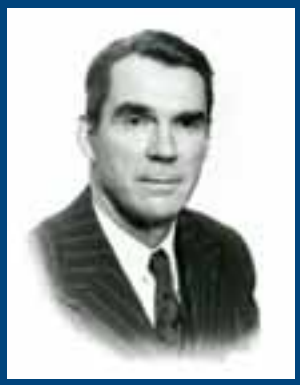



LUDWIG INSTITUTE FOR CANCER RESEARCH

Annual Research Highlights Report 2005

Index



I am persuaded that eventual mastery of cancer will come only from intense and unremitting scientific exploration over many decades.

Daniel K. Ludwig
December 17, 1974

Introduction

The Ludwig Institute for Cancer Research (LICR) is an international non-profit research institute dedicated to the fight against cancer. Through its Branches, affiliated laboratories and clinical trials sites, the LICR research enterprise spans the globe with activities across Asia, Australasia, Europe, and North and South America.

LICR is distinguishable from other cancer research institutes, not only by its size and global reach, but also by two fundamental attributes: its reliable, long-term funding perspective and the fact that it takes responsibility for the entire discovery continuum from the laboratory to the clinic.

A limitation of the traditional academic cancer research model is that funding is fragmented, overlapping and lacks strategic coordination. Universities and research institutes usually are not themselves able to finance a significant portion of the research conducted on their campuses. Rather, grants made directly to the individual investigators for individual projects are the principal source of funding for academic research. Consequently, the capacity of universities and institutes to influence the content or character of the work carried out by their faculty, or to bring together individuals or laboratories to create a larger, more synergistic research endeavor is diminished. The result is a system in which the research undertaken is predominantly defined by what can be achieved in a single laboratory within a funding cycle of, typically, three years. Though this research funding model has been effective, it supports a rather narrow range of disjointed activity. It does not encourage collaborative and coordinated undertakings or enable longer term, broad scale initiatives. In contrast, LICR funds over 70% of the cost of the research that it conducts. Though its most basic science is investigator-initiated, LICR encourages, enables and brings together multidisciplinary teams of investigators to tackle larger, more complex cancer challenges.

Many universities and academic research institutes lack the expertise, infrastructure and resources to take their laboratory discoveries into the clinic. To bridge that divide, they turn their work product over to companies that engage in drug development, a process driven by commercial considerations: time to product launch and share of market captured. Between the worlds of laboratory discovery and therapeutic utility, there is a missing link that disrupts the efficient exploration of knowledge for human benefit. That missing link is clinical discovery. LICR is convinced that the same systematic, investigative rigor that yielded the laboratory discovery in the first place should be applied in early-phase clinic trials to assess fully a discovery's therapeutic potential. It has, therefore, committed the resources and marshalled the capabilities that allow it to evaluate its promising basic discoveries in first-in-man clinical studies.

LICR is dedicated to making meaningful and lasting contributions to the understanding and clinical management of cancer. This report gives some highlights of the progress that LICR made to this end in 2005.

Antibody Targeting Program

The 806 Antibody from Laboratory to Clinical Discovery

Antibody-based therapies have long been an interest of the Ludwig Institute for Cancer Research. Antibodies that target signaling molecules that promote cancer cell growth currently represent one of the most promising areas in the development of new treatments for cancer. Pharmaceutical companies have recently brought to market several antibody therapies that effectively benefit patients by targeting the epidermal growth factor receptor (EGFR) (cetuximab), vascular endothelial growth factor (bevacizumab) or hematopoietic differentiation antigens (rituximab). Mutations or overexpression of EGFR are linked to over 50% of all cancers of epithelial cell origin, which makes this a very promising target for novel cancer therapies.

Some ten years ago, a collaboration between the San Diego and New York Branches was formed specifically to generate antibodies that target a mutant variant of EGFR, de2-7 EGFR (ΔEGFR, or EGFR variant III). The de2-7 EGFR mutant is found mostly in glioblastoma, a cancer that is intractable to virtually all conventional treatments, and is an excellent candidate for antibody targeting given that the variant is on the cell surface and is structurally distinct from normal, or 'wild-type', EGFR (wtEGFR). In the characterization process, one of those anti- de2-7 EGFR antibodies, 806, was found to also bind a subset of wtEGFR molecules, but *only* when the molecule was over-expressed, as in the case on many forms of carcinoma. The targeting of 806 to de2-7 EGFR and overexpressed wtEGFR, but not wtEGFR on normal tissue, makes this antibody unique, with less likelihood to cause toxic side-effects when used clinically and with the potential to have a far greater therapeutic index than other available agents that attach less selectively to all forms of EGFR.

LICR's Antibody Targeting Program has been investigating the 806 antibody in preclinical and clinical studies to assess its potential as a highly-specific EGFR-targeted therapy.

Conformation Analyses and New Epitopes for Antibody Targeting

LICR investigators have taken advantage of the unique binding specificity of 806 to further unravel the complexities of EGFR activation and its hyper-activity in cancer. A team from the Melbourne and New York Branches and Affiliates in New Haven have made major advances in our understanding of conformational epitopes in growth factors while investigating the structural basis for the specificity of 806. The team demonstrated that over-expression of EGFR leads to the accumulation of under-glycosylated EGFR in the cell's endoplasmic reticulum. Unexpectedly, this immature form of the receptor was also detected at the cell surface. Since under-glycosylated EGFR is primed for activation, it may contribute to spontaneous receptor activity and cancer cell growth. This body of work furthers our understanding of the activation and conformation of EGFR and also presents a new approach to generating potential anti-tumor antibodies with reduced targeting of normal tissues: the generation of antibodies specific to immature receptors.

Johns TG, Mellman I, Cartwright GA, Ritter G, Old LJ, Burgess AW, Scott AM. 'The antitumor monoclonal antibody 806 recognizes a high-mannose form of the EGF receptor that reaches the cell surface when cells over-express the receptor.' FASEB Journal 19(7):780-2, 2005.

Engineering & Characterization Of A Chimeric 806

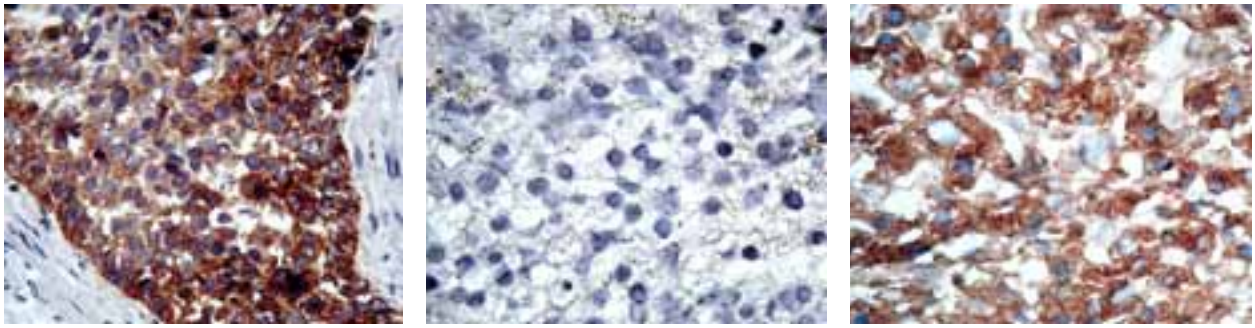
The original 806 was generated as a mouse antibody that may be immunogenic, *i.e.* cause the human immune system to react against the antibody therapy and clear it from the blood. Thus the mouse antibody was re-engineered by a team from the Melbourne Branch and Affiliates in Homburg to enable production of a chimeric antibody, ch806, appropriate for early-phase clinical trials.

Panousis C, Rayzman VM, Johns TG, Renner C, Liu Z, Cartwright G, Lee FT, Wang D, Gan H, Cao D, Kypridis A, Smyth FE, Brechbiel MW, Burgess AW, Old LJ, Scott AM. 'Engineering and characterisation of chimeric monoclonal antibody 806 (ch806) for targeted immunotherapy of tumours expressing de2-7 EGFR or amplified EGFR.' British Journal of Cancer 92(6):1069-77, 2005.

Anti-tumor Activity Of 806 In Xenografts

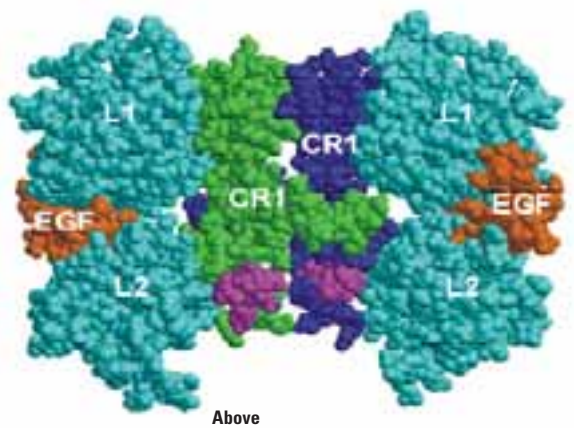
The treatment of human tumor xenografts in mice by research teams from the Melbourne, New York and San Diego Branches have shown that 806 has anti-tumor activity against glioma and against tumors with overexpressed EGFR. Further preclinical studies showed that combining 806 with a second form of anti-EGFR antibody or with a tyrosine kinase inhibitor specific to EGFR results in additive and, in some cases, synergistic anti-tumor activity.

Perera RM, Narita Y, Furnari FB, Gan HK, Murone C, Ahlkvist M, Luwor RB, Burgess AW, Stockert E, Jungbluth AA, Old LJ, Cavenee WK, Scott AM, Johns TG. 'Treatment of human tumor xenografts with monoclonal antibody 806 in combination with a prototypical epidermal growth factor receptor-specific antibody generates enhanced antitumor activity.' Clinical Cancer Research 11(17):6390-9, 2005.



Above
Left: Immunohistochemistry of a squamous cell carcinoma of the lung using LICR's monoclonal antibody (mAb) 806 shows staining (brown color) of cells that overexpress the epidermal growth factor receptor (EGFR). Non-cancerous cells that have normal levels or no EGFR are white or pale blue. The nuclei of the cells stain dark blue.

The advantage of 806 over other anti-EGFR mAbs is clear from immunohistochemistry on normal liver samples. The 806 mAb does not target normal cells (middle), whereas other anti-EGFR mAbs do bind to normal cells (right).



Above
Space-filled model of a ligand (EGF, in orange)-bound EGFR dimer stabilized by the interaction of the CR1-loop from each EGFR molecule. Reproduced from Johns et al. Journal of Biological Chemistry 279(29):30375-84, 2004.

The first clinical trial of 806 was initiated in 2005 at the Melbourne Branch. The trial is testing the safety of 806 in patients with carcinomas that express de2-7 EGFR or overexpress wtEGFR.

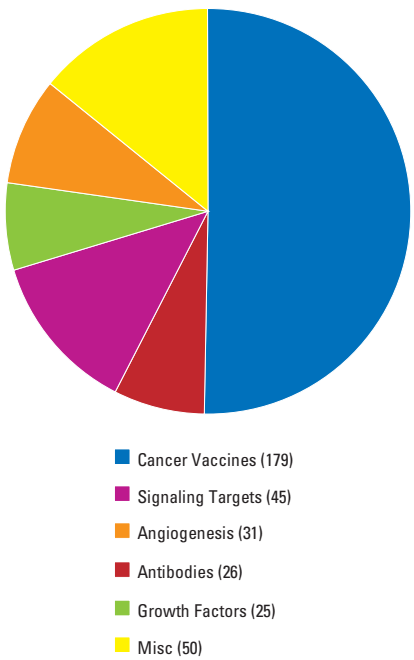
In 2005, the Institute had an externally funded budget of USD 26 million provided by governments, foundations and industry from around the world. This constituted 27% of the LICR 2005 annual operating budget of USD 95 million.

PIramed Enters R&D Collaboration With Genentech

LICR's first spin-off company, PIramed Ltd, was formed in 2003 to explore the therapeutic potential of PI 3-kinase research conducted at the UCL Branch and collaborators at Cancer Research UK and the Institute of Cancer Research. In 2005, PIramed entered into a research and development collaboration with one of the world's leading biotechnology companies, Genentech, Inc. This is believed to be one of the largest preclinical collaborations ever signed by a UK biotechnology company.

PIramed will conduct preclinical research with Genentech, and Genentech will be solely responsible for clinical development, regulatory approvals, manufacturing and commercialisation. PIramed has an exclusive licence to novel isoform-specific PI 3-kinase inhibitors from the tri-partite collaboration between LICR, Cancer Research UK and the Institute of Cancer Research and Yamanouchi Pharmaceutical Company which preceded formation of the Company. PIramed also has an exclusive licence for screening PI 3-kinase p110α from LICR.

On June 30, 2005, LICR's patent estate was 356 issued patents. The estate can be broken down into the research areas shown at right.



Gene Expression

Gene expression is the process by which the information in a gene is used to generate its functional product, usually a protein. First, the gene's DNA sequence is 'transcribed' into an RNA sequence under strict regulatory controls (see p7, "Transcription Regulation"). After processing of a primary RNA transcript, the resultant 'messenger RNA' (mRNA) is 'translated' into a protein. Measuring gene expression, usually by detecting the presence of mRNA transcripts, identifies genes active in particular cell processes or during tumorigenesis.

Identification of Cancer-Testis (CT) Genes

An LICR-led collaboration of academic and commercial groups used massively-parallel signature sequencing (MPSS) technology to identify 20 genes that encode putative cancer-testis (CT) antigens, proteins expressed in germ cells and in cancer cells and recognized by the immune system. It was shown that a substantial proportion of these CT or CT-like genes are found on the X-chromosome, and a new gene family of distinctive X-linked CT antigens was also discovered. These results were featured 'Research Highlights' in *Nature Reviews Cancer* in July. The team also used MPSS to elucidate the gene expression profiles of some 20000 genes in 32 human tissue samples. All of these data were made publicly-available at http://mpss.licr.org

Chen YT, Scanlan MJ, Venditti CA, Chua R, Theiler G, Stevenson BJ, Iseli C, Gure AO, Vasicek T, Strausberg RL, Jongeneel CV, Old LJ, Simpson AJG. 'Identification of distinctive cancer/testis-antigen genes by massively parallel signature sequencing.' Proceedings of the National Academy of Sciences USA 106:45-12, 2005.

Jongeneel CV, Delorenzi M, Iseli C, Zhou D, Haudenschild CD, Khrebtukova I, Kuznetsov D, Stevenson BJ, Strausberg RL, Simpson AJ, Vasicek TJ. 'An atlas of human gene expression from massively parallel signature sequencing (MPSS).' Genome Research 15(7):1007-14, 2005

Correlation Of Gene Expression And Prognosis

CT antigens are potential targets for immunotherapies (see pp14-15, 'Cancer Vaccine Program') and may also provide prognostic information. A study from the New York Branch and Affiliates in New York showed the expression of CT genes appears to correlate with a poor outcome in non-small cell lung cancer.

Gure AO, Chua R, Williamson B, Gonen M, Ferrera CA, Gnjatic S, Ritter G, Simpson AJ, Chen YT, Old LJ, Altorki NK. 'Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer.' Clinical Cancer Research 11(22):8055-62, 2005.

Early Diagnosis Of Cancer And Other Diseases

A study from the São Paulo Branch was featured on the cover of the American Association for Cancer Research's journal, *Cancer Research*. Gene expression profiles of esophageal and stomach cancers, which are thought to be caused by inflammation, and intestinal metaplasias (structural changes in the cells) were elucidated. The results have direct application for the diagnosis and understanding of Barrett's disease, which is characterized by intestinal metaplasia and which is a risk factor for adenocarcinoma of the esophagus.

Gomes LI, Esteves GH, Carvalho AF, Cristo EB, Hirata R Jr, Martins WK, Marques SM, Camargo LP, Brentani H, Pelosof A, Zitron C, Sallum RA, Montagnini A, Soares FA, Neves EJ, Reis LF. 'Expression profile of malignant and nonmalignant lesions of esophagus and stomach: differential activity of functional modules related to inflammation and lipid metabolism.' Cancer Research 65(16):7127-36, 2005.

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A new theory of cancer—gametic recapitulation

Data suggest that cancer-causing mutations can result in the reactivation of gene expression programs normally only active in germ cells. Could these transcription programs confer some of the central characteristics of malignancy?

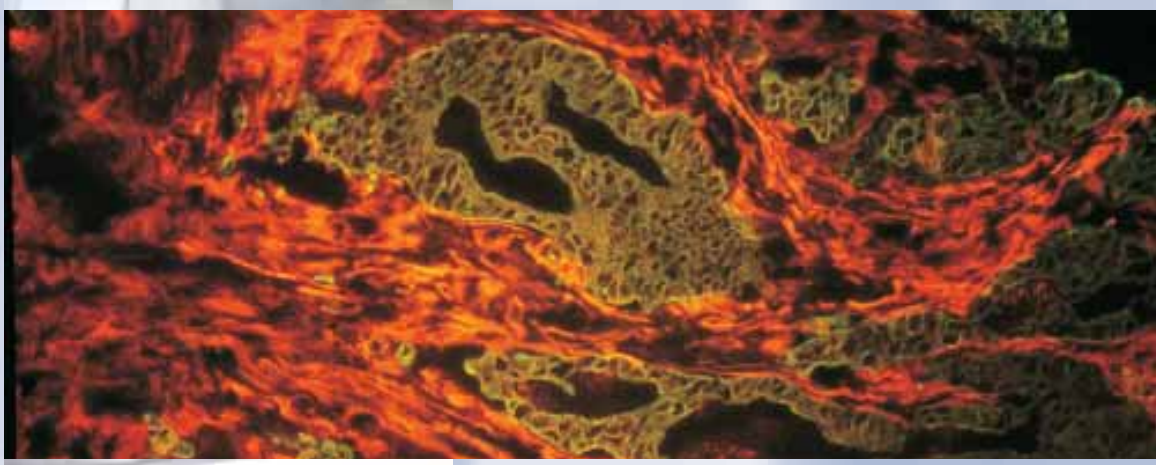
- Features of the development of germ cells and tumors share important biological similarities, including immortalization, migration, invasion, immune evasion, angiogenesis, hypomethylation of promoters, growth and differentiation.
- Human cancers so frequently produce trophoblastic hormones, that some (for example, chorionic gonadotropin) are used as prognostic indicators for a range of epithelial tumors.
- A growing number of proteins, the cancer-testis (CT) antigens, appear to be active only in germ cells, trophoblasts and tumors.

LICR scientists have proposed that reactivation of gene expression programs would result in gamete-specific products deleterious for the orderly requirements of normal somatic cells, but highly advantageous for the cancer cell. In this model, normal stem cells become altered not in the genes that directly control the cell cycle and proliferation, but in the genes that control germ-cell gene expression.

Simpson AJG, Caballero OL, Jungbluth A, Chen YT, Old LJ. 'Cancer/Testis Antigens, Gametogenesis and Cancer.' Nature Reviews Cancer 100:46-12, 2005.

Human Colon Cancer Initiative (HCCI)

LICR has undertaken to collect and generate molecular and clinical data with a view to developing methods for improving the treatment of colon cancer patients. The HCCI, which is based at the LICR Melbourne Branch, is a network of clinical and scientific collaborators who have now collected colon cancer specimens, blood and the relevant clinical data from hundreds of patients. In conjunction with Australia's largest scientific organization, the Commonwealth Scientific and Industrial Research Organization (CSIRO), both antibody and proteomics assays are being used to analyze the blood for potential prognostic and treatment biomarkers. Arrangements are being put in place to analyze the tumor DNA for mutations known to be associated with colon cancer.



The LICR Antibody Targeting Program is investigating the therapeutic potential of combining antibodies against cancer cells with antibodies against tumor stroma and tumor blood vessels. This figure shows immuno-fluorescent-labeled mAb A33 targeting colon cancer cells (yellow/green) and mAb F19 targeting the tumor stroma (orange/red), the connective tissue around the tumor cells.

In April 2005, the State Government of Victoria (Australia) awarded a $7 million grant to establish the Victorian Tissue Bank Initiative (VTBI), which will provide tissue specimens to the HCCI and other cancer research organizations in the State of Victoria. Together, LICR, CSIRO, the State Government of Victoria and the Royal Melbourne Hospital are supporting the development of sophisticated clinical informatics tools and the expansion of tissue collection to more than twenty hospitals in Australia and the USA.

Transcription Regulation

The regulation of transcription (the copying of DNA into RNA) is a critical first step in controlling the gene expression necessary for all cellular processes. Transcription is regulated by complex interactions between transcription factors, which bind to promoter sequences at the beginning of each gene, and proteins that bind to regulatory enhancer, repressor and/or insulator sequences in the genome. These regulatory sequences define the combinatorial codes that direct and specify gene expression patterns. Identification and characterization of these regulatory sequences are vital to understanding the complex patterns, or 'profiles,' of gene expression and elucidating the molecular basis of cancer.

Sterol Regulatory Element Binding Proteins

The SREBP family of transcription factors regulates genes involved in the synthesis of lipids, which are required for, amongst other things, the formation of cell membranes. LICR scientists from the Uppsala Branch analyzed the expression of key genes involved in lipid metabolism, and found that SREBP-mediated transcription was regulated during the cell cycle as a result of specific modifications of the SREBP proteins. The team also showed that the SREBP family is itself regulated by the SCFFbw7 protein, which has been shown to control several other proteins vital for cell cycle control, and also to be inactivated in cancers of the breast, endometrium, ovary and colon. The evidence supports the hypothesis that deregulation of lipid synthesis facilitates the growth and proliferation of cancer cells. The research also suggested that SCFFbw7 and its interaction with SREBPs may be an attractive target for developing new cholesterol-lowering therapies for the fight against cardiovascular disease.

Bengoechea-Alonso MT, Punga T, Ericsson J. 'Hyperphosphorylation regulates the activity of SREBP1 during mitosis.' Proceedings of the National Academy of Sciences USA 102(33):11681-6, 2005.

Sundqvist A, Bengoechea-Alonso MT, Ye X, Lukiyanchuk V, Jin J, Harper JW, Ericsson J. 'Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCFFbw7.' Cell Metabolism 1(6):379-91, 2005.

Whole Genome Promoter Mapping

A team from the San Diego Branch developed an efficient, new method to identify thousands of regulatory sequences, in a study that marks a major advance in decoding regulatory networks in the human genome. The study was conducted with a commercial partner and is part of a competitive National Human Genome Research Institute (USA) initiative, 'The ENCODE Project: ENCyclopedia of DNA Elements.'

Kim TH, Barrera LO, Zhen M, Qu C, Singer MA, Richmond TA, Wu Y, Green RD, Ren B. 'A high-resolution map of active promoters in the human genome.' Nature 436(7052):876-80, 2005.

Kim TH, Barrera LO, Qu C, Van Calcar S, Trinklein ND, Cooper SJ, Luna RM, Glass CK, Rosenfeld MG, Myers RM, Ren B. 'Direct isolation and identification of promoters in the human genome.' Genome Research 15(6):830-9, 2005.

Sox21 in Neuronal Differentiation

Research from the Stockholm Branch was featured on the cover of *Nature Neuroscience* in August. The stunning image showed fluorescent-labeled transcription factors in the spinal cord of an embryonic chick. The team discovered that Sox21 inhibits the Sox1-3 transcription factors that prevent the differentiation of neural cells. The balance of Sox21/Sox1-3 determines whether neural cells remain as precursors or differentiate into neurons. It is highly plausible that the mechanism governing neural stem cell differentiation has parallels in cancer stem cell differentiation.

Sandberg M, Kallstrom M, Muhr J. 'Sox21 promotes the progression of vertebrate neurogenesis.' Nature Neuroscience 8(9):995-1001, 2005.

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TGF-β Program

Transforming growth factor (TGF)-β is the archetypal member of a super-family of cytokines that regulate cell growth, differentiation, proliferation and apoptosis (programmed cell death), and also tumor invasiveness. By binding to type I (ALK) and type II receptors, TGF-β members trigger a complicated signal transduction system involving the selective activation and inhibition of intracellular proteins known as 'Smads', ultimately resulting in gene transcription by Smad protein complexes.

SMAD7 Levels Predict Prostate Cancer Therapy Response

The compound 2-methoxyestradiol (2-ME) is being investigated as a potential cancer therapy because of its capacity to inhibit tumor angiogenesis and cause cancer cell apoptosis. LICR investigators from the Uppsala Branch showed that the apoptotic effect of 2-ME is dependent upon the gene expression of Smad7, a TGF-β signaling pathway adaptor molecule, in prostate cancer cells. These findings suggest that measuring Smad7 levels in prostate cancer patients may indicate which patients will or will not benefit from treatment with 2-ME. The team also delineated the signaling pathways in TGF-β-induced apoptosis of prostate cancer cells, identifying crucial roles for Smad7 and β-catenin, a signaling protein mutated in many cancers.

Edlund S, Lee SY, Grimsby S, Zhang S, Aspenstrom P, Heldin CH, Landstrom M.'Interaction between Smad7 and beta-catenin: importance for transforming growth factor beta-induced apoptosis.' Molecular Cell Biology 25(4):1475-88, 2005.

Davoodpour P, Landstrom M.'2-Methoxyestradiol-induced apoptosis in prostate cancer cells requires Smad7.' Journal of Biological Chemistry 280(15):14773-9, 2005.

New TGF-β/cytokine Link In Gastric Cancer

TGF-β/Smad and cytokine/STAT are two powerful signaling pathways that have been implicated independently in cancer onset and progression. Investigators from the Melbourne Branch have established a novel link between these two pathways in the initiation and progression of tumor formation in the stomach. In the mouse, excessive activation of STAT3 induces the TGF-β inhibitor protein, Smad7, which causes epithelial cells to be released from TGF-β's cell cycle control. Importantly, gene expression profiles from human tumor samples suggest the same mechanism is occurring in human gastric cancer. These results add further weight to the argument for developing cancer therapies that target STAT3 and TGF-β.

Jenkins BJ, Grail D, Nheu T, Najdovska M, Wang B, Waring P, Inglese M, McLoughlin RM, Jones SA, Topley N, Baumann H, Judd LM, Giraud AS, Boussioutas A, Zhu HJ, Ernst M. 'Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling', Nature Medicine 11(8):845-52, 2005.

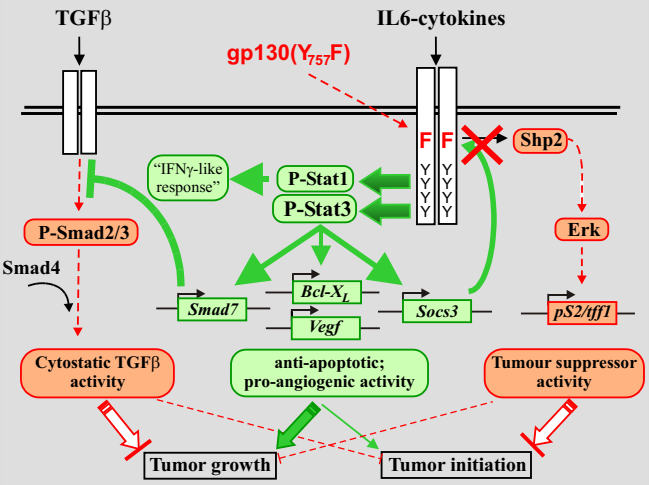


Diagram of proposed TGF-β/cytokine signaling in gastric cancer. Reproduced from Jenkins et al. *Nature Medicine* 11(8) :845-52, 2005.

TGF-β/Smad3 Counteracts BRCA-1

Mutations in the BRCA-1 gene, which regulates DNA damage repair, gene transcription and chromatin remodeling, confer susceptibility to early-onset familial breast and ovarian cancers. A team from the Uppsala Branch has shown that Smad3 binds to BRCA1 and suppresses BRCA1-dependent DNA repair in breast cancer cells.

Dubrovska A, Kanamoto T, Lomnytska M, Heldin CH, Volodko N, Souchelnytskyi S. "TGFbeta1/Smad3 counteracts BRCA1-dependent repair of DNA damage.' Oncogene 24(14):2289-97, 2005.

Phosphoproteome Analysis Of TGF-β

Investigators from the Uppsala Branch also conducted the first phosphoproteomic analysis of TGF-β stimulated cells and learned more about TGF-β-induced gene expression in breast cancer cells. A phosphoproteomic analysis detects proteins activated by 'phosphorylation', the addition of a phosphate group. Below is an image of proteins (appearing as black spots on a silver background) phosphorylated in response to TGF-β stimulation of breast cancer cells.

Stasyk T, Dubrovska A, Lomnytska M, Yakymovych I, Wernstedt C, Heldin CH, Hellman U, Souchelnytskyi S. 'Phosphoproteome profiling of transforming growth factor (TGF)-beta signaling: abrogation of TGFbeta1-dependent phosphorylation of transcription factor-II-I (TFII-I) enhances cooperation of TFII-I and Smad3 in transcription.' Molecular Biology of the Cell 16(10):4765-80, 2005.

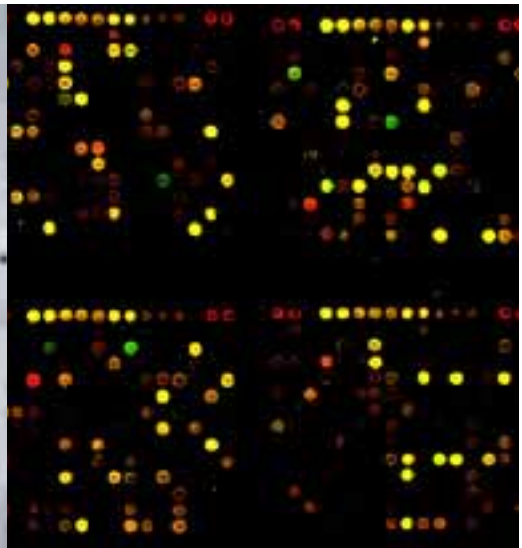
Smad7 and members of the TGF-β type I receptor family, ALK1-7, were discovered and characterized by investigators from the Uppsala Branch in the early 1990's. These results formed the basis of LICR's TGF-β Program, which integrates research from the Uppsala, Brussels and Melbourne Branches and Affiliates in Amsterdam and Tokyo.

Reprogramming Of Gene Expression In Tumor Invasion

Differentiation from an epithelial cell to a more motile mesenchymal cell—epithelial-mesenchymal transition (EMT)—is a first step in tumor invasion and metastasis. LICR investigators from the Uppsala Branch analyzed the gene expression profiles in various mouse and human epithelial cells following stimulation with different TGF-β family members. This study is the first step in the decryption of genetic networks downstream of TGF-β, which link cell proliferation and EMT; knowledge critical to identifying candidate targets for therapies that might prevent EMT.

Valcourt U, Kowanetz M, Niimi H, Heldin CH, Moustakas A. 'TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial-mesenchymal cell transition.' Molecular Biology of the Cell 16(4):1987-2002, 2005.

Below: An image of a two-dimensional gel detecting proteins (black spots and streaks) present in a breast cancer cell line following TGF-β stimulation.



Left: A close-up of a microarray used to compare gene expression in human epithelial cells that have been stimulated by TGF-β and those that have not. Genes that have elevated expression (red spots) are induced by TGF-β, while genes that have lowered expression (green spots) are repressed by TGF-β. Yellow spots identify genes that are not involved or controlled by TGF-β signaling.

Celebrating its '5th Birthday' in 2005, *Nature Immunology* selected several papers from each of its five years, that "helped drive the immunology field forward". One of these studies was conducted by a research team from the LICR New York Branch and Affiliates in Konstanz and Oxford as part of the Cancer Vaccine Collaborative (see p15).

Gadola SD, Zaccai NR, Harlos K, Shepherd D, Castro-Palomino JC, Ritter G, Schmidt RR, Jones EY, Cerundolo V. 'Structure of human CD1b with bound ligands at 2.3 Å, a maze for alkyl chains. Nature Immunology 3:721-6, 2002.

On June 30, 2005, there were 218 students being trained in LICR laboratories and a total of 919 LICR employees.

Myeloproliferative Diseases

Hematopoiesis is the formation of blood cells, and disruptions to this process can result in myeloproliferative disesases such as leukemia (abnormal proliferation of white blood cells), polycythemia vera (PV, excessive production of red blood cells), and thrombocytosis (excessive production of platelets). A group of signalling factors critical in hematopoiesis are the 'cytokines', which regulate cell growth and differentiation by binding to cell surface receptors that signal through the Janus kinases (JAKs) to activate the STAT family of transcription factors.

Hyperactive STAT3 In Thrombocytosis

The interleukin-6 (IL-6) cytokine family binds to the gp130 receptor to regulate cellular responses during hematopoiesis. Investigators from the Melbourne Branch found, in a mouse model, that mutated gp130 resulted in hyperactivation of STAT1 and STAT3 and caused a broad spectrum of hematopoietic abnormalities. The team showed that gp130-dependent STAT3, but not STAT1, hyperactivity is responsible for thrombocytosis, and enlargement of the spleen and lymph nodes in mice.

Jenkins BJ, Roberts AW, Najdovska M, Grail D, Ernst M. 'The threshold of gp130-dependent STAT3 signaling is critical for normal regulation of hematopoiesis.' Blood 105(9):3512-20, 2005.

Jagged1-dependent Notch Signaling

The conventional thinking was that Jagged1, a ligand for Notch receptors, was critical for renewal of the hematopoietic stem cells (HSC) from which blood and immune cells are formed. However a team from the Lausanne Branch showed that inactivating the *jagged1* gene in bone marrow does not impair HSC self-renewal. These data have thus confounded the paradigm that Jagged1-dependent Notch signaling is essential for hematopoiesis.

Mancini SJ, Mantei N, Dumortier A, Suter U, Macdonald HR, Radtke F. 'Jagged1-dependent Notch signaling is dispensable for hematopoietic stem cell self-renewal and differentiation.' Blood 105(6):2340-2005.

JAK Mutations In Human Disease

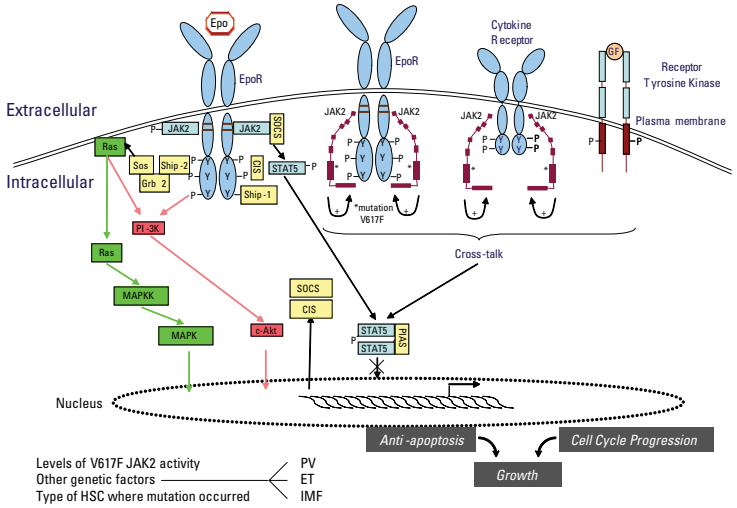
Scientists from the Brussels Branch were part of a collaborative study that identified a JAK2 mutation as being the principal cause of PV. The mutation constitutively activated JAK2 and is found in other myeloproliferative disorders, allowing a whole new classification of these disorders to be made. Subsequently, the LICR investigators showed that the homologous mutations in two other JAKs, JAK1 and Tyk2, led to constitutive activation of STAT3, STAT5 and several other signaling pathways implicated in cancer. These results suggest that JAK1 and Tyk2 are potential oncogenes, and should be investigated in various human cancers.

James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Bennaceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W. 'A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera.' Nature 434(7037):1144-8, 2005.

Staerk J, Kallin A, Demoulin JB, Vainchenker W, Constantinescu SN. 'JAK1 and Tyk2 activation by the homologous polycythemia vera JAK2 V617F mutation: Cross-talk with IGF1 receptor.' Journal of Biological Chemistry 280:41893-41899, 2005.

Janus Kinase 2 (JAK2) was originally discovered at the Melbourne Branch in 1992. It has subsequently been shown to play crucial roles in signal transduction, via cytokine, G protein-coupled and tyrosine kinase growth factor receptors, in mammalian development, physiology and disease. In fact, it appears that JAK2 is responsible for at least three different myeloproliferative disorders.

Harpur AG, Andres AC, Ziemiecki A, Aston RR, Wilks AF. 'JAK2, a third member of the JAK family of protein tyrosine kinases.' Oncogene 7(7):1347-53, 1992.



Diagrammatical representation of JAK2 signaling in myeloproliferative disorders. This LICR study was featured on the front cover of *The American Sociey of Hematology Education Program Book*, 2005.

Vainchenker W, Constantinescu SN. 'A Unique Activating Mutation in JAK2 (V617F) Is at the Origin of Polycythemia Vera and Allows a New Classification of Myeloproliferative Diseases.' Hematology (American Society of Hematology Education Program) 195-200, 2005.

Branches

Each of the Institute's Branches (see p25) is physically and functionally associated with a university or research institute and/or a non-profit hospital. This arrangement guarantees an academic environment conducive to laboratory discovery and provides access to local institutional resources and expertise in both the laboratory and the clinic.

Brussels, Belgium
Lausanne, Switzerland
University College London, London, UK
Melbourne, Australia
New York, USA
San Diego, USA
São Paulo, Brazil
Stockholm, Sweden
Uppsala, Sweden
London St Mary's, London, UK *(until July)*

James R. Kerr Program

The James R. Kerr Program enables interactions between LICR and leading investigators in countries that are scientifically talented but offer few opportunities for international collaboration in advanced cancer research. James R. Kerr Program investigators are located in the following cities:

Beijing, China
Cape Town, South Africa
Istanbul, Turkey
Kyiv, Ukraine
Moscow, Russia
Xi'an, China

Affiliates

LICR extends its knowledge and expertise, in addition to institutional affiliations at each Branch location, by recruiting outstanding research and clinical investigators as LICR Affiliates. Some of these Affiliates are active in LICR Clinical Trials (see p16). LICR Affiliates are located in the following cities:

Amsterdam, The Netherlands
Auckland, New Zealand
Buffalo, USA
Brussels, Belgium
Cambridge, UK
Frankfurt, Germany
Gunma, Japan
Helsinki, Finland
Homburg, Germany
Ithaca, USA
Konstanz, Germany
Kuopio, Finland
Kyoto, Japan
Lausanne, Switzerland
London, UK
Mie, Japan
New Haven, USA
New York, USA
Nijmegen, The Netherlands
Nagasaki, Japan
Okayama, Japan
Osaka, Japan
Oxford, UK
Philadelphia, USA
St Louis, USA
Tokyo, Japan
Zürich, Switzerland

Cancer Vaccine Program

The human immune system fights cancer partly through the production and activation of specialized immune cells; 'cytolytic T cells' (CTL) and 'helper T cells'. Each CTL population recognizes a different 'antigen' on the cancer cell surface and destroys that cell. Cancer may escape from immunological control if the rate of cancer growth outstrips the immune system's activity, or if mechanisms of immune modulation or suppression are activated, by the tumor, in the local tumor environment. Immunotherapies such as cancer vaccines are designed to tip the balance in favor of the patient's immune response by stimulating the production of specific T cells against the particular cancer antigen (or mix of antigens) in the vaccine.

LICR investigators from the Brussels Branch were the first to clone a human cancer antigen. This seminal research allowed the rational design of antigen-based cancer vaccines.

van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. 'A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma.' Science (1991) 254(5038):1643-7.

CTL Populations Reawakened By Cancer Vaccines

Investigators at the Brussels Branch reported, in back-to-back *Journal of Experimental Medicine* papers, that a cancer vaccine can specifically stimulate the production of antigen-specific CTLs and also non-specifically activate CTL populations produced spontaneously against multiple cancer antigens. This observation opens a new way of thinking about cancer vaccines having a role in reawakening multiple CTL populations that had been been functionally paralyzed, perhaps through immune modulation and suppression by the tumor.

Germeau C., Ma W., Schiavetti F., Lurquin C., Henry E., Vigneron N., Brasseur F., Lethe B., De Plaen E., Velu T., Boon T., Coulie P.G. High frequency of antitumor T cells in the blood of melanoma patients before and after vaccination with tumor antigens. Journal of Experimental Medicine (2005) 201(2):241-8

Lurquin C., Lethe B., De Plaen E., Corbiere V., Theate I., van Baren N., Coulie P.G., Boon T. Contrasting frequencies of antitumor and anti-vaccine T cells in metastases of a melanoma patient vaccinated with a MAGE tumor antigen. Journal of Experimental Medicine (2005) 201(2):249-57

Focus on Pro-tumor Treg Cells

The immune system is a powerful force for the destruction of invaders, but must be closely controlled so that autoimmunity (immune system attack on normal cells) does not occur. One mechanism of immune regulation is the production of regulatory T cells, 'Treg cells'. Treg cells suppress the activity of key immune system components directed towards normal cells, thus protecting them from destruction. However, Treg cells may also act to negate am immune response induced by cancer vaccines and thus 'protect' cancer cells. This year saw a concerted focus on the function and role of Treg cells in cancer by a team from the New York Branch and Affiliates in Mie and St Louis. Using mouse models, the team found that Treg cells promote immunological 'escape' of cancer cells by inhibiting 'natural killer' (NK) and NKT cells, and that the effect of Treg cells on anti-tumor responses is controlled by interferon-gamma (IFN-γ). The team also defined the specific protein sequences that are recognized by naturally occurring Treg cells. The team also showed that Treg cells suppress the generation and activation of antigen-specific T helper cells in humans. The study was conducted on samples from patients with a naturally occurring antibody response to the NY-ESO-1 cancer-testis (CT) antigen, from normal donors, and from patients without NY-ESO-1 antibodies. In patients and donors without NY-ESO-1 antibodies, depletion of Treg cells was required before NY-ESO-1-specific T helper cells could be induced. Together these data suggest that the inclusion of a compound that inhibits Treg function may be required before cancer vaccine therapies can be fully effective.

Nishikawa H, Kato T, Tawara I, Takemitsu T, Saito K, Wang L, Ikarashi Y, Wakasugi H, Nakayama T, Taniguchi M, Kuribayashi K, Old LJ, Shiku H. 'Accelerated chemically induced tumor development mediated by CD4+CD25+ regulatory T cells in wild-type hosts.' Proceedings of the National Academy of Sciences USA 102(26):9253-7, 2005.

Nishikawa H, Kato T, Tawara I, Ikeda H, Kuribayashi K, Allen PM, Schreiber RD, Old LJ, Shiku H. 'IFN-gamma controls the generation/activation of CD4+ CD25+ regulatory T cells in antitumor immune response.' Journal of Immunology 175(7):4433-40, 2005.

Nishikawa H, Kato T, Tawara I, Saito K, Ikeda H, Kuribayashi K, Allen PM, Schreiber RD, Sakaguchi S, Old LJ, Shiku H. 'Definition of target antigens for naturally occurring CD4(+) CD25(+) regulatory T cells.' Journal of Experimental Medicine 201(5):681-6, 2005.

The team also showed that Treg cells are involved in the generation and regulation of antigen-specific T helper cells. The study was conducted on samples from patients with a naturally occurring antibody response to the NY-ESO-1 cancer-testis (CT) antigen, from normal donors and from patients without NY-ESO-1 antibodies. In patients and donors without NY-ESO-1 antibodies, depletion of Treg cells is required before NY-ESO-1-specific T

helper cells can be induced. Thus cancer vaccine therapies may require the inclusion of a compound to inhibit Treg function in order to be fully effective.

Nishikawa H, Jager E, Ritter G, Old LJ, Gnjatic S. 'CD4+ CD25+ regulatory T cells control the induction of antigen-specific CD4+ helper T cell responses in cancer patients.' Blood 106(3):1008-11, 2005.

Tumor Infiltrating Lymphocytes As Prognostic Indicators

Evidence is mounting that a cancer patient's prognosis can be predicted by the presence of tumor infiltrating lymphocytes (TILs), the white blood cells that produce components of the immune system. A multi-national CVC team, which included investigators from the New York Branch and LICR Affiliates in New York and Buffalo, examined the precise location and nature of TIL subpopulations in 117 patients with epithelial ovarian cancer. Results of the detailed analyses indicate that specific ratios of CD8+/CD4+ TIL subtypes and CD8+/Treg were associated with a favorable prognosis, with the latter corresponding to a 70% increase in patient survival with epithelial ovarian cancer.

Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani, H, Old LJ, Odunsi K. 'Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proceedings of the National Academy of Sciences USA (2005) 102(51):XX

Immunotherapy For Multiple Myeloma

Multiple myeloma is an incurable disease, although some donor lymphocyte infusion has been efficacious in a small number of patients. LICR investigators from the New York Branch and Affiliates in New York have discovered that the majority of plasma cells transformed in multiple myeloma express the CT antigens, CT7 (or MAGE-C1) and/or MAGE-A3/6. The level of expression of the antigens also correlated with plasma cell proliferation, suggesting that each has a role in the etiology of the cancer (see also p5, 'A New Theory of Cancer—Gametic Recapitulation'). This study, published in *Blood* and highlighted by an accompanying commentary (*Blood* 106(1):5, 2005), presents these CT antigens as promising targets for future vaccine therapies for multiple myeloma.

Jungbluth AA, Ely S, DiLiberto M, Niesvizky R, Williamson B, Frosina D, Chen YT, Bhardwaj N, Chen-Kiang S, Old LJ, Cho HJ. 'The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation.' Blood 106(1):167-74, 2005.

First Cancer Vaccine Trial Of TLR9 Agonist

A team of academic and industry collaborators led by the Lausanne Branch conducted the first clinical trial of a synthetic Toll 9 receptor (TLR9) ligand agonist, CpG7909, designed to enhance the immunological response to a cancer antigen, Melan-A. TLR9 agonists can mimic the innate immune response, which is among the most fundamental pathways that protect the body from bacterial pathogens. The vaccine plus adjuvant induced CTL production that was an order of magnitude higher than patients who received the Melan-A antigen alone. The TLR9 ligand agonist is now being developed as an immune modulator in Phase III trials by a major pharmaceutical company.

Speiser D.E., Lienard D., Rufer N., Rubio-Godoy V., Rimoldi D., Lejeune F., Krieg A.M., Cerottini J.C., Romero P. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. Journal of Clinical Investigation (2005) 115(3):739-46.

Cancer Vaccine Collaborative (CVC)

The CVC is an innovative partnership between two not-for-profit institutions, the Cancer Research Institute and the Ludwig Institute for Cancer Research. 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 Program constitutes the LICR laboratory and clinical research conducted under the auspices of the CVC.

CANCER RESEARCH INSTITUTE

LUDWIG INSTITUTE FOR CANCER RESEARCH

Clinical Discovery

LICR believes that human benefit from laboratory research is derived most efficiently when early clinical studies are conducted to verify and explore discoveries in the human setting. Integrated laboratory and clinical research, or 'clinical discovery', is fundamental to the mission of LICR. Therefore, LICR sponsors and conducts its own early-phase, proof-of-concept clinical trials to explore the potential of new treatment strategies with maximum efficiency and innovation, while ensuring patient safety and appropriate compliance with regulatory guidelines.

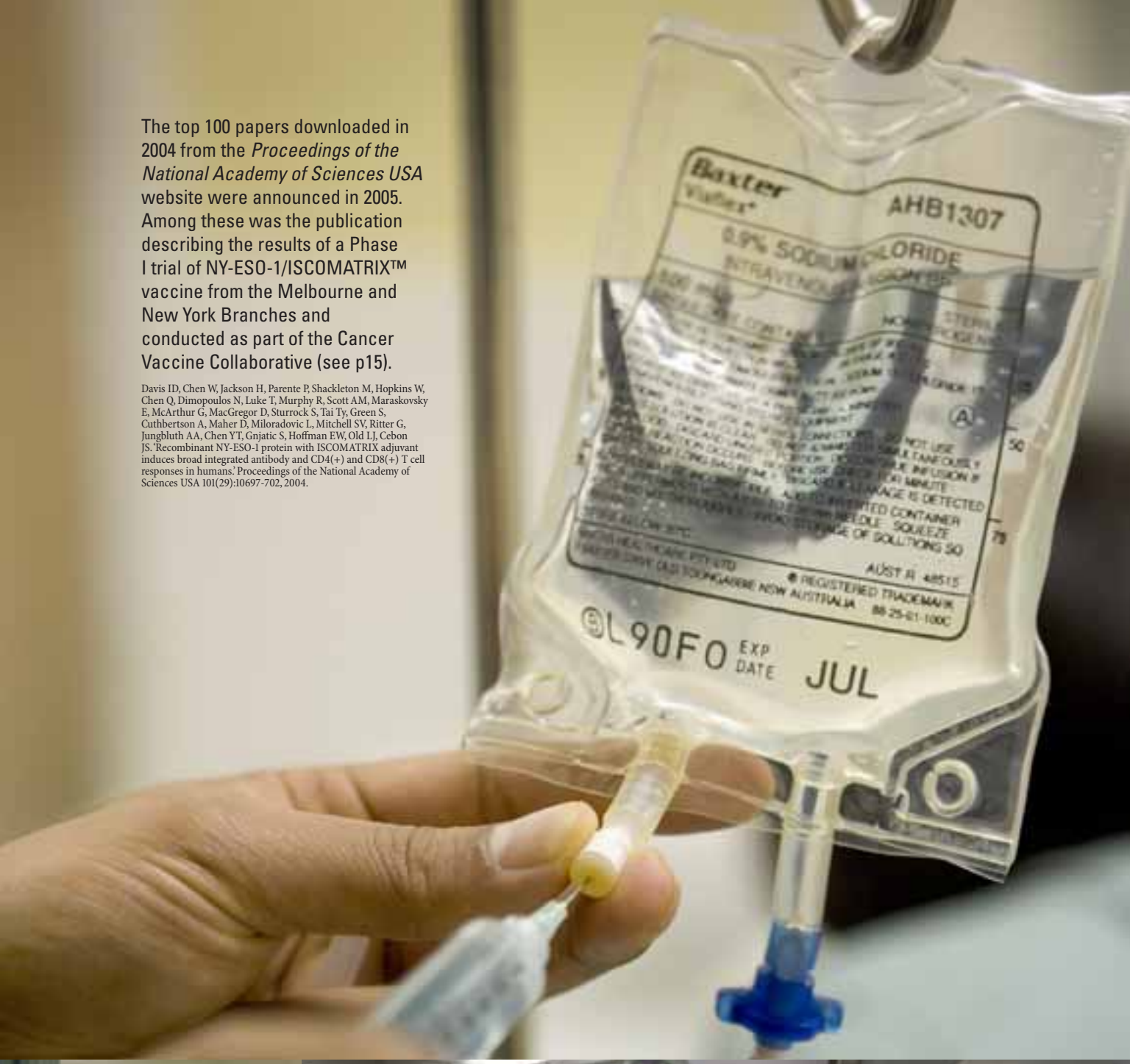
LICR initiated 10 early-phase clinical trials in 2005, bringing the total number of active LICR sponsored trials to 32. The clinical trials used expertise and reagents generated primarily through the Antibody Targeting (pp2-3) and Cancer Vaccine (pp14-15) Programs.

Active Clinical Sites In 2005

Brussels, Belgium	*Brussels Branch / Clinique Universitaires Saint-Luc*
Buffalo, USA	*Roswell Park Cancer Institute*
Frankfurt, Germany	*Krankenhaus Nordwest*
Gunma, Japan	*Gunma University School of Medicine*
Homburg, Germany	*University of Saarland Medical School*
Houston, USA	*M.D. Anderson Cancer Center*
Lausanne, Switzerland	*Lausanne Branch / Centre Hospitalier Universitaire Vaudois*
Melbourne, Australia	*Melbourne Branch / Austin Hospital*
Mie, Japan	*Mie University School of Medicine*
New York, USA	*New York Branch / Memorial Sloan-Kettering Cancer Center* *Columbia-Presbyterian Medical Center* *Weill Medical College of Cornell University*
Nijmegen, The Netherlands	*University Hospital Nijmegen*
Okayama, Japan	*Okayama University Medical School*
Osaka, Japan	*Osaka University Graduate School of Medicine*
Oxford, United Kingdom	*John Radcliffe Hospital*
Zürich, Switzerland	*University Hospital Zürich*

The top 100 papers downloaded in 2004 from the *Proceedings of the National Academy of Sciences USA* website were announced in 2005. Among these was the publication describing the results of a Phase I trial of NY-ESO-1/ISCOMATRIX™ vaccine from the Melbourne and New York Branches and conducted as part of the Cancer Vaccine Collaborative (see p15).

Davis ID, Chen W, Jackson H, Parente P, Shackleton M, Hopkins W, Chen Q, Dimopoulos N, Luke T, Murphy R, Scott AM, Maraskovsky E, McArthur G, MacGregor D, Sturrock S, Tai Ty, Green S, Cuthbertson A, Maher D, Miloradovic L, Mitchell SV, Ritter G, Jungbluth AA, Chen YT, Gnjatic S, Hoffman EW, Old LJ, Cebon JS. 'Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans.' Proceedings of the National Academy of Sciences USA 101(29):10697-702, 2004.



Angiogenesis Program

Angiogenesis, the process of forming new blood vessels, is central to wound healing and reproduction in the body. Tumor cells and stroma, the connective tissue around the tumor, can also stimulate angiogenesis, by secreting angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Without the blood and nutrients supplied by newly-generated blood vessels, it is thought that no tumor would grow to be more than a few millimeters in size. Lymphangiogenesis, a related process, is the formation of new lymphatic vessels. The blood and lymphatic vessels are thought to provide two of the principle routes by which cancer metastasizes (spreads) from the original tumor site.

VEGFR-3 Crucial For Lymphatic Metastasis

Following secretion by tumor cells, VEGF-C and VEGF-D promote lymphatic metastasis by binding to VEGFR-3 on endothelial cells and initiating the processes of lymphangiogenesis and tumor cell invasion into those new lymphatic vessels. Research conducted by Affiliates in Helsinki and Kuopio showed that inhibition of VEGFR-3 can block both lymphangiogenesis and tumor cell invasion in a mouse tumor model when VEGFR-3 inhibition is commenced soon after the tumor is transplanted. Although inhibition of VEGFR-3 at a later stage significantly reduced lymphangiogenesis, it could not block tumor cell invasion and spread. These data suggest that VEGF-based adjuvant therapies intended to prevent lymphatic metastasis following surgery may need to target both blood vessel angiogenesis as well as lymphangiogenesis.

He Y, Rajantie I, Pajusola K, Jeltsch M, Holopainen T, Yla-Herttuala S, Harding T, Jooss K, Takahashi T, Alitalo K. Vascular endothelial cell growth factor receptor 3-mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels. Cancer Research (2005) 65(11):4739-46.

Dissecting The Roles Of Lymphangiogenic Growth Factors

Vascular endothelial growth factor-D (VEGF-D) is a secreted lymphangiogenic protein known to play an important role in tumor-induced lymphangiogenesis and lymphatic metastasis. In order to elucidate the growth factor's role(s) in embryogenesis and normal adult tissues, investigators from the Melbourne Branch generated and analyzed a mouse model with an inactive VEGF-D gene. While the abundance of lymphatic vessels in the lung was reduced, suggesting VEGF-D has a role in the pulmonary lymphatic system, overall the mouse model's lymphatic vasculature was relatively normal. This indicates that VEGF-D appears to be dispensable for formation of the lymphatic vasculature during embryogenesis or that another VEGF can compensate for VEGF-D during development.

Baldwin ME, Halford MM, Roufail S, Williams RA, Hibbs ML, Grail D, Kubo H, Stacker SA, Achen MG. 'Vascular endothelial growth factor D is dispensable for development of the lymphatic system.' Molecular and Cellular Biology 25(6):2441-9, 2005.

Alitalo K, Tammela T, Petrova TV. 'Lymphangiogenesis in development and human disease.' Nature 438(7070):946-53, 2005.

PDGF-D And Heart Disease

Further research into PDGF-D, by the investigators at the Stockholm Branch who discovered the protein, has shown that overexpression of the angiogenic growth factor in the heart stimulates the proliferation of cardiac fibroblasts and arterial vascular smooth muscle cells (SMCs) in mice. This excess, uncontrolled expression of the protein results in cardiac fibrosis, with subsequent dilated cardiomyopathy and finally cardiac failure. The identification of PDGF-D as a key component in cardiac fibrosis and the first step in atherosclerosis renders it a promising target for potential cardiac disease therapies.

Ponten A, Folestad EB, Pietras K, Eriksson U. 'Platelet-derived growth factor D induces cardiac fibrosis and proliferation of vascular smooth muscle cells in heart-specific transgenic mice.' Circulation Research 97(10):1036-45, 2005.

PDGF-CC And Ischemia

Investigators from the Stockholm Branch characterized the processing of PDGF-CC, the secreted, latent form, into PDGF-C, which binds to its cognate receptor to act as an angiogenic growth factor. However a joint study between the Stockholm and Uppsala Branches and collaborators then showed that PDGF-CC itself induced differentiation (reorganization) of bone marrow cells into endothelial cells and SMCs and induced migration of those endothelial cells to areas of ischemia, where tissue damage has limited blood supply. The effects of PDGF-CC on vascular and muscle regeneration suggests that this growth factor may form the basis of novel strategies for the revascularization of damaged tissues, e.g. following cardiac failure or limb injuries.

Li X, Tjwa M, Moons L, Fons P, Noel A, Ny A, Zhou JM, Lennartsson J, Li H, Luttun A, Ponten A, Devy L, Bouche A, Oh H, Manderveld A, Blacher S, Communi D, Savi P, Bono F, Dewerchin M, Foidart JM, Autiero M, Herbert JM, Collen D, Heldin CH, Eriksson U, Carmeliet P. 'Revascularization of ischemic tissues by PDGF-CC via effects on endothelial cells and their progenitors.' Journal of Clinical Investigation 115(1):118-27, 2005.

Fredriksson L, Ehnman M, Fieber C, Eriksson U. 'Structural requirements for activation of latent platelet-derived growth factor CC by tissue plasminogen activator.' Journal of Biological Chemistry 280(29):26856-62, 2005.

For over seven years, LICR has coordinated and supported a global Program to research angiogenesis and lymphangiogenesis with the aim of developing therapies that could target both processes. The efforts of the Program investigators, drawn from the Melbourne, Stockholm and Uppsala Branches as well as Affiliates in Helsinki and Kuopio, have identified four of the five known vascular endothelial growth factors (VEGF-B, VEGF-C, VEGF-D and VEGF-E) and two of the four known platelet-derived growth factors (PDGF-C and PDGF-D) as well as a novel receptor, VEGF receptor-3 (VEGFR-3).

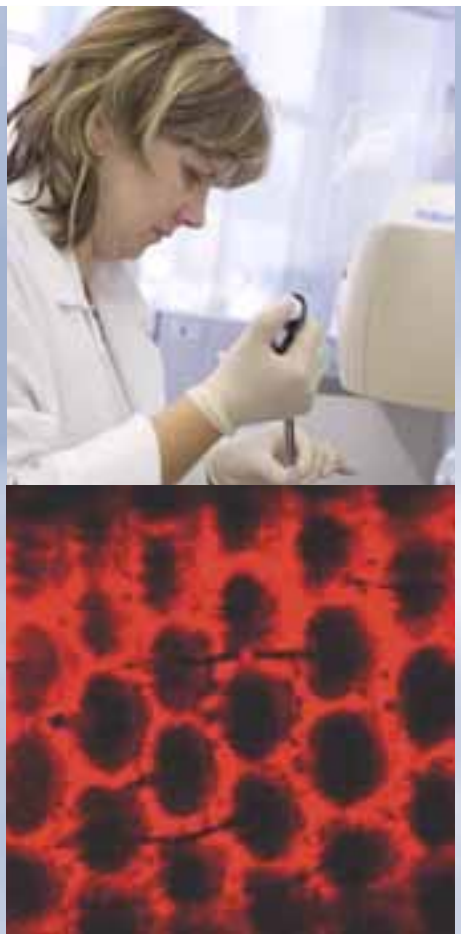
LICR has a singular focus on cancer but recognizes that some of its medical research findings may have therapeutic value for other human diseases. Thus LICR places great importance on supporting and facilitating, principally through the licensing of its intellectual property, the research and development of non-oncology therapies for human benefit. Several of these licensing relationships involve discoveries from the LICR's Angiogenesis Program.

Lymphatix Ltd Jumps To Life With Million Euro Seed Fund

A new spin-off company, Lymphatix Ltd, was launched in November 2005 with Helsinki University Funds providing €1 million to initiate operations. Lymphatix Ltd plans to develop and commercialize products based on VEGF-C and VEGF-D (licensed from LICR) as pro-angiogenic/lymphangiogenic factors in the treatment of human conditions with impaired blood supply (e.g. heart ischemia) or lymphoid drainage (e.g. edema).

VEGF-D Gene Therapy May Reduce Vascular Graft Access Surgeries

In 2005, Ark Therapeutics Group plc announced the first results from a Phase II clinical trial of a pro-angiogenic therapy, Trinam®, which utilizes VEGF-D licensed from LICR. Trinam® appears to prevent blood vessel blockage following vascular graft access surgery (insertion of an artificial blood vessel) required for patients with kidney failure to undergo dialysis.



Fluorescent staining of the superficial lymphatic network of the tails of VEGF-D-deficient mice. The honeycomb pattern formed by these lymphatic vessels is similar to that observed in wild-type mice. (Courtesy of the Melbourne Branch.)

Cell Cycle Checkpoints and Cancer

Genome integrity and cell growth and division are tightly regulated by cell cycle checkpoints that must be disrupted for tumor development and progression to occur. Many of the known oncogenes, such as p53, the function of which is lost in >50% of cancers, are part of the checkpoint apparatus. Many current cancer therapies, particularly chemotherapies, act by blocking the division of cells that are rapidly cycling, cancerous or not, which frequently result in side effects. Understanding the cell cycle may give clues for the design of new, cancer-specific therapies.

Transcription Factors Of The DNA Damage Checkpoint

The p53 family of transcription factors (p53, p63 and p73), responds to DNA damage during G1 and plays a key role in halting the cell cycle and initiating apoptosis (programmed cell death). Investigators at the University College London Branch found that E2F1, a member of the transcription factor family regulating the expression of multiple cell cycle control proteins, stimulates the apoptotic function of p53, but not p63 and p73. This is the first demonstration of p53 activity being regulated during the cell cycle by E2F/p53 interactions. The University College London team is also characterizing the ASPP protein family, which was discovered at the Branch and activates p53-mediated apoptosis. The team showed that both ASPP1 and ASPP2 are transcriptional targets of the E2F family, providing a mechanism by which E2F cooperates with p53 to induce apoptosis. The aim of this research is to explore the therapeutic potential of re-activating p53 function to cause apoptosis of cancer cells.

Fogal V, Hsieh JK, Royer C, Zhong S, Lu X. 'Cell cycle-dependent nuclear retention of p53 by E2F1 requires phosphorylation of p53 at Ser315.' EMBO Journal 24(15):2768-82, 2005.

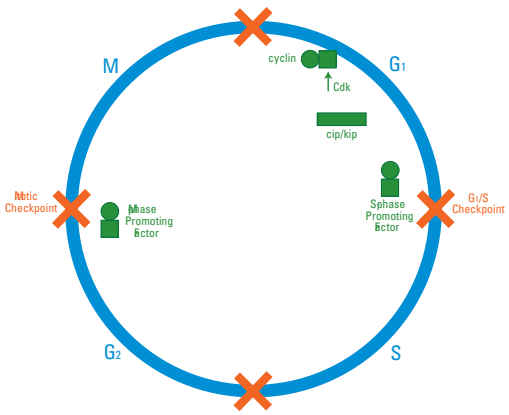
Fogal V, Kartasheva NN, Trigiante G, Llanos S, Yap D, Vousden KH, Lu X. 'ASPP1 and ASPP2 are new transcriptional targets of E2F.' Cell Death Differentiation 12(4):369-76, 2005.

G1/S Cyclin Checkpoints

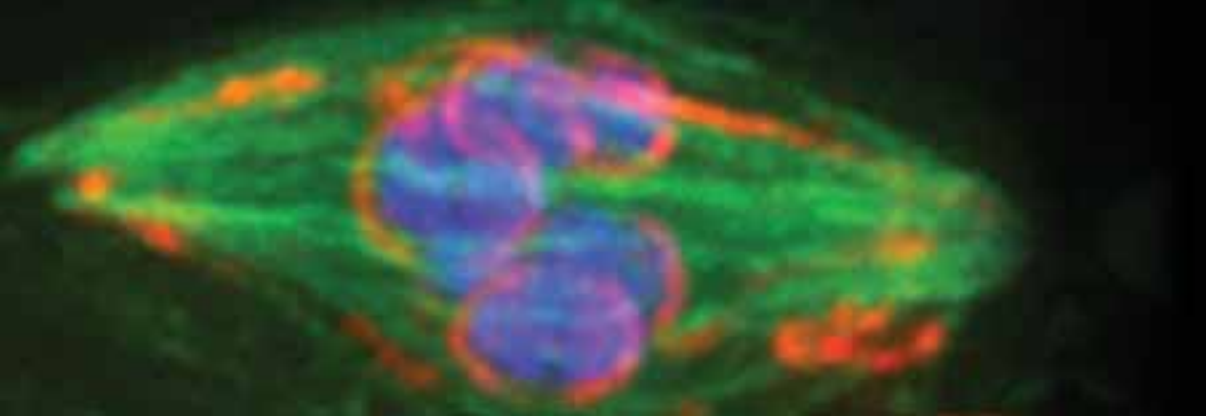
Cell cycle progression in the G1 and S phases is controlled by cyclin-dependent kinases (cdk) that are positively regulated by cyclins and negatively regulated by two families of cdk inhibitors, cip/kip and ink4. The expression levels of cdks are fairly constant, but they must be complexed with cyclins, the expression levels of which rise and fall as the cell cycle progresses, to be active. For example, cylin E/cdk2 kinase activity is important in G1, while the 'S phase promoting factor' includes the cyclin A/cdk2 complex. In 2005, a team from the Uppsala Branch found that all members of the TGF-β super-family (see pp8-9, 'TGF-β Program') can inhibit epithelial cell growth by inducing the expression of $p21^{cip1}$ to inhibit cyclin E/cdk2 kinase activity. Meanwhile, a team of investigators from the University College London Branch and the James R. Kerr Program in Xi'an, China, showed that the binding of different cyclins and cdks to $p27^{kip1}$, which also inhibits cyclin E/cdk2 kinase activity, is dependent on the posttranslational modification of $p27^{kip1}$. Understanding the regulation of $p21^{cip1}$ and $p27^{kip1}$ may provide new strategies to inhibit cancer cell growth through reactivation of the growth inhibitory activities of these proteins.

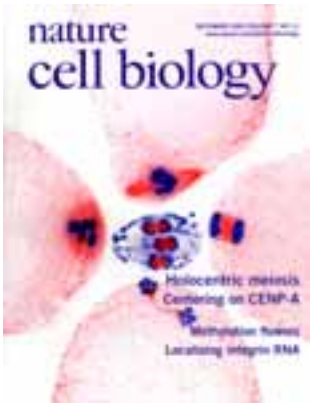
Pardali K, Kowanetz M, Heldin CH, Moustakas A. 'Smad pathway-specific transcriptional regulation of the cell cycle inhibitor p21(WAF1/Cip1).' Journal of Cell Physiology 204(1):260-72, 2005.

Zhang W, Bergamaschi D, Jin B, Lu X. 'Posttranslational modifications of p27kip1 determine its binding specificity to different cyclins and cyclin-dependent kinases in vivo.' Blood 105(9):3691-8, 2005.



Below: The cell cycle consists of four main phases: G_1, the growth and preparation of chromosomes for replication; *S*, the synthesis of DNA; G_2, protein production and growth in preparation for the next phase, and; *M*, for 'mitosis', the process by which chromosomes are divided into two daughter cells.





This image (above left) from the San Diego Branch shows outer kinetochore proteins forming cup-like structures on bivalent meiotic chromosomes, the DNA is blue, the KNL-1 protein is red and the spindle microtubules are green. The image is from a study featured on the December cover of *Nature Cell Biology* (above) (Monen *et al.* Nature Cell Biology 7:1248-55, 2005).
Reprinted by permission from Macmillan Publishers Ltd: Nature Cell Biology (Volume 7, Number 12), copyright 2005.

Mitotic Checkpoint And The Kinetochore

The 'mitotic spindle' is a network of microtubules that connect the kinetochore, at the centromere of the chromosome, to the centrosome, which separates chromosome pairs. The mitotic checkpoint halts cell cycle progression when even one chromosome has not properly attached to the mitotic spindle, thus ensuring that all chromosomes are divided equally between daughter cells. Scientists at the San Diego Branch have been investigating the mitotic checkpoint and the disruptions that cause aneuploidy, an abnormal number of chromosomes, which is a characteristic of cancer cells. The team identified the protein complex, ZW10-Rod, as the bridge between the kinetochore and the Mad1-Mad-2 heterodimer that forms the checkpoint's actual 'wait' signal. ZW10 recruits the Mad1-Mad2 complex to unattached kinetochores and then removes the complex after kinetochore attachment to the centromere. ZW10, Mad1 and Mad2 have all been shown to be mutated in colorectal cancers with gross aneuploidy.

Kops GJ, Kim Y, Weaver BA, Mao Y, McLeod I, Yates JR 3rd, Tagaya M, Cleveland DW. 'ZW10 links mitotic checkpoint signaling to the structural kinetochore.' Journal of Cell Biology 169(1):49-60, 2005.

Kinetochore And The CENP Family

The centromeric protein CENP-A is the foundation for the assembly of kinetochores, and its misregulation has been reported in colorectal cancers. Research by investigators at the San Diego Branch has found a surprising CENP-A independent mechanism that is responsible for segregating holocentric chromosomes in *C. elegans* meiosis. Further studies of this adaptation should provide insight into the mechanisms by which chromosomes are connected to the spindle apparatus and segregated during cell division. The San Diego Branch teams also focused on the characterization of the kinetochore proteins, CENP-E and CENP-F, finding that CENP-E is responsible for appropriately silencing the mitotic checkpoint 'wait' signal and that CENP-F is essential for efficient assembly of a stable microtubule-kinetochore interface.

Monen J, Maddox PS, Hyndman F, Oegema K, Desai A. 'Differential role of CENP-A in the segregation of holocentric C. elegans chromosomes during meiosis and mitosis.' Nature Cell Biology 7(12):1148-55, 2005.

Mao Y, Desai A, Cleveland DW. 'CENP-E silences BubR1-dependent mitotic checkpoint signaling.' Journal of Cell Biology 170(6):873-80, 2005.

Bomont P, Maddox P, Shah JV, Desai AB, Cleveland DW. 'Unstable microtubule capture at kinetochores depleted of the centromere-associated protein CENP-F.' EMBO Journal 24(22):3927-39, 2005.

Genome Instability As Future Diagnostic?

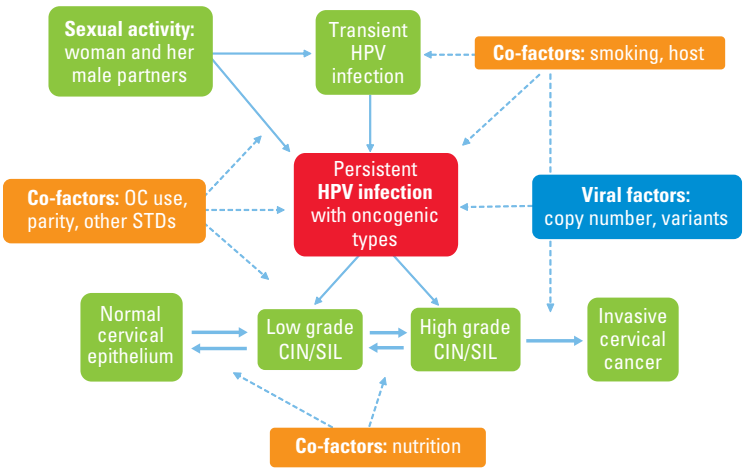
Mitotic checkpoint breakdown results in a phenotype of chromosomal instability (CIN), which involves changes in chromosome number (aneuploidy) or structure, and is seen in several hereditary cancer predisposition syndromes. However understanding the CIN phenotype has been complicated by the complexity of systematically organizing and characterizing the multiplicity of different genome rearrangements. Investigators from the San Diego Branch analyzed the phenotypes of different yeast mutations to correlate particular classes of genome rearrangements with disruptions in mechanistic pathways such as DNA recombination, telomerase activity or cell cycle checkpoints. Parallels revealed by the comparison of the yeast genome rearrangement classes with 47800 human cancer karyotypes suggests that genome rearrangements in human cancers might provide information about human carcinogenesis and which genes are best candidates for mutation screening in cancer.

Putnam CD, Pennaneach V, Kolodner RD. 'Saccharomyces cerevisiae as a model system to define the chromosomal instability phenotype.' Molecular Cell Biology 25(16):7226-38, 2005.

HPV Epidemiology

The availability of cervical and penile cancer samples from the Hospital do Cancer, the host institution of the São Paulo Branch, has allowed LICR investigators to analyze the natural history or 'epidemiology' of infection with Human Papillomavirus (HPV), the causative agent of cancers of the cervix, penis and anus. Understanding the epidemiology of HPV is an important step towards the development of strategies for preventing persistent infection and, ultimately, cervical and penile cancers.

The 'Ludwig/McGill cohort' was established in 1993 by the Ludwig Institute for Cancer Research and McGill University (Montreal, Canada) with a population of women in São Paulo. This cohort is one of the largest longitudinal studies of the natural history of HPV infection and risk of cervical cancer in the world.



LICR Leads First Clinical Trial of Quadrivalent HPV Vaccine Trial to Show Efficacy

The strong HPV research record from the team at the São Paulo Branch resulted in them being invited to lead a Phase II trial of Merck & Co. Inc.'s prophylactic HPV vaccine. The study was the first to demonstrate the safety, immunogenicity and efficacy of a quadrivalent HPV L1 vaccine containing Virus-like particles (VLPs) of HPV types 6 and 11, the two main causative agents of genital warts, and HPV types 16 and 18, responsible for about 70% of all cervical cancers. The Phase II trial was conducted in Brazil (where most of the volunteers were accrued by the LICR team), the USA and Northern Europe, with women who showed no sign of HPV infection prior to joining the trial. Over the two and a half years of follow-up after vaccination, the combined incidence of persistent infection from HPV 6, 11, 16, or 18 and related genital disease, including new cervical pre-cancers and genital warts, was reduced by 90 percent in women who received the vaccine compared with women who received a placebo. Although the study was not originally designed or powered to assess vaccine efficacy, meta-data suggested that the vaccine prevented cervical pre-cancers and genital warts caused by the HPV types 16, 18, 6 and 11 in all women to whom it was given. Data from a Phase III clinical trial that included the São Paulo team, later confirmed the high efficacy of the vaccine, which was submitted for regulatory review and licensing in December 2005.

Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, Wheeler CM, Koutsky LA, Malm C, Lehtinen M, Skjeldestad FE, Olsson SE, Steinwall M, Brown DR, Kurman RJ, Ronnett BM, Stoler MH, Ferenczy A, Harper DM, Tamms GM, Yu J, Lupinacci L, Railkar R, Taddeo FJ, Jansen KU, Esser MT, Sings HL, Saah AJ, Barr E. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. 'The Lancet Oncology' 6(5):271-8, 2005.

Notable Events

Dr. Lloyd J. Old, LICR's Scientific Director since 1988, stepped down from that position on December 31, 2005, to assume the responsibilities of Chairman of the Board. Dr. Old continues to be Director of the New York Branch and the Cancer Vaccine Collaborative (p15).

Mr. R. Palmer Baker Jr. held the post of Chairman of the LICR Board from 1995 to the end of 2005. Former counsel to Mr. Daniel K. Ludwig, Mr. Baker has been involved with the Institute since its inception.

Dr. A. Munro Neville, a staff member of 33 years, former LICR Associate-Director, founding Director of the Office of Intellectual Property, and Director of the Institute's Angiogenesis Program (pp18-19) retired in 2005.

The St Mary's London Branch was closed in July 2005 to consolidate LICR's operations in London at the University College London Branch. The St Mary's Branch Director, Dr. Paul J. Farrell, continues his research on Epstein-Barr Virus and its connection to cancer as an LICR Affiliate at the School of Medicine, Imperial College, London.

Dr. Ricardo R. Brentani retired as Director of the São Paulo Branch after 22 years in that position. Here Dr. Brentani (right) is pictured with the Australian Federal Government Minister for Education, Science and Training, Dr. Brendan Nelson, who toured the São Paulo Branch in April, 2005.

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