receptor, Nurr1, is essential for the generation of midbrain dopamine neurons. In the developing brain, Nurr1-induces a protein, Nrp1, that is involved in axon guidance and 'angiogenesis' (the formation of new blood vessels). Dopamine cells engineered *in vitro* may prove to be critical in the development of novel cell replacement strategies in Parkinson's disease, and analyses of their differentiation is likely to further our understanding of the differentiation of other cell types.

Andersson E, Tryggvason U, Deng Q, Friling S, Alekseenko Z, Robert B, Perlmann T, Ericson J. Identification of intrinsic determinants of midbrain dopamine neurons. Cell 2006 124(2):393-405.

Hermanson E, Borgius L, Bergsland M, Joodmardi E, Perlmann T. Neuropilin1 is a direct downstream target of Nurr1 in the developing brain stem. Journal of Neurochemistry 2006 97(5):1403-11.

Cell Proliferation

Cell growth and division are tightly regulated with genome integrity during the cell cycle. This ensures that cell proliferation occurs when the appropriate extracellular signals are received, and that each cell is replicated faithfully. Uncontrolled proliferation can be caused by deregulation of the cell cycle, through loss of function of cell cycle 'checkpoint' proteins, or by the failure of signaling pathways to appropriately transduce pro- and anti-proliferation signals. Many chemotherapy drugs specifically attack cells in a particular phase of the cell cycle. Some drugs inhibit proteins involved in DNA replication, while others inhibit proteins involved in the duplication and separation of chromosomes.

Cell Cycle Regulation for Cell Type-Specific Development

The p16Ink4a (p16) tumor suppressor protein is the archetypal member of the Ink4 family of cell cycle proteins known as cyclin-dependent kinase (CDK) inhibitors, which prevent entry into the cell cycle. A prevailing theory has held that p16 is not involved in normal cell proliferation, but is induced following cell stress, senescence or the activation of particular oncogenes. However, there is evidence to suggest that p16 might be involved in normal T cell proliferation. A team from the Lausanne Branch analyzed a mouse model lacking the *p16* gene and found that p16 deficiency results in increased numbers of T cells in the thymus and spleen. Experimental results showed that the increase in T cell number was not due to increased proliferation, but rather to decreased cell death. Absence of p16 reduced intrinsically regulated apoptosis (programmed cell death) of T cells *in vivo* as well as apoptosis induced by oxidative stress or DNA damage *in vitro*. The results indicate that p16 may play an important role as a cell type-specific survival regulator to ensure correct T cell development and homeostasis.

Bianchi T, Rufer N, MacDonald HR, Migliaccio M. The tumor suppressor p16Ink4a regulates T lymphocyte survival. Oncogene 2006 25(29):4110-5.

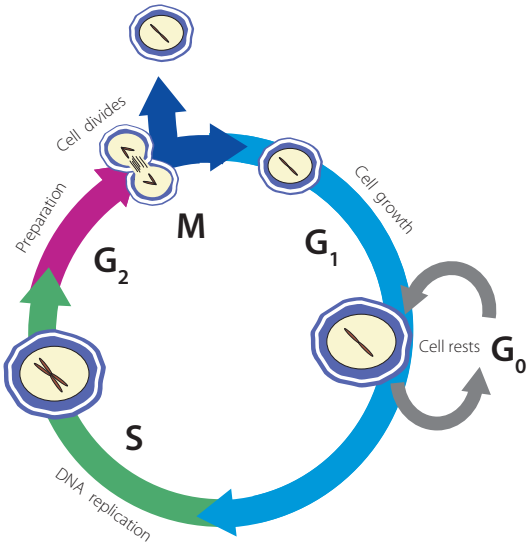
Cell Cycle and Lipid Synthesis Linked

Members of the sterol regulatory element-binding protein (SREBP) family of transcription factors control cholesterol and lipid metabolism, processes that are required to enlarge and create cell membranes during cell growth and proliferation. A team from the Uppsala Branch has shown that SREBP1 is phosphorylated by CDK1 during mitosis, thereby ensuring that a pool of active transcription factors is present to support lipid synthesis, and that inactivation of SREBP1 attenuated cell growth. This study, linking lipid synthesis with cell growth and proliferation, was featured on the cover of the journal *Cell Cycle*. The team also showed, in a separate study, that the activity of SREBP is regulated by its degradation following DNA binding and phosphorylation by the GSK-3β protein. The activity of GSK-3β is controlled by extracellular signals, which suggests that this may be one of the mechanistic links between growth factor signaling, lipid synthesis and cell growth and proliferation.

Bengoechea-Alonso MT, Ericsson J. Cdk1/cyclin B-mediated phosphorylation stabilizes SREBP1 during mitosis. Cell Cycle 2006 5(15):1708-18.

Punga T, Bengoechea-Alonso MT, Ericsson J. Phosphorylation and ubiquitination of the transcription factor sterol regulatory element-binding protein-1 in response to DNA binding. Journal of Biological Chemistry 2006 281(35):25278-86.

Many chemotherapy drugs specifically attack cells in a particular phase of the cell cycle. Some drugs inhibit proteins involved in DNA replication, while others inhibit proteins involved in the duplication and separation of chromosomes. The study of the cell cycle is thus vital to the application and optimization of chemotherapies.



Schematic of mammalian cell cycle. During the G1 phase, the cell increases in size, and then either exits the cell cycle to 'rest' in the G0 phase or continues to the S-phase. In the S-phase, DNA replication and repair occur, with a final check of genome integrity undertaken in the G2 phase. This occurs prior to the M-phase, in which the process of mitosis separates the chromosomes into two daughter cells.

Cell Cycle and Cell Morphology

The DNA damage and replication checkpoints maintain genome integrity by ensuring replication occurs correctly, prior to the cell exiting the S-phase of the cell cycle. In yeast, the loss of two checkpoint proteins, Mec1 and Rad53, cause DNA replication forks to collapse and partially replicated chromosomes to be partitioned inappropriately in the dividing cell. Mutations in the human 'homologs' (equivalents) of these proteins are associated with cancer; the rare Li Fraumeni syndrome, which greatly increases the risk of developing cancer, is caused by mutation of the human Rad53 homolog. Yeast cells divide via the formation of a bud, and while studying the division of Rad53 yeast mutants, LICR investigators discovered that the yeast's 'morphology' (shape) and cell walls were aberrant. A joint study by two San Diego teams showed that Rad53 interacts with proteins involved in bud growth and in 'cytokinesis,' the process whereby the cell 'cytoplasm' (the cell contents, excepting the nucleus that contains the genome) is divided as part of the formation of two separate cells. At least one of these proteins also appears to be involved in the response to DNA replication stress. These novel results—published in back-to-back papers in the *Journal of Cell Biology*—suggest that Rad53 also has a role in mediating polarized cell growth in response to DNA replication stress.

Enserink JM, Smolka MB, Zhou H, Kolodner RD. Checkpoint proteins control morphogenetic events during DNA replication stress in Saccharomyces cerevisiae. Journal of Cell Biology 2006 175(5):729-41.

Smolka MB, Chen SH, Maddox PS, Enserink JM, Albuquerque CP, Wei XX, Desai A, Kolodner RD, Zhou H. An FHA domain-mediated protein interaction network of Rad53 reveals its role in polarized cell growth. Journal of Cell Biology 2006 175(5):743-53.

S-phase Progression and DNA Checkpoints

Investigators at the San Diego Branch are using yeast as a model to study cell cycle progression. One research interest is the role of the different protein complexes involved in the assembly of 'chromatin,' the DNA/protein super-structure that packages the cell's genome. Without correct chromatin assembly, the cell is unable to progress through the S-phase of the cell cycle. Multi-protein chromatin assembly factor (CAF) complexes, CAF-I and RCAF, function synergistically for chromatin assembly, with the CAF-I complex interacting with RCAF via its Asf1 protein subunit. However, scientists have suspected that each CAF may have further, separate roles in DNA replication or repair, by regulating associated cell cycle checkpoints. Asf1 has been shown to interact with the checkpoint protein Rad53, while some mutants of the Cac1 protein, which is part of the CAF-I complex, activate the S-phase checkpoint. The San Diego Branch team engineered and analyzed yeast mutants lacking Asf1 or Cac1, and found that defects in the CAFs do not cause defects in the DNA replication or intra-S-phase DNA damage checkpoints. Rather, the progression of Asf1 mutants through S-phase is dependent on replication checkpoint proteins that stabilize the fork structure created during DNA replication. CAF-I mutants, meanwhile, appear to play a role in the detection of DNA damage, suggesting that activation, rather than regulation, of DNA damage checkpoints is the reason these cells fail to complete the cell cycle.

Kats ES, Albuquerque CP, Zhou H, Kolodner RD. Checkpoint functions are required for normal S-phase progression in Saccharomyces cerevisiae RCAF- and CAF-I-defective mutants. Proceedings of the National Academy of Sciences USA 2006 103(10):3710-5.

Cell Cycle Exit, ASPPs and p53

The tumor suppressor protein p53 is a major component of the DNA damage checkpoint that halts the cell cycle in G1 when DNA damage is detected. The protein is also a key player in the induction of apoptosis if DNA damage is not repaired. For the p53 system to fail, the products of both copies of the gene must be inactivated. More than half of all cancers have *p53* gene mutations, but the cause of the protein's non-function in the remaining cancers is not known. Several years ago, investigators from the London Branch discovered a p53-regulating protein family, the ASPP family, alterations in the expression of which might cause cancer formation in the absence of detectable p53 mutations. ASPP1 and ASPP2 stimulate p53's apoptotic function, while iASPP inhibits p53's function. In 2006, the team reported the generation and characterization of a mouse model lacking one or both copies of the *ASPP2* gene. Analyses of these mice, and of those crossed with mice lacking one or both copies of the *p53* gene, showed that *ASPP2*, like *p53*, is a tumor suppressor gene. This study is the first to provide genetic evidence that ASPP2 and p53 share overlapping functions in both embryological development and the suppression of tumors.

Vives V, Su J, Zhong S, Ratnayaka I, Slee E, Goldin R, Lu X. ASPP2 is a haploinsufficient tumor suppressor that cooperates with p53 to suppress tumor growth. Genes & Development 2006 20(10):1262-7.

ASPPs and the Genetic Basis of Cancer Susceptibility

In another study, the team assessed the interaction of ASPP proteins with two forms of p53, p53Pro72 and p53Arg72, which differ by one amino acid. The LICR team found that the ASPP family preferentially regulates the p53Pro72 over the p53Arg72 form. With the p53Pro72 form being more prevalent in ethnic groups from around the equator, there is a possibility that ASPP protein levels might contribute to variations in the cancer susceptibility observed in different ethnic groups.

Bergamaschi D, Samuels Y, Sullivan A, Zvelebil M, Breyssens H, Bisso A, Del Sal G, Syed N, Smith P, Gasco M, Crook T, Lu X. iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. Nature Genetics 2006 38(10):1133-41.

DNA Mismatch Repair

Cells have numerous mechanisms to preserve genome integrity. The identification and repair of mis-incorporated DNA bases is mediated by a complex network of proteins known as the DNA mismatch repair (MMR) system. Mutations in the genes encoding MMR proteins result in an increased risk of developing cancer. One such example is the RecQ-like DNA helicase family, defects in which constitute the genetic basis of some familial cancers. Helicases are proteins that unwind DNA during gene transcripion, DNA repair and replication, and 'homologous recombination' (HR), the process by which chromosomes exchange genetic material. Because of the complexity of human MMR, LICR investigators are using Saccharomyces cerevisiae *(yeast) models to dissect and understand the genetic and biochemical basis of mutations that cause familial cancer syndromes.*

DNA Mismatch Recognition

The first step in MMR is recognition of a mismatched DNA base. This involves a Msh2-Msh6 'dimer' (two physically interacting protein subunits) forming a ring around the DNA at the mismatched base, and then, upon binding of an ATP molecule, 'sliding' along the DNA. Three different models linking mismatch recognition to repair have been proposed with respect to the role of ATP binding to Msh2-Msh6, and whether or not ATP is hydrolysed to ADP in order to generate energy for the complex to slide along the DNA. A team from the San Diego Branch has now found that the Msh2-Msh6 dimer is converted to a sliding form when ATP binds simultaneously to both Msh2 and Msh6. This study is the first to show a communication between the two binding sites, with the Msh2 binding site having decreased affinity for ADP and increased affinity for ATP when ATP is bound to Msh6. The study is also the first to show that a DNA mismatch inhibits ATP hydrolysis specifically at the Msh6 site, thus allowing the Msh2 site to release ADP and bind ATP. Taken together, these results support the hypothesis that ATP hydrolysis is not required for the Msh2-Msh6 dimer to slide along the DNA. The team also performed a detailed characterization of three different dominant mutations in the Msh6 gene that affect the ATP binding site. The results reinforce the hypothesis that ATP binding and/or a conformational change is/are required for the sliding formation, and also suggests that ATP binding and/or a conformational change are required for assembly of the DNA-binding complex. This study defines two different biochemical defects that underlie dominant mutations in the *Msh6* gene.

Mazur DJ, Mendillo ML, Kolodner RD. Inhibition of Msh6 ATPase activity by mispaired DNA induces a Msh2(ATP)-Msh6(ATP) state capable of hydrolysis-independent movement along DNA. Molecular Cell 2006 22(1):39-49.

Hess MT, Mendillo ML, Mazur DJ, Kolodner RD. Biochemical basis for dominant mutations in the Saccharomyces cerevisiae MSH6 gene. Proceedings of the National Academy of Sciences USA 2006 103(3):558-63.

Unwinding the Roles of DNA Helicases

LICR investigators analyzed the roles of three different yeast DNA helicases, Sgs1, Srs2 and Rrm3, in the origin of gross chromosomal rearrangements (GCRs) and in activation of the DNA damage checkpoint in the cell cycle (see p11). To do this, the team deleted one or more of the helicases from mutants defective in either HR or checkpoint activation. The combinatorial analyses indicated that GCRs occurred in yeast lacking Sgs1 and Rrm3, but not in those lacking Srs2 and Rrm3. The results show that DNA helicases have distinct roles in maintaining genome integrity, functioning at the interface between DNA replication and repair. Since DNA helicases have been suggested as possible therapeutic targets to prevent cancer cell proliferation, detailed knowledge of their role(s) in DNA repair becomes vital to reduce the risk of side-effects from any potential therapy.

Schmidt KH, Kolodner RD. Suppression of spontaneous genome rearrangements in yeast DNA helicase mutants. Proceedings of the National Academy of Sciences USA 2006 103(48):18196-201.

Mutations in the human homologs of Sgs1 cause human cancer syndromes, thus characterization of the simpler yeast helicase is of great interest. To identify proteins involved in Sgs1-mediated DNA repair, the San Diego team analyzed the ability of multiple proteins implicated in the maintenance of genome integrity to prevent aberrant, spontaneous translocations in yeast lacking Sgs1. The team found that Sgs1, not MMR, was critical for suppressing chromosome translocations in cells with mutations in Rrm3, some of the checkpoint proteins and the chromatin assembly factor proteins Cac1 or Asf1 (see p11). The investigators also found that spontaneous translocations were often formed by multiple translocation events between similar DNA sequences from highly divergent genes. They hypothesize that this phenomenon results from Sgs1 having a unique role in preventing recombination between divergent DNA sequences.

Schmidt KH, Wu J, Kolodner RD. Control of translocations between highly diverged genes by Sgs1, the Saccharomyces cerevisiae homolog of the Bloom's syndrome protein. Molecular Cell Biology 2006 26(14):5406-20.

Chromosome Structure & Dynamics

Cancer is caused fundamentally by a loss of genome integrity that alters the structure, function and/or abundance of gene products. Genome integrity is maintained by the detection and repair of damage to DNA (see p13) and by the strict regulation of mitosis; the process of chromosome duplication and segregation to two identical daughter cells during cell division. Failure to accurately segregate chromosomes during cell division results in 'aneuploidy' (an incorrect number of chromosomes) in the daughter cells. The mitotic checkpoint prevents aneuploidy by detecting aberrant or missing attachments between spindle microtubules and kinetochores, multi-protein structures that assemble on the 'centromeres' (condensed and constricted regions) of duplicated chromosomes. Activation of the mitotic checkpoint delays cell cycle progression until all chromosomes are properly attached. Understanding how kinetochores assemble and interact with spindle microtubules—and how failures in this process trigger the mitotic checkpoint—are vital to understanding how aneuploidy occurs.

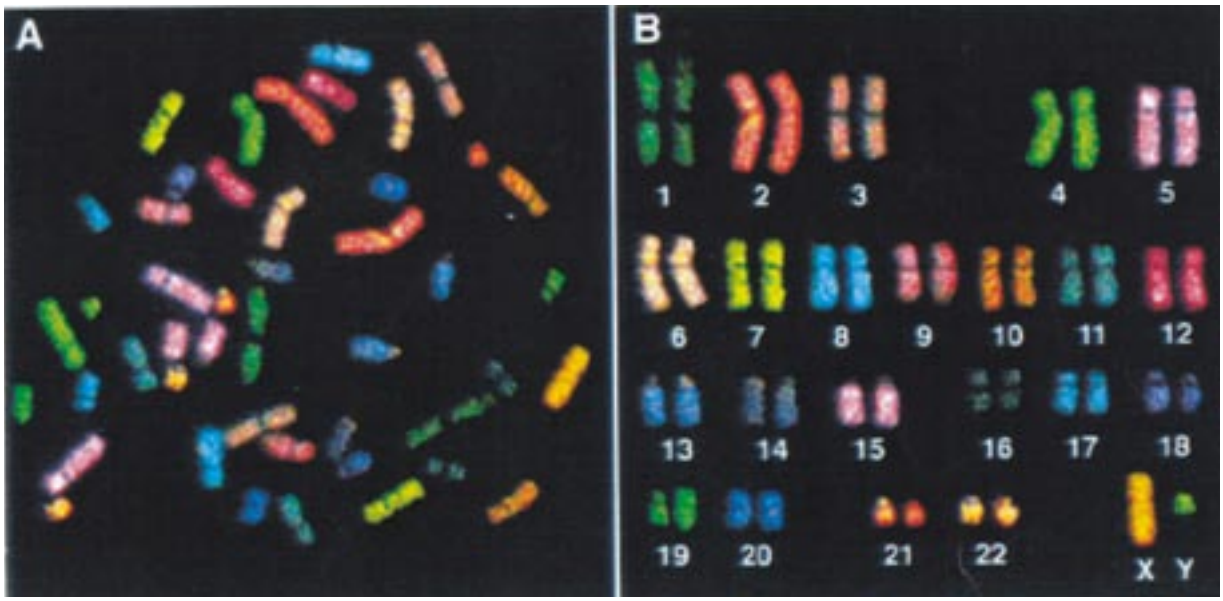
Microtubule Binding to the Kinetochore

In two landmark papers, a team from the San Diego Branch identified the proteins essential for both the kinetochore-microtubule interaction and the mechanism by which incorrect microtubule attachments are detected. While many proteins have been shown to affect the polymerization of microtubules or the stability of their attachment to the kinetochore, this study was the first to identify a network of 10 interacting proteins, the KMN network, that constitutes the essential core of the kinetochore's microtubule attachment site. The team uncovered the KMN network using a combination of biochemistry and RNAi-based screens in the worm *Caenhorabditis elegans (C. elegans)*. Subsequent reconstitution of the KMN network by expression of protein sub-complexes in bacteria, allowed the team to identify the components of the network that contribute to microtubule binding. The KMN network is comprised of the protein KNL-1, the Mis-12 protein complex, which includes the MIS-12, KBP-1, KPB-2 and KNL-3 protein subunits, and the Ndc80 complex of four proteins: Ndc80, Nuf2, Spc24 and Spc25. The findings suggest that KNL-1 and Mis12 complex together form a binding site for the Ndc80 complex. The KMN network thus has two distinct microtubule-binding activities: one involving the Ndc80/Nuf2 dimer within the Ndc80 complex, and one involving KNL-1. Current chemotherapies target microtubules in all cells of the body, which causes both side-effects and lowered drug efficacy. These kinetochore-forming proteins represent potential new targets for cancer chemotherapies, as their disruption will specifically target dividing cells.

Cheeseman IM, Chappie JS, Wilson-Kubalek EM, Desai A. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 2006 127(5):983-97.

The mitotic checkpoint detects the absence of kinetochore-microtubule attachments, but a mechanism to detect aberrant attachments is also required. The correction of improper microtubule attachments requires the Aurora B kinase, which detaches microtubules from the kinetochore, allowing microtubule binding and detachment to occur until an appropriate attachment is made. To detect aberrant attachments, Aurora B senses whether kinetochores are under tension. 'Biorientation,' which occurs when spindles pull the kinetochores in opposite directions, places the kinetochore-microtubule connection under tension, while 'syntelic attachments' (both kinetochores being pulled in the same direction) do not. Aurora B dissolves microtubule connections when the tension is not sensed, and the unoccupied kinetochore triggers the mitotic checkpoint. A collaboration between two groups at the San Diego Branch has now identified the centromere DNA/microtubule complex that acts as a tension sensor at the centromere. The San Diego team showed that a complex of two proteins, Bir1/Survivin and Sli15/INCENP, which controls Aurora B targeting and activation, also connects centromeric DNA to microtubules. When kinetochore tension is absent, the Bir1/Sli15 complex activates Aurora B, which phosphorylates components of both the core attachment and the tension-sensing complex to release the kinetochore from the microtubule. The team has postulated a model—based on these and other experimental studies—in which the core attachment is the Ndc80 complex described above. The Ndc80 complex binds directly to microtubules but its affinity is reduced by Aurora B-directed phosphorylation.

Sandall S, Severin F, McLeod IX, Yates JR 3rd, Oegema K, Hyman A, Desai A. A Bir1-Sli15 complex connects centromeres to microtubules and is required to sense kinetochore tension. Cell 2006 127(6):1179-91.



Visualization of human chromosomes in a cell arrested during mitosis (left image). There are two copies of chromosomes 1 to 22, while the sex chromosome pair may be XX (female) or XY (male), as shown in the right image. An aneuploid cell would have fewer or more than the 46 chromosomes shown in this example.

Nondisjunction and Aneuploidy

Aneuploidy is the most common characteristic of solid tumor cancer cells, and has been associated with acquired resistance to some cancer chemotherapies. However, it is not known whether aneuploidy contributes to, or is a consequence of, tumor formation. Precisely how aneuploidy occurs is also a subject of some debate. Nondisjunction, in which one pair of chromosomes fails to segregate accurately, was considered for many decades to result in one daughter cell gaining a chromosome (N+1) and the other daughter cell losing a chromosome (N-1). In 2005, this theory was challenged by researchers claiming that nondisjunction causes 'tetraploidy' (two sets of chromosomes in one cell, 4N). However, an LICR study from the San Diego Branch has refuted this theory. The group depleted the mitotic checkpoint protein CENP-E and analyzed the chromosome content of the resulting cells, a proportion of which made it through the cell cycle despite having chromosome segregation defects. The results showed that almost all instances of aneuploidy had gains or losses of one or two chromosomes, confirming that nondisjunction does indeed cause simple N+1/N-1 cells.

Weaver BA, Silk AD, Cleveland DW. Cell biology: nondisjunction, aneuploidy and tetraploidy. Nature 2006 442(7104):E9-10.

New Complexes to Stabilize the Kinetochore

The centromere is a region of 'chromatin,' the DNA/protein super-structure that packages the cell's genome, and is characterized by the presence of DNA complexed with the protein CENP-A. Investigators from the San Diego Branch this year discovered that CENP-A recruits a nucleosome associated complex (NAC) comprised of three previously unknown centromere proteins. The team also showed that seven new CENP-A nucleosomal distal (CAD) centromere components assemble on the CENP-A NAC. While the CENP-A NAC complex and associated CAD proteins are required for stabilizing the microtubule attachment, they do not appear to be involved in the mitotic checkpoint.

Foltz DR, Jansen LE, Black BE, Bailey AO, Yates JR 3rd, Cleveland DW. The human CENP-A centromeric nucleosome-associated complex. Nature Cell Biology 2006 8(5):458-69.

A second group from the San Diego Branch extended their work on kinetochore proteins identified in *C. elegans* to the analysis of the human equivalents. The team found that four proteins—Mis12, Dsn1, Nnf1 and Nsl1—form a discrete complex required for chromosome alignment. Cells depleted of the complex have kinetochore assembly defects, with reduced levels of inner and outer kinetochore proteins. The levels of a mitotic checkpoint protein, BubR1, were lower too, suggesting a cause for the compromised mitotic checkpoint function. The cells also exhibited defective chromosome biorientation.

Kline SL, Cheeseman IM, Hori T, Fukagawa T, Desai A. The human Mis12 complex is required for kinetochore assembly and proper chromosome segregation. Journal of Cell Biology 2006 173(1):9-17.

Expanding on Chromosome Condensation

As cells enter mitosis, their chromosomes are condensed to facilitate their segregation into daughter cells. A collaborative venture between two teams at the San Diego Branch developed a novel method to quantitatively analyze chromosome condensation in living cells. The ability to quantitatively evaluate the functional effects of disrupting specific proteins is invaluable for understanding mitosis. To monitor condensation in living cells, the team developed an image analysis based method to detect progressive changes in the fluorescence intensity distribution of 'green fluorescent protein' (GFP) fused to a chromatin protein. To analyze the effects of depleting key chromosomal proteins on condensation, the team used the *C. elegans* embryo as a model system. First, the LICR investigators showed that condensation occurs with biphasic kinetics; the first step (primary condensation) condenses diffuse chromatin into discrete linear chromosomes, and the second step (secondary condensation) compacts the chromosomes into even shorter structures. When condensin complexes, which are critical for proper condensation, were disrupted, the primary phase of chromatin compaction failed, and discrete chromosomes were not formed at all. Surprisingly, the team found that depleting CENP-A delayed chromatin condensation and also resulted in a failure of discrete chromosome formation. Depletion of the protein CENP-C, which blocks recruitment of all kinetochore proteins excepting CENP-A, had only a slowed primary phase and discrete chromosomes were able to form. Taken together, these findings suggest that CENP-A has a role in condensation that is independent of its role in kinetochore assembly.

The paper describing this study was designated a 'Must Read,' by '*Faculty of 1000 Biology*,' a group of over 1000 scientists that highlight the most interesting papers in biology.

Maddox PS, Portier N, Desai A, Oegema K. Molecular analysis of mitotic chromosome condensation using a quantitative time-resolved fluorescence microscopy assay. Proceedings of the National Academy of Sciences USA 2006 103(41):15097-102.

New Host Institution for São Paulo Branch

The São Paulo Branch, established at the Hospital do Cancer in 1983, commenced relocation to a new host institution in the city of São Paulo, the Hospital Alemão Oswaldo Cruz (HAOC) in 2006.

The HAOC is recognized as being one of the best hospitals in São Paulo.

Analyzing Immunological Responses Following Cervical Cancer Vaccination

Investigators at the São Paulo Branch were instrumental in clinical research on a vaccine against four strains of human papillomavirus (HPV) that cause genital warts and 70% of cervical cancer cases. The vaccine, Gardasil™ (Merck & Co.), was approved by regulatory bodies around the world in 2006.

This year, São Paulo Branch investigators led two studies that reported results from a clinical trial designed to assess both the 'immunogenicity' (ability to stimulate an immunological response) and the duration of the clinical efficacy of the HPV vaccine. The first study analyzed the immunogenicity of the vaccine in more than 1000 women over three years. The vaccine was highly efficacious against genital warts and cervical intraneoplasia (the precursor to cervical cancer) caused by the four HPV types, and the clinical efficacy extended over the three years of the study. The second study tracked a subset of more than 240 of these women through five years. The results revealed that the vaccine reduced 96% of persistent infection or disease resulting from the HPV types in the vaccine, and that the immunological protection persisted for at least five years. Characterizing the nature and duration of immunological responses will assist in decision-making about when, and how frequently, women should be immunized to protect them against cervical cancer.

Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, Brown DR, Ferenczy A, Harper DM, Koutsky LA, Kurman RJ, Lehtinen M, Malm C, Olsson SE, Ronnett BM, Skjeldestad FE, Steinwall M, Stoler MH, Wheeler CM, Taddeo FJ, Yu J, Lupinacci L, Railkar R, Marchese R, Esser MT, Bryan J, Jansen KU, Sings HL, Tamms GM, Saah AJ, Barr E. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine 2006 24(27-28):5571-83.

Villa LL, Costa RL, Petta CA, Andrade RP, Paavonen J, Iversen OE, Olsson SE, Hoye J, Steinwall M, Riis-Johannessen G, Andersson-Ellstrom A, Elfgren K, Krogh G, Lehtinen M, Malm C, Tamms GM, Giacoletti K, Lupinacci L, Railkar R, Taddeo FJ, Bryan J, Esser MT, Sings HL, Saah AJ, Barr E. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. British Journal of Cancer 2006 95(11):1459-66.

LICR Cancer Initiatives

Much of our understanding of cancer has come from the detailed and systematic laboratory-based analysis of the structure, function and interactions of single molecules. There is still much to be learned from the approach of carefully dissecting signaling pathways in cultured cells and/or model organisms to make and explore cancer research findings. However, LICR believes that it is imperative for continued progress in our understanding and control of human cancer to study the disease in the light of the reality that it affects human tissues composed of multiple, interactive human cells.

LICR has established 'Cancer Initiatives,' within which LICR investigators meet and share data and knowledge relevant to the tumor-type being studied. Investigators in the Cancer Initiatives have access to pedigreed tumor samples that are matched with normal samples from the same patient in many cases. The objective of each Cancer Initiative is to understand the details of tumor biology in the context of the organ in which the tumor arises.

LICR Brain Cancer Initiative

Most primary brain tumors, *i.e.* those that arise in the brain, originate from glia cells and are thus known as 'gliomas.' The most common and most malignant of the gliomas is the form 'gliobastoma multiforme' (GBM), which accounts for 12-15% of all intracranial tumors world-wide. The causes of GBM are not known, the prognosis is generally extremely poor and there have been no major advances in the disease's treatment for several decades.

PI3K Mutations in Gliobastoma Multiforme

Several studies have reported varying incidences of mutations in the gene *PIK3CA*, which encodes the p110a PI3K subunit (see pp40-44), in adult cases of GBM. In 2006, the first paper from 'Ludwig Cancer' (see pp4-5) described a study in which *PIK3CA* was sequenced in samples from 73 pediatric and adult GBM patients. The team—formed by investigators from the Ludwig Center at Johns Hopkins, the New York Branch, and Affiliates in São Paulo (Brazil)—found that *PIK3CA* mutations were present in 21% and 17% of pediatric and adult samples, respectively. This was the first study to find *PIK3CA* mutations in pediatric GBM, and several of the mutations detected in the study had not been described previously. The significant incidence of *PIK3CA* mutations suggests that therapeutic targeting of the PI3K pathway may be of value for patients with GBM.

Gallia GL, Rand V, Siu IM, Eberhart CG, James CD, Marie SK, Oba-Shinjo SM, Carlotti CG, Caballero OL, Simpson AJ, Brock MV, Massion PP, Carson BS Sr, Riggins GJ. PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. Molecular Cancer Research 2006 4(10):709-14.

We now know that cancer is a term that describes multiple diseases. And we have reached the point where tumors must be considered as organs, with each tumor type having its own structure and environment. LICR believes that further progress in cancer control will come from comprehensive studies of specific tumor-types in the cancer patient.

LICR Breast Cancer Initiative

Breast cancer is the most common form of cancer in women, and is the second most common cause of cancer death in women (after lung cancer). Advances have been made in breast cancer survival, with early diagnosis, better adjuvant therapies, and increased understanding of the impact of life-style on prevention, all having an effect on mortality and morbidity. However, breast cancer still causes enormous sociological, psychological and economic burdens, and more effective means of detection and treatment are required.

Detecting Early Genetic Changes in Breast Cancer

A collaboration between the London and São Paulo Branches has investigated DNA methylation in breast cancer samples. DNA methylation, a chemical modification of DNA in which methyl groups are added to the DNA base, cytosine (C), helps to maintain genome stability and normal gene expression. Hypomethylation, a decrease in DNA methylation, is associated with genome instability and is frequently found in tumor samples. The LICR team compared the methylation profiles of normal and breast cancer samples, and identified a novel, differentially methylated DNA fragment, SATR-1, that was hypomethylated in 86% of the breast tumors sampled. Assessment of the methylation status of the DNA around this fragment showed variable hypomethylation. The team's hypothesis—based on statistical analyses of the degree of hypomethylation—is that SATR-1 hypomethylation frequently occurs in the early stages of breast tumor development. Identification of early-stage changes might allow the development of more effective diagnostic tests than currently available.

Costa FF, Paixao VA, Cavalher FP, Ribeiro KB, Cunha IW, Rinck JA Jr, O'Hare M, Mackay A, Soares FA, Brentani RR, Camargo AA. SATR-1 hypomethylation is a common and early event in breast cancer. Cancer Genetics and Cytogenetics 2006 165(2):135-43.

Breast Cancer Markers for Diagnostic Tests

Investigators from the Uppsala Branch used sophisticated proteomics technologies to attempt to identify new markers for breast and ovarian cancers. Comparing blood samples from 79 women with breast cancer, 39 women with ovarian cancer and 31 women without cancer, the team found three proteins that were aberrantly expressed only in the samples from women with cancer. Further testing on the validity of these markers for cancer diagnosis is required. However, the results are encouraging for the development of relatively non-invasive, multi-parameter diagnostic/prognostic tests using blood protein profiling.

Lomnytska M, Dubrovska A, Hellman U, Volodko N, Souchelnytskyi S. Increased expression of cSHMT, Tbx3 and utrophin in plasma of ovarian and breast cancer patients. International Journal of Cancer 2006 118(2):412-21.

Setting the Transcriptome of Normal and Malignant Breast Epithelial Cells

A landmark paper from the Breast Cancer Initiative has established the 'transcriptome' (gene expression profile) of normal and malignant epithelial breast cells. The LICR team—composed of investigators from the London, New York and Lausanne Branches and Affiliates in London (UK)—purified and enriched specific cell types from normal and cancer samples. The team then used five different expression profiling technologies to identify gene transcripts differentially expressed in the samples. Consolidating the data from all five profiling experiments—the first study to utilize such an extensive multi-platform approach—allowed the investigators to produce and refine a master data set that provides a basis for the identification of possible new targets for breast cancer diagnosis, prognosis and therapy.

The first gene from the study to be analyzed in detail is *periostin*, one of a group of up-regulated genes believed to be involved in skeletal development and also breast cancer metastasis. The expression of the Periostin protein was analyzed by immunohistochemistry on additional breast tissue samples, and was found to correlate with poor outcome in a cohort of women with estrogen receptor (ER)-positive tumors.

Grigoriadis A, Mackay A, Reis-Filho JS, Steele D, Iseli C, Stevenson BJ, Jongeneel CV, Valgeirsson H, Fenwick K, Iravani M, Leao M, Simpson AJ, Strausberg RL, Jat PS, Ashworth A, Neville AM, O'Hare MJ. Establishment of the epithelial-specific transcriptome of normal and malignant human breast cells based on MPSS and array expression data. Breast Cancer Research 2006 8(5):R56.

Tumor Banks and Cancer Research

A central need of any truly effective cancer research enterprise is the establishment of documented and characterized tumor banks containing tumor specimens and 'matched' normal tissue and blood from cancer patients. The American Association for Cancer Research reports that a 2002 think-tank cited "a shortage of high-quality human tumor and tissue samples (as) the top barrier to progress in cancer research" (CR Magazine: Collaborations -> Results. Vol 1(1) 2006). Although tumor banks exist, few have specimens that have been fully 'pedigreed' with respect to detailed information about the tumor, the patient, the course of the disease and the patient's response to therapy, and most are focused on one tumor type only. Comparing samples from the same patient removes artefacts caused by gender, race, age, environment (exposure to toxins), lifestyle (smoking, obesity), cancer therapy and so on. With matched samples, the potentially tiny changes detected in DNA, RNA, proteins or biochemical signaling can be confidently ascribed to involvement in cancer metastasis.

Tissue banks are created and maintained by nearly all hospitals that have specialized surgeons. The surgeons who collect the tissues—and not the hospital that stores them—are typically in charge of the banks, although the hospital and its internal review board scrutinize and approve projects that use the tissues. LICR has brokered access to several tumor banks via collaborative agreements with physicians in charge of the banks. Central coordination ensures that strict regulatory oversight is maintained on the use of human samples.

This model creates a partnership that harnesses the physician's knowledge of the patients and the disease with the scientist's research expertise and technologies. The physician supplies access to the tissues, and participates in the conceptual development of the study and interpretation of the results. The LICR investigator is also involved in conceptual development and interpretation and, additionally, conducts the experiments on the tissues/samples. Including the physicians, surgeons and pathologists responsible for the construction of the tissue banks leads to these health professionals being integral parts of the overall study rather than simply resource providers. Gaining their first-hand experience of the disease and current treatments enhances the potential of the entire initiative.

GlaxoSmithKline/LICR License & Collaboration

In 2006, LICR and GlaxoSmithKline (GSK), the world's second-largest pharmaceutical company, announced the signing of an agreement whereby GSK licensed a substantial portfolio of cancer antigens from LICR. Cancer antigens are molecular fragments displayed on the surface of cancer cells and recognized by the human immune system. This licensing agreement follows a long-standing collaboration between both parties on GSK's investigational MAGE-A3 Antigen Specific Cancer Immunotherapeutic (ASCI)—another term for a therapeutic cancer vaccine—in non-small cell lung cancer (NSCLC).

The Cancer Vaccine Collaborative (see p33) conducted a pilot study of the same MAGE-A3 vaccine, also with a NSCLC patient population, from which a detailed survey of the immunological response to the vaccine was obtained. The resulting immunological data provided strong evidence in support of combining GSK's proprietary adjuvant with the MAGE-A3 cancer antigen in a trial designed to assess clinical endpoints.

GSK announced both the agreement and the interim results of a randomized phase II trial of the MAGE-A3 ASCI at the 2006 Annual Meeting of the American Society of Clinical Oncology (ASCO) in June. In the trial, NSCLC patients who had their primary tumors removed surgically were given either the ASCI or a placebo. Interim analyses indicated that there was a 33 percent relative reduction in risk of cancer recurrence in patients treated with MAGE-A3 ASCI compared to patients receiving the placebo. GSK is planning to initiate a Phase III trial in 2007.

Cancer Antigen Identification and Characterization

One of the obstacles in treating cancer is that cancer cells appear to be almost identical to normal cells. Conventional chemotherapeutic drugs are unable to differentiate between cancer cells and normal cells, and thus frequently cause side-effects by also targeting healthy tissues. The human immune system is able to distinguish between normal and cancer cells by recognizing 'cancer (or tumor) antigens,' molecules generated by the cancer cell and presented, on the cell surface, to the immune system. LICR is investigating two immunotherapy modalities based on cancer antigens. The first is based on antibodies that bind to the antigen to visualize the tumor (when labeled with a radioisotope), deliver a cytotoxic pay-load (when conjugated with a radioisotope or toxin), or induce an immune response against the cancer cell. The second modality is that of therapeutic cancer vaccines that induce an immune response against the cell displaying a cancer antigen. The identification and analysis of expression of cancer antigens is necessary to determine which antibody or which vaccine constitution will be most effective for particular tumor types.

New Cancer/Testis Antigen Family Discovered

Cancer/testis (CT) antigens are a subgroup of cancer antigens 'expressed' (produced) in normal germline cells (testis, placenta and embryological ovary) and in various types of tumors. Because of their restricted expression in normal tissues, several therapeutic cancer vaccines based on CT antigens are in early-phase clinical trials, with some of those clinical trials being conducted by LICR (see 'Cancer Vaccines,' pp30-33). A collaboration between investigators at the New York and São Paulo Branches has discovered and characterized a new family of CT antigens. The archetypal member is the protein CTSP-1, which is expressed in normal testis and in a variety of different tumor types, particularly melanoma, prostate and lung. Other members of the CTSP gene family also have a highly restricted expression pattern. Antibodies against the gene family members were detected in the plasma of 10% of 141 patients with cancer—particularly those patients with prostate, thyroid and breast tumors—indicating that the antigen is capable of eliciting spontaneous immune responses. Taken together, these results suggest that CTSP-1 is a promising candidate for cancer immunotherapy.

Parmigiani RB, Bettoni F, Vibranovski MD, Lopes MH, Martins WK, Cunha IW, Soares FA, Simpson AJ, de Souza SJ, Camargo AA. Characterization of a cancer/testis (CT) antigen gene family capable of eliciting humoral response in cancer patients. Proceedings of the National Academy of Sciences USA 2006 103(48):18066-71.

Physical Interaction Between Cancer/Testis Antigens

CT antigens can be divided into two groups; those whose genes are encoded on the X chromosome (CT-X antigens) and those that are not (non-X CT antigens). CT-X genes are often expressed in a coordinated manner in cancer cells, and their expression is associated with poor outcome in several tumor types. A collaboration between investigators at the New York and São Paulo Branches and Affiliates in New York (USA) found that two CT-X genes, MAGE-C1 and NY-ESO-1, physically interact and co-localize in melanoma cells. This is the first report of direct interaction between CT antigens. The relevance of this finding for cancer is being explored.

Cho HJ, Caballero OL, Gnjatic S, Andrade VC, Colleoni GW, Vettore AL, Outtz HH, Fortunato S, Altorki N, Ferrera CA, Chua R, Jungbluth AA, Chen YT, Old LJ, Simpson AJ. Physical interaction of two cancer-testis antigens, MAGE-C1 (CT7) and NY-ESO-1 (CT6). Cancer Immunity 2006 6:12.

Cancer/Testis Antigens in Bladder Cancer

Investigators from the New York Branch and Affiliates in Houston (USA) measured the expression of nine CT antigens in a panel of tumor samples from 95 patients with high-grade urothelial carcinoma, a tumor-type that accounts for more than 90% of all bladder cancers. The team found that at least one of the nine CT antigens was expressed in 77% of the cancer samples, with 61% expressing more than one CT antigen. Additionally, the expression of one of the antigens, CT10, appeared to correlate with disease-free survival of the patients, indicating that it may have prognostic value. These findings indicate a cancer vaccine targeting several CT antigens may be of therapeutic value for patients with bladder cancers.

Sharma P, Shen Y, Wen S, Bajorin DF, Reuter VE, Old LJ, Jungbluth AA. Cancer-testis antigens: expression and correlation with survival in human urothelial carcinoma. Clinical Cancer Research 2006 12(18):5442-7.

Characterization of Cancer Antigen Expression in Melanoma

The Cancer Vaccine Collaborative (see p33) is testing multiple melanoma vaccines based on CT antigens or 'differentiation' antigens, which are expressed in normal and malignant cells derived from one particular cell type. Investigators at the Melbourne Center have completed a large study to characterize the expression of three CT antigens, MAGE-A1, MAGE-A4 and NY-ESO-1, plus three differentiation antigens, gp100, tyrosinase and Melan-A, in melanoma. First, the team found that CT antigens are not highly expressed in desmoplastic melanoma, a rare form of melanoma, suggesting that a therapeutic cancer vaccine may not be efficacious for this form of cancer. The team then studied a series of over 500 malignant melanoma samples taken from both primary (original) tumors and metastatic (*i.e.* those that had spread from the primary) tumors, including multiple, sequential samples from 86 patients. This is the largest published series of primary and metastatic melanoma samples in which the expression of different classes of cancer antigens has been evaluated. Crucially, the results from the study reveal that antigen expression changes as the cancer metastasizes: the expression of some antigens is lost and the expression of others begins to be detectable. Taken together, the results of these studies provide critical information for selecting patients and antigens for immunotherapy clinical trials for melanoma.

Lim E, Browning J, MacGregor D, Davis ID, Cebon JS. Desmoplastic melanoma: comparison of expression of differentiation antigens and cancer testis antigens. Melanoma Research 2006 16(4):347-55.

Barrow C, Browning J, MacGregor D, Davis ID, Sturrock S, Jungbluth AA, Cebon J. Tumor antigen expression in melanoma varies according to antigen and stage. Clinical Cancer Research 2006 12(3 Pt 1):764-71.

An Inducible Mouse Model of Melanoma

In 2006, investigators from the Brussels Branch generated a mouse model in which melanoma that expresses a defined CT antigen can be induced. This genetic engineering approach may provide an invaluable preclinical model to study tumors. Most preclinical models available today are based on either spontaneous (mouse) melanomas that don't express a defined antigen or on transplantable tumors that express a defined antigen. The problem with transplantable tumors is that they have not developed in the natural tissue microenvironment and thus may not recapitulate the long-term interaction between the cancer cells, the immune system and the host tissues. This model can be used to study basic cancer immunology mechanisms, including the tumor's resistance to the immune system and tumor rejection following vaccination.

Huijbers IJ, Krimpenfort P, Chomez P, van der Valk MA, Song JY, Inderberg-Suso EM, Schmitt-Verhulst AM, Berns A, Van den Eynde BJ. An inducible mouse model of melanoma expressing a defined tumor antigen. Cancer Research 2006 66(6):3278-86.

Cancer Antigen Processing

To fight cancer or infection, the immune system must first identify cells as either 'self' or 'foreign,' with the latter category including self cells that have become malignant or infected with a pathogen. The basis of recognition of self and foreign cells lies in 'antigens,' molecules or molecular fragments that are displayed on the cell surface—within the major histocompatibility complex (MHC)—for recognition and binding by T cells. Antigenic 'peptides' (protein fragments) are generated from proteins that are expressed by pathogens or cancer cells, but not by normal cells. Understanding how proteins are processed into antigenic peptides can inform the development and optimization of vaccines for both pathogens and cancer.

Differential Antigen Processing

The multi-protein complex responsible for antigen processing is known as the proteasome. There are two proteasome forms: one is the standard proteasome found in most cells, while the other is the immunoproteasome, which differs from the standard proteasome by three protein subunits (see figure). The immunoproteasome-specific subunits are induced by the cytokine, interferon γ (IFNγ), in most cell types, but are expressed constitutively in dendritic cells (DCs). DCs are the cells that stimulate antigen-specific CD8+ T cell responses. The immunoproteasome was long considered to be the form that preferentially produces antigenic peptides, in part because of its higher protein-cleaving activity. While some antigenic peptides are indeed produced preferentially by the immunoproteasome, a 2000 LICR study showed that others are more efficiently produced by the standard proteasome. Two more LICR studies have now elucidated the mechanism of differential processing by the two proteasome forms.

The prevailing view has been that antigen production is dependent on the efficiency with which a full-length protein is cleaved to form the antigenic peptide. However, a study from the Brussels Branch has shown that both proteasome forms destroy certain antigens by cleaving full-length proteins within the antigenic peptide. These results have important implications for designing cancer vaccines (see pp30-33), in particular the form of antigen used in the vaccine and also the modality of the vaccine delivery. For example, two of the peptides shown to be destroyed by the immunoproteasome, $gp100_{209\text{-}217}$ and $tyrosinase_{369\text{-}377}$, are in clinical trials as candidate therapeutic cancer vaccines. These results suggest that an immunization procedure that bypasses DC, and thus immunoproteasome exposure, is required to elicit an antigen-specific CD8+ T cell response with these vaccines.

Chapiro J, Claverol S, Piette F, Ma W, Stroobant V, Guillaume B, Gairin JE, Morel S, Burlet-Schiltz O, Monsarrat B, Boon T, Van den Eynde BJ. Destructive cleavage of antigenic peptides either by the immunoproteasome or by the standard proteasome results in differential antigen presentation. Journal of Immunology 2006 176(2):1053-61.

A collaborative study, between the Lausanne and Brussels Branches and Affiliates in New York (USA), investigated the impact of differential processing of the Melan-A protein *in vivo*. Melan-A is a 'differentiation' antigen, being expressed in normal and malignant cells derived from melanocytes (pigment-producing skin cells). Since mouse and human Melan-A have the same expression pattern and share a high degree of sequence identity, differential antigen processing can be analyzed in a mouse model that lacks immunoproteasome subunits. Melan-A, which is in early-phase clinical trials for a therapeutic melanoma vaccine, elicits weak CD8+ T cell responses after immunization of normal mice. However, Melan-A elicits potent T cell responses when it is processed by DCs lacking immunoproteasomes. Interestingly, the 2006 Brussels Branch study (left) found that antigenic peptides from ubiquitous or differentiation proteins were processed poorly by the immunoproteasome, in contrast to cancer antigen proteins that are less 'self-like.' This *in vivo* study indicates that poor processing of 'self-like' antigenic peptides by the immunoproteasome might be the mechanism preventing spontaneous T cell responses against self antigens. Vaccine modalities requiring antigen processing by DCs will have to incorporate these new findings.

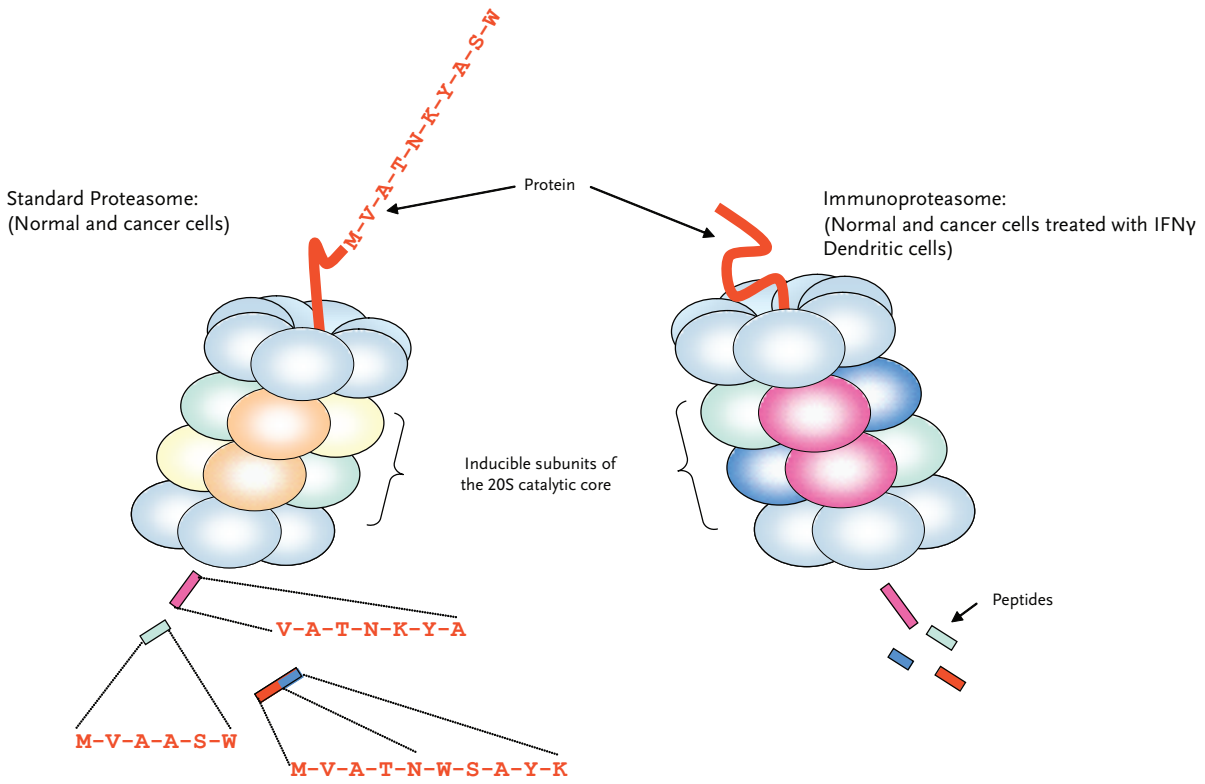
Chapatte L, Ayyoub M, Morel S, Peitrequin AL, Levy N, Servis C, Van den Eynde BJ, Valmori D, Levy F. Processing of tumor-associated antigen by the proteasomes of dendritic cells controls in vivo T-cell responses. Cancer Research 2006 66(10):5461-8.

The Immunoproteasome and Immunodominance

Antigen processing is a factor in determining 'immunodominance,' the phenomenon of a hierarchy based on the magnitude of the CD8+ T cell response elicited by each 'epitope' (the specific sequence, within the antigenic peptide, that is recognized by the T cell). Antigen processing creates, with varying efficiencies, a variety of peptides and thus plays a major role in establishing CD8+ T cell immunodominance and 'repertoire' (range of T cells each recognizing a different epitope). Other factors that may influence immunodominance are the levels of antigen/MHC complexes on antigen presenting cells, such as DC, and the 'avidity' (strength) of T cell binding to each antigen/MHC complex.

A team from the Melbourne Center used mouse models deficient in various immunoproteasome subunits to investigate how the level of antigen presentation and the T cell repertoire are governed in the well-defined immunodominance hierarchy that follows infection with the influenza A virus. The study showed that antigen presentation and the repertoire of CD8+ T cells work together to shape the immunodominance hierarchy. A mathematical model that interprets how hierarchies arise and change was generated. The team also demonstrated that increasing antigen presentation can overcome an impaired repertoire. Both of these study outputs provide valuable information for devising vaccination strategies to manipulate immunodominance.

Pang KC, Sanders MT, Monaco JJ, Doherty PC, Turner SJ, Chen W. Immunoproteasome subunit deficiencies impact differentially on two immunodominant influenza virus-specific CD8+ T cell responses. Journal of Immunology 2006 177(11):7680-8.



Immunodominance and Cancer Vaccines

The immunodominance of many pathogens has been established, but little is known about the immunodominance of cancer antigen epitopes. In 2006, two complementary studies analyzed the immunodominance hierarchy of the CT antigen, NY-ESO-1, which is in early-phase clinical trials as part of the Cancer Vaccine Collaborative (p33). Both studies analyzed the antigen-specific CD4+ and CD8+ T cell responses, as well as the different forms of the MHC molecules presenting the antigenic peptides.

The first study, conducted by a collaboration between the Melbourne Center and the Lausanne and New York Branches, comprehensively analyzed NY-ESO-1-specific T cells in a patient who had a spontaneous T cell response to the antigen. The responses for both CD4+ and CD8+ T cells were broad and hierarchical, similar to a typical antiviral response. The second study, a collaboration between the Lausanne and New York Branches and Affiliates in New York (USA) and Frankfurt (Germany) analyzed the blood of melanoma patients, some of whom had received a NY-ESO-1 vaccine, to identify NY-ESO-1 epitopes and assess antigen processing *in vitro*. The investigators discovered three immunodominant regions, each of which was shown to undergo efficient proteasomal processing. These immunodominant regions co-localized with sequences that have significant binding potential for multiple MHC molecules. The results suggest the co-localization of putative MHC binding and proteasome cleavage sites in a protein's amino acid sequence might identify immunodominant regions in cancer antigens.

Taken together, these studies suggest another strategy for the design of new cancer vaccines. Engineering a protein that strategically combines immunodominant CD8+ and CD4+ epitopes with affinity for the most common MHC molecules might yield a vaccine that serves a wide population of prospective patients and is also easier to produce than the full length protein. Importantly, combining immunodominant epitopes from several different cancer antigens might increase the efficacy of therapeutic cancer vaccines while diminishing the cancer cell's ability to down-regulate the production of cancer antigen expression in order to evade immune responses.

Jackson H, Dimopoulos N, Mifsud NA, Tai TY, Chen Q, Svobodova S, Browning J, Luescher I, Stockert L, Old LJ, Davis ID, Cebon J, Chen W. Striking immunodominance hierarchy of naturally occurring CD8+ and CD4+ T cell responses to tumor antigen NY-ESO-1. Journal of Immunology 2006 176(10):5908-17.

Valmori D, Levy F, Godefroy E, Scotto L, Souleimanian NE, Karbach J, Tosello V, Hesdorffer CS, Old LJ, Jager E, Ayyoub M. Epitope clustering in regions undergoing efficient proteasomal processing defines immunodominant CTL regions of a tumor antigen. Clinical Immunology 2006 122(2):163-72.

Antigen splicing: Pivotal LICR studies upset a paradigm

Genes have stretches of protein-coding DNA sequences interspersed with stretches of non-coding DNA sequences. When a gene is expressed, the entire DNA sequence is transcribed into RNA and the RNA is then 'spliced' such that the non-coding sequences are removed and the coding sequences are joined in a linear fashion. The resultant 'messenger RNA' (mRNA) molecule is the template upon which the protein's amino acid sequence is based. RNA splicing has long been thought to be the only process in which the 'DNA to RNA to protein' sequence linearity is disturbed.

However, investigators from the Brussels Branch were the first to show—in 2004—that the proteasome is able to splice peptides together such that the antigen sequence is non-contiguous, *i.e.* a small segment of the original protein sequence is omitted. In 2006, the team extended this ground-breaking research to establish that antigen processing can also splice peptides together such that the antigen sequence is not linear with the DNA sequence of the original protein, *i.e.* the peptide fragments can be scrambled or flipped before being spliced together.

These findings are of great interest because they demonstrate a variation in what was thought to be a fundamental feature of the universal genetic code. They are also significant for human disease. First, synthetic peptides being investigated in early-phase clinical trials of therapeutic cancer vaccines—and immune monitoring technologies to monitor their effects—are typically designed based on the gene sequence of cancer-specific proteins. However, modification by post-translational splicing means that antigen sequences are not predictable. More importantly, this mechanism significantly extends the number of antigenic peptides that can be produced from a single protein, and therefore widens the applicability of peptide vaccines against cancer and infectious diseases.

Vigneron N, Stroobant V, Chapiro J, Ooms A, Degiovanni G, Morel S, van der Bruggen P, Boon T, Van den Eynde BJ. An antigenic peptide produced by peptide splicing in the proteasome. Science 2004 304(5670):587-90.

Warren EH, Vigneron NJ, Gavin MA, Coulie PG, Stroobant V, Dalet A, Tykodi SS, Xuereb SM, Mito JK, Riddell SR, Van den Eynde BJ. An antigen produced by splicing of noncontiguous peptides in the reverse order. Science 2006 313(5792):1444-7.

T Cell Development and Function

To be able to develop clinically effective cancer vaccines, one must understand the molecular mechanisms behind the recognition of cancer cells by the immune system and the tumor's response to immunological attack. These are both largely mediated by T cells, or T lymphocytes, a group of white blood cells that are part of the immune response. There are several different T cell subsets, each with a distinct function in the immune response and each characterized by the presence of different cell surface markers. Of particular interest are the cytolytic CD8+ T cells (CTL) that destroy cancer (and virally infected) cells with the assistance of CD4+ helper T cells that secrete 'cytokines' (proteins that regulate the immune response). It is these types of T cells that are monitored routinely as part of LICR's cancer vaccine development (see pp30-33).

T Cell Differentiation: Markers and Functional Capacity

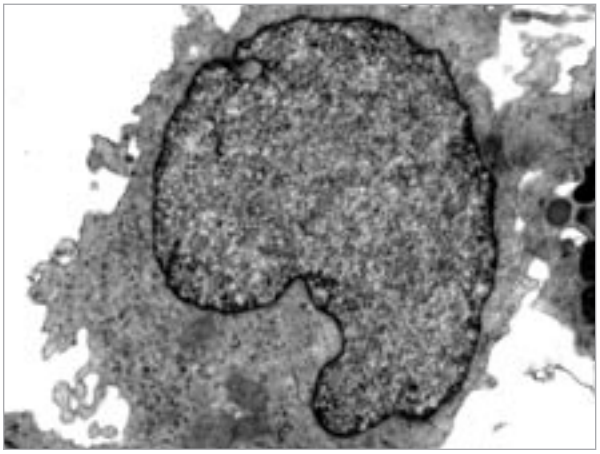
Upon their first stimulation with antigen, naïve (unspecialized) T cells differentiate into CD8+ effector T cells with cytolytic ability. This differentiation is marked by changes in the expression of cell surface molecules. The most widely-held model for the differentiation of T cells is based on the expression of the cell surface molecules CCR7 and CD45RA. The CD45RA+ CCR7- effector T cells were presented, in this model, as the ultimate stage of differentiation of CD8+ T cells. These 'terminally differentiated' effector cells were proposed as the most effective for the destruction of tumor cells and virus-infected cells, and were thought to have little capacity for proliferation. However, when investigators at the Brussels Branch studied the time-course of CCR7 and CD45RA expression on human CTL clones and blood CD8+ T cells following antigen stimulation, their findings did not support this model. The team found that CD45RA expression on CD8+ effector T cells is not a marker of terminal differentiation, incapacity to proliferate or particularly high lytic potential, but rather is indicative of the time since antigen exposure. This new concept leads to a reinterpretation of the significance of the presence of CD45RA+CCR7- effector CD8+ T cells in patients affected by viral infections or by cancer. These findings also have implications for evaluating the efficacy of immunization of cancer patients.

Carrasco J, Godelaine D, Van Pel A, Boon T, van der Bruggen P. CD45RA on human CD8 T cells is sensitive to the time elapsed since the last antigenic stimulation. Blood 2006 108(9):2897-905.

Homeostasis of Memory T cells

Antigen-specific memory CD8+ T cells are frequently identified by elevated expression of cell surface 'activation' markers, CD44 and CD122. However, mice that have not been immunized also have a subset of CD8+ T cells that express these markers, so-called $CD44^{high}$ CD8+ T cells. Antigen-specific and $CD44^{high}$ CD8+ T cells both appear to require the cytokine IL-15 for 'homeostasis' (maintenance of the cell population), but the downstream signal transduction pathways involved in homeostasis are not known. Investigators at the Lausanne Branch generated a mouse model lacking one of the two 'alleles' (copies) of *c-myc*, an oncogene that regulates cell proliferation, differentiation and 'apoptosis' (programmed cell death), to study its effect on T cell development and function. The loss of one *c-myc* allele caused the mice to have a decreased growth rate, reduced organ size and, surprisingly, deficiency of the $CD44^{high}$ CD8+ T cell population. This corresponded with a loss of homeostatic proliferation of these cells and a loss of response to IL-15 stimulation. These results identify *c-myc* as a novel downstream target of IL-15 signaling in the proliferation of memory and $CD44^{high}$ CD8+ T cells.

Bianchi T, Gasser S, Trumpp A, MacDonald HR. c-Myc acts downstream of IL-15 in the regulation of memory CD8 T-cell homeostasis. Blood 2006 107(10):3992-9.



An electron micrograph of a CD8+ cytotoxic T cell.

Nature's 24 Milestones in Cancer: LICR Investigators Cited in Five of 24

The editors of the journals *Nature*, *Nature Medicine* and *Nature Reviews Cancer* have given their opinion on the '24 Milestones in Cancer Research' since the end of the nineteenth century. 'Original Research Papers' authored by LICR investigators feature in five of the 24 Milestones, with another two Milestones quoting papers authored by LICR investigators as selected 'Further Reading.'

Nature Milestones Cancer. Nature Reviews Cancer 2006 6, S7-S22.

The ATLANTIC Philanthropies

A group of LICR scientists has been awarded a grant of USD18 million over three years for clinical discovery projects in the *Antibody Targeting, Cancer Vaccine* and *Signaling Programs*. The grant is from the international foundation, *The Atlantic Philanthropies*, which has an ultimate goal of making lasting changes in the lives of disadvantaged and vulnerable people.

The partnership between *The Atlantic Philanthropies* and LICR will use the Institute's infrastructure and operating know-how to extend its successful model of vaccine and antibody development into tumor types that have not benefited from advances to date. The program will also permit LICR to extend current Branch research projects into entirely new initiatives exploring small molecule inhibitors. This is an area in which LICR has strong laboratory experience and the research has matured to the point where preclinical and clinical investigation of novel therapies is now warranted.

Cancer Vaccines

The immune system can distinguish between normal and cancer cells, and be induced to selectively destroy the latter. However, cancer may escape from immunological control if mechanisms of immune modulation or suppression are activated in the local tumor environment. LICR is using laboratory and clinical research to explore cancer vaccines as a therapeutic modality to induce a strong and sustained response against cancer antigens. The ideal response to a cancer vaccine would be 'integrated,' meaning that all components of the adaptive immune system—CD8+ T cells, CD4+ T cells and antibodies—are generated to target the antigen. The development of an effective vaccine thus requires the ability to induce and monitor both T cell and antibody responses to defined antigens

Cancer Vaccine Components

Antigen - the selection of the antigen is crucial to the efficacy of the cancer vaccine. The ideal antigen is expressed in a high percentage of cells in a tumor and has restricted expression in normal cells (see pp22-23).

Antigen Constitution - the form of the antigen—a full-length protein, a 'peptide' (protein fragment), or DNA or RNA that encodes the protein or peptide—determines the delivery of the antigen and might have implications for how effectively the antigen is processed (see pp24-26) and presented.

Adjuvant - an agent that is added to the vaccine in order to augment the immune response to the antigen.

Delivery - vaccine delivery methods range from particle-mediated epidermal delivery—'shooting' particles coated with antigen DNA into the skin using pressurized air—to oral ingestion of an engineered bacterium.

Detection of CD8+ T Cell Responses to Vaccination *Ex Vivo*

The monitoring of vaccine-induced immune responses has typically involved the *in vitro* expansion of antigen-specific CD4+ and CD8+ T cells due to the very low numbers of T cells stimulated. However, this process involves culturing T cells in the laboratory for some time, which might lead to culture-induced changes in the T cell 'repertoire' (the range of antigen-specific T cells present) and T cell activation and differentiation (see p27). Investigators at the Lausanne Branch have now managed to achieve direct *ex vivo* detection of stimulated T cells without the need for laboratory culture. The team used a vaccine combining the adjuvant, Montanide, and synthetic peptides against the CT antigens, NY-ESO-1 and MAGE-A10, and the differentiation antigen, Melan-A (see pp22-23), to induce antigen-specific CD8+ T cells that were detectable *ex vivo*. This is the first study to show that peptide vaccines against CT antigens are able to induce *ex vivo* responses.

Baumgaertner P, Rufer N, Devevre E, Derre L, Rimoldi D, Geldhof C, Voelter V, Lienard D, Romero P, Speiser DE. Ex vivo detectable human CD8 T-cell responses to cancer-testis antigens. Cancer Research 2006 66(4):1912-6.

The Lausanne Branch team identified a single T cell clone that dominated the CD8+ T cell response to the Melan-A antigen and conducted a comprehensive analysis of the clone's properties. The CD8+ T cell clone arose prior to vaccination and its levels increased in the blood as the patient was vaccinated serially over 3.5 years. The clone was able to kill tumor cells and showed efficient homing to secondary tumors that had 'metastasized' (spread) from the original tumor site. This is the first study to show strong expansion of a cancer antigen specific T cell clonotype, which is comparable to a protective T cell response against viruses. It also indicates that serial vaccination is an important factor in the optimization of cancer immunization.

Speiser DE, Baumgaertner P, Barbey C, Rubio-Godoy V, Moulin A, Corthesy P, Devevre E, Dietrich PY, Rimoldi D, Lienard D, Cerottini JC, Romero P, Rufer N. A novel approach to characterize clonality and differentiation of human melanoma-specific T cell responses: spontaneous priming and efficient boosting by vaccination. Journal of Immunology 2006 177(2):1338-48.

Discovery of New Melan-A-specific CD4+ T Cell Epitopes

Melan-A is a possible target for therapeutic cancer vaccines against melanoma due to its restricted expression in melanoma cells and in normal (but non-essential) melanocytes. However, there has been little analysis of the repertoire of antigen-specific CD4+ T cells induced in response to the antigen. Investigators at the Lausanne Branch have now discovered two new Melan-A 'epitopes' (regions of the antigen sequence recognized by T cells) using CD4+ T cells from melanoma patients. The LICR team was also able to detect these antigen-specific CD4+ T cells *ex vivo* in the peripheral blood of melanoma patients. Further characterization showed that these new CD4+ T cell clones recognize both melanoma cells that express Melan-A and virus cells that have been engineered to artificially express the Melan-A sequences. These results suggest that the epitopes are naturally processed within the cell and presented on the cell surface for immunological recognition. The findings from this study will allow for more comprehensive monitoring of naturally occurring and vaccine-induced CD4+ T cell responses to Melan-A.

Bioley G, Jandus C, Tuyaerts S, Rimoldi D, Kwok WW, Speiser DE, Tiercy JM, Thielemans K, Cerottini JC, Romero P. Melan-A/MART-1-specific CD4 T cells in melanoma patients: identification of new epitopes and ex vivo visualization of specific T cells by MHC class II tetramers. Journal of Immunology 2006 177(10):6769-79.

Tumor Suppression of Peptide Vaccine Responses

Following an earlier finding that the presence of high levels of CD4+ CD25+ regulatory T cells (Tregs) in tumors correlates with a poor prognosis for ovarian cancer patients, investigators from the New York Branch and Affiliates in Buffalo (USA) have begun to investigate the relationship between antigen-specific CD4+ cells and Tregs. The clinical research was conducted on samples from ovarian cancer patients that had received a vaccine with a peptide, NY-ESO-$1_{157\text{-}170}$, from the cancer testis antigen, NY-ESO-1. The team assessed the origin and 'avidity' (efficacy of antigen recognition) of CD4+ T cells specific for that fragment of NY-ESO-1 in vaccinated patients. The team found that vaccine-induced CD4+ T cells had low avidity for the naturally processed NY-ESO-1 protein and were not particularly susceptible to Treg suppression. Importantly, they also found that spontaneously occurring, pre-existing CD4+ T cells with high avidity for the full-length NY-ESO-1 protein were suppressed by Tregs. These data suggest that the peptide vaccine cannot activate the pre-existing antigen-specific T cell precursor population, possibly because it is unable to overcome Treg action. Results from another study, from the Lausanne Branch, further explained T cell modulation or suppression within the tumor environment. Following vaccination with a Melan-A peptide vaccine, the Lausanne team showed that active, melanoma-specific CD8+ T cells were detectable *ex vivo* and in tumor metastases. However, the CD8+ T cells in the tumor differed from the blood T cells in functional capacity. Compounds that overcome tumor suppression of the immune response, *e.g.* by depleting Treg cells, might be necessary for successful immunological destruction of existing tumors.

Nishikawa H, Qian F, Tsuji T, Ritter G, Old LJ, Gnjatic S, Odunsi K. Influence of CD4+CD25+ regulatory T cells on low/high-avidity CD4+ T cells following peptide vaccination. Journal of Immunology 2006 176(10):6340-6.

Appay V, Jandus C, Voelter V, Reynard S, Coupland SE, Rimoldi D, Lienard D, Guillaume P, Krieg AM, Cerottini JC, Romero P, Leyvraz S, Rufer N, Speiser DE. New generation vaccine induces effective melanoma-specific CD8+ T cells in the circulation but not in the tumor site. Journal of Immunology 2006 177(3):1670-8.

Toward the Use of Bacteria in Cancer Vaccine Delivery

The bacterium, *Salmonella typhimurium (S. typhimurium),* is best known for causing typhoid fever, the historically devastating plague that is today controlled with antibiotics. Investigators from the New York Branch and Affiliates in Frankfurt (Germany), Mie (Japan) and New York (USA) assessed the ability of a strain of *S. typhimurium*, which had been engineered to produce and secrete the NY-ESO-1 CT antigen, to deliver a cancer antigen locally or systemically in a model system. This approach is attractive as it is expected the bacterium will act as a natural adjuvant to the immune system to strengthen the response against the antigen. NY-ESO-1-specific CD4+ and CD8+ T cells were produced when the bacterium was cultured *in vitro* with blood samples from patients with several tumor types. Following ingestion of the bacterium, established NY-ESO-1-expressing tumors 'regressed' (decreased in size) in mice. Furthermore, vaccination with the bacterium construct was accompanied by T cell responses against at least two other unrelated cancer antigens present in the tumor, suggesting that the NY-ESO-1 specific immunity triggered a cascade of anti-tumor responses. These preclinical analyses also showed that, as expected, the *S. typhimurium*-NY-ESO-1 vaccine was not toxic. These results open the way for a possible Phase I clinical trial of this intriguing new strategy for cancer vaccine delivery.

Nishikawa H, Sato E, Briones G, Chen LM, Matsuo M, Nagata Y, Ritter G, Jager E, Nomura H, Kondo S, Tawara I, Kato T, Shiku H, Old LJ, Galan JE, Gnjatic S. In vivo antigen delivery by a Salmonella typhimurium type III secretion system for therapeutic cancer vaccines. Journal of Clinical Investigation 2006 116(7):1946-54.

Vaccinia- and Fowlpox-Based Cancer Vaccines Induce Integrated Patient Responses

Vaccinia and fowlpox, members of the virus family, *Poxviridae*, are both highly immunogenic and capable of acting as a 'nucleic acid vector' (a vehicle for delivering DNA or RNA to a cell). LICR investigators assessed the ability of vaccinia and fowlpox that express the NY-ESO-1 cancer antigen sequence to induce an antigen-specific immune response in cancer patients. The Phase I trial—conducted by investigators from the New York Branch and Affiliates in Frankfurt (Germany), New York (USA) and Zurich (Switzerland)—included 36 patients with a variety of different tumors. The vaccines, given at different dose levels and at monthly intervals for at least four months, were well-tolerated and induced an integrated, NY-ESO-1-specific immune response in the majority of the patients.

Jager E, Karbach J, Gnjatic S, Neumann A, Bender A, Valmori D, Ayyoub M, Ritter E, Ritter G, Jager D, Panicali D, Hoffman E, Pan L, Oettgen H, Old LJ, Knuth A. Recombinant vaccinia/fowlpox NY-ESO-1 vaccines induce both humoral and cellular NY-ESO-1-specific immune responses in cancer patients. Proceedings of the National Academy of Sciences USA 2006 103(39):14453-8.

New Method to Prime Dendritic Cells for Immunotherapy

Dendritic cells (DCs) are immune cells that process and present antigens to other cells of the immune system. LICR investigators are exploring the possibility that 'immature' (not yet exposed to antigen) DCs can be collected from patients, loaded with antigens, and re-injected—as mature, antigen presenting cells—as a means of immunizing against cancer. A collaboration between the Melbourne Center and New York Branch developed a rapid method to generate large numbers of DCs loaded with peptides from the cancer-testis antigens NY-ESO-1, MAGE-A4 and MAGE-A10. Immature, 'mobilized' (stimulated to expand in population size) DCs were obtained from the blood of patients with 'resected' (surgically removed) melanoma and loaded overnight with the antigens, under Good Manufacturing Practice conditions at a specialized facility at the Melbourne Center. The DCs were then injected back into the patients over a course of several months. The team found that this method of vaccination was safe and also primed immune responses, particularly the production of antigen-specific CD8+ T cells, in melanoma patients.

Davis ID, Chen Q, Morris L, Quirk J, Stanley M, Tavarnesi ML, Parente P, Cavicchiolo T, Hopkins W, Jackson H, Dimopoulos N, Tai TY, MacGregor D, Browning J, Svobodova S, Caron D, Maraskovsky E, Old LJ, Chen W, Cebon J. Blood dendritic cells generated with Flt3 ligand and CD40 ligand prime CD8+ T cells efficiently in cancer patients. Journal of Immunotherapy 2006 29(5):499-511.

Dendritic Cells and Gene Therapy

Lentiviruses are members of the retrovirus family, which have an RNA genome rather than a DNA genome. LICR investigators from the Lausanne Branch engineered a lentivirus-based vector to deliver part of the Melan-A cancer antigen sequence, and assessed its ability to induce an antigen-specific response in mice. The lentivector induced a strong antigen-specific CD8+ T cell response that could be detected *ex vivo* and also re-activated, with a peptide vaccine, several months later. The results of the study also suggest that the lentivector induced a stronger and more sustained response than vaccination with the corresponding Melan-A peptide combined with the adjuvants, Montanide and CpG.

Chapatte L, Colombetti S, Cerottini JC, Levy F. Efficient induction of tumor antigen-specific CD8+ memory T cells by recombinant lentivectors. Cancer Research 2006 66(2):1155-60.

Cancer Vaccine Collaborative (CVC)

The CVC is an innovative partnership between two non-profit institutions, the Cancer Research Institute (CRI) and the Ludwig Institute for Cancer Research (LICR). Each of these institutions has a long and distinguished history in the field of cancer immunology and each is committed to translating laboratory discoveries in this field into therapeutic cancer vaccines. The cancer vaccine studies described in this report constitute a selection of publications from the LICR laboratory and clinical research conducted under the auspices of the CVC.

In 2004, the CVC completed a Phase I trial of the NY-ESO-1/ISCOMATRIX™ melanoma vaccine that showed antigen-specific immunological responses correlating with extended patient survival. Because of the study design and the small number of patients, the results are not considered conclusive of clinical efficacy. In 2006, therefore, the CVC initiated a randomized Phase II trial—at 18 sites in Australia, New Zealand and the United Kingdom—to evaluate further the clinical efficacy of the NY-ESO-1/ISCOMATRIX™ vaccine. In four months, 100 patients with resected melanoma and minimal residual disease were recruited. Half of the patients will receive a placebo and the other half will receive the vaccine. The patients will be followed for 18 months to assess relapse and survival.

Targeted Antibodies

An antibody is a protein that binds selectively to a cell-surface molecule. Antibodies are produced naturally by the immune system, but synthetic 'monoclonal antibodies' (mAbs) can also be designed and produced in the laboratory. LICR is generating and analyzing mAbs for cancer therapies.

Assessing Combinatorial Therapies with Targeted Antibodies

Radioimmunotherapy (RIT) is the use of a mAb to deliver a toxic dose of radiation direct to cancer cells. A limitation of RIT is the difficulty in achieving a strong dose of radiation within solid tumors, without damaging normal tissue or bone marrow. Investigators at the Melbourne Center are assessing a combined-modality RIT (CMRIT) that might reduce the dose required for efficacy. In this study, the team combined the Yttrium^{90} (^{90}Y) radiolabelled mAb ^{90}Y-hu3S193, which binds to the Le^{Y} blood group antigen, with the chemotherapy drug paclitaxel. Paclitaxel arrests cells in the G2/M phase of the cell cycle (see pp10-2), rendering them more sensitive to radiation-induced DNA damage and 'apoptosis' (programmed cell death). In a preclinical mouse model of breast cancer, CMRIT significantly reduced the size of the breast tumors, even at a low RIT dose. The next step will be to conduct an early-phase clinical trial to assess both the safety of CMRIT and whether the increased efficacy observed in the mouse model extends to human breast cancers.

Kelly MP, Lee FT, Smyth FE, Brechbiel MW, Scott AM. Enhanced efficacy of 90Y-radiolabeled anti-Lewis Y humanized monoclonal antibody hu3S193 and paclitaxel combined-modality radioimmunotherapy in a breast cancer model. Journal of Nuclear Medicine 2006 47(4):716-25.

Antibodies targeting cancer-specific antigens or signaling molecules that promote tumorigenesis represent one of the most promising therapeutic approaches for cancer today. LICR has explored the safety and targeting of seven mAbs in early-phase clinical trials. Six have been licensed for commercial development.

Antibody Targeting in Gastric Tumors

A team of LICR investigators from the New York Branch, Melbourne Center and Affiliates in Kyoto and Gunma (Japan) used biopsies to characterize the binding and targeting properties of the huA33 mAb, which binds to the A33 cancer antigen, in patients with gastric cancer. A week prior to surgery, 13 patients were given a single infusion of huA33 radiolabeled with Iodine^{131} (^{131}I-huA33). Each patient received one of four different dose levels. A whole body scan of each patient was then conducted to localize the ^{131}I-huA33 *in vivo*. After surgery, the distribution and amount of the A33 antigen, and localization of the huA33 binding, was measured in the 'resected' (surgically-removed) tumor samples. The results showed that huA33 has excellent targeting *in vivo*, and was co-localized with the A33 antigen in the gastric cancer tumors. The mAb is a potential therapy for advanced gastric cancer that is resistant to chemotherapy, and might also be useful as an 'adjuvant,' an additional treatment given to lower the chances of relapse after surgery has removed all detectable tumor.

Sakamoto J, Oriuchi N, Mochiki E, Asao T, Scott AM, Hoffman EW, Jungbluth AA, Matsui T, Lee FT, Papenfuss A, Kuwano H, Takahashi T, Endo K, Old LJ. A phase I radioimmunolocalization trial of humanized monoclonal antibody huA33 in patients with gastric carcinoma. Cancer Science 2006 97(11):1248-54.

Using Antibodies to Deliver Therapeutic-loaded Nanoparticles

One therapeutic approach under investigation is the use of antibodies as vehicles to selectively deliver cytotoxic molecules—so-called 'magic bullets'—to cancer cells. A team from the Melbourne Branch and Melbourne Center assessed the ability of a particle coated with huA33 to target the A33 antigen. This approach seeks to take advantage of the internalization mechanisms activated by huA33 binding to A33, to induce the cell to internalize a particle containing, or comprised of, a toxin. The investigators showed that particles coated with huA33 mAb bound to the A33 antigen in colon cancer cell lines with a high degree of specificity, and were internalized by those cells.

Cortez C, Tomaskovic-Crook E, Johnston APR, Radt B, Cody SH, Scott AM, Nice EC, Heath JK, Caruso F. Targeting and Uptake of Multilayered Particles to Colorectal Cancer. Advanced Materials 2006 18:1998-2003.

Melbourne Centre for Clinical Sciences

In 2006, a new concept was introduced to the LICR structure with the formation of the "LICR Melbourne Centre for Clinical Sciences" in Melbourne, Australia. LICR Centers will have a primary focus on clinical and translational, rather than laboratory, research, which is in contrast to LICR Branches that have a primary focus on laboratory and translational research.

The Melbourne Center groups are conducting preclinical and clinical research in growth factor signaling and cancer immunology as part of the Institute's research into the therapeutic modalities of small molecule inhibitors, antibodies and cancer vaccines. The Melbourne Center also houses the LICR 'Biological Distribution Facility,' for the storage and distribution of antibodies and cancer vaccines, and the repository of the 'Good Manufacturing Practice' (GMP) documentation for LICR's clinical trials.

The Center was formed by the administrative separation of the 'Parkville' and 'Austin' campuses of the Melbourne Branch. The host institution is 'Austin Health,' a major provider of tertiary health services, professional education and medical research in the northeast of Melbourne

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Epidermal Growth Factor (EGF): Preclinical Analyses of a Small Molecule Inhibitor

LICR has a long history of research on the epidermal growth factor receptor (EGFR), which is over-expressed or activated in approximately 50% of cancers of epithelial cell origin. This research has thus far produced two different candidate therapies, each at a different stage of translation from the laboratory to the clinic. The first is an antibody, 806, which targets a particular EGFR mutant present in a form of brain tumor known as glioblastoma (see p18), and also targets the normal EGFR when the receptor is overexpressed. Preclinical analyses of the second potential therapy, a small molecule inhibitor of EGFR, AG1478, were completed this year by a collaboration between the Melbourne Branch and Melbourne Center. These preclinical analyses demonstrated a dose-dependent inhibition of EGFR signaling and provide essential data for the design of dose regimens for future clinical studies with EGFR kinase inhibitors.

Ellis AG, Doherty MM, Walker F, Weinstock J, Nerrie M, Vitali A, Murphy R, Johns TG, Scott AM, Levitzki A, McLachlan G, Webster LK, Burgess AW, Nice EC. Preclinical analysis of the analinoquinazoline AG1478, a specific small molecule inhibitor of EGF receptor tyrosine kinase. Biochemical Pharmacology 2006 71(10):1422-34.

Transforming Growth Factor β

The transforming growth factor β (TGFβ) family is a group of growth factor 'ligands' that bind to receptors on the cell surface and activate a multiplicity of normal cell processes. TGFβ signaling also contributes to cancer cell invasiveness and metastasis. TGFβ, the archetype of the ligand family, activates the downstream Smad protein family that regulates the expression of different gene networks required for cell proliferation, differentiation and 'apoptosis' (programmed cell death). LICR investigators are dissecting the signaling pathways downstream of TGFβ and the other ligands in the family in search of new approaches for cancer diagnosis and control.

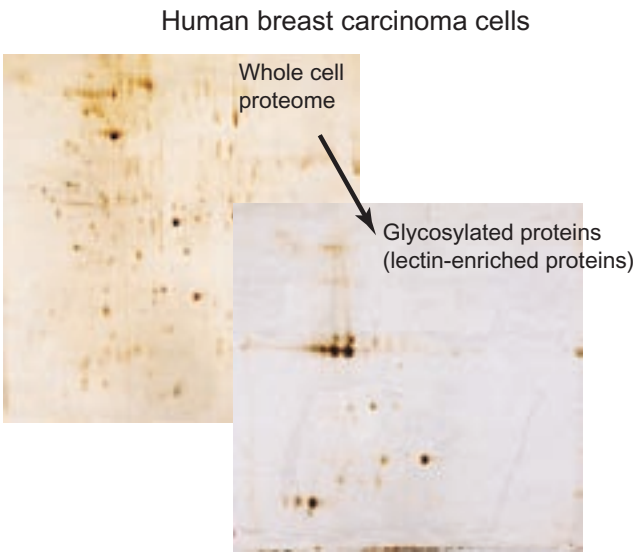
Smad2 and Smad3 Transcription

TGFβ signaling phosphorylates the 'R-Smad' proteins Smad2 and Smad3, which form a complex with Smad4 and translocate to the cell nucleus. The R-Smads shuttle in and out of the nucleus in response to changes in TGFβ signaling. LICR investigators from the Uppsala Branch this year discovered a novel nuclear export pathway for Smad3, which is mediated by a protein sequence common to several other Smad proteins. The export relies on the protein exportin4, which also exports a specific cofactor involved in protein translation, and the Ran GTPase, an enzyme that hydrolyses GTP for the energy required for various cell processes, including nuclear export.

Kurisaki A, Kurisaki K, Kowanetz M, Sugino H, Yoneda Y, Heldin CH, Moustakas A. The mechanism of nuclear export of Smad3 involves exportin 4 and Ran. Molecular Cell Biology 2006 26(4):1318-32.

Once in the nucleus, the Smad complex interacts with transcription coactivators and/or corepressors to regulate TGFβ target gene expression. A second team from the Uppsala Branch has discovered that the coactivators p300 and CBP 'acetylate' (add an acetyl group to) Smad2 and Smad3 in a TGFβ-dependent manner. Another coactivator, PCAF, acetylates only Smad2. The p300/CBP complex regulates a large number of transcription factors, including some of those involved in cell cycle progression (see pp10-12). The acetylation of R-Smads promotes their DNA-binding affinity, thereby enhancing Smad-mediated transcriptional activity. Acetylation of Smad molecules by coactivators could represent a new approach for regulating TGFβ signaling.

Simonsson M, Kanduri M, Gronroos E, Heldin CH, Ericsson J. The DNA binding activities of Smad2 and Smad3 are regulated by coactivator-mediated acetylation. Journal of Biological Chemistry 2006 281(52):39870-80.



Above: '*O*-glycosylation' (the addition of sugar residues to a protein) is a post-translational protein modification (PTM) that has been shown to regulate the function of certain proteins. An Uppsala Branch team studied *O*-glycosylation in response to TGFβ stimulation and identified the proteins undergoing PTM as being involved in cell survival, apoptosis, cell trafficking and RNA processing. Thus far, the results of the study have identified a new mechanism by which TGFβ may protect cells from apoptosis.

Iwahana H, Yakymovych I, Dubrovska A, Hellman U, Souchelnytskyi S. Glycoproteome profiling of transforming growth factor-beta (TGFbeta) signaling: nonglycosylated cell death-inducing DFF-like effector A inhibits TGFbeta1-dependent apoptosis. Proteomics 2006 6(23):6168-80.

Smad7 and Apoptosis

Smad7 inhibits Smad signaling by binding to activated TGFβ receptors and preventing activation of the R-Smads. Several years ago, investigators from the Uppsala Branch showed that TGFβ induces apoptosis via Smad7 and the signaling protein p38. Further investigation by the team has now shown that TGFβ-induced apoptosis is also dependent on p53, a tumor-suppressor protein that regulates cell growth and proliferation, and causes apoptosis when DNA damage is detected during the cell cycle (see p12). This TGFβ/p53-mediated apoptosis is also dependent on another protein that responds to DNA damage during the cell cycle, ATM. The study's results suggest that Smad7 acts as a scaffold protein to facilitate the formation of the p38/p53/ATM/Smad7 complex and the sequential activation of the components in the ATM-p53 pathway to induce apoptosis in response to DNA damage. Silencing Smad7 activity resulted in mitotic spindle defects and disruption of the cell cycle checkpoints; irregularities that are required for cancer onset and progression. These findings suggest that TGFβ and Smad7 play an important role in maintaining genome integrity in epithelial cells. The expression of Smad7 might be useful, following further confirmatory analyses, as a novel biomarker to predict tumor progression in patients with breast, colon and prostate cancers.

Zhang S, Ekman M, Thakur N, Bu S, Davoodpour P, Grimsby S, Tagami S, Heldin CH, Landstrom M. TGFbeta1-induced activation of ATM and p53 mediates apoptosis in a Smad7-dependent manner. Cell Cycle 2006 5(23):2787-95.

TGFβ Signaling in the Immune System

To investigate the developmental and physiological role(s) of Smad7 *in vivo*, an LICR team engineered a mouse model lacking the half of the Smad7 protein that inhibits TGFβ signaling. The resulting mice were smaller than wild-type mice, indicating that Smad7 has an important role during embryological development. Smad7 also has a role in the immunological response, with an immune system stimulant inducing altered B cell responses in the mice. B cells lacking Smad7's inhibitory function had higher rates of apoptosis and lower proliferation compared to normal B cells. Investigation of the effects of Smad7 on other immune system components is ongoing.

Li R, Rosendahl A, Brodin G, Cheng AM, Ahgren A, Sundquist C, Kulkarni S, Pawson T, Heldin CH, Heuchel RL. Deletion of exon I of SMAD7 in mice results in altered B cell responses. Journal of Immunology 2006 176(11):6777-84.

First Study to Show New Type of Functional Receptor Cross-Talk

The bone morphogenetic proteins (BMPs) are part of the TGFβ ligand family and regulate differentiation, cell proliferation and apoptosis via activation of the BMP-specific Smad proteins, Smad1, Smad5 and Smad8. The transmembrane BMP type II receptor (BMPR-II), to which BMPs bind, activates signaling by phosphorylating serine and threonine residues in the amino acid sequence of downstream signaling proteins. A collaborative study between two groups from the Uppsala Branch has this year shown that BMPR-II interacts and cooperates functionally with c-kit, a transmembrane receptor that activates signaling by phosphorylating tyrosine residues in downstream signaling proteins. It is well known that there is functional cross-talk between the downstream signaling proteins activated by serine and threonine kinase receptors and those activated by tyrosine kinase receptors. However, this is the first study to show evidence of the different receptor types forming a functional complex upon ligand binding.

Hassel S, Yakymovych M, Hellman U, Ronnstrand L, Knaus P, Souchelnytskyi S. Interaction and functional cooperation between the serine/threonine kinase bone morphogenetic protein type II receptor with the tyrosine kinase stem cell factor receptor. Journal of Cell Physiology 2006 206(2):457-67.

Platelet-Derived Growth Factor

The platelet-derived growth factor (PDGF) family is a group of ligands that stimulate cell proliferation, survival and migration through the activation of two PDGF receptors, PDGFRα and PDGFRβ. PDGFRs are receptor tyrosine kinases; they initiate signal transduction by phosphorylating tyrosine residues in the amino acid sequences of downstream proteins. The PDGF family consists of four protein chains, PDGF-A, -B, -C and -D, that form five biologically active 'dimers' (two functionally and physically linked proteins): PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, PDGF-DD. Two of the four ligands, PDGF-C and PDGF-D, were discovered at the Stockholm Branch as part of the LICR Angiogenesis Program. PDGFs secreted by tumors contribute to induction of angiogenesis and lymphangiogenesis, the formation of new blood and lymphatic vessels, respectively. These processes provide routes for cancer cells that have detached from the tumor to 'metastasize' (spread) around the body (see pp6-7). PDGF signaling is thus central to metastasis, the ultimate cause of most cancer deaths

Receptor Down-Regulation and Recycling

In response to binding of PDGF-B or PDGF-D chains, the PDGFRβ dimerizes and 'autophosphorylates' (catalyzes the addition of phosphate groups to its own amino acid sequence) to become active. PDGF signaling ceases when the receptor is down-regulated, either by 'dephosphorylation' (removal of the phosphate groups by a tyrosine phosphatase protein) or by degradation of the receptor, in lysosomes or proteasomes, subsequent to internalization by the cell. The receptor can also be recycled to the cell surface following internalization. Receptors are marked for degradation by 'ubiquitination,' a process in which the ubiquitin ligase protein c-Cbl adds ubiquitin groups to the receptor's amino acid sequence. Investigators at the Uppsala Branch are studying PDGFR down-regulation to understand whether, and how, the disruption of receptor down-regulation allows cancer cells to proliferate continuously, or tumors to promote angiogenesis, in the absence of growth factor ligands. In 2006, the team identified a scaffold protein, Alix, that modulates PDGFRβ down-regulation by facilitating the interaction between the receptor and c-Cbl. The LICR team also found that over-expression of Alix reduced the rate of clearance of PDGFRβ from the cell surface, but it is not yet clear if this was caused by decreased internalization or increased receptor recycling.

Lennartsson J, Wardega P, Engstrom U, Hellman U, Heldin CH. Alix facilitates the interaction between c-Cbl and platelet-derived growth factor beta-receptor and thereby modulates receptor down-regulation. Journal of Biological Chemistry 2006 281(51):39152-8.

In a second study, the team analyzed the role of the tyrosine phosphatase TC-PTP in receptor down-regulation. TC-PTP had previously been shown to dephosphorylate PDGFRβ, and the Uppsala Branch team extended this observation to show that increased PDGFRβ phosphorylation occurs, primarily at the cell surface, in the absence of TC-PTP. Further analysis showed that the loss of TC-PTP caused the fate of the internalized PDGFRβ to shift from being degraded to being recycled to the cell surface. In addition, loss of TC-PTP specifically induced recycling of PDGFRββ homodimers and PDGFRαβ heterodimers, but not PGDFRαα homodimers. This paper is the first to provide evidence that a tyrosine phosphatase is involved in growth factor receptor trafficking and is also the first to show that there is differential trafficking between PDGFR family members.

Karlsson S, Kowanetz K, Sandin A, Persson C, Ostman A, Heldin CH, Hellberg C. Loss of T-cell protein tyrosine phosphatase induces recycling of the platelet-derived growth factor (PDGF) beta-receptor but not the PDGF alpha-receptor. Molecular Biology of the Cell 2006 17(11):4846-55.

A New Signaling Partnership

The S6K protein, a key player in the regulation of cell growth, has been implicated in the onset and progression of several cancer types. S6K is activated by signaling proteins recruited to activated receptors, which sequentially phosphorylate serine and threonine residues in the S6K amino acid sequence. Investigators at the London Branch have now found that S6K can interact directly with PDGFR but is, in this case, activated by the phosphorylation of tyrosine residues in the S6K sequence. This mechanism of activation is independent of that mediated by serine/threonine phosphorylation. The novel mechanism suggests that PDGF signaling might regulate hitherto unsuspected S6K functions.

Rebholz H, Panasyuk G, Fenton T, Nemazanyy I, Valovka T, Flajolet M, Ronnstrand L, Stephens L, West A, Gout IT. Receptor association and tyrosine phosphorylation of S6 kinases. FEBS Journal 2006 273(9):2023-36.

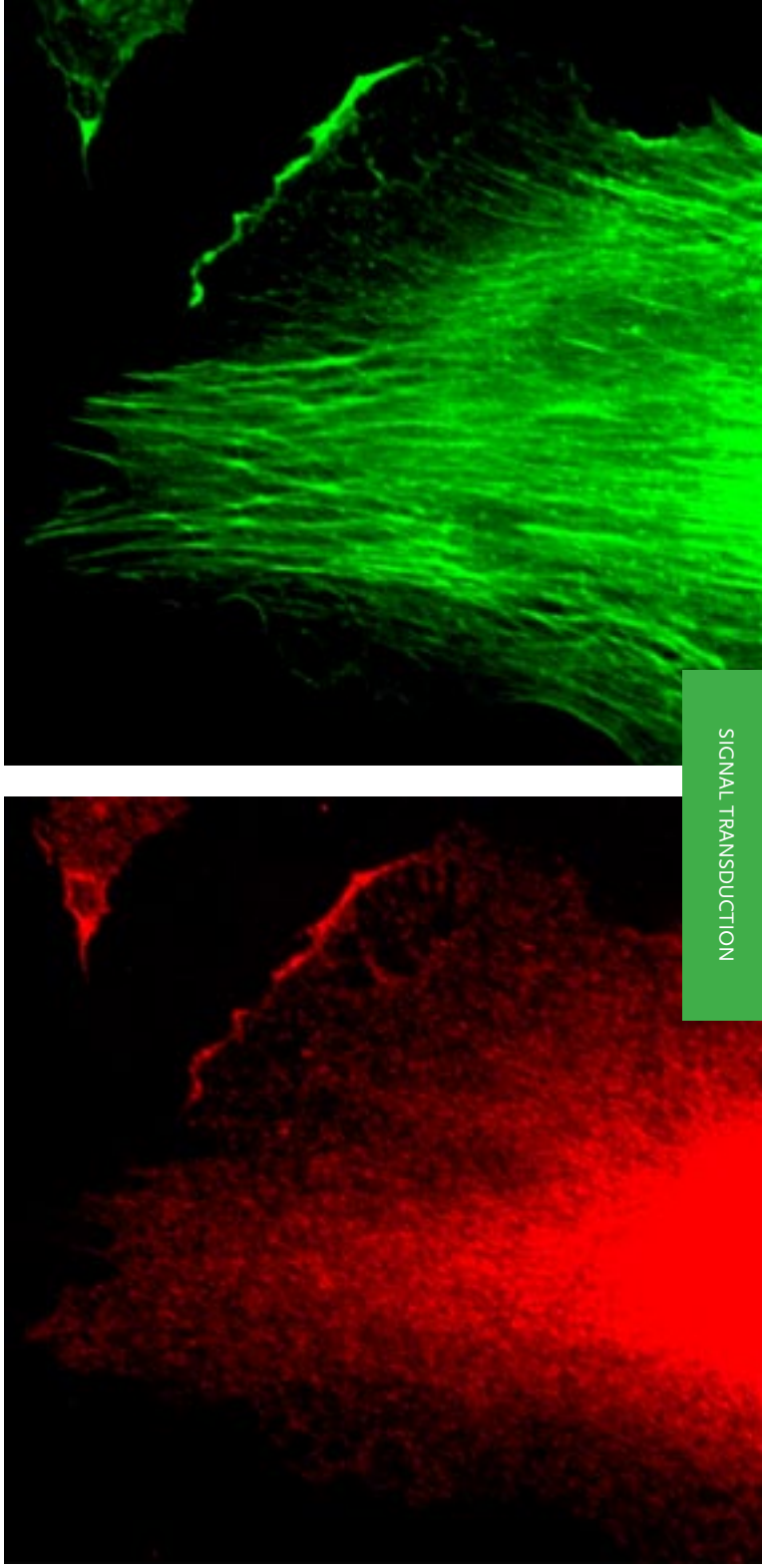
PDGF Signaling and Cell Migration

'Chemotaxis' is cell migration towards a 'chemoattractant.' PDGFs are chemoattractants that induce chemotaxis of several cell types, including smooth muscle cells that form blood and lymph vessels. Investigators from the Uppsala Branch, dissecting PDGF ligand-specific signaling, focused on the activation of the signaling protein JNK during PDGF-BB-mediated chemotaxis of fibroblast cells. JNK, which is activated by PDGF, epidermal growth factor (EGF) and transforming growth factor β (see pp36-37), mediates a signaling pathway that is thought to be central to inflammation, cell 'differentiation' (specialization in function and structure) and apoptosis. The LICR team found that chemotaxis mediated by PDGF-BB was dependent on both PI3K (see p42) and JNK. JNK co-localized at the membrane ruffles that form as the cell's actin cytoskeleton reorganizes to physically move the leading edge of the cell forward. The team also assessed PDGFRβ/PI3K/JNK migration in cell lines from glioblastoma, a highly invasive brain cancer (see p18). The team found that inhibiting JNK greatly reduced PDGF-BB-mediated chemotaxis of the cells, thus suggesting JNK inhibition may be a strategy to address the invasiveness of glioblastomas.

Amagasaki K, Kaneto H, Heldin CH, Lennartsson J. c-Jun N-terminal kinase is necessary for platelet-derived growth factor-mediated chemotaxis in primary fibroblasts. Journal of Biological Chemistry 2006 281(31):22173-9.

In a second study, the Uppsala Branch team discovered that PDGF-BB-induced migration of fibroblasts is suppressed by hyaluronan. Hyaluronan is an extracellular matrix molecule that, when bound to cell surface adhesion receptor, CD44, controls cell migration, proliferation and differentiation. Hyaluronan is also an important component of 'stroma,' the tissue that surrounds and physically supports the tumor. The team found that hyaluronan-activated CD44 inhibits the activation of PDGFRβ by PDGF-BB, probably by activating an unknown tyrosine phosphatase that dephosphorylates PDGFRβ. Extracellular matrix proteins and growth factors, such as hyaluronan and PDGF-BB, are known to cooperate in processes such as wound healing, but this study suggests that growth factors and extracellular matrix molecules might also act antagonistically to control the growth and motility of cells.

Li L, Heldin CH, Heldin P. Inhibition of platelet-derived growth factor-BB-induced receptor activation and fibroblast migration by hyaluronan activation of CD44. Journal of Biological Chemistry 2006 281(36):26512-9.



These images, from the Uppsala Branch, show the proteins Jnk (red) and actin (green) co-localizing in the membrane ruffles of a cell during PDGF-BB-induced chemotaxis.

PI3K Signaling: A Retrospective

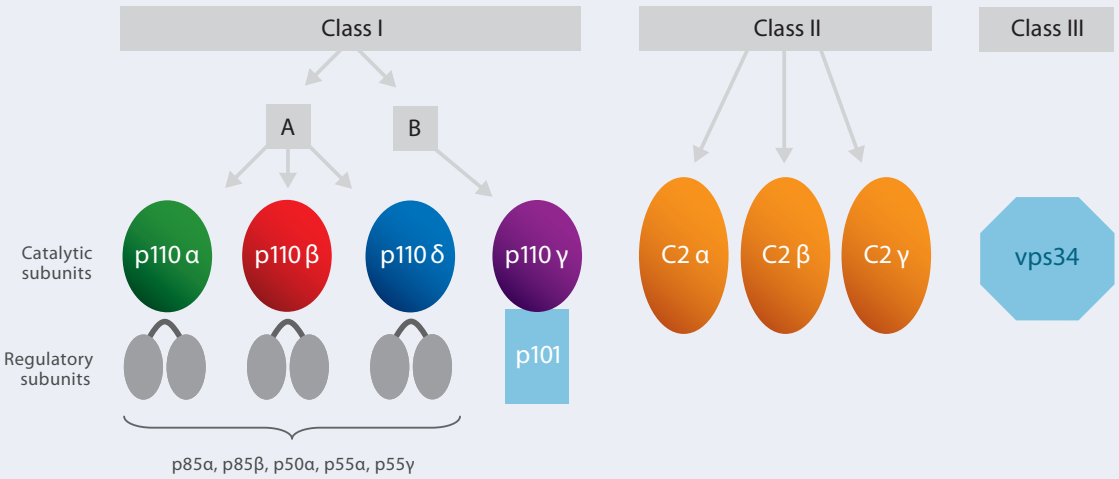
The development of potential new therapies based on detailed understanding of a complex family of enzymes

In response to stimulation of cell surface receptors, PI 3-kinases (PI3Ks) 'phosphorylate' (activate by addition of phosphate groups) lipids that act as second messengers to transmit signals inside the cell. PI3K signaling activates all cell processes deregulated in cancer, including cell growth, proliferation and migration. The PTEN tumor suppressor (see p45) 'dephosphorylates' (inactivates by removal of phosphate groups) the lipids phosphorylated by PI3Ks. Deregulation of PI3K signaling is a feature common to most, if not all, cancers, and can occur in two different ways. The first is through loss-of-function of PTEN. The second is through constitutive PI3K signaling resulting from the mutation or overexpression of PI3K itself, or of upstream cell surface receptors. In particular, these are platelet-derived growth factor (PDGF) receptor (see pp38-9), the insulin-like growth factor (IGF) receptor, and members of the epidermal growth factor receptor (EGFR) family: ErbB-1/EGFR, which is activated or overexpressed in over half of all cancers of epithelial origin, ErbB-3/HER3, and ErbB-2/HER2, which is implicated in approximately twenty-five percent of breast cancers. Intra-cellular signaling proteins have also been implicated in constitutive PI3K activity, the most well known of these is the Ras oncogene, which is mutated in approximately twenty-five percent of all cancers.

The PI3K Lipid Kinase Family

LICR investigators at the London Branch established the internationally adopted nomenclature for the PI3K family by classifying the various PI3K subunits based on their structure and function.

Class I_A enzymes consist of any one of the 'catalytic' subunits—p110α, p110β, or p110δ—complexed with any one of the 'regulatory' subunits—p85α or -β, p50α, or p55α or-γ. Only one Class I_B PI3K enzyme exists, and this is made up of the p110γ catalytic and the p101 or p87/84 regulatory subunit. There are three Class II PI3Ks—C2α, C2β, and C2γ—and one Class III PI3K, Vps34. The p110a, p110δ, p110g, C2α and C2β and Vps34 proteins, plus most of their adaptor proteins, were discovered by LICR investigators at the London Branch. The Branch investigators also cloned the PI3K isoforms in the fruit-fly, *Drosophila melanogaster*. Because there is only one isoform in each of class I, II and III, *D. melanogaster* has proved to be an excellent model organism in which to dissect and analyze PI3K function.



Zvelebil M, McDougall L., Volinia S, Vanhaesebroeck B, Panayotou G, Gout I, Steim, R, Koga H, Leevers S, Das P, Salim K, Linacre J, Panaretou C, Wetzker R, Waterfield M. Structural and Functional diversity of phosphatidylinositide-3 kinases. Philosophical Transactions of the Royal Society of London 1995 351:217-223.

Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends in Biochemical Sciences 1997 22(7):267-72.

Vanhaesebroeck B, Waterfield MD. Signaling by distinct classes of phosphoinositide 3-kinases. Experimental Cell Research 1999 253(1):239-254.

The PI3K signaling pathway is the most frequently subverted pathway in human cancer. LICR investigators were among the pioneers of PI3K research, and have been leaders in the field since its inception.

Otsu M, Hiles I, Gout I, Fry MJ, Ruiz-Larrea F, Panayotou G, Thompson A, Dhand R, Hsuan J, Totty N, Smith AD, Morgan SJ, Courtneidge SA, Parker PJ, Waterfield MD. Characterization of two 85 kd proteins that associate with receptor tyrosine kinases, middle-T/pp60c-src complexes, and PI3-kinase. Cell 1991 65(1):91-104.

Hiles ID, Otsu M, Volinia S, Fry MJ, Gout I, Dhand R, Panayotou G, Ruiz-Larrea F, Thompson A, Totty NF, Hsuan JJ, Courtneidge SA, Parker PJ, Waterfield MD. Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. Cell 1992 70(3):419-29.

MacDougall LK, Domin J, Waterfield MD. A family of phosphoinositide 3-kinases in Drosophila identifies a new mediator of signal transduction. Current Biology 1995 5(12):1404-15.

Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nurnberg B, Gierschik P, Seedorf K, Hsuan JJ, Waterfield MD, Wetzker R. Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science 1995 269(5224):690-3.

Volinia S, Dhand R, Vanhaesebroeck B, MacDougall LK, Stein R, Zvelebil MJ, Domin J, Panaretou C, Waterfield MD. A human phosphatidylinositol 3-kinase complex related to the yeast Vps34p-Vps15p protein sorting system. EMBO Journal 1995 14(14):3339-48.

Leevers SJ, Weinkove D, MacDougall LK, Hafen E, Waterfield MD. The Drosophila phosphoinositide 3-kinase Dp110 promotes cell growth. EMBO Journal 1996 15(23):6584-94.

Domin J, Pages F, Volinia S, Rittenhouse SE, Zvelebil MJ, Stein RC, Waterfield MD. Cloning of a human phosphoinositide 3-kinase with a C2 domain that displays reduced sensitivity to the inhibitor wortmannin. Biochemical Journal 1997 326 (Pt 1):139-147.

Linassier C, MacDougall LK, Domin J, Waterfield MD. Molecular cloning and biochemical characterization of a Drosophila phosphatidylinositol-specific phosphoinositide 3-kinase. Biochemistry Journal 1997 321 (Pt 3):849-56.

Panaretou C, Domin J, Cockcroft S, Waterfield MD. Characterization of p150, an adaptor protein for the human phosphatidylinositol (PtdIns) 3-kinase. Substrate presentation by phosphatidylinositol transferprotein to the p150.Ptdins 3-kinase complex. Journal of Biological Chemistry 1997 272(4):2477-85.

Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, Higashi K, Volinia S, Downward J, and Waterfield MD. P110delta, a novel phosphoinositide 3-kinase in leukocytes. Proceedings of the National Academy of Sciences USA 1997 94(9):4330-4335.

Weinkove D, Leevers SJ, MacDougall LK, Waterfield MD. p60 is an adaptor for the Drosophila phosphoinositide 3-kinase, Dp110. Journal of Biological Chemistry 1997 272(23):14606-10.

Arcaro A, Volinia S, Zvelebil MJ, Stein R, Watton SJ, Layton MJ, Gout I, Ahmadi K, Downward J, Waterfield MD. Human phosphoinositide 3-kinase C2beta, the role of calcium and the C2 domain in enzyme activity. Journal of Biological Chemistry 1998 273(49):33082-33090.

PI3K and Growth Factor Signaling

Research from the LICR San Diego, London and Uppsala Branches has shed light on PI3K signaling mediated by specific growth factors. A selection of LICR research papers relating only to signaling downstream of the PDGFβ receptor (see pp38-39) includes:

PI3K mediates cell migration, the central process in the spread of cancer (see 'Metastasis,' pp6-7). This finding from the investigators at the Uppsala Branch alerted the field to the potential therapeutic strategy of inhibiting PI3K activity to prevent metastasis;

Wennstrom S, Siegbahn A, Yokote K, Arvidsson AK, Heldin CH, Mori S, Claesson-Welsh L. Membrane ruffling and chemotaxis transduced by the PDGF beta-receptor require the binding site for phosphatidylinositol 3' kinase. Oncogene 1994 9(2):651-60.

PI3K regulates 'interstitial fluid homeostasis' (the maintenance of fluid between cells). The Uppsala Branch teams subsequently showed that lowering interstitial pressure—by inhibiting PDGFR-PI3K signaling—enhances the efficacy of some chemotherapies;

Heuchel R, Berg A, Tallquist M, Ahlen K, Reed RK, Rubin K, Claesson-Welsh L, Heldin CH, Soriano P. Platelet-derived growth factor beta receptor regulates interstitial fluid homeostasis through phosphatidylinositol-3' kinase signaling. Proceedings of the National Academy of Sciences USA 1999 96(20):11410-11415.

Pietras K, Rubin K, Sjoblom T, Buchdunger E, Sjoquist M, Heldin CH, Ostman A. Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Research 2002 62(19):5476-5484.

PI3K induces the synthesis of lipids for new cell membranes by activating genes controlled by sterol regulatory element binding proteins (SREBP) (see p10);

Demoulin JB, Ericsson J, Kallin A, Rorsman C, Ronnstrand L, Heldin CH. Platelet-derived Growth Factor Stimulates Membrane Lipid Synthesis Through Activation of Phosphatidylinositol 3-Kinase and Sterol Regulatory Element-binding Proteins. Journal of Biological Chemistry 2004 279(34):35392-35402.

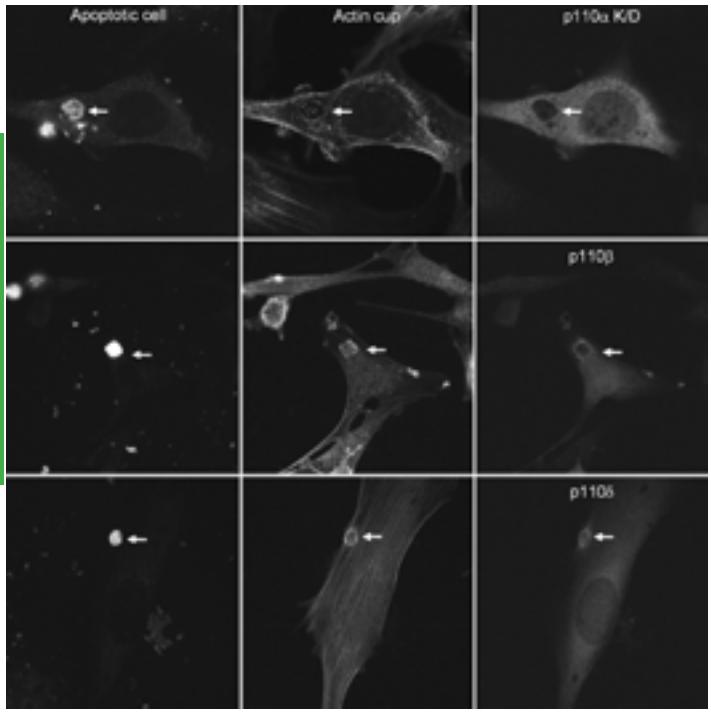
PI3K induces expression of vascular endothelial growth factor (VEGF), a key molecule involved in angiogenesis. This finding, from the San Diego Branch, links two areas of therapeutic interest within LICR. LICR's intellectual property portfolios relating to both PI3K and VEGF-induced angiogenesis are being developed by several of the Institute's commercial partners (see 'Intellectual Property & Licensing,' pp46-47).

Wang D, Huang HJ, Kazlauskas A, Cavenee WK. Induction of vascular endothelial growth factor expression in endothelial cells by platelet-derived growth factor through the activation of phosphatidylinositol 3-kinase. Cancer Research 1999 59(7):1464-72.

PI3Ks and Cell Migration

A collaboration between multiple groups from the London Branch first demonstrated that class I_A PI3Ks have distinct biological roles. This finding was revealed with the use of a 'macrophage' (immune system scavenging cell) model to dissect PI3K-mediated migration.

Vanhaesebroeck B, Jones GE, Allen WE, Zicha D, Hooshmand-Rad R, Sawyer C, Wells C, Waterfield MD, Ridley AJ. Distinct PI(3)Ks mediate mitogenic signalling and cell migration in macrophages. Nature Cell Biology 1999 (1):69-71.



PI3Ks p110α and p110δ are recruited, and co-localize with actin, during the 'phagocytosis' (ingestion of cells and cell debris) of apoptotic cells by macrophages. [Figure courtesy of Dr. Yann Leverrier]

Additional studies have now shown that the particular PI3K isoform involved in migration is dependent on the receptor(s) activated, and that different PI3K isoforms are involved at each stage in the migration process. For example, migration toward the 'chemoattractant' (a chemical that makes cells move towards it) MCP-1, which activates G-protein coupled receptors (GPCRs), is dependent on PI3Kγ. However, migration towards CSF-1, which activates receptor tyrosine kinases (RTKs), is more dependent on PI3Kδ, although PI3Kγ also contributes. Interestingly, initial actin reorganization following stimulation of GPCRs or RTKs is not dependent on PI3Kγ. However, this isoform is needed for optimal migration. A 2006 study has now revealed that CSF-1 and PI3K regulate the formation of cellular structures required for adhesion in macrophages, which could explain, in part, how PI3Ks contribute to migration.

Jones GE, Prigmore E, Calvez R, Hogan C, Dunn GA, Hirsch E, Wymann MP, Ridley AJ. Requirement for PI 3-kinase gamma in macrophage migration to MCP-1 and CSF-1. Experimental Cell Research 2003 290(1):120-131.

Weiss-Haljiti C, Pasquali C, Ji H, Gillieron C, Chabert C, Curchod ML, Hirsch E, Ridley AJ, van Huijsduijnen RH, Camps M, Rommel C. Involvement of phosphoinositide 3-kinase gamma, Rac, and PAK signaling in chemokine-induced macrophage migration. Journal of Biological Chemistry 2004 279(41):43273-84.

Wheeler AP, Smith SD, Ridley AJ. CSF-1 and PI 3-kinase regulate podosome distribution and assembly in macrophages. Cell Motility and Cytoskeleton 2006 63(3):132-40.

PI3K-mediated migration in transformed human cells also involves multiple PI3K isoforms. The migration *in vitro* of certain breast cancer cells that overexpress EGFR can be blocked by specific inhibition of p110δ. On the other hand, an epithelial carcinoma cell line that overexpresses EGFR appears to require a class II PI3K, C2β, in cell migration *in vitro*. In response to EGF signaling, C2β is recruited to the receptor and becomes part of a multi-protein complex that activates Rac-dependent signaling (see p6), thereby regulating cytoskeletal organization and cell migration.

Sawyer C, Sturge J, Bennett DC, O'Hare MJ, Allen WE, Bain J, Jones GE, Vanhaesebroeck B. Regulation of breast cancer cell chemotaxis by the phosphoinositide 3-kinase p110delta. Cancer Research 2003 63(7):1667-1675.

Katso RM, Pardo OE, Palamidessi A, Franz CM, Marinov M, De Laurentiis A, Downward J, Scita G, Ridley AJ, Waterfield MD, Arcaro A. Phosphoinositide 3-Kinase C2beta regulates cytoskeletal organization and cell migration via Rac-dependent mechanisms. Molecular Biology of the Cell 2006 17(9):3729-44.

PI3K and Immunology

Expression of the p110δ and p110γ isoforms is highly enriched in white blood cells, with p110δ also expressed in solid tumors such as breast and melanoma. The restricted expression of p110δ in white blood cells in healthy individuals led the London Branch scientists to investigate PI3K signaling in immunology. By specifically inactivating the p110δ PI3K in mice, the team was able to show that PI3K signaling plays an important role in the development and function of B and T lymphocytes, and in the anaphylactic allergic responses. These results suggest that too much PI3K activity could lead to autoimmunity and leukemia, while a reduction in PI3K activity could lead to immunodeficiencies. Consequently, it is thought that PI3K inhibitors might be of use to prevent organ transplant rejection and certain autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis. These findings have inspired pharmaceutical companies to develop small molecule inhibitors against p110δ for use as anti-inflammatory agents, as well as for oncology, especially the treatment of leukemias.

Okkenhaug K, Patton DT, Bilancio A, Garcon F, Rowan WC, Vanhaesebroeck B. The p110delta isoform of phosphoinositide 3-kinase controls clonal expansion and differentiation of Th cells. Journal of Immunology 2006 177(8):5122-8.

Bilancio A, Okkenhaug K, Camps M, Emery JL, Ruckle T, Rommel C, Vanhaesebroeck B. Key role of the p110{delta} isoform of PI3K in B cell antigen and IL4 receptor signalling - comparative analysis of genetic and pharmacological interference with p110{delta} function in B cells. Blood 2005 107(2):642-50.

Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, Fox R, Bruce I, Walker C, Sawyer C, Okkenhaug K, Finan P, Vanhaesebroeck B. Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. Nature 2004 431(7011):1007-1011.

Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, Vanhaesebroeck B. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science 2002 297(5583):1031-1034.

PI3K in Growth and Diabetes

A decade ago, a study by the London Branch revealed that cell growth in *D. melanogaster* was mediated specifically by class I PI3Ks. However, the specific role of the archetypal PI3K molecule, p110α, which has been shown to be mutated or overexpressed in cancer, has remained elusive until now. In 2006, investigators from the London Branch used a new approach to generate a PI3K mouse model: inactivating, rather than removing, the gene of interest. This was done specifically to ensure that other members of the PI3K family would not compensate for the absence of the p110α protein. The resultant mice were smaller, but ate more and had increased levels of body fat. Additionally, the mice had raised insulin levels and were glucose-intolerant. Thus it appears that p110α controls the action of insulin and other key hormonal signals that play roles in growth, diabetes and obesity.

These data show that the p110α isoform plays a key role in insulin receptor signaling (IRS), which could explain the selective activation of p110α, over the other PI3K isoforms, in cancer. It is possible that p110α mutation or overexpression allows cancer cells to hijack the IRS system to up-regulate pathways involved in cell metabolism, growth and transformation.

Leevers SJ, Weinkove D, MacDougall LK, Hafen E, Waterfield MD. The Drosophila phosphoinositide 3-kinase Dp110 promotes cell growth. EMBO Journal 1996 15(23):6584-94.

Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B. Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. Nature 2006 441(7091):366-370.

The ubiquity of PI3K signaling in normal cell processes means that careful assessment of toxicity to normal tissues will be necessary during translational and clinical development. However, the LICR mouse model with inactivated PI3K p110α—which mimics the effect of systemic administration with a p110α-specific drug—has provided vital information for assessing potential side-effects from PI3K inhibition. The finding that these mice, despite having dampened insulin signaling, showed no signs of developing diabetes or had any severe metabolic disturbances, is welcome news for advocates of isoform-specific therapeutic strategies.

PI3K Isoform-specific Inhibitors for Acute Myeloid Leukemia

Investigators from the London Branch conducted preclinical analyses of a p110δ-specific inhibitor for the treatment of acute myeloid leukemia (AML). The analyses indicated that this PI3K isoform impacts on AML cell proliferation and resistance to chemotherapy. Importantly, selective p110δ inhibition had no toxicity for the normal progenitor cells, which was in contrast to a broad spectrum PI3K inhibitor. Additionally, the p110δ inhibitor had a synergistic effect when combined with a standard AML chemotherapeutic agent. These results suggest that further investigation of p110δ-specific inhibitors for AML, which has a poor prognosis, is warranted.

Billottet C, Grandage VL, Gale RE, Quattropani A, Rommel C, Vanhaesebroeck B, Khwaja A. A selective inhibitor of the p110delta isoform of PI 3-kinase inhibits AML cell proliferation and survival and increases the cytotoxic effects of VP16. Oncogene 2006 25(50):6648-59.

Quantifying Signaling Pathway Activation for Personalized Cancer Therapy

Two teams at the London Branch this year published a study describing the development of a sensitive quantification method for intracellular signaling based on the measurement of PI3K-dependent protein kinase activity in cells. Robust and simple methods, such as the methodology described, can be used to assess whether PI3K activation is a factor in an individual patient's cancer. With this information, meaningful decisions can be made with respect to incorporating PI3K-inhibition in a therapeutic strategy for that patient. This quantification approach can theoretically be extended to other signaling pathways implicated in cancer and other diseases.

Cutillas PR, Khwaja A, Graupera M, Pearce W, Gharbi S, Waterfield M, Vanhaesebroeck B. Ultrasensitive and absolute quantification of the phosphoinositide 3-kinase/Akt signal transduction pathway by mass spectrometry. Proceedings of the National Academy of Sciences USA 2006 103(24):8959-64.

Spin-off Success for a Model of Integrated Laboratory and Translational Research

LICR's first spin-off company, PIramed Ltd (see p47), was formed to develop PI3K inhibitors generated through a translational research program initiated by the London Branch. The collaboration—between academic partners, LICR, Imperial Cancer Research Fund (now Cancer Research UK) and the Institute for Cancer Research (both London, UK), and industry partner, Yamanouchi Pharmaceutical Company (now Astellas Pharma, Japan)—generated multiple lead compounds that were screened for isoform specificity and also physiological effect. This approach of combining fundamental science with translational work has advanced our knowledge of PI3K signaling in cancer and other human diseases and has also enabled the industrial development team to make informed decisions, based on solid science.

LICR's commitment to a long-term and substantial investment in the PI3K Program has demonstrated how laboratory discoveries can be translated into applications for human benefit. The investment—in staff, fundamental and translational research, academic and industry relationships, intellectual property and technology licensing, and the careful management of their integration—ultimately attracted venture capital funds and significant stage II pharmaceutical interest for the current development of anti-PI3K therapies for both cancer and inflammatory diseases

New Mechanism of Control for PTEN, the PI3K Antagonist

Loss of the genomic region containing the PTEN gene occurs in multiple cancers, and mutations in the PTEN gene cause several hereditary cancer predisposition syndromes. PTEN's tumor-suppressor function has been primarily linked solely to its role as an antagonist of PI3K signaling. However, the mechanisms that control PTEN activity itself have remained largely elusive.

In 2006, a pivotal study from a San Diego Branch team showed that PTEN physically and functionally interacts with the protein PCAF. PCAF regulates gene transcription via interaction with the p300/CBP proteins that bind to sequence-specific transcription factors typically involved in cell growth and 'differentiation' (specialization). The LICR investigators found that PCAF 'acetylates' (adds an acetyl group to) PTEN, reducing that protein's lipid phosphatase activity, in response to growth factor signaling. This rapid, reversible regulation of PTEN's activity allows cells to progress through the cell cycle in response to growth factor stimulation. This study was the first to describe acetylation as a novel mechanism for regulating enzymatic function and cell proliferation, and places PTEN at the linkage point of growth factor signaling and gene expression.

The paper was selected as a *Journal of Biological Chemistry* 'Paper of the Week,' meaning that it was judged by the *Journal*'s Editorial Board members and Associate Editors to rank in the top 1% of reviewed papers for significance and overall importance. This distinction is awarded to 50-100 of the 6600 papers published each year by the *Journal of Biological Chemistry*, the most cited biomedical research journal in the world.

Okumura K, Mendoza M, Bachoo RM, DePinho RA, Cavenee WK, Furnari FB. PCAF modulates PTEN activity. 2006 Journal of Biological Chemistry 281(36):26562-8.

In 1998, LICR investigators from the San Diego Branch showed that PTEN's lipid, but not protein, phosphatase activity was required to suppress cancer cell proliferation. PTEN blocks the progression of cells through the G1 phase of the cell cycle (see p11). This was the first study to elucidate the mechanism behind PTEN's tumor-suppressor function.

Furnari FB, Huang HJ, Cavenee WK. The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. Cancer Research 1998 58(22):5002-5008.

Intellectual Property & Licensing

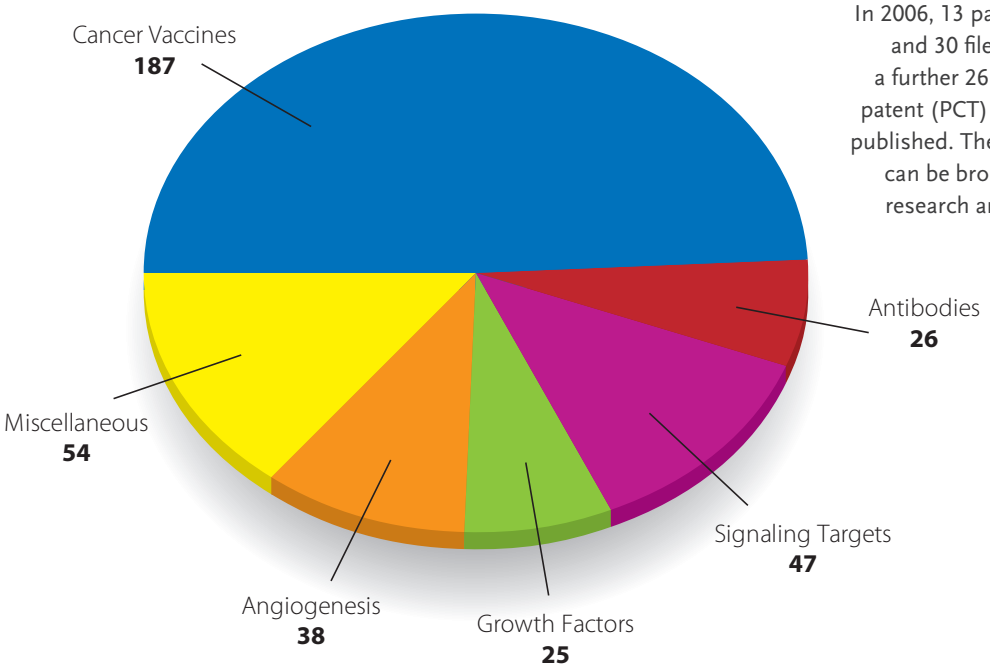
LICR's mission is to understand and control human cancer by translating its discoveries into applications for human benefit. To reach the general public and be of human benefit, these applications must undergo clinical development—rigorous testing in large Phase III trials and regulatory approval—and be commercialized, which involves large-scale manufacture, marketing, distribution and sales.

It is the purview of pharmaceutical and biotechnology companies to perform clinical development and commercialization. Therefore, the Institute must work with industry to accomplish its mission. A for-profit company will not commit hundreds of millions of dollars, and many years, to develop a discovery, without the expectation of a financial return. The protection and eventual licensing of intellectual property (IP) generated by laboratory and clinical discoveries is thus a fundamental part of any commitment to benefit humankind through cancer research.

The LICR's Office of Intellectual Property, established in 1989, takes responsibility for the filing, prosecution and maintenance of patents and their subsequent licensing. The Office's interactions with industry are guided by principles (see opposite) designed to both protect the ethos and integrity of the Institute, and to ensure—as much as is possible—that LICR discoveries are actively pursued as cancer diagnostic or therapeutic agents.

A central feature of its strategy to develop and commercialize LICR research discoveries is the Institute's commitment to perform laboratory and clinical evaluations that are able to both engage industry interest and guide future clinical development strategies. In particular, early-phase clinical trials that explore the investigational agent and the basic science around its use in the human setting have enhanced substantially the Institute's negotiating position in licensing discussions. Through clinical discovery, the investigational agent can be demonstrated to be viable in the eyes of the commercial world, and particularly to partners who can take them more widely into the clinic

LICR has licensing agreements with more than 20 companies in six countries. Licensing partners include GlaxoSmithKline, the world's second-largest pharmaceutical company, which licensed a large portfolio of cancer antigens from LICR in 2006 (see p21).



LICR US patents by category

In 2006, 13 patents were issued and 30 filed in the USA, and a further 26 new international patent (PCT) applications were published. The US patent estate can be broken down into the research areas shown at left.

Guiding Principles for LICR Licensing Interactions

Academic issues and concerns are the dominant factors in exploiting commercial opportunities.

Interactions with commercial firms must be designed to protect the Institute's reputation and endowment, and to further the Institute's research into the cause and cure of cancer.

Companies are to be provided only with license rights commensurate with their capacity (financial, research, marketing, administrative, organization and proprietary rights), and the companies are expected to exploit discoveries in a timely fashion for the public benefit.

Arrangements are to be structured and managed so that no one company acquires a dominant influence on Institute activities.

LICR has a singular focus on cancer but recognizes that some of its medical research findings may have therapeutic value for other human diseases. The Institute places great importance on supporting and facilitating, principally through the out-licensing of its intellectual property, the research and development of non-oncology therapies for human benefit. Presently, these include potential treatments for amyotrophic lateral sclerosis (ALS, or 'Lou Gehrig's Disease'), asthma, chronic pulmonary obstructive disease, edema, lymphedema and heart ischemia, and to prevent blood vessel blockage following vascular graft access surgery.

LICR Spin-off Companies

Spin-off companies are commercial enterprise vehicles that license technologies generated from LICR research findings. An equity/ownership position allows LICR to have input into the further development of Institute findings.

PIramed Ltd (UK, 2003) was formed to explore the therapeutic potential of PI3K research (pp40-44) from the LICR London Branch and collaborators at Cancer Research UK and Institute of Cancer Research. In 2005, *PIramed* entered into a research and development collaboration with Genentech, Inc., in what was believed to be one of the largest preclinical collaborations ever signed by a UK biotechnology company. In 2006, PIramed Ltd was named, by analyst newsletter *FierceBiotech*, as one of the top 15 emerging biotechnology companies in the UK.

Lymphatix (Finland, 2004) was formed to develop and commercialize therapy opportunities, for non-cancer indications, that arose from discoveries made by the LICR's global Angiogenesis Program. Agonists for vascular endothelial growth factor (VEGF)-C and VEGF-D will be assessed for the treatment of medical conditions with impaired blood supply or lymphoid drainage. Lymphatix was launched in collaboration with Licentia Ltd, the technology transfer arm of the University of Helsinki.

Vegenics (Australia, 2006) was launched in collaboration with Licentia Ltd, the technology transfer arm of the University of Helsinki, to develop and commercialize cancer therapies based on discoveries from LICR's Angiogenesis Program. Antagonists for vascular endothelial growth factor (VEGF)-C and VEGF-D will be assessed as cancer therapeutic and diagnostic agents.

Life Science Pharmaceuticals (USA, 2006) was formed to develop and commercialize three targeted antibodies from LICR's global Antibody Targeting Program. These antibodies—A33, F19 and 806—are indicated for the possible treatment and/or diagnosis of a variety of cancers, including head and neck, breast, colorectal, esophageal, and non-small cell lung (NSCLC) cancers, squamous cell carcinoma, and glioblastoma (brain tumors).

Recepta (Brazil, 2006) was formed to develop and commercialize four targeted antibodies, from LICR's global Antibody Targeting Program. The antibodies—A34, Le^b, MX35 and 3S193—are indicated for the possible treatment and/or diagnosis of a variety of cancers, including breast, colorectal, esophageal, and ovarian cancers. Recepta is the first Brazilian oncology start-up company to initiate clinical trials, and has attracted substantial support from that country's federal government.

Notable Events

Thierry Boon, Ph.D., Director of the LICR Brussels Branch, was elected as a Foreign Associate of National Academy of Sciences USA.

Jean-Charles Cerottini, M.D., founding Director of the LICR Lausanne Branch in 1971, and one of LICR's longest-serving staff, retired in 2006. Dr. Cerottini was presented with the D.K. Ludwig Award by the LICR Board of Directors in June. Dr. H. Robson MacDonald was appointed as the Acting Branch Director.

Don Cleveland, Ph.D., a Member at the LICR San Diego Branch, was elected as a Member of the National Academy of Sciences, USA, and also as a Member of the American Academy of Arts and Sciences, USA.

Carl-Henrik Heldin, Ph.D., Director of the Uppsala Branch, was elected as a Member of the Scan Balt Academy and also as a Foreign Member of the Finnish Scientific Society. He also gave the 2006 K.J. Öbrink Lecture at Uppsala University.

Richard Kolodner, Ph.D., Executive Director for Laboratory Sciences & Technology and Member at the LICR San Diego Branch, received the Katharine Berkan Judd Award Lectureship from the Memorial Sloan-Kettering Cancer Center in New York, USA, and the '2006 Annual Faculty Award for Excellence in Research' from the Vice-Chancellor of the University of California, San Diego School of Medicine, San Diego CA, USA.

Ralf Pettersson, M.D., Ph.D., founding Director of the LICR Stockholm Branch in 1985, stepped down from that position in mid-2006 to focus on his research. Dr. Pettersson has been a member of the Nobel Prize Committee, the working body that determines the Nobel Prize in Physiology or Medicine, for 17 years (Adjunct Member 1990-4, 2001-6; Member 1995-2000) and has held the roles of Vice-Chairman (1997) and Chairman (1998-2000) of the Committee.

Thomas Perlmann, Ph.D., who was appointed Acting Director of the LICR Stockholm Branch in 2006, was elected one of the 50 members of the Nobel Assembly, which awards the Nobel Prize in Physiology or Medicine. He was also named as the 2006 Umesono Lecturer at The Salk Institute, San Diego CA, USA.

Andrew M. Scott, M.D., was named Director of the new 'Melbourne Centre for Clinical Sciences.' Dr. Scott is also Director of the Centre for Positron Emission Tomography, Department of Nuclear Medicine, Austin Health, Melbourne. Appointed as a Member in 2006, Dr. Scott originally joined the Melbourne Branch as an Associate Member in 2000, and was appointed Associate Melbourne Branch Director - Clinical Program in 2002.

Luisa L. Villa, Ph.D., was appointed Director of the LICR São Paulo Branch in July, 2006. A Member since 1998, Dr. Villa originally joined the Branch in 1983 as a senior investigator. Dr. Villa is recognized internationally for her studies on human papillomavirus (HPV) and its etiology in cancer, and her contributions to the testing of prophylactic vaccines against the virus.

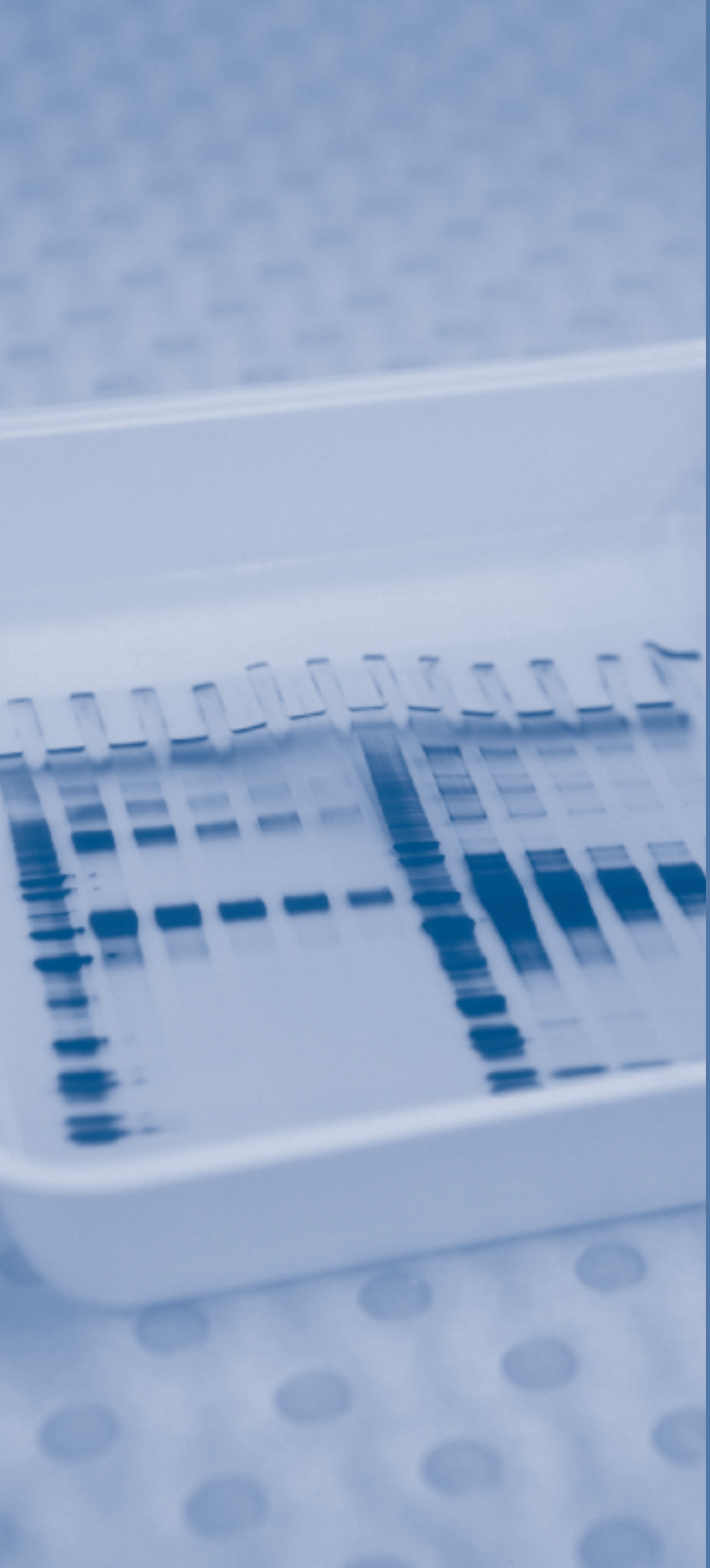
Werner Held, Ph.D., an Associate Member at the LICR Lausanne Branch, was one of the three investigators awarded the '2006 Leenard's Prize for the Promotion of Scientific Research' by the Leenard's Foundation, Lausanne, Switzerland.

Karen Oegema, Ph.D., an Assistant Member at the LICR San Diego Branch, was named the '2006 Woman in Cell Biology Junior Awardee' by the American Society for Cell Biology.

Ken Pang, M.D., a Ph.D. student at the LICR Melbourne Center, received a 'High Commendation' in the '2006 Premier's Awards for Medical Research in the State of Victoria.' Dr. Pang (right) is pictured receiving the award from The Honourable Steve Bracks MP, the Premier of the State of Victoria.

Serhiy Souchelnytskyi, Ph.D., an Assistant Member at the LICR Uppsala Branch, was awarded a High Cancer Research Position by the Swedish Cancer Society. Only two or three are awarded each year.

Messrs Georges-André Cuendet (left) and R. Palmer Baker Jr. retired from the LICR Board of Directors on June 30, 2006. Both were presented with the D.K. Ludwig Award, in recognition of their extraordinary years of service to the Institute.



As of 30th June 2006, LICR investigators had been awarded a total of USD 30 million in external funding from governments and foundations around the world. This constituted 30% of the LICR's annual operating budget of USD 100 million.

On 30th June 2006, there were 221 students being trained in LICR laboratories and a total of 888 LICR employees.

LICR investigators published more than 390 original research articles and research reviews in 2006

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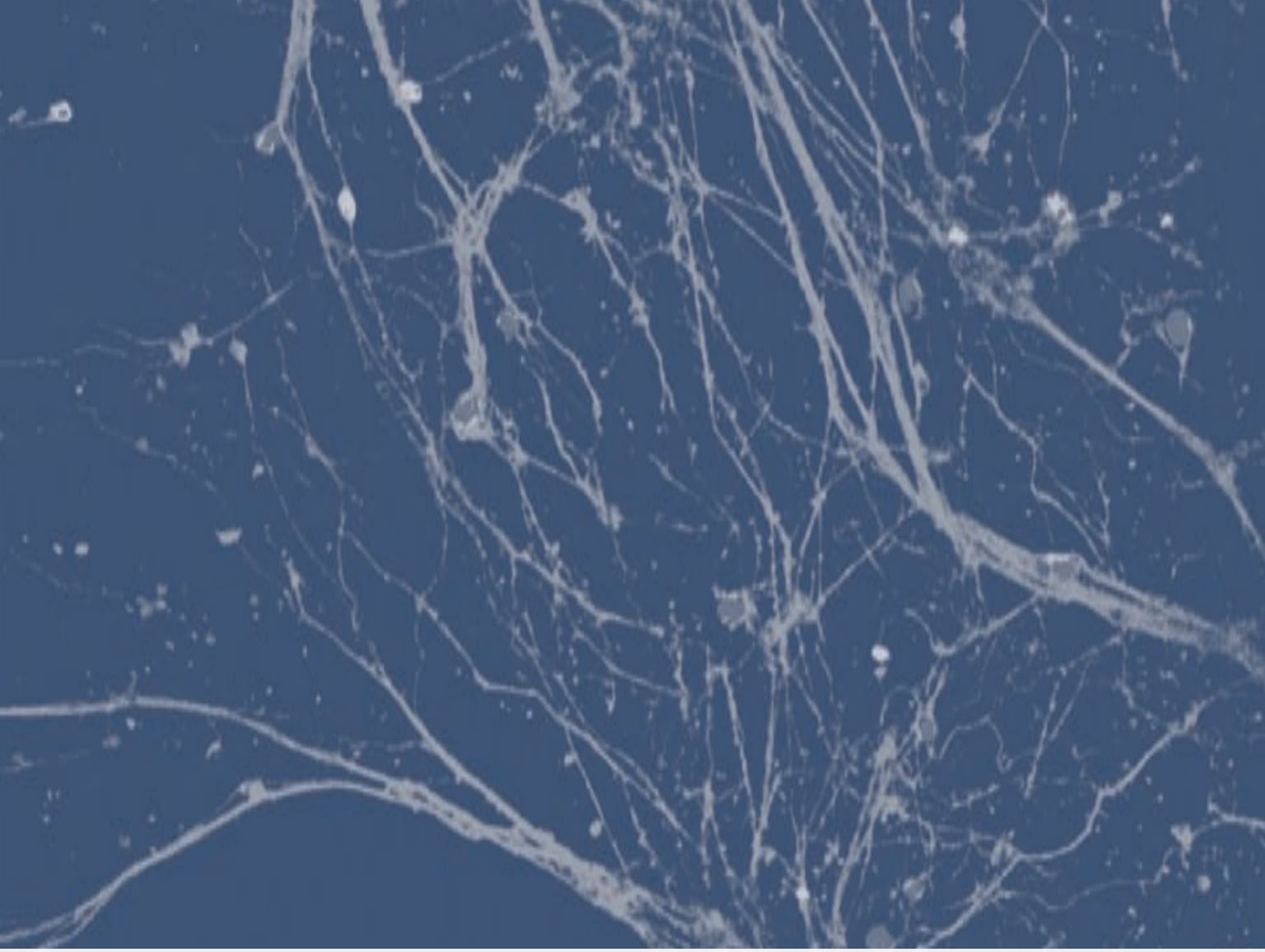

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