The Ludwig Institute For Cancer Research

Annual Research Report
2004

Publication date: May, 2005

Table of Contents

The Ludwig Institute for Cancer Research (LICR) is the largest international academic Institute dedicated to the fight against cancer. With ten Branches in seven countries across Australasia, Europe, and North and South America, and numerous Affiliates across Asia, Australasia, Europe, and North and South America, the scientific network that is the Ludwig Institute for Cancer Research quite literally covers the globe. The Institute is not only associated with established and world-renowned academic institutions, but has also, through its James R. Kerr Program, expanded its support into countries such as Brazil, China, Russia and Turkey, which are scientifically talented but have little opportunity for international collaboration in advanced cancer research.

The uniqueness of the Ludwig Institute for Cancer Research lies not only in its size and scale, but also in its philosophy and ability to drive its results from the world of academia into the world of the clinic. The Institute has accumulated a staggering portfolio of reagents, knowledge, expertise, and intellectual property, and, unusually for an academic institution, has also actively assembled the personnel, facilities, and practices necessary to patent, license, and thus fully develop the most promising aspects of that portfolio into cancer therapies. The Institute believes that the same science-driven investigative rigor that yielded the academic discoveries should, indeed must, be applied in the clinic to fully develop the therapeutic potential of any discovery.

The goal of the LICR is to make a real contribution to the clinical management of cancer. This report summarizes the progress that LICR investigators made to that end in 2004.

In the 33 years since the Ludwig Institute for Cancer Research (LICR) was founded, it has grown to become the largest international academic institute dedicated to the fight against cancer. The research of the Institute is carried out at ten Branches in seven countries around the world, and also in cooperation with an international network of affiliated scientists and clinicians. This organizational structure maximizes the Institute's opportunities to interact with a broad range of different laboratory and clinical environments, and facilitates the worldwide recruitment of outstanding scientists.

The research orientation of each Branch of the LICR is defined by the Branch Director in the context of the overall objectives of the Institute. Each Branch is staffed to enable it to address complex biological problems related to cancer, and provide a critical mass of scientists with expertise in relevant scientific disciplines. Branches have formal associations with University Hospitals, which allows not only a close interaction between laboratory and clinical scientists, but also the provision of clinical resources required for basic research, and the Institute's Clinical Trials Program.

The quality of research conducted by LICR scientists is monitored on an ongoing basis by the Institute's Scientific Directorate and Scientific Committee, and is independently assessed through external peer-review processes. A commitment to programmatic-based research has resulted in the establishment of numerous collaborations between Branches and LICR Affiliates, individual investigators who are experts in fields that complement the research objectives of the Institute's Programs. The LICR also benefits from affiliations, through the James R. Kerr Program, with investigators in Brazil, China, Russia, South Africa, Turkey, and Ukraine; countries that are scientifically talented but have fewer opportunities for international collaboration in advanced cancer research. All of these activities extend the Institute's scientific global reach, in accordance with the wishes of our founder, Mr. Daniel K. Ludwig.

The following scientific progress report summarizes several examples of LICR's research Programs, based on studies published in 2004 by LICR Branch staff members and Affiliates.

Genetics Discipline

Carcinogenesis disrupts important cell processes, for example migration, growth or apoptosis (programmed cell death), that control, and are controlled by, the 'expression' of genes. A gene is expressed when its DNA is 'transcribed' into mRNA, which in turn is translated into a protein. Measuring gene expression changes associated with the execution of cellular processes can indicate which genes are involved in a particular process. This is important to understand fundamental cell biology, and also to understand how the processes are corrupted by and/or contribute to cancer. Programs in the discipline of Genetics include: Gene Discovery, Function, and Expression; Mutation and Epigenetic Screening; Cancer Genomics (Gene Expression Profiling, and Genome Annotation); and Cancer Epidemiology.

Gene Expression Profiling Program

Gene expression profiling measures the increase or decrease of a gene's mRNA in a cancer cell relative to the level of that gene's mRNA level in a normal cell. These analyses are frequently performed using microarrays, which measure the gene expression of hundreds or thousands of genes at one time. Gene expression differences in cancer samples can indicate which genes are involved in cell processes important in cancer. Expression differences can also identify expression patterns, or 'markers', that can be used for diagnosis and prognosis of individual cancer types, and perhaps one day for predicting responses to particular therapies.

In 1999, LICR formed a Microarray Consortium with Cancer Research, UK (London, UK) and the Wellcome Trust Sanger Institute (Cambridge, UK) in order to produce microarrays for LICR research. Groups at the University College London (UCL) and Uppsala Branches used these microarrays to analyze signaling pathways that are frequently disrupted in cancer. Investigators at the UCL Branch used a model breast cancer cell system (generated by the LICR Breast Cancer Initiative) to study the over-expression of the HER-2 (ErbB-2) receptor tyrosine kinase. HER-2, which is over-expressed in approximately 25% of all breast cancers, is a signaling partner of the epidermal growth factor receptor (EGFR, see 'Signal Transduction Program'), a target of one of the potential therapies being evaluated within the LICR's 'Antibody Targeting Program'. Microarray analyses identified gene expression changes likely to drive the proliferation and anchorage-independent growth (an important requirement for metastatic spread of cancer) of breast cancer cells that over-express HER-2. Uppsala Branch scientists used microarrays to further their research on dissecting the role of the transforming growth factors beta (TGF-beta), which have both tumor suppressing and tumor progressing roles. The team compared the signaling induced by TGF-beta 1 ligand, TGF-beta 1, which inhibits growth of epithelial cells and induces their differentiation ('pro-cancerous'), and another member of the TGF-beta ligand family, bone morphogenetic protein (BMP)-7, which weakly affects growth and does not induce differentiation. Both ligands signal through the same differentiation pathway, which is dependent on the transcription factor, *Smad4*. Initial studies have determined that the physiological effects of TGF-beta 1 and BMP-7 are due, at least in part, to *Smad4*-dependent regulation of the genes *Id2* and *Id3*; TGF-beta 1 down-regulates the expression of *Id2* and *Id3*, while BMP-7 up-regulates their expression. The team is now using RNA interference (RNAi) technology to further analyze the role of key genes in epithelial cell growth and differentiation. Further studies of genes identified in microarray analyses will improve our understanding of the HER-2 and TGF-beta pathways, which are particularly important in breast and epithelial cancers, respectively. Furthermore, both pathways are comprised of signaling molecules identified as future therapeutic targets for LICR, thus these expression analyses may also indicate the appropriateness of targeting these pathways (in terms of potential side-effects) and/or identify new targets for inhibition.

LICR also initiated its own microarray facility, at the São Paulo Branch, to take advantage of the hundreds of thousands of mRNA clones generated through the 'Human Cancer Genome Project'; a four year collaboration between LICR and the State of São Paulo Research Foundation (FAPESP). The LICR's Breast Cancer Initiative, comprised of investigators from the UCL and São Paulo Branches and the London (Sutton) Affiliate Center, used these microarrays to identify novel prognostic markers for breast cancer in samples of two different breast cell types. The team found that the expression patterns of particular genes correlated with survival in breast cancer patients; for example the expression of the gene, *SPARC (osteonectin),* was a marker of poor prognosis. The team is now further analyzing the results to identify potential novel diagnostic and prognostic markers that will be tested in prospective studies of breast cancer patients. Finally, investigators at the São Paulo Branch utilized these microarrays to identify molecular classifiers for gastric cancer and nonmalignant diseases of the gastric mucosa. Gastric cancer has a high mortality rate, due primarily to the late diagnosis of the disease. The São Paulo Branch investigators identified 376 genes that were altered in gastric cancer, and showed that the expression patterns within these genes could diagnose and distinguish between pre-cancerous gastric lesions and gastric cancer. These findings will now be applied to a prospective study of patients with pre-cancerous lesions to test the validity of using these expression profiles for early diagnosis of gastric cancer.

At the London St Mary's Branch, investigators studied gene expression, using more traditional and specific methods, to continue characterization of the ASPP1 and ASPP2 proteins. The gene expression analyses showed that the ASPP1 and ASPP2 proteins, which was discovered at the Branch in 2001, activate all three members of the 'p53 tumor suppressor family': p53, p63 and p73. The p53 protein is mutated in approximately 35-40% of all cancers, and causes pre-cancerous cells to undergo apoptosis when activated by molecules that detect corrupted cellular processes. To date, few proteins have been shown to activate the p63 and p73 proteins, which are rarely mutated in cancer and also control apoptosis. These results are the first to identify a common activator for all three family members, and thus first to provide evidence that there is (at least) one common cellular regulator of the entire family. This knowledge is important when considering how cancer cells block the p53 family's tumor suppressor activity, and investigating therapeutic strategies devised to reactivate tumor suppression.

Biochemistry Discipline

Cells interact with their environment by sending and receiving signals that initiate and terminate cellular processes such as cell division, growth, differentiation, migration and survival. External signals activate receptors on the cell surface, which in turn activate intracellular signal transduction cascades that regulate cellular processes. Cancer cells have abnormal signal generation and reception, which allow them to grow out of control, escape apoptosis, and invade other tissues. Programs in the discipline of Biochemistry include: Signal Transduction (Receptor Kinases and Phosphatases, Non-Receptor Kinases and Phosphatases, Nuclear Receptors, and Cytokines); and Protein Chemistry (Mass Spectrometry, Proteomics, and Structural Modeling).

Signal Transduction Program - Receptors

Receptors are cell surface proteins that, upon ligand binding and activation, initiate signal transduction. In the case of receptor tyrosine kinases (RTK) this signal transduction occurs through the addition of phosphate groups to tyrosine amino acids in intracellular signaling molecules. This cascade of signaling molecule activation ultimately catalyzes the cellular processes corrupted or subverted by cancer.

LICR scientists have made significant and substantial contributions to the discovery of receptors, intracellular signaling molecules, and the dissection of the complex interactions between multiple signaling pathways. This has led to the identification of a number of potential targets for therapeutic intervention. LICR staff and Affiliates continue to identify additional members of key pathways, their interactions with other signaling molecules, and their involvement/s in cellular processes.

The epidermal growth factor receptor (EGFR) is part of the ErbB RTK family of EGFR (ErbB-1), HER-2 (ErbB-2, see 'Gene Expression Profiling Program'), ErbB-3 and ErbB-4. Upon the binding of a growth factor ligand, an EGFR molecule dimerizes with another member of the ErbB family, and the active dimer then initiates signal transduction. Mutations in, or over-expression of, EGFR molecules are common in cancer, with estimates placing the rate as high as 50% of all epithelial cancers having EGFR aberrations. One EGFR mutation that has been studied extensively by LICR investigators at the Melbourne, New York, San Diego and Stockholm Branches is the delta2-7 EGFR mutation, which is commonly found in glioma (brain tumors). The mutant has a large deletion that results in the receptor having a different conformation and being constitutively active, thus conferring a growth advantage to the cancer cells. To investigate the effect of the receptor's conformation on EGFR regulation, a team from the Melbourne Branch constructed EGFR molecules with specific mutations. The team found that, amongst other things, mutations that simply disrupt the inactive receptor's conformation do not result in spontaneous or constitutive receptor activation. Another Melbourne Branch team showed that the delta2-7 EGFR mutant also dimerizes with 'wild-type' (normal) EGFR receptors, and is able to activate the wild-type EGFR receptors in the absence of ligand. These findings of co-activation are directly relevant to the study and possible treatment of glioma, as the delta2-7 EGFR mutant and wild-type EGFR receptors are expressed together in these tumors. This information may be particularly important for one of LICR's most promising therapies, the monoclonal antibody c806, which was generated specifically to recognize the delta2-7 EGFR mutant. The c806 antibody has been shown to also target wild-type EGFR, but only when it is over-expressed and it is now being studied extensively in the laboratory, as part of the 'Antibody Targeting Program', and in the clinic, as part of the 'Clinical Trials Program' (a Phase I clinical trial was initiated in late 2004). A fundamental knowledge of the antibody's target and the signaling cascades that it may or may not disrupt is vital for interpreting clinical research results and enabling the further investigation of c806 and other potential anti-EGFR therapies.

Another receptor being investigated by LICR scientists is the Nurr1 receptor, a nuclear receptor that also acts as a transcription factor to express genes essential for the development of midbrain dopaminergic neurons. While a relationship between Nurr1 and cancer *per se* has not been established, research on the receptor is providing vital data that may be applied to RTKs that do have a primary association with carcinogenesis. The Nurr1 studies being conducted at the Stockholm Branch, like the studies of EGFR at the Melbourne Branch, have focused on receptor activation and structure. Although Nurr1 is an 'orphan' receptor, meaning that it has no known ligands, there are two regions of the protein known to be important for activation. The LICR investigators constructed Nurr1 molecules with specific mutations in these regions to determine which amino acids were important for the receptor's activation. During the course of this study, the team also found that a major signaling pathway that is frequently implicated in cancer, the mitogen-activated protein kinase (MAPK) pathway, positively affects Nurr1's transcriptional activity. In a separate study, the team showed that Nurr1 is able to dimerize with, and signal through, the retinoid X receptor (RXR), which is the target of a new class of anti-cancer therapies currently being marketed. The relationship between Nurr1 and RXR is currently being assessed further.

Cell Biology Discipline

Research in Cell Biology examines how the function and structure of the cell is affected through the disruption of cellular processes by cancer. Programs in the discipline of Cell Biology include: Angiogenesis; Cell Migration and Metastasis (Rho GTPases and Cell Polarity); Cell Cycle and Apoptosis (Cell Cycle and Apoptosis, and Mitosis and The Centromere).

Mitosis and The Centromere Program

Mitosis is the process by which chromosomes are replicated accurately and segregated evenly into the two daughter cells generated when a cell divides. To segregate the chromosomes, a 'centrosome' at each end of the cell produces microtubule spindles that attach to 'kinetochores', macromolecular assemblies bound to each chromosome. The kinetochore is attached to a structural subunit of the chromosome, known as the 'centromere'. The spindles, under the control of the centrosome, pull the chromosomes into two identical sets, one for each new daughter cell. When mitosis fails, the daughter cells receive too many or too few chromosomes. This is known as aneuploidy; one of the primary characteristics of a cancer cell.

Investigators at the San Diego Branch have been analyzing the mechanisms behind chromosome segregation, and in particular the proteins and macromolecular structures that constitute the centromere, centrosome and kinetochore. One team has been working to clarify the poorly understood mechanisms through which centrosomes are formed from basic structures known as centrioles. The group devised a sophisticated fluorescence microscopy-based assay to analyze centriole assembly in the embryos of *Caenorhabditis elegans* (worm model organism). The results have identified one protein crucial for centrosome duplication, and have elucidated the roles of three more proteins found in centrioles. A second team, investigating the constitution of the kinetochore using the same model organism, identified a subset of 10 related proteins at the kinetochore-microtubule interface. Seven of these proteins were previously not known to be part of the kinetochore. The results indicated that there are three groups of proteins that contribute to the kinetochore-microtubule interface, each of which makes a distinct contribution to the superstructure. The investigators subsequently identified the equivalent set of proteins in human cells; four of which are novel, uncharacterized proteins.

Finally, the mitotic spindle checkpoint, which is controlled by the kinetochore, is the point at which the cell cycle is paused to ensure that all chromosomes have been accurately replicated and segregated before cell growth continues. A third team of investigators at the San Diego Branch have been studying the known kinetochore proteins and have shown that the release of certain proteins constitutes part of the process that generates the signals to pause the cell cycle. The team also found that reducing the levels of the checkpoint proteins BubR1 and Mad2, or preventing BubR1's kinase activity, causes apoptosis of the cell. These findings suggest that reducing the levels of particular checkpoint proteins or inhibiting signaling from BubR1 may be approaches to destroying rapidly growing cancer cells.

LICR investigators, through their analyses of the proteins of the centrosome, centromere and kinetochore, and their contributions to mitosis, have furthered our understanding of both how aneuploidy occurs and how it contributes to cancer initiation and progression. This fundamental information is important to understand the effects of existing cancer therapies (some chemotherapies for example) that interfere with mitosis, and to perhaps assist in the strategic development of therapies that more effectively target cancer cells and not normal cells.

Immunology Discipline

The immune system has a remarkable capacity for fending off infectious diseases, and it has become clear that these same defenses can recognize and destroy cancer cells. LICR is working on treatment strategies, such as targeted antibodies and cancer vaccines, which harness the body's immune system to more effectively and specifically fight cancer cells. An understanding of the role of the immune system in cancer development and the development of cancer therapies based on immunologic principles continue to be major objectives of LICR, which is, arguably, the largest academic center in the world for cancer immunology studies. Programs in the discipline of Immunology include: Cancer Vaccine (Characterization of Human Immune Response, and Vaccine Constitution and Development); Cellular and Molecular Immunology (Adaptive Immune Response, and Innate Immune Response); Cancer Antigen Discovery (Cancer Antigen Identification, and Cancer Antigen Characterization); and Antibody Targeting (Antibody Characterization, and Antibody Engineering).

Cancer Vaccine Program - Characterization of the Human Immune Response

A successful cancer vaccine needs to either induce the immune system to recognize tumor cells as abnormal, or strengthen a spontaneous immune response such that it is sufficient to halt tumor growth or spread. Once the mechanisms for inducing appropriate, consistent and robust human immune responses are understood, the effects of such responses on human tumors and, hence, the potential of cancer vaccines can be rationally evaluated and optimized.

LICR is actively investigating potential cancer vaccines in early-phase clinical trials, and concurrently characterizing the human immune response to immunization with tumor-associated antigens to understand when and why immunization succeeds or fails to vaccinate against cancer cells. Fundamental information on the processes behind the immune response, or lack thereof, will allow a better understanding of how to optimize vaccines. For example, investigators at the LICR Lausanne Branch analyzed the function of tumor-specific CD8+ T cells, the so-called 'cytolytic T cells' (CTLs) for their capacity to destroy cancer cells following immunization. Although tumor-specific CTLs are generated either spontaneously or induced in response to immunization, they rarely cause complete tumor eradication. The team studied the functional efficacy of CTLs specific for the Melan-A/MART-1 melanoma antigen, and found that although the CTLs in the blood displayed strong functionality, the CTLs in the tumor and in the metastatic lymph nodes were not functional. These important findings confirm that factors in the microenvironments of the tumor and metastatic lymph nodes are blunting the CTL response and thus blocking the complete eradication of the tumor cells. The team is now trying to identify these factors, and devise ways of repressing the inhibition of CTL function.

In 2004, there was considerable progress made in characterizing the CD8+ and CD4+ T cell 'repertoires' induced by immunization with different antigens in different cancers. A T cell repertoire is constituted by the profile of different 'clonotypes', each of which utilize distinct T cell receptors to recognize a variety of different sequences, or 'epitopes', in the vaccine antigen. A team at the Brussels Branch, working in collaboration with a research group at their host institution, found that immunization with a peptide fragment derived of the tumor associated MAGE-3 antigen induced a repertoire of over 100 different CD8+ (CTL) clonotypes. Their findings also suggested the clonotypes potentially associated with tumor regression may be those produced at very low levels. The T cell repertoire generated by the NY-ESO-1/ISCOMATRIX™ vaccine in melanoma clinical trials at the Melbourne Branch (see Clinical Trials Program below) was studied jointly by the Melbourne and New York Branches. The team found that the vaccine induced a

broad range of CD8+ and CD4+ clonotypes, many with specificity for previously unidentified immunogenic epitopes of NY-ESO-1. Another team, comprised of investigators from the New York Branch and the New York (Columbia) Affiliate Center, analyzed CD4+ epitopes derived from the SSX-2 cancer antigen in melanoma patients. They found a CD4+ T cell clonotype corresponding to a dominant SSX-2 epitope in the blood and tumor-infiltrating lymphocytes in melanoma patients, but not in healthy donors.

These LICR studies have shown that each antigen produces multiple CTL clonotypes; some of which are more dominant than others (i.e. produced in greater abundance) and some of which are present at near undetectable levels. Studies investigating the relationships between particular NY-ESO-1 T cell epitopes and a) cytokine release in ovarian cancer (New York Branch and Buffalo Affiliate Center) and b) expression of human leukocyte antigen (HLA) genes in various cancers (New York Branch, and Frankfurt and Zürich Affiliate Centers) were also published in 2004. Thus research on the T cell repertoire is now focusing on dissecting which, if any, clonotypes correlate with tumor regression, with the aim being to then investigate how to increase the generation of those particular clonotypes.

The James R Kerr Program

The late James R. Kerr was introduced to the LICR by Mr. Ludwig; Mr. Kerr served as Chairman of the LICR. The James R. Kerr Program was initiated to foster scientific collaborations with talented scientists from countries that have had limited opportunities for funding cancer research studies. The LICR has established collaborative research projects at leading academic centers in China, Ukraine, Russia, South Africa, Turkey and Brazil.

In 2004 much of the focus of the program was to concentrate on integrating the projects of Kerr Program Scientists with the activities of the Institute as a whole. In Turkey scientists collaborate with researchers at the New York Branch to examine the role of paraneoplastic antigens in small cell lung cancer. This collaborative investigation was extended to Russian members of the Kerr Program, who were conducting similar studies. In 2005 these three groups plan to collaborate on a lung cancer program that will investigate the role of paraneoplastic antigens as diagnostic tools in the detection of lung cancer.

In China the LICR Xi'an Monoclonal Antibody Facility produces monoclonal antibody reagents for LICR branch members and affiliate institution members throughout the world. Researchers at Peking University are working towards conducting the first LICR clinical vaccine trial for cancer patients in China. As part of the Institute's Antibody Program researchers in the Ukraine are working with scientists in London and Germany to produce recombinant proteins and monoclonal antibodies targeted to cell surface antigens present on cancer cells.

In 2004 the LICR began a major initiative to create a Bioinformatics Program for cancer researchers called BRAIN. Kerr Bioinformaticians from Brazil, Russia and South Africa were major contributors to this initiative.

The LICR James R. Kerr Program will continue to support talented cancer researchers who contribute to integrative scientific collaborations within the Programs of the Institute.

Clinical Trials Program

The importance of a new discovery in cancer research cannot be assessed until its clinical impact has been established. Accordingly, the Institute is committed to taking its discoveries from the laboratory into the clinic within a collaborative academic environment, and following the principles of strict academic rigor. The global LICR clinical activities are coordinated centrally by the Office of Clinical Trials Management in New York.

LICR currently sponsors 41 early-phase clinical trials primarily using the expertise and reagents generated through the Antibody Targeting and Cancer Vaccine Programs. These early-phase clinical trials are designed to allow the Institute to examine the clinical potential of new treatment strategies with maximum efficiency and flexibility, whilst ensuring patient safety, regulatory compliance, and the control of its intellectual property.

Cancer Vaccines

The NY-ESO-1 cancer antigen, discovered at the LICR New York Branch, is frequently expressed in a variety of human tumors, and has been shown to be spontaneously immunogenic. Through the Cancer Vaccine Collaborative (CVC), a partnership established between LICR and the Cancer Research Institute (CRI), many different formulations of NY-ESO-1-based vaccines have been studied in parallel, LICR-sponsored early-phase clinical trials. Investigating vaccine variables in parallel trials allows a comparison of their ability to induce and sustain an immunological response to NY-ESO-1. For example, in various trials, the NY-ESO-1 antigen has been delivered in the form of peptide, protein, or DNA, with different adjuvants (compounds that stimulate the immune system), and by different delivery methods, such as fowlpox, vaccinia, particle-mediated epidermal delivery, or simple intra-muscular injection.

In 2000, LICR initiated a clinical trial of NY-ESO-1 protein complexed with the proprietary ISCOMATRIX™ adjuvant from the Australian biotechnology company, CSL Limited. The planning and management of the trial were coordinated by the LICR Offices of Clinical Trials Management and Protocol Review, with assistance from the LICR Office of Intellectual Property and Technology Licensing (for negotiations with CSL Limited). The study was conducted at the LICR Melbourne Branch with collaborative support from the LICR New York Branch. The trial involved 46 patients, most with melanoma, who were given three monthly injections following surgical removal of their tumor. In the double-blind study, some patients received different doses of the NY-ESO-1/ISCOMATRIX™ vaccine, some received the NY-ESO-1 protein without the ISCOMATRIX™ complex, and some received an inactive placebo.

Detailed studies of the patients' immune responses revealed that the NY-ESO-1/ISCOMATRIX™ vaccine, in contrast to vaccination with the placebo, induced the production of antibodies, and CD8+ and CD4+ T cells that target the NY-ESO-1 antigen. Vaccination with the NY-ESO-1 protein alone induced fewer responses than the NY-ESO-1/ISCOMATRIX™ complex. In a review of the patients two years later, the researchers found that cancer recurred in five out of seven patients who received the placebo and nine out of 16 who received just the NY-ESO-1 protein. However, of the patients who received the NY-ESO-1/ISCOMATRIX™ vaccine, 14 out of 19 remained cancer-free. While the results are very encouraging, it must be pointed out that this type of retrospective analysis is considered less exact than if it had been done in a planned, prospective fashion. The LICR Melbourne team, together with the staff of the LICR Offices of Clinical Trials Management, Protocol Review and Intellectual Property and Technology Licensing are in the process of planning a randomized Phase II study that will prospectively analyze the efficacy of the NY-ESO-1/ISCOMATRIX™ vaccine.

Clinical Trials Centers

The LICR conducts a centrally managed, coordinated, global network of Phase I and II clinical trials that utilize standardized monitoring and technologies to compare single trial variables in parallel.

The following LICR Clinical Trials Centers had active trials in 2004:

Australia

- Austin Health (LICR Melbourne Branch), Melbourne, Australia
- Peter MacCallum Cancer Center, Melbourne, Australia

Europe

- Clinique Universitaires Saint-Luc (LICR Brussels Branch), Brussels, Belgium
- Krankenhaus Nordwest, Frankfurt, Germany
- Centre Hospitalier Universitaire Vaudois (LICR Lausanne Branch), Lausanne, Switzerland
- University Hospital Nijmegen, Nijmegen, Netherlands
- University Hospital Zürich, Zürich, Switzerland

North America

- Memorial Sloan-Kettering Cancer Center (LICR New York Branch), New York, USA
- Weill Medical College of Cornell University, New York, USA
- Columbia-Presbyterian Medical Center, New York, USA
- Roswell Park Cancer Institute, Buffalo, USA

Biological Production Facilities

There are extensive standardization and quality control criteria that must be met when producing clinical reagents for use in human clinical trials. These "current Good Manufacturing Practices" (cGMP) ensure the safety of investigational agents and compliance with legal and regulatory, and licensing requirements. To translate discoveries rapidly and effectively into investigational agents for clinical trials, a bioproduction facility (BPF) must be able to produce material that meets these high standards.

The BPF in Melbourne utilizes eukaryotic cell production. In 2004, the BPF focused on the production of the monoclonal antibody c806; the first clinical trial of this antibody was initiated in December. The facility also vialed and dispensed several antibodies and vaccine reagents for LICR clinical trials. The facility further serves as a documentation center and depot for LICR clinical investigation agents including the conduct of ongoing stability studies.

The Cornell University/LICR BPF at Ithaca, New York utilizes bacterial and yeast expression systems to produce reagents. In 2004, the BPF developed a GMP production process (expression in the yeast, *Pichia pastoris*, and downstream purification) for a single A33 chain antibody construct, which will be manufactured in 2005. In addition, several other proteins, including NY-ESO-1, SSX2, MAGE-3 and Melan A, all of which are required for LICR's Cancer Caccine Program trials, have entered process development, with NY-ESO-1 protein being the most advanced. The production campaign for NY-ESO-1 protein is scheduled for the second quarter of 2005.

Intellectual Property Program

To ensure that the Institute is able to capitalize on its discoveries, a vigorous patent protection policy has been pursued. In 2004, 29 patents were issued to LICR in the United States of America with corresponding issued and published world-wide patents, and a further 32 new priority patents were filed. Most of these patents are related to growth factors, cytokines, signaling molecules, antibodies and human tumor antigens.

The research efforts of LICR have resulted in a series of unique scientific discoveries, leading to the establishment of a significant, intellectual property portfolio. As an example, the Antigen Discovery Program underlies several other LICR Programs, including the Antibody Targeting, Bioinformatics, Genomics, and Cancer Vaccine Programs. Intense endeavors are now underway to bring these discoveries to the attention of the pharmaceutical and biotechnology industries as candidates for licensing and the development of future cancer therapies.

To date, certain antigens, such as those of the MAGE family and the NY-ESO-1 antigen discovered as part of the Antigen Discovery Program, have been licensed to pharmaceutical or biotechnology partners for development as cancer vaccines. In most cases, only specific utilities for these antigens have been licensed as appropriate to the partner's expertise and proprietary technology.

Academic Matters

During 2004, LICR investigators published in excess of 300 papers in peer-reviewed journals.

The quality of the Institute's science continued to be internationally recognized. In the last year, the following distinctions and awards were received:

- Dr. Johan Ericsson (Uppsala Branch) awarded the Fernstrom Young Scientist Award;
- Dr. Buzz Baum (University College London Branch) awarded a Young Investigator Award from the European Molecular Biology Organization (EMBO);
- Dr. Lloyd Old (New York Branch) awarded the Johns Hopkins University President's Medal;
- Dr. Lloyd Old (New York Branch) awarded the Stanford University School of Medicine's Dean's Medal;
- Dr. Anne Ridley (University College London Branch) awarded the 'Grand Prize', the 'Liliane Bettencourt pour les Sciences du Vivant' from the Bettencourt Schueller Foundation; and
- Dr. Webster Cavenee (San Diego Branch) awarded the 2004 Annual Faculty Award for Excellence, University of California, San Diego (UCSD).

Formal academic review to assess the quality and impact of the research carried out by nine Institute staff members was conducted in 2004 by the Institute's Scientific Committee and Scientific Directorate.

Nine staff members underwent external review for Associate Member rank and were promoted:

- Dr. Freddy Radtke (Lausanne Branch)
- Dr. Joan Heath (Melbourne Branch)
- Dr. Matthias Ernst (Melbourne Branch)
- Dr. Margaret Hibbs (Melbourne Branch)
- Dr. Marc Achen (Melbourne Branch)
- Dr. Achim Jungbluth (New York Branch)
- Dr. Luiz Reis (São Paulo Branch)
- Dr. Aristidis Moustakas (Uppsala Branch)
- Dr. Johan Ericsson (Uppsala Branch)

Two staff members underwent review for Associate Investigator rank, and were appointed:

- Dr. Darrin Smith (Melbourne Branch)
- Dr. Brian Stevenson (Lausanne Branch)

The following appointment, to Assistant Member rank, was also made:

- Dr. Jonas Muhr (Stockholm Branch)

Mr. R. Palmer Baker, Jr.
Chairman

Mr. Georges-Andre Cuendet

Mr. Olivier Dunant

Dr. Adolf E. Kammerer

Mr. Pierre Languetin

Mr. Edward A. McDermott, Jr.

Dr. Lloyd J. Old

Sir Derek Roberts

Dr. Jane Royston

Mr. Alfred Berger

Mr. John D. Gordan III

Mr. Richard D.J. Walker
Secretary To The Board

Scientific Committee

Dr. Lloyd J. Old
Chairman

Dr. Douglas T. Fearon	*University of Cambridge, Cambridge, England*
Dr. Samuel Hellman	*University of Chicago, Chicago, U.S.A.*
Dr. George Klein	*Karolinska Institute, Stockholm, Sweden*
Dr. Ira Mellman	*Yale University, New Haven, U.S.A. (until September)*
Dr. Lucille Shapiro	*Stanford University, Stanford, U.S.A.*
Dr. Phillip Sharp	*Massachusetts Institute of Technology, Cambridge, U.S.A.*
Dr. Harald Zur Hausen	*Deutsches Krebschforschungszentrum, Heidelberg, Germany*

Dr. A. Munro Neville
Secretary To The Committee

Ludwig Institut für Krebsforschung Stadelhoferstrasse 22 8001 Zurich, Switzerland	*Registered Address*
Postfach 8024 Zurich, Switzerland	*Postal Address*
Telephone: [41] (0)1-267 6262 Fax: [41] (0)1-267 6200	
Mr. R. Palmer Baker Jr.	*Chairman*
Dr. Lloyd J. Old	*Institute Director, and Chief Executive Officer (until September)*
Mr. Edward A. McDermott, Jr.	*President, and Senior Executive Officer (from September)*
Dr. A. Munro Neville	*Associate Director (until October)* *Special Assistant to the President (from October)*
Dr. George D. Demetri	*Associate Director - Clinical (from September)*
Dr. Richard D. Kolodner	*Associate Director - Scientific (from September)*
Dr. Andrew J.G. Simpson	*Associate Director - Programs (from September)*
Dr. Jonathan C.A. Skipper	*Associate Director - Intellectual Property & Technology Licensing (from October)*
Mr. Richard D.J. Walker	*Chief Financial Officer*

Milbank, Tweed, Hadley & McCloy, New York Niederer, Kraft & Frey, Zurich	*Counsels*
KPMG Fides Peat, Zurich	*Auditors*
Credit Suisse, Zurich National Westminster Bank PLC, London HSBC Bank USA, New York	*Principal Bankers*
Australia: A.R.B.N. 001 379 344 Belgium: 415.420 Switzerland: CH-020.3.916.330-2 United Kingdom: fc007198 United States of America: EIN 23-712-1131	*Company Registration Numbers*

Ludwig Institute for Cancer Research
605 Third Avenue / 33rd Floor
New York, NY 10158 U.S.A.
Telephone: [1] 212 450 1500
Fax: [1] 212 450 1555

Address

Mr. R. Palmer Baker, Jr.	*Chairman*
Ms. Karen A. Hyland	*Executive Assistant*
Dr Lloyd J. Old	*Institute Director, and Chief Executive Officer (until September)*
Ms Nancy Leyrer	*Assistant to Director*
Mr. Edward A. McDermott, Jr.	*President, and Senior Executive Officer (from September)*
Ms. Kathy Meaghan	*Assistant to President*
Ms. Nadette Bulgin	*Deputy Assistant to President*
Dr. George D. Demetri	*Associate Director - Clinical (from September)*
Dr. Richard D. Kolodner	*Associate Director - Scientific (from September)*
Dr. Andrew J.G. Simpson	*Associate Director - Programs (from September)* *Director, James R. Kerr Program*
Dr. Jonathan C.A. Skipper	*Associate Director - Intellectual Property & Technology Licensing (from October)*

Office of Academic Review

Dr. Ellen Puré	*Director*
Ms. Susan Andrews	*Assistant to Director*

Office of Clinical Trials Management

Dr. Eric W. Hoffman	*Director*
Dr. Gerd Ritter	*Assistant Director*
Dr. Ralph Venhaus	*Head of Clinical Affairs*
Dr. Linda Pan	*Clinical Programs Associate*
Ms. Julie Andrew	*Clinical Project Manager*
Ms. Kristin Iversen,	*Clinical Project Manager (until September)*
Mr. John C-K. Tang	*Clinical Data Management*
Ms. Elisa Plante	*IT Portfolio*
Dr. MaryJane Rafii	*Head of Regulatory Affairs and Safety*
Mr. Gary O'Donnell	*Manager Drug Safety*
Ms. June Swirz	*Assistant to Director*
Ms. Elisa Ruiz	*Executive Secretary*

Office of Communications

Dr. Sarah L. White	*Director (from July)*
Mr. Joshua Carr	*Electronic Communications*

Office of Intellectual Property

Dr. Jonathan C. Skipper	*Director (from October)*
Dr. Pär Olsson	*Scientific Administrative Officer*
Dr. Mona Jhaveri	*Scientific Administrative Officer*

Office of Program Development (until September)

Dr. Jonathan C. Skipper	*Director*

Office of the Protocol Review Committee

Dr. Herbert F. Oettgen	*Chairman*
Ms. Nancy Leyrer	*Secretary*

Ludwig Institute for Cancer Research
Horatio House
77-85 Fulham Palace Road / 5th Floor South
London, W6 8JC, United Kingdom
Telephone: [44] (0)20 8735 9240
Fax / Main: [44] (0)20 8741 4990
Fax / Second: [44] (0)20 8741 5884

Address

Office of Intellectual Property

Dr. A. Munro Neville	*Director (until October)* *Special Assistant to the President (from October)*
Ms. Linda A. Lenthall	*Assistant to Dr. Neville*
Dr. Deborah Carter	*Associate Director*
Dr. Pauline Stasiak	*Senior Scientific Administrative Officer*
Dr. Amy (Lam) Jeffs	*Scientific Administrative Officer*
Mr. Ben Williams	*Scientific Administrative Assistant*
Ms. Brenda Goulder	*Secretary (until May)*
Ms. Angela Burningham	*Secretary (from June)*

Office of Technology Licensing

Dr. Robert Burns	*Director*

Ludwig Institut für Krebsforschung *Address*
Stadelhoferstrasse 22
8001 Zürich, Switzerland

Postfach: 8024 Zürich, Switzerland *Postal Address*

Telephone: [41] (0)1 267 62 62
Fax / Main: [41] (0)1 267 62 00

Mr. Richard D.J. Walker *Chief Financial Officer*
Ms. Andrea Keller *Assistant to Chief Financial Officer*

Mr. Odd Aardalsbakke *Deputy Chief Financial Officer*
Ms. Patricia McCormick *Assistant Controller IP*
Mr. Darren Shearer *Assistant Controller IP*

Mr. Martin Born *Financial Controller*
Ms. Paola Maio *Accountant*
Mr. Reto Schläfle *Accountant (until September)*
Ms. Monica Hess *Accountant (from November)*

Mr. Steve Birnbaum *Manager IT Finance*

Mr. Richard McCleary *Budget and Planning Manager/Internal Auditor*

Ms. Karin Genhart *Secretary*

Ludwig Institute for Cancer Research
UNIL - BEP
Dorigny
1015 Lausanne
Switzerland

Telephone: [41] (0)21 692 4060
Fax: [41] (0)21 692 4065

Address

Office of Information Technology

Dr. C. Victor Jongeneel	*Director*
Ms. Jocelyne Muller	*Secretary/Administrator*
Dr. Christian Iseli	*Systems Manager*
Dr. Dmitri Kuznetsov	*Biocomputing Specialist*
Dr. Brian Stevenson	*Bioinformatics Specialist*
Dr. Yves Stauffer	*Postdoctoral Fellow (until February)*
Mr. Gregory Theiler	*Graduate Student*

"Cancer Immunity" Journal

Dr. Monique Zahn	*Editorial Assistant*

Brussels Branch of Human Cancer Cell Genetics
Avenue Hippocrate 74, UCL 7459
1200 Brussels, Belgium
Tel: [32] (0)2 764 74 59
Fax: [32] (0)2 762 94 05

Dr. Thierry Boon *Branch Director*

Lausanne Branch of Immunology
Chemin des Boveresses 155
1066 Epalinges s/Lausanne, Switzerland
Tel: [41] (0)21 692 59 66
Fax: [41] (0)21 653 44 74

Dr. Jean-Charles Cerottini *Branch Director*

London St Mary's Branch of Molecular Virology
Imperial College of Science Technology and Medicine/St Mary's Campus
Norfolk Place, W2 1PG, London, UK
Tel: [44] (0)20 7724 55 22
Fax: [44] (0)20 7724 85 86

Dr. Paul J. Farrell *Branch Director*

London University College Branch of Cell and Molecular Biology
Courtauld Building, 91 Riding House Street
W1W 7BS, London UK
Tel: [44] (0)20 7878 4000
Fax: [44] (0)20 7878 4040

Dr. Michael D. Waterfield *Branch Director (until September)*
Dr. Xin Lu *Branch Director (from September)*

Melbourne Branch of Tumor Biology
PO Box 2008 Royal Melbourne Hospital
Parkville, Victoria 3050, Australia
Tel: [61] (0)3 9341 31 55
Fax: [61] (0)3 9341 31 04

Dr Antony W. Burgess *Branch Director*

New York Branch of Human Cancer Immunology
Memorial Sloan-Kettering Cancer Center
1275 York Avenue, Box 32
New York, NY 10021-6007 USA
Tel: [1] 212 639 8600
Fax: [1] 212 717 3100

Dr Lloyd J. Old *Branch Director*

San Diego Branch of Cancer Genetics
University of California San Diego
9500 Gilman Drive
La Jolla, CA 92093-0660 USA
Tel: [1] 858 552 4920
Fax: [1] 858 534 7750

Dr Webster K. Cavenee *Branch Director*

São Paulo Branch of Cancer Genetics
Rua Prof. Antonio Prudente
109-4 Andar, Liberdade 01509-010
São Paolo, SP, Brazil
Tel: [55] (0xx) 11 33 88 32 00
Fax: [55] (0xx) 11 32 07 70 01

Dr Ricardo R. Brentani *Branch Director*

Stockholm Branch of Molecular and Cell Biology
Karolinska Institute, Box 240
S-17177 Stockholm, Sweden
Tel: [46] (0)8 524 871 00
Fax: [46] (0)8 33 28 12

Dr Ralf E. Pettersson *Branch Director*

Uppsala Branch of Growth Regulation
Biomedical Center, Box 595
S-751 24 Uppsala, Sweden
Tel: [46] (0)18 16 04 00
Fax: [46] (0)18 16 04 20

Dr. Carl-Henrik Heldin *Branch Director*

LUDWIG INSTITUTE FOR CANCER RESEARCH

Brussels Branch
of Human Cancer Cell Genetics

Brussels, Belgium

Staff List

Boon, Thierry *Director, Member*

Pre-clinical Research Resources Group

Warnier, Guy *Veterinarian and Group Head*
Matthews, Jean-Pierre *Technical Assistant, part time*
Sirichanto, Phone Praseuth *Technical Assistant*
Souvannaraj, Keota *Technical Assistant*
Souvannaraj, Keoprasith *Technical Assistant, part time*

Therapeutic Vaccination Group

Marchand, Marchand *Senior Clinical Investigator*
van Baren, Nicolas *Clinical Investigator*
Verfaille, Christian, M.D. *Clinical Project Manager*
Degueldre, Jérôme *Clinical Research Associate*

Immunotherapy Analysis Group

Van Pel, Aline *Associate Member*
Colau, Didier *Senior Investigator*
Godelaine, Danièle *Senior Investigator*
Brasseur, Francis *Associate Investigator*
Lethé, Bernard *Associate Investigator*
Lurquin, Christophe *Associate Investigator*
Bastin, David *Technician*
Ha Thi, Vinh *Technician*
Letellier, Marie-Claire *Technician*
Panagiotakopoulos, Maria *Technician*
Swinarska, Magdalena *Technician*
Carrasco, Javier *Graduate Student*

Human Tumor Antigen Group

van der Bruggen, Pierre *Member*
Stroobant, Vincent *Postdoctoral Fellow*
Wildmann, Claude *Research Associate*
Zhang, Yi *Postdoctoral Fellow (until November)*
Renkvist, Nicolina *Postdoctoral Fellow*
Sun, Zhaojun *Visiting Scientist*
Demotte, Nathalie *Technician*
Ottaviani, Sabrina *Technician*
Tollet, Bénédicte *Technician*
Nicolay, Hugues *Graduate Student (until February)*

Tumor Immunology and Antigen Processing Group

Van den Eynde, Benoît *Member*
Uyttenhove, Catherine *Senior Investigator*
Chapiro, Jacques *Postdoctoral Fellow*
Ma, Wenbin *Postdoctoral Fellow (until April)*
Théate, Ivan *Postdoctoral Fellow, part time*

Pilotte, Luc *Research Associate*
Donckers, Dominique *Technician*
Piette, Fanny *Technician (until October)*
Huijbers, Ivo *Graduate Student*
Guillaume, Benoit *Graduate Student*
Naslund, Tanja *Graduate Student (until February)*
Parmentier, Nicolas *Graduate Student*
Vigneron, Nathalie *Graduate Student*

Tumor Genetics Group

De Plaen, Etienne *Assistant Member*
De Smet, Charles *Assistant Member*
Blondiaux, Claudine *Technician*
Kholmanskikh, Olga *Graduate Student*
Loriot, Axelle *Graduate Student*

Cytokine Group

Renauld, Jean-Christophe *Member*
Van Snick, Jacques *Member*
Louahed, Jamila *Assistant Member*
Dumoutier, Laure *Postdoctoral Fellow*
de Lestré, Brigitte *Technician*
Stevens, Monique *Technician*
Van Roost, Emiel *Technician*
Knoops, Laurent *Graduate Student(until September)*
Steenwinckel, Valérie *Graduate Student*
Tounsi, Amel *Graduate Student (from September)*

Signal Transduction Group

Constantinescu, Stefan *Assistant Member*
Moucadel, Virginie *Postdoctoral fellow*
Kubatzky, Katharina *Postdoctoral fellow*
Li, Mingli *Technician (until August)*
Yin, Yan *Technician (from September)*
Royer, Johan *Graduate Student*
Staerck, Judith *Graduate Student*

Technical Support

Lejeune, Joseph *Head of Bioinformatics Unit*
Van Cleve, Jacques *Research Assistant in Bioinformatics (from May)*
Wauters, Paul *Laboratory Manager*
Tonon, André *FACS Operator*
Germis, Jean-Luc *Technician*
Orviz, Manuel *Technical Assistant*
Van Goetsenhoven, Antoinette *Technical Assistant*
Ahloumou, Mohamed *Laboratory Helper*
Hamed, Mustafa *Laboratory Helper*
Jebari, Samira *Laboratory Helper*
Mahieu-Gaudy, Pascale *Laboratory Helper*

Marianini, Encarnacion *Laboratory Helper*
Reyes Prieto, Rafaela *Laboratory Helper*

Administration & Secretariat

Florean, Dario *Administrator*
Bien, Aline *Accounting and Administrative Assistant*
Hoppe, Sylvie *Scientific Secretary*
Krack, Nathalie *Librarian*
Lemoine, Carine *Scientific and Administrative Assistant, part time*
Roks, Arnaud *Accounting Assistant*
Schoonheydt, Geneviève *Administrative Assistant*
Winand, Valérie *Scientific Secretary*
Verhelst, Jean-Claude *Office Clerk (from April)*

Director's Report

The Brussels Branch specializes in cancer immunology and cancer genetics. The notion that the immune system might be enlisted to rid the body of cancer draws on past work at the Branch, which revealed that most human tumors bear antigens that can be recognized by cytotoxic T lymphocytes (CTLs). Some of these antigens are highly tumor-specific, while others are expressed on certain normal cells. A number of antigens have been found on many different types of tumors, suggesting that a therapeutic strategy targeting such antigens could be used to treat a wide range of cancers. The Brussels Branch continues the search for tumor antigens, and evaluates their therapeutic potential in vaccine trials of cancer patients.

The Brussels Branch is also involved in research on the immunological functions of several cytokines, particularly IL-9 and IL-22 which have been discovered at the Branch. The signaling pathways and genes induced by these cytokines are also studied.

Thierry Boon

Research Report

Therapeutic Vaccination Group

Because of their tumor specificity, MAGE antigens are promising candidates for cancer vaccine development. The Group, led by Marie Marchand and Nicolas Van Baren, designs and conducts clinical trials investigating either MAGE peptides, recombinant MAGE proteins, or a recombinant ALVAC virus encoding a *MAGE* minigene. All these cancer vaccines are very well tolerated by the patients. Tumor regressions are observed in 10 to 20% of the vaccinated patients with metastatic melanoma, with complete or partial clinical responses being observed in 5 to 10% of the patients.

While it is possible to derive CTL that recognize and kill autologous tumor cells *in vitro*, the way to induce an effective CTL response against a MAGE antigen in cancer patients is not yet known. Clinical vaccination trials have two main objectives. The first objective is to assess the effectiveness of various vaccination modalities; by following the clinical evolution of the tumor, by

analyzing whether a specific CTL response to the vaccine antigen occurred, and by determining whether immunological and clinical responses are correlated. The second objective is to analyze T lymphocytes and tumor samples collected at different time points during vaccination to better understand what happens in patients who experience regression of metastatic lesions, and why this does not happen in the majority of patients with overall disease progression. This knowledge will be used to design new vaccination modalities.

Immunotherapy Analysis Group

The Group led by Drs. Aline Van Pel and Danièle Godelaine, in collaboration with Drs. Pierre Coulie (Institute of Cellular Pathlogy, Université Catholique de Louvain, Belgium) and Kris Thielemans (Vrije Universiteit Brussel, Belgium) is pursuing vaccination of melanoma cancer patients with tumor-specific MAGE-3.A1 antigen as peptide alone, as recombinant ALVAC canarypox, or with autologous dendritic cells pulsed with the antigenic peptide. The immune response of each vaccination approach, joined to a possible correlation with clinical outcome, has remained the main research interest. The Group has already described how the well established HLA-peptide tetramer approach allowed the follow up of the MAGE-3.A1 anti-vaccine CTL response, through the isolation of CTL clones and the genetic analysis of T cell receptor (TCR) sequences. For some of these patients, tumor cell lines were established from tumor samples removed by surgery. From these metastases, tumor infiltrating lymphocytes were also isolated and cloned. These anti-tumor CTL clones were found to be directed against epitopes unrelated to the vaccine, such as mutated or differentiation antigens. In one patient, a strong immune reactivity against epitopes of the MAGE-C2 protein expressed by the tumor was found. Following the characterization of their TCR, a genetic analysis has demonstrated the amplification of some of the CTL clones, seen not only in the metastases but also in the blood. Some of these CTL clones were highly enriched in tumor samples relative to the blood. Such expansion of several anti-tumor CTL clones, including some directed against MAGE-C2 epitopes, was confirmed in another clinically responding patient after vaccination with MAGE-3.A1 antigen. Future research will focus on the understanding of this phenomenon by analyzing other patients including clinical non-responders.

Human Tumor Antigen Group

The Group, led by Dr. Pierre van der Bruggen, is defining antigenic peptides encoded by "cancer-testis" (CT) genes like those of the *MAGE* family, which are expressed by cells from many different cancer types. Therapeutic vaccination of cancer patients with MAGE peptides is now in progress, and the identification of additional antigenic peptides is important to increase the range of patients eligible for therapy and to provide tools for a reliable monitoring of the immune response.

Stimulation of CD8+ T lymphocytes with antigen-presenting dendritic cells infected with viruses carrying *MAGE* genes has led to the identification of a large number of new antigenic peptides presented by HLA class I molecules. Similarly, stimulation of CD4+ T cells with dendritic cells pulsed with a MAGE protein has revealed new antigenic peptides presented by HLA class II molecules. The finding that almost every cancer patient whose tumor expresses a *MAGE* gene has at least one HLA molecule presenting a MAGE antigenic peptide suggests numerous possibilities for therapeutic vaccination. Efforts are currently devoted to set up assays that accurately monitor CD4+ T cell responses to cancer vaccines. The Group recently validated the use of the first HLA-DP4 tetramer, which was folded with a MAGE-3 peptide for patients vaccinated with peptides. The Group has also validated a quantitative approach to isolate anti-vaccine T cells directed against all possible HLA-peptide combinations that could be targeted by the response for patients vaccinated with a protein.

Functional defects of T cells are also being studied, with the observation that human CTL clones lose their specific cytolytic activity and cytokine production under certain stimulation conditions. These inactive CTL simultaneously lose their labeling by an HLA-peptide tetramer, although the amount of TCR-CD3 at their surface is not reduced. These results suggest the existence of a new type of functional defect of CTL.

Tumor Immunology and Antigen Processing Group

Building on the molecular definition of tumor antigens recognized by T cells, the Group of Dr. Benoît Van den Eynde mainly focuses on two aspects of tumor immunology; the processing of tumor antigens and the study of animal models to optimize cancer immunotherapy and evaluate tumor resistance mechanisms. The Group also recently described several new tumor antigens, which are encoded by cancer-germline gene *MAGE-C2* or derived from melanocytic protein gp100.

Tumor antigens recognized by CTL consist of peptides that are presented by major histocompatibility complex (MHC) molecules at the cell surface and derive from intracellular proteins that are degraded by the proteasome. The intracellular pathway leading from the protein to the peptide/MHC complex is known as "antigen processing". The Group recently described a new mode of production of antigenic peptides by the proteasome, based on that apparatus cutting and pasting peptide fragments to form a new spliced peptide. The first example was a peptide derived from human melanocyte protein gp100. This antigenic peptide is nine amino acids long and is produced by the splicing of two fragments that were initially non-contiguous in the parental protein. The splicing made by the proteasome is tightly coupled to the proteolytic reaction, and appears to occur by transpeptidation involving an acyl-enzyme intermediate. The Group is currently working on a second example of spliced peptide, where the two fragments are rearranged before splicing. The processing differences between the standard proteasome, which is present in most cells, and the immunoproteasome, which is found in dendritic cells and in cells exposed to interferon-gamma, are also being studied. Several tumor antigens were found to be processed differently by the two proteasome types, usually because of a preferential cleavage made by one or the other proteasome within the antigenic peptide itself.

Translation of knowledge about tumor antigens into efficient cancer immunotherapy requires additional studies on the various strategies that can be used. Some of these studies can be performed in preclinical animal models. The currently available murine models are limited by the fact they are based on transplantation of tumor cells, grown *in vitro*, into a healthy animal. This does not recapitulate the long-term host/tumor relationship that occurs in humans when a tumor slowly develops within a normal tissue. To circumvent this limitation and obtain more relevant information from such preclinical models, the Group has designed a new mouse melanoma model in which tumors expressing a given antigen can be induced using a transgenic system based on Cre-lox recombination. This involves a transgenic mouse strain with an inducible expression of ras and an inducible inactivation of the tumor-suppressor genes of the INK4A locus. The induction is based on recombinase Cre-ER, which is under the control of the tyrosinase promoter. Thus, after topical treatment with tamoxifen, CreER will recombine the transgenes in melanocytes, thereby inactivating INK4A and activating ras. In addition, the induced melanomas will express the model tumor antigen encoded by gene *P1A*, which is also activated by recombination of the transgene. The results show that about 33% of the treated mice develop cutaneous melanomas expressing P1A. These tumors grow slowly and progressively, without causing distant metastases. This model should prove useful to optimize immunotherapy.

Tumor Genetics Group

Human tumors express specific CT antigens arising from the activation of genes, such as *MAGE, BAGE, GAGE* and *LAGE/NY-ESO1*, which are normally expressed only in germ cells. As germ cells are not subject to scrutiny by the immune system, antigens encoded by these genes are strictly tumor-specific. The Group of Drs. Etienne De Plaen and Charles De Smet is trying to identify new genes specifically expressed in tumors and germ cells. Screening procedures based on differential expression profiling allowed the isolation of several genes with cancer and germline specific expression. Most of these genes are typically expressed in spermatogonia, the pre-meiotic stage of sperm development, and are located on the X chromosome

Additionally, efforts are also devoted to determining the function of CT genes. To analyze the functions of a MAGE protein, MAGE-A1, Dr. Etienne De Plaen and his team are searching for binding partners of this protein. An interaction between MAGE-A1 and transcriptional regulator SKIP was discovered using yeast two-hybrid screening. SKIP is an adaptor protein that connects DNA binding proteins to proteins that activate or repress transcription. The results suggest that, by binding to SKIP and recruiting histone deacetylase 1, the MAGE-A1 protein present in the nucleus represses transcription. The Group is now trying to identify the genes that are regulated by MAGE-A1 by using an inducible transfected *MAGE-A1* gene and the microarray technology.

Dr. Charles De Smet and his team are studying the mechanisms leading to the activation of CT genes in tumors. The Group has previously shown that these genes rely primarily on DNA methylation for their repression in normal somatic tissues, and that their activation in tumors is a consequence of the overall genome demethylation process that often accompanies tumorigenesis. The Group is now focusing on the mechanisms of demethylation of these genes in tumors. Stable activation of CT genes in tumors does not require a permanent demethylating activity, but depends on the presence of specific transcription factors that maintain the promoter region unmethylated. Antisense-mediated knock-down experiments indicated that DNMT1 is the primary DNA methyltransferase maintaining methylation of cancer-germline genes. Transient down-regulation of DNMT1 induced stable activation of cancer-germline genes, supporting the view that hypomethylation of these genes in tumors results from a historical event of demethylation.

Finally, the Group is investigating the gene expression profile of tumor samples and tumor cell lines obtained from melanoma patients who received experimental cancer vaccines. Using microarray and quantitative RT-PCR, the group is trying to identify genes involved in the resistance of tumors to CTL.

Cytokine Group

Led by Dr. Jean-Christophe Renauld, the Group studies the biology of interleukin-9 (IL-9) and IL-22, two cytokines discovered at the Branch. IL-9 is a Th2 cytokine that plays a role in immune responses against intestinal parasites and asthma. IL-22, originally identified as a gene induced by IL-9 in T lymphocytes, upregulates the production of acute phase reagents in liver. Its activity in inflammatory responses is modulated by a specific antagonist, the IL-22 binding protein. The role of IL-9 and IL-22 in inflammation is currently being investigated using transgenic and gene-targeted mice for these cytokines and their receptors.

First, IL-9 transgenic mice, that have a high level of this cytokine in all tissues, are characterized by a high susceptibility to the development of T cell lymphomas. Another major aspect of IL-9 biology is its effect on the growth and differentiation of mast cells. IL-9 transgenic mice show increased numbers of mast cells in the gut and airways. Finally, a puzzling activity of IL-9 is a selective increase in the peritoneal B1b cell subpopulation. Although the specificity of these cells is far from clear, they might be related to some auto-immune processes. In line with the oncogenic

activity of IL-9 in transgenic mice, this cytokine was shown to be a potent anti-apoptotic factor for T cell lymphomas. The anti-apoptotic effect of IL-9 does not involve MAP-kinases but is mediated by the JAK/STAT pathway. Therefore, the Group is now focusing on the characterization of genes whose expression is regulated by IL-9 through the activation of STAT transcription factors.

A study of cytokine function based on auto-vaccination of mice with mouse cytokines linked to non-self proteins or helper peptides was initiated several years ago using IL-9. It has now been extended to IL-12, whose excessive production has been linked to several auto-immune diseases. Auto-vaccination against IL-12 was found to completely protect against some forms of murine experimental auto-immune encephalomyelitis at the expense of reduced resistance against an intracellular parasite such as *Leishmania major*.

Coupling of human IL-9 to a carrier protein was also instrumental in the development of anti-human IL-9 monoclonal antibodies with potent inhibitory activity. These antibodies allowed the first detection of human IL-9 protein production by peripheral blood mononuclear cells (PBMC) stimulated with allergen or helminthic infection. A systematic screening of human plasma samples showed that, while no IL-9 could be detected in sera from normal individuals, it was found in approximately 40 % of patients with Hodgkin's Disease and correlated with nodular sclerosis subtype and negative prognostic factors.

Signal Transduction Group

The Group, led by Dr. Stefan Constantinescu, studies: the mechanisms by which Janus kinases (JAKs) act as chaperones for cytokine receptor traffic; the structure and function of cytokine receptors; and JAK-STAT signaling in blood formation and oncogenesis. During 2004, the Group focused on the structure and function of the thrombopoietin (TpoR) and erythropoietin receptors (EpoR) and their involvement in myeloproliferative syndromes. An extended cysteine scanning mutagenesis of the EpoR juxtamembrane extracellular (JM) and transmembrane (TM) regions led to the isolation of three novel constitutively active EpoR mutants and to the identification of a helix cap structure at the junction between the JM and TM regions. This sequence may become a target for screening of small molecule activators/ inhibitors of the EpoR.

A novel approach was established to study signaling by distinct dimeric conformations of cytokine receptors. Fusion proteins were engineered between a dimeric coiled-coil and the TM and cytoplasmic domains of cytokine receptors in such a way that all seven possible relative orientations of the dimeric receptors were imposed. Using this approach, several active dimeric conformations of the TpoR were identified. As a function of the dimeric conformation, the TpoR appears to activate distinct signaling pathways at different stages of hematopoietic development. The Group is now attempting to ascribe the signaling molecules responsible for this differential signaling. The mechanisms by which STAT proteins become constitutively activated and how they function in the nucleus of transformed hematopoietic or patient-derived leukemia cells are also being studied.

In collaboration with the laboratory of Dr. William Vainchenker (Institut Gustave Roussy, Villejuif), the Group has contributed to the discovery of a point mutation of the pseudokinase domain of JAK2, which is present in a majority of Polycythemia Vera patients and in patients with essential thrombocythemia and idiopathic myelofibrosis. Signaling downstream of this JAK2 mutant and its effects on cytokine receptor traffic and erythroid/myeloid differentiation are major focuses.

Publications

Primary Research Articles

1. Bartlett N.W., Dumoutier L., Renauld J.-C., Kotenko S., McVey C., Lee H.-J., Smith, G.L. A new member of the interleukin 10-related cytokine family encoded by a poxvirus. Journal of General Virology (2004) 85:1401-1412.
2. Bertrand M., Huijbers I., Chomez P., De Backer O. Comparative expression analysis of the MageD genes during embryongenesis and brain development. Developmental Dynamics (2004) 230:325-334.
3. Bonehill A., Heirman C., Tuyaerts S., Michiels A., Breckpot K., Brasseur F., Zhang Y., van der Bruggen P., Thielemans K. Messenger RNA-electroporated dendritic cells presenting MAGE-A3 simultaneously in HLA class I and class II molecules. The Journal of Immunology (2004) 172:6511-6512.
4. Breckpot K., Heirman C., De Greef C., van der Bruggen P., Thielemans K. Identification of new antigenic peptide presented by HLA-Cw7 and encoded by several MAGE genes using dendritic cells transduced with lentivirus. The Journal of Immunology (2004) 172:2232-2237.
5. Brenner C., Deplus R., Didelot C., Loriot A., Viré E., De Smet C., Gutierrez A., Danovi D., Bernard D., Boon T., Pelicci P-G., Amati B., Kouzarides T., de Launoit Y., Di Croce L., Fuks F. Myc represses transcription through recruitment of DNA methyltransferase corepressor. The EMBO Journal (2004) 24:336-346.
6. Corbière V., Nicolay H., Russo V., Stroobant V., Brichard V., Boon T., and van der Bruggen P. Identification of a MAGE-1 peptide recognized by cytolytic T lymphocytes on HLA-B*5701 tumors. Tissue Antigens (2004) 63:453-457.
7. De Smet C., Loriot A., Boon T. A promoter-dependent mechanism leading to selective hypomethylation within the 5' region of gene MAGE-A1 in tumor cells. Molecular and Cellular Biology (2004) 24:4781-4790.
8. Dullaers M., Breckpot K., Van Meirvenne S., Bonehill A., Tyaerts S., Michiels A., Straetman L., Heirman C, De Greef C., van der Bruggen P., Thielemans K. Side-by-side comparison of lentivirally transduced and mRNA-electroporated dendritic cells: implications for cancer immunotherapy protocols. Molecular Therapy (2004) 10:768-779.
9. Dumoutier L., Tounsi A., Michiels T., Sommereyns C., Kotenko S.V., Renauld J.C. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/interferon-lambda 1: similarities with type I interferon signaling. Journal of Biological Chemistry (2004) 279:32269-32274.
10. Hör S., Pirzer H., Dumoutier L, Bauer F., Wittmann S., Sticht H., Renauld J.-C., de Waal-Malefyt R., Fickenscher H. The T-cell lymphokine interleukin-26 targets epithelial cells through the interleukin-20 receptor 1 and interleukin-10 receptor 2 chains. Journal of Biological Chemistry (2004) 279:33343-33351.
11. Knoops L., Louahed J., Renauld J.C. IL-9-induced expansion of B-1b cells restores numbers but not function of B-1 lymphocytes in xid mice. The Journal of Immunology (2004) 172:6101-6106.

12. Laduron S., Deplus R., Zhou S., Kholmanskikh O., Godelaine D., De Smet C., Hayward D., Fuks F., Boon T., De Plaen E. MAGE-A1 interacts with adaptor skip and the deacetylase HDAC1 to repress transcription. Nucleic Acids Research (2004) 32:4340-4350.

13. Liénard D., Rimoldi D., Marchand M., Dietrich P-Y., van Baren N., Geldhof C., Batard P., Guillaume P., Ayyoub M., Pittet M., Zippelius A., Fleischhauer K., Lejeune F., Cerottini J-C., Romero P., Speiser D. Ex vivo detectable activation of Melan-A-specific T cells correlating with inflammatory skin reactions in melanoma patients vaccinated with peptides in IFA. Cancer Immunity (2004) 4:4.

14. Lombry C., Marteleur A., Arras M., Lison D., Louahed J., Renauld J.-C., Préat V., Vanbever R. Local and systemic immune responses to intratracheal instillation of antigen and DNA vaccines in mice. Pharmaceutical Research (2004) 21:127-135.

15. Lonchay C., van der Bruggen P., Connerotte T., Hanagiri T., Coulie P., Colau D., Lucas S., Van Pel A., Thielemans K., van Baren N., Boon T. Correlation between tumor regression and T cell responses in melanoma patients vaccinated with a MAGE antigen. Proceedings of the National Academy of Sciences USA (2004) 101:14631-14638.

16. Ma W., Germeau C., Vigneron N., Maernoudt A.-S., Morel S., Boon T., Coulie P., and Van den Eynde B. Two new tumor-specific antigenic peptides encoded by gene Mage-C2 and presented to cytolytic T lymphocytes by HLA-A2. International Journal of Cancer (2004) 109:698-702.

17. Probst-Kepper M., Hans-Jürgen H., Herrmann H., Janke V., Ocklenburg F., Klempnauer J., Van den Eynde B., Weiss S. Conformational restraints and flexibility of 14-meric peptides in complex with HLA-B*3501. The Journal of Immunology (2004) 173:5610-5616.

18. Royer Y., Menu C., Liu X., Constantinescu S.N. High-throughput gateway licistronic retroviral vectors for stable expression in mammalian cells: exploring the biologic effects of STAT5 overexpression. DNA and Cell Biology (2004) 23:355-3652004.

19. Schultz E., Schuler-Thurner B., Stroobant V., Jenne L., Berger T., Thielemanns K., van der Bruggen P., Schuler G. Functional analysis of tumor-specific Th cell responses detected in melanoma patients after dendritic cell-based immunotherapy. The Journal of Immunology (2004) 172:1304-1310.

20. Shanker A., Auphan-Anezin N., Chomez P., Giraudo L., Van den Eynde B., Schmitt-Verhulst A.-M. Thymocyte-intrinsic genetic factors influence CD8 T cell lineage commitment and affect selection of a tumor-reactive TCR. The Journal of Immunology (2004) 172:5069-5077.

21. Sheikh F., Baurain VV., Lewis-Antes A., Shah NK., Smirnov SV., Anantha S., Dickensheets H., Dumoutier L., Renauld JC., Zdanov A., Donnelly RP., and Kotenko SV. Cutting edge : IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. The Journal of Immunology (2004) 172:2006-2010.

22. Stephens P., Hunter C., Bignell G., Edkins S., Davies H., Teague J., Stevens C., O'Meara S., Smith R., Parker A., Barthorpe A., Blow M., Brackenbury L., Butler A., Clarke O., Cole J., Dicks E., Dike A., Drozd A., Edwards K., Forbes S., Foster R., Gray K., Greenman C., Halliday K., Hills K., Kosmidou V., Lugg R., Menzies A., Perry J., Petty R., Raine K., Ratford L., Shepherd R., Small A., Stephens Y., Tofts C., Varian J., West S., Widaa S., Yates A., Brasseur F., Cooper C.S., Flanagan A.M., Knowles M., Leung S.Y., Louis D.N., Looijenga L.H., Malkowicz B., Pierotti M.A., Teh B., Chenevix-Trench G., Weber B.L., Yuen S.T., Harris G., Goldstraw P., Nicholson A.G., Futreal P.A., Wooster R. Stratton M.R. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature (2004) 431:525-526.

23. Uyttenhove C., Arendse B., Stroobant V., Brombacher F., Van Snick J. Development of an anti-IL-12 p40 auto-vaccine : protection in experimental autoimmune encephalomyelitis at the expense of increased sensitivity to infection. European Journal of Immunology (2004) 34:3572-3581.

24. Van Pesch, V., Lanaya, H., Renauld, J.-C., Michiels T. Characterization of the murine interferon-alpha gene family. Journal of Virology (2004) 78:8219-8228.

25. Vantomme V., Dantinne C., Amrani N., Permanne P. Gheysen D., Bruck C., Stoter G., Britten M., Keilholz U., Lamers C., Marchand M., Delire M. and Gueguen M. Immunologic Analysis of a Phase I/II Study of Vaccination with MAGE-3 Protein Combined with the AS02B Adjuvant in Patients with MAGE-3-Positive Tumors. Journal of Immunotherapy (2004) 27:124-135.

26. Vialle-Castellano A., Laduron S., De Plaen E., Jost E., Dupont S., Ameye G., Michaux L., Coulie P.G., Olive D., Boon T., and van Baren N. A gene expressed exclusively in acute B lymphoblastic leukemia. Genomics (2004) 83:85-94.

27. Vialle-Castellano A., Gaugler B., Mohty M., Isnardon D., Van Baren N., Olive D. Abundant expression of fibronectin is a major feature of leukemic dendritic cells differentiated from patients with acute myeloid leukemia. Leukemia (2004) 18:426-433.

28. Vigneron N., Stroobant V., Chapiro J., Ooms A., Degiovanni G., Morel S., van der Bruggen P., Boon T., and Van den Eynde B. An antigenic peptide produced by peptide splicing in the proteasome. Science (2004) 304:587-590,

Reviews / Commentaries / Book Chapters

1. Knoops L., Renauld J-C. IL-9 and its receptor : from signal transduction to tumorigenesis. Growth Factors (2004) 22:207-215.

2. Ghaffari S., Constantinescu S. Erythropoietin and Erythropoietin Receptor: History, Structure, Interactions and Intracellular Signal Transduction. Encyclopedia of Molecular Medicine (Editor T. Creighton) John Wiley and Sons Inc. 2004, DOI:10.1002/0471203076.emm2011

LUDWIG INSTITUTE FOR CANCER RESEARCH

Lausanne Branch of Immunology

Lausanne, Switzerland

Staff List

Cerottini, Jean-Charles *Director, Member*

Cell Fate Determination Group

Radtke, Freddy *Associate Member (SNF Professor)*
Dumortier, Alexis *Postdoctoral Fellow (from May)*
Mancini, Stéphane *Postdoctoral Fellow (until August)*
Lathion, Stéphanie *Postdoctoral Fellow (until September)*
Besseyrias, Valérie *Research Associate*
Cobas, Monica *Graduate Student*
Riccio, Orbicia *Graduate Student*
Vauclair, Sophie *Graduate Student*

Developmental Immunology Group

MacDonald, H. Robson *Associate Director, Member*
Wilson, Anne *Associate Investigator*
Ernst, Bettina *Postdoctoral Fellow (until July)*
Schümann, Jens *Postdoctoral Fellow*
Ferrero, Isabel *Research Associate*
Lees, Rosemary K. *Research Associate*
Bianchi, Teresa *Graduate Student*

Innate Immunity Group

Held, Werner *Associate Member*
Chalifour, Anick *Postdoctoral Fellow*
Coudert, Jérôme *Postdoctoral Fellow*
Scarpellino, Leonardo *Senior Technical Assistant*
Goux, Delphine *Graduate Student*
Jeannet, Grégoire *Graduate Student (from April)*

Molecular Immunology Group

Luescher, Immanuel F. *Member*
Guillaume, Philippe *Assistant Investigator (Tetramer Production Facility)*
Angelov, Georgiev *Postdoctoral Fellow (from December)*
Cebecauer, Marek *Postdoctoral Fellow*
Goffin, Laurence *Postdoctoral Fellow*
Mark, Silke *Postdoctoral Fellow*
Neff, Laurence *Postdoctoral Fellow*
Cornaz, Rolf *Technical Assistant (Tetramer Production Facility)*

Antigen Processing Group

Lévy, Frédéric *Associate Member (SNF Professor)*
Colombetti, Sara *Postdoctoral Fellow (from March)*
Lindholm, Cecilia *Postdoctoral Fellow*
Lévy, Nicole *Senior Technical Assistant*
Peitrequin, Anne-Lise *Senior Technical Assistant*
Chapatte, Laurence *Graduate Student*
Viatte, Sébastien *Graduate Student*

Molecular Tumor Immunology Group

Cerottini, Jean-Charles *Director, Member*
Rimoldi, Donata *Associate Investigator*
Michielin, Olivier *Postdoctoral Fellow*
Leimgruber, Antoine *Research Associate (from July)*
Mühlethaler, Katja *Senior Technical Assistant*
Salvi, Susanne *Senior Technical Assistant*
Fagerberg, Theres *Graduate Student*

Clinical Tumor Immunology Group

Romero, Pedro *Member*
Speiser, Daniel E. *Associate Member*
Alves, Pedro *Postdoctoral Fellow*
Barbey, Catherine *Postdoctoral Fellow (from April)*
Derre, Laurent *Postdoctoral Fellow (from September)*
Mahnke, Yolanda *Postdoctoral Fellow*
Tabiasco, Julie *Postdoctoral Fellow (until September)*
Baumgärtner Meier, Petra *Research Associate*
Meraldi, Verena *Research Associate (until August)*
Milesi, Renate *Senior Technical Assistant*
Montandon, Nicole *Senior Technical Assistant*
Baroffio, Céline *Technical Assistant*
Bioley, Gilles *Graduate Student*
Bricard, Gabriel *Graduate Student*
Jandus, Camilla *Graduate Student (from September)*

Clinical Trials Program

Speiser, Daniel E. *Associate Member*
Liénard, Danielle *Clinical Investigator*
Lejeune, Ferdy *Affiliate Clinical Investigator*
Geldhof, Christine *Clinical Research Associate*
Minaidis, Danielle *Research Nurse*

Technical Support

Zaech, Pierre *Technical Engineer (until August)*
Devêvre, Estelle *Technical Services*
Grosjean, Frédéric *Technical Services (from June)*

Administration & Secretariat

Méan, Marie-Madeleine *Administrator*
Seravalli, Capucine *Accounting Chief (until February)*
Marouani, Iris *Accounting Assistant*
Nogaré, Nicole *Accounting Assistant*
Lopez, Celia *Secretary to Director*
Poncelet, Audrey *Secretary*
Van Overloop, Martine *Secretary*

Branch Director's Report

The Lausanne Branch has continued to focus its activities on the molecular and cellular mechanisms that regulate immunity mediated by T cells, natural killer (NK) cells and NKT cells. Given the complexities of the innate and adaptive immune systems, a significant part of the laboratory research program involves studies in mice that aim to elucidate of the variety of recognition strategies used by lymphoid cells. As a result of the recent progress in the identification of tumor antigens recognized by CD8+ T cells in cancer patients, a strong emphasis of the Branch laboratory research program deals with the basics of human cancer immunology. This program is closely integrated with our growing clinical program, which is conducted as a part of LICR's Cancer Vaccine and Clinical Trials Programs.

In addition to the longstanding interactions established with our local partners, such as the Department of Biochemistry (University of Lausanne), the Swiss Institute of Experimental Cancer Research (ISREC), the Lausanne unit of the Swiss Institute of Bioinformatics, and the Multidisciplinary Oncology Center (CePO) at the University Hospital of Lausanne (CHUV), new collaborative work has been implemented with several groups of the Swiss Federal Institute of Technology Lausanne (EPFL). Thanks to this partnership with local research and clinical academic institutions, the Branch is in a unique position to translate the insights and discoveries generated through basic immunology research into clinical investigation and applications.

It is noteworthy that this local inter-institutional network will be considerably strengthened in the near future following the decision made this year by the University of Lausanne, the EPFL and the CHUV to create a comprehensive cancer center in partnership with ISREC and LICR. As this development will greatly enhance and broaden our research capability and, hence, offer new research opportunities to the Branch, we look forward with great enthusiasm to the next phase of our activities.

Jean-Charles Cerottini

Research Report

Cell Fate Determination Group

The Cell Fate Determination Group, led by Dr. Freddy Radtke, is interested in the molecular mechanisms controlling stem cell maintenance, lineage commitment and differentiation in self-renewing systems such as the hematopoietic system, the skin and the gut. In addition, this Group investigates the potential role of these mechanisms in tumorigenesis. Current attention is focused on the Notch signaling pathway, which regulates numerous binary cell fate decisions in diverse organisms. Previous studies by the group using conditional gene targeting strategy have established an essential role for Notch1 in specifying T cell lineage. In addition, a novel function for Notch1 has been uncovered at a later stage of T cell development, where it is involved in the control of VDJ rearrangement at the T cell receptor (TCR) beta locus. In the skin an unexpected function of Notch1 as a tumor suppressor gene has been discovered. In the intestinal tract Notch signaling is required for the maintenance of undifferentiated proliferative cells in crypts thereby acting as a gatekeeper of stem/progenitor cells.

Developmental Immunology Group

The Developmental Immunology Group, led by Dr. Rob MacDonald, continues to be interested in the development of T cells and in particular a subset of unconventional T cells known as NKT cells. NKT cells have recently emerged as an important regulatory T cell subset implicated in autoimmunity and tumor immunity. In contrast to conventional T cells, NKT cells utilize a semi-invariant αβTCR to recognize glycolipids presented by the monomorphic CD1d molecule. In order to study thymic selection of NKT cells, the Group has generated transgenic mice expressing CD1d either on CD4+ CD8+ cortical thymocytes or on thymic dendritic cells. Positive selection of NKT cells was found to be mediated exclusively by cortical thymocytes whereas negative selection was mediated primarily (but not exclusively) by dendritic cells. Thus, like conventional T cells, the NKT cell repertoire is shaped by both positive and negative selection.

A more recent interest of the group is the role of the proto-oncogene c-myc in hematopoiesis. In collaboration with Dr. Andreas Trumpp (ISREC), the Group has used conditional gene targeting to delete c-myc in adult bone marrow. As expected from the well known function of c-myc in proliferation, all differentiated cell lineages (myeloid, erythroid and lymphoid) disappeared with time in c-myc-deficient bone marrow. Unexpectedly, however, hematopoietic stem cells not only persisted but actually increased in number. These studies indicate that c-myc plays a pivotal role in maintaining the balance between hematopoietic stem cells self-renewal and differentiation.

Innate Immunity Group

The Innate Immunity Group, led by Dr. Werner Held, studies the development and function of NK cells. NK cells play important roles in innate immunity to infection and tumor cells. NK cell-mediated lysis of target cells is regulated by a dual receptor system, which integrates signals from activating receptors and inhibitory receptors. The latter interact with Major Histocompatibility Complex (MHC) class I molecules expressed on target cells. Surprisingly the Group found that the inhibitory Ly49A NK cell receptor not only binds to the MHC class I ligand expressed in *trans* (i.e. on potential target cells) but is constitutively associated with MHC class I in *cis* (i.e. on the same cell). *Cis* association and *trans* interaction occur via the same binding site. Consequently, *cis* association limits the number of Ly49A receptors available for binding MHC class I on target cells, thereby dampening NK cell inhibition via Ly49A. By lowering the threshold at which NK cell activation exceeds NK cell inhibition, *cis* interaction seems to allow an optimal discrimination of normal and infected or transformed host cells.

Molecular Immunology Group

The main research interest of the Molecular Immunology Group, led by Dr. Immanuel Luescher, is focused on the molecular mechanisms involved in CD8+ cytolytic T lymphocyte (CTL) recognition of, and activation by, peptide-MHC (pMHC) complexes. The current research effort is based on the preparation of well defined soluble pMHC complexes and their testing on CD8+ T cells. Initially the Group prepared and studied dimeric pMHC complexes containing linkers of different length connecting the α3 C-termini of the two pMHC entities. Findings obtained include: (i) pMHC dimers containing short linkers (10-30 Å) efficiently bind to and activate cloned CTL, whereas those containing long linkers do not; (ii) pMHC dimers containing short, but not those containing long linkers, induce rapid death of activated, but not naïve, CD8+ T cells. This effect is not based on classical death mechanisms (e.g. granzyme/perforin-, Fas-, TRAIL- or TNFR-mediated), but relies on cell activation-dependent mitochondrial dysfunction and most likely involves Bcl2 pro-apoptotic family members (e.g. BIM and BNIP3); (iii) pMHC complexes containing long rigid linkers not only fail to trigger CD8+ T cells but effectively inhibit CTL-mediated cytotoxicity; (iv) based on cell activation and binding studies, FRET experiments and

computer assisted docking experiments, the Group discovered a binary binding mode by which two TCR molecules engage in an anti-parallel manner to two pMHC complexes facing each other with their constant domains. Subsequent studies with well defined tetramers and octameric pMHC complexes confirmed these findings. Notably, it was found that octameric pMHC complexes with sub-nanomolar binding constants that contain short linkers induce CTL death, whereas those containing long rigid linkers completely inhibit CTL-mediated cytotoxicity. This work, done in collaboration with groups at EPFL, uses single molecule microscopy as a new tool to investigate peptide presentation by antigen-presenting cells as well as peptide recognition by CTL on a molecular level.

Antigen Processing Group

The Antigen Processing Group, led by Dr. Frédéric Lévy, is involved in the analysis of the intracellular events that contribute to the production of CTL-defined tumor antigens. The group has shown previously that the HLA-A*0201-restricted immunodominant peptide derived from Melan-A, the target of vaccine trials in the Branch, is inefficiently processed in cells expressing the immunoproteasomes, a type of proteasome that is constitutively expressed in dendritic cells and induced in many other cells upon exposure to IFN-γ. Immunization of HLA-A2 transgenic mice with third generation recombinant lentiviral vectors expressing the minimal antigenic determinant of Melan-A induced a potent T cell response, which was detectable *ex vivo*. In contrast, only a modest response was detected after immunization with lentivectors containing the sequence of the full length Melan-A protein. To test whether the expression of immunoproteasomes contributes to this poor response, the group performed similar experiments in HLA-A2 transgenic mice in which the gene coding for LMP2, one of the catalytic subunits of the immunoproteasome, has been deleted. These mice were generated in collaboration with the group of Dr. Benoît Van den Eynde (LICR Brussels Branch). Contrary to the response observed in HLA-A2 transgenic mice, immunization of these HLA-A2 LMP2-/- mice with lentivectors containing the full-length Melan-A sequence resulted in a T cell response that was indistinguishable, both in amplitude and quality, from the response after immunization with lentivectors containing the sequence of the minimal determinant. These results demonstrate that the expression of immunoproteasomes negatively affects the processing of Melan-A, thereby influencing the anti-Melan-A T cell response *in vivo*.

As part of the Cancer Antigen Discovery Collaborative established between LICR and the Cancer Research Institute (CRI), a project aiming at the identification of new candidate peptide tumor antigens in colorectal cancer has recently been launched. To this end, the group has collected pairs of normal and neoplastic colorectal samples from over 30 patients undergoing surgery at the CHUV. In those samples, the Group first monitored the expression of several CT antigens, including NY-ESO-1, SSX-2, MAGE-A3, MAGE-A4, MAGE-A10 and the recently characterized NXF2 by end-point PCR. For all those genes, the relative expression frequencies were lower than 20%, limiting their use for immunotherapeutic treatments of colorectal cancers. In collaboration with Drs. Victor Jongeneel (LICR Office of Information Technology), Pedro Romero and Daniel Speiser, the Group has initiated a large-scale gene analysis of pairs of colorectal samples and has identified several potential targets.

Molecular Tumor Immunology Group

Research activities of the Molecular Tumor Immunology group, led by Dr. Jean-Charles Cerottini, are devoted to the characterization of human tumor antigens that are recognized by CD8+ T cells from melanoma patients. One project aims at identifying the function and regulation of the melanocyte lineage specific protein Melan-A, a small transmembrane protein that is the target of vaccine trials in the Branch. Melan-A is present in the trans-Golgi network and in early stage melanosomes of pigmented cells. The Group has generated Melan-A knock-down cells using

lentivirus-delivered siRNA as well as Melan-A over-expressing cells. Characterization of these cells showed that Melan-A, in contrast to other melanocyte-specific proteins, has a negative role in pigmentation. Expression of tyrosinase, the key enzyme in melanin synthesis, was not altered in Melan-A knock-down or overexpressing cells, suggesting that Melan-A may affect melanogenesis by modifying the melanosomal environment. In addition, it has been found that Melan-A is mono-ubiquitylated and interacts with two members of the HECT-E3 ubiquitin ligase family. Blocking Melan-A ubiquitylation decreased the half-life of the protein and affected its localization. Interestingly, this also influenced melanin content in melanocytic cells. The results suggest a role of ubiquitylation in the sorting of Melan-A from melanosomes to multi-vesicular bodies. These findings also highlight the importance of ubiquitylation processes in the physiology of pigmented cells.

Another project, which is being carried out in collaboration with the Swiss Institute of Bioinformatics, is focused on the development of molecular modeling techniques, such as homology modeling, molecular dynamics and free energy simulations, to design optimized peptides for cancer vaccines as well as optimized specific TCR sequences for adoptive transfer. In particular, TCR sequences that compose the tumor specific repertoire of human CD8+ T cells have been modeled to allow structural comparison and identification of key structural motifs. In parallel, a residue-based free energy decomposition has been carried out to identify the relative importance of each residue of the CDR loops to the TCR affinity. Based on both methods, rational TCR modifications are proposed and evaluated *in silico* with the same methods. Since the kinetic parameters of TCR binding are also key for T cell activation, the Group has established a new method based on non-equilibrium statistical mechanics to compute the binding free energy profile of the TCR. The method has been evaluated on a test system for which the experimental kinetic parameters were reproduced with good accuracy and is now used to evaluate interesting modified TCR sequences. Once promising candidates have been determined with the *in silico* approach, an *in vitro* validation will be carried out using soluble molecules and Biacore techniques.

Clinical Tumor Immunology Group

The Clinical Tumor Immunology Group, led by Dr. Pedro Romero, continued its studies on both naturally acquired and vaccine induced CD8+ T cell responses to well defined melanoma tumor antigens. To this end, the Group is investigating the relationship between the T cell surface phenotype and functional stage of differentiation. While it is widely accepted that T cells not previously exposed to antigen in the periphery co-express the high molecular weight isoform of CD45, CD45RA, and the SLC/ELC chemokines receptor CCR7, the Group has identified a subset of cord blood CD8+ T cells that does not express the latter molecule. Such subset is rare or does not exist at all in peripheral T cells in adult individuals. Detailed characterization of these cells conclusively showed that they were naïve T cells and already present in the mature single CD8+ thymocyte population. These cells may represent precursors of naïve T cells programmed to enter peripheral tissues (e.g. the skin) that may play a role in the acquisition of peripheral tolerance during the neonatal phase of life, such as those demonstrated in mouse models. In 2004, the Group also concluded a major study assessing the functional properties of antigen-experienced CD8+ T cells in various tissues obtained from a relatively large group of cancer patients. Taking advantage of the high frequencies of Melan-A-specific T cells often occurring in HLA-A2 patients with advanced melanoma, the Group extensively phenotyped these cells and isolated them directly from freshly dissociated tumor masses for functional assays, such as lytic activity, cytokine secretion and expression of effector function associated genes. It should be stressed that this study is unique in that it avoids *in vitro* expansion of the antigen specific T cells, thus allowing the acquisition of information on the functional status of T cells infiltrating human tumors. The major finding was that T cells recovered from tumor masses are hyporesponsive. Indeed, they

have reduced levels of perforin and lytic activity and are unable to release IFN-γ upon antigen challenge. However, they are still able to produce IFN-γ when the stimulus bypasses the T cell receptor. Importantly, hyporesponsiveness is reversible upon short term *in vitro* culture. The cells also display significant levels of proliferation when cultured in the presence of cytokines. An additional interesting finding was that the T cells with the same antigen specificity recovered from the circulating compartment from the same patients appeared functionally competent, pointing to a tumor associated state of functional tolerance. These findings may help explain, at least in part, the paradox of tumor progression in patients with apparently vigorous responses of circulating tumor antigen-specific T cells. They raise two important issues. First, the critical importance of monitoring T cell function, and not just T cell frequencies, in the tumor microenvironment. And second, the need to identify strategies to overcome blunting of the specific T cell response within the tumor for vaccines to be clinically effective.

As part of LICR's Cancer Vaccine Program a series of clinical trials of specific immunotherapy of cancer are conducted by the subgroup led by Dr. Daniel Speiser. The objective of the program is to pursue the step-by-step development of optimized peptide-based cancer vaccines. Melanoma patients have been immunized with peptides, adjuvants and novel immune stimulatory substances, particularly CpG oligodeoxynucleotides. Peptides under investigation are the HLA-A*0201 restricted antigens Melan-A, tyrosinase, NY-ESO-1 and MAGE-A10. Immune responses are assessed *ex vivo*, allowing the quantification of activated T cells and the direct quantitative and qualitative characterization of their molecular and functional properties. The results obtained so far show that, unlike patients receiving peptide in saline (who had no detectable immune responses), peptide incorporation in Incomplete's Freund's (IFA) adjuvant leads to relatively strong T cell activation in about half of the patients. Patients treated with peptide plus the newly developed CpG oligodeoxynucleotides 7909 in IFA reached about 10 times higher T cell frequencies, and thus exhibited a considerably stronger T cell expansion than that obtained so far with synthetic T cell vaccines used at low peptide doses. The goal is to induce protective T cell responses leading to cancer regression and/or disease stabilization. This is based on the straight forward assumption that protective T cells express high affinity TCRs, undergo strong clonotypic bursts (leading to high frequencies *in vivo*), home to diseased tissue and efficiently exert effector functions (cytotoxicity, cytokine production) *in situ*. The Group is directly testing this assumption by applying cellular and molecular immune monitoring approaches. In particular, new cell sorting strategies are now combined with PCR-based techniques for the analysis of single antigen-specific T cells, including T cell receptor CDR3β-spectratyping and as well as expression of transcripts encoding molecules associated with T cell functions.

Publications

Primary Research Articles

1. Ayyoub, M., Hesdorffer, C.S., Metthez, G., Stevanovic, S., Ritter, G., Chen, Y.-T., Old, L.J., Speiser, D., Cerottini, J.-C. and Valmori, D. Identification of an SSX-2 epitope presented by dendritic cells to circulating autologous CD4+ T cells. Journal of Immunology (2004) 172:7206-7211.

2. Ayyoub, M., Hesdorffer, C.S., Montes, M., Merlo, A., Speiser, D., Rimoldi, D., Cerottini, J.-C., Ritter, G., Scanlan, M., Old, L.J., and Valmori, D. An immunodominant SSX-2-derived epitope recognized by CD4+ T cells in association with HLA-DR. Journal of Clinical Investigation (2004) 113:1225-1233.

3. Chapatte, L., Servis, C., Valmori, D., Burlet-Schiltz, O., Dayer, J., Monsarrat, B., Romero, P. and Lévy, F. Final antigenic Melan-A peptides produced directly by the proteasomes are preferentially selected for presentation by HLA-A*0201 in melanoma cells. Journal of Immunology (2004) 173:6033-6040.

4. Cobas, M., Wilson, A., Ernst, B., Mancini, S.J.C., MacDonald, H.R., Kemler, R. and Radtke, F. β-catenin is dispensable for hematopoiesis and lymphopoiesis. Journal of Experimental Medicine (2004) 199:221-229.

5. Didierlaurent, A., Ferrero, I., Otten, L.A., Dubois, B., Reinhardt, M., Carlsen, H., Blomhoff, R., Akira, S., Kraehenbuhl, J.-P. and Sirard, J.-C. Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response. Journal of Immunology (2004) 172:6922-6930.

6. Doucey, M.-A., Scarpellino, L., Zimmer, J., Guillaume, P., Luescher, I.F., Bron, C. and Held, W. Cis association of Ly49A with MHC class I restricts natural killer cell inhibition. Nature Immunology (2004) 5:328-336.

7. Gakamsky, D.M., Luescher, I.F. and Pecht, I. T cell receptor-ligand interactions: a conformational preequilibrium or an induced fit. Proceedings of the National Academy of Sciences USA (2004) 101:9063-9066.

8. Gross, D.-A., Graff-Dubois, S., Opolon, P., Cornet, S., Alves, P., Bennaceur-Griscelli, A., Faure, O., Guillaume, P., Firat, H., Chouaib, S., Lemonnier, F.A., Davoust, J., Miconnet, I., Vonderheide, R.H. and Kosmatopoulos, K. High vaccination efficiency of low-affinity epitopes in antitumor immunotherapy. Journal of Clinical Investigation (2004) 113:425-433.

9. Hauert, J., Fernandez-Carneado, J., Michielin, O., Mathieu, S., Grell, D., Schapira, M., Spertini, O., Mutter, M., Tuchscherer, G. and Kovacsovics, T. A template-assembled synthetic protein surface mimetic of the von Willebrand factor A1 domain inhibits botrocetin-induced platelet aggregation. ChemBioChem (2004) 5:856-864.

10. Höflinger, S., Kesavan, K., Fuxa, M., Hutter, C., Heavey, B., Radtke, F. and Busslinger, M. Analysis of Notch1function by in vitro T cell differentiation of *Pax5* mutant lymphoid progenitors. Journal of Immunology (2004) 173:3935-3944.

11. Li, C., Grosdidier, A., Crambert, G., Horisberger, J.-D., Michielin, O. and Geering, K. Structural and functional interaction sites between Na, K-ATPase and FXYD proteins. Journal Biological Chemistry (2004) 279:38895-38902.

12. Liénard, D., Rimoldi, D., Marchand, M., Dietrich, P.-Y., van Baren, N., Geldhof, C., Batard, P., Guillaume, P., Ayyoub, M., Pittet, M.J., Zippelius, A., Fleischhauer, K., Lejeune, F., Cerottini, J.-C., Romero, P. and Speiser, D.E. *Ex vivo* detectable activation of Melan-A-specific T cells correlating with inflammatory skin reactions in melanoma patients vaccinated with peptides in IFA. Cancer Immunity [serial online] 4:4.URL:http://www.cancerimmunity.org/v4p4/040404.htm

13. Meidenbauer, N., Zippelius, A., Pittet, M.J., Laumer, M., Vogl, S., Heymann, J., Rehli, M., Seliger, B., Schwarz, S., Le Gal, F.-A., Dietrich, P.Y., Andreesen, R., Romero, P. and Mackensen, A. High frequency of functionally active Melan-A-specific T cells in a patient with progressive immunoproteasome-deficient melanoma. Cancer Research (2004) 64:6319-6326.

14. Meijer, S.L., Dols, A., Hu, H.-M., Chu, Y., Romero, P., Urba, W.J. and Fox, B.A. Reduced L-selectin ($CD62L^{Low}$) expression identifies tumor-specific type 1 T cells from lymph nodes draining an autologous tumor cell vaccine. Cellular Immunology (2004) 227:93-102.

15. Michielin, O., Udry, E., Périard, D., Matzinger, O., Lobrinus, J.A. and Stupp, R. Irinotecan-induced interstitial pneumonia. Lancet Oncology (2004) 5:322-324.

16. Movassagh, M., Spatz, A., Davoust, J., Lebecque, S., Romero, P., Pittet, M., Rimoldi, D., Liénard, D., Gugerli, O., Ferradini, L., Robert, C., Avril, M.-F., Zitvogel, L, and Angevin, E. Selective accumulation of mature DC-lamp+ dendritic cells in tumor sites is associated with efficient T-cell-mediated antitumor response and control of metastatic dissemination in melanoma. Cancer Research (2004) 64:2192-2198.

17. Nagorsen, D., Servis, C., Lévy, N., Provenzano, M., Dudley, M.E., Marincola, F.M. and Lévy, F. Proteasomal cleavage does not determine immunogenicity of gp100-derived peptides $gp100_{209\text{-}217}$ and $gp100_{209\text{-}217T210M}$. Cancer Immunology Immunotherapy (2004) 53:817-824.

18. Romero, P., Cerottini, J.-C. and Speiser, D.E. Monitoring tumor antigen specific T-cell responses in cancer patients and phase I clinical trials of peptide-based vaccination. Cancer Immunology Immunotherapy (2004) 53:249-255.

19. Rothenfusser, S., Hornung, V., Ayyoub, M., Britsch, S., Towarowski, A., Krug, A., Sarris, A., Lubenow, N., Speiser, D., Endres, S. and Hartmann, G. CpG-A and CpG-B oligonucleotides differentially enhance human peptide-specific primary and memory CD8+ T-cell responses in vitro. Blood (2004) 103:2162-2169.

20. Speiser, D.E., Pittet, M.J., Guillaume, P., Lubenow, N., Hoffman, E., Cerottini, J.-C. and Romero, P. *Ex vivo* analysis of human antigen-specific CD8+ T-cell responses: Quality assessment of fluorescent HLA-A2 multimer and interferon-gamma ELISPOT assays for patient immune monitoring. Journal of Immunotherapy (2004) 27:298-308.

21. Tacchini-Cottier, F., Allenbach, C., Otten, L.A. and Radtke F. Notch1 expression on T cells is not required for CD4+ T helper differentiation. European Journal of Immunology (2004) 34:1588-1596.

22. Tardivel, A., Tinel, A., Lens, S., Steiner, Q.-G., Sauberli, E., Wilson, A., Mackay, F., Rolink, A.G., Beermann, F., Tschopp, J. and Schneider, P. The anti-apoptotic factor Bcl-2 can functionally substitute for the B cell survival but not for the marginal zone B cell differentiation activity of BAFF. European Journal of Immunology (2004) 34:509-518.

23. Teixeiro, E., Daniels, M.A., Hausmann, B., Schrum, A.G., Naeher, D., Luescher, I., Thome, M., Bragado, R. and Palmer, E. T cell division and death are segregated by mutation of TCRβ chain constant domains. Immunity (2004) 21:515-526.

24. Walker, E.B., Haley, D., Miller, W., Floyd, K., Wisner, K.P., Sanjuan, N., Maecker, H., Romero, P., Hu, H.-M., Alvord, W.G., Smith II, J.W., Fox, B.A. and Urba, W.J. $gp100_{209\text{-}2M}$ peptide immunization of human lymphocyte antigen-A2+ stage I-III melanoma patients induces significant increase in antigen-specific effector and long-term memory CD8+ T cells. Clinical Cancer Research (2004) 10:668-680.

25. Wilson, A., Murphy, M.J., Oskarsson, T., Kaloulis, K., Bettess, M.D., Oser, G.M., Pasche, A.-C., Knabenhans, C., MacDonald, H.R. and Trumpp, A. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes & Development (2004) 18:2747-2763.

26. Yoon, K., Nery, S., Rutlin, M.L., Radtke, F., Fishell, G. and Gaiano, N. Fibroblast growth factor receptor signaling promotes radical glial identity and interacts with Notch1 signaling in telencephalic progenitors. Journal of Neurosciences (2004) 24:9497-9506.

27. Zippelius, A., Batard, P., Rubio-Godoy, V., Bioley, G., Liénard, D., Lejeune, F., Rimoldi, D., Guillaume, P., Meidenbauer, N., Mackensen, A., Rufer, N., Lubenow, N., Speiser, D., Cerottini, J.-C., Romero, P. and Pittet, M.J. Effector function of human tumor-specific CD8 T cells in melanoma lesions: A state of local functional tolerance. Cancer Research (2004) 64:2865-2873.

28. Zippelius, A., Bioley, G., Le Gal, F.-A., Rufer, N., Brandes, M., Batard, P., De Smedt, M., Plum, J., Speiser, D.E., Cerottini, J.-C., Dietrich, P.-Y., Romero, P. and Pittet, M.J. Human thymus exports naive CD8 T cells that can home to nonlymphoid tissues. Journal of Immunology (2004) 172:2773-2777.

29. Montjovent, M.-O., Burri, N., Mark, S., Federici, E., Scaletta, C., Zambelli, P.-Y., Hohlfeld, P., Leyvraz, P.-F., Applegate, L.L. and Pioletti, D.P. 2004. Fetal bone cells for tissue engineering. Bone 35:1323-1333.

30. Lahav, R., Suvà, M.-L., Rimoldi, D., Patterson, P.H. and Stamenkovic, I. 2004. Endothelin receptor B inhibition triggers apoptosis and enhances angiogenesis in melanomas. Cancer Res. 64:8945-8953.

Reviews / Commentaries / Book Chapters

29. Godfrey, D.I., MacDonald, H.R., Kronenberg, M., Smyth, M.J. and Van Kaer, L. NKT cells: What's in a name? Nature Reviews Immunology (2004) 4:231-237.

30. Lejeune, F.J., Matter, M., Guggisberg, D., Cerottini, J.-P. and Liénard, D. Prise en charge du mélanome malin cutané au stade de tumeur primaire. Medicine & Hygiene (2004) 2504:2256-2263.

31. Radtke, F., Wilson, A. and MacDonald, H.R. Notch signalling in T-and B-cell development. Current Opinions in Immunology (2004) 16:174-179.

32. Radtke, F., Wilson, A., Mancini, S.J.C. and MacDonald, H.R. Notch regulation of lymphocyte development and function. Nature Immunology (2004) 5:247-253.

LUDWIG INSTITUTE FOR CANCER RESEARCH

London St. Mary's Branch of Molecular Virology

London, England

Staff List

Farrell, Paul J *Director, Member*

Epstein-Barr Virus and Cell Growth Control Group

Farrell, Paul J *Member, Professor of Tumor Virology*
Crook, Timothy *Associate Clinical Investigator*
Spender, Lindsay *Assistant Investigator (until November)*
Bodelon, Gustavo *Postdoctoral Fellow (from July)*
Smith, Paul *Postdoctoral Fellow (from May)*
Syed, Nelofer *Technician*
Attard, Marlene *Technician*
Elgueta, Claudio *Technician*
Sullivan, Alex *Technician (until September)*
Al-Mozaini, Maha *Student*
Amon, Wolfgang *Student*
Lucchesi, Walter *Student (from November)*
Pattle, Samuel *Student*
Whiteman, Hannah *Student*

Tumor Suppressor Gene Group (until September)

Lu, Xin *Member, Professor of Cancer Biology*
Bergamaschi, Daniele *Postdoctoral Fellow*
Llanos, Susannah *Postdoctoral Fellow (until March)*
Samuels-Lev, Yardena *Postdoctoral Fellow (until May)*
Vives, Virginie *Postdoctoral Fellow*
Zhong, Shan *Senior Technician*
Ratnayaka, Indrikah *Technician*
Slee, Elizabeth *Technician*
Fogal, Valentina *Student*
Gillotin, Sebastien *Student*
Godin, Nadia *Student*
Su, Jian *Student*

Oncogene Group

Watson, Roger *Reader, Imperial College (BBSRC)*
Tavner, Fiona *Postdoctoral Fellow*
Pearce, Jacqueline *Student*

B Lymphocyte Biology and Epstein-Barr Virus Group

Allday, Martin *Professor, Imperial College, (Wellcome Trust, MRC)*
O'Nions, Jenny *Postdoctoral Fellow*
Sourisseau, Tony *Postdoctoral Fellow (until May)*
Spyer, Moira *Postdoctoral Fellow*
Parker, Gillian *Research Assistant*
Anderton, Emma *Student (from October)*
Leao, Maria *Student (until October)*
Young, Paul *Student*

Technical Support

Coulson, Lee *Laboratory Aide*
Zlobecki, Martin *Laboratory Aide (until September)*

Administration & Secretariat

Curtis, Julie *Administrator*
De Felice, John *Laboratory Manager*
Sabina Jankowska *Secretary*

Branch Director's Report

The London St Mary's Branch studies the molecular mechanisms that give rise to cancer and the role of viruses, particularly Epstein-Barr virus (EBV), in human cancer. The cell biology groups investigate the regulation and functions of key proteins involved in control of the cell cycle and apoptosis. The virology program at the St Mary's Branch seeks to understand the mechanisms by which EBV causes cell proliferation and the role of the virus in cancer. In the longer term, we hope to take advantage of this knowledge to develop new diagnostic procedures or cancer therapies based on the molecular mechanisms we have discovered or the presence of EBV in tumor cells.

In our EBV research, we are identifying the key cell genes and cell cycle controls that are regulated by the virus as it causes cell proliferation. The regulation of the Runx3 tumor suppressor gene by EBNA-2 and the control of CDK2 activity by EBNA-3C have been investigated. The molecular biology of the p53 tumor suppressor protein, its family members p63 and p73 and their regulation by the ASPP proteins have also seen substantial progress. Identification of the full length iASPP gene structure has revealed an additional level of regulation and the complexities of specific phosphorylation controls of p53 and the p27 cyclin dependent kinase inhibitor have been clarified. Our studies of the regulation and function of the B-Myb proto-oncogene have also progressed through our creation of specific knock in mice with targeted mutations in the promoter that allow determination of the in vivo properties of this gene.

Members of the London St Mary's Branch have also coordinated two of the LICR programs, that provide support to the Institute worldwide. The Microarray Consortium conducted jointly with the Wellcome Trust Sanger Institute and Cancer Research UK has been extended to the end of 2005; it provides human and mouse gene expression profiling microarrays to several Branches. The Xi'an Monoclonal Antibody Facility at the 4th Military Medical School in Xi'an, China, continues to produce high quality monoclonal antibodies for many LICR Branches.

This year has seen major changes in the London St Mary's Branch. Dr. Xin Lu was promoted to Director of the London University College Branch and her group moved there in September. At the end of the year, Dr Tim Crook and some of his colleagues moved to Breakthrough Breast Cancer Research at the Institute for Cancer Research in London, where Dr Crook will establish a new research group. These are prestigious appointments and we congratulate them both on their success. In view of the close relationship of the remaining research with the adjacent Department of Virology of Imperial College Faculty of Medicine, it has therefore been decided to discontinue the London St Mary's Branch in 2005. The Epstein-Barr Virus and Cell Growth Control Group will continue as an LICR Affiliate Center within the Department of Virology, Imperial College (UK).

In its 18 years of operation the London St Mary's Branch made outstanding advances in the fields of molecular virology, tumor suppressor gene and oncogene research. Some of these discoveries will have direct applications in medical science. The Branch also trained 50 graduate students and many of the students, post-docs and group leaders have gone on to senior research positions. It has been successful and a truly enjoyable place to do science.

Paul J. Farrell

Research Report

Epstein-Barr Virus and Cell Growth Control Group

Dr Paul Farrell's Group investigates the functions of EBV genes expressed in human cancers associated with EBV and the control of viral latency and reactivation. The purpose is to identify the basis for novel approaches to eliminate cells infected by EBV, particularly the tumor cells of EBV-associated cancers.

EBV infection of human B lymphocytes causes cell proliferation. In B lymphocytes, the viral EBNA2 transcription factor activates cell gene expression, and the group has identified some of the important target cell genes for EBNA2, such as c-Myc, RUNX3 and a PI3 kinase regulatory subunit. The Group identified a new mechanism of regulatory interaction, such that RUNX3 regulates RUNX1 expression, contributing to the mutually exclusive expression of RUNX3 and RUNX1 in human B lymphoid cell lines. RUNX3 repressed the RUNX1 P1 promoter by binding specifically to conserved RUNX sites near the transcription start of the promoter. 'Small interfering RNA' (siRNA) inhibition of RUNX3 in lymphoblastoid cells resulted in increased RUNX1 expression, indicating that continuous expression of physiological levels of RUNX3 is required to maintain repression. Furthermore expression of RUNX3 was required for efficient proliferation of B cells immortalised by EBV. Cross-regulation between different RUNX family members is therefore a means of controlling RUNX protein expression and must now be considered in the interpretation of pathological changes due to loss of RUNX3 tumor suppressor function, or following gene duplication or translocation events.

The group is also studying viral genes expressed in cancers such as the EBER RNAs and BART genes in order to determine their function. The latent viral infection present in the lymphocytes and EBV-associated cancers can reactivate to the productive virus cycle in which infectious virus particles are secreted. Episomal reporter plasmids containing the EBV *ori*P sequence and stably transfected into Akata Burkitt's lymphoma cells have been used to analyse EBV lytic cycle gene regulation. First, the Group found that the Zp promoter, but not the Rp promoter, of EBV can be activated in the absence of protein synthesis in these *oriP* plasmids, casting doubt on the immediate early status of Rp. An additional level of regulation of Zp was implied by analysis of a mutation of the ZV element. Secondly, the analyses of late lytic cycle promoters revealed that the correct relative timing, dependence on ori lyt in *cis*, and sensitivity to inhibitors of DNA replication was reconstituted on the *oriP* plasmids. Late promoter luciferase activity from *oriP* plasmids also incorporating a replication-competent ori lyt was PAA-sensitive, a hallmark of EBV late genes. A minimal ori lyt, which only replicates weakly, was sufficient to confer late timing of expression specifically on late promoters. Finally, deletion analysis of EBV late promoter sequences upstream of the transcription start site confirmed that sequences between –49 and +30 are sufficient for late gene expression, which is dependent on ori lyt in *cis*. However, the TATT version of the TATA box found in many late genes was not essential for late expression.

Dr Tim Crook's group is attempting to link molecular changes detectable in human cancer samples to clinical outcome. Molecular genetic markers that would predict the outcome of cancer therapy would be very valuable in targeting treatment to those patients who will respond. They might also suggest trials of treatments that would be better for subgroups of patients, but cannot presently be used because they are not suitable for all patients. The EBV Group previously found a relationship between a polymorphism at codon 72 of the p53 tumor suppressor gene (72P) and outcome of chemotherapy in head and neck cancer in tumors where the p53 had a cancer related mutation. The Group has now investigated how the same polymorphism in wild-type p53 (72R) modulates response to chemotherapy *in vitro* and *in vivo*. In inducible systems, and in cells expressing the endogenous protein, expression of 72P wild-type p53 results in a predominant G1 arrest, with only minor apoptosis, at drug concentrations causing extensive apoptosis in cells expressing the 72R wild-type variant. The superior apoptosis-inducing activity of the 72R form correlates with more efficient induction of specific apoptosis-associated genes, and is maximal in the presence of serine 46. *In vivo*, the outcome of chemo-radiotherapy of squamous carcinomas is more favourable in cancers retaining a wild-type 72R allele, such cases having higher response rates and longer survival than those with wild-type 72P. Together, these results reveal that this single nucleotide polymorphism (SNP) is an important determinant of response to anticancer agents in cells expressing wild-type p53.

B lymphocyte Biology and Epstein-Barr Virus Group

Dr Martin Allday's Group has continued to investigate the deregulation of cell cycle checkpoints by latent EBV infection. EBV acts downstream of p53 to over-ride the p53/pRb-mediated G1 checkpoint activated by genotoxic stress. It appears that EBV either targets p21WAF1 or Cdk2 and the Group is currently trying to establish which EBV gene product is responsible and by what mechanism. A surprising discovery was that suppression of this checkpoint is dependent on the type of DNA damage sustained by B cells. In normal B cells driven to proliferate by T cell-derived mitogens, p21WAF1 protein accumulates after treatment with both cross-linking agents (cisplatin and melphalan) and agents that produce double-strand breaks (γ gamma-irradation and bleomycin). However in B cells driven to proliferate by EBV, although p21WAF1 levels increase after double strand breaks (DSBs), cross-linking agents fail to stimulate an increase in p21WAF1. EBV apparently only modifies the response to certain types of DNA damage (cross-links). The basis for this differential response and the possible role of DNA-repair mechanisms is currently being investigated.

In addition to targeting a G1 checkpoint by acting downstream of p53 and upstream of pRb, recent data obtained by the Group suggest that EBV can also disrupt a mitotic checkpoint. Latent infection of Burkitt's lymphoma-derived cells with either B95-8 or P3HR1 strains of EBV is associated with failure of the cells to arrest in metaphase after treatment with nocodazole. This indicates that EBV interferes with the regulation of the mitotic spindle checkpoint. Since previously showing that over-expression of the latent nuclear antigen EBNA3C alone also suppresses the mitotic spindle checkpoint, the Group is now focusing on this function of EBNA3C. The interaction between EBNA3C and its relative EBNA3A with the cellular co-repressor of transcription C-terminal binding protein (CtBP) is also being studied. The interaction of both these proteins with CtBP is necessary for their ability to act as oncogenes in cooperation with Ha-Ras. The Group recently discovered that CtBP also binds to Meq, the nuclear antigen of another oncogenic herpesvirus Marek's Disease Virus (MDV). In collaboration with Dr Venugopal Nair (Institute of Animal Health, Compton) it was shown that the Meq-CtBP interaction is necessary for tumor induction in chickens by MDV. The role of CtBP in the oncogenic behaviour of these two unrelated tumor-associated herpesviruses is now a major interest of the Group.

Tumor Suppressor Gene Group

The objective of this Group, led by Dr. Xin Lu, is to develop new strategies to suppress tumor growth. In particular, the continuation of its studies on the regulation of p53 mediated apoptosis by E2F and the ASPP family of proteins.

ASPP1 and ASPP2 interact with p53 and enhance its ability to induce apoptosis by selectively elevating the expression of pro-apoptotic p53-responsive genes. The DNA binding and transactivation function of p53 are required for ASPP1 and ASPP2 to stimulate the apoptotic functions of p53. Mdm2 and mdmx, two inhibitors of p53, prevent ASPP1 and ASPP2 from stimulating the apoptotic function of p53 by binding and inhibiting the transcriptional activity of p53. Importantly, mdm2 and mdmx can prevent the stimulatory effects of ASPP1 and ASPP2 without targeting p53 for degradation. Additionally, ASPP1 and ASPP2 induce apoptosis independently of p53. By binding to p63 and p73 in vitro and in vivo, ASPP1 and ASPP2 stimulate the transactivation and apoptotic function of p63 and p73. Hence ASPP1 and ASPP2 are the first two identified common activators of all p53 family members suggesting that ASPP1 and ASPP2 could suppress tumor growth even in tumors expressing mutant p53.

In addition to p53, the tumor suppressor Rb pathway is frequently inactivated in human cancer. This often results in an increase in the transcriptional activity of the E2F family of transcription factors that regulates the expression of a number of genes whose products are involved in cell cycle control, DNA replication and apoptosis. Interestingly, E2F-1 binds *in vivo* the promoters of ASPP1 and ASPP2 genes, and E2F-1, 2 and 3 all activate the isolated ASPP1 and 2 promoters. Over-expression of E2F-1 increased the expression levels of ASPP1 and ASPP2 mRNA and proteins. The identification of ASPP1 and ASPP2 genes as transcriptional targets of E2F provides another mechanism by which E2F co-operates with p53 to induce apoptosis.

The third member of the ASPP family is iASPP, which binds and inhibits the apoptotic function of p53. iASPP is also the most conserved member of the ASPP family. The group's recent identification of a longer form of iASPP(RAII), which at 828 amino acids is more than twice the size of the iASPP(RAI) protein originally reported, provided a novel insight into the regulation of iASPP function. Whereas the shorter iASPP(RAI) is predominantly nuclear, if expressed separately, the N-terminus of iASPP is entirely cytoplasmic. The longer 828 amino acid iASPP is located in both the cytoplasm and the nucleus. This more complex subcellular localisation of the longer iASPP protein means that it may be subject to greater regulation and provides another layer in the control of p53-induced apoptosis.

Despite the wealth of information on the regulation of wild type p53 function by phosphorylation, little is known about the biological effect of phosphorylation on mutant p53. The Group has shown that p53H175 is phosphorylated like wild-type p53 in cells of the same background. Rat embryo fibroblasts were more potently transformed by Ser392 non-phosphorylatable forms of p53 mutants, p53H175A392 and p53W248A392 in co-operation with the ras oncogene, than by p53H175S392 and p53W248S392. p53H175A392 also had an enhanced ability to confer cellular resistance to the cytotoxic effect of cisplatin and UV. Ser392 unphosphorylated p53 was present in human breast tumors expressing mutant p53 including p53H175. The results demonstrated a novel function of Ser392 phosphorylation in regulating the oncogenic function of mutant p53.

Finally, using two dimensional gel electrophoresis analysis, the group observed that cell cycle entry is associated with a significant increase in $p27^{kip1}$ phosphorylation in human primary B cells. Different cyclins and cdks interact with distinct post-translationally modified isoforms of $p27^{kip1}$ *in vivo*. Cyclin E but not cyclin A selectively interacts with phosphorylated $p27^{kip1}$ isoforms while cyclin D1 and D2 favour unphosphorylated $p27^{kip1}$ isoforms *in vivo*. Interestingly, cyclin D3 and

cdk4 selectively interact with phosphorylated $p27^{kip1}$ in BL40 cells. Among all D-type cyclin/cdk4 and cdk6 complexes, Cyclin D3/cdk4 is most active in sequestering the inhibitory activity of $p27^{kip1}$ *in vitro* in a cyclinE/cdk2 kinase assay. This novel feature of the binding specificity of $p27^{kip1}$ to cyclins and cdks *in vivo* is relevant to the over-expression of cyclin D3 in the presence of high levels of $p27^{kip1}$ observed in human B cell lymphomas with adverse clinical outcome.

Oncogene Group

Dr Roger Watson's Group continued its study of cell cycle transcriptional regulation. One focus of this work is the B-Myb transcription factor, whose gene is transcriptionally regulated during the cell cycle by E2F transcription factor activity. Notably, B-Myb is required for early embryonic development and itself is implicated in regulating cell proliferation and differentiation. By targeting mouse embryonic stem (ES) cells, the Group knocked-in a mutation of the E2F regulatory element within the B-myb promoter. Heterozygous and homozygous mice carrying this mutation have subsequently been generated. *B-myb* expression was found to be deregulated in mouse embryo fibroblasts carrying the mutation, and the effects of this on mouse development are currently being studied. The Group is also using this system to study regulation of this promoter by E2F/pRb complexes during the cell cycle. As a by-product of this work, mice carrying a hypomorphic allele of B-myb have been obtained. Consistent with gene knockout experiments, heterozygous mice are fully viable, but there is a pronounced deficiency during development of homozygotes. Study of this phenotype should further illuminate the role of B-Myb during embryonic development.

Publications

Primary Research Articles

1. Amon W., Binné U.K., Bryant H., Jenkins P.J., Elgueta Karstegl C., Farrell P.J. Lytic cycle gene regulation of Epstein-Barr virus. Journal of Virology (2004) 78:13460-13469.
2. Bergamaschi D., Samuels Y., Jin B., Duraisingham S., Crook T., Lu X. ASPP1 and ASPP2 are common activators of the p53 family members p63 and p73. Molecular and Cellular Biology (2004) 24:1341-1350.
3. Brown V.L., Harwood C.A., Crook T., Kelsell D.P., Proby C.M. p16INK4a and p14ARF tumour suppressor genes are commonly inactivated in cutaneous squamous cell carcinoma. Journal of Investigative Dermatology (2004) 122:1284-1292.
4. O'Nions, J., Allday M.J. Proliferation and differentiation in isogenic populations of peripheral B cells activated by Epstein-Barr virus or T cell-derived mitogens. Journal of General Virology (2004) 85: 881-895.
5. Sullivan A., Syed N., Gasco M., Bergamaschi D., Trigiante G., Attard M., Hiller L., Farrell P.J., Smith P., Lu X., Crook T. Polymorphism in wild-type p53 modulates response to chemotherapy in vitro and in vivo. Oncogene (2004) 23:3328-3337.
6. Yap D.B.S., Hsieh J-K., Zhong S., Heath V., Gusterson B., Crook T., Lu X. Ser392 phosphorylation regulates the oncogenic function of mutant p53. Cancer Research (2004) 64: 4749-4754.

Reviews / Commentaries / Book Chapters

1. O'Nions J., Allday M.J. Deregulation of the cell cycle by Epstein-Barr virus. Advances in Cancer Research 92: 119-178, 2004. , Elsevier Publishers, (eds. Klein and Vande Woude)
2. Slee E., O'Connor D., Lu X. To die or not to die: how does p53 decide? Oncogene (2004) 23:2809-2818.
3. Watson R.J. B-Myb: a highly regulated member of the Myb transcription factor family. in "Myb Transcription Factors: Their Role in Growth, Differentiation and Disease" Frampton, Jon (Ed.), Kluwer Academic Publishers, Dordrecht, The Netherlands, pp181-200, 2004.

London University College Branch
of Cell and Molecular Biology

London, England

Staff List

Waterfield, Michael D. *Member, Director (until September)*
Lu, Xin Member, *Director (from September)*

Bioinformatics and Computational Biology Laboratory

Zvelebil, Marketa *Senior Investigator*
Bursteinsas, Borisas *Postdoctoral Fellow (from August)*
Ng, Aylwin *Postdoctoral Fellow (from September)*
Lykostratis, Kostas *Graduate Student*
Sims, David *Graduate Student (from August)*
Gao Qiong, Alice Scientific Officer
Mollison, Ewan *Scientific Officer (from September)*

Breast Cancer Laboratory

O'Hare, Mike *Group Leader*
Fulford, Laura *Clinical Fellow*
Kim, Jong-Bin *Graduate Student*
Grigoriadis, Anita *Scientific Officer*

Cell Shape and Polarity Laboratory

Baum, Buzz *Assistant Member*
Craig, Gavin *Postdoctoral Fellow*
Duchek, Peter *Postdoctoral Fellow*
Kunda, Patricia *Postdoctoral Fellow*
Sims, David *Graduate Student*
Dominguez, Veronica *Scientific Officer (until November)*
Liu, Tao *Scientific Officer (from November)*

Cell Signalling Laboratory

Vanhaesebroeck, Bart *Assistant Member*
Bilancio, Antonio *Postdoctoral Fellow*
Billottet, Clotilde *Postdoctoral Fellow (from April)*
Cutillas, Pedro *Postdoctoral Fellow*
Foukas Lazaros *Postdoctoral Fellow*
Graupera, Mariona *Postdoctoral Fellow*
Osborne, Nicola *Postdoctoral Fellow (from April)*
Papakonstanti, Evangelia *Postdoctoral Fellow*
Ali, Khaled *Graduate Student*
Geering, Barbara *Graduate Student*
Nock, Gemma *Graduate Student (from October)*
Kok, Klaartje *Visiting Graduate Student (from March)*
Woods, Lizzie *Graduate Student*
Pearce, Wayne *Scientific Officer*
Peskett, Emma *Scientific Officer*

Cellular and Molecular Biology Laboratory

Ridley, Anne *Member*
Entwistle, Alan *Research Associate*

Cernuda-Morollon, Eva *Postdoctoral Fellow*
Heasman, Sarah *Postdoctoral Fellow (from October)*
Ivetic, Alex *Postdoctoral Fellow*
Millan, Jaime *Postdoctoral Fellow*
Pajari, Anne-Maria, *Postdoctoral Fellow (from August)*
McKenzie, Jenny *Postdoctoral Fellow (from October)*
Reymond, Nicolas *Postodoctoral Fellow (from October)*
Riento, Kirsi *Postdoctoral Fellow*
Takesono, Aya *Postdoctoral Fellow*
Villalonga, Priam *Postdoctoral Fellow*
Garg, Ritu *Postdoctoral Fellow*
Bhavsar, Parag *Postdoctoral Fellow*
Bright, Michael *Graduate Student (from October)*
Christoloudou, Maria *Graduate Student*
Gruenewald, Jana *Gruaduate Student (from October)*
McKenzie, Jenny *Graduate Student (until July)*
Smith, Stephen *Graduate Student*
Wheeler, Ann *Graduate Student*

Drosophila Morphogenesis Laboratory

Barrett, Kathy *Assistant Member*
Gardano, Laura *Postdoctoral Fellow*
Nikolaidou, Kelly *Postdoctoral Fellow*
Escott, Gareth *Graduate student*
Hiley, Charly *Graduate student*
Kitsou, Effie, *Graduate student*
Batchelor, Emily *Scientific Officer*

Proteomics Laboratory

Naaby-Hansen, Soren *Assistant Member*
Warnasuriya, Gayathri *Postdoctoral Fellow (until March)*
Hastie, Claire *Graduate Student (until September)*

Structural Biology Laboratory (until September)

Driscoll, Paul *Associate Member*
Vines, David *Postdoctoral Fellow*
Sankar, Andrew *Postdoctoral Fellow*

Tumor Suppresor Group (from September)

Lu, Xin *Director, Member*
Bergamaschi, Daniele *Postdoctoral Fellow*
Vives, Virginie *Postdoctoral Fellow*
Zhang, Wenqing *Postdoctoral Fellow*
Breyssens, Hilde *Graduate Student (from October)*
Gillotin, Sebastien, *Graduate Student*
Royer, Christophe, *Graduate Student*
Sottocorlona, Roberta *Graduate Student (from October)*
Su, Jian *Graduate Student*
Ratnayuka, Indrika *Scientific Officer*

Slee, Elizabeth *Scientific Officer*
Sullivan, Alex *Research Associate*
Zhong, Shan *Higher Scientific Officer*

Proteomics Section Cruciform Annex

Waterfield, Michael *Director, Member*

Bioanalytical Chemistry Laboratory

Cramer, Rainer *Assistant Member*
Thompson, Andrew *Postdoctoral Fellow (until August)*
Gerrits, Albert *Graduate Student*
Jacob, Richard *Graduate Student*
Cutillas, Pedro *Graduate Student (until April)*
Vilasi, Annalisa *Graduate Student (until March)*
Calnan, Denis *Higher Scientific Officer*
Corless, Steve *Higher Scientific Officer*
Saxton, Malcolm *Scientific Officer (until August)*

Cancer Biochemistry Laboratory

Timms, John *Assistant Member*
Weeks, Mark *Postdoctoral Fellow*
Bertani, Mariana *Graduate Student*
Chan, Hong-Lin *Graduate Student*
Jovceva, Eleonora *Graduate Student*
Sinclair, John *Scientific Officer*

Cancer Translational Proteomics Laboratory

Waterfield, Michael *Member, Head of Proteomics Unit*
Barnouin, Karin *Postdoctoral Fellow (until June)*
Gharbi, Severine *Postdoctoral Fellow*
Akpan, Akunna *Laboratory Manager*

Technical Support

Scrace, Geoff *Research Associate*
Mistry, Kay *Laboratory Manager*
Williams, Simon *Assistant Scientific Officer*
Garady, Adam *Security (until July)*
McCreadie, James *Security*
Zlobecki, Martin *Security (from September)*

Administration & Secretariat

Anning, Richard *Administrator*
Emakpose, Loretta *Administrative Assistant*
Ward, Alex *Administrative Assistant*
Stone, Deborah *Director's Assistant*
Barnsley, Sarah *Scientific Administrator*

Branch Director's Report

During 2004, the directorship of the Branch was handed over from Dr. Michael D. Waterfield, the Director for the last 18 years, to Dr. Xin Lu. The research focus of the Groups in the Branch, during Dr. Waterfield's tenure, was cell signal transduction in cell growth, proliferation, death and migration. Many significant basic and applied advances in cancer research were made during this time.

Progress in cell signaling made by the individual research Groups during 2004 is summarized in each Group's research reports. The importance of some of these discoveries attracted worldwide recognition. Of particular note were the awards to Branch Group Leaders Dr. Anne Ridley, who received the Lillian Bettencourt Life Sciences Award prize for her work in cell biology, and Dr. Buzz Baum, who was inducted into the EMBO YIP (EMBO Young Investigator Programme).

The appointment of Dr. Xin Lu as the new Director of the UCL Branch represents a great opportunity to extend the Branch's research interests into the area of tumor suppression as an extension of her special interest in tumor suppressor genes including p53. The addition of this new area of expertise will ensure a future focus of the Branch on the identification of molecular targets and new strategies to suppress tumor growth and metastasis. This will be achieved through focusing studies on the interactions in signaling pathways that are involved in tumor development, tumor suppression, cell migration and metastasis.

Mike Waterfield and Xin Lu

Research Report

Bioinformatics and Computational Biology Group

The Bioinformatics and Computational Biology Group, led by Dr Marketa Zvelebil, has changed focus in the last year. From mainly being involved in analysing proteomics data and designing a LIMS database, which is now complete, the group has switched focus to designing and constructing a European Union (EU) grant-funded Knowledge Base. The Group is designing three Knowledge Bases with specific expertise; 'FlyNet' for *Drosophila* information, in collaboration with Dr. Buzz Baum's Group; 'CAIN' specific for cancer, in collaboration with Dr. Anne Ridley's Group; and 'MAIN', specific for inflammation. The databases will be used to write programs to assist in the analysis and understanding of novel experimental data. These Knowledge Bases will be linked to a main global LICR database called 'BRAIN' (Bioinformatics Resource for the Analysis of Information on Neoplasia), which is being constructed in collaboration with other LICR Branches, the LICR Office of Information Technology, James R. Kerr Program Investigators and LICR Affiliate Centers.

A large part of the Group's research is now concentrated on delineating specific protein-protein interactions, the interactions between proteins and other non-protein compounds (e.g. lipids and small molecules) and demarcating specific pathways, such as the integrin signaling pathways and the chemokine-regulated migration pathways. The Group is also building quantitative mathematical models for these pathways, which will be refined iteratively alongside experimental data. From this, the Group can perturb the model to simulate changes in the dynamics reflecting the different patho-physiological states. This provides a dynamic picture of the processes involved in the specific pathways and can be used to predict the system-wide effects of either activation or inhibition of any proteins, or components, involved in the pathway.

In addition to this, the Group continues to develop novel analytical programs for data such as MPSS and for the analysis and correlation of multivariate data from different experiments. There is also a strong and successful ongoing research programme into three-dimensional modelling of protein structure and ligand interaction.

Cell Shape and Polarity Group

The Cell Shape and Polarity laboratory, led by Dr. Buzz Baum, is using *Drosophila* genetics and double stranded (ds) RNA-mediated interference (RNAi) to study the role of the actin cytoskeleton in cell and tissue morphogenesis. Ultimately, this work aims to shed light on the molecular events underlying the loss of tissue organization and deregulation of cytoskeleton dynamics during tumorigenesis and metastasis.

Much of the work in the lab relies on the use of RNAi in *Drosophila* cell culture. The Group helped pioneer this technology and, since then, has used it to screen the entire *Drosophila* genome for genes regulating actin cytoskeletal organisation and cell shape. In the last year, this approach has identified many novel, conserved actin regulators whose function is being actively characterized in motile and non-motile *Drosophila* cells and, in collaboration with Dr. Anne Ridley, in mammalian cell culture models of metastasis. To make sense of these enormous phenotypic data sets, a database has been designed in collaboration with the Bioinformatics and Computational Biology Group, which enables users to browse though images of ds RNA-treated cells and to group genes based on RNAi phenotype, expression profile and protein interactions. By cross-correlating data in this way, the Group hopes to define the distinguishing molecular features that determine whether or not a given cell will migrate.

Over the last year the Group has also set up a unique system in which to study the cell biological roles of genes identified in RNAi screens in a tissue and developmental context. Importantly, using an epithelial tissue within the *Drosophila* pupae, changes in cellular dynamics and epithelial organization can be followed in adjacent clones of mutant and wild-type tissue at high resolution in the living animal. In the coming year, the Group will use this system to study the molecular and cellular events that underlie remodeling of cell junctions and the ECM during tumor cell metastasis in the fly.

Cell Signaling Group

The Cell Signaling Group, led by Dr Bart Vanhaesebroeck, aims to understand the roles and mechanism of action of the phosphatidylinositol 3-kinase (PI3K) isoforms that signal downstream of tyrosine kinases and Ras.

PI3Ks control a variety of biological responses in normal cells and, together with the p53 pathway, is one of the most frequently deregulated signaling pathways in cancer. Mammals have eight distinct isoforms of PI3K, the roles of which are largely unknown. Therapeutic intervention with PI3K signaling will have to be targeted at selected isoforms, given that global inhibition of all PI3Ks by small molecule inhibitors is expected to result in excessive toxicity to normal tissues, many of which are also dependent on this pathway.

Work in the Cell Signaling Group combines cell biological, pharmacological and biochemical approaches with studies at the organism level, using the mouse as a model system. More recently, the Group has also started to integrate primary human cancer samples in their analyses (in collaboration with Dr. Asim Khwaja, Department of Haematology, University College London).

In 2004, the Group continued to focus on the p110δ isoform of PI3K, which was isolated at the Branch several years ago. The p110δ was identified as a key target for therapeutic intervention in inflammation and allergy. Phenotypic characterization of mice targeted in other isoforms of PI3K has also progressed well, with new phenotypes highly relevant to cancer being uncovered. The

Group's observations indicate that PI3K isoform function is specific and gene dosage-dependent, and cannot be readily compensated by other PI3K isoforms. This work has also revealed that receptors and signaling pathways in untransformed cells often critically dependent on just one isoform of PI3K. This is a remarkable and unexpected finding, and the Group is in the process of investigating whether this also applies to oncogenes that can utilize PI3K activity to promote malignant transformation. Cells derived from these gene-targeted mice, in combination with pharmacological approaches, are further used to dissect the molecular mechanisms of isoform-specific signaling by PI3K enzymes.

Cellular and Molecular Biology Group

The Cellular and Molecular Biology Group, led by Dr. Anne Ridley, is focused on investigating the intracellular signaling pathways underlying cell migration, which are important for the invasion and metastasis of tumor cells, in angiogenesis, and for leukocyte recruitment to tumors.

The Group's research is concentrated on signaling by the Rho family of GTPases, which are key regulators of cell adhesion and migration, using a combination of cell and molecular biological approaches. The Group found that the Rho family member RhoE, whose levels are altered in a variety of human tumors, not only regulates the actin cytoskeleton and thereby enhances cell migration, but also independently inhibits cell cycle progression and Ras-induced cell transformation.

Work on the cell adhesion protein p120ctn has revealed that it activates the Rho GTPase Rac and affects both the actin and microtubule cytoskeletons to regulate cell shape. In addition, the Group has begun to investigate the roles of closely related Rho GTPase isoforms in cell migration, for example showing that macrophages express more Rac1 than Rac2, yet Rac1 deletion does not impair migration.

Together, this work expands the understanding of the multiple functions of individual Rho GTPases and their associated signaling proteins in cells.

Drosophila Morphogenesis

The Drosophila Morphogenesis Group, led by Dr Kathy Barrett, has been continuing to focus its research into the control of cell shape and motility.

The control of cell shape and motility is of prime importance in development, resistance to disease, and tumorigenesis. The mechanisms used in this control include tight regulation of the actin and microtubule cytoskeleton. There is increasing evidence that all aspects of cytoskeletal regulation involve Rho GTPase based signal transduction pathways. In the last decade, research into these signaling pathways has progressed into *in vivo* models, including mouse, *C. elegans,* and *Drosophila*. Using these models and recent advances in microscopy, it has become possible to visualize in real time changes in cell shape and migration. This has dramatically increased the understanding of the role of Rho GTPase signaling in these processes. The exact mechanisms for this type of control, however, are still unknown. The Group used the powerful genetics of *Drosophila* together with *in vivo* real time imaging to elucidate the role of Rho GTPase signaling in the regulation of cell shape and motility.

Using live imaging in *Drosophila* the Group has identified some of the molecules involved in specific aspects of cell shape change and has demonstrated that these molecules are used reiteratively for this particular alteration in cell shape. In addition, the Group has contributed significantly to answering a major question in the field relating to the means by which individual Rho proteins are able to distinguish between the multitude of different outcomes of their activation. A collaboration with Dr Mark Miodownik (King's College, London) is continuing in a pioneering study to model mathematically the cell shape changes involved in development. The

Group has successfully concluded the initial stages of the model-building and will begin to test the model over the next year. These studies will incorporate the effect of mutations in the genes that control morphogenesis and provide unique insights into morphogenetic mechanisms.

Structural Biology Group

The Structural Biology Group, led by Dr Paul Driscoll, continued to explore the interaction of the death domains of the tumor necrosis factor (TNF) superfamily receptor Fas and the adaptor protein FADD. For the first time the Group was able to generate a nuclear magnetic resonance (NMR)-tractable form of the wild-type Fas death domain and study its interaction with the FADD death domain by NMR and other physical methods. It was demonstrated that the two small proteins combine to make a large 'aggregate' complex that is soluble, but only partially visible by NMR. This behavior was found to be common to many single residue mutant Fas death domain constructs.

As part of the effort to establish whether this 'aggregation' represents a physiologically relevant event, the Group has established a collaboration with Dr Huseyin Mehmet (Imperial College, London) in which wild-type and mutant forms of the full length Fas receptor were transfected into a cell line previously unresponsive to the agonist Fas-ligand. The apoptotic response of a subset of cell-lines engineered to express variants of the Fas receptors appears to correlate with *in vitro* Fas-DD/FADD-DD binding behavior detected by NMR, suggesting that what is observed by NMR may not represent a non-specific phenomenon, and may correlate with the suggestion that Fas receptor signaling requires high order oligomerization. Further work is required to establish the stoichiometry of the complexes observed by NMR, as well as the overall size and concentration dependence, for which quantitative dynamic light scattering and translation diffusion measurements monitored as a function of ionic strength and buffer osmolarity, are underway.

In other work, the Group has obtained crystals and a preliminary 3D structure of the Phox homology (PH) domain of a class II PI3K. Additionally, as a result of developing a factorial screening system for the refolding of disulphide-containing protein domainsm, the Group has solved the three-dimensional solution structure of the N-terminal scavenger receptor cysteine-rich (SRCR) of the T- and B-cell antigen CD5.

Tumor Suppression Group

The focus of the Tumor Suppression Group, led by Dr. Xin Lu, is to develop new strategies to suppress tumor growth. In particular, the laboratory continued their studies on the regulation of p53-mediated apoptosis by E2F and the ASPP family of proteins.

Three members of the ASPP family have been identified: ASPP1, ASPP2 and iASPP. ASPP1 and ASPP2 interact with p53 and enhance its ability to induce apoptosis. Mdm2 and mdmx, two inhibitors of p53, prevent ASPP1 and ASPP2 from stimulating the apoptotic function of p53. Additionally, ASPP1 and ASPP2 induce apoptosis independently of p53 by binding to p63 and p73 *in vitro* and *in vivo*. For this reason, ASPP1 and ASPP2 are the first two identified common activators of all p53 family members, suggesting that ASPP1 and ASPP2 could suppress tumor growth even in tumors expressing mutant p53. The third member of the ASPP family, iASPP, which binds and inhibits the apoptotic function of p53, is also the most conserved member of the ASPP family.

In addition to p53, the tumor suppressor Rb pathway is frequently inactivated in human cancer. This often results in an increase in the transcriptional activity of the E2F family of transcription factors that regulates the expression of a number of genes whose products are involved in cell cycle control, DNA replication and apoptosis. Overexpression of E2F-1 increased the expression levels of ASPP1 and ASPP2 mRNA and proteins. The identification of ASPP1 and ASPP2 genes as

transcriptional targets of E2F provides another mechanism by which E2F co-operates with p53 to induce apoptosis.

Despite a wealth of information on the regulation of wild type p53 function by phosphorylation, nothing is known about the biological effect of phosphorylation on mutant p53. However, it was recently demonstrated that a novel function of Ser392 phosphorylation in regulating the oncogenic function of mutant p53.

Bioanalytical Chemistry Group

The Bioanalytical Chemistry Group, led by Dr. Rainer Cramer, made significant progress in the development of new methodologies in mass spectrometry (MS). The novel chemical peptide derivatization strategies were further developed and partially tested on complex proteome samples. Although more work is required, the initial data show enormous potential for automated high-sensitivity analysis of entire proteomes and has already led to some early publications and an award.

The novel liquid UV-MALDI methodologies and protocols, which have been recently developed, also promise exciting new improvements in biological mass spectrometry. Using a liquid matrix instead of the conventional solid matrices means that some of the fundamental properties of MALDI inherently change. Thus, using new protocols, many areas of MALDI-MS analysis potentially benefit from this fundamental change in the MALDI methodology. These areas include quantification, on-line reaction monitoring, ion flux stability, automation of MALDI data acquisition and mining, analytical versatility and LC coupling to MALDI-TOF-MS.

The Group also started work on developing methods for large-scale population screening for better cancer diagnostics using proteomic patterns by MALDI-MS of human blood serum. The ideas behind the work have attracted essential funds from the Medical Research Council of the UK to continue the evaluation of protocols and techniques for a successful ovarian cancer screening test.

Cancer Proteomics Group

The Cancer Proteomics Group, led by Dr John Timms, is focused on elucidating the mechanisms of cancer cell signaling and identifying cancer-specific markers using quantitative proteomics and microarray technology. An ongoing focus is the functional dissection of the ErbB receptor tyrosine kinase signaling network, particularly the role played by ErbB2 in promoting tumor progression. The Group has used parallel proteomic and differential mRNA expression analysis in a model cell system to show how specific genes are regulated downstream of ErbB2 and in response to specific ErbB ligands. One novel finding of this work is the down-regulation of interferon-dependent signaling by ErbB2 to promote cellular proliferation. The Group is now extending this work by examining proteomic changes associated with ErbB2 overexpression and metastatic state in breast tumor samples. Selected targets from these studies will be further characterised functionally using biochemical and cell biological methods in collaboration with Drs. Buzz Baum and Anne Ridley. Future efforts are directed towards the application of quantitative MS methods to provide higher coverage and sensitivity in the proteomic analysis of cancer cell signaling events.

Research is also focused on elucidating the signaling networks and mechanisms involved in determining cell shape, adhesion and motility. Projects include the identification of protein changes associated with induction of the small G protein RhoE (with Dr. Anne Ridley) and the combination of RNAi, 2D-DIGE and MS to examine the effects of knocking down the expression of specific cancer-associated and cytoskeletal-regulating genes in a *Drosophila* cell model (with Dr Buzz Baum). It is well known that aberrant redox regulation plays an important role in tumorigenesis, and so the Group is also focusing on characterising the molecular events associated

with oxidative stress. These studies have included the application of semi-automated, quantitative proteomics and the development and application of a novel cysteine-labelling 2D-DIGE strategy to identify proteins that are subject to redox modification. By linking protein data from these experiments with parallel mRNA and metabolomic data, the Group has been able to provide a more detailed picture of the molecular processes that are affected by oxidative stress.

Publications

Primary Research Articles

1. Alam T.N., O'Hare M.J., Laczko I., Freeman A., Al-Beidh F., Masters J.R., Hudson D.L. Differential expression of CD44 during human prostate epithelial cell differentiation. Journal of Histochemistry and Cytochemistry (2004) 52(8):1083-90.
2. Aleyasin H., Cregan S.P., Iyirhiaro G., O'Hare M.J., Callaghan S.M., Slack R.S., Park D.S. Nuclear factor-(kappa)B modulates the p53 response in neurons exposed to DNA damage. Journal of Neuroscience (2004) 24(12):2963-73.
3. Ali K., Bilancio A., Thomas M., Pearce W., Gilfillan A.M., 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):1007-11.
4. Bailly K., Ridley A.J., Hall S.M., Haworth S.G. RhoA activation by hypoxia in pulmonary arterial smooth muscle cells is age and site specific. Circulation Research (2004) 94(10):1383-91.
5. Baum B., Craig G. RNAi in a postmodern, postgenomic era. Oncogene (2004) 23(51):8336-9.
6. Bergamaschi D., Samuels Y., Jin B., Duraisingham S., Crook T., Lu, X. ASPP1 and ASPP2: common activators of p53 family members. Molecular Cell Biology (2004) 24(3):1341-50.
7. Biyasheva A., Svitkina T., Kunda P., Baum B., Borisy G. Cascade pathway of filopodia formation downstream of SCAR. Journal of Cell Science (2004) 117(6):837-48.
8. Campbell M., Allen W.E., Sawyer C., Vanhaesebroeck B., Trimble E.R. Glucose-potentiated chemotaxis in human vascular smooth muscle is dependent on cross-talk between the PI3K and MAPK signaling pathways. Circulation Research (2004) 95(4):380-8.
9. Clarke C.L., Sandle J., Parry S.C., Reis-Filho J.S., O'Hare M.J., Lakhani S.R. Cytokeratin 5/6 in normal human breast: lack of evidence for a stem cell phenotype. Journal of Pathology (2004) 204(2):147-52.
10. Cohen-Gonsaud M., Barthe P., Pommier F., Harris R., Driscoll P.C., Keep N.H., Roumestand C. (1)H, (15)N, and (13)C chemical shift assignments of the resuscitation promoting factor domain of Rv1009 from Mycobacterium tuberculosis. Journal of Biomolecular NMR (2004) 30(3):373-4.
11. Costa F.F., Verbisck N.V., Salim A.C., Ierardi D.F., Pires L.C., Sasahara R.M., Sogayar M.C., Zanata S.M., Mackay A., O'Hare M., Soares F., Simpson A.J., Camargo A.A. (2004). Epigenetic silencing of the adhesion molecule ADAM23 is highly frequent in breast tumors. Oncogene (2004) 23(7):1481-8.

12. Cramer, R., Saxton, M., Barnouin, K. Sample preparation of gel electrophoretically separated protein binding partners for analysis by mass spectrometry. Methods Molecular Biology (2004) 261:499-510.

13. Cutillas P.R., Chalkley R.J., Hansen K.C., Cramer R., Norden A.G., Waterfield M.D., Burlingame A.L., Unwin, R.J. The urinary proteome in Fanconi syndrome implies specificity in the reabsorption of proteins by renal proximal tubule cells. American Journal of Physiology - Renal Physiology (2004) 287(3):F353-64.

14. Cutillas P.R., Norden A.G., Cramer R., Burlingame, A.L., Unwin, R.J. Urinary proteomics of renal Fanconi syndrome. Contributions to Nephrology (2004) 141:155-69.

15. Fauth C., O'Hare M.J., Lederer G., Jat P.S., Speicher M.R. Order of genetic events is critical determinant of aberrations in chromosome count and structure. Genes Chromosomes Cancer (2004) 40(4):298-306.

16. Franz C.M., Ridley A.J. p120 catenin associates with microtubules: inverse relationship between microtubule binding and Rho GTPase regulation. Journal of Biological Chemistry (2004) 279(8):6588-94.

17. Harris R., Esposito D., Sankar A., Maman J.D., Hinks J.A., Pearl L.H., Driscoll, P.C. The 3D solution structure of the C-terminal region of Ku86 (Ku86CTR). Journal of Molecular Biology (2004) 335(2):573-82.

18. Ivetic A., Florey O., Deka J., Haskard D.O., Ager A., Ridley A.J. Mutagenesis of the ezrin-radixin-moesin binding domain of L-selectin tail affects shedding, microvillar positioning, and leukocyte tethering. Journal of Biological Chemistry (2004) 279(32):33263-72.

19. Jones C., Mackay A., Grigoriadis A., Cossu A., Reis-Filho J.S., Fulford L., Dexter T., Davies S., Bulmer K., Ford E., Parry S., Budroni M., Palmieri G., Neville A.M., O'Hare M.J., Lakhani S.R. Expression profiling of purified normal human luminal and myoepithelial breast cells: identification of novel prognostic markers for breast cancer. Cancer Research (2004) 64(9):3037-45.

20. Lecca D., Piras G., Driscoll P., Giorgi O., Corda M.G. A differential activation of dopamine output in the shell and core of the nucleus accumbens is associated with the motor responses to addictive drugs: a brain dialysis study in Roman high- and low-avoidance rats. Neuropharmacology (2004) 46(5), 688-99.

21. MacDougall L.K., Gagou M.E., Leevers S.J., Hafen E., Waterfield, M.D. Targeted expression of the class II phosphoinositide 3-kinase in Drosophila melanogaster reveals lipid kinase-dependent effects on patterning and interactions with receptor signaling pathways. Molecular Cell Biology (2004) 24(2):796-808.

22. Magalhaes B.S., Harris R., Plevin M.J., Driscoll P.C. Backbone 1H, 13C, and 15N resonance assignments for a 29 kD monomeric variant of Pseudomonas aeruginosa dimethylarginine dimethylaminohydrolase. Journal of Biomolecular NMR (2004) 29(3):463-4.

23. Nagano K., Masters J.R., Akpan A., Yang A., Corless S., Wood C., Hastie C., Zvelebil M., Cramer R., Naaby-Hansen S. Differential protein synthesis and expression levels in normal and neoplastic human prostate cells and their regulation by type I and II interferons. Oncogene (2004) 23(9):1693-703.

24. Nikolaidou K.K., Barrett K. A Rho GTPase signaling pathway is used reiteratively in epithelial folding and potentially selects the outcome of Rho activation. Current Biology (2004) 14(20):1822-6.

25. Norden A.G., Sharratt P., Cutillas P.R., Cramer R., Gardner S.C., Unwin, R.J. Quantitative amino acid and proteomic analysis: very low excretion of polypeptides >750 Da in normal urine. Kidney International (2004) 66(5):1994-2003.

26. Palmieri C., Saji S., Sakaguchi H., Cheng G., Sunters A., O'Hare M.J., Warner M., Gustafsson J.A., Coombes R.C., Lam, E.W. The expression of oestrogen receptor (ER)-beta and its variants, but not ERalpha, in adult human mammary fibroblasts. Journal of Molecular Endocrinology (2004) 33(1):35-50.

27. Plevin M.J., Magalhaes B.S., Harris R., Sankar A., Perkins S.J., Driscoll P.C. Characterization and manipulation of the Pseudomonas aeruginosa dimethylarginine dimethylaminohydrolase monomer--dimer equilibrium. Journal of Molecular Biology (2004) 341(1):171-84.

28. Reif K., Okkenhaug K., Sasaki T., Penninger J.M., Vanhaesebroeck B., Cyster, J.G. Cutting edge: differential roles for phosphoinositide 3-kinases, p110gamma and p110delta, in lymphocyte chemotaxis and homing. Journal of Immunology (2004) 173(4):2236-40.

29. Rosenblatt J., Cramer L.P., Baum B., McGee K.M. Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. Cell (2004) 117(3):361-72.

30. Slee E.A., Gillotin S., Bergamaschi D., Royer C., Llanos S., Ali S., Jin B., Trigiante G., Lu X. The N-terminus of a novel isoform of human iASPP is required for its cytoplasmic localization. Oncogene (2004) 23(56):9007-16.

31. Smith P.D., O'Hare M.J., Park D.S. Emerging pathogenic role for cyclin dependent kinases in neurodegeneration. Cell Cycle (2004) 3(3):289-91.

32. Smith P.D., O'Hare M.J., Park, D.S. CDKs: taking on a role as mediators of dopaminergic loss in Parkinson's disease. Trends in Molecular Medicine (2004) 10(9):445-51.

33. Sullivan A., Syed N., Gasco M., Bergamaschi D., Trigiante G., Attard M., Hiller L., Farrell P.J., Smith P., Lu X., Crook T. Polymorphism in wild-type p53 modulates response to chemotherapy in vitro and in vivo. Oncogene (2004) 23(19):3328-37.

34. van Nieuw Amerongen G.P., Natarajan K., Yin G., Hoefen R.J., Osawa M., Haendeler J., Ridley A.J., Fujiwara K., van Hinsbergh V.W., Berk B.C. GIT1 mediates thrombin signaling in endothelial cells: role in turnover of RhoA-type focal adhesions. Circulation Research (2004) 94(8):1041-9.

35. Villalonga P., Guasch R. M., Riento K., Ridley A.J. RhoE inhibits cell cycle progression and Ras-induced transformation. Molecular Cell Biology (2004) 24(18):7829-40.

36. Weiss-Haljiti C., Pasquali C., Ji H., Gillieron C., Chabert C., Curchod M.L., Hirsch E., Ridley A.J., van Huijsduijnen R.H., 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.

37. Wells C.M., Ridley A.J. Analysis of cell migration using the dunn chemotaxis chamber and time-lapse microscopy. Methods Molecular Biology (2004) 294:31-42.

38. Wells C.M., Walmsley M., Ooi S., Tybulewicz V., Ridley A.J. Rac1-deficient macrophages exhibit defects in cell spreading and membrane ruffling but not migration. Journal of Cell Science (2004) 117(7):1259-68.

39. White S.L., Gharbi S., Bertani M.F., Chan H.L., Waterfield M.D., Timms J.F. Cellular responses to ErbB-2 overexpression in human mammary luminal epithelial cells: comparison of mRNA and protein expression. British Journal of Cancer (2004) 90(1):173-81.

40. Yap D.B., Hsieh J.K., Zhong S., Heath V., Gusterson B., Crook T., Lu X. Ser392 phosphorylation regulates the oncogenic function of mutant p53. Cancer Research (2004) 64(14):4749-54.

41. Zhukovskaya N.V., Fukuzawa M., Tsujioka M., Jermyn K.A., Kawata T., Abe T., Zvelebil M., Williams J.G. Dd-STATb, a Dictyostelium STAT protein with a highly aberrant SH2 domain, functions as a regulator of gene expression during growth and early development. Development (2004) 131(2):447-58.

Reviews / Commentaries / Book Chapters

1. Ivetic A., Ridley A.J. Ezrin/radixin/moesin proteins and Rho GTPase signalling in leucocytes. Immunology (2004) 112(2):165-76.

2. Ivetic, A., and Ridley, A. J. (2004). The telling tail of L-selectin. Biochem Soc Trans 32(Pt 6), 1118-21.

3. Kim J.B., O'Hare M.J., Stein R. Models of breast cancer: is merging human and animal models the future? Breast Cancer Research (2004) 6(1):22-30.

4. Kim J.B., Stein R., O'Hare, M.J. Three-dimensional in vitro tissue culture models of breast cancer-- a review. Breast Cancer Research Treatments (2004) 85(3):281-91.

5. Okkenhaug K., Bilancio A., Emery J.L., Vanhaesebroeck, B. Phosphoinositide 3-kinase in T cell activation and survival. Biochemical Society Transactions (2004) 32(2):332-5.

6. Ridley A. J. Rho proteins and cancer. Breast Cancer Research and Treatment (2004) 84(1):13-9.

7. Ridley A. J. Pulling back to move forward. Cell (2004) 116(3):357-8.

8. Ridley A. J., Hall A. Snails, Swiss, and serum: the solution for Rac 'n' Rho. Cell (2004) 116(2 Suppl):S23-7.

9. Slee E.A., O'Connor D.J., Lu X. To die or not to die: how does p53 decide? Oncogene (2004) 23(16):2809-18.

10. Vanhaesebroeck B., Rohn J.L., Waterfield M.D. Gene targeting: attention to detail. Cell (2004) 118(3):274-6.

11. Wheeler A.P., Ridley A.J. Why three Rho proteins? RhoA, RhoB, RhoC, and cell motility. Experimental Cell Research (2004) 301(1):43-9.

12. Williams J.G., Zvelebil, M. SH2 domains in plants imply new signalling scenarios. Trends in Plant Science (2004) 9(4):161-3.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Melbourne Branch of Tumor Biology

Melbourne, Australia

Staff List

Burgess, Antony *Member, Branch Director*
Scott, Andrew *Associate Member, Associate Director, Clinical Program*
Stacker, Steven *Associate Member, Associate Director, Scientific Program*

Angiogenesis Laboratory

Achen, Marc *Associate Member, Head (from April)*
Stacker, Steven *Assistant Member, Head*
Davydova, Natalia *Assistant Investigator*
Macheda, Maria *Postdoctoral Fellow*
Caesar, Carol *Senior Research Officer*
Inder, Rachel *Senior Research Officer*
Roufail, Sally *Senior Research Officer*
McDonald, Narelle *Research Officer (until June)*
Vranes, Dimitria *Research Officer (until October)*
Zhang, You-Fang *Research Officer (from November)*
Kugathasan, Kumudhini *Graduate Student (until November)*
McColl, Brad *Graduate Student*
Loughran, Stephen *Honours Student*
Farnsworth, Rae *Honours Student*
Williams, Steven *Undergraduate Student*

Colon Molecular & Cell Biology Laboratory

Ernst, Matthias *Associate Member, Head*
Heath, Joan *Associate Member, Head*
Abud, Helen *Assistant Investigator*
De Jong-Curtain, Tanya *Postdoctoral Fellow (from April)*
Jenkins, Brendan *Postdoctoral Fellow*
Trotter, Andrew *Postdoctoral Fellow (from May)*
Verkade, Heather *Postdoctoral Fellow (from May)*
Ng, Anne Yee-Lee *Postdoctoral Fellow (until February)*
Samuel, Michael *Postdoctoral Fellow (from December)*
Grail, Dianne *Chief Research Officer*
Liu, Yong-Shu *Chief Research Officer*
Inglese, Melissa *Senior Research Officer (until February)*
White, Sara *Senior Research Officer*
Najdovska, Meri *Research Officer (from March)*
Smith, Natasha *Research Officer*
Richardson, Elsbeth *Research Assistant*
Christou, Aliki *Visiting Research Assistant*
Bateman, Trudie *Graduate Student*
Tomaskovic-Crook, Eva *Graduate Student*
Blits, Marjolein Visiting *Student (until April)*
Johnsson, Anna-Karin *Visiting Student (until February)*
Mittler, Lutz *Visiting Student (September to July)*
Zanterna, Alt *Sabbatical Visitor (until July)*

Cytokine Biology Laboratory

Lieschke, Graham *Assistant Member, Head*
Layton, Judith *Senior Investigator*
McPhee, Dora *Senior Research Officer*
Varma, Sony *Senior Research Officer*
Hayman, John *Research Officer*
Kapitany, Luke *Research Officer*
Hogan, Benjamin *Graduate Student (until November)*
Crowhurst, Meredith *Graduate Student*
Boonchalermvichian, Chaiyaporn *Visiting Student*

Epithelial Biochemistry Laboratory

Burgess, Antony *Member, Branch Director, Head*
Nice, Edouard *Associate Member*
Walker, Francesca *Associate Investigator*
Catimel, Bruno *Assistant Investigator*
Clayton, Andrew *Research Associate*
Faux, Maree *Assistant Investigator*
Stenvers, Kaye *Postdoctoral Fellow*
Zhu, Hong-Jian *Assistant Investigator*
Lin, Feng *Peptide Chemist(until June)*
Cody, Stephen *Microscopy Facility Manager*
Condron, Melanie *Senior Research Officer*
Nerrie, Maureen *Senior Research Officer*
Nheo, Thao *Senior Research Officer*
Orchard, Suzanne *Senior Research Officer*
Rothacker, Julie *Senior Research Officer*
Weinstock, Janet *Senior Research Officer*
Zhang, Hui-Hua *Senior Research Officer*
Catimel, Jenny *Research Officer (from May)*
Gras, Emma *Research Officer*
Henderson, Christine *Research Officer (from March)*
Ross, Janine *Research Officer*
Tursky, Melinda *Research Officer (until November)*
Lees, Emma *Visiting Research Assistant (from October)*
Kleikemp, Sabine *Graduate Student (until March)*
Wang, Bo *Graduate Student*
Xiao, Yi (Connie) *Graduate Student (from March)*
Zamurs, Laura *Graduate Student*
Dahl, Staffan *Summer Student (until August)*
Baumann, Lucy *Visiting Student (until July)*
Hartig, Nicole *Visiting Student (from August)*
Peter, Sandblad *Visiting Student (until February)*
Sevenson, Lena *Visiting Student (until July)*
Karrlander, Maria *Visiting Student (from September)*

Joint ProteomicS Laboratory

(previously Joint Protein Structure Laboratory)

(A Joint Venture of the Ludwig Institute and The Walter and Eliza Hall Instutute of Medical Research)

Simpson, Richard *Member, Head*
Reid, Gavin *Assistant Member (until June)*
Hong, Ji *Assistant Investigator*
Layton, Meredith *Assistant Investigator (from March)*
Bernhard, Oliver *Postdoctoral Fellow (from August)*
Stensballe, Allan *Postdoctoral Fellow (from August)*
Kapp, Eugene *Senior Data Analyst*
Eddes, James *Data Manager*
Frecklington, *David Senior Research Officer*
Roberts, Kade *Postdoctoral Fellow (until August)*
Church, Nicole *Research Officer*
Ahn, Sung-Min *Graduate Student (from May)*
Goode, Robert *Graduate Student (from February)*
Greening, David *Graduate Student (from February)*
Skandarajah, Anita *MD Student (from February)*
Mathias, Rommel *Honours Student*
Tascone, Philip *Honours Student*
Na, Un Visiting *Student (from July)*
Ungan, Dennis *Visiting Student (from September)*

Joint ProteomicS Facility

Moritz, Robert *Proteomics Facility Manager*
Tu, Guo-Fen *Assistant Investigator*
Clippingdale, Andrew *Proteomics Research Officer*
Connolly, Lisa *Proteomics Project Service Manager*
Gilbert, Sam *Jnr. Mass Spectrom. Technician*
Kapp, Eugene *Senior Data Analyst*
Eddes, James *Senior Data Manager*

Signal Transduction Laboratory

Hibbs, Margaret *Associate Member, Head*
Harder, Ken *Research Associate*
Kemp, Joanna *Postdoctoral Fellow*
Quilici, Cathy *Chief Research Officer*
Inglese, Melissa *Senior Research Officer (from March)*
Kontouri, Nicole *Senior Research Officer*
Zlatic, Kristina *Senior Research Officer*
Casagranda, Franca *Chief Research Officer (from May)*
Turner, Amanda *Research Officer (until February)*
Beavitt, Sarah-Jane *Graduate Student (until November)*
Tsantikos, Evelyn *Undergraduate Student (from July)*
Magnusson, Kristina *Visiting Student (from March)*
Lundgren-May, Therese *Visiting Student (from August)*

Tumor Supressor Laboratory

Maruta, Hiroshi *Associate Member, Head*
Nheu, Thao Vuong *Senior Research Officer*
Hirokawa, Yumiko *Senior Research Officer*
Huynh, John *Summer Student (until January)*

Bioinformatics

Hall, Nathan *Bio-Informatics Officer*
Amos, Sally *Undergraduate Student*
Norton, Chris *Summer Student*

Information Technology Centre (Parkville Campus)

Thege, Greg *I.T. Manager*
Runting, Andrew *I.S. Manager*
Lay, Sami *I.T. Officer*
Houlis, Jim *Help Desk Support Officer (from August)*
Mathews, Peter *Information Officer/Librarian*

Technical Support (Parkville Campus)

Trainor, Robert *Laboratory Manager (from August)*
Burgess, Rosie *Purchasing Officer (from June)*
Cole, Robin *Equipment Officer, Maintenance*
Daws, Douglas *Equipment Officer, Maintenance*
Feakes, Valerie *Senior Research Officer, Histology*
Vaughan, Sarah *Media Technician (from March)*
Hughes, Andrew *Senior Research Officer, Aquarium*
Helman, Tracy *Animal Facility Manager (until October)*
Peirce, Susan *Chief Veterinary Officer, Animal Ethics*
Arnold, Melissa *Technical Officer, Animal Facility*
Thorne, Teleah *Technical Officer, Animal Facility*
Cohen, Jaqueline *Technical Assistant, Animal Facility*
Kuykhoven, Keilly *Technical Assistant, Animal Facility*
Teal, Bianca *Technical Assistant, Animal Facility*
Webster, Gaye *Technical Assistant, Animal Facility*
Maney, Wayne *Technical Assistant, Wash up*
Topham, Helen *Technical Assistant, Wash up*
Zammit, Carmen *Technical Assistant, Wash up*

Administration & Secretariat (Parkville Campus)

Tsai, Kim *General Manager*
Catanzariti, Nadia *Financial Accountant*
Lancuba, Sofia *Accounts Officer*
White, Sandra *Payroll/HR Officer (from May)*
Clark, Pauline *Human Resources Manger*
Perra, Cristina *Human Resources Officer*
Thumwood, Cassandra *Scientific Administrative Officer*
Jones, Pamela *Personal Assistant*
Pakin, Simone *Secretary (until April)*
Stevens, Wendy *Receptionist*

Makris, Polytimi *Administrative Assistant/ Receptionist*
Near, Christine *Animal Ethics Officer*

MELBOURNE BRANCH CLINICAL PROGRAM

Human Colon Cancer Initiative

(A joint venture of the Ludwig Institute, The Royal Melbourne Hospital, The Western Hospital and CSIRO)

Gibbs, Peter *Senior Clinical Research Fellow*
Johns, Julie *Data Manager/Tissue Bank Coordinator (from March)*
Chapman, Matthew *Medical Scientist (from August)*

Joint Austin Ludwig Medical Oncology Unit

Cebon, Jonathan *Associate Member, Head*
Davis, Ian *Assistant Member*
Barrow, Catherine *Clinical Research Fellow*
Tebbutt, Niall *Senior Clinical Research Fellow*
Rudolph, Anne *Personal Assistant (until October)*
Laura Woodings *Acting Personal Assistant (from October)*

CANCER VACCINE PROGRAM

Cancer Vaccine Laboratory

Cebon, Jonathan *Associate Member, Head*
Maraskovsky, Eugene *Assistant Member, LICR Associate*
Schnurr, Max *Postdoctoral Fellow (until March)*
Robson, Neil *Postdoctoral Fellow (from May)*
Shin, Amanda *Research Officer*
Toy, Tracey *Research Officer*
Gedye, Craig *Graduate Student (from February)*
John, Tom *Graduate Student (from February)*
Lioe, Hadi *Graduate Student (until June)*
Helling, Imke *Visiting Student (from September)*

Dendritic Cell Laboratory

Davis, Ian *Assistant Member, Head*
Morris, Leone *Senior Research Officer*
Quirk, Juliet *Senior Research Officer*
Seddon, Lauren *Senior Research Officer*

T-Cell Laboratory

Chen, Weisan *Assistant Member, Head*
Qiyuan, Chen *Assistant Investigator*
Jackson, Heather *Manager – Clinical Trials Monitoring*
Dimopoulos, Nektaria *Senior Research Officer*
Tai, Tsin Yee *Senior Research Officer*
Sanders, Megan *Senior Research Officer (from April)*
Mifsud, Nicole *Senior Research Officer (from February)*
Kennedy, Gina *Research Officer*

Bethune, Barbara *Blood Processor*
Swift, Karen *Animal Technician*
Pang, Ken *Graduate Student*

TUMOR TARGETING PROGRAM

Tumor Targeting Laboratory

Scott, Andrew *Associate Member, Head*
Fook-Thean, Lee *Associate Investigator*
Zhanqi, Liu, *Assistant Investigator*
Gill, Sanjeev *Clinical Research Fellow (from February)*
Rayzman, Veronika *Postdoctoral Fellow*
Rigopoulos, Angela *Senior Research Officer*
Mountain, Angela *Senior Research Officer*
Spirkoska, Violeta *Senior Research Officer*
Wang, Dongmao *Senior Research Officer*
Hall, Cathrine *Senior Research Officer (until September)*
Ardipradja, Katherine *Research Officer (from March)*
Kelly, Marcus *Graduate Student*
Marinic, Anna *Graduate Student*
Lawrentschuk, Nathan *Visiting Graduate Student (from July)*

Oncogenic Signaling Laboratory

Johns, Terrance *Assistant Member, Head*
Cao, Diana *Senior Research Officer*
Vitali, Angela *Senior Research Officer*
Tavarnesi, Meri *Research Officer (until August)*
Gan, Hui *Graduate Student*
Perera, Rushika *Graduate Student (until April)*
Cvrljevic, Anna *Honours Student*
Angerosa, Julie *Honours Student*
Ahlquvist, Marika *Visiting Student (March to July)*

Biological Production Facility

Rubira, Michael *Quality Assurance/Facilities Manager*
Murphy, Roger *Quality Control Manager/Protein Chemist*
Perani, Angelo *Senior Cell Biologist*
Jois, Jennifer *Senior Research Officer/ Cell Biologist*
O'Hoy, Kim *Cell Biologist*
Cartwright, Glenn *Purification Development Scientist*
Kypridis, Anna *Purification Process Scientist*
Cornehls, Jeff *Quality Compliance Officer*
Tran, Hung *Quality Control Officer (from June)*
Howes, Linda *Quality Control Scientist (April to May)*

Cancer Clinical Trials Centre

Hopkins, Wendie Senior Research Nurse
Gibbs, Sharen Research Nurse
Goldie, Heather Research Nurse
Lee, Sunanta Data Systems Developer

Joint Pathology/ Tissue Bank Group

MacGregor, Duncan, *Director, Dept. Anatomical Pathology, LICR Associate*
Murone, Carmel *Scientist, Anatomical Pathology*
Sturrock, Sue *Scientist, Anatomical Pathology*
Browning, Judy *Scientist, Anatomical Pathology*

Technical Support (Austin Campus)

Fox, Simon *Assistant Laboratory Manager*
Koul, Usha *Technical Assistant, Wash up*
Wapshott, Yvonne *Technical Assistant, Wash Up*

Administration & Secretariat (Austin Campus)

Tsai, Kim *General Manager*
Sharples, Catherine *Clinical Operations Manager*
Lewis, Kath *Personal Assistant to Associate director*
Smyth, Fiona *Scientific Administrative Officer*
Mazzeo, Mary *Financial Accountant*
Humphrey, Susan *Accounts Officer (until October)*
Lazarovska, Vanessa *Receptionist*
Cox, Shayne *P/C Network Support Officer*
Houlis, Jim Help *Desk Support Officer (from August)*

Clinical Trials Australia

(formerly the Centre for Developmental Cancer Therapeutics (CDCT)

(A joint venture of the Ludwig Institute, The Walter and Eliza Hall Institute of Medical Research, The Austin Hospital, the Western Hospital, The Royal Melbourne Hospital and Peter MacCallum Cancer Institute)

Rosenthal, Mark *Director, LICR Associate*
Alt, Carole *Chief Operations Officer*
Raunow, Heike *Research Nurse*

Branch Director's Report

Research at the Melbourne Branch continues to concentrate on the characterization of the cytokines, growth factors and receptor signaling events that influence the growth of human tumors. Our basic research combines with our clinical program to create new opportunities for the development of more effective anti-cancer treatments.

Animal models of gastrointestinal biology developed by the Colon Molecular and Cell Biology Laboratory are creating new opportunities to identify morphogenic factors in the intestine and to understand the interactions between two powerful cytokine networks: gp130 and TGF-β signaling. Through the Branch's zebrafish resource, we are in a position to efficiently analyze the genes responsible for several unusual phenotypes in the intestine, and also in the hemopoeitic system by the Cytokine Biology Laboratory. We have continued investigating the complexity of cytokine activated cell signaling in the Signal Transduction Laboratory, particularly the role of the intracellular lyn tyrosine kinase in the activation of inhibitory feedback loops. Dissection of the pathways involved in RAS/PAK1 activation and their role in oncogenesis continues to be of

particular interest in our Tumor Suppressor Laboratory. Studies by the Angiogenesis Laboratory are defining the roles of VEGF-C and VEGF-D, the growth factors that control the lymphatic vessel infiltration into tumors, in the spread of tumor cells.

The Epidermal Growth Factor Receptor (EGFR), a receptor system known to be involved in colon cancer, continues to be a central focus across both the Austin and the Parkville campuses of the Branch. The Epithelial Biochemistry Laboratory is involved in the analysis of EGFR and another signaling pathway implicated in colon cancer, the wnt/β-catenin/apc/axin system. The Oncogenic Signaling Laboratory, which was established within the Tumor Targeting Program, is also studying EGFR. The preclinical characterization of ch806, a novel anti-EGFR antibody, has been completed and a Phase I single dose escalation study has commenced in patients with advanced tumors expressing the 806 antigen.

The Melbourne Branch Clinical Program has continued to make considerable progress over the last 12 months in both laboratory and clinical research. Observations from a NY-ESO-1/ ISCOMATRIX™ vaccine trial, conducted in collaboration with CSL Ltd, are being extended in an international study that will test the vaccine's clinical efficacy. Ongoing cancer vaccine clinical studies are supported by the T Cell Laboratory. The GMP-grade Dendritic Cell (DC) Facility commenced a clinical trial investigating the role of DCs in vaccination against cancer antigens. Our Phase I clinical trials with recombinant antibodies cG250 (renal cancer) and hu3S193 (colon, lung and breast cancer) have been completed and a trial investigating huA33 radioimmunotherapy with capecitabine (colon cancer) are ongoing and showing encouraging results. In mid 2004 an antibody drug candidate developed by a team of researchers at the Melbourne Branch was exclusively in-licensed by KaloBios Pharmaceuticals for the treatment of autoimmune diseases. The first generation antibody is planned to enter human clinical trials by the second half of 2005.

During 2004 the Institute continued its partnership with Austin Health for the creation and fundraising for the new Olivia Newton-John Cancer Centre, which will incorporate LICR research and clinical programs. Our tissue bank program has continued and an Institute-wide Human Colon Cancer Initiative (HCCI) was commenced with the prospective collection of colon cancer specimens from multiple hospitals throughout Melbourne. Our participation in the Human Proteome Organization (HUPO) through JPSL, our Joint ProteomicS Laboratory, has allowed the application state-of-the-art proteomic techniques to the identification of low abundance proteins in plasma or serum from colon cancer patients.

Antony W. Burgess

Research Report
Parkville Campus

Angiogenesis Laboratory

Tumor angiogenesis facilitates growth of solid tumors and spread of malignant cells via blood vessels to distant parts of the body (metastasis). Lymphangiogenesis (growth of lymphatic vessels) in cancer promotes metastatic spread via lymphatic vessels. Therefore, anti-angiogenesis and anti lymphangiogenesis are emerging themes in the development of novel cancer therapeutics. The Angiogenesis Laboratory, led by Drs. Steven Stacker and Marc Achen, seeks to determine the molecular mechanisms that regulate formation of blood vessels and lymphatics in cancer and to determine which mechanisms control the interaction of tumor cells with lymphatic endothelium

and promote lymphogenous spread. A major focus of this work is the vascular endothelial growth factor (VEGF) family of glycoproteins. Previously, the Group identified the fourth VEGF family member, VEGF-D, and demonstrated that it stimulates angiogenesis, lymphangiogenesis and spread of malignant tumor cells via the lymphatics. VEGF-D can be secreted in a relatively inactive form which is subject to proteolytic processing that dramatically enhances receptor-binding affinity. The Group has recently shown that the serine protease plasmin and a range of other enzymes are capable of activating VEGF-D and the closely-related VEGF-C. Further studies of the activation of these growth factors and their mechanisms of signaling in cancer will facilitate approaches for blocking tumor angiogenesis and lymphangiogenesis. Future studies will involve exploring the role of multiple VEGFs using cell culture systems and animal tumor models.

Another research focus of the Angiogenesis Laboratory is the Ryk receptor, a member of the Receptor Tyrosine Kinase (RTK) family. Ryk has been recently shown to bind members of the Wnt family of morphogens. Biochemical studies of Ryk have shown that it associates with other RTK family members, the Eph receptors, to form signaling complexes. The Group's studies of Ryk-deficient mice have indicated the key role for Ryk in craniofacial and cardiovascular development. Further studies are aimed at determining the importance of Ryk in mammalian Wnt signaling in development and cancer.

Colon Molecular and Cell Biology Laboratory

The studies of the Colon Molecular and Cell Biology Laboratory, led by Drs. Matthias Ernst and Joan Heath, are designed to identify and characterize the properties of genes and pathways involved in colorectal cancer. A basic premise to our research is the concept that cancer recapitulates abnormal organ development. The Group has developed a gene discovery program in zebrafish to identify genes that govern intestinal development and is using reverse genetics approaches in mice to evaluate findings in the context of mammalian models of gastrointestinal disease.

The zebrafish studies have reached an exciting stage with the identification of a panel of 15 zebrafish mutants with defects in endoderm/intestine formation, such as hyperproliferation, lack of cell polarity, abnormal migration and excess apoptosis. The underlying mutant genes are all in the early stages of positional cloning. Once identified, the effects of these genes on epithelial cell behavior will be studied using a relatively accessible *in vitro* electroporation/embryonic gut culture system developed in the laboratory.

Cytokine Biology Laboratory

The Cytokine Biology Laboratory, led by Dr. Graham Lieschke, focuses on fundamental processes that control white blood cell development, growth and differentiation. Genetic approaches available in the zebrafish model organism are exploited, as a basis for understanding the perturbation of these processes in leukaemogenesis. A forward genetic chemical mutagenesis screen for myeloid failure in zebrafish has been brought to completion, and by the end of 2004, approximately 20 stable pedigrees of mutant zebrafish with defective leukocyte development had been recovered. Descriptive, genetic and molecular characterization of this uniquely large set of myeloid mutants is proceeding. Employing powerful transient techniques for reverse genetic function analysis in zebrafish, an analysis of the function of the *gcm2* (glial cells missing 2) transcription factor was completed, and demonstrated a surprising parallel between the molecular pathways regulating parathyroid gland development in mammals and gill development in fish.

Epithelial Biochemistry Laboratory,

The Epithelial Biochemistry Laboratory, led by Dr. Antony Burgess, continues to focus on the biochemistry and biology of colon cancer. The Group's research on the cell surface receptors that influence differentiation, motility, adherence and survival has implicated a central role for the EGFR family, integrin signaling and the transforming growth factor β receptor (TGFβR). The relationship of EGFR untethering and oligomerization to initial ligand binding and signaling processes is now being studied. Interestingly, the results indicate that laminin-10 stimulation synergizes with EGFR signaling to stimulate survival pathways in colonic cell lines.

The Group's research on the biochemistry of apc has allowed the generation of cell lines with apc tagged with green fluorescent protein, and this chimeric molecule clearly localizes to the same regions of the filopodia as apc. Using real time fluorescence microscopy and auto-correlation microscopy a direct interaction between axin and apc has been observed, and the proteins associated with both of these proteins in colonic cells are being characterized. It is becoming clearer that the apc system filters signaling from wnt/frizzled stimulation, however, the relationship of this regulatory system to the initial cell biological events of colon cancer has yet to be unraveled.

It is known that epithelial-mesenchyme transition (EMT) is integral to the invasive processes. Research by the Group has determined TGFβ signaling co-operates with ras-MAPK activation during both the initial phases of EMT and in the late stages when the metastatic tumor cells invade distant organs.

Signal Transduction Laboratory

The Lyn tyrosine kinase is a unique member of the Src family of protein tyrosine kinases that predominantly regulate signals through inhibitory receptors and promote signal termination. Studies in the Signal Transduction Laboratory, led by Dr. Margaret Hibbs, have identified Lyn as a tumor suppressor; Lyn deficient mice have a myeloproliferative disorder and develop macrophage tumors, whereas mice expressing a gain-of-function mutation in Lyn show no predisposition towards tumor development. Biochemical characterization of macrophages from both mouse mutants has shown that Lyn plays a critical role in the regulation of inhibitory signaling within the myeloid lineage. The Group's hypothesis is that reduction of inhibitory signaling may underlie the myeloproliferative disorder that predisposes the mice to tumor development.

In fact, the Group has now shown that loss of Lyn results in a stem/progenitor cell-intrinsic defect leading to an age-dependent increase in myeloid, erythroid and primitive hematopoietic progenitors that is independent of autoimmune disease. Lyn plays essential inhibitory roles within hematopoietic cells by recruiting the 5' inositol phosphatases, SHP-1 and SHIP-1, to the plasma membrane. A comparison of hematopoiesis in Motheaten and SHIP-$1^{-/-}$ mice showed similar defects in the erythroid and myeloid compartments, suggesting an intimate relationship between Lyn, SHP-1 and SHIP-1 in regulating hematopoiesis. Lyn appears to be dispensable for erythropoietin-dependent phosphorylation of these two phosphatases and for Jak/STAT/MAP kinase pathway activation in immature primary erythroid cells, implying that defects in these pathways do not underlie the severe alterations in erythropoiesis observed.

The Group has also demonstrated that Lyn is a critical negative regulator of T_H2 immune responses in an asthma mouse model. Lyn-deficient mice developed a severe, persistent inflammatory asthma-like syndrome with lung eosinophilia, mast cell hyper-degranulation, intensified bronchospasm, hyper IgE, and T_H2 immune deviation. The Group confirmed that Lyn, although not expressed in T cells, is expressed in DCs, key initiators of T cell-dependent immune responses,. These results explain how Lyn, which is not expressed in T cells, can influence T cell functional phenotype.

Joint ProteomicS Laboratory

The Joint ProteomicS Laboratory (JPSL), led by Dr. Richard Simpson, is a joint initiative of LICR and the Walter and Eliza Hall Institute of Medical Research (http://www.wehi.edu.au).

Through the Joint Proteomics Research Laboratory, JPSL is currently focussed on cutting-edge analytical protein chemistry to analyze early oncogenic changes in colon cancer. Networks of interacting proteins and other molecules mediate the functions of living cells by controlling gene (and protein) expression. Some of these proteins are secreted into the blood and collectively constitute a molecular fingerprint whose individual protein components exhibit specified levels of expression. The Group's hypothesis is that various sub-types of colon cancer will have a unique blood fingerprint of secreted proteins. Thus, a number of proteomics approaches are being used to analyze early oncogenic changes in colon cancer to identify diagnostic biomarkers of disease initiation, progression and the monitoring of various treatment regimes.

The second arm of JPSL, the Joint Proteomics Service Facility (JPSF) was officially launched in May 2004. JPSF has established a world-class high-throughput protein identification and analysis facility for use by scientists from both parent institutions. The Facility's six scientists have streamlined a number of mass spectrometry (MS) protein analysis operations to allow access to high-throughput, high-quality, state-of-the-art protein identification. Scientists have access to a full range of chromatography, electrophoretic, advanced MS and data mining techniques with JPSF's proteomic platforms being based on high-resolution MS/MS (e.g., Ion-Trap and hybrid quadrupole/Time-of-Flight mass spectrometers) and innovative in-house, automated computer technologies that allow reliable and sensitive identification of both proteins and peptides. Protein services have been extended to include protein separations using 1D and 2D electrophoresis, high performance gel filtration and reversed-phase chromatography. The JPSF protein identification software is supported by the world's best integrated protein sequence database prepared by colleagues from the LICR Office of Information Technology (Lausanne).

Tumor Suppressor Laboratory

In 2004, the research efforts of the Tumor Suppressor Laboratory, led by Dr. Hiroshi Maruta, concentrated on investigating the therapeutic effects of the drug FK228 on the human breast cancers and NF (neurofibromatosis) in animal models. The ring peptide FK228 is a potent histone deacetylase (HDAC) inhibitor that is currently in Phase II trials for a number of cancers, but not breast cancer or NF. FK228 is known to activate at least two tumor suppressor genes coding gelsolin (a PIP3-sequestering protein) and p21/WAF1 (a CDK-inhibitor), thereby suppressing RAS transformation. The Group found that FK228 inhibits RAS-induced activation of the kinase PAK1 through gelsolin. Furthermore, it was demonstrated that FK228 is able to inhibit the growth of both human breast and NF1/NF2-deficient cancer cells in culture. These results suggested that PAK1 activity is required for the malignant growth of these lines since normal cells were unaffected by this drug. Subsequently, FK228 was shown to inhibit the growth NF1-deficient tumor cell line, MPNST in animal models. These observations suggest that FK228 may have a very broad applicability in cancer since it appears to inhibit central signaling mechanisms employed by cancer cells.

Austin Hospital Campus

Tumor Targeting Program

Tumor Targeting Laboratory

In 2004, the development of therapeutics and the biology of EGFR was a major focus of research program of the Tumor Targeting Laboratory, led by Dr. Andrew Scott. Other areas of interest included a collaborative study with Dr Paul Ramsland (Austin Research Institute, Melbourne) that solved the 3D structure of the humanized monoclonal antibody hu3S193 interacting with its target cancer antigen Lewis Y. The intracellular trafficking mechanism of this antibody was evaluated using confocal microscopy. In addition, the Group explored the immunobiology of recombinant antibodies for the therapy of metastatic melanoma (with Dr Paul Ramsland).

Throughout 2004, several projects of note were continued: research into colon cancer biology and therapeutics; the identification of new antigen targets in colon cancer through proteomics; the exploration of Carbon-11 labeled EGFR tyrosine kinase inhibitors; an A33 antigen knock-in mouse model (in collaboration with Dr. Matthias Ernst, Colon Biology Laboratory), and; pre-clinical studies with mAb IIIA4 and an ephrinA5-Fc construct directed against the Eph A3 receptor in collaboration with Dr Martin Lackmann (Department of Biochemistry and Molecular Biology, Monash University, Melbourne) and Dr Andrew Boyd (Queensland Institute for Medical Research, Brisbane).

The development of novel antibodies to CD59, fibroblast activation protein (FAP), Tissue Factor, anti-idiotype antibodies, and targets on ErbB family members were also ongoing. Preclinical radioimmunotherapy studies in animal models of solid and haematopoetic cancers are under investigation with tumor targeting monoclonal antibodies radiolabeled with the alpha emitting radioconjugate, ^{213}Bi-CHX-A"-DTPA-, or gamma emitting Lutitium-177. Studies in models of breast and renal carcinomas have also demonstrated the enhancement of radioimmunotherapy when combined with EGFR tyrosine kinase inhibition.

Oncogenic Signaling Laboratory

The Oncogenic Signaling Laboratory, led by Dr. Terrance Johns, was created at the beginning of 2004 and is an integral component of the Tumor Targeting Program. The Group's research focus is on the identification and characterization of cell surface tyrosine kinase receptors that promote the survival and/or growth of cancer cells with the view of developing therapeutic antibodies to these receptors. In particular, there are two key areas of interest. (1) mAb 806, a novel antibody that recognizes a mutant EGFR commonly expressed in glioma known as the delta2-7 EGFR (de2-7 EGFR or EGFRvIIII) and a subset of the wild type EGFR found in cells that over-express the receptor. The Group identified the epitope recognized by mAb 806 and demonstrated that this epitope is only exposed in a transient form of the EGFR that occurs as the receptor moves from its inactive to active state. Unsurprisingly, the epitope is constitutively exposed in the de2-7 EGFR mutation. (2) Analysis of the role of the ligand-independent mutant of the EGFR, de2-7 EGFR, in development of glioma. Interestingly, the de2 7 EGFR mediates a growth advantage *in vivo* when expressed in glioma cells but has only a small effect on growth *in vitro*. During the past year the Group has clearly shown that the phosphorylation, and hence activation, of the de2-7 EGFR is dramatically up-regulated *in vivo*. This up-regulation is mediated by the binding of extracellular components (e.g. collagen) to integrin-β1, which is expressed on the surface of glioma cells. The signaling pathways downstream of integrin-β1 that are responsible for this observation are currently being mapped.

Clinical Trials

A first-in-man Phase I trial of the Lewis Y targeting antibody, hu3S193 was completed, and demonstrated impressive results with tumor targeting and immune function. A Phase I trial of ^{131}I-huA33 in combination with capecitabine in patients with metastatic colorectal cancer has continued, and a first-in-man Phase I trial of the EGFR targeting antibody, ch806, was commenced.

Biological Production Facility

The LICR is unique in having established a Biological Production Facility to manufacture study drugs under Good Manufacturing Practice (GMP) conditions for LICR sponsored trials worldwide. A GMP purification campaign of the chimeric monoclonal antibody ch806 was completed in 2004, with product release for the first-in-man Phase I trial. Alternatives for cell line development and production systems are being investigated within the facility for a number of antibody constructs. Several antibodies and peptides were formulated and vialed, and the Biological Production Facility continues to supply the LICR international Clinical Trials Program with reagents and material from the facility's extensive inventory of antibodies, proteins and peptides.

Centre for Positron Emission Tomography

The research program in molecular imaging involved clinical studies in glioma, melanoma, lymphoma, renal, lung and colon cancer. Research studies in tumor hypoxia imaging in glioma, lung and renal cancer patients demonstrated unique data on this important component of tumor metabolism. The radiochemistry research continued with the application of a novel method of ^{11}C labeling signaling inhibitor compounds.

Cancer Vaccine Program

Cancer Vaccine Laboratory

In 2004, the Cancer Vaccine Laboratory, led by Dr. Jonathan Cebon, concluded a clinical trial in collaboration with the biotechnology company CSL Ltd, with the protein tumor antigen NY ESO 1 formulated in the adjuvant ISCOMATRIX™. Vaccination induced an integrated and broad based immune response against NY-ESO-1 that involved CD4+ and CD8+ T lymphocytes as well as high titre antibodies. The vaccine appeared to prevent recurrence of melanoma in patients with disease that had been removed surgically but still carried a high risk of recurrence. This has prompted plans for a randomized clinical trial at major Australian & UK centers to formally evaluate the vaccine's efficacy. Other clinical protocols that will extend LICR's knowledge of NY-ESO-1 include a Phase II trial evaluating the impact of this vaccine in patients with measurable melanoma, a study of the cutaneous and circulating memory T cell responses in previously vaccinated patients, and the potential for DCs to enhance the immune effects of the vaccine. Laboratory studies have evaluated a variety of different DC subsets generated *in vitro* or isolated from patients, and the impact of exogenous factors on their ontogeny, activation and function. Central to these studies were the collaborative links with the clinic and the T Cell Laboratory that provided the epitope-specific CD4+ and CD8+ T cells required to test DC function. The focus on DC biology has included studies into nucleotide receptors, DC cytokine production and antigen 'cross-presentation'. These have provided a crucial foundation for understanding the mechanisms that

underlie the immune response to the NY-ESO-1 vaccine and provided the clinical DC team with insights for novel clinical approaches for immunotherapy.

Other highlights for 2004 include establishment of a regional node of the Cancer Vaccine Collaborative, a joint LICR and Cancer Research Institute (CRI) initiative, with Drs. Rod Dunbar and Mike Findlay in Auckland, New Zealand, and a collaboration with the Victorian Transplantation and Immunogenetics Service to study antigen and human leukocyte antigen (HLA) expression in cancer with a particular focus on the impact of vaccination.

Dendritic Cell Facility (DCF)

DCs are critical in the initiation and maintenance of immune responses, and the Cancer Vaccine and T Cell Laboratories at the LICR Austin campus have been investigating the biology of DC for several years. Many murine studies, as well as a rapidly enlarging literature of human clinical trials, have shown that vaccination using DC induces potent immune responses against a variety of target antigens including tumor-associated antigens. A previous clinical trial using Flt3 ligand, a growth factor that mobilizes DC into blood and tissues, has provided important data and, based on this, the LICR Dendritic Cell Facility (DCF) was built in order to continue the investigation of the role of DC in vaccination of humans against cancer antigens. The DCF is a GMP-grade facility designed to support the production of DCs for clinical use in the Cancer Vaccine Program. The first clinical trial was completed in 2003; the second commenced in late 2004 and will conclude in Q2 2005. This trial involves a novel method of DC production from peripheral blood without cytokine mobilization, using magnetic beads linked to the BDCA-1 antibody and the Miltenyi Biotec CliniMACS®plus Instrument. This trial also builds upon our previous experience using recombinant NY-ESO-1 formulated in ISCOMATRIX™ adjuvant (CSL Ltd.). Results from the current trial thus far indicate that the BDCA-1 selection system is able to derive sufficient numbers of DC from non-mobilized leukapheresis specimens to allow culture, activation, loading with NY-ESO-1 ISCOMATRIX™ and injection back into patients. No significant adverse events have been observed. Immunological analyses will include skin testing against the NY-ESO-1 protein and against antigen-pulsed DC, anti-NY-ESO-1 antibodies, and blood T cell responses against NY-ESO-1. T cell responses will be performed using a validated method developed by Dr Weisan Chen, head of the T cell Laboratory, and used in the trial of the NY-ESO-1 protein complexed with ISCOMATRIX™ conducted by the Cancer Vaccine Laboratory.

T Cell Laboratory

The research of the T Cell Laboratory, led by Dr. Weisan Chen, has focused on two areas: monitoring antigen-specific T cell responses in patients from cancer vaccine clinical trials; and using the influenza mouse model to explore improvements in cancer vaccine design and to understand T cell immunodominance and memory induction.

Sample assessments from LICR clinical trials have led to a number of novel observations during 2004. These included: vaccination induced immunodominant response to a 13mer antigenic epitope from NY-ESO-1, which represents the longest tumor epitope recognized by CD8+ T cells; discovering the first immunodominant HLA-B1801-restricted T cell epitope from NY-ESO-1, and; demonstrated clear immunodominant responses amongst T cells specific to multiple CD4+ and CD8+ epitopes from a patient with naturally occurring anti-tumor immune responses. These findings are under further investigation. In addition, through the establishment of mammalian Chinese hamster ovary (CHO) cells expressing NY-ESO-1, the Group has clearly demonstrated that antibody responses observed in patients from the initial Phase I study of NY-ESO-1/ ISCOMATRIX™ were indeed anti-NY-ESO-1 responses and not responses to bacterial components of the recombinant protein.

The formal establishment and expansion of the T Cell Laboratory has assisted research into improving vaccine design and understanding T cell immunodominance and memory induction. By using multiple immunoproteasome subunit knockout mice, the Group has shown that an immunodominant T cell response can be rendered as subdominant if antigen presentation is reduced to 50%. Investigations with transgenic and knockout animals and the influenza model system have continued to explore the contribution of the immunoproteasome to CD8+ T cell differentiation and memory induction. The Group is working on a T cell direct priming model in the absence of cross-priming, which could potentially enable the dissection of the independent contribution of the two priming modes during influenza infection. The search is continuing for the most immunodominant T cell epitope of anti-influenza responses in the BALB/c mice.

Joint Austin Ludwig Oncology Unit

The Joint Oncology Unit is one of Australia's foremost units devoted to clinical care and research where the focus is the development of new therapies. Whilst the LICR's Cancer Vaccine and Antibody Targeting Programs are central to clinical research activities, a number of other emerging cancer treatments are also evaluated at the Austin Campus. Highlights of these include early phase trials performed as a principal site for Cancer Trials Australia (*http://www.cancertrialsaustralia.com*) as well as later phase trials, often undertaken as a participant of one of the Australian cooperative trials groups. The Joint Oncology Unit is a major Australian centre for post graduate training in Medical Oncology for the University of Melbourne. In conjunction with Austin Health, the Joint Oncology Unit has also participated in an appeal to build the "Olivia Newton John Cancer Centre" at the hospital.

Further highlights for 2004 include: clinical trials with a variety of new cancer immunotherapeutics; Pentrys vaccine in prostate cancer; a HER2 vaccine in breast cancer; G oligodeoxynucleotide in renal cancer; the monoclonal antibodies huA33 in advanced colorectal cancer; and G250 in renal cancer and Cetuximab in colorectal cancer. In addition there were clinical trials using: the tyrosine kinase receptor inhibitors SU 11248 in conjunction with positron emission tomography (PET) scanning; ZD 1839 (Iressa) in lung and head & neck cancer; a VEGF receptor kinase inhibitor in hepatocellular carcinoma; and BAY 43 9006 in renal cancer. The Joint Oncology Unit actively participated in over 30 clinical trials run by collaborative groups such as the Australia New Zealand (ANZ) Breast Trials Group, the Australasian Leukaemia and Lymphoma Group (ALLG), the Trans-Tasman Radiation Oncology Group (TROG) and the Australasian Gastrointestinal Trials Group (AGITG). The Unit also has an ongoing interest in functional imaging and PET through a collaboration with the Department of PET and Nuclear Medicine.

Royal Melbourne / Western Hospitals

Major developments over 2004 include the successful establishment of tumor tissue banking and robust clinical data collection from patients with colorectal cancer at Western Hospital and at Melbourne Private Hospital. Over 250 tumor samples with matching clinical data have been collected. In addition, blood samples are now being collected on all consenting patients prior to surgery, which will prove a very valuable resource for the Human Colon Cancer Initiative. Another major achievement has been the successful linking of all data collected from the Western, Royal Melbourne and Austin Health, allowing for deidentified data from these three sites to be combined and interrogated. This now occurs in an ethically approved manner that does not compromise patient privacy. This clinical data is now also linked to the upgraded tissue bank databases at each of the sites and key research databases within LICR and the hospitals. This will facilitate the identification of important prognostic and predictive factors in colorectal cancer, which should enable a significant improvement in the outcomes of patients with colorectal cancer.

Publications

Primary Research Articles

1. Abud H.E., Heath J.K. Detecting apoptosis during the formation of polarized intestinal epithelium in organ culture. Cell Death and Differentiation (2004) 11(7): 788-89.
2. Abud H.E., Lock P., Heath J.K. Efficient gene transfer into the epithelial cell layer of embryonic mouse intestine using low-voltage electroporation. Gastroenterology (2004) 126(7): 1779-87.
3. Ackermann U., Tochon-Danguy H.J., Scott A.M. BF3 etherate catalysed formation of [11C]-methyl esters: a novel radiolabelling technique. Journal of Labelled Compounds & Radiopharmaceuticals (2004) 47: 523-30.
4. Basu S., Zhang H.H., Quilici C., Dunn A.R. Candida albicans can stimulate stromal cells resulting in enhanced granulopoiesis. Stem Cells and Development (2004) 13(1): 39-50.
5. Bauer A., Adrian N., Williamson B., Panousis C, Fadle N., Smerd J., Fettah I., Scott A.M., Pfreundschuh M., Renner C. Targeted Bioactivity of Membrane-Anchored TNF by an Antibody-Derived TNF Fusion Protein. Journal of Immunology (2004) 172(6): 3930-3939.
6. Chen Q., Jackson H., Parente P, Luke T., Rizkalla M., Tai T.Y., Zhu H.C., Mifsud N.A., Dimopoulos N., Masterman K-A., Hopkins W., Goldie H., Maraskovsky E., Green S., Miloradovic L., McCluskey J., Old L.J., Davis I.D., Cebon J., Chen W. Immunodominant CD4+ responses identified in a patient vaccinated with full-length NY-ESO-1 formulated with ISCOMATRIX adjuvant. Proceedings of the National Academy of Sciences of the USA (2004) 101(25): 9363-8.
7. Chen W., Masterman K-A., Basta S., Haeryfar S.M.M., Dimopoulos N., Knowles B., Bennink J.R. Cross-priming of CD8+ T cells by viral and tumor antigens is a robust phenomenon. European Journal of Immunology (2004) 34: 194-9.
8. Chen W., Pang K., Masterman K-A., Kennedy G., Basta S., Dimopoulos N., Hornung F., Smyth M., Bennink J.R., Yewdell J.W. Reversal in the immunodominance hierarchy in secondary CD8+ T cell responses to influenza A virus: roles for cross-presentation and lysis-independent immunodomination. Journal of Immunology (2004) 173(8): 5021-7.
9. Cheung N.S., Choy M.S., Halliwell B., Teo T.S., Bay B.H., Lee A.Y., Qi R.Z., Koh V.H., Whiteman M., Koay E.S., Chiu L.L., Zhu H-J., Wong K.P., Beart P.M., Cheng H C. Lactacystin-induced apoptosis of cultured mouse cortical neurons is associated with accumulation of PTEN in the detergent-resistant membrane fraction. Cellular and Molecular Life Sciences (2004) 61(15): 1926-34.
10. Chong Y-P., Mulhern T.D., Zhu H-J., Fujita D.J., Bjorge J.D., Tantiongco J.P., Sotirellis N., Lio D.S., Scholz G., Cheng H-C. A novel non-catalytic mechanism employed by the C-terminal Src-homologous kinase to inhibit Src-family kinase activity. Journal of Biological Chemistry (2004) 279(20): 20752-66.
11. Davis I.D., Chen W., Jackson H., Parente P., Shackleton M., Hopkins W., Chen Q., Dimopoulos N, Luke T., Murphy R., Scott A.M., Maraskovsky E., McArthur G., MacGregor D., Sturrock S., Tai T.Y., Green S., Cuthbertson A., Maher D., Miloradovic L., Mitchell S.V., Ritter G., Jungbluth A.A., Chen Y.T., Gnjatic S., Hoffman E.W., Old L.J., Cebon J.S. 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 of the USA (2004) 101(29): 10697-702.

12. Ernst M., Jenkins B.J. Acquiring signalling specificity from the cytokine receptor gp130. Trends in Genetics (2004) 20 (1): 23-32.

13. Faux M.C., Ross J.L., Meeker C., Johns T.G., Ji H., Simpson R.J., Layton M.J., Burgess A.W. Restoration of full-length adenomatous polyposis coli (APC) protein in a colon cancer cell line enhances cell adhesion. Journal of Cell Science (2004) 117(3): 427-39.

14. Foo S.S., Mitchell P.L., Berlangieri S.U., Smith C.L., Scott A.M. Positron emission tomography scanning in the assessment of patients with lymphoma. Internal Medicine Journal (2004) 34(7): 388-97.

15. Foo S.S., Ramdave S., Berlangieri S.U., Scott A.M. Detection of occult bone metastases of lung cancer with fluorine-18 fluorodeoxyglucose positron emission tomography. Australasian Radiology (2004) 48(2): 2004 214-6.

16. Harder K.W., Quilici C., Naik E., Inglese M., Kountouri N., Turner A., Zlatic K, Tarlinton D.M., an Hibbs M.L. Perturbed myelo/erythropoiesis in Lyn-deficient mice is similar to that in mice lacking the inhibitory phosphatases SHP-1 and SHIP-1. Blood (2004) 104(13): 3901-10.

17. He H., Hirokawa Y., Gazit A., Yamashita Y., Mano H., Kawakami Y., Hsieh C-Y., Kung H-J., Lessene G., Baell J., Levitzki A., Maruta H. The Tyr-kinase inhibitor AG879, that blocks the ETK-PAK1 interaction, suppresses the RAS-induced PAK1 activation and malignant transformation. Cancer Biology & Therapy (2004) 3(1): 96-101.

18. Held G., Matsuo M., Epel M, Gnjatic S., Ritter G., Lee S-Y., Tai T.Y., Cohen C.J., Old L.J., Pfreundschuh M., Reiter Y., Hoogenboom H.R., Renner C. Dissecting cytotoxic T cell responses towards the NY-ESO-1 protein by peptide/MHC-specific antibody fragments. European Journal of Immunology (2004) 34(10): 2919-29.

19. Hirokawa Y., Tikoo A., Huynh Y, Hanermann C.O., Giovannini M., Xiao G-H., Testa J.R., Wood J., Maruta H. A clue to the therapy of neurofibromatosis type 2: NF2/merlin is a PAK1 inhibitor. Cancer Journal (2004) 10(1): 20-26.

20. Hogan B.M., Danks J.A., Layton J.E., Hall N.E., Heath J.K., Lieschke G.J. Duplicate zebrafish pth genes are expressed along the lateral line and in the central nervous system during embryogenesis. Endocrinology (2004) 146(2): 547-51.

21. Hogan B.M., Hunter M.P., Oates A.C., Crowhurst M.O., Hall N.E., Heath J.K., Prince V.E., Lieschke G.J. Zebrafish gcm2 is required for gill filament budding from pharyngeal ectoderm. Developmental Biology (2004) 276(2): 508-22.

22. Hughes B.G., Yip D., Chao M.W., Gibbs P., Carroll S., Goldstein D., Burmeister B., Karapetis C. Audit of postoperative chemoradiotherapy as adjuvant therapy for resected gastroesophageal adenocarcinoma: an Australian multicentre experience. ANZ Journal of Surgery (2004) 74(11): 951-6.

23. Issa W., Tochon-Danguy H.J., Lambert J., Sachinidis J.I., Ackermann U., Liu Z., Scott A.M. Synthesis and evaluation of a thymidine analog for positron emission tomography study of tumor DNA proliferation in vivo. Nuclear Medicine and Biology (2004) 31(7):2004 839-49.

24. Jackson H.M., Dimopoulos N., Chen Q., Luke T., Yee Tai T., Maraskovsky E., Old L.J., Davis I.D., Cebon J., Chen W. A robust human T-cell culture method suitable for monitoring CD8+

and CD4+ T-cell responses from cancer clinical trial samples. Journal of Immunological Methods (2004) 291(1-2): 51-62.

25. Jefford M., Kiffer J.D., Somers G., Daniel F.J., Davis I.D. Metastatic basal cell carcinoma: rapid symptomatic response to cisplatin and paclitaxel. Australian and New Zealand Journal of Surgery (2004) 74(8): 2004 704-5.

26. Jefford M., Michael M., Rosenthal M.A., Davis I.D., Green M., McClure M., Smith J., Waite B., Zalcberg J. A novel combination of Cisplatin, irinotecan, and capecitabine in patients with advanced cancer. Investigational New Drugs (2004) 22(2):185-92.

27. Jenkins B.J., Grail D., Inglese M., Quilici C., Bozinovski S., Wong P., Ernst M. Imbalanced gp130-dependent signaling in macrophages alters macrophage colony-stimulating factor responsiveness via regulation of c-fms expression. Molecular and Cellular Biology (2004) 24(4):1453-63.

28. Johns T.G., Adams T.E., Cochran J.R., Hall N.E., Hoyne P.A., Rothacker J., Richards K.M., Kim Y.S., Nice E.C., Walker F., Ritter G., Jungbluth A.A., Old L.J., Ward C.W., Burgess A.W., Wittrup K.D., Scott A.M. Identification of the epitope for the epidermal growth factor receptor-specific monoclonal antibody 806 reveals that it preferentially recognizes an untethered form of the receptor. Journal of Biological Chemistry (2004) 279(29):30375-84.

29. Judd L.M., Alderman B.M., Howlett M, Schulkes A., Dow C., Moverley J., Grail D., Jenkins B.J., Ernst M., Giraud A.S. Gastric cancer development in mice lacking the SHP2 binding site on the IL-6 family co-receptor gp130. Gastroenterology (2004) 126(1):196-207.

30. Kanellis J., Levidiotis V., Khong T., Cox A.J., Stacker S.A., Gilbert R.E., Cooper M.E., Power D.A. A study of VEGF and its receptors in two rat models of proteinuria. Nephron Physiology (2004) 96(1):26-36.

31. Kloft C., Graefe E.U., Tanswell P., Scott A.M., Hofheinz R., Amelsburg A., Karlsson M.O. Population pharmacokinetics of sibrotuzumab, a novel therapeutic monoclonal antibody, in cancer patients. Investigational New Drugs (2004) 22(1):39-52.

32. Lioe H., O'Hair R.A.J., Reid G.E. A mass spectrometric and molecular orbital study of H2O loss from protonated tryptophan and oxidized tryptophan derivatives. Rapid Communications in Mass Spectrometry (2004) 18(9):978-88.

33. Luft T., Maraskovsky E., Schnurr M., Knebel K., Kirsch M., Gorner M., Skoda R., Ho A.D., Nawroth P., Bierhaus A. Tuning the volume of the immune response: strength and persistence of stimulation determine migration and cytokine secretion of dendritic cells. Blood (2004) 104(4):1066-74.

34. Luwor R.B., Zhu H-J., Walker F., Vitali A.A., Perera R.M., Burgess A.W., Scott A.M., Johns T.G. The tumor-specific de2-7 epidermal growth factor receptor (EGFR) promotes cells survival and heterodimerizes with the wild-type EGFR. Oncogene (2004) 23(36): 6095-104.

35. Maraskovsky E., Sjolander S., Drane D., Schnurr M., Le T.T.L., Mateo L., Luft T., Masterman K-A., Tai T.Y., Chen Q.Y., Green S., Sjolander A., Pearse M., Lemonnier F.A., Lemonnier F.A., Chen W., Cebon J., Suhrbier A. NY-ESO-1 protein formulated in ISCOMATRIX adjuvant is a potent anticancer vaccine inducing both humoral and CD8+ T-cell-mediated immunity and protection against NY-ESO-1+ tumors. Clinical Cancer Research (2004) 10: 2879-90.

36. Mawdsley D.J., Cooper H.M., Hogan B.M., Cody S.H., Lieschke G.J., Heath J.K. The Netrin receptor Neogenin is required for neural tube formation and somitogenesis in zebrafish. Developmental Biology (2004) 269: 302-15.

37. Maxwell M.J., Yuan Y., Anderson K.E., Hibbs M.L., Salem H.H., Jackson S.C. SHIP1 and Lyn kinase negatively regulate integrin alpha IIbbeta 3 signaling in platelets. Journal of Biological Chemistry (2004) 279(31): 32196-204.

38. Miller M.E., Michaylira C.Z., Simmons J.G., Ney D.M., Dahly E.M., Heath J.K., Lund P.K. Suppressor of cytokine signaling-2: a growth hormone-inducible inhibitor of intestinal epithelial cell proliferation. Gastroenterology (2004) 127(2): 570-81.

39. Mitchell P.L., Basser R.L., Chipman M., Grigg A., Mansfield R., Cebon J., Davis I.D., Appia F., Green M. A phase II study of escalated-dose docetaxel with granulocyte colony-stimulating factor support in patients with advanced breast cancer. Annals of Oncology (2004) 15(4):585-9.

40. Moritz R.L., Ji H., Schutz F., Connolly L.M., Kapp E.A., Speed T.J., Simpson R.J. A proteome strategy for fractionating proteins and peptides using continuous free-flow electrophoresis coupled off-line to reversed-phase high-performance liquid chromatography. Analytical Chemistry (2004) 76(16):4811-24.

41. Nakagawa T., Lan H.Y., Zhu H-J., Kang D.H., Schreiner G.F., Johnson R.J. Differential regulation of VEGF by TGF-beta and hypoxia in rat proximal tubular cells. American Journal of Physiology, Renal Physiology (2004) 287(4):F658-64.

42. Nakagawa T., Li J.H., Garcia G., Mu W., Piek E., Bottinger E.P., Chen Y., Zhu H-J., Kang D.H., Schreiner G.F., Lan H.Y., Johnson R.J. TGF-beta induces proangiogenic and antiangiogenic factors via parallel but distinct Smad pathways. Kidney International (2004) 66(2):605-13.

43. Nheu T., He H., Hirokawa Y., Walker F.E., Wood J., Maruta H. PAK is essential for RAS-induced upregulation of cyclin D1 during the G1 to S transition. Cell Cycle (2004) 3(1):71-4.

44. Odom S., Gomex G., Kovarova M., Furumoto Y., Ryan J.J., Wright H.V., Gonzalez-Espinosa C., Hibbs M.L., Harder K.W., Rivera J. Negative Regulation of Immunoglobulin E-dependent Allergic Responses by Lyn Kinase. Journal of Experimental Medicine (2004) 199(11):1491-502.

45. Olayioye M.A., Hoffmann P., Pomorski T., Armes J., Simpson R.J., Kemp B.E., Linderman G.J., Visvader J.E. The phosphoprotein StarD10 is overexpressed in breast cancer and cooperates with ErbB receptors in cellular transformation. Cancer Research (2004) 64(10):3538-44.

46. Palmer J., Ernst M., Hammacher A., Hertzog P.J. Constitutive activation of gp130 leads to neuroendocrine differentiation in vitro and in vivo. Prostate (2004) 62(3):282-9.

47. Pedrioli P.G., Eng J.K., Hubley R., Vogelzang M., Deutsch E.W., Raught B., Pratt B., Nilsson E., Angeletti R.H., Apweiler R., Cheung K., Costello C.E., Hermjakob H., Huang S., Julian R.K., Kapp E.A., McComb M.E., Oliver S.G., Omenn G., Paton N.W., Simpson R., Smith R., Taylor C.F., Zhu W., Aebersold R. A common open representation of mass spectrometry data and its application to proteomics research. Nature Biotechnology (2004) 22(11):1459-66.

48. Putra L.J., Lawrentschuk N., Ballok Z., Hannah A., Poon A., Tauro A., Davis I.D., Hicks R.J., Bolton D.M., Scott A.M. 18F-fluorodeoxyglucose positron emission tomography in evaluation of germ cell tumor after chemotherapy. Urology (2004) 64(6):1202-7.

49. Ramsay R.G., Micallef S.J., Willams B., Lightower S., Vincan E., Heath J.K., Fernley R., Mantamadiotis T., Bertoncello I. Colony-stimulating factor-1 promotes clonogenic growth of normal murine colonic crypt epithelial cells in vitro. Journal of Interferon & Cytokine Research (2004) 24(7):416-27.

50. Ramsland P.A., Farrugia W., Bradford T.M., Hogarth P.M., Scott A.M. Structural convergence of antibody binding of carbohydrate determinants in Lewis Y tumor antigens. Journal of Molecular Biology (2004) 340(4):809-18.

51. Reid G.E, Roberts K.D., Kapp E.A., Simpson R.J. Statistical and mechanistic approaches to understanding the gas-phase fragmentation behavior of methionine sulfoxide containing peptides. Journal of Proteome Research (2004) 3(4):751-9.

52. Rutanen J., Rissanen T.T., Markkanen J.E., Gruchala M., Kivela A., Hedman M, Hedman A., Heikura T., Orden M.R., Stacker S.A., Achen M.G., Hartikainen J., Ylä-Herttuala S. Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart. Circulation (2004) 109(8):1029-35.

53. Schnurr M., Toy T., Shin A., Hartmann G., Rothenfusser S., Soellner J., Davis I.D., Cebon J.S., Maraskovsky E. Role of adenosine receptors in regulating chemotaxis and cytokine production of plasmacytoid dendritic cells. Blood (2004) 103(4):1391-7.

54. Sedelies K.A., Sayers T.J., Edwards K.M., Chen W., Pellicci D.G., Godfrey D.I., Trapani J.A. Discordant regulation of granzyme H and granzyme B expression in human lymphocytes. Journal of Biological Chemistry (2004) 279(25): 26581-7.

55. Shackleton M., Davis I.D., Hopkins W., Jackson H., Dimopoulos N., Tai T.Y., Chen Q., Parente P., Jefford M., Masterman K-A., Caron D., Chen W., Maraskovsky E., Cebon J. The impact of imiquimod, a Toll-like receptor-7 ligand (TLR7L), on the immunogenicity of melanoma peptide vaccination with adjuvant Flt3 ligand. Cancer Immunity (2004) 4:9.

56. Sims N.A., Jenkins B.J., Quinn J.M., Nakamura A., Glatt M., Gillespie M.T., Ernst M., Martin T.J. Glycoprotein 130 regulates bone turnover and bone size by distinct downstream signaling pathways. Journal of Clinical Investigation (2004) 113(3):379-89.

57. Souriau C., Rothacker J., Hoogenboom H.R., Nice E.C. Human antibody fragments specific for the epidermal growth factor receptor selected from large non-immunized phage display libraries. Growth Factors (2004) 22:185-94.

58. Vaughan H.A., St Clair F., Scanlan M.J., Chen Y.T., Maraskovsky E., Sizeland A., Old L.J., Cebon J. The humoral immune response to head and neck cancer antigens as defined by the serological analysis of tumor antigens by recombinant cDNA expression cloning. Cancer Immunity (2004) 4:5.

59. Vaughan H.A., Svobodova S., MacGregor D., Jungbluth A.A., Browning J., Davis I.D., Parente P., Chen Y.T., Stockert E., Old L.J., Cebon J. Immunohistochemical and molecular analysis of human melanomas for expression of the human cancer-testis antigens NY-ESO-1 and LAGE-1. Clinical Cancer Research (2004) 10(24):8396-404.

60. Walker F., Orchard S., Jorissen R.N., Hall N.E., Zhang H.H., Hoyne P.A., Adams T.E., Johns T.G., Ward C., Garrett T.P.J., Zhu H-J., Nerrie M., Scott A.M., Nice E., Burgess A.W. CR1/CR2 interactions modulate the functions of the cell surface epidermal growth factor receptor. Journal of Biological Chemistry (2004) 279(21):22387-98.

61. Webb A.I., Dunstone M.A., Chen W., Aguilar M.L., Chen Q., Jackson H., Sliwkowski M.X., Kjer-Nielsen L., Beddoe T., McCluskey J., Rossjohn J., and Purcell A.W. Functional and structural characteristics of NY-ESO-1-related HLA A2-restricted epitopes and the design of a novel immunogenic analogue. Journal of Biological Chemistry (2004) 279(22):23438-46.

62. Whitehead J.P., Simpson F., Hill M.M., Thomas E., Connolly L.M., Collart F., Simpson R.J., James D.E. Insulin and oleate promote translocation of inosine-5' monophosphate dehydrogenase to lipid bodies. Traffic (2004) 5(10):739-49.

63. Zeng Y., Opeskin K., Baldwin M.E., Horvarth L.G., Achen M.G., Stacker S.A., Sutherland R.L., Williams E.D. Expression of vascular endothelial growth factor receptor-3 by lymphatic endothelial cells is associated with lymph node metastasis in prostate cancer. Clinical Cancer Research (2004) 10(15):5137-44.

64. Zhao L., Hart S., Chen J., Melenhorst J.J., Bierie B., Ernst M., Stewart C., Schaper F., Heinrich P.C., Ullrich A., Robinson G.W., Hennighausen L. Mammary gland remodeling depends on gp130 signaling through Stat3 and MAPK. Journal of Biological Chemistry (2004) 279(42):44093-100

Reviews / Commentaries / Book Chapters

1. Abdel-Dayem H., Scott A.M., Macapinlac H., Atay S. Thallium-201 choloride: a tumour imaging agent. In: Nuclear Medicine in Clinical Diagnosis and Treatment (3rd Ed). Ell, P.J., Gambir, S (Eds), Churchill Livingstone, London, pp71-82 (2004).

2. Abud H.E. Shaping developing tissues by apoptosis. Cell Death and Differentiation (2004) 11(8):797-9.

3. Foo S.S., Abbott D.F., Lawrentschuk N., Scott A.M. Functional imaging of intratumoral hypoxia. Molecular Imaging and Biology (2004) 6 (5):291-305.

4. Lieschke G.J. Animal models of hematopoietic growth factor perturbations in physiology and pathology. In: Cancer Drug Discovery and development; Hematopoietic Growth Factors in Oncology: Basic Science and Clinical Therapeutics. Morstyn, G., Foote, M.A., Lieschke, G.J. (Eds), Human Press, Totowa, NJ, pp11-44 (2004).

5. Maruta H., Hirokawa Y. Signal therapy of neurofibromatosis (NF). Genome Medicine (Tokyo) (2004) 4:185-92.

6. McColl B.K., Stacker S.A., Achen M.G. Molecular regulation of the VEGF family - inducers of angiogenesis and lymphangiogenesis. Acta Pathologica, Microbiologica et Immunologica Scandinavica (2004) 112(7-8):463-80.

7. Nice E., Aguilar M.I. Micropreparative HPLC of peptides and proteins. Methods in Molecular Biology (2004) 251:165-76.

8. Nice E.C., Aguilar M.I. Multidimensional HPLC purification of proteins. Methods in Molecular Biology (2004) 251: 177-82.

9. Scott A.M., Poon A. PET imaging of brain tumours. In: Nuclear Medicine in Clinical Diagnosis and Treatment (3rd Ed). Ell, P.J., Gambir, S (Eds), Churchill Livingstone, London, pp345-59 (2004).

10. Simpson R.J. International proteomics initiatives: technological challenges. European Pharmaceutical Review (2004) 9: 25-36.

11. Simpson R.J. Purifying proteins for proteomics: a laboratory manual. (2004) New York Publisher: Cold Spring Harbour Laboratory Press Editors: R.J. Simpson.

12. Simpson R.J., Dorow D.S. Proteomic approaches for biomarker discovery in colorectal cancer. In: Biomedical applications of proteomics. Sanchez, J.-C., Corthals, G., Hochstrasser, D.F. (Eds), Wiley-VCH, Weinheim, New York, pp111-131 (2004)

13. Stacker S.A., Hughes R.A., Achen M.G. Molecular targeting of lymphatics for therapy. Current Pharmaceutical Design (2004) 10:65-74.

14. Stacker S.A., Williams R.A., Achen M.G. Lymphangiogenic growth factors as markers of tumor metastasis. Acta Pathologica, Microbiologica et Immunologica Scandinavica (2004) 112(7-8):539-49.

15. Van der Wall H., Clarke S., Scott A.M., Murray I.P.C. Evaluation primary bone tumours. In: Nuclear Medicine in Clinical Diagnosis and Treatment (3rd Ed). Ell, P.J., Gambir, S (Eds), Churchill Livingstone, London, pp 623-40 (2004).

16. Carr, C.D., Moritz R.L. Role of Reversed-phase High-Performance Liquid Chromatography in Protein Isolation and Purification. In: Purifying Proteins for Proteomics: A Laboratory Manual. Simpson, R.J. (Ed), Cold Spring Harbor Laboratory Press, pp 179-208 (2004).

17. Brockelhurst, K., Courey, A.J., Gul, S., Lin, S-H., Moritz, R.L. Affinity and Immunoaffinity chromatography. In: Purifying Proteins for Proteomics: A Laboratory Manual. Simpson, R.J. (Ed) Cold Spring Harbor Laboratory Press, pp 221-274 (2004).

18. Wong S., Gibbs P., Chao M.W., Jones I., McLaughlin S., Tjandra J.J., Faragher I., Green M. Carcinoma of the anal canal: a local experience and review of the literature. ANZ Journal of Surgery (2004) 74(7): 541-6.

19. Chao M.W., Tjandra J.J., Gibbs P., McLaughlin S. How safe is adjuvant chemotherapy and radiotherapy for rectal cancer? Asian Journal of Surgery (2004) 27(2): 147-61.

20. Gibbs P., Chao M.W., Jones I.T., Yip D. Evidence supports adjuvant radiation therapy in selected cases with rectal cancer. ANZ Journal of Surgery (2004) 74(3): 152-7.

LUDWIG INSTITUTE FOR CANCER RESEARCH

New York Branch
of Human Cancer Immunology

New York, USA

Staff List

Old, Lloyd J. *Director, Member*
Ritter, Gerd *Associate Director, Associate Member*
Jungbluth, Achim *Associate Member*
Gnjatic, Sacha *Assistant Member*
Gure, Ali *Assistant Member*
Scanlan, Matthew *Assistant Member (until March)*
Caballero, Otavia *Associate Investigator (from October)*
Lee, Frank K.N. *Assistant Investigator*
Lee, Sang-Yull *Assistant Investigator (until July)*
O'donoghue, Joseph *Assistant Investigator*
Vannelli, Todd *Assistant Investigator*
Sharma, Padmanee *Clinical Fellow (until June)*
Chen, Lin-Chi *Clinical Fellow (from July)*
Nishikawa, Hiroyoshi *Research Fellow*
Sato, Eiichi *Postdoctoral Fellow*
Cohen, Leonard *Research Assistant, Assistant Laboratory Administrator*
Fortunato, Sheila *Research Assistant*
Williams, Clarence *Research Assistant*
Yin, Beatrice *Research Assistant, Laboratory Manager*
Chua, Ramon *Senior Research Technician (from July)*
Frosina, Denise *Senior Research Technician*
Ritter, Erika *Senior Research Technician*
Santiago, Darren *Senior Research Technician (until July)*
Eastlake- Wade, Susannah *Research Technician (until May and from September)*
Kelly, David *Research Technician*
Castelli, Sandra *Research Technician (from November)*
Grgas, Anthony *Research Technician (from July)*
Lee, Woojung *Research Technician, (from August)*
Krapivinisky, Evgeniya *Research Technician*
Murray, Anne *Research Technician*
Venditti, Charis *Research Technician*
Villalobos, Cristina *Research Technician (until November)*
Mark, George *Consultant (from March)*

LICR Associates

Chen, Yao-T. *Professor of Pathology and Laboratory Medicine, Weill Medical College of Cornell University*
Valmori, Danila *Associate Professor of Medical Science and Surgical Science, Columbia-Presbyterian Medical School*
Ayyoub, Maha *Assistant Professor of Medical Science and Surgical Science, Columbia-Presbyterian Medical School*

Secretariat

Rios, Jannette *Administrative Assistant*

Branch Director's Report

The major objective of the New York Branch is the identification and characterization of suitable antigenic targets for antibody-based and vaccine-based immunotherapies of human cancer. The New York Branch, the smallest of the ten LICR Branches, utilizes a fully integrated and multidisciplinary approach, with the individual research groups - Antigen Discovery, Biochemistry, Pathology/Morphology, T Cell Immunology and Serological Typing, and Clinical Trials Groups - operating in close collaboration with each other. The highly coordinated activities of the research groups, and their interactions with selected external academic and industrial research partners, are designed such that new discoveries are rapidly transferred into early phase clinical evaluation in cancer patients. Thus the New York Branch is an integral part of the LICR's Clinical Trials Program for cancer immunotherapy with monoclonal antibodies (mAbs) and vaccines.

Over the past decade, the Branch has identified and characterized a series of cell surface antigens for the LICR's Antibody Targeting Program. Six antigens have been selected for early phase clinical evaluation, and clinical trials with mAbs against those antigens are ongoing, or soon to commence, within the LICR Clinical Trials Program. Currently, a major focus is on the generation and development of non-immunogenic human, chimeric or veneered antibody constructs for clinical evaluation. The protocols developed for the clinical study of the newer generation of antibody constructs are designed to provide data on pharmacokinetic and quantitative biodistribution properties, tumor localization and imaging characteristics, immunogenicity, and therapeutic activity of antibody alone and in combination with, or as a carrier of, cytotoxic agents.

As part of LICR's Cancer Antigen Discovery and Cancer Vaccine Programs, the discovery and characterization of new targets is a continuing objective of the Branch with particular emphasis on gene products having characteristics of cancer/testis (CT) antigens. In addition, the Branch's research groups provide critical laboratory support to Branch investigators and LICR Affiliates involved in the Clinical Trials Program in order to monitor, optimize, and characterize the immune responses to cancer vaccines. The Antigen Discovery and Pathology Groups perform the antigen typing of tumors to determine the potential eligibility of a patient to participate in clinical trials, the Biochemistry Group generates monitoring agents and works closely with the LICR Bioprocess Laboratory at Ithaca in the generation of clinical trial reagents, and the T Cell Immunology and Serological Typing Group monitors humoral and cellular immune responses of patients enrolled in the cancer vaccine trials.

The Branch is involved in the immunological monitoring for nearly all clinical trials performed under the auspices of the Cancer Vaccine Collaborative (CVC) which was established in partnership with the Cancer Research Institute (New York, USA) in 2002. Furthermore, the Branch serves as immunological reference laboratory for the LICR's Clinical Trials Program, as well as a training laboratory for immunological monitoring and molecular typing techniques required for the LICR's Cancer Vaccine and Antibody Targeting Programs.

Lloyd J. Old & Gerd Ritter

Research Report

Antibody Development and Antibody-based Immunotherapy

Antigens identified by the New York Branch that are being explored as antigenic targets for antibody-based immunotherapy include: A33, a 43 K glycoprotein with selective expression in normal and malignant epithelium of the gastrointestinal tract (recognized by mAb A33); G250, a glycoprotein expressed by a high percentage of renal cancers (recognized by mAb G250); Le^y, an oligosaccharide epitope expressed on glycolipids and glycoproteins by a wide range of epithelial cancers (recognized by mAb 3S193); GD3, a ganglioside with high expression in melanoma and other neuroectodermal tumors (recognized by mAb KM871); FAP-α, a 95 K glycoprotein strongly expressed in the stromal fibroblasts of epithelial cancers (recognized by mAb F19 and mAb BIBH1); and a novel variant form (transitional untethered receptor) of the epidermal growth factor receptor (EGFR, recognized by mAb 806), which is expressed by a proportion of brain cancers and other tumor types.

First generation chimeric or humanized monoclonal antibodies have been generated to these target antigens and Phase I clinical studies within the LICR Clinical Trials Program have demonstrated impressive selectivity and tumor targeting in antigen expressing cancer patients. In collaboration with the LICR Melbourne Branch and Dr. Christoph Renner (LICR Homburg Affiliate Center, Germany), antibody engineering has been initiated to refine and optimize desired characteristics of these antibodies. Establishment of stable cell lines capable of supporting large scale manufacturing of these antibodies has also been undertaken. Synthetic genes encoding the antibody heavy and light chain variable regions and the human heavy and light chain constant regions (IgG1 and kappa) were constructed to contain codon-optimized exons interspersed by truncated versions of their natural introns. Expression vectors capable of directing the production of recombinant antibody molecules for mAbs A33 806 and F19 were constructed, cloned and sequence verified. Small scale production of each antibody is underway to facilitate their ultimate characterization for specificity and affinity.

Mouse mAb 806 was initially raised, as part of a collaboration with the LICR San Diego Branch, to recognize a truncated (de2-7 deletion) form of EGFR found predominantly in glioblastoma. Analysis of mAb 806 gave rise to the surprising finding that mAb 806 showed specificity for overexpressed/amplified EGFR. In a panel of normal tissues mAb 806 showed little or no reactivity with tissues known to express wildtype levels of EGFR, including liver and skin. In contrast, tumors known to overexpress EGFR, e.g. glioblastoma, squamous cell carcinoma of the lung, and head and neck, urinary, and bladder cancers showed strong reactivity by immunohistochemistry. In collaboration with the Melbourne Branch and Dr. Dane Wittrup at Massachusetts's Institute of Technology (Massachusetts, USA), the epitope recognized by mAb 806 has now been identified to be contained within a short cysteine loop (amino acids 287-302) in the EGFR. This epitope becomes only available to mAb 806 binding in a transitional form of the EGFR that occurs when the receptor changes from an inactive tethered into an active dimeric untethered configuration. In case of the truncated (de2-7 deletion) EGFR mutation, the receptor is constitutively untethered and thus can be recognized by mAb 806. The mAb 806 is now undergoing further preclinical characterization at the Melbourne Branch, and a chimeric version of this antibody generated at the LICR's Biological Production Facility (BPF) in Melbourne will enter LICR early phase clinical trials in 2005. A humanized version of mAb 806 is being developed through antibody engineering.

In a series of Phase I clinical trials, humanized mAb A33 has shown good targeting in patients with advanced colorectal cancer and some clinical responses have been observed with the antibody alone or in combination with chemotherapy (BOF-Strep). Based on these

initial observations follow-up studies combining humanized mAb hu A33 with various chemotherapeutic regimens (huAb A33 and capecitabine at the Melbourne Branch; huAb A33 and 5FU plus leucovorin plus oxaliplatin in Affiliate Centers in Frankfurt, Homburg and Zurich) have been initiated. A significant number of patients developed human anti-human antibodies (HAHA) against hu A33, in particular when the antibody was given alone in repeated doses. Thus, several new human, veneered or chimeric IgG1 A33 antibodies have been generated in collaboration with Dr. Renner, to overcome the high frequency of HAHA induction, which accelerated antibody serum clearance, blocked tumor targeting and consequently abrogated tumor response. In order to select the best candidate for immunotherapy the genetically engineered antibodies were subjected to a detailed analysis at the New York Branch, including specificity, cell-surface-reactivity, binding affinity, antigenicity and immunological effector functions. Selected antibodies were then further analyzed at the Melbourne Branch for radiolabeling characteristics and serum stability and for biodistribution and pharmacokinetics in vivo in animal xenograft models.

A fractionated radioimmunotherapy Phase I trial with ^{131}I-cG250 in metastatic clear cell renal cancer was carried out at the LICR New York (Memorial Sloan-Kettering Cancer Center, MSKCC) Affiliate Center to determine the maximum tolerated whole body radiation absorbed dose (MTD). Fifteen patients with measurable metastatic renal cancer were studied. Targeting to known disease > 2 cm in diameter was noted in all patients. Dose-limiting toxicity was hematopoietic and the maximum tolerated dose was 0.75 Gy. The tumor adsorbed radiation dose from non-myeloablative amounts of ^{131}I-cG250 was inadequate to expect significant clinical responses. In collaboration with Affiliate Dr. Egbert Oosterwijk and colleagues (Nijmegen Affiliate Center, The Netherlands) radiometal-labeled cG250, which may have greater therapeutic potential, is being investigated. Furthermore, a new construct combining the high targeting capabilities of mAb G250 with a potent biological effector molecule, TNF, has been developed in collaboration with Drs. Renner and Oosterwijk. The construct is currently being characterized *in vitro* and *in vivo* and the hybridoma is being evaluated for the production of clinical grade material at the Melbourne BPF.

Branch investigators recently found that Le^{y} antigen is highly expressed in small cell lung cancer and a multiple dose targeting study of hu3S193 in patients with small cell lung cancer has been initiated at MSKCC. The primary objective of this study is to determine the targeting, tissue distribution, and pharmacokinetics of hu3s193 trace-labeled with ^{111}In.

A new Positron Emission Tomography (PET) Program has been established, as part of the Antibody Targeting Program, at the Branch's host institution, MSKCC, with LICR Affiliates Drs. Chaitanya Divgi, Steve Larsen and Ron Finn. This program will allow quantitative assessment of radiolabeled antibody tumor targeting in real time. The ultimate goal is to replace invasive biopsies to measure tumor antibody uptake. The initial focus is on hu A33, hu 3S193, and ch G250 and a first clinical trial has been initiated with ^{124}I-labeled hu A33 in patients with colorectal cancer. To investigate whether a smaller size antibody construct may improve the ration of tumor to non-tumor targeting a clinical-grade scFv A33 construct is being produced at the LICR Bioprocess Laboratory at Cornell University, Ithaca (see also Cornell Affiliate Center report) and is expected to enter clinical testing in 2005. Availability of appropriate chelating agents that will sequester a radioactive metal ion and can easily be combined with an antibody or other molecules of interest is critical for this program. In collaboration with LICR Affiliates Dr. Richard Schmidt (Konstanz Affiliate Center, Germany) and Dr. Peter Smith-Jones (New York (MSKCC) Affiliate Center, USA) a novel bifunctional chelating agent, TAME-Hex A, with high stability for Ga (III) ions has been designed and synthesized.

Antigen Discovery and Characterization

The Antigen Discovery Group has identified a large array of distinct antigens using serological screening of cDNA expression cloning (SEREX) (for details see the LICR Cancer Immunome Database; http://www2.licr.org/CancerImmunomeDB/). These antigens fall into different categories including differentiation antigens, splice variants, mutational antigens, overexpressed/amplified antigens, and cancer-testis (CT) antigens. Identification of new antigenic targets for cancer vaccines continued as a major focus of the Branch in 2004. Two studies were launched to identify new CT antigens using bioinformatics approaches, one based on the evaluation of public EST database, and the other based on the analysis of MPSS (massively parallel signature sequencing) data derived from normal tissues and from CT-rich cell lines.

In the MPSS study, data from a battery of normal human tissues and from two cell lines were analyzed, identifying ~1000 genes that were predominantly expressed in testis. Additional rounds of *in silico* filtering of these genes and RT-PCR validation confirmed 20 potential CT genes, with several of them exhibiting expression in five or more of the 21 cancer cell lines examined. One of these genes, designated CT45, belongs to a family that comprises six highly similar members that clustered in tandem within a 125kb region on Xq26.3. CT45 was found to be expressed in 13 of 21 cancer cell lines and eight of 29 lung cancer specimens examined.

In the EST-based study, a list of 371 genes with testis-dominant expression was compiled through the analysis of publicly available EST databases. An initial selection of 20 candidate genes was evaluated by RT-PCR, and several new CT candidate genes were identified. One of these, CT46 is expressed strongly in testis, weakly in placenta, with the highest level of expression in other tissues <1% of testicular expression. CT46 gene was expressed in ~30% of various carcinomas examined, with ~10% showing expression at levels >10% of the testicular level of expression. CT46 is a single-copy gene on chromosome 1q21.3 that encodes a HORMA domain-containing protein, presumably expressed at meiotic phase of the germ cells.

In addition to the identification of new CT genes, the Branch continued to pursue the understanding of CT antigen gene expression in tumors. There are a considerable number of genes that have expression restricted to germ cells and malignant tumors (CT antigens), possibly due to a recapitulation of the germ line gene expression program in cancer. Formation of the male gamete occurs in sequential mitotic, which can be undertaken for spermatogonial stem cell renewal or spermatogonial proliferation toward meiosis, meiotic and postmeiotic phases. The transcript profiles of mammalian male rat and mouse germ cells have been studied for several groups using c-DNA microarrays, and genes involved in controlling these specialized mitotic and meiotic cycles of mammalian germ cell differentiation are being identified. To understand the phenomenon of germ line gene expression in tumors, genes that are germ cell-specific or differentially expressed in different stages of the germ line development and also expressed in cancer tissues are being identified. Using the data available, hundreds of germ cell-specific genes or genes differentially expressed in the germ line and also expressed in cancer tissues are being identified using bioinformatics tools. The question of whether the tumor expression of the newly identified genes and also of the known CT antigens contributes to carcinogenesis is also being explored. For the establishment of the relative contribution of the CT antigens in conferring growth advantage to normal cells, the Group is performing classic cell growth and transformation assays in mouse embryonic fibroblast cells (NIH/3T3) and immortalized normal human cells transfected with recombinant plasmids. *NY-ESO-1, SYCP-1* and *MAGEA3* have been cloned and are being tested in these assays. Also, to reveal the correlation between over expression of the genes identified and biological features of cancer cell lines, recombinant plasmids producing hairpin small interfering RNA (siRNA) to target the mRNAs using a vector-based RNA interference technology are being constructed. Furthermore, as a prototype, the SSX4 promoter was characterized and it is now

being utilized as a model to study genetic and epigenetic influences in the regulation of CT expression. A large collection of non-small cell lung cancer specimens have also been analyzed for CT expression. Results indicate that CT genes were expressed in a coordinated fashion, and that high CT gene expression is a poor prognostic indicator, often correlating to other parameter of adverse clinical outcome and to poor overall survival.

For an antigen to serve as a potential target for immunotherapy, it is critical to obtain information on its tissue expression not only on the RNA level, but also on the protein level. Novel mAbs are being prepared for newly discovered antigens to use in comprehensive immunohistochemical expression analyses performed by the Pathology/Morphology Group. The mAbs are prepared by the Branch's Biochemistry Group, or in collaboration through the James R. Kerr Program with Dr. Boquan Jin (Xi'an, China) and/or Dr. Ivan Gout (Kiev, Ukraine). Extensive immunohistochemical analyses of a range of CT antigens, including MAGE-A1, MAGE-A3, MAGE-A10, NY-ESO-1, SSX2, SSX4, CT7, CT10, CT16, and non-CT antigens NY-BR-1 and Rab38 has been carried out in a large panel of normal and malignant tissues. Normal tissue expression of CT antigens was generally restricted to cells undergoing early phases of spermatogenesis. A proportion (20-40%) of a wide range of different tumor types express CT antigens in a heterogeneous/homogeneous fashion.

Earlier work with SOX Group B and ZIC2 two non-CT neuroectodermal antigens, previously identified by SEREX of small cell lung cancer (SCLC), has also been extended. The analysis of a large number of sera from SCLC patients (with or without paraneoplastic neurological disease) for seroreactivity against these antigens is being performed. This study will help shed light on the role that such antibody responses might have on patients' clinical outcome, also providing crucial information that would determine the feasibility of these antigens as potential therapeutic vaccine targets in these patients.

Cancer Vaccines

NY-ESO-1, which was discovered in the Branch using SEREX, represents a particularly promising new target antigen for vaccine development, because of its capacity to simultaneously induce both humoral and cellular immune responses in a proportion of cancer patients having NY-ESO-1-expressing tumors. A major part of the Branch's research activities on cancer vaccines is focused on this particular antigen, and several clinical vaccine trials targeting the NY-ESO-1 antigen have now been initiated. Besides safety, the main objective of these trials is to assess NY-ESO-1-specific immunity following vaccination with NY-ESO-1. For this purpose, novel types of assays have been developed, allowing sensitive and specific monitoring of humoral and both CD4 and CD8 cellular immune responses to NY-ESO-1. These developments in the monitoring techniques help define the picture of naturally occurring immune responses to NY-ESO-1 in cancer patients and provide the basis for understanding and comparing the nature of responses achieved by immunization.

Through a network of collaborations, the potential of patients with gastric, hepatocellular, breast, or esophageal cancers for participation in clinical studies with NY-ESO-1 have been assessed. With Drs. Wei-Feng Chen and Yu Wang at the Beijing Affiliate Center (China), expression of NY-ESO-1 in liver and gastric cancer was found respectively in 51% and 12% of patients, and concomitant humoral and cellular immunity to NY-ESO-1 was often observed. With the team led by Dr. Eiichi Nakayama at the Okayama Affiliate Center (Japan), esophageal and breast tumors were found to express NY-ESO-1 respectively in 33% and 42% of patients, correlating again with the ability to elicit immune responses.

Based on similar studies already performed in lung cancer, several LICR-sponsored vaccine trials with lung cancer patients are ongoing in collaboration with Dr. Nasser Altorki at Weill Medical College at Cornell University. In one of these trials, cancer patients were vaccinated with

HLA-A2-restricted peptides 157-165 from NY-ESO-1 in incomplete Freund's adjuvant and CpG. Dr. Elke Jäger (Frankfurt Clinical Trials Center), in close collaboration with Dr. Alexander Knuth (Zürich Clinical Trials Center), is coordinating a second site for this study. In addition, a cancer vaccine trial with peptide 157-165 from NY-ESO-1 in incomplete Freund's adjuvant has been initiated with Dr. Jakob Dupont (MSKCC Clinical Trials Center) in patients with ovarian cancer.

Together with Dr. Kunle Odunsi (Buffalo Clinical Trials Center, USA) and Dr. Elke Jäger, the $CD4^+$ T cell response to HLA-DP4-restricted epitope 150-174 from NY-ESO-1 has been analyzed in detail. Ovarian cancer patients with NY-ESO-1 antibody were found to have both Th1- and Th2-type pre-existing responses to this peptide. However, there was no association between the presence of NY-ESO-1 antibody and HLA-DP4 status. Based on these findings, a study has been initiated at the Buffalo Center with peptide 157-170 from NY-ESO-1 (also present in LAGE-1), to elicit or boost CD4 and potentially CD8 T cell responses in ovarian cancer patients with tumors expressing NY-ESO-1 or LAGE-1.

To assess the potential of vaccines with full-length proteins, a trial was initiated with Drs. Dean Bajorin and Padmanee Sharma at MSKCC, using NY-ESO-1 protein plus the adjuvant, BCG, in the hope of eliciting or augmenting humoral and cellular immune responses in bladder cancer patients at high risk of relapse after cystectomy. Promising immunological and clinical results had already been observed in a trial conducted at the Melbourne Branch, where vaccination with NY-ESO-1 protein formulated in ISCOMatrix™ adjuvant showed broad integrated immune responses.

Another full-length protein vaccine trial with promising results was conducted by Dr. Altorki in patients with non-small cell lung cancer. The antigen used was MAGE-3, the most commonly expressed CT antigen that represents a prime target for cancer vaccines, despite infrequent natural occurrence of MAGE-3-specific immune responses *in vivo*. Strong humoral responses to the protein in eight out of nine patients vaccinated with MAGE-3 in the presence of saponin-based adjuvant AS02B were observed, whereas only marginal titers were achieved without adjuvant. Cellular CD4 T cell responses were found in four patients who became seropositive to MAGE-3 as a result of vaccination. Fifteen patients from this original study have been re-enrolled to receive additional cycles of MAGE-3 protein vaccination with adjuvant. The potential priming or boosting effect with broader monitoring capacity taking into account any potential epitopes will be assessed. These developments provide the framework for further evaluating integrated immune responses in vaccine settings and for optimizing these responses for clinical benefit.

To better understand potential pathways leading to protein immunization, the mechanism of cross-presentation of exogenous antigens by antigen presenting cells is also being studied by the T cell Group. In collaboration with Dr. Ira Mellman (New Haven Affiliate Center, USA), immune complexes of NY-ESO-1 were studied for their capacity to be presented to T cells by antigen-presenting cells. Langerhans-like cells were found to be able to cross-present immune complexes in an interferon-γ dependent fashion, by a unique mechanism that could not be attributed to either antigen uptake nor processing machinery but did correlate with ability of the cell to mature. Mechanistic elements of NY-ESO-1 presentation were also addressed, together with Dr. Renner, with the development of mAb reagents directed to peptide 157-165 from NY-ESO-1 in the context of the HLA-A2 molecule. This mAb was able to directly label cells expressing NY-ESO-1 and HLA-A2, adding an element to the panoply of tools for monitoring NY-ESO-1 immunity. The focus is now on defining immunity to NY-ESO-1 at the tumor site and exploring whether potential regulatory T cell activity accounts for the generation and lack of activation of NY-ESO-1-specific cellular responses.

The role of CD1d restricted NKT cells on the quality and size of the adaptive immune response to soluble antigens is also of interest. In collaboration with LICR Affiliates Drs. Richard Schmidt (Konstanz Affiliate Center, Germany) and Vincenzo Cerundolo (Oxford Affiliate Center, UK),

novel analogs of the glycosphingolipid alpha-GalCer have been synthesized and studied for their capability to enhance immune responses to cancer antigens *in vitro* and *in vivo*. In addition, the structural relationship between human CD1d and alpha-GalCer has been explored. This mechanism will be explored in future clinical trials to modulate immune responses induced by vaccination with cancer antigens.

Publications

Primary Research Articles

1. Atanackovic D., Altorki N.K., Stockert E., Williamson B., Jungbluth A.A., Ritter E., Santiago D., Ferrara C.A., Matsuo M., Selvakumar A., Dupont B., Chen Y.T., Hoffman E.W., Ritter G., Old L.J., Gnjatic S. Vaccine-induced CD4+ T cell responses to MAGE-3 protein in lung cancer patients. Journal of Immunology (2004) 172(5):3289-96.
2. Arslantas E., Smith-Jones P.M., Ritter G., Schmidt R.R. TAME-HexA – A novel bifunctional chelating agent for radioimmunoimaging. European Journal Organic Chemistry (2004) 3979-3984.
3. Ayyoub M., Hesdorffer C.S., Montes M., Merlo A., Speiser D., Rimoldi D., Cerottini J.C., Ritter G., Scanlan M.J., Old L.J., Valmori D. An immunodominant SSX-2-derived epitope recognized by CD4+ T cells in association with HLA-DR. Journal for Clinical Investigation (2004) 113(8):1225-33.
4. Ayyoub M., Hesdorffer C.S., Metthez G., Stevanovic S., Ritter G., Chen Y.T., Old L.J., Speiser D., Cerottini J.C., Valmori D. Identification of an SSX-2 epitope presented by dendritic cells to circulating autologous CD4+ T cells. Journal of Immunology (2004) 172 (11): 7206-11.
5. Busam K.J., Wolchok J., Jungbluth A.A., Chapman P. Diffuse melanosis after chemotherapy-induced tumor lysis syndrome in a patient with metastatic melanoma. Journal of Cutaneous Pathology (2004) 31(3):274-80.
6. Campagnolo C., Meyers K.J., Ryan T., Atkinson R.C., Chen Y.T., Scanlan M.J., Ritter G., Old L.J., Batt C.A. Real-Time, label-free monitoring of tumor antigen and serum antibody interactions. Journal Biochemical and Biophysical Methods (2004) 61(3):283-98.
7. Chen Y.T., Gure A.O., Scanlan M.J. Serological Analysis of Expression cDNA Libraries (SEREX): An Immunoscreening Technique for Identifying Immunogenic Tumor Antigens. Methods in Molecular Medicine (2004) 103:207-16.
8. Chen Q., Jackson H., Parente P., Luke T., Rizkalla M., Tai T.Y., Zhu H.C., Mifsud N.A., Dimopoulos N., Masterman K.A., Hopkins W., Goldie H., Maraskovsky E., Green S., Miloradovic L., McCluskey J., Old L.J., Davis I.D., Cebon J., Chen W. Immunodominant CD4+ responses identified in a patient vaccinated with full-length NY-ESO-1 formulated with ISCOMATRIX adjuvant. Proceedings of the National Academy of Sciences USA (2004) 101(25):9363-8.
9. Damasceno L.M., Pla I., Chang H-J., Cohen L., Ritter G., Old L.J., Batt C.A. An optimized fermentation process for very high production of a single chain Fv antibody fragment in Pichia pastoris. Protein Expression and Purification (2004) 37:18-26.

10. Dani M.A., Dani S.U., Lima S.P., Martinez A., Rossi B.M., Soares F., Zago M.A., Simpson A.J. Less DeltamtDNA4977 than normal in various types of tumors suggests that cancer cells are essentially free of this mutation. Genetics and Molecular Research (2004) 3(3):395-409.

11. Davis I.D., Chen W., Jackson H., Parente P., Shackleton M., Hopkins W., Chen Q., Dimopoulos N., Luke T., Murphy R., Scott A.M., Maraskovsky E., McArthur G., MacGregor D., Sturrock S., Tai T.Y., Green S., Cuthbertson A., Maher D., Miloradovic L., Mitchell S.V., Ritter G., Jungbluth A.A., Chen Y.T., Gnjatic S., Hoffman E.W., Old L.J., Cebon J.S. 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 (2004) 20;101(29):10697-702.

12. Deckert P.M., Bornmann W.G., Ritter G., Williams C. Jr, Franke J., Keilholz U., Thiel E., Old L.J., Bertino J.R., Welt S. Specific tumour localisation of a huA33 antibody--carboxypeptidase A conjugate and activation of methotrexate-phenylalanine. International Journal of Oncology (2004) 24(5):1289-95.

13. Divgi C.R., O'Donoghue J.A., Welt S., O'Neel J., Finn R., Motzer R.J., Jungbluth A., Hoffman E., Ritter G., Larson S.M., Old L.J. Phase I clinical trial with fractionated radioimmunotherapy using 131I-labeled chimeric G250 in metastatic renal cancer. Journal of Nuclear Medicine (2004) 45(8):1412-21.

14. Ferreira E.N., Pires L.C., Parmigiani R.B., Bettoni F., Puga R.D., Pinheiro D.G., Andrade L.E., Cruz L.O., Degaki T.L., Faria M. Jr, Festa F., Giannella-Neto D., Giorgi R.R., Goldman G.H., Granja F., Gruber A., Hackel C., Henrique-Silva F., Malnic B,. Manzini C.V., Marie S.K., Martinez-Rossi N.M., Oba-Shinjo S.M., Pardini M.I., Rahal P., Rainho C.A., Rogatto S.R., Romano C.M., Rodrigues V., Sales M.M., Savoldi M., da Silva I.D., da Silva N.P., de Souza S.J., Tajara E.H., Silva W.A. Jr, Simpson A.J., Sogayar M.C., Camargo A.A., Carraro D.M. Identification and complete sequencing of novel human transcripts through the use of mouse orthologs and testis cDNA sequences. Genetics and Molecular Research (2004) 3(4):493-511.

15. Fujita S., Wada H., Jungbluth A.A., Sato S., Nakata T., Noguchi Y., Doki Y., Yasui M., Sugita Y., Yasuda T., Yano M., Ono T., Chen Y.T., Higashiyama M., Gnjatic S., Old L.J., Nakayama E., Monden M. NY-ESO-1 expression and immunogenicity in esophageal cancer. Clinical Cancer Research (2004) 10(19):6551-8.

16. Held G., Matsuo M., Epel M., Gnjatic S., Ritter G., Lee S.Y., Tai T.Y., Cohen C.J., Old L.J., Pfreundschuh M., Reiter Y., Hoogenboom H.R., Renner C. Dissecting cytotoxic T cell responses towards the NY-ESO-1 protein by peptide/MHC-specific antibody fragments. European Journal of Immunology (2004) 34(10):2919-29.

17. Huarte E., Karbach J., Gnjatic S., Bender A., Jäger D., Arand M., Atanackoviv D., Skipper J., Ritter G., Chen Y.T. , Old L.J., Knuth A., Jäger E. HLA-DP4 expression and immunity to NY-ESO-1: correlation and characterization of cytotoxic CD4+ CD25- CD8- T cell clones. Cancer Immunity (2004) 4:15.

18. Jackson H.M., Dimopoulos N., Chen Q., Luke T., Yee Tai T., Maraskovsky E., Old L.J., Davis I.D., Cebon J., Chen W. A robust human T-cell culture method suitable for monitoring CD8+ and CD4+ T-cell responses from cancer clinical trial samples. Journal of Immunological Methods (2004) 291(1-2):51-62.

19. Johns T.G., Adams T.E., Cochran J.R., Hall N.E., Hoyne P.A., Olsen M.J., Kim Y.S., Rothacker J., Nice E.C., Walker F., Ritter G., Jungbluth A.A., Old L.J., Ward C.W., Burgess A.W., Wittrup K.D., Scott A.M. Identification of the Epitope for the Epidermal Growth Factor Receptor-

specific Monoclonal Antibody 806 Reveals That It Preferentially Recognizes an Untethered Form of the Receptor. Journal of Biological Chemistry (2004) 279(29):30375-30384.

20. Joosten C.E., Cohen L.S., Ritter G., Batt C.A., Shuler M.L. Glycosylation profiles of the human colorectal cancer A33 antigen naturally expressed in the human colorectal cancer cell line SW1222 and expressed as recombinant protein in different insect cell lines. Biotechnology Progress (2004) 20(4):1273-9.

21. Lee S.Y., Williamson B., Caballero O.L., Chen Y.T., Scanlan M.J., Ritter G., Jongeneel C.V., Simpson A.J., Old L.J. Identification of the gonad-specific anion transporter SLCO6A1 as a cancer/testis (CT) antigen expressed in human lung cancer. Cancer Immunity (2004) 17:4.

22. Lopes L.F., Piccoli Fde S., Paixao V.A., Latorre Mdo R., Camargo B., Simpson A.J., Caballero O.L. Association of CYP3A4 genotype with detection of Vgamma/Jbeta trans-rearrangements in the peripheral blood leukocytes of pediatric cancer patients undergoing chemotherapy for ALL. Leukemia Research (2004) 28(12):1281-6.

23. Matsuo M., Nagata Y., Sato E., Atanackovic D., Valmori D., Chen Y.T., Ritter G., Mellman I., Old L.J., Gnjatic S. IFN-gamma enables cross-presentation of exogenous protein antigen in human Langerhans cells by potentiating maturation. Proceedings of the National Academy of Sciences USA (2004) 101(40):14467-72.

24. Prasad M.L., Jungbluth A.A., Patel S.G., Iversen K., Hoshaw-Woodard S., Busam K.J. Expression and significance of cancer testis antigens in primary mucosal melanoma of the head and neck. Head Neck (2004) 26(12):1053-7.

25. Qian F., Gnjatic S., Jager E., Santiago D., Jungbluth A., Grande C., Schneider S., Keitz B., Driscoll D., Ritter G., Lele S., Sood A., Old L.J., Odunsi K. Th1/Th2 CD4+ T cell responses against NY-ESO-1 in HLA-DPB1*0401/0402 patients with epithelial ovarian cancer. Cancer Immunity (2004) 4:12.

26. Schreiber R.D., Old L.J., Hayday A.C., Smyth M.J. Response to 'A cancer immunosurveillance controversy. Nature Immunology (2004) 5(1):4-5.

27. Shang X.Y., Chen H.S., Zhang H.G., Pang X.W., Qiao H., Peng J.R., Qin L.L., Fei R., Mei M.H., Leng X.S., Gnjatic S., Ritter G., Simpson A.J., Old L.J., Chen W.F. The spontaneous CD8+ T-cell response to HLA-A2-restricted NY-ESO-1b peptide in hepatocellular carcinoma patients. Clinical Cancer Research (2004) 10(20):6946-55.

28. Sugita Y., Wada H., Fujita S., Nakata T., Sato S., Noguchi Y., Jungbluth A.A., Yamaguchi M., Chen Y.T., Stockert E., Gnjatic S., Williamson B., Scanlan M.J., Ono T., Sakita I., Yasui M., Miyoshi Y., Tamaki Y., Matsuura N., Noguchi S., Old L.J., Nakayama E., Monden M. NY-ESO-1 expression and immunogenicity in malignant and benign breast tumors. Cancer Research (2004) 64(6):2199-20.

29. Suzuki H., Katayama N., Ikuta Y., Mukai K., Fujieda A., Mitani H., Araki H., Miyashita H., Hoshino N., Nishikawa H., Nishii K., Minami N., Shiku H. Activities of granulocyte-macrophage colony-stimulating factor and interleukin-3 on monocytes. American Journal of Hematology (2004) 75(4):179-89.

30. Tammela J., Jungbluth A.A., Qian F., Santiago D., Scanlan M.J., Keitz B., Driscoll D., Rodabaugh K., Lele S., Old L.J., Odunsi K. SCP-1 cancer/testis antigen is a prognostic indicator and a candidate target for immunotherapy in epithelial ovarian cancer. Cancer Immunity (2004) 18:4:10.

31. Vaughan H.A., St Clair F., Scanlan M.J., Chen Y.T., Maraskovsky E., Sizeland A., Old L.J.,

Cebon J. The humoral immune response to head and neck cancer antigens as defined by the serological analysis of tumor antigens by recombinant cDNA expression cloning. Cancer Immunity (2004) 16:4:5.

32. Vaughan H.A., Svobodova S., MacGregor D., Sturrock S., Jungbluth A.A., Browning J., Davis I.D., Parente P., Chen Y.T., Stockert E., St Clair F., Old L.J., Cebon J. Immunohistochemical and molecular analysis of human melanomas for expression of the human cancer-testis antigens NY-ESO-1 and LAGE-1. Clinical Cancer Research (2004) 10(24):8396-404.
33. Wang Y., Wu X.J., Zhao A.L., Yuan Y.H., Chen Y.T., Jungbluth A.A., Gnjatic S., Santiago D., Ritter G., Chen W.F., Old L.J., Ji J.F. Cancer/testis antigen expression and autologous humoral immunity to NY-ESO-1 in gastric cancer. Cancer Immunity (2004) 4:11.
34. Wang Y., Liang Z., Yuan Y.H., Han Y., Liu Y.X., Liu N., Chen Y.T., Ritter G., Old L.J., Zhang L.J. Expression of multiple cancer-testis antigen genes in non-small cell lung cancer treated by chemotherapy prior surgery. National Medical Journal of China (2004) 84(6):464-8.

Reviews / Commentaries / Book Chapters

1. Luftl M., Schuler G., Jungbluth A.A. Melanoma or not? Cancer testis antigens may help. British Journal of Dermatology (2004) 51(6):1213-8.
2. Dunn G.P., Old L.J., Schreiber R.D. The three Es of cancer immunoediting. Annual Review Immunology (2004) 22:329-60.
3. Dunn G.P., Old L.J., Schreiber R.D. The immunobiology of cancer immunosurveillance and immunoediting. Immunity (2004) 21(2):137-48.
4. Scanlan M.J., Simpson A.J., Old L.J. The cancer/testis genes: review, standardization, and commentary. Cancer Immunity (2004) 23:4:1.
5. Strausberg R.L., Simpson A.J., Old L.J., Riggins G.J. Oncogenomics and the development of new cancer therapies. Nature (2004) 429:469-474.
6. Skipper J., Hoffman E.W., O'Donnell-Tormey J., Old L.J. Translation of cancer immunotherapies. Nature Medicine (2004) 10(11):1154-5.

LUDWIG INSTITUTE FOR CANCER RESEARCH

San Diego Branch
of Cancer Genetics

San Diego, USA

Staff List

Cavenee, Webster K. *Director, Member*

Laboratory Of Tumor Biology

Cavenee, Webster K. *Member*

Section of Human Carcinogenesis

Furnari, Frank *Assistant Investigator*
Okumura, Koichi *Postgraduate Researcher*
Bonavia, Rudy *Postdoctoral Fellow*
Lin, Xinyi *Postgraduate Researcher*
Mukasa, Akitake *Postdoctoral Fellow*
Harding, Timothy *Associate Research Staff (from May)*
Steele, Julianne *Associated Research Staff (until May)*

Section of Molecular Cytogenetics

Arden, Karen *Associate Investigator*
Hosaka, Taisuke *Postdoctoral Fellow*
Lu, Wen *Postdoctoral Fellow*
Boyer, Antonia *Associate Research Staff*
Bishop, David *Associate Research Staff (until July)*
Smith, Wendy *Graduate Student*

Laboratory of Cancer Genetics

Kolodner, Richard *Member*
Enserink, Jorrit *Postdoctoral Fellow (from May)*
Gazzoli, Isabella *Post Graduate Researcher (until June)*
Hess, Martin *Postdoctoral Fellow*
Huang, Meng Er *Visiting Research Scientist I*
Mazur, Daniel *Postdoctoral Fellow*
Pennaneach, Vincent *Post Graduate Researcher*
Putnam, Christopher *Postdoctoral Fellow*
Schmidt, Kristina *Post Graduate Researcher*
Cassel, Dena *Associate Research Staff (until April)*
Harries, Karin Cecelia *Associate Research Staff*
Martinez-Barragan, Sandra *Associate Research Staff*
Mueller, James *Associate Research Staff*
Nguyen, Ann Thu *Laboratory Support Staff*
Valerio, Gabriel *Laboratory Support Staff (June to September)*
Harrington, Jill *Graduate Student*
Kats, Ellen *Graduate Student*
Mendillo, Marc *Graduate Student*
Nardin, Stephanie *Graduate Student*
Shell, Scarlet *Graduate Student*

Laboratory of Cell Biology

Cleveland, Don *Member*
Black, Ben *Postdoctoral Fellow*
Boillee, Severine *Postdoctoral Fellow*
Bomont, Pascale *Postdoctoral Fellow*
Foltz, Dan *Postdoctoral Fellow*
Fujimoto, Jiro *Visiting Research Scholar I*
Garcia, Michael *Postdoctoral Fellow*
Jansen, Lars *Postdoctoral Fellow (from February)*
Kops, Geert *Postdoctoral Fellow (until November)*
Lobsiger, Christian *Postgraduate Researcher*
Mao, Yinghui *Postdoctoral Fellow*
McAlonis-Downes, Melissa *Associate Research Staff (from May)*
Miller, Timothy *Postdoctoral Fellow*
Shah, Jagesh *Postdoctoral Fellow*
Van de Velde, Christine *Postgraduate Researcher*
Weaver, Beth *Postdoctoral Fellow*
Wang, Yin *Associate Research Staff*
Ward, Christopher *Associate Research Staff (until August)*
Yamanaka, Koji *Postgraduate Researcher*
Kim, Yumi *Graduate Student (from July)*
Roy, Barnali *Undergraduate Intern (until September)*
Roy, Tamal *Undergraduate Intern (from September)*
Silk, Alain *Graduate Student*

Laboratory of Chromosome Biology

Desai, Arshad *Assistant Member*
Cheeseman, Iain *Postdoctoral Fellow*
Kline-Smith, Susan *Postdoctoral Fellow (from July)*
Maddox, Paul *Postdoctoral Fellow*
Hyndman, Francie *Associate Research Staff*
Essex, Anthony *Graduate Student (from July)*
Monen, Joost *Graduate Student*
Sandall, Sharsti *Graduate Student*

Laboratory of Gene Regulation

Ren, Bing *Assistant Member*
Kim, Tae Hoon *Postdoctoral Fellow*
Li, Zirong *Post Graduate Researcher*
Wang, Kun *Postdoctoral Fellow (from February)*
Ching, Christina *Laboratory Support Staff (from January)*
Erickson, Lindsay *Laboratory Support Staff (from January)*
Ke, Eugene *Graduate Student (from September)*
Barrera, Leah *Graduate Student*
Heintzman, Nathaniel *Graduate Student*
Maynard, Nathaniel *Graduate Student*
Stuart, Rhona *Associate Research Staff (from May)*
Qu, Chunxu *Associate Research Staff*
Van Calcar, Sara *Associate Research Staff*

Laboratory of Mitotic Mechanisms

Oegema, Karen *Assistant Member*
Audhya, Anjon *Postdoctoral Fellow*
Dammermann, Alexander *Postdoctoral Fellow*
Maddox, Amy *Postdoctoral Fellow*
Yong, Ji *Laboratory Support Staff (from July)*
Lewellyn, Lindsay *Graduate Student (from July)*
Portier, Nathan *Graduate Student*
Davis, Rochelle *Undergraduate Intern*
Hsien, Jennifer *Undergraduate Intern (until September)*

Laboratory of Proteomic Biology

Zhou, Huilin *Assistant Member*
Gao, Lu *Post Graduate Researcher*
Smolka, Marcus *Postdoctoral Fellow*
Zhou, Weidong *Post Graduate Researcher*
Ryan, Jennifer *Associated Research Staff*
Chang, Justin *Graduate Student (until June)*
Ponte-de-Albuquerque, Claudio *Graduate Student*
Chen, Sheng-hong *Graduate Student (from July)*
Kwo, Henry *Undergraduate Intern (until May)*
Xiao, Wei *Undergraduate Intern (June to December)*

Technical Support

Reigle, Brian *Technical Support LAN Services*
Weger, John *Research Associate (Core Sequencing) (until June)*
Ness, Stefanie *Associated Research Staff (Core Sequencing)*
Wu, Joann *Associated Research Staff (Core Sequencing)*

Administration & Secretariat

Peacock, Dianne *Branch Administrator*
McPhail, Natalie *Branch Controller*
Goulding, Ruth *Branch Grants Accounting Manager*
Munoz-Mack, Alma *Branch Grants Accounting Manager*
Cramer, Michelle *Branch Human Resources Manager (until April)*
Gray, Barbara *Accounts Payable Specialist*
Inglese, Jane *Payroll and Benefits Manager*
Cardiel, Blanca A. *Grants Accountant*
Garrett, Delvin *Grants Accountant*
Callahan, Kimberly *GL Accountant (until May)*
Vergara, Christy *Administrative Assistant*
Birk, Heather *Administrative Assistant*
Kern, Eric *Administrative Assistant*
Montgomery, Ann *Administrative Assistant*
Van Roekel, Alexis *Administrative Assistant*

Branch Director's Report

This year was the first for some time that the Branch has not been bringing in new laboratories. We await the triage that will occur upon the opening of the new UCSD School of Pharmaceutical Sciences building and the resultant acquisition of our next tranche of space in our present building. That pause has allowed us to assure that our Assistant Members receive the attention and mentoring they need to solidify the establishment of their research groups. This has been successfully accomplished: each of them is publishing papers in high visibility journals, each has secured external competitive funding and each has attracted a group of outstanding graduate students and postdoctoral fellows. The Branch is well-placed for its ongoing success in the future with these stellar groups. It is also clear that the Branch continues as a vibrant and challenging environment for training and internationally recognized research.

During 2004, we have continued to make discoveries of importance; some of these are summarized in the text of this Report. Branch staff received international awards and honors and we continue in roles of significance in scientific societies and journal editorial and program review responsibilities. Many postdoctoral fellows and graduate students have moved on to independent positions in academia or industry after having finished their training with us. Moreover, there are increasing examples of collaborative interactions between and among our research groups both in the Branch and with other national and international investigators. Another measure of our success is the large and steadily increasing level of competitive grant support won by Branch staff. The quality, accomplishment and breadth of expertise of Branch staff has been praised and welcomed by The Dean of our Medical School, Dr. Edward Holmes, as well as the Chairs of our academic Departments, and our partnership with the University has never been stronger. We look forward to our future with great optimism.

Webster K. Cavenee

Research Report

Laboratory of Tumor Biology

The Laboratory is headed by Dr. Webster Cavenee and comprises two Sections with complementary interests. The *Section of Molecular Cytogenetics* is led by Dr. Karen Arden and is focused on three general areas. The first is the etiologic role of the alveolar rhabdomyosarcoma-associated PAX3-FKHR fusion gene. Discoveries this year suggest this tumor-specific fusion gene, together with additional specific genetic changes, play a significant role in the malignant conversion of normal cells. Ongoing progress is being made to identify PAX3-FOXO1 target genes and to investigate the mechanisms by which these genes are regulated. The second is the normal function of the forkhead FOXO sub-family of genes, each of which has been associated with cancer. Foxos are regulated by multiple protein modifications, such as phosphorylation (by many kinases including Akt, SKG, CK1, DYRK1, IKK, ROCK), acetylation (by CBP/p300, PCAF), deacetylation (by SIRT1), and ubiquitination. Progress this year has focused on the regulation of these transcription factors by signaling pathways other than those previously described. The third area is the identification of new markers of prostate cancer. Work published this year not only identified new markers of prostate cancer but also suggested that tumor-associated benign prostatic hypertropy (BPH) and stroma exhibit changes in gene expression that can be used to determine, indirectly, the presence of tumor cells in the prostate. The *Section of Human*

Carcinogenesis, led by Dr. Frank Furnari, is investigating two prevalent genetic alterations involved in advanced stage glioma; the commonly amplified and truncated epidermal growth factor receptor gene (ΔEGFR) and mutation of the phosphatase and tensin (PTEN) homology gene. In the case of the ΔEGFR, its importance in driving tumor maintenance and initiation has been discovered using a doxycycline-regulation *in vivo*. To identify mechanisms whereby the PTEN protein might be regulated or aid in the regulation of transformation through protein-protein interactions, several interacting proteins have been identified and characterized. One example is MSP58 (58-kDa microspherule protein). Introduction of MSP58 into *pten*$^{(-/-)}$ mouse embryonic fibroblasts (MEFs) resulted in transformation, however concurrent introduction of wild-type PTEN caused a dramatic reduction in the number of MSP58-induced colonies. This inhibition required interaction with PTEN but not for it to be catalytically active.

Laboratory of Cancer Genetics

The Laboratory, headed by Dr. Richard D. Kolodner is focused on three general areas: 1) the use of the yeast *Saccharomyces cerevisiae* as a model organism for identification of genes that function to suppress the accumulation of mutations and other types of genome rearrangements in DNA; 2) the development of mice containing mutations in genes that suppress the accumulation of mutations and other types of genome rearrangements in DNA and the analysis of the role of these genes in suppressing cancer susceptibility; and, 3) gaining an understanding of the role of defects in these genes in inherited and sporadic cancers in humans. Genetic studies have led to the demonstration that germ line mutations in human genes encoding the major DNA mismatch repair system cause an inherited cancer susceptibility syndrome, HNPCC. In addition, some sporadic cancers develop defects in the mismatch repair gene *MLH1* at an early step in tumorigenesis. Using the *S. cerevisiae* model system, the laboratory has now overproduced and purified most of the proteins known to function in mismatch repair and has begun to characterize them. A key result that has come from these studies has been the development of conditions under which the MLH1-PMS1 complex can assemble onto the MSH2-MSH6 complex that has been loaded onto a mispaired base in DNA thus reconstituting a key step in the recognition of mispaired bases in DNA. In the continuation of human genetics studies, a scheme for the genetic analysis of HNPCC families has been developed that allows detection of disease causing germ line mutations in mismatch repair genes in more than 95% of bona fide HNPCC families. To understand the other major type of cancers, which are characterized by ongoing genome instability, the Laboratory has developed a novel mutator assay in *S. cerevisiae*, which allows detection of genome rearrangements and has used this assay to define the genes and pathways that prevent genome instability. Recent experiments using this system have shown that enzymes that detoxify reactive oxygen species play a key role in preventing genome instability and have defined the key mechanisms by which rearranged chromosomes are formed. Finally, by making targeted mutations in mice, it has been demonstrated that a Rfa1 mutation, found to cause increased genome instability in *S. cerevisiae*, causes both increased genome instability and increased development of cancers in mice.

Laboratory of Cell Biology

The Laboratory, headed by Dr. Don Cleveland, is focused on two general areas: how centromeres are specified and how they function to faithfully deliver one copy of each chromosome into each daughter cell at cell division; and, the molecular genetics of axonal growth and mechanisms of human motor neuron disease, especially the disease, familiarly known as Lou Gehrig's disease, or amyotrophic lateral sclerosis (ALS). Although the centromere governs chromosomal inheritance, this unique genetic element is not defined by DNA sequence. In the last year, the Laboratory demonstrated that a piece of the centromere-specifying epigenetic mark is through assembly of centromeric chromatin that is structurally more rigid than other nucleosomes. Centromeres are

also the signaling device for the mitotic checkpoint, the major mechanism controlling cell cycle advance during mitosis. The Laboratory has identified the mechanism of activation and silencing of this essential mammalian checkpoint and demonstrated that inhibition of the checkpoint is a promising target for cancer therapy. A second interest is in the mechanism leading to selective killing of motor neurons in ALS. In almost every example of inherited human neurodegenerative disease, the question of selective vulnerability of individual neurons has been perplexing, since the mutant gene products are widely expressed. Using modeling in mice, selective killing of motor neurons in inherited ALS was demonstrated to be accompanied by damage from disease causing mutant proteins aggregating onto and damaging mitochondria. Since this occurs only in tissues at risk, this implies selective differences in mitochondria within those cells, offering an explanation for selectivity in cell killing from these ubiquitously expressed mutants.

Laboratory of Chromosome Biology

The Laboratory, headed by Dr. Arshad Desai, is focused on understanding the mechanisms that segregate chromosomes during cell division. Specifically, the group is analyzing kinetochores, macromolecular assemblies that form on chromosomes to connect them to spindle microtubules during cell division. The kinetochore-microtubule connection is central to accurate segregation of chromosomes and prevention of aneuploidy. This interface is also the target of anti-mitotic chemotherapeutic drugs, such as taxol. In the past year, the laboratory has identified a conserved protein network, comprised of 10 interacting proteins, that is required for assembly and function of the outer domains of the kinetochore that interact with spindle microtubules. Using *in vivo* functional assays, the laboratory has defined the precise contributions of each component to kinetochore function in *Caenorhabditis elegans* and is extending analysis of selected homologous proteins to human cells. The mechanisms that specify kinetochore formation at a localized site on chromosomes are also being studied. The specification event involves formation of specialized chromatin containing a histone H3 variant. This specialized chromatin domain is proposed to be propagated by DNA replication and epigenetically direct assembly of kinetochores. The laboratory has evidence suggesting that deposition of this histone variant occurs *de novo* following fertilization in *C. elegans*, allowing unprecedented access for analysis of its targeting. Using an unbiased functional genomic strategy, a novel protein involved in this deposition was identified and the process of defining its mechanism of action has begun. Additionally, a requirement for the specialized chromatin formed by this histone variant in mitotic chromosome structure in the *C. elegans* embryo has been identified.

Laboratory of Gene Regulation

The Laboratory, headed by Dr. Bing Ren, is interested in understanding how the complex gene regulatory networks in mammalian cells control cellular proliferation and differentiation. The research is divided into two general areas: (1) Identification and characterization of transcriptional regulatory elements, such promoters, enhancers, repressor/silencing elements, in the human genome; (2) Finding cellular target genes of various oncogenic pathways in an effort to understand the molecular mechanisms of tumorigenesis. In 2004, the Laboratory used an unbiased genome wide location analysis approach to identify the target genes for the β-catenin pathway in colon cancers. One of the novel target genes identified encodes endothelin-1, a small peptide known to be overexpressed in colon cancers and many other solid tumors. The Laboratory further demonstrated that Endothelin-1 plays a key role in β-catenin's oncogenic transforming activity, as it maintains cells survival and has an anti-apoptosis function. The discovery provided a basis for using Endothelin-1 as an early diagnostic marker for colon cancers, and point to the values of Endothelin-1 receptor antagonists as potential therapeutics for treating the disease. The Laboratory also developed genome-tiling arrays to systematically map human promoters in the

genome. More than 10,000 active promoters in the primary human fibroblast IMR90 cells were identified. Analysis of the sequence features of these promoters revealed a general link between human promoters and CpG island sequences, and that the TATA box is not a general feature of human promoters.

Laboratory of Mitotic Mechanisms

The Laboratory, headed by Dr. Karen Oegema, is focused on understanding the morphogenetic transformations required for cell division. The Laboratory uses the early embryo of the soil nematode *Caenorhabditis elegans* as a model system because of the advantages it offers for the molecular analysis of mitosis. RNA-mediated interference (RNAi) in *C. elegans* makes it feasible to analyze the first mitotic division of embryos depleted of any targeted gene product. The group is combining RNAi-based functional genomics with single-cell high-resolution microscopy assays and biochemical characterization of native protein complexes to study three aspects of cell division: centrosome duplication and maturation; cleavage furrow assembly and membrane dynamics during cytokinesis; and, kinetochore specification and assembly (a collaboration with Dr. Arshad Desai). In 2004, the roles of two homologs of the actin binding protein anillin were characterized. One is required for cortical contractility during early embryogenesis and contributes to the stability of the cleavage furrow. The second anillin is specifically required for gonad structure and oogenesis. A novel fluorescence-microscopy based assay to monitor chromosome condensation was also developed. This assay was used to show that the mitotic kinase Aurora A, frequently overexpressed in cancers, is required to maintain appropriate timing between chromosome condensation and nuclear envelope breakdown. This function is at least in part independent of its role in centrosome assembly. A previously uncharacterized protein that links cytokinesis with the metabolism of RNAs maternally loaded into oocytes was also characterized.

Laboratory of Proteomic Biology

The Laboratory, headed by Dr. Huilin Zhou, focuses on two general areas: 1) signal transduction mechanisms of the DNA damage response in yeast and human cells; and 2) development of proteomic technologies for the identification of kinase substrates and detection of dynamic protein-protein interactions by mass spectrometry. Using *S. cerevisiae*, the Laboratory has applied a new quantitative proteomics method, N-isotag technology, to identify and quantify DNA damage induced phosphorylation to the key components of the kinase cascade in the DNA damage response. They include Rad53, Dun1, Rad9, Mrc1, Mec1 and others. The Laboratory is currently investigating the regulation and function of these proteins, using a combination of *in vitro* biochemistry and *in vivo* genetic and cell biological analyses. A new proteomic technology, PATH, has been developed and applied to the analysis of Rad53 in yeast and Chk2 in human, and a number of novel regulatory proteins and substrates of these kinases, which include proteins involved in gene transcription and cell division, have been identified. The Laboratory has begun to investigate the biochemical basis of substrate recognition for Rad53 and Chk2, and to dissect their functions in detail. The PATH technology is expected to be generally useful to characterize transient protein-protein interactions frequently found in kinases, phosphatase and other enzymes. The laboratory is beginning to apply the PATH technology to study signaling pathways in the DNA damage response and cell cycle regulation.

Publications

Primary Research Articles

1. Audhya A, Loewith R, Parsons AB, Gao L, Tabuchi M, Zhou H, Boone C, Hall MN and Emr SD. Genome-wide Lethality Screen Identifies New PI4,5P(2) Effectors that Regulate the Actin Cytoskeleton. The EMBO Journal (2004) 23:3747-57.
2. Bandipalliam B, Garber J, Kolodner RD and Syngal S. Clinical Presentation Correlates With the Type of Mismatch Repair Gene Involved in HNPCC. Gastroenterology (2004) 126: 936-7.
3. Black BE, Foltz DR, Chakravarthy S, Luger K, Woods VL and Cleveland DW. Structural Determinants for Generating Centromeric Chromatin. Nature (2004) 430: 578-82.
4. Cheeseman I, Niessen S, Anderson S, Hyndman F, Yates J, Oegema K and Desai A. A Conserved Protein Network Controls Assembly of the Outer Kinetochore and Its Ability to Sustain Tension. Genes and Development (2004) 18:2255-68.
5. Cheeseman I and Desai A. A Combined Approach for the Localization and Tandem Affinity Purification of Protein Complexes from Metazoans. Science (2005) STKE. pl1.
6. Dammerman A, Pelletier L, Muller-Reichert T, Desai A and Oegema K. (2004) Centriole Assembly Requires Both Centriolar and Pericentriolar Material Proteins. Developmental Cell (2004) 7:815-29.
7. ENCODE Project Consortium. The ENCODE (ENCyclopedia Of DNA Elements) Project. Science (2004) 306: 636-40.
8. Hosaka T, Biggs III WH, Tieu D, Boyer AD, Varki NM, Cavenee WK and Arden KC. Disruption of Forkhead Transcription Factor (Foxo) Family Members in Mice Reveals Their Functional Diversification. Proceedings of the National Academy of Sciences USA (2004) 101: 2975-80.
9. Kim TH, Xiong H, Zhang Z and Ren B. Beta-Catenin Activates the Growth Factor Endothelin-1 in Colon Cancer Cells. Oncogene. (2004)Nov 22; [Epub ahead of print]
10. Kops GJPL, Foltz DR and Cleveland DW. Lethality to Human Cancer Cells Through Massive Chromosome Loss by Inactivation of the Mitotic Checkpoint. Proceedings of the National Academy of Sciences USA (2004) 101: 8699-704.
11. Liu J, Lillo C, Jonsson PA, Vande Velde C, Miller TM, Subramaniam JR, Rothstein JD, Marklund S, Andersen PM, Brannstrom T, Wong PC, Williams DS and Cleveland DW. Toxicity of Familial ALS-linked SOD1 Mutants from Selective Recruitment to Spinal Mitochondria. Neuron (2004) 43: 5-17.
12. Mazina OM, Mazin AV, Nakagawa T, Kolodner RD and Kowalczykowski SC. The *Saccharomyces cerevisiae* Mer3 Helicase Stimulates 3' to 5' DNA Heteroduplex Extension in Rad51-Mediated Pairing. Cell (2004) 117: 47-56.
13. Mitchison TJ, Maddox P, Groen A, Cameron L, Perlman Z, Ohi R, Desai A, Salmon ED and Kapoor TM. Bipolarization and Poleward Flux Correlate During Xenopus Extract Spindle Assembly. Molecular Biology of the Cell (2004) 15:5603-15.
14. Myung K, Smith S and Kolodner RD. Mitotic Checkpoints Function in the Formation of Gross Chromosomal Rearrangements in Saccharomyces Cerevisiae Proceedings of the National Academy of Sciences USA (2004) 101: 15980-5.

15. Nishikawa R, Suigiyama T, Narita Y, Furnari F, Cavenee WK and Matsutani M. Immunohistochemical Analysis of the Mutant Epidermal Growth Factor, ΔEGFR, in Glioblastoma, Brain Pathology (2004) 21: 53-6.

16. Pennaneach V and Kolodner RD. Recombination and the Tel1 and Mec1 Checkpoints Differentially Effect Genome Rearrangements Driven by Telomere Dysfunction in *Saccharomyces cerevisiae*. Nature Genetics (2004) 36: 612-7.

17. Putnam CD, Pennaneach V and Kolodner RD. Chromosome Healing Through Terminal Deletions Generated by De Novo Telomere Additions in Saccharomyces Cerevisiae. Proceedings of the National Academy of Sciences USA (2004) 101: 13262-7.

18. Schmidt KH and Kolodner RD. Requirement of Rrm3 Helicase for Recombinational Repair of Spontaneous DNA Lesions in Cells Lacking Srs2 or Sgs1 Helicases. Molecular and Cellular Biology (2004) 24: 3213-26.

19. Schmidt MHH, Furnari FB, Cavenee WK, Dikic I and Bogler O. Alix/AIP1 Interacts Constitutively with EGFR and Antagonizes Its Downregulation By the Cbl-SETA/CIN85 Complex. Molecular and Cellular Biology (2004) 24: 8981-93.

20. Shah JV, Botvinick E, Bonday Z, Furnari F, Berns M and Cleveland DW. Dynamics of centromere and kinetochore proteins: implications for checkpoint signaling and silencing. Current Biology (2004) 14: 942-52.

21. Stuart RO, Wachsman W, Berry CC, Wang-Rodriquez J, Wasserman L, Masys D, Arden K, Goodison S, McClelland M, Wang Y, Sawyers A, Kalcheva I, Tarin D and Mercola D. *In Silico* Cell-Type-Associated Patterns of Gene Expression in Prostate Cancer. Proceedings of the National Academy of Sciences USA (2004) 101: 615-20.

22. Tajima A, Hess MT, Cabrera BL, Kolodner RD and Carethers JM. The Mismatch Repair Complex hMutSα Recognizes 5-Fluoruracil-modified DNA: Implications for Chemosensitivity and Resistance. Gastroenterology (2004) 127:1678-84.

23. Tornaletti S, Maeda LS, Kolodner RD and Hanawalt PC. Effect of 8-Oxoguanine on Transcription Elongation by T7 RNA Polymerase and Mammalian RNA Polymerase II. DNA Repair (2004) 3:483-94.

24. Vande Velde C, Garcia ML, Yin X, Trapp BD and Cleveland DW. The Neuroprotective Factor Wlds Does Not Attenuate Mutant SOD1-mediated Motor Neuron Disease. NeuroMolecular Medicine (2004) 5: 193-204.

25. Wang H, Hu X, Ding X, Dou Z, Yang Z, Shaw AW, Teng M, Cleveland DW, Goldberg ML, Niu L, and Yao X. Human Zwint-1 Specifies Localization of Zweste White 10 to Kinetochores and is Essential for Mitotic Checkpoint Signaling. Journal of Biological Chemistry (2004) 279: 54590-8.

26. Yang G, Scherer SJ, Shell S, Yang K, Kim M, Lipkin M, Kucherlapati R, Kolodner RD and Edelmann, W. Dominant Effects of the Msh6T1217D Amino Acid Substitution of DNA Mismatch Repair, DNA Damage Responses and Cancer Susceptibility. Cancer Cell (2004) 6: 139-50.

27. Zhou W, Ryan JJ and Zhou H. Global Analyses of Sumoylated Proteins in Saccharomyces Cerevisiae. Induction of Protein Sumoylation by Cellular Stresses. Journal of Biological Chemistry (2004) 279: 32262-8.

Reviews / Commentaries / Book Chapters

1. Accili D and Arden KC. FoxOs at the Crossroads of Cellular Metabolism, Differentiation and Transformation. Cell (2004) 117: 421-6.
2. Arden KC. FoxO: Linking New Signaling Pathways. Molecular Cell (2004) 14: 416-8.
3. Bögler O and Cavenee WK. Methylation and Genomic Damage in Gliomas. In: Genomic and Molecular Neuro-Oncology. W Zhang and G Fuller, eds. (2004) Jones and Bartlett Publishers, Sudbury, MA, pp. 3-16.
4. Boillee S and Cleveland DW. Gene Therapy for ALS Delivers. Trends in Neurosciences (2004) 27: 235-8.
5. Bruijn LI, Miller TI and Cleveland DW. Unraveling the Mechanisms Involved in Motor Neuron Degeneration in ALS. Annual Review of Neurosciences (2004) 27: 723-49.
6. Cavenee WK. Tumor Suppressor Genes. In: Cancer Research – An Encyclopedic Reference. (2004) Springer Verlag Publishers, Berlin.
7. Cheeseman I and Desai A. Cell Division: AAAtacking the Mitotic Spindle. Current Biology (2004) 14:R70-2.
8. Cheeseman I and Desai A. Are You Feeling Tense? Nature (2004) 428:32-3. (News & Views)
9. Encyclopedia of Biological Chemistry. (2004). Vol. I-IV. (Eds., Lane MD, Lennarz WJ, Carafolli E, Cleveland DW, Dixon JE, Eexton JH, and Modrich P) Elsevier, Oxford.
10. Kline-Smith S and Desai A. Kinesins as Microtubule Disassembly Enzymes. In: Encyclopedia of Biological Chemistry (2004) 2: 517-21.
11. Kolodner RD. Eukaryotic DNA Mismatch Repair: Biochemical Mechanisms and Cancer Genetics. In: Encyclopedia of Biochemistry. W Lennarz and MD Lane, eds. Academic Press, Elsevier Science, USA, San Diego (2004) 1: 675-82.
12. Maddox PS, Oegema K, Desai A and Cheeseman I. "Holo"er Than Thou: Chromosome Segregation and Kinetochore Function in C. elegans. Chromosome Research (2004) 12:641-53.
13. Ren B and Dynlacht BD. Use of Chromatin Immunoprecipitation Assays in Genome-Wide Location Analysis of Mammalian Transcription Factors. In: Methods in Enzymology (2004) 376: 304-15.
14. Zhou H, Boyle R and Aebersold R. Quantitative protein analysis by solid phase isotope tagging and mass spectrometry. Methods in Molecular Biology (2004) 261: 511-8.

LUDWIG INSTITUTE FOR CANCER RESEARCH

São Paulo Branch
of Cancer Biology and Epidemiology

São Paulo, Brazil

Staff List

Brentani, Ricardo R. *Member, Director*

Cell and Molecular Biology Group

Brentani, Ricardo R. *Member, Director*
Castanheiras, Débora *Visiting Scientist*
Ferreira, Severino da Silva *Technician*
Machado, Cleiton Fagundes *Technician (from January)*
Nomizo, Regina *Technician*
Nonogaki, Suely *Technician*
Silva, Miyuki Fukuda da *Technician*
Castro, Rosa Maria R. P. S. *Graduate Student*
Couto, Cinthia Guiso da Cunha *Graduate Student*
Freitas, Adriana R.O. *Graduate Student*
Hajj, Glaucia Noeli Maroso *Graduate Student*
Kindlmann, Kathleen *Graduate Student (until February)*
Landemberger, Michele Christine *Graduate Student*
Lima, Flávia Regina Souza *Graduate Student*
Lopes, Marilene Hohmuth *Graduate Student*
Mancini, Gabriel Lourenço *Graduate Student (from November)*
Muras, Angelita Gonzalez *Graduate Student*
Arantes, Camila Pinto *Under Graduate Student*
Baucia, Fernanda Bignardi *Under Graduate Student (from March)*
Lembke, Carolina Gimiliani *Under Graduate Student (until July)*
Takiishi, Tatiana *Under Graduate Student*

Cancer Genetics Group

Caballero, Otávia L. S. *Assistant Member (until September)*
Oliveira, André Luiz Vettore de *Senior Investigator*
Lopes, Luis Fernando *Visiting Scientist*
Carvalho, Fabrício de *Technician*
Cunha, Danielle Renzoni da *Technician*
Martins, Dinamar A. Gaspar *Techinician (from May)*
Moura, Ricardo Pereira de *Technician*
Paixão, Valéria Aparecida *Technician*
Seixas, Andrea Quintella de Andrade *Technician*
Bulgarelli, Adriana *Graduate Student*
Dellamano, Márcia *Graduate Student (until October)*
Felix, Roberta Spetic *Graduate Student (from March)*
Góes, Fernanda Costa Grizzo de Sampaio *Graduate Student (until October)*
Kagohara, Luciane Tsukamoto *Graduate Student (until February)*
Kagohara, Luciane Tsukamoto *Graduate Student (from May)*
Maschietto, Mariana *Graduate Student*
Okuda, Erika Araki *Graduate Student (from December)*
Oliveira, Mariana Brait Rodrigues do *Graduate Student (until March)*
Pinto, Luciana Wermersbach *Graduate Student (from October)*
Sredni, Simone Treiger *Graduate Student (until August)*
Vidal, Daniel Onofre *Graduate Student*

Achatz, Maria Isabel *Under Graduate Student (from August)*
Maistro, Simone *Under Graduate Student (from Novermber)*
Mello, Barbara Pereira de *Under Graduate Student (until June)*

Laboratory of Molecular Biology and Genomics

Camargo, Anamaria Aranha *Assistant Member*
Carraro, Dirce Maria *Senior Investigator*
Bastos, Elen Pereira *Technician*
Costa, Maria Cristina Ferreira *Technician*
Filho, Fernando Camargo *Technican*
Kaiano, Jane Haruko L. *Technician*
Monteiro, Elisangela *Technician (until February)*
Padovani, Reimar *Techinician*
Pires, Lilian Campos *Technician*
Poli, Monica Caamaño Cristóvão *Technician (from August)*
Salim, Anna Christina de Matos *Technician (until February)*
Trevisan, Andrea *Technician (from August)*
Bettoni, Fabiana *Graduate Student (from April)*
Castro, Nádia Pereira de *Graduate Student (from March)*
Conde, Sandro José *Graduate Student (from April)*
Costa, Fabricio Falconi *Graduate Student (until July)*
Ierardi, Daniela Filippini *Graduate Student (from October)*
Klassen, Giseli *Graduate Student*
Parmigiani, Raphael Bessa *Graduate Student (from April)*
Rangel, Maria Cristina Rodrigues *Graduate Student*
Salim, Anna Christina de Matos *GraduateStudent (from March)*
Silva, Ana Paula Medeiros *GraduateStudent*
Trevisan, Andrea *Graduate Student (until July)*
Verbisck, Newton Valério *Graduate Student*
Cavalher, Felicia Peterson *Under Graduate Student (from March)*
Ferreira, Elisa Napolitano e *Under Graduate Student*
Geraldo, Murilo Vieira *Under Graduate Student*
Granato, Mariana Facchini *Under Graduate Student (until January)*
Pineda, Paulo Henrique Baldan *Under Graduate Student (from August)*
Saraiva, Thiago Felcar *Under Graduate Student (from August)*

Computational Biology Group

Souza, Sandro José *Associate member*
Nishyama Jr., Milton Yutaka *Technician (from June)*
Oliveira, Rodrigo Soares *Technician (until September)*
Barbosa, Elza Helena Andrade *Graduate Student*
Galante, Pedro A. F. *Graduate Student (from April)*
Leerkes, Maarten *Graduate Student (until May)*
Possik, Patricia Abrao *Graduate Student (from April)*
Sakabe, Noboru Jo *Graduate Student*
Slager, Natanja Sara Kirschbaum *Graduate Student*
Souza, Jorge Estefano Santana de *GraduateStudent*
Souza, Robson Francisco de *Graduate Student (from June)*
Vibranovski, Maria Dulcetti *Graduate Student*
Carvalho, Patricia Marra *Under Graduate Student (from June)*

Molecular Immunology Group

Reis, Luiz Fernando Lima *Associate member (from July)*
Carvalho, Alex Fiorini de *Senior Investigator*
Carvalho, Maria Eudoxia Pilloto de *Technician (from August)*
Dias, Adriana Abalen M. *Technician*
Marques, Sarah Martins *Technician*
Mendonça Neto, Chamberlein E. *Technician*
Mota, Louise Danielle de Carvalho *Technician*
Patrao, Diogo Ferreira da Costa *Technician (from April)*
Santos, Mariana Martins Siqueira *Technician*
Silva, André Abreu da *Technician*
Spilborghs, Graziela Machado G. Turco *Technician*
Abrantes, Eduardo Fernandes *Graduate Student (until May)*
Barreto, Bianca Diana *Graduate Student*
Diniz, Suzana Nogueira *Graduate Student (until May)*
Gomes, Luciana Inácia *Graduate Student*
Martins, Waleska Kerllen *Graduate Student*
Mello, Barbara Pereira de *Graduate Student (from August)*
Muto, Nair Hideko *Graduate Student*
Pagotto, Ana Helena *Graduate Student*
Stolf, Beatriz Simonsen *Graduate Student (until January)*
Termini, Lara *Graduate Student*
Coló, Anna Estela Luiza *Under Graduate Student*
Marques, Mariana Morato *Under Graduate Student*
Neves Filho, Emerson *Under Graduate Student (until January)*

Virology Group

Villa, Luisa Lina *Member*
Andreoli, Maria Antonieta Avilla *Technician*
Costa, Maria Cecília *Technician*
Ferreira, Silvaneide A. *Technician*
Lepique, Ana Paula *Technician (from March)*
Miyamura, Romulo A. *Technician*
Paula, Lenice Galan De *Nurse*
Pereira Sobrinho, João S. *Technician*
Boccardo, Enrique Mario *Technician*
Pinto, Maria Luiza B. G. *Nurse*
Prado, Jose Carlos Mann *Technician*
Santos, Monica I. R. *Technician*
Baldi, Carina Vitoria Manzini *Graduate Student (from August)*
Bolpetti, Aline do Nascimento *Graduate Student*
Sichero, Laura C. S. *Graduate Student*
Junes, Katiana de S. *Graduate Student*
Rabachini, Tatiana *Graduate Student*
Silvestre, Rodrigo Vellasco D. *Graduate Student (until October)*
Souza, Patricia Savio de *Graduate Student*
Thomman, Patrícia *Graduate Student*
Godoy, Fabiana de *Under Graduate Student (from August)*
Nicastro, Gianlucca Gonçalves *Under Graduate Student (from August)*

Technical Support

Carneiro, Izabel Costa *Laboratory Worker*
Gonçalves, Wanderley Lourenço *Assistant Animal Keeper*
Lima, Ricardo Roberto Ferreira *Animal Keeper*
Mendonça, Roseli de Souza *Laboratory Worker*
Monteiro, Luiz Antonio *Maintenance Assistant*
Nascimento, Carlos F. *Technician*
Oliveira, Ederval Machado Cardoso de *Assistant Animal Keeper (from March)*
Sampaio, Sirleia Miranda *Laboratory Worker*
Silva, Domingos S. M. *Assistant Animal Keeper (until March)*
Silva, Eudes *Maintenance Supervisor*
Silva, Francisco Sampaio *Maintenance Assistant*
Sousa, Leonel C. B. *Photographic Artist*

Administration & Secretariat

Campos, Paulo Roberto *Administrator*
Capelucci, Renata Juliana Souza *Office Clerk*
Coelho, Viviani Ap. Martins *Accountant*
Cristino Júnior, Elias Lopes *Office Boy*
Fietzek, Birgit *Receptionist*
Hering, Maria Stella Leme *Research Secretary (Virology)*
Lopes, Fernanda Marques Conceição *Research Secretary (Human Cancer Genome)*
Monteiro, Jaqueline *Executive Secretary*
Paraguassú, Erica *Office Clerk*
Pereira, Ana Cláudia *Research Secretary (Computational Biology)*
Pereira, Carla Regina F. *Secretary*
Ribeiro, Eliane Lopes *Research Secretary (Cancer Genetics)*
Rocha, Roseli Z. *Importations*
Rocha, Juliana Freitas *Office clerk*
Rocha, Rubens Antonio da *Storekeeper*
Rodrigues, Aline Pacifico *Research Secretary (Microarray)*
Silva, Raquel Hessel da *Project Clerk (Virology)*
Sousa, Andréa Ramos de *Office Clerk*
Souza, Raquel Rodrigues de *Office Clerk*
Souza, Maria Conceição *General Services*
Walter, Antje Luise *Assistant Administrator*

Branch Director's Report

The São Paulo Branch's expertise in genomics projects is internationally recognized through ORESTES, and now the Transcript Finishing Initiative (TFI), both of which were part of the Human Cancer Genome Project; a colaboration partly-funded by FAPESP (The State of São Paulo) and LICR. The Branch has also been involved in other genome sequencing and expression projects, including the successful implementation of a virtual genomics institute named ONSA (Organization for Nucleotide Sequencing and Analysis). Genomics has continued to be a keen interest, with the analysis of tumor-specific splice variants and the down regulation of ADAM23 by methylation.

The intensive collaboration with our host institution, the Hospital do Cancer, has led to the collection of over 8000 fully pedigreed specimens, the setting up of tools such as tissue arrays, cDNA microarrays fully exploiting the ORESTES clone collection, RNA banks with over 1000 specimens and a laser dissection facility. Coupled to powerful bioinformatics, this pipeline has already begun to yield fruits, the highlight of which was featured on the cover of the journal Cancer Research in 2004.

The work on human papilloma viruses (HPV) is now focusing on detection in males, and the immune responses associated with the HPV infectioun and cervical neoplasia. This involves the study of positive as well as negative associations between HPV infection, cervical neoplasia risk, and alleles and haplotypes of MHC class II genes. We also observed that titers of neutralizing antibodies are more correlated with HPV-16 infections than IqG titers. This has led to important involvement in clinical trials of quadrivalent HPV vaccine with Merck, Sharp and Dohme.

Finally, it is becoming increasingly clear that the prion protein (PrPcP) may have a role in epilepsy and cognitive function. We have contributed to the elucidation of PrPcP's role in neuronal differentiation mediated by its interactions with extracellular glycoproteins laminin and vitronectin,as well as its neuroprotective effect through its interaction with extendin or STI1. The analysis of PrPc is therefore crucial for furthering the understanding of several neurological disorders.

Ricardo R. Brentani

Research Report

Cellular And Molecular Biology Group

The Cellular and Molecular Biology Group, led by Dr. Ricardo Brentani, continues to evaluate the importance of the Cellular Prion protein (PrP^c) and its ligands, laminin and Stress Inducible Protein 1 (STI1), in differentiation and protection against programmed cell death. The Group previously demonstrated that STI1-PrP^c interaction activates cAMP-dependent protein kinase A (PKA) and Mitogen Activated Protein Kinase (MAPK). PKA activation has been related to protection against cell death and it has now been demonstrated that MAPK activity induced by PrP^c-STI-1 association is responsible for neuronal differentiation. These results demonstrate that two parallel effects of the PrPc-STI1 interaction, neuritogenesis and neuroprotection, are mediated by distinct signaling pathways. The Group also characterized an additional PrP^c ligand, vitronectin, an extracellular matrix and serum protein. Both PrPc and vitronectin are highly expressed and co-localized in dorsal root ganglia neurons, and their interaction induces axonal growth. Using functional assays, the Group demonstrated that the lack of this interaction in PrP^c null mice is compensated by integrin activation.

Using PrP^c gene ablated mice the Group showed that cellular prion has an important function on neuronal plasticity. Another important role is PrP^c's protection against oxidative stress. Mitochondrial respiration is a useful parameter for the determination of cellular metabolic rate and it is a major source of reactive oxygen species. However, it was determined baseline mitochondrial respiration in different brain of wild-type and PrP^c ablated mice is similar. Thus, mitochondrial respiration might not be directly related to the higher oxidative stress previously observed in brains from PrP^c ablated mice.

The importance of PrP^c is being evaluated in human diseases other than transmissible spongiform encephalopathies. An important achievement was the use of denaturing high performance liquid chromatography (HPLC) to evaluate polymorphisms and mutations in the gene for the PrP^c protein. The Group reported that a PrP^c polymorphism at codon 171 (Asn —> Ser), absent in controls, was found to be heterozygous in 13% of patients with malformations of cortical development.

Computational Biology Group

The Computational Biology Group, led by Dr. Sandro Souza, has continued its studies on the human transcriptome. In 2004, alternative splicing, and its significance, was one major focus of interest. While there is some evidence indicating that at least some of the events are biologically meaningful, the high prevalence of alternative splicing has raised the possibility that the transcripts are spurious products of the splicing machinery. The Group approached this problem through a large-scale analysis of 'intron retention'. Using several bioinformatics tools, thousands of intron retention events in the human transcriptome were characterized. These events showed a biased distribution regarding several features, which strongly suggests that a significant fraction of intron retention events have biological significance.

The Group also identified splicing variants and splicing factors, components of the spliceosome, differentially expressed in several tumors, as evaluated by SAGE (Serial Analysis of Gene Expression) and microarray. Efforts to fully explore data from LICR SAGE and MPSS (Massively Parallel Signature Sequencing) initiatives have continued. In collaboration with the Molecular Biology and Genomics Group, the influence of single nucleotide polymorphisms (SNPs) on the tag to gene assignment in SAGE and MPSS experiments was evaluated. All the resulting data and analyses were made available through SAGE Genie (http://cgap.nci.nih.gov/SAGE).

The Group also extended its collaborations in large-scale bioinformatics/genomics projects, with active participation in the H-invitational jamboree, which provided full annotation for more than 20,000 human genes. As a consequence, the Group has also participated in the launch of H-Angel, a multi-centric initiative to develop a platform for storage and visualization of gene expression data. In 2004, the Group also organized the second International Conference on Bioinformatics and Computational Biology (www.icobicobi.com.br). Together with other groups in the State of São Paulo, the Computational Biology Group has generated and analyzed thousands of ORESTES (Open Reading Frames Expressed Sequence Tags) from honeybee, and finalized the Transcript Finishing Initiative, one of the extensions of the LICR's Human Cancer Genome Project.

Laboratory of Cancer Genetics

The Laboratory of Cancer Genetics, led by Dr. Otávia Caballero, has been working on the identification of potential tumor targets through methylation profiling and the analysis of gene expression. Once new targets are identified, retrospective studies using well-characterized paraffin-embedded tissues will be used for target validation and clinical correlation.

Methylation-associated silencing of gene promoters is considered an important mechanism of tumor suppressor gene inactivation, and because hypermethylation can be reverted chemically, it holds great promise as a therapeutic approach. The methylation profile of candidate genes in 71 pediatric patients with Acute Lymphoblastic Leukemia (ALL) clearly showed a correlation between hypermethylation of the *CALCA* gene, and overall survival two years after diagnosis with central nervous system (CNS) infiltration. A similar analysis has been conducted on four candidate genes in bladder cancer, with at least one displaying hypermethylation in 66 of 73 bladder tumors. This high prevalence is being further investigated. Finally, DNA methylation

changes are well documented in adult patients with ALL, acute myeloid leukemia (AML) and adult myelodysplastic syndromes (MDS), a heterogeneous group of clonal hematopoietic stem cell disorders. However, DNA methylation has been poorly investigated in pediatric MDS patients. The Group investigated promoter methylation of 14 genes in 25 bone marrow samples from pediatric MDS patients, and found that the methylation patterns were similar in childhood and adult MDS. Thus the methylation of certain genes may be used as a tool to improve the diagnosis and treatment of pediatric MDS patients.

The global analysis of the tumor cell transcriptome allows the identification of genes with characteristic and specific expression in tumor cells. The Group used SAGE analyses to identify genes differentially expressed in chemo-sensitive and chemo-resistant blastemal component Wilms' Tumors (WT), breast cancer and normal breast tissue, glioblastomas and normal brain. These results are currently being confirmed using Real Time-PCR (RT-PCR) and/or tissue microarray analyses. However, the results have indicated several interesting candidates for prognostic and diagnostic utility, for example five potential molecular markers for resistance to chemotherapy in blastemal WT have been identified.

Laboratory of Molecular Biology and Genomics

The Laboratory of Molecular Biology and Genomics, led by Dr. Anamaria Camargo, is analyzing differentially methylated sequences in breast tumors. Differential Methylation Hybridization (DMH) was used, in collaboration with Drs. Michael Cleveland and Yipeng Wang (Sidney Kimmel Cancer Center, San Diego, USA), to determine the methylation status of 3813 promoter regions of cancer related genes in breast cancer samples. Additionally, Methylation-Sensitive Arbitrarily Primed PCR (MS-APPCR) indicated the promoter region of ADAM23, a member of the ADAM (A Disintegrin And Metalloprotease Domain) family of cell surface glycoproteins, was differentially methylated in breast tumors. Downregulation of the mRNA expression levels of the promoters of ADAMs 12, 23 and 33 were found to correlate directly with promoter hypermethylation in breast cell lines. The Group showed that ADAM23 hypermethylation in 109 ductal invasive primary breast tumors correlated significantly with tumor stage, number of positive lymph nodes and the occurrence of distant metastases. Similar studies for ADAM 12 and 33 will be pursued. Patients with ADAM23 hypermethylation had a significantly shorter metastasis-free and overall survival. The diagnostic and prognostic potential of differentially methylated genes will eventually be evaluated in large collections of tumor samples provided by the A.C.Camargo Hopsital, São Paulo, Brazil.

The Group is also applying gene expression data to biological questions. Data generated by the LICR MPSS initiative are being used to identify transcriptional changes associated with ERBB2 overexpression, which is observed in 25-30% of breast cancers. Apparently novel transcripts with differential expression were converted into cDNA fragments corresponding to 10 human transcripts, four of which were confirmed to be differentially expressed in the ERBB2 transfected cell line. Functional characterization of these novel genes will be pursued. Finally, the LICR Transcriptome Database was used to identify novel cancer testis (CT) antigens for immunotherapy. A total of 1,255 candidates were identified and 94 are currently being analyzed by RT-PCR. In order to validate these candidates as novel CT antigens, the Group is also evaluating the presence of specific antibodies in serum from cancer patients.

Virology Group

The Group, led by Dr. Luisa Villa, is using epidemiology, from the 2 000 women being followed over a ten year period as part of the Ludwig/McGill cohort, and molecular techniques to understand the association between cervical human papillomavirus (HPV) infection and the risk

of cervical neoplasia. This understanding is the first step towards the development of strategies for preventing genital HPV disease and, ultimately, cervical cancer. The Group has previously shown that intratypic variation of HPV-16 is an important predictor of progression to clinically relevant cervical lesions. This epidemiologic observation was corroborated by studies of the transcriptional activity from the natural promoters of the different variants detected in the cohort that demonstrated an enhanced P97 promoter activity for the non-European variants when compared to the HPV-16 prototype.

Another important aspect was the investigation of the immune responses associated with HPV infections and risk of cervical neoplasia. The Group has described positive and negative associations between some alleles and haplotypes of MHC Class II genes (HLA-DRB1, -DQA1, and –DQB1) and cervical cancer, precursor lesions, and HPV infections. Concerning humoral responses, both serum IgG levels and more recently neutralizing antibodies (Nabs) were tested in the specimens from the cohort study. Preliminary analyses indicate that measures of NAbs against HPV-16 are a more specific indicator of HPV-16 infections than total IgG titers. All samples from the Ludwig/McGill cohort are being retested with these assays, to better understand the level and type of immune responses that will eventually protect women against HPV through vaccination. In this respect, the Group is collaborating with Merck, Sharp & Dohme in designing and supervising clinical trials of a quadrivalent HPV (6, 11, 16, 18) L1 vaccine.

Finally, the Group is investigating the level of micronutrients and antioxidants in the sera from women of the Ludwig/McGill cohort. The primary observation, that consumption of certain carotenoids as well as higher endogenous retinoic acid concentrations in the serum levels, may decrease the duration of an oncogenic HPV infection thereby decreasing risk of cervical disease in high-risk women. Additional studies are underway.

Molecular Immunology Group

The Group, led by Dr. Luis Reis, is now completed devoted to the analysis of expression profile of tumors. During the last two years a strong bioinformatics infrastructure has been established with Hospital do Cancer, integrating tumor bank clinical information, clone collection and array production, quality control and data analysis. Critical protocols for mRNA amplification essential for analysis of biopsy-derived samples have also been established. With this infrastructure in place, the Group is now addressing issues that are relevant for the daily life of cancer patients such as early diagnosis and prognosis.

Molecular classifiers were identified that may be important in the discrimination of patients with intestinal metaplasia, a nonmalignant lesion, which has higher risk of malignant transformation. Since the risk for developing of adenocarcinomas of the esophagus is also associated with presence of intestinal metaplasia of the distal esophagus, known as Barret's disease, the Group is investigating the molecular events underlining the transformation of squamous to columnar tissue in the distal esophagus and the malignant transformation of intestinal metaplasia into adenocarcinomas in both organs.

The Group is continuing to explore the expression profile of malignant and non-malignant lesions of the thyroid gland. This is important to better understand the molecular events related to proliferative disorders of the gland (goiter and adenomas) and to identify molecular markers that could help in the precise distinction between adenomas and follicular carcinomas prior to surgery. The Group has also defined predictors of responsiveness to the clinical treatment of locally advanced squamous cell carcinoma of the larynx. This strategy is now a major effort in the management of these tumors since it can avoid laryngectomy, thus reducing morbidity and improving quality of life.

Publications

Primary Research Articles

1. Baiocchi O.C., Colleoni G.W.B., Caballero O.L., Vettore A.L., Andrade A.L., Pestana J.O.M. Quantification of Epstein-Barr viral load and determination of a cut off value to predict the risk of posttransplant lymphoproliferative disease in a renal-transplant cohort. Journal Haematologica (2004) 89(3):366-8.
2. Boccardo E., Noya F., Broker T., Chow L., Villa L.L. HPV 18 confers resistance to TNF-alpha in organotypic cultures of human keratinocytes. Virology (2004) 328(2):233-243.
3. Borojevic R., Roela R.A., Rodarte R.S., Thiago L.S., Pasini F.S., Conti F.M., Rossi M.I.D., Reis L.F.L.R., Lopes L.F., Brentani M.M. Bone marrow stroma in childhood myelodysplastics syndrome: composition, ability to sustain hematopoiesis in vitro, and altered gene expression. Leukemia Research (2004) 28:831-844.
4. Calsa Junior T, Carraro DM, Benatti MR, Barbosa AC, Kitajima JP, Carrer H.- Structural features and transcript-editing analysis of sugarcane (Saccharum officinarum L.) chloroplast genome. Current Genetics (2004) 46(6):366-73.
5. Carneiro C.R.W., Postol E., Nomizo R., Reis L.F.L., Brentani R.R. Identification of Enolase as a Laminin-Binding protein on the surface of Staphylococcus Aureus. Microbes and Infection (2004) 6:604-608.
6. Carraro D.M., Camargo A.A., Salim A.C.M., Gonzaga L., Costa G.C., Vasconcelos A.T.R., Simpson A.J.G. Closure of rRNA related gaps in the *Chromobacterium violaceum* genome with the PCR assisted counting extension (pace) protocol. Genetic And Molecular Research (2004) 3(1):53-63.
7. Castro R.M.P.S., Landemberger M.C., Walz R., Carlotti Jr. C.G., Huang N., Cunha D.R., Moura R., Caballero O.L., Sakamoto A.C., Nitrini R., Brentani R.R., Martins V.R. High capacity and low cost detection of prion protein gene variant alleles through denaturing HPLC methodology. Journal Neuroscience Methods (2004) 139:263-269.
8. Coelho F.R.G., Prado J.C.M., Pereira Sobrinho J.S., Hamada G., Landman G., Pinto C.A., Nonogaki S., Villa L.L. Estrogen and progesterone receptors in human papilloma virus-related cervical neoplasia. Brazilian Journal of Medical and Biological Research (2004) 37(1):83-88.
9. Cortezzi S.S., Provazzi P.J., Sobrinho J.S., Mann-Prado J.C., Reis P.M.P., Freitas S.E.N., Gósi Fo J.F., Fukuyama E., Cordeiro J.A., Cury P.M., Maniglia J.V., Villa L.L., Tajara E.H., Rahal P. Analysis of Human Papillomavirus (HPV) Prevalence and TP53 Polymorphism in Head and Neck Squamous Cell Carcinomas. Cancer Genetic and Cytogenetic (2004) 150:44-49.
10. Costa F., Verbisck N.V., Salim A.C.M., Ierardi D.F., Pires L.C., Sasahara R.M., Colin C., Sogayar M.C., Zanata S.M., Mackay A., Soares F.A., Simpson A.J.G., Camargo A.A. Epigenetic silencing of the adhesion molecule ADAM23 is highly frequent in breast tumors. Oncogene (2004) 23:1481-1488.
11. de Pieri C., Beltramini L.M., Selistre-de-Araujo H.S., Vettore A.L., da Silva Arruda P., Oliva G., de Souza D.H. Overexpression, purification, and biochemical characterization GumC, an enzyme involved in the biosynthesis of exopolysaccha by Xylella fastidiosa. Protein Expression and Purification (2004) 34(2):223-8.

12. Diniz S.N., Nomizo R.., Cisalpino P.S., Teixeira M.M., Bown G.D., Mantovani A., Gordon S., Reis L.F.L.R., Dias A.A.M. Improved phagocytosis in periotenal macrophages from TSG-14/ PTX3 transgenic mice. Journal of Leukocyte Biology (2004) 75(4):649-56.

13. Ferreira E., Pires L.C., Parmigiani R.B., Bettoni F., Puga R.D., Pinheiro D.G., Andrade L.E.C., Cruz L.O., Degaki T.L., Faria Jr. M., Festa F., Giannella-Neto D., Giorgi R.R., Goldman G.H., Granja F., Gruber A., Hackel C., Henrique-Silva F., Malnic B., Manzini C.V.B., Marie S.K.N., Martinez-Rossi N.M., Oba-Shinjo S.M., Pardini M.I.M.C., Rahal P., Rainho C.A., Rogatto S.R., Romano C.M., Rodrigues V., Sales M.M., Savoldi M., da Silva I.D.C.G., da Silva N.P., de Souza S.J., Tajara E.H., Silva Jr. W.A., Simpson A.J.G., Sogayar M.C., Camargo A.A., Carraro D.M. Identification and complete sequencing of novel human transcripts through the use of mouse orthologs and testis cDNA sequences. Genetics and Molecular Research (2004) 3:493-511.

14. Galante P.A.F., Sakabe N.J., Kirschbaum-Slager N., de Souza S.J. Detection and evaluation of intron retention events in the human transcriptome RNA (2004) 10(5):757-65.

15. Imanishi T., Itoh T., Suzuki Y., O'Donovan C., Fukuchi S., Koyanagi K.O., Barrero R.A., Tamura T., Yamaguchi-Kabata Y., Tanino M., Yura K., Miyazaki S., Ikeo K., Homma K., Kasprzyk A., Nishikawa T., Hirakawa M., Thierry-Mieg J., Thierry-Mieg D., Ashurst J., Jia L., Nakao M., Thomas M.A., Mulder N., Karavidopoulou Y., Jin L., Kim S., Yasuda T., Lenhard B., Eveno E., Suzuki Y., Yamasaki C., Takeda J., Gough C., Hilton P., Fujii Y., Sakai H., Tanaka S., Amid C., Bellgard M., Bonaldo Mde F., Bono H., Bromberg SK., Brookes A.J., Bruford E., Carninci P., Chelala C., Couillault C., de Souza S.J., Debily M.A., Devignes M.D., Dubchak I., Endo T., Estreicher A., Eyras E., Fukami-Kobayashi K., Gopinath G.R., Graudens E., Hahn Y., Han M., Han Z.G., Hanada K., Hanaoka H., Harada E., Hashimoto K., Hinz U., Hirai M., Hishiki T., Hopkinson I., Imbeaud S., Inoko H., Kanapin A., Kaneko Y., Kasukawa T., Kelso J., Kersey P., Kikuno R., Kimura K., Korn B., Kuryshev V., Makalowska I., Makino T., Mano S., Mariage-Samson R., Mashima J., Matsuda H., Mewes H.W., Minoshima S., Nagai K., Nagasaki H., Nagata N., Nigam R., Ogasawara O., Ohara O., Ohtsubo M., Okada N., Okido T., Oota S., Ota M., Ota T., Otsuki T., Piatier-Tonneau D., Poustka A., Ren S.X., Saitou N., Sakai K., Sakamoto S., Sakate R., Schupp I., Servant F., Sherry S., Shiba R., Shimizu N., Shimoyama M., Simpson A.J., Soares B., Steward C., Suwa M., Suzuki M., Takahashi A., Tamiya G., Tanaka H., Taylor T., Terwilliger J.D., Unneberg P., Veeramachaneni V., Watanabe S., Wilming L., Yasuda N., Yoo H.S., Stodolsky M., Makalowski W., Go M., Nakai K., Takagi T., Kanehisa M., Sakaki Y., Quackenbush J., Okazaki Y., Hayashizaki Y., Hide W., Chakraborty R., Nishikawa K., Sugawara H., Tateno Y., Chen Z., Oishi M., Tonellato P., Apweiler R., Okubo K., Wagner L,. Wiemann S., Strausberg R.L., Isogai T., Auffray C., Nomura N., Gojobori T., Sugano S. Integrative annotation of 21,037 human genes validated by full-length cDNA clones – Public Library of Sciences Biology (2004) 2(6):856-75.

16. Junqueira A.C., Lessinger A.C., Torres T.T., da Silva F.R., Vettore A.L., Arruda P., Azeredo Espin A.M. The mitochondrial genome of the blowfly Chrysomya chloropyga (Diptera: Calliphoridae).Gene (2004) 15:339-7-15.

17. Kirschbaum-Slager N., Lopes G.M.P., Galante P.A.F., Riggins G.J., de Souza S.J. Splicing factors are differentially expressed in tumors. Genetics and Molecular Research (2004) 3:512-520.

18. Lopes L.F., Piccoli F.S., Paixão V.A.- Latorre M.R.D.O., Tornout J.V., de Camargo B., Simpson A.J., Caballero O.L. The CYP 3A4 gen and the Vγ/ Jβ trans-rearrangement in pediatric ALL patients undergoing chemotherapy. Are they biologic markers related to secondary leukemia and genotoxicity? –Leukemia Research (2004) 28(12):1281-6.

19. Maglio L.E., Perez M.F., Martins V.R., Brentani R.R., Ramirez O.A. Hippocampal synaptic plasticity in mice devoid of cellular prion protein. Molecular Brain Research (2004) 131(1-2):58-64.

20. Meireles I.S., Cristo E.B., Cavalho A.F., Hirata Jr R., Pelosof A., Gomes L.I., Martins W.K., Begnami M.D., Zitron C., Montagnini A.L., Soares F.A., Neves E.J., Reis L.F.L. Molecular classifiers for Gastric cancer and pre-malignat diseases of the gastric mucosa. Cancer Research (2004) 64(4):1255-65.

21. Monteiro-Vitorello C.B., Camargo L.E., Van Sluys M.A., Kitajima J.P., Truffi D., do Amaral A.M., Harakava R., de Oliveira JC, Wood D, de Oliveira MC, Miyaki C, Takita MA, da Silva AC, Furlan LR, Carraro DM, Camarotte G, Almeida NF Jr, Carrer H, Coutinho LL, El-Dorry HA, Ferro MI, Gagliardi PR, Giglioti E, Goldman MH, Goldman GH, Kimura ET, Ferro ES, Kuramae EE, Lemos EG, Lemos MV, Mauro SM, Machado MA, Marino CL, Menck CF, Nunes LR, Oliveira RC, Pereira GG, Siqueira W, de Souza AA, Tsai SM, Zanca AS, Simpson AJ, Brumbley SM, Setubal JC.- The genome sequence of the gram-positive sugarcane pathogen Leifsonia xyli subsp. xyli. Molecular Plant-Microbe Interaction (2004) 17(8):827-36.

22. Muniz J.R., Alves C.A., de Pieri C., Beltramini L.M., Selistre-de-Araujo H.S., Vettore A.L., da Silva F.R., Arruda P., Garrat R.C., Oliva G., de Souza D.H. Overexpression, purification, biochemical characterization, and molecular modeling of recombinant GDP-mannosyltransferase (GumH) from Xylella fastidiosa. Biochemical and Biophysical Research Communications 315(2):485-92.

23. Nishimoto I.N., Pinheiro N.A., Rogatto S.R., Carvalho A.L., Caballero O.L., Simpson A.J.G., Kowalski L.P. Alchool dehydrogenase 3 genotype as a risk factor for upper aerodigestive tract cancer. Archives of Otolaryngology Head and Neck Surgery (2004) 130(1): 78-82.

24. Nishimoto I.N., Pinheiro N.A., Rogatto S.R., Carvalho A.L., Simpson A.J.G., Caballero O.L., Kowalski L.P. Cyclin D1 gene polymorphism as a risk factor for squamous cell carcinoma of the upper aerodigestive system in non-alcoholics. Oral Oncology (2004) 40(6):604-610.

25. Nunes F.M., Valente V., Sousa J.F., Cunha M.A., Pinheiro D.G., Maia R.M., Araujo D.D., Costa M.C., Martins W.K., Carvalho A.F., Monesi N., Nascimento A.M., Peixoto P.M., Silva M.F., Ramos R.G., Reis L.F., Dias-Neto E., Souza S.J., Simpson A.J., Zago M.A., Soares A.E., Bitondi M.M., Espreafico E.M., Espindola F.S., Paco-Larson M.L., Simoes Z.L., Hartfelder K., Silva Jr. W.A. The use of Open Reading frame ESTs (ORESTES) for analysis of the honey bee transcriptome. BMC Genomics (2004) 5(1):84.

26. Parmigiani R.B., Magalhaes G.S., Galante P.A., Manzini C.V., Camargo A.A., Malnic B. A novel human G protein-coupled receptor is overexpressed in prostate cancer. Genetics and Molecular Research (2004) 3:521-31.

27. Peres Lopes G.M., de Souza S.J. Dissecting the human spliceosome through bioinformatics and proteomics approaches. Journal of Bioinformatics and Computational Biology (2004) 1(4):743-750.

28. Pinto A.P., Schlecht N.F., Pintos J., Kaiano J., Franco E.L., Crum C.P., Villa L.L. Prognostic significance of lymph node variables and human papillomavirus DNA in invasive vulvar carcinoma. Gynecology Oncology (2004) 92(3):856-65.

29. Poli M.C.C., Villa L.L., Colella R., Deheinzelin D. Molecular evidence of tumour cell removal from salvaged blood after irradiation and leucocyte depletion. Transfusion (2004) 14:151-155.

30. Sakabe N.J., Vibranovski M.D., de Souza S.J. A bioinformatics analysis of alternative exon usage in human genes coding for extracellular matrix proteins. Genetic Molecular Research (2004) 3:532-540.

31. Siegel E.M, Craft N.E., Roe D.J., Duarte-Franco E., Villa L.L., Franco E.L., Giuliano A.R. Temporal variation and identification of factors associated with endogenous retinoic isomers in serum from Brazilians women. Cancer Epidemiology, Biomarkers & Prevention (2004) 13(11):1693-1703.

32. Silva A.P., Chen J., Carraro D.M., Wang S.M., Camargo A.A. Generation of longer 3' cDNA fragments from massively parallel signature sequencing tags. Nucleic Acids Research (2004) 32(12):e94.

33. Silva, A.P., De Souza, J.E., Galante, P.A., Riggins, G.J., De Souza, S.J., Camargo, A.A. The impact of SNPs on the interpretation of SAGE and MPSS experimental data. Nucleic Acids Research (2004) 32:6104-10.

34. Simpson A.J., Camargo A.A., Ferro J.A., Parra J., Vasconcelos A.T. Coordinated, network-based research as a strategic component science in Brazil. Genetic Molecular Research (2004) 3(1):18-25.

35. Sogayar M.C., Camargo A.A., Bettoni F., Carraro D.M., Pires L.C., Parmigiani R.B., Ferreira E.N., de Sa Moreira E., do Rosario D de O Latorre M., Simpson A.J., Cruz L.O., Degaki T.L., Festa F., Massirer K.B., Sogayar M.C., Filho F.C., Camargo L.P., Cunha M.A., De Souza S.J., Faria Jr. M., Giuliatti S., Kopp L., de Oliveira P.S., Paiva P.B., Pereira A.A., Pinheiro D.G., Puga R.D.S., de Souza J.E., Albuquerque D.M., Andrade L.E., Baia G.S., Briones M.R., Cavaleiro-Luna A.M., Cerutti J.M., Costa F.F., Costanzi-Strauss E., Espreafico E.M., Ferrasi A.C., Ferro E.S., Fortes M.A., Furchi J.R., Giannella-Neto D., Goldman G.H., Goldman M.H., Gruber A., Guimaraes G.S., Hackel C., Henrique-Silva F., Kimura E.T., Leoni S.G., Macedo C., Malnic B., Manzini B.C.V., Marie S.K., Martinez-Rossi N.M., Menossi M., Miracca E.C., Nagai M.A., Nobrega F.G., Nobrega M.P., Oba-Shinjo S.M., Oliveira M.K., Orabona G.M., Otsuka A.Y., Paco-Larson M.L., Paixao B.M., Pandolfi J.R., Pardini M.I., Passos Bueno M.R., Passos G.A., Pesquero J.B., Pessoa J.G., Rahal P., Rainho C.A., Reis C.P., Ricca T.I., Rodrigues V., Rogatto S.R., Romano C.M., Romeiro J.G., Rossi A., Sa R.G., Sales M.M., Sant'Anna S.C., Santarosa P.L., Segato F., Silva Jr. W.A., Silva I.D., Silva N.P., Soares-Costa A., Sonati M.F., Strauss B.E., Tajara E.H., Valentini S.R., Villanova F.E., Ward L.S., Zanette D.L. Ludwig-FAPESP Transcript Finishing Initiative. A transcript finishing initiative for closing gaps in the human transcriptome. Genome Research (2004) 14(7):1413-23.

36. Sredni S.T., Cunha I.W., Magrin J., Pinto C.A.L., Lopes L.F.- Endodermal sinus tumor of the parotid gland in a child: a case report. Pediatric and Development Pathology (2004) 7(1):77-80.

37. Sredni S.T., Camargo B., Caballero O.L., Neves J.I., Soares F.A. – Pan-cytokeratin immunoexpression in Wilms' tumors: a simple approach for understanding tumor epithelial differentiation. São Paulo Medical Journal (2004) 122(4):181-3.

38. Vencio R.Z., Brentani H., Patrao D.F., Pereira C.A. Bayesian model accounting for within-class biological variability serial nalysis of gene expression (SAGE). BMC Bioinformatics (2004) 5(1):119.

39. Vicentz M., Cara F.A., Okura V.K., da Silva F.R., Pedrosa G.L., Hemerly A., Capella A.N., Marins M., Ferreira P.C., Franca S.C., Grivet L., Vettore A.L., Kemper E.L., Burnquist W.L., Targon M.L., Siqueira W.J., Kuramae E.E., Camargo L.E. Evaluation of monocot and eudicot divergence using the sugarcane transcriptome. Plant Physiology (2004) 134(3):951-9.

40. Waitzberg A.F., Nonogaki S., Nishimoto I.N., Kowalski L.P., Miguel R.E., Brentani R.R., Brentani M.M. Clinical significance of c-myc and p53 expression in head and neck squamous cell carcinomas. Cancer Detection Prevention (2004) 28(3):178-186.

41. Walz R., Castro R.M.R.P.S., Landemberger M.C., Velasco T.R., Terra-Bustamante V.C., Bastos A.C., Bianchin M., Wichert-Ana L., Araújo D., Alexandre Jr. V., Santos A.C., Machado H.R, Carlotti Jr. C.G., Brentani R.R., Martins V.R., Sakamoto A.C. Cortical Malformations are associated with a rare polymorphism of cellular prion protein. Neurology (2004) 63:557-560.

Reviews / Commentaries / Book Chapters

1. Prado M.A., Silva J.A., Magalhães A.C., Prado V., Linden R., Martins V.R., Brentani R.R. PrPc on the road: trafficking of the cellular prion protein. Journal of Neurochemistry (2004) 88:769-781.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Stockholm Branch
of Molecular and Cellular Biology

Stockholm, Sweden

Staff List

Pettersson Ralf. F. *Director, Member*

Protein Transport Group

Pettersson, Ralf. F. *Head*
Fuxe, Jonas *Postdoctoral Fellow, part-time (until July)*
Neve, Etienne *Postdoctoral Fellow*
Sollerbrant, Kerstin *Postdoctoral Fellow*
Bergström, Anita *Senior Technician*
Mirza, Momina *Graduate Student*
Raschperger, Elisabeth *Graduate Student*
Vincent, Theresa *Graduate Student*
Överby, A. *Graduate Student*

Developmental Biology Group

Eriksson, Ulf *Head, Member*
Li, Xuri *Assistant Investigator*
Fieber, Christina *Postdoctoral Fellow*
Li, Hong *Postdoctoral Fellow*
Zhou, Jianming *Postdoctoral Fellow*
Rezaian, Soheilla *Technician*
Åkerblom, Barbara *Senior Technician (until July)*
Ehnman, Monika *Graduate Student*
Folestad, Erika *Graduate Student*
Fredriksson, Linda *Graduate Student*
Lidén, Martin *Graduate Student*
Pontén, Annica *Graduate Student*
Wang, Xun *Graduate Student*

Yeast Cell and Molecular Biology Group

Ljungdahl, Per O. *Head, Associate Member*
Andersson, Jessica *Postdoctoral Fellow (until September)*
Heessen, Stijn *Postdoctoral Fellow*
Martinez Rodriguez, Paula *Postdoctoral Fellow*
Nyman, Tomas *Postdoctoral Fellow*
Andréasson, Claes *Graduate Student*
Boban, Mirta *Graduate Student*
Kota, Jhansi *Graduate Student*

Gene Expression Group

Perlmann, Thomas *Head, Member*
Benoit, Gerard *Postdoctoral Fellow*
Borgius, Lotta *Postdoctoral Fellow*
Malewicz, Michal *Postdoctoral Fellow*
Petersson, Susanna *Postdoctoral Fellow*
Werme, Martin *Postdoctoral Fellow*
Wu, Siqin *Postdoctoral Fellow*
Joodmardi, Eliza *Technician*

Friling, Stina *Graduate Student*
Hermanson, Elisabeth *Graduate Student*
Kadkhodaei, Banafsheh *Graduate Student*
Lengquist, Johan *Graduate Student*
Nordzell, Mariette *Graduate Student (until September)*
Volakakis, Nikolaos *Graduate Student*

Neurogenesis Group

Muhr, Jonas *Head, Assistant Member*
Bergsland, Maria *Graduate Student*
Källström, Magdalena *GraduateStudent*
Sandberg, Magnus *GraduateStudent*

Technical Support

Tollman, Inger *Laboratory Manager*
Anderling, Mats *Systems Administrator*
Enrione Barrera, Erika *Laboratory Assistant*

Administration & Secretariat

Linderholm, Charlotte *Administrator*
Freidenfelt, Birgitta *Secretary and Assistant to the Administrator*

Branch Director's Report

The research programs of the five groups at the Stockholm Branch span quite broad areas in the general field of cell and molecular biology. Research interests during the past year have centered on the following topics: Regulation of export of membrane proteins from the endoplasmic reticulum (ER); function of the coxsackie- and adenovirus receptor (CAR/CXADR); mechanisms of cellular nutrient assessments and metabolic compartmentalization in yeast; cell biology of retinoids (vitamin A compounds); mechanisms of action of nuclear receptors, including the retinoid receptors and a subclass of orphan receptors, particularly in the context of the central nervous system, and; characterization of novel growth factors within the vascular endothelial growth factor (VEGF) / platelet-derived growth factor (PDGF) family regulating the formation of new blood vessels (angiogenesis).

A new Group, headed by Dr. Jonas Muhr, was established at the beginning of the year. The focus of the group is to unravel fundamental principles regulating growth and differentiation of neural stem cells. Exciting progress has been made in elucidating the role of a set of Sox transcription factors in this regulation. The Group led by Dr. Thomas Perlmann has continued its studies on the role of orphan nuclear receptors, notably the Nurr1 receptor, which is involved in the differentiation of neurons into dopamine-producing neurons. The program has broadened to include the identification of additional factors of importance in this process, and during the year, two additional transcription factors critical for the specification of dopamine cells were identified. The Group led by Dr. Ulf Eriksson has identified tissue plasminogen activator as the protease activating the novel growth factor PDGF-C, first discovered by the group. In addition, exciting roles have been established for PDGF-CC in the revascularization of the post-ischemic heart and limb in mice. The Group headed by the Director has found further evidence that CAR plays as an

important role as a component of tight junctions in epithelial cells. Finally, the Group headed by Dr. Per O. Ljungdahl has made major advances in uncovering the mechanisms by which yeast cells assess and respond to the altered availability of amino acids in the environment.

Ralf F. Pettersson

Research Report

Protein Transport Group

During the past year, the Group, headed by Dr. Ralf F. Pettersson, has focused on three separate projects: (i) the role of transmembrane lectin receptors in the export of soluble glycoproteins from the ER in mammalian cells, (ii) the analyses of the normal function of CAR/CXADR, and (iii) the development of a reverse genetics system for members of the *Bunyaviridae* family of RNA viruses.

(i) The Group has continued to explore the structure of ERGIC-53/p58, a lectin-like receptor facilitating the export of a subset of glycoproteins from the ER to the Golgi complex. Having solved the crystal structure of the calcium-bound form of the carbohydrate recognition domain to 1.46Å resolution in 2003, the recent focus has been on understanding the basis for the oligomeric (dimer/hexamer) structure of the receptor. Biochemical analyses indicate that the basic unit of the receptor is a disulfide-linked homodimer. Three such dimers assemble into a stable non-disulfide-linked hexamer that is likely to be the active, cargo-binding receptor recycling between the ER and Golgi complex via the intermediate compartment.

(ii) A few years ago, the Group initiated a new program to analyze the normal cellular function of CAR. This receptor is required for the uptake of adenoviruses and coxsackie B viruses into cells. CAR is a member of a growing family of cell surface proteins (called the CTX-family) belonging to the Ig superfamily. Members include CTX, JAM A-D, A33, ESAM, BT-IgSF, and CLMP, the latter identified by the group three years ago. In a yeast two-hybrid screen, the PDZ-domain protein called LNX-1 (Ligand of Numb protein X-1) was previously found to bind to the cytoplasmic tail of CAR. During the year, LNX-2, a homologue of LNX-1, was similarly found to interact with CAR. In both cases, the C-terminal end of CAR binds to the second (out of four) PDZ-domain of the two homologs. The functional role of the CAR-LNX interaction remains to be established. CAR was found to localize to tight junctions of epithelial cells in many organs of mice, and CAR levels appeared to correlate with the "tightness" of tight junctions.

(iii) The Group's long-standing interest in the molecular and cell biology of a large family of RNA viruses, the *Bunyaviridae*, has recently resulted in the development of a polymerase I-dependent "reverse genetics system". Using this new system, the focus during the year has been on the identification of the packaging signals enabling the virus to package its genome into virions. During these studies, it was found that virus-like particles (VLPs) were released to high titers from cells transfected with only the genes expressing the viral structural proteins (the nucleocapsid protein N, the glycoproteins G1 and G2, the RNA polymerase L), and reporter genes encoding the green fluorescent protein (GFP) or chloramphenicol acetyl transferase (CAT) flanked by the viral promoter and RNA packaging sequences. These VLPs are now being used to analyze structural motifs in the cytoplasmic tail of the G1 glycoportein important for virus particle formation (budding) in the Golgi complex.

Yeast Cell and Molecular Biology Group

The Yeast Group, led by Dr. Per O. Ljungdahl, has continued to explore the molecular basis of how eukaryotic cells assess the availability of amino acids present in their growth environment. The overall goal has been to use yeast as a model system to build understandable paradigms, and thereby to make lasting contributions towards understanding this basic and unsolved biological problem. Efforts are focused on two distinct, but interrelated areas of cell biology. Work in the first area is aimed at elucidating how yeast cells perceive and respond to the presence of extracellular amino acids. The Group has identified the primary sensor components that function at the plasma membrane (PM) and has defined positive and negative regulatory circuits that control the activity of downstream effector molecules. The goal of the second area is to define the mechanisms governing the exit of polytopic membrane proteins, i.e. proteins with multiple transmembrane segments, from the ER. Understanding membrane protein biogenesis is a prerequisite to fully understand the mechanisms involved in nutrient assessments; the proteins that facilitate amino acid uptake and that comprise the receptor component of the extracellular amino acid sensor are polytopic proteins.

During the last year three noteworthy advances in our understanding of amino acid based signaling in yeast were made. First, the Group found that the PM localized amino acid sensor, the SPS-sensor, possesses proteolytic activity. The sensor component Ssy5p is a novel "chymotrypsin-like" protease. The Group has found that Ssy5p is activated in two steps. In the first step, like many other proteases, Ssy5p cleaves itself by autolysis. However, unlike other proteases, this cleavage does not result in an active protease. Strikingly, this initial processing event merely enables the primed but inactive catalytic domain to assemble with the other SPS-sensor components. The second activation step, which leads to transcription factor processing, the actual output of SPS-sensor signaling, requires the other SPS-sensor components and ligand (amino acid) binding. Second, we have uncovered a unique protein complex situated in the inner nuclear membrane that negatively modulates amino acid uptake by squelching the expression of amino acid permease genes. This complex, designated the Asi complex, is not associated with nuclear pores but somehow negatively controls the capacity of two SPS-sensor-regulated transcription factors to enter the nucleus and gain access to SPS-sensor-regulated promoters. These findings are shedding light on an entirely novel mode of regulating gene expression in eukaryotic cells. Third, the Group has shown that Shr3p, an integral component of the ER membrane, specifically prevents amino acid permeases from aggregating, and thus plays a critical role in assisting these transport proteins to fold and correctly attain tertiary structures required for ER exit. Shr3p is the founding member of a unique class of integral ER membrane proteins that function as membrane-localized chaperones. The Group has established that in cells individually lacking one of these ER proteins, discrete sets of polytopic membrane proteins, their cognate substrates, aggregate and consequently fail to exit the ER. Strikingly, aggregation is specifically limited to their cognate substrates. These findings indicate that polytopic membrane proteins require highly selective membrane-localized chaperones to overcome common structural constraints associated with membrane insertion and folding.

Developmental Biology Group

The research program of the Group, led by Dr. Ulf Eriksson, focuses on the biology of members of the VEGF/PDGF family, and their role in angiogenesis, tissue repair, and tumorigenesis.

The VEGF-B project. VEGF-B is abundantly expressed in metabolically highly active organs such as the heart, muscle, brown fat, and brain. VEGF-B is a poor mitogen for endothelial cells *in vitro*, and its physiological role has remained obscure. Surprisingly, VEGF-B is required for efficient tumor growth, and the Group is currently analyzing the mechanisms underlying

this requirement. At least part of the growth stimulating effect of VEGF-B is a promotion of the tumor-stromal reaction observed in most tumors. In mice with experimentally induced ischemia of the heart, VEGF-B treatment results in an enhanced revascularization of the heart (in collaboration with Dr. Peter Carmeliet, University of Leuven, Belgium), and myocardial injection of adenoviruses encoding VEGF-B in normal pig heart induced a strong angiogenic response (in collaboration with Dr. Seppo Ylä-Herttuala, LICR Kuopio Affiliate Center, Finland). Similar treatments in ischemic mouse and rabbit hind limb muscle failed to induce significant angiogenesis. The results show that organ-specific therapeutic responses can be generated, and that VEGF-B can be considered a heart-specific angiogenic factor.

The PDGF project. Two genes encoding novel members of the PDGF family have been identified. These growth factors, denoted PDGF-CC and PDGF-DD, are latent and require proteolytic activation to become capable of binding to and activating the two known PDGF-receptors (α and β, respectively). Both growth factors are expressed in tumors. The Group identified tissue plasminogen activator (tPA) as a specific activator of PDGF-CC, but not of PDGF-DD. Normal primary mouse fibroblasts in culture express both PDGF-CC and tPA, and the Group's studies with tPA-deficient fibroblasts showed that their growth was dependent on an autocrine/paracrine growth-stimulatory loop involving tPA-mediated activation of PDGF-CC. These findings suggest a novel role of tPA in tissue growth and maintenance separate from its previous suggested unique role in the fibrinolytic system. The interaction between PDGF-CC and tPA suggests that proteases related to tPA can activate PDGF-DD, and efforts are undertaken to identify these proteases. The plasminogen/plasminogen activator system is of high relevance in several pathological conditions, particularly in cancer, and it is likely that further analyses of the activating proteases will provide interesting results on the functions of both novel PDGFs in cancer. Transgenic overexpression of both growth factors in heart induced extensive fibrosis and cardiac hypertrophy with vascular changes. The results showed that PDGF-DD is a potent mitogen for vascular smooth muscle cells raising the possibility that it may play an important role in atherosclerosis and restenosis. The potential therapeutic roles of the novel PDGFs in the treatment of tissue ischemia and regeneration are also explored. PDGF-CC treatment in the heart infarction and hind limb mouse models results in stimulation of angiogenesis, and in muscle regeneration in the limb. The biological effects of PDGF-CC are, at least in part, the result of a stimulation of vascular progenitor cells. Similar studies involving PDGF-DD are underway. The Group's studies so far suggest that the novel PDGFs may be of therapeutic value in cardiovascular and other diseases.

Gene Expression Group

The Gene Expression Group, led by Dr. Thomas Perlmann, is analyzing nuclear receptors; ligand-regulated transcription factors that bind steroid hormones, thyroid hormone, retinoids and other small and lipophilic signaling molecules. These receptors are fascinating proteins for several different reasons: First, they are excellent tools for understanding how genes are regulated since small molecule ligands can be used to switch these transcription factors between active and inactive states. Second, classical nuclear receptor signaling pathways, e.g. steroid hormone and retinoid receptors, influence many biological pathways important in development and adult physiology. Accordingly, their significance in disease, including cancer, is critical. Third, nuclear receptors include a large number of related but less well characterized orphan receptors lacking identified ligands. The existence of these proteins is intriguing and suggests that additional unexplored nuclear receptor-mediated signaling pathways remain to be characterized.

Nurr1 is an orphan nuclear receptor, previously shown by the group to be critically involved in dopamine cell development. Dopamine cells are clinically important since they degenerate in

Parkinson's disease and are also involved in several other disorders, including schizophrenia. An important goal is to establish methods allowing engineering of dopamine neurons from stem cells. These studies are important for two reasons: First, *in vitro* generated dopamine cells offer excellent opportunities to study how immature cells are maintained as stem cells and how they differentiate into dopamine cells. Second, *in vitro* engineered dopamine cells can prove to be critical in the development of novel cell replacement strategies in Parkinson's disease. The Group has increased its efforts to identify additional factors of importance in this process and in the previous year two additional transcription factors that are critical in the specification of dopamine cells were identified. The characterization of these novel factors is combined with studies of stem cells in ongoing experiments.

The Group has also continued its characterization of novel ligands activating a nuclear receptor (RXR) that heterodimerizes with Nurr1. These ligands are enriched in the developing and mature central nervous system and the group has previously shown that they promote the survival of neurons. Finally, based on previous structural studies, the Group is focusing on characterizing a novel mechanism whereby the nuclear receptor can activate transcription

Stem Cell Biology Group

The main aim of the Stem Cell Biology Group, led by Dr. Jonas Muhr, is to unveil fundamental principles regulating growth and differentiation of neural stem cells (NSCs) in the developing vertebrate central nervous system (CNS). Important aims are to characterize the molecular pathways that maintain neural stem cells in an undifferentiated proliferative state, and to find out how these pathways are counteracted by genetic programs directing cells towards terminal neuronal differentiation.

To address these issues the Group has focused on the role of a set of HMG-box transcription factors of the Sox gene family in the neurogenesis process in chick embryos, having previously demonstrated that Sox1, Sox2 and Sox3 (Sox1-3) block the differentiation process of NSCs and act to maintain neural cells in an undifferentiated proliferative state. These findings have led to the conclusion that the suppression of Sox1-3 activities is a prerequisite for the ability of NSCs to differentiate into neurons. The Group has continued to study these processes and found that another HMG-box protein, Sox21, has the opposite activity as compared to Sox1-3, promoting NSCs to differentiate. Interestingly, Sox21 mediates this function by counteracting the activity of Sox1-3 proteins. Thus, the selection of whether NSCs remain as progenitor cells, or become committed to differentiation, appears to depend on the balance between Sox21 and Sox1-3 activities. The Group is currently examining how, at the molecular level, Sox1-3 and Sox21 mediate their distinct functions. Moreover, in an additional project work is underway to decipher the genetic pathways establishing a neuronal phenotype of differentiating NSCs.

Publications

Primary Research Articles

1. Andréasson C., Neve E.P.A., Ljungdahl P.O. Four permeases import proline and the toxic proline analog azetidine-2-carboxylate into yeast. Yeast (2004) 21:193-199.
2. Andréasson C., Ljungdahl P.O. The N-Terminal Regulatory Domain of Stp1p Is Modular and, Fused to an Artificial Transcription Factor, Confers Full Ssy1p-Ptr3p-Ssy5p Sensor Control. Molecular and Cellular Biology (2004) 24:7503-7513.

3. Codina A., Benoit G., Gooch J.T., Neuhaus D., Perlmann T., Schwabe W.R. Identification of a novel co-regulator interaction surface on the ligand-binding domain of Nurr1 using NMR footprinting. Journal of Biological Chemistry (2004) 279:53338-53345.

4. Flick K., Katz A., Överby A., Feldmann H., Pettersson R.F., Flick R. Functional analysis of the noncoding regions of the Uukuniemi virus (*Bunyaviridae*) RNA Segments. Journal of Virology (2004) 78:11726-11738.

5. Fredriksson L., Li H., Fieber C., Li X., Eriksson U. Tissue plasminogen activator is a potent activator of PDGF-CC. The EMBO Journal (2004) 23:3793-3802.

6. Furuhashi M., Sjöblom T., Abrahamsson A., Engelsen J., Micke P., Li H., Bergsten-Folestad E., Eriksson U., Heuchel R., Betsholtz C., Heldin C-H., Östman, A. Platelet-derived growth factor production by B16 melanoma cells leads to increased pericyte abundance in tumors and an associated increase in tumor growth rate. Cancer Research (2004) 64:2725-2733.

7. Lengqvist J., Mata De Urquiza A., Bergman A.C., Willson T.M., Sjövall J., Perlmann T., Griffiths W.J. Polyunsaturated fatty acids including docosahexaenoic acid arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain. Molecular & Cellular Proteomics (2004) 3:692-703.

8. Martinez P., Ljungdahl P.O. An ER packaging chaperone determines the amino acid uptake capacity and virulence of *candida albicans*. Molecular Microbiology (2004) 51:371-384.

9. Nordzell M., Aarnisalo P., Benoit G., Castro D.S., Perlmann T. Defining an N-terminal activation domain of the orphan nuclear receptor Nurr1. Biochemical and Biophysical Research Communications (2004) 313:205-211.

10. Nsegbe E., Wallén-Mackenzie Å., Dauger S., Roux J.C., Shvarev Y., Lagercrantz H., Pelmann T., Herlenius E. Congenital hypoventilation and impaired hypoxic response in Nurr1 mutant mice. Journal of Physiology (2004) 556:43-59.

11. Raschperger E., Engström U., Pettersson R.F., Fuxe J. CLMP, a novel member of the CTX family and a new component of the epithelial tight junction. Journal of Biological Chemistry (2004) 279:796-804.

12. Shaw C., Holland P., Sinnreich M., Allen C., Sollerbrant K., Karpati G., Nalbantoglu J. Isoform-specific expression of the Coxsackie and adenovirus receptor (CAR) in neuromuscular junction and cardiac intercalated discs. BMC Cell Biology (2004) 5:42.

13. Uutela M., Wirzenius M., Paavonen K., Rajantie I., He Y., Karpanen T., Lohela M., Wiig H., Salven P., Pajusola K., Eriksson U., Alitalo K. PDGF-D induces macrophage recruitment, increased interstitial pressure and blood vessel maturation during angiogenesis. Blood (2004) 104:3198-3204.

14. Vincent T., Pettersson R.F., Crystal R.G., Leopold P.L. Cytokine-mediated downregulation of coxsackievirus-adenovirus receptor in endothelial cells. Journal of Virology (2004) 78:8047-58.

Reviews / Commentaries / Book Chapters

1. Benoit G., Malewicz M., Perlmann T. Digging deep into the pockets of orphan nuclear receptors: insights from structural studies. Trends in Cell Biology (2004) 14:369-76.

2. Fredriksson L., Li H., Eriksson U. The PDGF family: four gene products form five dimeric isoforms. Cytokine and Growth Factor Reviews (2004) 15:197-204.

3. Nyman T., Kota J., Ljungdahl P.O. Ancillary proteins in membrane targeting of transporters. In: Topics in Current Genetics – Molecular Mechanisms Controlling Transmembrane Transport (eds. E. Boles and R. Krämer), pp 207-234 Springer-Verlag, Heidelberg, 2004.
4. Philipson L., Pettersson R.F. The coxsackie-adenovirus receptor - a new receptor in the immunoglobulin family involved in cell adhesion. In: Adenoviruses: model and vectors in virus-host interactions, W Doerfler and P Böhm (eds). Current Topics in Microbiology and Immunology (2004) 273:87-111.
5. Perlmann T., Wallén-Mackenzie Å. Nurr1, and orphan nuclear receptor with essential functions in developing dopamine cells. Cell & Tissue Research (2004) 318:45-52.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Uppsala Branch
of Growth Regulation

Uppsala, Sweden

Staff List

Heldin, Carl-Henrik *Director*

PDGF Signaling Group

Heldin, Carl-Henrik *Group Head*

Section of Translational Research

Hellberg, Carina *Assistant Investigator, Section Head*
Furuhashi, Masao *Postdoctoral Fellow (until June)*
Hasumi, Yoko *Postdoctoral Fellow (from April)*
Kowanetz, Katarzyna *Postdoctoral Fellow*
Bäckström, Gudrun *Senior Technical Assistant*
Sandström Leppänen *Jill, Technical Assistant*
Karlsson, Susann *Graduate Student (from April)*
Klosowska-Wardega, Agnieszka *Graduate Student (from July)*

Section for Signal Transduction

Lennartsson, Johan *Postdoctoral Fellow, Section Head*
Amagasaki, Kenichi *Postdoctoral Fellow (from September)*
Chiara, Federica *Postdoctoral Fellow (until February)*
Rorsman, Charlotte *Senior Technical Assistant*
Kallin, Anders *Graduate Student (until January)*
Wardega, Piotr *Graduate Student (from July)*
Gembarska, Agnieszka *GraduateStudent (March to December)*

Cytoskeletal Regulation Group

Aspenström, Pontus *Associate Investigator, Group Head*
Hájková, Lucie *Postdoctoral Fellow (until April)*
Pacholsky, Dirk *Postdoctoral Fellow (from June)*
Ruusala, Aino *Senior Technical Assistant*
Fransson, Åsa *Graduate Student*
Johansson, Ann-Sofi *Graduate Student*

Gene Targeting Group

Heuchel, Rainer *Assistant Member, Group Head*
Li, Ronggui *Postdoctoral Fellow*
Åhgren, Aive *Senior Technical Assistant*
Zieba, Agata *Graduate Student (from September)*

TGF β Signaling Group

Moustakas, Aristidis *Associate Member, Group Head*
Niimi, Hideki *Postdoctoral Fellow*
Thuault, Sylvie *Postdoctoral Fellow (from February)*
Valcourt, Ulrich *Postdoctoral Fellow (until June)*
Morén, Anita *Senior Technical Assistant*
Kowanetz, Marcin *Graduate Student*
Lönn, Peter *Graduate Student (from July)*
Pardali, Katerina *Graduate Student*

Integrated Signaling Group

Souchelnytskyi, Serhiy *Assistant Member, Group Head*
Dubrovska, Anna *Postdoctoral Fellow*
Iwahana, Hiroyuki *Postdoctoral Fellow (until March)*
Yakymovych, Ihor *Visiting Scientist*
Yakymovych, Mariya *Visiting Scientist*
Bhaskaran, Nimesh *Graduate Student (from May)*
Woksepp, Hanna *Graduate Student (from February)*

Apoptotic Signaling Group

Landström, Maréne *Assistant Member, Group Head*
Edlund, Sofia *Postdoctoral Fellow (until May)*
Lee, So Young *Postdoctoral Fellow*
Zhang, Shouting *Postdoctoral Fellow*
Grimsby, Susanne *Senior Technical Assistant*
Ekman, Maria *Graduate Student (from August)*
Cavallini, Nicola *Student (February to April)*

Gene Expression Group

Ericsson, Johan *Associate Member, Group Head*
Grönroos, Eva *Assistant Investigator*
Bengoechea Alonso *Maria Teresa, Postdoctoral Fellow*
Kanduri, Meena *Postdoctoral Fellow (from May)*
Punga, Tanel *Postdoctoral Fellow (from April)*
Sundqvist, Anders *Postdoctoral Fellow*
Lukiyanchuk, Vasyl *Graduate Student*
Simonsson, Maria *Graduate Student*

Matrix Biology Group

Heldin, Paraskevi *Associate Investigator, Group Head*
Bagchi, Sonchita *Graduate Student (from August)*
Asteriou, Trias *Postdoctoral Fellow (until June)*
Li, Yuejuan *Postdoctoral Fellow*
Kamiryo, Masaru *Graduate student*
Li, Lingli *Graduate Student*

Protein Structure Group

Hellman, Ulf *Member, Group Head*
Conrotto, Paolo *Graduate Student (from April)*
Engström, Ulla *Senior Technical Assistant*
Wernstedt, Christer *Senior Technical Assistant*

Technical Support

Ejdesjö, Bengt *Purchasing Officer*
Hedberg, Ulf *IT support*
Hermansson, Lars-Erik *Service Engineer*
Pettersson, Gullbritt *Laboratory Assistant*
Schönquist, Inger *Laboratory Assistant*

Administration & Secretariat

Hallin, Eva *Finance and Administration Manager*
Schiller, Ingegärd *Secretary*

Branch Director's Report

The aim of the research at the Uppsala Branch is to elucidate the signaling pathways that control cell growth and migration in cells. As malignant cells show perturbations of such pathways, we hope that our results will reveal suitable targets for the development of signal transduction modulators that can be used for treatment of cancer.

Important themes of our research are, as before, platelet-derived growth factor (PDGF) and transforming growth factor β (TGF β). PDGF isoforms are major mitogens for connective tissue cells and certain other cell types, and are implicated in autocrine as well as paracrine stimulation in tumors. Important goals are to elucidate the mechanisms of signal transduction downstream of PDGF receptors and to explore the clinical utility of PDGF antagonists. TGF β family members have important roles in controlling differentiation during the embryonal development, as they inhibit the growth of most cell types. In cancer, the role of TGF β is complicated; initially it is a tumor suppressor through its ability to inhibit growth and stimulate apoptosis of cells, but at later stages of tumor progression TGF β promotes tumorigenesis, e.g. by changing the differentiated state of cells to a more invasive one and through indirect effects that include stimulation of angiogenesis and suppression of the immune system. In addition to elucidating the molecular mechanism of TGF β action, an important aim of our work is to explore whether TGF β antagonists can be used for treatment of advanced cancer.

The work at our Branch is performed in nine different groups that work on various aspects of signal transduction. The groups complement each others in terms of technical skills. Thus, in addition to conventional cell and molecular biology methods, we have access to technology in proteomics and mass spectrometry, microarray analyses, advanced microscopy and mouse genetics

Carl-Henrik Heldin

Research Report

PDGF Signaling Group

The work in the PDGF Signaling Group, led by Dr. Carl-Henrik Heldin, is aimed at elucidating the mechanisms of signal transduction via PDGF receptors, as well as exploring the clinical utility of PDGF antagonists.

The PDGF Signal Transduction Section, headed by Dr. Johan Lennartsson, attempts to clarify signaling pathways activated by PDGF and how this activation results in diverse cellular responses. These studies involve detailed analyses of differences in signaling downstream of the two PDGF receptor isoforms, PDGFRα and PDGFRβ. There are two different approaches, one involving DNA microarray analysis of genes induced following activation of the two receptor isoforms, and the other involving purification and identification of proteins phosphorylated by the receptors.

A biological read-out that differs between PDGFRα and β is the ability to induce chemotaxis. By disrupting various signaling pathways and investigating how this reflects on the ability of the PDGFRs to mediate chemotaxis, the Group can identify key proteins in this process. These proteins are further analyzed in regard to PDGFR isoform specific signaling. Combined, these studies will shed light on the unique functional abilities of the PDGFRα and β. In addition, the Group is also studying the effect of the activation loop tyrosine in the PDGFRβ on the receptor's kinase activity and its ability to induce signal transduction.

The Translational Research Section, headed by Dr, Carina Hellberg, investigates the therapeutic targeting of PDGF receptors on tumor stromal cells, such as fibroblasts and pericytes. Current studies investigate the molecular mechanisms underlying the PDGF-induced increase in tumor interstitial fluid pressure (IFP). The theory that lowering of tumor IFP using PDGF receptor antagonists could increase the tumor uptake of therapeutic antibodies, as well as reduce tumor hypoxia, is being investigated. Furthermore, PDGF dependent pericyte recruitment contributes to tumor angiogenesis and growth, thus the possibility that PDGF antagonist-mediated pericyte targeting could constitute an anti-angiogenic approach, either by itself or in combination with agents targeting endothelial cells, is being explored. Additionally, regulation of the termination of PDGF β-receptor signal transduction by receptor dephosphorylation, internalization and subsequent degradation, is under investigation.

Cytoskeletal Regulation Group

The work within the Cytoskeletal Regulation Group, led by Dr. Pontus Aspenström, is aimed at elucidating signaling pathways that control cell growth and migration during normal physiological conditions, as well as during disease. The Group has focused on the Rho GTPases, a gene family of 23 members in human cells. The studies have shown that the effects on the actin filament system evoked by the different Rho GTPases, are more intricate than previously recognized. In addition, several members of the Rho GTPases have roles distinct from regulating the organization of the actin filament system. For instance, Miro has important roles in regulating mitochondrial homeostasis. Furthermore, the Group has studied pathways that activate or modulate the activity of the Rho GTPases and has found that Smad7 is required for the TGF-β induced activation of Cdc42 as well as for the concomitant reorganization of the actin filament system. Finally, the Group is studying the verprolins, which are WASP-binding proteins involved in actin reorganization and endocytosis.

Gene Targeting Group

The Gene Targeting Group, led by Dr. Rainer Heuchel, develops mouse genetic models to explore the *in vivo* importance of specific signaling pathways initiated by different growth factors. In order to investigate the possible involvement of the PDGF β-receptor in disease, two mouse models with gain of function mutations in the activation loop of the kinase domain, which have been found as oncogenic mutations in other tyrosine kinase receptors, were generated. The first introduced mutation did not cause an overt phenotype in the mouse, despite the fact that the mutant PDGF β-receptor conferred increased anti-apoptotic signaling and increased migratory behavior of isolated mouse embryonic fibroblasts in a ligand-independent manner. A second, more potent mutation of the same amino acid residue was not compatible with embryonic development and highlighted the importance of proper PDGF signaling during embryogenesis.

The group also investigates TGF-β signaling in *in vivo* models. In order to elucidate the *in vivo* function of Smad7, a negative regulator of TGF-β signaling, the Smad7 gene was targeted in mice in collaboration with Dr. Anthony Pawson (Ontario Genomics Institute, Canada). Mutant mice showed skeletal malformations and a changed B cell response due to increased TGF-β signaling in the absence of down-modulating Smad7.

TGF-β Signaling Group

The TGF-β Signaling Group, led by Dr. Aristidis Moustakas, investigates signaling pathways and gene networks that regulate cell growth and differentiation via TGF-β, and processes that contribute to tumor cell invasiveness and metastasis. The Group has described new mechanisms of Smad3 protein nucleocytoplasmic shuttling, and of Smad4 ubiquitination by Smurfs. The Group also screens for novel ubiquitin ligases and de-ubiquitinases that regulate Smad4 function and proteins that interact with Smad4 in an ubiquitin-dependent manner. Using large-scale gene expression analysis, the Group identified new target genes that regulate either cell growth or epithelial-to-mesenchymal transition (EMT), a prerequisite for carcinoma metastasis. A model that explains signaling specificity by TGF-β members was subsequently developed. TGF-βs suppresses robustly epithelial cell growth and induces potently EMT by inducing the expression of the cell cycle inhibitor p21 and by repressing the expression of the Id2 and Id3 transcriptional regulators. Bone morphogenetic proteins (BMPs) weakly suppress epithelial growth and fail to induce EMT because they induce sustained Id2/Id3 levels, which antagonize directly the effects of p21, at least at the level of cell cycle regulation.

Integrated Signaling Group

The main objective in the Integrated Signaling Group, led by Dr. Serhiy Souchelnystskyi, is to explore molecular mechanisms of cellular carcinogenic transformation. Proteomics, which provides a comprehensive overview of changes in protein expression and activities, is being used to unveil cancer-related changes in cells, and intracellular signaling mechanisms of TGF-β. The Group's proteomics platform is based on two-dimensional gel electrophoresis for separation of proteins, dedicated computer-aided image analysis for detection of changes, and mass spectrometry for identification of selected proteins. Proteome expression maps of human breast epithelial cells have been constructed, and significant changes in cell proteomes have been observed between primary, immortal and tumorigenic cells. Proteomics can also identify markers to detect and monitor cancer. The Group has detected aberrant expression of three proteins in patients with breast and ovarian cancer, with the presence of these proteins in the plasma correlating with the early stages of cancer.

The Group has also identified more than 300 proteins regulated by TGF-β in DNA damage repair and DNA damage-induced signaling. The findings define novel molecular mechanisms of TGFβ-dependent regulation of the maintenance of genomic DNA integrity. The possibility of developing specific kinase inhibitors of TGF-β receptors is also being explored, with the aim of developing compounds useful for the treatment of diseases such as cancer and diabetes, and nephrotic and fibrotic conditions.

Apoptotic Signaling Group

The work in the Apoptotic Signaling Group, led by Dr. Maréne Landström, aims at elucidating the molecular mechanisms whereby TGF-β induces apoptosis. TGF-β and Wnt signals are important regulators of cell fate during embryogenesis, as well as in tumor progression. The Group has found that Smad7 in the TGF β signaling pathway interacts with β catenin and lymphoid enhancer binding factor 1/T cell-specific factor, transcriptional regulators in the Wnt signaling pathway, in a TGF-β-dependent manner. Furthermore, with the use of siRNA and anti-sense techniques, the Group has shown that Smad7 expression is required for TGF-β-induced stabilization of β catenin, increase of c Myc and subsequent apoptosis in human prostate cancer cells, as well as in immortalized human keratinocytes. Interestingly, Smad7 together with the MAP kinase p38 was observed to regulate the activity of Akt and glycogen synthetase kinase 3β, which in turns leads to the stabilization of β catenin. The Group has previously demonstrated that Smad7 is

required for TGF-β-induced activation of p38 and subsequent apoptosis. Moreover, Smad7 was found to be also required for 2 methoxyestradiol-induced apoptosis in prostate cancer cells. 2 methoxyestradiol is an endogenous metabolite of estradiol 17β, and is used in clinical trials for treatment of a variety of advanced cancers.

Gene Expression Group

The Gene Expression Group, led by Dr. Johan Ericsson, is interested in how post-translational modifications regulate the activity of transcription factors. Most proteins are targeted by some type of post-translational modification, such as phosphorylation, acetylation or ubiquitination. These modifications function as molecular switches that control the activity of proteins. Elucidating the mechanisms involved in controlling these switches will be a major challenge in the post-genomic era. The Group has identified novel pathways that regulate the activity of proteins associated with major human diseases. Most of this work has been focused on two groups of proteins, the Smad and SREBP families of transcriptional regulators. The Group has demonstrated that competition between acetylation and ubiquitination of overlapping lysine residues is a novel pathway to regulate protein stability. In addition, transcription-dependent degradation was identified as a novel pathway to regulate gene expression. Work is currently underway to define the signals involved in these pathways, since they may represent general mechanisms to control the function of proteins. Finally, the Group recently identified YY1 as a novel regulator of the p53 tumor suppressor and the role of YY1 in p53-dependent processes is currently being characterized.

Matrix Biology Group

The research in the Matrix Biology Group, led by Dr. Paraskevi Heldin, is focused on understanding the role of hyaluronan in the microenvironment of tumor cells for the progression of tumors. Until now it has not been clear how hyaluronan, produced by tumor cells or adjacent non-cancer stromal cells, affect tumorigenesis. The Group has recently shown that hyaluronan synthesizing enzymes promote tumorigenicity, whereas degrading enzymes suppress tumor development. The Group also found that aggressive breast cancer cells, in which individual hyaluronan synthases have been knocked-down by siRNA techniques, have the intrinsic ability to stimulate compensatory mechanisms in order to maintain their hyaluronan production. In a microarray-based effort, the gene profile induced by hyaluronan fragments in endothelial cells was characterized. One of the induced components was the cytokine Gro1, which was shown to be essential for the effects of hyaluronan fragments on endothelial cell differentiation. Additionally, the Group is studying how hyaluronan-CD44 interactions affect cellular behavior including cell adhesion and motility, proliferation, and differentiation. The aim is to elucidate how signals induced by hyaluronan binding to CD44 are interrelated with signals induced by PDGF-BB and TGF-β.

Protein Structure Group

The Protein Structure Group, led by Dr. Ulf Hellman, continues its close interaction with all other Groups at the Branch, with the major tasks being to identify and analyze proteins. The Group has developed sophisticated peptide syntheses, such that it is now possible to produce various modifications on specific amino acids in a peptide. This has been especially useful in the case of phosphorylation on threonines, serines or tyrosines, and acetylation of lysines. The Group is also identifying phosphorylation sites in proteins using 32P orthophosphate labeling and Edman degradation in amino acid sequence analyzers. Combined with phosphor imaging the sensitivity is far better than that obtained by mass spectrometry-based methods. Finally, the Group also operates a Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-ToF-MS) instrument. Its main use is in peptide mass fingerprinting (PMF), which

has totally replaced Edman chemistry for protein identification - a consequence of the growing number of sequenced genomes and the technical development of MS. In spite of the efficiency of PMF, it is often necessary to perform *de novo* amino acid sequencing. This is efficiently done in the MALDI TOF/TOF, using a derivatization protocol which enhances specific fragmentation during Post Source Decay (PSD).

Publications

Primary Research Articles

1. 1. Agüero F., Noé G., Hellman U., Repetto Y., Zaha A., Cazzulo J.J. Purification, cloning, and expression of the mitochondrial malate dehydrogenase (mMDH) from protoscolices of Echinococcus granulosus. Molecular & Biochemical Parasitology (2004) 137:207-214.
2. All-Ericsson C., Girnita L., Müller-Brunotte A., Brodin B., Seregard S., Östman A., Larsson O. c-Kit-dependent growth of uveal melanoma cells: a potential therapeutic target? Investigative Ophthalmology & Visual Science (2004) 45:2075-2082.
3. Alvarado-Kristensson M., Melander F., Leandersson K., Rönnstrand L., Wernstedt C., Andersson T. p38-MAPK signals survival by phosphorylation of caspase-8 and caspase-3 in human neutrophils. The Journal of Experimental Medicine (2004) 199:449-458.
4. Aspenström P. The mammalian verprolin homologue WIRE participates in receptor-mediated endocytosis and regulation of the actin filament system by distinct mechanisms. Experimental Cell Research (2004) 298:485-498.
5. Aspenström P., Fransson Å., Saras J. Rho GTPases have diverse effects on the organization of the actin filament system. Biochemical Journal (2004) 377: 327–337.
6. Ben-Saadon R., Fajerman I., Ziv T., Hellman U., Schwartz A.L., Ciechanover A. The tumor suppressor protein $p16^{INK4a}$ and the human papillomavirus oncoprotein-58 E7 are naturally occurring lysine-less proteins that are degraded by the ubiquitin system. Direct evidence for ubiquitination at the N terminal residue. The Journal of Biological Chemistry (2004) 279: 41414-41421.
7. Bergström J., Murphy C.L., Weiss D.T., Solomon A., Sletten K., Hellman U., Westermark P. Two different types of amyloid deposits--apolipoprotein A-IV and transthyretin--in a patient with systemic amyloidosis. Laboratory Investigation (2004) 84:981 988.
8. Chiara F., Bishayee S., Heldin C-H., Demoulin J-B. Autoinhibition of the platelet-derived growth factor β receptor tyrosine kinase by its C-terminal tail. The Journal of Biological Chemistry (2004) 279: 19732-19738.
9. Chiara F., Goumans M-J., Forsberg H., Åhgren A., Rasola A., Aspenström P., Wernstedt C., Hellberg C., Heldin C-H., Heuchel R. A Gain of function mutation in the activation loop of platelet-derived growth factor β-receptor deregulates its kinase activity. The Journal of Biological Chemistry (2004) 279: 42516 42527.
10. Davoodpour P., Bergström M., Landström M. Effects of 2-methoxyestradiol on proliferation, apoptosis and PET-tracer uptake in human prostate cancer cell aggregates. Nuclear Medicine & Biology (2004) 31: 867-874.

11. Demoulin J-B., Ericsson J., Kallin A., Rorsman C., Rönnstrand L., Heldin C-H. Platelet-derived growth factor stimulates membrane lipid synthesis through activation of phosphatidylinositol 3-kinase and sterol regulatory element-binding proteins. The Journal of Biological Chemistry (2004) 279:35392-35402.

12. Dudas M., Nagy A., Laping N.J., Moustakas A., Kaartinen V. Tgf-β3-induced palatal fusion is mediated by Alk 5/Smad pathway. Developmental Biology (2004) 266:96 108.

13. Edlund S., Landström M., Heldin C-H., Aspenström P. Smad7 is required for TGF-β-induced activation of the small GTPase Cdc42. Journal of Cell Science (2004) 117:1835-1847.

14. Furuhashi M., Sjöblom T., Abramsson A., Ellingsen J., Micke P., Li H., Bergsten-Folestad E., Eriksson U., Heuchel R., Betsholtz C., Heldin C-H., Östman A. Platelet-derived growth factor production by B16 melanoma cells leads to increased pericyte abundance in tumors and an associated increase in tumor growth rate. Cancer Research (2004) 64:2725-2733.

15. Galindo M., Varela N., Espinoza I., Toro G.C., Hellman U., Wernstedt C., Galanti N. Chromatin from two classes of platyhelminthes display both protist H1 and higher eukaryote core histones. FEBS Letters (2004) 567: 225-229.

16. Grimsby S., Jaensson H., Dubrovska A., Lomnytska M., Hellman U., Souchelnytskyi S. Proteomics-based identification of proteins interacting with Smad3: SREBP-2 forms a complex with Smad3 and inhibits its transcriptional activity. FEBS Letters (2004) 577:93-100.

17. Grönroos E., Terentiev A.A., Punga T., Ericsson J. YY1 inhibits the activation of the p53 tumor suppressor in response to genotoxic stress. Proceedings of the National Academy of Sciences USA (2004) 101: 12165-12170.

18. Göransson V., Johnsson C., Jacobson A., Heldin P., Hällgren R., Hansell P. Renal hyaluronan accumulation and hyaluronan synthase expression after ischaemia-reperfusion injury in the rat. Nephrology, Dialysis, Transplantation (2004) 19:823-830.

19. Haglund K., Ivankovic-Dikic I., Shimokawa N., Kruh G.D., Dikic I. Recruitment of Pyk2 and Cbl to lipid rafts mediates signals important for actin reorganization in growing neurites. Journal of Cell Science (2004) 117:2557-2568.

20. Hassel S., Eichner A., Yakymovych M., Hellman U., Knaus P., Souchelnytskyi S. Proteins associated with type II bone morphogenetic protein receptor (BMPR-II) and identified by two-dimensional gel electrophoresis and mass spectrometry. Proteomics (2004) 4:1346-1358.

21. Ibarrola N., Kratchmarova I., Nakajima D., Schiemann W.P., Moustakas A., Pandey A., Mann M. Cloning of a novel signaling molecule, AMSH-2, that potentiates transforming growth factor β signaling. BioMed Central Cell Biology (2004) 5: 2.

22. Jaffe A.B., Aspenström P., Hall A. Human CNK1 acts as a scaffold protein, linking Rho and Ras signal transduction pathways. Molecular and Cellular Biology 24:1736 1746.

23. Kahata K., Hayashi M., Asaka M., Hellman U., Kitagawa H., Yanagisawa J., Kato S., Imamura T., Miyazono K. Regulation of transforming growth factor-beta and bone morphogenetic protein signalling by transcriptional coactivator GCN5. Genes to Cells (2004) 9:143-151.

24. Kallin A., Demoulin J-B., Nishida K., Hirano T., Rönnstrand L., Heldin C H. Gab1 contributes to cytoskeletal reorganization and chemotaxis in response to platelet-derived growth factor. The Journal of Biological Chemistry (2004) 279:17897-17904.

25. Klint P., Hellman U., Wernstedt C., Åman P., Ron D., Claesson-Welsh L. Translocated in liposarcoma (TLS) is a substrate for fibroblast growth factor receptor-1. Cellular Signalling (2004) 16:515-520.

26. Kowanetz K., Crosetto N., Haglund K., Schmidt M.H., Heldin C-H., Dikic I. Suppressors of T-cell receptor signaling Sts-1 and Sts-2 bind to Cbl and inhibit endocytosis of receptor tyrosine kinases. The Journal of Biological Chemistry (2004) 279:32786-32795.

27. Kowanetz K., Husnjak K., Höller D., Kowanetz M., Soubeyran P., Hirsch D., Schmidt M.H., Pavelic K., De Camilli P., Randazzo P.A., Dikic I. CIN85 associates with multiple effectors controlling intracellular trafficking of epidermal growth factor receptors. Molecular Biology of the Cell (2004) 15: 3155-3166.

28. Kowanetz M., Valcourt U., Bergström R., Heldin C-H., Moustakas A. Id2 and Id3 define the potency of cell proliferation and differentiation responses to transforming growth factor β and bone morphogenetic protein. Molecular and Cellular Biology (2004) 24:4241-4254.

29. Leppänen O., Rutanen J., Hiltunen M.O., Rissanen T.T., Turunen M.P., Sjöblom T., Brüggen J., Bäckström G., Carlsson M., Buchdunger E., Bergqvist D., Alitalo K., Heldin C-H., Östman A., Ylä-Herttuala S. Oral imatinib mesylate (STI571/gleevec) improves the efficacy of local intravascular vascular endothelial growth factor-C gene transfer in reducing neointimal growth in hypercholesterolemic rabbits. Circulation (2004) 109: 1140-1146.

30. Li L., Bu S., Bäckström T., Landström M., Ulmsten U., Fu X. Induction of apoptosis and G2/M arrest by 2 methoxyestradiol in human cervical cancer HeLaS3 cells. Anticancer Research (2004) 24: 873-880.

31. Lomnytska M., Lukiyanchuk V., Hellman U., Souchelnytskyi S. Transforming growth factor-β1-regulated proteins in human endothelial cells identified by two-dimensional gel electrophoresis and mass spectrometry. Proteomics (2004) 4:995-1006.

32. Lundström A., Gallio M., Englund C., Steneberg P., Hemphälä J., Aspenström P., Keleman K., Falileeva L., Dickson B., Samakovlis C. Vilse, a conserved Rac/Cdc42 GAP mediating Robo repulsion in tracheal cells and axons. Genes & Development (2004) 18:2161-2171.

33. Micke P., Bjørnsen T., Scheidl S., Strömberg S., Demoulin J-B., Pontén F., Östman A., Lindahl P., Busch C. A fluid cover medium provides superior morphology and preserves RNA integrity in tissue sections for laser microdissection and pressure catapulting. The Journal of Pathology (2004) 202:130-138.

34. Neuschäfer-Rube F., Hermosilla R., Rehwald M., Rönnstrand L., Schülein R., Wernstedt C., Püschel G.P. Identification of a Ser/Thr cluster in the C terminal domain of the human prostaglandin receptor EP4 that is essential for agonist-induced beta-arrestin1 recruitment but differs from the apparent principal phosphorylation site. Biochemical Journal (2004) 379:573-585.

35. Pandey R.R., Ceribelli M., Singh P.B., Ericsson J., Mantovani R., Kanduri C. NF-Y regulates the antisense promoter, bidirectional silencing and differential epigenetic marks of the Kcnq1 imprinting control region. The Journal of Biological Chemistry (2004) 279:52685 - 52693.

36. Persson C., Sjöblom T., Groen A., Kappert K., Engström U., Hellman U., Heldin C-H., den Hertog J., Östman A. Preferential oxidation of the second phosphatase domain of RPTPα revealed by a new antibody-based method for detection of oxidized PTPs. Proceedings of the National Academy of Sciences USA (2004) 101:1886-1891.

37. Persson C., Sävenhed C., Bourdeau A., Tremblay M.L., Markova B., Böhmer F.D., Haj F.G., Neel B.G., Elson A., Heldin C-H., Rönnstrand L., Östman A., Hellberg C. Site-selective regulation of platelet-derived growth factor β receptor tyrosine phosphorylation by T-cell protein tyrosine phosphatase. Molecular and Cellular Biology (2004) 24:2190-2201.

38. Raschperger E., Engström U., Pettersson R.F., Fuxe J. CLMP, a novel member of the CTX family and a new component of epithelial tight junctions. The Journal of Biological Chemistry (2004) 279:796-804.

39. Richnau N., Fransson Å., Farsad K., Aspenström P. RICH-1 has a BIN/Amphiphysin/Rvsp domain responsible for binding to membrane lipids and tubulation of liposomes. Biochemical and Biophysical Research Communications (2004) 320:1034-1042.

40. Ruusala A., Aspenström P. Isolation and characterisation of DOCK8, a member of the DOCK180-related regulators of cell morphology. FEBS Letters (2004) 572: 159-166.

41. Saras J., Wollberg P., Aspenström P. Wrch1 is a GTPase-deficient Cdc42 like protein with unusual binding characteristics and cellular effects. Experimental Cell Research (2004) 299: 356-369.

42. Shimokawa N., Qiu C-H., Seki T., Dikic I., Koibuchi N. Phosphorylation of JNK is involved in regulation of H+-induced c-Jun expression. Cellular Signalling (2004) 16: 723-729.

43. Sjöblom T., Yakymovych I., Heldin C-H., Östman A., Souchelnytskyi S. Smad2 suppresses the growth of Mv1Lu cells subcutaneously inoculated in mice. European Journal of Cancer (2004) 40: 267-274.

44. Szymkiewicz I., Destaing O., Jurdic P., Dikic I. SH3P2 in complex with Cbl and Src. FEBS Letters (2004) 565: 33-38.

45. Yakymovych I., Heldin C-H., Souchelnytskyi S. Smad2 phosphorylation by type I receptor: Contribution of arginine 462 and cysteine 463 in the C-terminus of Smad2 for specificity. The Journal of Biological Chemistry (2004) 279: 35781-35787.

46. Zernii E.Y., Tikhomirova N.K., Hellman U., Philippov P.P., Senin II. Annexins in bovine photoreceptors. Annexins (2004) 1: 44-50.

47. Zolessi F.R., Durán R., Engström U., Cerveñansky C., Hellman U., Arruti C. Identification of the chicken MARCKS phosphorylation site specific for differentiating neurons as Ser 25 using a monoclonal antibody and mass spectrometry. Journal of Proteome Research (2004) 3: 84-90.

48. Zuberovic A., Ullsten S., Hellman U., Markides K.E., Bergquist J. Capillary electrophoresis off-line matrix-assisted laser desorption/ionisation mass spectrometry of intact and digested proteins using cationic-coated capillaries. Rapid Communications in Mass Spectrometry (2004) 18: 2946-2952.

Reviews / Commentaries / Book Chapters

1. Aspenström P. Integration of signalling pathways regulated by small GTPases and calcium. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research (2004) 1742:51-58.

2. Heldin C-H ed. Thematic issue on platelet-derived growth factor. Cytokine & Growth Factor Reviews (2004) 15: 195-289.

3. Heldin C-H. Development and possible clinical use of antagonists for PDGF and TGF-β. Uppsala Journal of Medical Science (2004) 109:165-178.

4. Heldin C-H., Rubin K., Pietras K., Östman A. High interstitial fluid pressure - an obstacle in cancer therapy. Nature Reviews Cancer (2004) 4:806-813.

5. Heldin C-H. Platelet-derived growth factor - an introduction. Cytokine & Growth Factor Reviews (2004) 15:195-196.

6. Höller D., Dikic I. Receptor endocytosis via ubiquitin-dependent and -independent pathways. Biochemical Pharmacology (2004) 67:1013-1017.

7. Micke P., Östman A. Tumour-stroma interaction: cancer-associated fibroblasts as novel targets in anti-cancer therapy? Lung Cancer (2004) 45: S163–S175.

8. Östman A., Heldin C-H. Platelet-derived growth factor (PDGF). In: Encyclopedia of Endocrine Diseases, Martini L, ed. Academic Press, San Diego, USA, (2004) 4: 690-696.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Affiliate Research Reports

Peter ten Dijke, Ph.D.

Division of Cellular Biochemistry
The Netherlands Cancer Institute
Amsterdam, The Netherlands

Research Report

Transforming growth factor-β1 (TGF-β1) is a secreted homodimeric protein that regulates numerous responses, such as proliferation, differentiation, migration and apoptosis. TGF-β1 is the prototypic member of a family of over 35 structurally related pleiotropic cytokines. Members of the TGF-β superfamily have crucial roles in development and tissue homeostasis. Perturbation of their signaling has been implicated in several developmental disorders and human diseases, including cancer, fibrosis and auto-immune disorders. The aims of our research are to elucidate the molecular mechanisms by which TGF-β family members elicit their cellular effects via their serine/threonine kinase receptors and intracellular Smad effectors and to generate animal models for human diseases caused by subverted TGF-β family signaling.

Mechanisms underlying TGF-β stimulated tumor progression and metastasis

Cancer cells are often selectively insensitive to TGF-β-induced growth inhibition and apoptosis. Furthermore, TGF-β has been shown to function as a tumor promoter in late phases of tumorigenesis. Carcinomas, which often secrete high amounts of TGF-β, respond to it by enhanced invasion and metastasis. TGF-β may mediate these effects directly on tumor cells via subverted Smad-dependent or independent responses. To obtain more insight into the mechanisms that underlie TGF-β-induced invasion and metastasis of tumor cells, we (in collaboration with Dr. C. Löwik, Leiden University Medical Center, Leiden) have developed a mouse model of bone metastasis of MDA-MB241 breast tumor cells and PC3 prostate cancer cells. Both tumor types are injected intracardially to enable circulation of the tumor cells throughout the body. The method results in selective invasion and outgrowth of tumor cells in bone, causing either osteolytic (in the case of MDA MB 231) or osteosclerotic lesions (in the case of PC3 cells). The cells have been stably transfected with a CMV-driven luciferase reporter plasmid to allow in vivo monitoring using non-invasive imaging. Analysis of the effect of overexpression or knockdown of specific TGF-β family signaling components on metastasis is in progress.

Regulating endothelial cell behavior by TGF-β

TGF-β regulates vascular development and maintenance. TGF-β has both pro and anti-angiogenic properties of which the molecular mechanisms are unclear. Using in vitro studies, we have demonstrated that TGF-β activates two distinct type I TGF-β receptors, i.e. ALK1 and ALK5, which have opposite effects on endothelial cells. TGF-β/ALK1 signaling via Smad1/5 stimulates endothelial cell migration, proliferation, tube formation and invasion, whereas TGF-β/ALK5 signaling via Smad2/3 inhibits these processes. Interestingly, we have recently demonstrated that ligand binding and intracellular kinase domains of ALK5 are required for TGF-β/ALK1 signaling. TGF-β/ALK5 pathway can elicit an antagonistic signal via direct interaction and activation of ALK1. Activated ALK1 not only induced a biological response opposite from ALK5, but also directly inhibited ALK5/Smad signaling. The requirement for ALK5 in ALK1 activation and the counteractive interplay between ALK5 and ALK1 provides the endothelial cell with an intricately regulated TGF-β controlled switch, which will determine whether its fate is quiescence or active migration and proliferation. Furthermore, the endothelial specific accessory TGF-β receptor endoglin was found to be required for proliferation of endothelial cells and efficient TGF-β/ALK1 signaling.

Regulating osteoblast differentiation by bone morphogenic proteins (BMPs) and secreted antagonists

BMPs have an important role in controlling mesenchymal cell fate. The critical target genes by which BMPs mediate cell fate, however, are poorly characterized. We performed transcriptional profiling of mesenchymal cells upon ectopic expression of one of three distinct BMP type I receptors in a constitutively active mutant form. Interestingly, all three type I receptors induced identical transcriptional profiles. Many extracellular matrix genes were upregulated, muscle-related genes downregulated and transcription factors/signaling components modulated. These target genes, some of them unexpected, may offer new insights into the mechanism by which BMPs regulate mesenchymal cell fate.

Sclerosteosis, a skeletal disorder characterized by high bone mass due to increased osteoblast activity, is caused by loss of the SOST gene product, sclerostin, a member of the DAN family with BMP antagonist activity. In collaboration with Dr. C. Löwik, we found that SOST/sclerostin is expressed exclusively by osteocytes in bone and inhibits differentiation and mineralization of osteoblastic cells. Although sclerostin shares some of the actions of the classical BMP antagonist noggin, we demonstrated that it also has actions distinctly different from it. In contrast to noggin, sclerostin did not inhibit BMP-induced alkaline phosphatase activity of mouse myoblastic C2C12 cells. In addition, sclerostin had no effect on BMP-stimulated Smad phosphorylation and direct transcriptional activation of BMP response element reporter constructs. Its unique localization and action on osteoblasts suggest that sclerostin may be the previously proposed osteocyte-derived factor that is transported to osteoblasts at the bone surface and inhibits bone formation.

Publications

Primary Research Articles

1. Carvalho R.L.S., Jonker L., Goumans M-J., Larsson J., Bouwman P., Karlsson S., Ten Dijke P., Arthur H.M., Mummery C.L. Defective paracrine signalling by TGFβ in yolk sac vasculature of endoglin mutant mice: a paradigm for HHT. Development (2004) 131:6237-6247.
2. Chuva de Sousa Lopes S.M., Feijen A., Korving J., Korchynskyi O., Larsson J., Karlsson S., Ten Dijke P., Lyons K.M., Goldschmeding R., Doevedans P., Mummery C.L. CTGF expression and Smad signaling during mouse heart development and myocardial infarction. Developmental Dynamics (2004) 231:542-50.
3. Itoh F., Itoh S., Goumans M-J., Valdimarsdottir G., Iso T., Dotto G.P., Hamamori Y., Kedes L., Kato M., Ten Dijke P. Synergy and antagonism between Notch and BMP receptor signaling pathways in endothelial cells. The EMBO Journal (2004) 23:541-551.
4. Lebrin F., Goumans M-J., Jonker L., Carvalho R.L.C., Valdimarsdottir G., Thorikay M., Mummery C., Arthur H.M., Ten Dijke P. Endoglin promotes endothelial cell proliferation and TGF-β/ALK1 signal transduction. The EMBO Journal (2004) 23:4018-4028.
5. Lutz M., Krieglstein K., Schmitt S., Ten Dijke P., Sebald W., Wizenmann A., Knaus P. Nerve growth factor mediates activation of the Smad pathway in PC12 cells. European Journal of Biochemistry (2004) 271:920-931.
6. Mazerbourg S., Klein C., Roh J., Kaivo-Oja N., Mottershead D.G., Korchynskyi O., Ritvos O., Hsueh A.J. Growth differentiation factor-9 signaling is mediated by the type I receptor, activin receptor-like kinase 5. Molecular Endocrinology (2004) 18:653-665.
7. Monteiro R.M., Chuva de Sousa Lopes S.M., Korchynskyi O., Ten Dijke P., Myummery C.L. Spatio-temporal activation of Smad1 and Smad5 in vivo: monitoring transcriptional activity of Smad proteins. Journal of Cell Science (2004) 117:4653-4663.

8. Piek E., Van Dinther M., Parks W.T., Sallee J.M., Bottinger E.P., Roberts A.B., Ten Dijke P. RLP, a novel Ras-like protein, is an immediate early TGF-β target gene that negatively regulates transcriptional activity induced by TGF-β. Biochemical Journal (2004) 383:187-199.

9. Van Bezooijen R.L., Roelen B.A., Visser A., Van Der Wee-Pals L., De Wilt E., Karperien M., Hamersma H., Papapoulos S.E., Ten Dijke P., Lowik C.W. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. Journal of Experimental Medicine (2004) 199:805-814.

Reviews / Commentaries / Book Chapters

1. Ten Dijke P., Hill C.S. New insights into TGF-β-Smad signalling. Trends in Biochemical Sciences (2004) 29:265-273.

2. Ten Dijke P., Piek E. Receptor serine/threonine kinases. In: The encyclopedia of endocrinology and endocrine diseases. Ed. L. Martini, Academic Press (2004) 4, 174-180.

V. Peter Collins, M.D., B.Ch., F.R.C.Path., F.Med. Sci.

Department of Pathology
University of Cambridge
Cambridge, UK

Research Report

The research aim of the group is to define the molecular mechanisms involved in the oncogenesis and progression of common primary human brain tumors. A knowledge of these mechanisms will form the basis for the identification of prognostic markers, indicators of sensitivity and resistance to therapy, and the development of logical, innovative and specific treatment modalities. The research focuses on characterizing the genomic abnormalities and transcriptosome in a large panel of histologically well-characterized tumors from patients with careful follow up.

Establishing genome wide copy number analysis

The group has in the past characterized many genetic abnormalities using restriction fragment length polymorphism (RFLP) and microsatellite analyses and thus identified a number of tumor suppressor genes and oncogenes involved in the oncogenesis and progression of human gliomas. However these techniques are labor intensive, require a considerable amount of material and do not permit an overall assessment of the copy number changes throughout the genome in series of individual tumors. Genomic array techniques (array CGH) provide such a possibility and require small amounts of DNA from each case. During 2004, we have been both developing and validating this technology and applying it to our tumor series. Currently we have a 1Mb array of the whole genome with the addition of clones containing known tumor suppressor genes and oncogenes, and tile path arrays for chromosomes 1, 6, 7, 10, and 22.

The tile path array for chromosome 22 has been used to analyze 126 astrocytic gliomas (5 astrocytomas, 29 anaplastic astrocytomas and 92 glioblastomas). The data were combined with our accumulated data from microsatellite analysis permitting a detailed picture of the status of chromosome 22 in these tumors. The findings indicate that the arrangements of chromosome 22 are very complex with some tumors having combinations of deletions and reduplications of regions of the retained chromosome copy. The most interesting finding was in two tumors that had small overlapping homozygous deletions involving only three genes. Currently mutation analysis of these genes in cases with only one retained allele is ongoing to identify the potential tumor suppressor gene.

Detailed studies of chromosomes 1, 6, 7 and 10 are ongoing for the astrocytic gliomas as well as the use of the 1 Mb array to get an overview of the copy number changes in the other frequent human brain tumors including 100 oligodendrogliomas, 50 ependymomas, 35 medulloblastomas and 50 pilocytoíc astrocytomas.

Expression analysis

In parallel with the genomic array methodology we have established expression array technology using the Affymetrix platform. More than 60 astrocytic tumors with excellent quality mRNA have been studied using the U133A chip. We are currently analyzing the data and correlating the findings with the 1Mb genomic array data on the same cases. Correlations with the clinical data from these cases will be carried out at a later date.

Studies of promoter hypermethylation

Good prognosis in anaplastic oligodendroglioma patients treated with alkylating agents is associated with allelic loss on 1p and 19q, but the molecular mechanisms responsible for this relationship are as yet unknown. Expression of the DNA repair enzyme O(6)-methylguanine DNA methyltransferase (MGMT) may conferr resistance to DNA-alkylating drugs commonly used in the treatment of anaplastic oligodendrogliomas and other malignant gliomas. We analysed 52 oligodendroglial tumors for MGMT promoter methylation, as well as mRNA and protein expression using sequencing of sodium bisulfite-modified DNA to determine the methylation status of 25 CpG sites within the MGMT promoter. In 88% we detected MGMT promoter hypermethylation as defined by methylation of more than 50% of the sequenced CpG sites. RT-PCR showed reduced MGMT mRNA levels in the majority of tumors with hypermethylation and immunohistochemical analysis showed no or very few MGMT positive tumor cells. MGMT promoter hypermethylation was significantly more frequent in tumors with loss of heterozygosity on chromosome arms 1p and 19q as compared to tumors without allelic losses on these chromosomes arms.

PDGFRA promoter polymorphisms

Platelet derived growth factor receptor alpha (PDGFRA) expression is limited to some neural progenitor cells in normal brain but typical in a variety of brain tumors. The molecular mechanisms are not known, but as amplification is uncommon, changes in transcriptional regulation are assumed. We investigated the link between single nucleotide polymorphisms (SNPs) within the PDGFRA gene promoter and the occurrence of brain tumors (medulloblastomas, supratentorial primitive neuroectodermal tumors (PNETs), ependymal tumors, astrocytomas, oligodendrogliomas, and mixed gliomas). There are five different promoter haplotypes. We found a significant over-representation of the H2delta haplotype in both PNET (10-fold) and ependymoma (6.5-fold) patient groups. The precise functional role in PDGFRA gene transcription for the H2delta haplotype is not known, but we could show that the H2delta haplotype disrupts the binding of the transcription factor ZNF148 as compared to the other five promoter haplotypes. The findings suggest that this may play a role in the oncogenesis of these tumor types.

Replication timing of human chromosome 6

Genomic microarrays have been used to assess DNA replication timing in a variety of eukaryotic organisms. Using the tile path genomic array of chromosome 6 we developed we assessed the replication timing of chromosome 6. We could define the replication timing map of the whole of chromosome 6 in general, and the MHC region in particular. Positive correlations were observed between replication timing and a number of genomic features including GC content, repeat content and transcriptional activity.

Publications

Reviews / Commentaries / Book Chapters

1. Collins V. P. Brain tumours: classification and genes. Journal of Neurology, Neurosurgery & Psychiatry (2004) 75 Suppl 2:ii2-11.
2. Ichimura K., Ohgaki H., Kleihues P., Collins V. P. Molecular pathogenesis of astrocytic tumours. Journal of Neurooncology (2004) 70:137-160.
3. Reifenberger G., Collins V. P. Pathology and molecular genetics of astrocytic gliomas. Journal of Molecular Medicine (2004) 82:656-670.

Kari Alitalo, Ph.D.

Haartman Institute and Biomedicum Helsinki
University of Helsinki
Helsinki, Finland

Research Report

Angiogenesis and permeability of blood vessels are regulated by vascular endothelial growth factor (VEGF) via its two receptors VEGFR-1 and VEGFR-2. The VEGFR-3 receptor does not bind VEGF and its expression becomes restricted mainly to lymphatic endothelia during development. We have found that homozygous VEGFR-3 targeted mice die around midgestation due to failure of cardiovascular development. We have also purified and cloned the VEGFR-3 ligand, VEGF-C. Transgenic mice expressing VEGF-C show evidence of lymphangiogenesis and VEGF-C knockout mice have defective lymphatic vessels. The proteolytically processed form of VEGF-C binds also to VEGFR-2 and is angiogenic. VEGF-D is closely related to VEGF-C, similarly processed and binds to the same receptors. Thus VEGF-C and VEGF-D appear to be both angiogenic and lymphangiogenic growth factors. VEGF-C overexpression led to lymphangiogenesis and growth of the draining lymphatic vessels, intralymphatic tumor growth and lymph node metastasis in several tumor models. Furthermore, soluble VEGFR-3, which blocked embryonic lymphangiogenesis, also blocked lymphatic metastasis in breast and lung cancer models. These results together with recent clinical cancer studies suggest that paracrine signal transduction between tumor cells and the lymphatic endothelium may be involved in lymphatic metastasis of human cancers.

Reduced expression of neural cell adhesion molecule (NCAM) has been implicated in the progression to tumor malignancy in cancer patients. We have shown that tumors of NCAM-deficient Rip1Tag2 transgenic mice exhibit up-regulated expression of the lymphangiogenic factors vascular endothelial growth factor (VEGF)-C and -D and, with it, increased lymphangiogenesis. Repression of VEGF-C and -D function by adenoviral expression of a soluble form of their cognate receptor, VEGF receptor-3, results in reduced tumor lymphangiogenesis and lymph node metastasis. The results indicate that the loss of NCAM function causes lymph node metastasis via VEGF-C- and VEGF-D-mediated lymphangiogenesis.

We have shown by gene expression microarrays that neoplastic cells of Kaposi sarcoma are closely related to lymphatic endothelial cells (LECs) and that Kaposi sarcoma herpesvirus (KSHV) infects both LECs and blood vascular endothelial cells (BECs) *in vitro*. The gene expression microarray profiles of infected LECs and BECs show that KSHV induces transcriptional reprogramming of both cell types. These data show that the gene expression profile of Kaposi sarcoma resembles that of LECs, that KSHV induces a transcriptional drift in both LECs and BECs, and that lymphangiogenic molecules are involved in the pathogenesis of Kaposi sarcoma.

Bone marrow (BM)-derived cells are thought to participate in the growth of blood vessels during postnatal vascular regeneration and tumor growth, a process previously attributed to stem and precursor cells differentiating to endothelial cells. We used multichannel laser scanning confocal microscopy of whole-mounted tissues to study angiogenesis in chimeric mice created by reconstituting C57BL mice with genetically marked syngeneic BM. We show that BM-derived endothelial cells do not significantly contribute to tumor- or cytokine-induced neoangiogenesis. Instead, BM-derived periendothelial vascular mural cells were persistently detected at sites of tumor- or vascular endothelial growth factor-induced angiogenesis. Subpopulations of these cells expressed the pericyte-specific NG2 proteoglycan, or the hematopoietic markers CD11b and CD45, but did not detectably express the smooth muscle markers smooth muscle alpha-actin or desmin. Thus, the major contribution of the BM to angiogenic processes is not endothelial, but

may come from progenitors for periendothelial vascular mural and hematopoietic effector cells. We did not find significant incorporation of genetically marked BM-derived cells in lymphatic vessels during tumor- or vascular endothelial growth factor C-induced lymphangiogenesis. The degree of tumor lymphangiogenesis correlated with lymphatic vessel density in the peritumoral area, and despite tumor lymphangiogenesis, lymphatic metastasis failed to occur in gene-targeted vascular endothelial growth factor C(+/-) mice that have hypoplasia of the lymphatic network. Our data demonstrate that during tumor lymphangiogenesis and cancer cell dissemination via the lymphatics, the newly formed lymphatic vessels sprout from the pre-existing local lymphatic network with little if any incorporation of BM-derived endothelial progenitor cells.

Platelet-derived growth factor-D (PDGF-D) is a recently characterized member of the PDGF family with unknown *in vivo* functions. We investigated the effects of PDGF-D in transgenic mice by expressing it in basal epidermal cells and then analyzed skin histology, interstitial fluid pressure, and wound healing. When compared with control mice, PDGF-D transgenic mice displayed increased numbers of macrophages and elevated interstitial fluid pressure in the dermis. Wound healing in the transgenic mice was characterized by increased cell density and enhanced recruitment of macrophages. Macrophage recruitment was also the characteristic response when PDGF-D was expressed in skeletal muscle or ear by an adeno-associated virus vector. Combined expression of PDGF-D with VEGF-E led to increased pericyte/smooth muscle cell coating of the VEGF-E-induced vessels and inhibition of the vascular leakiness that accompanies VEGF-E-induced angiogenesis. These results show that full-length PDGF-D is activated in tissues and is capable of increasing interstitial fluid pressure and macrophage recruitment and the maturation of blood vessels in angiogenic processes.

Several endothelial growth factors induce both blood and lymphatic angiogenesis. However, a systematic comparative study of the impact of these factors on vascular morphology and function has been lacking. In this study, we report a quantitative analysis of the structure and macromolecular permeability of fibroblast growth factor (FGF)-2-, VEGF-A-, and VEGF-C-induced blood and lymphatic vessels. Our results show that VEGF-A stimulated formation of disorganized, nascent vasculatures as a result of fusion of blood capillaries into premature plexuses with only a few lymphatic vessels. Ultrastructural analysis revealed that VEGF-A-induced blood vessels contained high numbers of endothelial fenestrations that mediated high permeability to ferritin, whereas the FGF-2-induced blood vessels lacked vascular fenestrations and showed only little leakage of ferritin. VEGF-C induced approximately equal amounts of blood and lymphatic capillaries with endothelial fenestrations present only on blood capillaries, mediating a medium level of ferritin leakage into the perivascular space. No endothelial fenestrations were found in FGF-2-, VEGF-A-, or VEGF-C-induced lymphatic vessels. These findings highlight the structural and functional differences between blood and lymphatic vessels induced by FGF-2, VEGF-A, and VEGF-C. Such information is important to consider in development of novel therapeutic strategies using these angiogenic factors.

Lymphatic vessels are essential for immune surveillance, tissue fluid homeostasis and fat absorption. Defects in lymphatic vessel formation or function cause lymphedema. We have shown that the VEGF-C is required for the initial steps in lymphatic development. In VEGF-C-/- mice, endothelial cells commit to the lymphatic lineage but do not sprout to form lymph vessels. Sprouting was rescued by VEGF-C and VEGF-D but not by VEGF, indicating VEGF receptor 3 specificity. The lack of lymphatic vessels resulted in prenatal death due to fluid accumulation in tissues, and VEGF-C+/- mice developed cutaneous lymphatic hypoplasia and lymphedema. Our results indicate that VEGF-C is the paracrine factor essential for lymphangiogenesis, and show that both VEGF-C alleles are required for normal lymphatic development. However, in zebrafish reducing VEGF-C levels using morpholino antisense oligonucleotides, or through overexpression

of a soluble form of the VEGF-C receptor, VEGFR-3, affects the coalescence of endodermal cells in the anterior midline, leading to the formation of a forked gut tube and the duplication of the liver and pancreatic buds. Further analyses indicate that VEGF-C is additionally required for the initial formation of the dorsal endoderm. We also demonstrate that VEGF-C is required for vasculogenesis as well as angiogenesis in the zebrafish embryo. These data argue for a requirement of VEGF-C in the developing vasculature and, more surprisingly, implicate VEGF-C signalling in two distinct steps during endoderm development, first during the initial differentiation of the dorsal endoderm, and second in the coalescence of the anterior endoderm to the midline.

Lymphatic vessels are essential for the removal of interstitial fluid and prevention of tissue edema. Lymphatic capillaries lack associated mural cells, and collecting lymphatic vessels have valves, which prevent lymph backflow. In lymphedema-distichiasis (LD), lymphatic vessel function fails because of mutations affecting the forkhead transcription factor FOXC2. We report that Foxc2(-/-) mice show abnormal lymphatic vascular patterning, increased pericyte investment of lymphatic vessels, agenesis of valves and lymphatic dysfunction. In addition, an abnormally large proportion of skin lymphatic vessels was covered with smooth muscle cells in individuals with LD and in mice heterozygous for Foxc2 and for the gene encoding lymphatic endothelial receptor, VEGFR-3 (also known as Flt4). Our data show that Foxc2 is essential for the morphogenesis of lymphatic valves and the establishment of a pericyte-free lymphatic capillary network and that it cooperates with VEGFR-3 in the latter process. Our results indicate that an abnormal interaction between the lymphatic endothelial cells and pericytes, as well as valve defects, underlie the pathogenesis of LD.

Publications

Primary Research Articles

1. Cao R., Eriksson A., Kubo H., Alitalo K., Cao Y., Thyberg J. Comparative Evaluation of FGF-2-, VEGF-A-, and VEGF-C-Induced Angiogenesis, Lymphangiogenesis, Vascular Fenestrations, and Permeability. Circulation Research (2004) 94:664-70.
2. Crnic I., Strittmatter K., Cavallaro U., Kopfstein L., Jussila L., Alitalo K., Christofori G. Loss of neural cell adhesion molecule induces tumor metastasis by up-regulating lymphangiogenesis. Cancer Research (2004) 64:8630-8.
3. He Y., Rajantie I., Ilmonen M., Makinen T., Karkkainen M.J., Haiko P., Salven P., Alitalo K. Preexisting lymphatic endothelium but not endothelial progenitor cells are essential for tumor lymphangiogenesis and lymphatic metastasis. Cancer Research (2004) 64:3737-40.
4. Karkkainen M.J., Haiko P., Sainio K., Partanen J., Taipale J., Petrova T.V., Jeltsch M., Jackson D.G., Talikka M., Rauvala H., Betsholtz C., Alitalo K. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nature Immunology (2004) 5:74-80.
5. Nilsson I., Rolny C., Wu Y., Pytowski B., Hicklin D., Alitalo K., Claesson-Welsh L., Wennstrom S. Vascular endothelial growth factor receptor-3 in hypoxia-induced vascular development. FASEB Journal (2004) 18:1507-15.
6. Ober E.A., Olofsson B., Makinen T., Jin S.W., Shoji W., Koh G.Y., Alitalo K., Stainier D.Y. VEGF-C is required for vascular development and endoderm morphogenesis in zebrafish. EMBO Reports (2004) 5: 78-84.
7. Petrova T.V., Karpanen T., Norrmen C., Mellor R., Tamakoshi T., Finegold D., Ferrell R., Kerjaschki D., Mortimer P., Yla-Herttuala S., Miura N., Alitalo K. Defective valves and

abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. Nature Medicine (2004) 10:974-81.

8. Rajantie I., Ilmonen M., Alminaite A., Ozerdem U., Alitalo K., Salven P. Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood (2004) 104:2084-6.

9. Saaristo A., Tammela T., Timonen J., Yla-Herttuala S., Tukiainen E., Asko-Seljavaara S., Alitalo K. Vascular endothelial growth factor-C gene therapy restores lymphatic flow across incision wounds. FASEB Journal (2004) 18:1707-9.

10. Uutela M., Wirzenius M., Paavonen K., Rajantie I., He Y., Karpanen T., Lohela M., Wiig H., Salven P., Pajusola K., Eriksson U., Alitalo K. PDGF-D induces macrophage recruitment, increased interstitial pressure and blood vessel maturation during angiogenesis. Blood (2004) 104:3198-204.

11. Wang H.W., Trotter M.W., Lagos D., Bourboulia D., Henderson S., Makinen T., Elliman S., Flanagan A.M., Alitalo K., Boshoff C. Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma. Nature Genetics (2004) 36:687-93.

Michael Pfreundschuh, M.D. & Christoph Renner, M.D., Ph.D.

University of Saarland
Homburg, Germany

Research Report

Our group in Homburg has a major focus on tumor immunology, with its main activities in the area of tumor antigen identification using the SEREX technology and antibody development. All these efforts are closely linked and integrated into the main LICR Programs and done in cooperation with other LICR Affiliates. The specific activities can be summarised under the following four areas.

Serological identification of new tumor antigens using eukaryotic SEREX technology in a yeast cell surface display system

The transfer of the SEREX technology into an eukaryotic expression system (Recombinant antigen expression on yeast surface, RAYS) has allowed us to extend the current SEREX antigen repertoire and identify potential candidate genes recognized by the immune system because of their conformational structure or tertiary modification (e.g. glycosylation). Analyzing a breast cancer library, many genes known to be over-expressed in breast cancer were isolated and are currently being further evaluated as potential target antigens. Using this methodology a new mucine variant has also been identified. The RAYS analysis is currently being extended to prostate/pancreatic/colon cancer, Hodgkin's lymphoma and mantle cell leukemia.

Analysis of expression and serological immune responses in patients with pancreatic cancer against known cancer testis (CT) antigens

Expression analysis, by RT-PCR, of known CT antigens in tumor tissue from patients with pancreatic cancer has revealed that SCP-1 and GAGE are expressed at high levels and might represent potential targets for vaccine purposes. Humoral immune response against cancer testis antigen SCP-1 was found in 14 out of 96 pancreatic cancer patients. Only patients with pT status 3 or 4 demonstrated sero-reactivity against SCP-1, whereas none of the patients with pT status 1 or 2 had a humoral immune response against SCP-1.

Identification of MHC class I and II restricted peptides for vaccine purposes

Searching for promiscuous MHC-II-restricted peptides that might be suitable as a CD4+ stimulating vaccine for many patients, we used the SYFPEITHI algorithm and identified one pentadecamer epitope for SSX-2 (p45-59) and one for NY-ESO-1 (p134-148), respectively which induced specific CD4+ T-cell responses. The SSX-2 peptide was restricted by the HLA-DRB1 subtypes *0701, *1101 and *1302, which have a cumulative prevalence of approximately 25% in the Caucasian population. CD4+ T-cell responses specific for the NY-ESO-1 peptide (p134-148) were restricted to the HLA-DRB1 subtypes *0101, *0301, *0401, and *0701, which have a cumulative prevalence of 40% in the Caucasian population. The DR restriction of both pentadecamers was demonstrated by inhibition with an HLA-DR antibody and a functional peptide displacement titration assay with the pan-DR-binding T-helper epitope PADRE as the competitor. The natural processing and presentation of these epitopes was demonstrated by T-cell recognition of NY-ESO-1/SSX-2+ melanoma cell lines.

Selection of MHC-peptide specific antibodies for the analysis of presentation and targeting of CT-antigen derived peptides

MHC-peptide antibodies are a new class of antibodies that allow for the specific detection of antigen derived peptides presented in a predefined MHC context. As an example, we have

generated antibodies that recognize specifically the NY-ESO$_{157-165}$-peptide/HLA-A0201 complex. Peptide variants such as the NY-ESO-1$_{157-167}$ peptide or the cryptic NY-ESO$_{159-167}$ peptide were not recognized. Moreover, the antibody recognizes specifically the NY-ESO-1$_{157-165}$ peptide displayed on NY-ESO-1/HLA-A2 positive tumor cells and blocks recognition by NY-ESO-1$_{157-167}$ peptide specific CD8(+) T cells. Over the last 12 months, the repertoire of MHC-peptide specific antibodies has been extended to other NY-ESO-1 derived epitopes, Melan-A, SSX-2, G250 and Influenza-Matrix-protein and CMV derived peptides. Having this set of antibodies, the detailed analysis of the process of peptide presentation and their potential use as target antigens for therapeutic antibodies will be the major focus of our future activities.

Generation of biological active antibodies

To increase the cytolytic activity of monoclonal antibodies, we decided to generate modified antibodies and fusion proteins using the antibody molecule as carrier for biologically active compounds such as tumor necrosis factor (TNF). Two different target antigens, one being expressed in the tumor surrounding stroma and one directly on the surface of renal cancer cells, were used as models. For both antigenic systems, high biological activity in vivo of the TNF moiety could be demonstrated by the induction of apoptosis in tumor cells, up-regulation of tissue factor expression in endothelial cells and increased migratory capacity of macrophages. Treating FAP-positive tumor xenografts in mice confirmed the activity of the construct and revealed a massive infiltrate of macrophages at the rim of the tumor. Our main efforts in this area are currently directed towards a better understanding of the different role TNF receptor 1 (TNF-R1) and TNF-R2 play in this model system and the generation of a high level expressing hybridoma cell line suitable for production of materials for clinical trials.

Publications

Primary Research Articles

1. Held G., Matsuo M., Gnjatic S., Ritter G., Lee S-Y., Tai T-Y., Cohen C., Old L.J., Pfreundschuh M., Hoogenboom H.R., Renner C. Dissecting cytotoxic T cell responses towards the NY-ESO-1 protein by peptide-MHC specific antibody fragments. European Journal of Immunology (2004) 34(10):2919-29.
2. Kubuschok B., Xie X., Jesnowski R., Preuss K.D., Romeike B.F., Neumann F., Regitz E., Pistorius G., Schilling M., Scheunemann P., Izbicki J.R., Lohr J.M., Pfreundschuh M. Expression of cancer testis antigens in pancreatic carcinoma cell lines, pancreatic adenocarcinoma and chronic pancreatitis. International Journal of Cancer (2004) 109(4): 568-75.
3. Neumann F., Wagner C., Stevanovic S., Kubuschok B., Schormann C., Mischo A., Ertan K., Schmidt W., Pfreundschuh M. Identification of an HLA-DR-restricted peptide epitope with a promiscuous binding pattern derived from the cancer testis antigen HOM-MEL-40/SSX2. International Journal of Cancer (2004) 112(4):661-8.
4. Neumann F., Wagner C., Kubuschok B., Stevanovic S., Rammensee H.G., Pfreundschuh M. Identification of an antigenic peptide derived from the cancer-testis antigen NY-ESO-1 binding to a broad range of HLA-DR subtypes. Cancer Immunology Immunotherapy (2004) 53(7):589-99.
5. Bauer S., Adrian N., Williamson B., Panousis C., Fadle N., Smerd J., Fettah I., Scott A.M., Pfreundschuh M., Renner C. Targeted Bioactivity of Membrane Anchored TNF by an Antibody Derived TNF Fusion Protein. Journal of Immunology (2004) 172(6):3930-9.

Richard R. Schmidt, Ph.D.

Fachbereich Chemie
Universität Konstanz
Konstanz, Germany

Research Report

In 2004, the Affiliate Center in Konstanz worked essentially in three different areas which were partly supported by the Ludwig Institute for Cancer Research.

(1) Synthesis of α-Galactosyl Ceramide and Structurally Modified Mimetics for Immunological Studies.

CD1 proteins are a lineage of antigen-presenting molecules with unusually hydrophobic ligand-binding grooves that present glycolipid antigens to T-cells. A marine-sponge derived glycolipid α-galactopyranosyl ceramide (αGalCer), with C18-phytosphingosine as one of the lipid moieties and a saturated C26 fatty acyl chain as the second lipid moiety, is such a potent stimulator of human T-cells. Stimulation occurs through binding of the glycolipid by CD1d, presentation to T-cells, and formation of a CD1d-glycolipid-T-cell receptor complex.

(a) Synthesis of αGalCer and a truncated analogue. αGalCer has obviously important biological properties, therefore access to this compound for immunological studies is required. Previously, we performed a successful synthesis for this compound, which was repeated (a total of about 14 synthetic steps) in order to provide material to Drs. V. Cerundolo (Oxford Affiliate Center), G. Ritter (New York Branch), and D. Speiser (Lausanne Branch) as part of the LICR Cancer Vaccine Program. This synthetic work was also extended to a truncated analogue with a C9-phytosphingosine residue for use in biological studies.

(b) Synthesis of structural mimetics of αGalCer. The great interest in the biological properties of αGalCer led to the synthesis of some structurally closely related analogues, but with varying success. The aim of our work was to replace the readily cleavable glycosidic linkage of αGalCer by a biologically much more stable ether linkage, which should also avoid a dramatic increase in the synthetic complexity. Hence, it was decided to replace the α-D-galacotpyranosyl residue by inositol, D-glycerol, L-threitol and L-arabinitol. These compounds (six in total) were successfully obtained, and provided to Dr. Cerundolo at the Oxford Affiliate Center for biological studies.

The natural ligand of such T-cell receptor expressing cells has not yet been defined. Therefore, a search for possible candidates is now being undertaken, based on (possibly) mammalian compounds that are structurally related to αGalCer.

(2) Synthesis of Bifunctional Chelating Agents for Radioimmunotherapy (RIT)

Bifunctional chelating agents continue to be an interesting topic in medicinal research because of their usefulness in the diagnosis and therapy of cancer diseases. Radioimmuno-imaging (RII) and radioimmunotherapy (RIT) rely on the ability of a molecule with a chelating functionality that will sequester a radioactive metal ion to combine with a monoclonal antibody or any other receptor-specific substrate. This approach allows radiopharmaceuticals to be delivered specifically to malignant tissue while minimizing the risk of unspecific irradiation of sane tissue.

Chelating agents useful for RII and RIT should be able to form metal-chelates with high thermodynamic stability as well as high kinetic inertness *in vivo*, to reduce intoxication arising from the loss of the radioactive (heavy-)metal ion. Derivatives of diethylenetriamino-pentaacetic acid (DTPA) and some others have been used for this purpose at present, but new chelators with improved or altered physical properties for different biological applications are desirable.

Astonishingly, no bifunctional chelating agents based on tripodal 1,1,1-tris(aminomethyl) ethane (TAME), an excellent chelating agent, have been reported. Therefore, two novel bifunctional chelating agents (named TAME-Hex A and B) have been designed and synthesized by the Konstanz Center. These chelators showed very good stability with gallium(III) ions and thus are highly effective candidates for use in RII, especially in positron emission tomography (PET). This work was performed in collaboration with Drs. P. Smith-Jones (New York (Memorial Sloan-Kettering Cancer Center) Affiliate Center) and G. Ritter (New York Branch) as part of the LICR Antibody Targeting Program. A provisional patent application of this work was filed and thereafter part of this work was also published.

We have also designed and synthesized successfully novel DTPA derived bifunctional chelating agents that should have particularly good chelating properties. Therefore, we are looking forward to the characterization of the biophysical properties of these compounds.

(3) Synthesis of Saponin QS 21 Analogs as Immune Adjuvants.

Saponins are complex amphiphilic glycolipid conjugates which are mainly of plant origin. A particularly interesting class of saponins has been isolated from the bark of the *Quillaja Saponaria* Molina tree, which grows mainly in South America. The most famous of these is QS 21, a bisdesmosidic triterpenoid of a very complex structure, which is the most potent saponin immune adjuvant known to date.

For structure-activity relationship and toxicity studies, about 30 to 40 structurally simpler derivatives were synthesized by the Konstanz Center in 2003. In 2004, the main goal was to synthesize partial structures of saponin QS 21. Based on the general structure drawing, saponin QS 21 can be disconnected into two parts: the northwest part, consisting of a Xylβ(1-3)[Galβ(1-2)]GlcUA trisaccharide residue linked to the 3-O of triterpene quillaic acid, and the southeast part, consisting of a Xylβ(1-3)Xylβ(1-4)Rhaα(1-2)Fuc tetrasaccharide, having a stereochemically complex arabinofuranosyl-octanoyl-octanoyl substituent, linked to the C-28 carboxylate group of quillaic acid. In the last year, several structural analogs of the northwest part were successfully synthesized and made available for immunological studies. It is interesting to note that one structurally related compound exhibited antimycobacterial activity. Also the highly challenging task, namely the synthesis of the southeast part, was successfully performed. Studies to combine the two parts to full size QS 21, or a closely related derivative, are now underway.

Publications

Primary Research Articles

1. J.D. Silk, I.F. Hermans, U. Gileadi, T.W. Chong, D. Shepherd, M. Salio, B. Mathew, R.R. Schmidt, S.J. Lunt, K.J. Williams, I.J. Stratford, A.L. Harris, V. Cerundolo. Utilizing the adjuvant properties of CD1d-dependent NK T cells in T cell-mediated immunotherapy. Journal Clinical Investigation (2004) 114:1800-1811.
2. E. Arslantas, P. Smith-Jones, G. Ritter, R.R. Schmidt. TAME-Hex A - A Novel Bifunctional Chelating Agent for Radioimmunoimaging. European Journal of Organic Chemistry (2004) 3979-3984.

Seppo Ylä-Herttuala, M.D., Ph.D., FESC

A.I.Virtanen Institute
University of Kuopio
Kuopio, Finland

Research Report

During the last year we have tested the therapeutic properties of vascular endothelial growth factor (VEGF), placental growth factor (PLGF) and the mature short form of VEGF-D in pig myocardium using percutaneous, catheter-mediated delivery of adenoviruses. It was found that intraventricular delivery of adenoviruses encoding the mature short form of VEGF-D was equally efficient as VEGF-A in causing capillary enlargement and improved blood flow in pig myocardium. Adenoviruses encoding VEGF-B_{167}, VEGF-B_{186} and PLGF were also effective in causing capillary enlargement in the pig myocardium. The effects were dose-dependent and optimal dose for the treatment of pig myocardium has been established. No alterations have been found in clinical chemistry parameters. Our results suggest that members of the VEGF family may be potential candidates for the treatment of severe coronary heart disease and myocardial ischemia.

Since members of the VEGF family may potentially enhance atherogenesis via neovascularization of atherosclerotic lesions, we have analyzed the effects of adenoviruses encoding VEGF-A, VEGF-B_{167}, VEGF-C and VEGF-D in hyper-cholesterolemic, atherosclerosis-prone transgenic mice. Adenoviruses were administered via tail vein injection and increased levels of all VEGF proteins were detected in mouse plasma. The results show that the presence of VEGFs in systemic circulation does not enhance atherogenesis in atherosclerosis-prone mice. No evidence of increased angiogenesis was found in atherosclerotic lesions or in aortic tissue. The results are important when considering clinical use of the members of the VEGF family in various patient populations.

Effects of the overexpression of angiopoietin-1 using adenoviral gene delivery have been analyzed in perivascular tissue. It was found that angiopoietin-1 can induce angiogenesis and neovascularization around arteries. However, angiopoietin-1 did not induce vascular wall thickening in rabbits which may be a favourable property as compared to some other vascular growth factors.

We have also studied the efficacy of VEGF-C adenovirus administered together with platelet-derived growth factor (PDGF) receptor tyrosine kinase inhibitior Gleevec in rabbit balloon denutation restenosis model. It was found that Gleevec treatment improved the long-term antirestenotic effect of VEGF-C. Gleevec treatment alone did not produce any long-lasting antirestenotic effects. The results suggest that combination of VEGF-C gene therapy with inhibition of PDGF signalling may be useful for the limitation of restenosis after vascular manipulations.

As part of collaborative work with other LICR laboratories, A.I.Virtanen Institute virus vector laboratory has produced several adenoviral vectors encoding members of the VEGF family, decoy VEGF receptors, PDGFs, angiopoietin-1 and some other constructs to Dr. Kari Alitalo (Helsinki Affiliate Center), Dr. Ulf Eriksson (LICR Stockholm Branch) and other LICR laboratories. Also, we have produced several lots of human clinical grade ad-ESO and ad-MAGE for LICR clinical investigators at the New York Branch and the Frankfurt Affiliate Center.

Publications

Primary Research Articles

1. Rutanen J., Rissanen T.T., Markkanen J.E., Gruchala M., Silvennoinen P., Kivelä A., Hedman A., Hedman M., Heikura T., Orden M-R., Stacker S.A., Achen M.G., Hartikainen J., Ylä-Herttuala S. Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart. Circulation (2004) 109:1029-1035.
2. Leppänen O., Rutanen J., Hiltunen M.O., Rissanen T.T., Turunen M.P., Sjöblom T., Brüggen J., Backström G., Carlsson M., Buchdunger E., Bergqvist D., Alitalo K., Heldin C-H., Östman A., Ylä-Herttuala S. Oral imatinib mesylate (STI571/Gleevec) improves the efficacy of local intravascular vascular endothelial growth factor-C gene transfer in reducing neointimal growth in hypercholesterolemic rabbits. Circulation (2004) 109:1140-1146.

Parmjit Jat, Ph.D.

Institute of Neurology
University College London
London, UK

Research Report

Minimally immortalized human mammary luminal epithelial cells

Mammary cell lines have previously been generated by immortalizing purified normal human mammary luminal epithelial cells with SV40 T antigen. Even though such lines proved extremely useful, they were far from ideal because they possessed gross karyotypic rearrangements since expression of T antigen by itself did not directly immortalize these cells but extended their finite life span during which the telomeres continue to shorten and eventually resulted in a telomere-shortening induced 'crisis'. These cell lines had emerged by spontaneous reactivation of telomerase, or alternative lengthening of telomeres.

The identification that telomere maintenance was a major determinant of replicative lifespan in human cells and thus of the immortalized state led us to demonstrate that reconstitution of telomerase activity or SV40 T antigen alone was not sufficient for immortalization of adult human mammary fibroblasts and endothelial cells. However a combination of both genes resulted in the efficient generation of immortal cell lines irrespective of the order in which or when they were introduced but did affect genomic stability.

Our finding that T antigen interacts with Bub1, a spindle assembly checkpoint protein, suggested a potential explanation for the chromosome aberrations and aneuploidy. After demonstrating that interaction with Bub1 was not required for the ability of T antigen to immortalize rat embryo fibroblasts, we constructed a triple mutant of SV40 T antigen that does not interact with Bub1, does not bind to DNA and is thermolabile: U19dl89-97tsA58.

Introduction of U19dl89-97tsA58 T antigen in conjunction with hTERT into purified populations of luminal epithelial cells followed by sequential passaging of the transduced cells at clonal densities to deplete the extended lifespan has yielded immortal cultures of luminal cells. Karyotypic analysis has indicated that the cells are stable and diploid. Moreover, the cells are conditional for growth; inactivation of T antigen results in a rapid cessation of growth even though telomerase is constitutively active.

More detailed karyotypic and comparative genomic hybridization (CGH) analysis of these cells is now underway. Expression profiling is also being done to compare them to normal luminal cells and to determine the basal transcriptional profile. These cells should represent minimally immortalized counterparts of luminal epithelial cells.

We will first determine the expression level of the breast cancer candidates, identified by LICR's Massively Parallel Signature Sequencing (MPSS) initiative, in these cells as part of LICR's Breast Cancer Initiative. Genes identified to be up regulated in breast cancer will be constitutively over-expressed whereas genes identified to be down-regulated will be silenced by RNA interference. The resulting cultures will be analysed for changes in morphology, growth, biology, physiology, transformation parameters and expression profiled.

Publications

Primary Research Articles

1. Fauth C., O'Hare M.J., Lederer G., Jat P.S., Speicher M.R. Order of genetic events is critical determinant of aberrations in chromosome count and structure. Genes Chromosomes Cancer (2004) 40:298-306.
2. Cotsiki M., Lock R.L., Cheng Y., Williams G.L., Zhao J., Perera D., Freire R., Entwistle A., Golemis E., Roberts T.M., Jat P.S., Gjoerup O. V. SV40 large T antigen targets the spindle assembly checkpoint protein Bub1. Proceedings of the National Academy of Sciences USA (2004) 101:947-952.

Sunil R Lakhani M.D., F.R.C. Path.

Molecular Pathology Laboratory
Institute for Cancer Research
London UK

Research Report

The classical pathological data in making a cancer related diagnosis include (sub)classification, grade (differentiation), stage (extent of disease), and prognostic and predictive factors. The overall objective of the Group is to utilise molecular techniques in conjunction with traditional pathological data to extend the understanding of the disease process and hence provide information to aid patient management. The work in the Molecular Pathology Laboratory is therefore of a translational nature.

The two major areas of work fall into 'Molecular Genetic Classification of Breast Cancer' and molecular pathology of the 'Multistep Model'. Much of this work is being undertaken as part of LICR's Breast Cancer Initiative, with Drs. M.J. O'Hare (LICR University College London Branch), P. Jat (LICR London Affiliate Centre) and A.M. Neville (LICR London Office).

Molecular Genetic Classification of Breast Cancer

Molecular pathology of basal/myoepithelial lesions within the breast.

The classification, behaviour, prognosis and carcinogenic pathways of these tumors are essentially unknown, making clinical management decisions of these tumors a challenge.

We have demonstrated that basal breast cancers have a distinct morphology and metastatic patterns and also a distinct genotype. Further, the basal tumors are heterogenous and we are currently attempting to define the sub-groups of basal tumors which have differing prognosis. Work is also on-going to establish whether these tumors respond to conventional chemotherapy (in collaboration with Professor Ian Smith, Royal Marsden Hospital, London).

Familial Breast & Ovarian Cancer

The pathology and molecular pathology of familial breast cancers has been carried out as a European based consortium activity. This work is now being translated to clinical practice to identify high-risk women. It also links with the basal project above. We have demonstrated that approximately 70% of BRCA1 related tumors express basal keratins.

Multistep carcinogenesis

The screening program has led to an increase in the identification of early precancerous and 'borderline' lesions. We are now investigating a recently recognised screen-detected lesion referred to as 'Columnar Cell Hyperplasia'(CCH).

Columnar Cell Lesions

CCH are increasingly seen in core biopsies but their significance and hence subsequent management (to carry out further excision or not) remains unclear. The objective is to understand the biological (clonal) nature of the lesions, their inter-relationships with other higher grade lesions such as ductal carcinoma in situ (DCIS) and hence suggest possible management strategies. We have recently completed this study and demonstrated that many CCH lesions are indeed clonal and related to higher grade lesions.

Normal Breast Tissue

We are studying normal breast tissues to investigate the frequency and distribution of changes within the breast. The data show that genetic changes in the form of loss of heterohyzogosity (LOH) does occur but is rare in normal tissue and when it does, it tends to segregate to certain parts of the breast. We have also found that in patients with BRCA1 germline mutation, the LOH

at the BRCA1 locus randomly affects the mutant or wild-type allele.

Recently it has been suggested that CK5/6 expression in the absence of CK19 or SMA identifies the putative stem cell in the adult breast. We have carried out a thorough study to investigate this issue using frozen and paraffin material and shown that this is incorrect. In frozen samples, no CK5/6 only + cells are seen unlike paraffin material, suggesting that the previous observation was an artefact of antigen retrieval. This has important implications for all models based on paraffin tissue alone.

Publications

Primary Research Articles

1. Angele S., Jones C., Reis Filho J.S., Fulford L.G., Treilleux I., Lakhani S.R, Hall J. Expression of ATM, p53, and the MRE11-Rad50-NBS1 complex in myoepithelial cells from benign and malignant proliferations of the breast. Journal of Clinical Pathology (2004) 57:1179-1184.
2. Clarke C.L., Sandle J., Parry S.C., Reis-Filho J.S., O'Hare M.J., Lakhani S.R. Cytokeratin 5/6 in normal human breast: lack of evidence for a stem cell phenotype. Journal of Pathology (2004) 204:147-152.
3. Jones C., Ford E., Gillett C., Ryder K., Merrett S., Reis-Filho J.S., Fulford L.G., Hanby A., Lakhani S.R. Molecular cytogenetic identification of subgroups of grade III invasive ductal breast carcinomas with different clinical outcomes. Clinical Cancer Research (2004) 10:5988-5997.
4. Jones C., Mackay A., Grigoriadis A., Cossu A., Reis-Filho J.S., Fulford L., Dexter T., Davies S., Bulmer K., Ford E., Parry S., Budroni M., Palmieri G., Neville A.M., O'Hare M.J., Lakhani S.R. Expression profiling of purified normal human luminal and myoepithelial breast cells: identification of novel prognostic markers for breast cancer. Cancer Research (2004) 64:3037-3045.
6. Lakhani S.R., Manek S., Penault-Llorca F., Flanagan A., Arnout L., Merrett S., McGuffog L., Steele D., Devilee P., Klijn J.G., Meijers-Heijboer H., Radice P., Pilotti S., Nevanlinna H., Butzow R., Sobol H., Jacquemier J., Lyonet D.S., Neuhausen S.L., Weber B., Wagner T., Winqvist R., Bignon Y.J., Monti F., Schmitt F., Lenoir G., Seitz S., Hamman U., Pharoah P., Lane G., Ponder B., Bishop D.T., Easton D.F. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. Clinical Cancer Research (2004) 10:2473-2481.
7. Millis R.R., Pinder S.E., Ryder K., Howitt R., Lakhani S.R. Grade of recurrent in situ and invasive carcinoma following treatment of pure ductal carcinoma in situ of the breast. British Journal of Cancer (2004) 90:1538-1542.
8. Reis-Filho J.S., Fulford L.G., Crebassa B., Carpentier S., Lakhani S.R. Collagenous spherulosis in an adenomyoepithelioma of the breast. Journal of Clinical Pathology (2004) 57:83-86.
9. Simpson P.T., Gale T., Reis-Filho J.S., Jones C., Parry S., Steele D., Cossu A., Budroni M., Palmieri G., Lakhani S.R. Distribution and significance of 14-3-3sigma, a novel myoepithelial marker, in normal, benign, and malignant breast tissue. Journal of Pathology (2004) 202:274-285.

Reviews / Commentaries / Book Chapters

1. Lakhani S.R. Putting the brakes on cylindromatosis? New England Journal of Medicine (2004) 350:187-188.

Ira Mellman, Ph.D.

Department of Cell Biology
Yale University
New Haven, USA

Research Report

Efforts in New Haven focus on how an understanding of membrane dynamics can provide insight into critical features of cell biology and immunology directly relevant to cancer. Thus our work during the past year has not only been concerned with fundamental problems of cell organization and function, but also with a number of specific problems including antigen presentation and vaccine development, the interaction of therapeutic antibodies with cancer cells in culture and *in vivo*, and the morphogenesis of colon cancer. A number of projects have been conducted in coordination, or in close collaboration, with LICR Branches and other Affiliate Centers, in direct or indirect support of the Antibody Targeting and Cancer Vaccine Programs. A number of other relevant projects have been conducted independently. Our report will concentrate on those aspects of our work that are most closely associated with ongoing LICR activities.

Antigen presentation by dendritic cells

Dendritic cells (DCs) are now well recognized as playing an essential role in the initiation of nearly all antigen-specific immune responses, including the maintenance of peripheral tolerance. As such, their direct or indirect importance to any efforts designed at modulating the immune response to cancer is self-evident. During the past year, our group continued to devote a considerable effort to understanding the remarkable range of specializations and features that contribute the DC's exquisite capacity for antigen processing, presentation, and stimulation of T cells, NK cells, and B cells.

Lysosomal function in DCs is carefully regulated. Presentation by DCs of antigens on major histocompatibility complex (MHC) class II molecules (and during cross-presentation even class I molecules) typically depends on the ability of DC endocytic organelles to accumulate and carefully degrade incoming antigens. A major effort extending back several years led us this past year to a careful comparison of lysosomal compartments in DCs versus other cell types. Remarkably and counterintuitively, we found that DC lysosomes are greatly attenuated with respect to their content of lysosomal proteases, the enzymes required for the processing of protein antigens. Macrophages, for example, express up to 100-fold higher levels of a wide range of proteases. Interestingly, all other lysosomal hydrolases (glycosidases, nucleases, lipases, etc.) are expressed comparably in both cell types. Function evidence obtained both in cell culture, and *in vivo* (mice), demonstrate that the restricted capacity of DCs to degrade internalized antigens facilitates antigen presentation in at least several important ways, such as bestowing on DCs the ability to disseminate antigen throughout the immune system. Antigens captured in the periphery remain largely undigested in DCs after their arrival in the T cell regions of lymph nodes, even if the trek requires several days. In addition, the limited capacity for proteolysis actually enhances the ability of DCs to form immunogenic peptide-MHC class II complexes. Finally, by limiting proteolytic potential, we can now understand why DCs are so effective at controlling their ability to process antigens as a function of maturation state (which, we found previously, results in enhanced lysosomal acidification and enhanced catabolic activity). Nearly all DC populations (human and mouse) examined to date exhibit a reduced expression of lysosomal proteases, as to B cells, another important professional antigen presenting cell. Among DCs, a sole exception to the rule can be found in the case of IL-4/GM-CSF-derived DCs from human monocytes. These cells are far more reminiscent of macrophages and monocytes in terms of their lysosomal phenotype. If, as

we believe we have shown (see below), there was an important functional advantage to having attenuated protease levels, then targeting this DC population for vaccine therapy would not be optimal.

Antigen stability determines immunogenicity. Long ago, a collaborator, Dr. Rolf Zinkernagel, suggested that the physical properties of antigens themselves would greatly influence their own immunogenicity. Although evidence was lacking, the fact that DCs expressed remarkably low levels of proteases suggested to us that antigen stability might be one such feature. To test this idea, and also to indirectly assess whether restricted proteolysis in DCs was important for antigen presentation *in vivo*, we devised an approach whereby otherwise identical model antigens were rendered more or less sensitive to lysosomal proteolysis by covalent or non-covalent modifications. Spectacular results were obtained by monitoring T cell, DTH, and antibody responses for several such protein and haptenic antigens, showing a direct correlation between antigen stability in DCs with the induction of robust immune responses. Poor, unstable antigens could also be rendered more stable by cross-linking and fixation. Finally, strategies that further depress lysosomal function further facilitate presentation. Many adjuvant mixtures or carrier proteins (Freund's, KLH) appear to reduce lysosomal proteolysis and may account for at least some of the ability exhibited by these agents in enhancing immune responses. The implications of these studies as a consideration for vaccine design are clear, and are topics for discussion among members of the Vaccine Program and at the Yale Cancer Center. We hope collaborative human trials based on these findings will be implemented in the next 1-2 years.

Mechanisms of cross-presentation. One of the most important, and elusive, features of DC function is their ability to "cross present" exogenous antigens on MHC class I molecules. In a continuing collaboration with members of the LICR New York Branch, we have been investigating the features of human DCs that endow them with the ability to perform this remarkable function. For example, interferon-gamma has been identified as enabling cross presentation activity *in vitro*. These insights will be important not only for the consideration of vaccine strategies, but also for probing the cell biological mechanisms of cross presentation. In this regard, it is also worth noting that most of the immunological community has assumed an intimate role for the endoplasmic reticulum (ER) in cross presentation based on work published from a group in Montreal claiming, in our view prematurely, that the ER directly fused with the plasma membrane during phagocytosis. In recent collaborative studies, we have applied virtually our entire armamentarium of techniques (live cell imaging, quantitative immuno-electron microscopy, functional biochemistry assays, biophysical fluorescence assays) to evaluate this novel mechanism of phagocytosis. Simply put, we could find absolutely no evidence in favor of direct ER involvement; even our preliminary reports of these findings have caused an interesting "stir in the academy".

Cell biology of epithelial cancers: merging basic biology with antibody therapy

Our group has long been interested in the fundamental mechanisms responsible for generating polarity of individual epithelial cells, and for the overall morphogenesis of epithelial tissues. Last year we made substantial progress in understanding the basic cell biology of polarity by demonstrating an essential role for endocytic organelles (specifically "recycling endosomes") in generating polarity on the secretory pathway. Tying several years of work together, as well as provocative suggestions dating back over a decade, we have finally provided direct evidence for the long suspected but elusive intersection between the endocytic and secretory pathways. Such work provides an essential context for understanding the behavior of cancer cells, particularly in the case of epithelial cancers: what is the sequence of cellular events that lead to the formation of epithelial tumors? How do tumor susceptibility genes and mutations manifest themselves at the cellular level, the only level that really matters? How do the cellular features of cancer cells and tumor stroma create opportunities for therapeutic intervention? With this in mind, and in close

collaboration with Drs. Chaitanya Divgi and Steve Larsen at the LICR New York (Memorial Sloan-Kettering Cancer Center) Affiliate Center, as well as with key members of the New York Branch (Drs. Gerd Ritter and Lloyd .J.Old) and the Melbourne Branch (Dr. Joan Heath), we initiated a major effort to examine the cell biology of colon cancer in 2004. These studies include important studies performed under the auspices of the LICR Antibody Targeting Program to evaluate the mechanism of action of A33 and F19.

Colon cancer cell biology. Dr. Cecile Chalouni with the help of Anisyah Alyahya and the surgical pathology section at the Yale Cancer Center (Jose Costa, Director) have been working to perfect approaches to the collection and microscopic analysis of normal and cancerous tissue from human patients. The quality of the images now being collected are improving to the point where the detailed cell biology of normal and tumor cells can be evaluated at the macromolecular level, looking for alterations is cell organization, polarity, the distribution of proteins encoded by important tumor susceptibility genes, etc. Some are so good that already one such image was selected as "Cell of the Month" by Nature in the past year. Painstaking work, it is absolutely essential that specimen collection and preservation be optimized at every stage in order to enable definitive analysis. The superb microscopy suite maintained by Dr. Derek Toomre at the Affiliate Center in New Haven is also essential to conducting this work.

Interaction of A33 with normal and tumor tissue. One major goal is to determine how and why monoclonal antibody A33 targets colon cancer despite recognizing its cognate antigen even on normal colonic epithelium. Initial studies were performed in cell culture, using both fixed and live cells. Results have shown that A33 is poorly internalized by the majority of colon cancer cell lines, and when internalization does occur, it is "atypical": apparently marking a pathway taken by junctional complex components that avoids conventional endocytic organelles. Such a pathway may also occur *in vivo*. With the group at the New York (MSKCC) Affiliate Center, we have performed exploratory studies to localize A33 in patients who received the antibody several days prior to bowel resection. Thus far, our attempts have been unsuccessful due to the presence of large amounts of endogenous human IgG. We are devising alterations to the original protocol to provide epitope tagged A33 that could enable its selective detection at the cellular level. New single chain A33 constructs, perhaps coupled to quantum dots, are also under development in collaboration with Dr. Carl Batt at the LICR Ithaca Affiliate Center/Biological Production Facility. Current data are consistent with the view that A33 is lost from normal tissue by the constitutive turnover of gut epithelia; retention at tumor sites may occur due to the progressive loss of continuity of tumor tissue with the gut lumen.

Interaction of F19 with stromal cells. The LICR antibody F19 (FAP-alpha) has the remarkable capacity to target tumor stroma. We have investigated whether this antibody does so by binding or binding followed by endocytosis. Evidence from cell culture studies of human fibroblasts has thus far indicated that endocytosis of F19 is at best an inefficient process.

APC and microtubule dynamics in epithelial cells. APC has long been appreciated as an important tumor susceptibility gene in colon cancer, yet the cell biological basis for why it contributes to cancer remains poorly described. Dr. Rushika Perera (originally from the Melbourne Branch) together with Dr. Derek Toomre has begun studying this problem from the point of view of APC control of microtubule function. By applying modern, quantitative imaging techniques in their studies of live cells, they have established assays that will enable an evaluation of the phenotype of known APC mutations on microtubule function and epithelial cell biology.

Publications

Primary Research Articles

1. Ayala M.I. Yeaman C. Wright J.R., Ang A., Maeda Y., Mellman I., Nelson W.J. Malhotra V. Protein kinase D (PKD) regulates basolateral, but not apical, plasma membrane exit from the trans Golgi network. Nature Cell Biology (2004) 6:106-112.
2. Duffield A., Fölsch H., Mellman I., Caplan M. Differential sorting of the H,K-ATPase β-subunit in MDCK cells and LLC-PK1 cells is independent of μ1B adaptin expression. Traffic (2004) 5:449-461.
3. Chang H.C., Hull M., Mellman I. The J-domain protein Rme-8 interacts with Hsc70 to control clathrin-dependent endocytosis in *Drosophila*. Journal of Cell Biology (2004) 164:1055-1064.
4. Steinman R.M., Mellman I. Immunotherapy: bewitched, bothered and bewildered no more. Science (2004) 305:197-200.
5. Matsuo M., Nagata Y., Sato E., Atanackovic D., Valmori D., Chen Y.-T., Ritter G., Mellman I., Old L.J., Gnjatic S. IFN-g enables cross-presentation of exogenous protein antigen in human Langerhans cells by potentiating maturation. Proceedings of the National Academies of Science USA (2004) 101:14467-14472.
6. Ang A.L., Taguchi T., Francis S., Fölsch H., Murrells L., Pypaert M., Warren G., Mellman I. Recycling endosomes can serve as intermediates during transport of VSV G protein to the plasma membrane of MDCK cells. Journal of Cell Biology (2004) 167:531-543.
7. zum Buschenfelde C.M., Unternaehrer J., Mellman I., Bottomly K. Regulated recruitment of MHC class II and costimulatory molecules to lipid rafts in dendritic cells. The Journal of Immunology (2004) 173:6119-6124.

Joseph Schlessinger, Ph.D.

Yale University School of Medicine
New Haven, Connecticut, USA

Research Report

As part of our long-term goal to obtain a comprehensive view of the intracellular signaling pathways that are activated by receptor tyrosine kinases (RTKs) including epidermal growth factor (EGF) and fibrolast growth factor (FGF)-receptor, we have prepared connection maps for the signaling pathways that are activated via the EGFR and FGFR. Maps are available at Science's Signal Transduction Knowledge Environment. We have shown that both receptors stimulate a similar complement of intracellular signaling pathways. However, whereas activated EGFRs function as the main platform for recruitment of signaling proteins, signaling through the FGFRs is mediated primarily by assembly of a multidocking protein complex. Moreover, FGFR signaling is subject to additional intracellular and extracellular control mechanisms that do not affect EGFR signaling. The differential circuitry of the intracellular networks that are activated by EGFR and FGFR may affect signal specificity and physiological responses.

We have previously demonstrated that the docking protein Gab1 recruits PI-3 kinase (PI3K) and forms a complex with tyrosine phosphorylated FRS2α in response to FGF stimulation resulting in activation of the PI3K/Akt-dependent cell survival pathway. As part of our goal to compare signaling pathways which are activated by EGFR and FGFR, we have studied the role of Gab1 in signaling via EGFR. We used fibroblasts isolated from Gab1-/- mouse embryos to explore the mechanism of EGF stimulation of the PI3K/Akt anti-apoptotic cell signaling pathway. We have shown that Gab1 is essential for EGF stimulation of PI3K and Akt in these cells and that these responses are mediated by complex formation between p85, the regulatory subunit of PI3K, and three canonical tyrosine phosphorylation sites on Gab1. Complex formation between Gab1 and the protein tyrosine phosphatase Shp2 negatively regulates Gab1 mediated PI3K and Akt activation following EGF-receptor stimulation. We also demonstrate that tyrosine phosphorylation of ErbB3 may lead to recruitment and activation of PI3K and Akt in Gab1-/- MEFs. The primary mechanism of EGF-induced stimulation of the PI3K/Akt anti-apoptotic pathway occurs via the docking protein Gab1. However, in cells expressing ErbB3, EGF and neuroregulin can stimulate PI3K and Akt activation in a Gab1-dependent or Gab1-independent manner.

Analysis of the role of FRS2α in mouse development.

We have characterized the effect of FRS2α deficiency on mouse development. *Frs2*α-null mouse embryos have a defect in anterior-posterior (A-P) axis formation, and are developmentally retarded resulting in embryonic lethality by E8.0. FRS2α is essential for the maintenance of self-renewing trophoblast stem (TS) cells in response to FGF4 in the extraembryonic ectoderm (ExE) that gives rise to tissues of the placenta. By analyzing chimeric embryos, we found that FRS2α also plays a role in cell movement through the primitive streak during gastrulation. In addition, we have shown experimentally that Bmp4 expression in TS cells is controlled by MAP kinase dependent FGF4 stimulation. Moreover, both the expression of Bmp4 in ExE and activation of Smad1/5 in epiblasts are reduced in *Frs2*α-null embryos. These experiments demonstrate that FRS2α plays a critical role in mediating multiple processes during embryonic development and reveal a potential new link between FGF and Bmp4 signaling pathways in early embryogenesis.

Shp2 recruitment by FRS2α in FGF signaling plays an important role in lens and retina development.

Early development of the lens and retina depends upon reciprocal inductive interactions between the embryonic surface ectoderm and the underlying neuroepithelium of the optic vesicle. FGF signaling has been implicated in this signal exchange. After FGF stimulation, tyrosine-phosphorylated FRS2α recruits four molecules of the adaptor protein Grb2 and two molecules of the protein tyrosine phosphatase Shp2, resulting in activation of the Ras/MAP-kinase and PI3K/Akt signaling pathways. We have explored the role of signaling pathways downstream of FRS2α in eye development by analyzing the phenotypes of mice that carry point mutations in either the Grb2-(*Frs2α*^{4F}) or the Shp2-binding sites (*Frs2α*^{2F}) of FRS2. Although *Frs2α*$^{4F/4F}$ mice exhibited normal early eye development, all *Frs2α*$^{2F/2F}$ embryos were defective in eye development and showed anophthalmia or microphthalmia. Consistent with the critical role of FRS2 in FGF signaling, the level of activated extracellular signal-regulated kinase in *Frs2α*$^{2F/2F}$ embryos was significantly lower than that observed in wild-type embryos. Furthermore, expression of Pax6 and Six3, molecular markers for lens induction, were decreased in the *Frs2α*$^{2F/2F}$ presumptive lens ectoderm. Similarly, the expression of Chx10 and Bmp4, genes required for retinal precursor proliferation and for lens development, respectively, was also decreased in the optic vesicles of *Frs2α*$^{2F/2F}$ mice. These experiments demonstrate that intracellular signals that depend on specific tyrosine residues in FRS2α lie upstream of gene products critical for induction of lens and retina.

Development of a new scaffold-based approach for identification of new families of drug candidates.

In collaboration with scientist at Plexxikon, we have developed a new scaffold-based drug discovery approach for the identification of new drug candidates. This approach has been applied for the identification of new compounds that inhibit the activity of FGFRs as well as other tyrosine and serine/threonine kinases. In our first study, we have shown how this method can be used for the development of new families of inhibitors for phosphodiesterases. We also compare the mechanism of activation of the newly identified PDE inhibitor family to known drugs that act by blocking the activities of PDE's.

Cyclic nucleotide phosphodiesterases (PDEs) comprise a large family of enzymes that regulate a variety of cellular processes. The scaffold-based drug design approach starts with low affinity screening of a low molecular weight compound library followed by high throughput co-crystallography of the newly identified compounds to select scaffold compounds that exhibit a dominant binding mode and have appropriate substitution sites. The 4000-fold improvement in the potency achieved within two rounds of chemical synthesis demonstrated the robustness of this scaffold-based approach for the identification of new inhibitors that can be further developed into drug candidates. We have also obtained crystal structures of the catalytic domains of PDE4B, PDE4D and PDE5A with ten different inhibitors including drug candidates, cilomilast and roflumilast, for respiratory diseases. These co-crystal structures reveal a common scheme of inhibitor binding to the PDEs: (i) A hydrophobic clamp formed by highly conserved hydrophobic residues that sandwich the inhibitor in the active site; (ii) hydrogen bonding to an invariant glutamine that controls the orientation of inhibitor binding.

Publications

Primary Research Articles

1. Lamothe B., Yamada M.,Schaeper U., Birchmeier W., Lax I., Schlessinger J. The docking protein Gab1 is an essential component of an indirect mechanism for FGF stimulation of the PI 3-kinase/Akt anti-apoptotic pathway. Molecular and Cellular Biology (2004) 24:5657-5666.
2. Zhang K.Y., Card G. L., Suzuki Y., Artis D.R., Fong D., Gillette S., Hsieh D., Neiman J., West B.L., Milburn M.V., Kim S-H., Schlessinger J., Bollag G.. A novel 'glutamine switch' mechanism for nucleotide selectivity of phosphodiesterases. Molecular Cell (2004) 15:279-286.
3. Mattoon D., Lamothe B., Lax I., Schlessinger J. The docking protein Gab1 is the primary mediator of EGF-stimulated activation of the PI-3K/Akt cell survival pathway. BMC Biology (2004) 2:24.
4. Schlessinger J. Common and distinct elements in signaling via EGF and FGF receptors. Science (2004) 306:1506-1507.
5. Gotoh N., Ito M., Yamamoto S., Yoshino I., Song N., Wang Y., Lax I., Schlessinger J., Shibuya M., Lang R.A. Tyrosine phosphorylation sites on FRS2α responsible for Shp2 recruitment are critical for induction of lens and retina. Proceedings of the National Academies of Science USA (2004) 101:17144-17149.
6. Card G.L., England B.P., Suzuki Y., Fong D., Powell B., Lee B., Luu C., Tabrizizad M., Gillette S., Ibrahim P.N., Artis D.R., Bollag G., Milburn M.V., Kim S-H., Schlessinger J., Zhang K.Y. J.. Structural basis for the activity of drugs that inhibit phosphodiesterases. Structure (2004) 12:2233-2247.

Graham Warren, Ph.D.

Yale University Medical School
New Haven, Connecticut, USA

Research Report

Cell Cycle Regulation of the Golgi Apparatus

The central feature of the Golgi apparatus is the stack of closely-apposed and flattened cisternae that mediate the post-translational processing of transiting cargo. In mammals, several hundred stacks are stitched together, generating a ribbon-like structure that resides close to the nucleus and near to the centrosomes. This location begs the question as to how this organelle is partitioned equally to the two daughter cells during mitosis.

Work by us and others over a number of years have shown that the Golgi undergoes a dramatic fragmentation into vesicles and clusters of vesicles at the onset of mitosis. What happens next has been the subject of debate. One model postulates that these fragments become dispersed throughout the mitotic cell cytoplasm thereby ensuring that equal amounts are delivered to each daughter cell. Another postulates the merger of these fragments with the ER, which then mediates the partitioning process.

In order to address this problem we have recently generated BSC-1 cell lines that express both ER and Golgi proteins tagged with different GFP variants. This has allowed us to follow, in considerable detail, the movements of these different membrane compartments throughout mitosis using video fluorescence microscopy. The results show clearly that these two organelles do not merge at any time. To confirm these results we exploited a chance observation by others, that filipin III fragments ER but not Golgi membranes and so immobilizes proteins in the ER. This allowed us to show that the Golgi marker was never present in the ER during mitosis, except when it was delivered there deliberately by brefeldin A.

Further progress was also made in studying the role played by GRASP65 in mitotic fragmentation of the Golgi apparatus. GRASP65 was first identified by us as an N-myristoylated, cytoplasmic protein that was involved in stacking Golgi cisternae at the end of mitosis. More recently we were able to show that beads coated with recombinant GRASP65 aggregated in a mitotically regulated fashion, showing that this protein is capable of linking adjacent surfaces. We have now taken this analysis to the next step, determining which of the phosphorylation sites in this protein are important for the regulation of cisternal stacking. We find that the N-terminal GRASP domain is responsible for the trans pairing that brings surfaces together. The C-terminal domain contains multiple phosphorylation sites for both CDK1 and polo-like kinases. This C-terminal domain appears to stop the N-terminal domain from forming too strong an interaction so that phosphorylation tips the balance, breaking the trans oligomers of GRASP65 and thereby unstacking Golgi cisternae. This in turn leads to extensive tubulation and vesiculation, followed by dispersal throughout the mitotic cell cytoplasm.

Publications

Primary Research Articles

1. Axelsson M.A.B., Warren G. Rapid, ER-independent diffusion of the mitotic Golgi haze. Molecular Biology Cell (2004) 15:1843-1852.

Ellen Puré, Ph.D.

Wistar Institute
Philadelphia, Pennsylvania

Research Report

Inflammation is typically a self-limiting reparative response to infection or injury but severe acute inflammation and persistent inflammation can lead to tissue injury. Indeed, inflammation is now recognized to be a critical component of a wide variety of diseases ranging from acute septic shock to chronic inflammatory diseases and cancer. Inflammation and cancer are characterized by activation of local stromal cells, leukocyte infiltration and, turnover and remodeling of extracellular matrix. Our laboratory is studying the cell-cell and cell-matrix interactions that regulate the inflammatory response and tumor cell growth and metastasis. Our major accomplishments over the past year include: 1) Characterization of a novel mechanism of regulation of interleukin 12 (IL-12) production; 2) Defining a role for host CD44 in tumor metastasis; and 3) Defining a role of CD44 in the recruitment of inflammatory cells to tumors following immunotherapy.

A novel mechanism of regulation of IL-12

IL-12 is a key mediator of type 1 inflammatory responses, including the production of interferon-gamma, that can contribute to disease pathogenesis. Thus our recent discovery of a novel 12/15-lipoxygenase dependent pathway leading to the production of IL-12 has important implications and provides new potential therapeutic targets for the treatment of inflammation. We found that IL-12 production *in vitro* was markedly reduced in macrophages from 12/15-lipoxygenase deficient mice (Alox mice) compared to wild-type mice. Furthermore, IL-12 production in wild-type macrophages is sensitive to an inhibitor of 12/15-lipoxygenase. Interestingly, we found that 12/15-LO regulation of IL-12 production is cell-type restricted. Thus, in contrast to the defect in IL-12 production by 12/15-LO-deficient peritoneal macrophages *in vitro*, and macrophages in atherosclerotic lesions *in vivo*, we found that bone marrow-derived macrophages and dendritic cells from 12/15-LO deficient mice produce comparable levels of IL-12 compared to bone marrow-derived wild-type macrophages and dendritic cells. Based on preliminary studies, the degree to which IL-12 production depends on 12/15-LO in the various cell types appears to correlate with the expression of 12/15-LO.

We also found that regulation of IL-12 by 12/15-LO is stimuli specific as 12/15-LO-deficient macrophages produced normal levels of IL-12 in response to *in vitro* stimulation with bacterial CpG and STAg, the immunodominant antigen of toxoplasma gondii, while the response to LPS and hyaluronan were markedly reduced. Thus, the dependence on12/15-LO for IL-12 production appears to vary depending on the particular receptor mediating stimulation of macrophages. Interestingly, priming of macrophages with interferon-gamma resulted in production of IL-12 predominantly via the 12/15-LO dependent pathway regardless of the agent subsequently used to stimulate macrophages. Although the mechanisms of priming are not completely defined, priming by interferon-gamma is known to involve the activation of STAT1 dependent transcription factors including ICSBP. This is of interest since we have reported that the defect in IL-12 production in 12/15-LO-deficient macrophages is associated with a decrease in nuclear ICSBP levels. More recently, we have found that the reduction in nuclear ICSBP correlates with a reduction in the physical association of ICSBP as well as NF-κB with the IL-12p40 promoter as shown using chromatin immunoprecipitation assays.

In summary, the major accomplishments for the past year have been: 1) To demonstrate that the role of 12/15-lipoxygenase in mediating production of IL-12 is both cell type and stumilus

dependent; 2) To demonstrate that priming with interferon-gamma promotes production of IL-12 through predominantly a 12/15-LO dependent pathway; and 3) To demonstrate that 12/15-LO can regulate the assembly and or stability of the physical association of ICSBP and NF-kB within the complex of transcription factors and co-factors that induce IL-12p40 promoter activity.

Role of CD44 in Tumor-Host Interactions

The ultimate goal of immunotherapeutic approaches to cancer is to activate inflammatory cells that home to tumors and destroy the tumor cells. We have begun to investigate the role of CD44 in tumor infiltration by inflammatory cells. CD44 is expressed by most cells, including leukocytes, and mediates cell-cell and cell-matrix interactions. It has been implicated in regulation of migration of neutrophils, macrophages and T cells to sites of inflammation by regulating leukocyte adhesion to vascular endothelium. Based on our knowledge of CD44, we hypothesized that a lack of CD44 in the tumor host would impair leukocyte homing into tumors after immunotherapy and thereby allow tumors to grow more rapidly. We tested this hypothesis using a model of immunogene therapy established by our collaborator, Dr. Steve Albelda (Philadelphia, PA.). Flank TC-1 lung epithelial cell tumors were established and then injected with a recombinant adenovirus encoding murine IFNβ (Ad.IFNβ). TC-1 tumor growth prior to "immunotherapy" was identical in wild-type and CD44-deficient mice. Surprisingly, although Ad.IFNβ had only a modest effect in wild-type mice, it markedly reduced tumor growth in CD44-/- mice. The enhanced inhibition of tumor growth was associated with a greater accumulation of neutrophils within tumors and CD8+ T cells. Furthermore, depletion of neutrophils prior to treatment with Ad.IFNβ abrogated the effects on tumor cells growth. These data suggest that neutrophils actively participate in the anti-tumor immune response, either directly or indirectly, in this model. We are currently pursuing these findings at a mechanistic level.

In other studies, we have investigated the role of CD44 and its principle ligand hyaluronan in tumor metastasis. We used anti-CD44 monoclonal antibodies and HA inhibitory peptides to investigate the role of host CD44 in several steps in metastasis. Blocking CD44 and HA mediated interactions affected the initial attachment of tumor cells to the pulmonary vessel wall as well as tumor cell survival post attachment. Our data indicate that host CD44 may have a role in tumor metastases.

Publications

Primary Research Articles

1. Kothapalli D., Fuki I., Ali K., Stewart S.A.., Zhao L., Yahil R., Kwiatkowski D., Hawthorne E.A., FitzGerald G.A., Phillips M.C., Lund-Katz S., Puré E., Rader D.J., Assoian R.K. Antimitogenic effects of HDL and apoE mediated by Cox-2-dependent IP activation. Journal of Clinical Investigation (2004) 113:609-18.
2. Cichy J., Puré E. Cytokines regulate the affinity of soluble CD44 for. FEBS Letters (2004) 556:69-74.
3. Sague S.L., Tato C., Puré E., Hunter C.A. The regulation and activation of CD44 by natural killer (NK) cells and its role in the production of IFN-γ. Journal of Interferon and Cytokine Research (2004) 24:301-309.
4. Lavens D., Faust E., Lu F., Jacob M., Leta M., Lieberman P., Puré E. Identification of protein tyrosine kinases required for B cell receptor-mediated activation of an Epstein Barr Virus immediate early gene promoter. Journal of Virology (2004) 78:8543-51.

Robert D. Schreiber, Ph.D.

Department of Pathology and Immunology
Washington University School of Medicine
St. Louis, Missouri

Research Report

Identification of Tumor Antigens of Tumors From Immunodeficient Mice

The central focus of this project is to understand the process of cancer immunoediting at the molecular level. We envisage this process as one that not only protects the host against tumor development but also sculpts the immunogenicity of tumors that from in immunocompetent hosts thereby rendering them more able to escape immune detection and elimination. Since tumors isolated from immunodeficient mice have not been exposed to the sculpting actions of adaptive immunity, we are testing the hypothesis that these unedited tumors may express unique antigens that have not been seen before because most of the tumor antigens that have been currently characterized were identified from edited tumors derived from immunocompetent hosts. Two studies were completed on this project this year and both focused on identifying the roles of the interferons (IFN) in specifying tumor immunogenicity.

One study stemmed from our previous finding that tumor cell immunogenicity was critically linked to tumor cell responsiveness to interferon-gamma (IFN-γ). Based on this observation we predicted that tumor cells from immunocompetent mice might be rendered IFN-γ insensitive as a result of a cancer immunoediting process and thereby evade further immune detection and elimination. Preliminary support for this hypothesis came from our finding that 23% of long-term human lung adenocarcinoma cell lines were insensitive to IFN-γ. In some cases, tumor cell insensitivity to IFN-γ was caused by the lack of expression of critical components of the IFN-γ receptor-signaling complex e.g., the IFNGR1 subunit of the IFN-γ receptor or JAK1, the tyrosine kinase that associates with the intracellular domain of IFNGR1. In other cases, tumor cell insensitivity to IFN-γ resulted from the production of abnormal forms of JAK2, a second functionally critical tyrosine kinase that associates with the IFNGR2 subunit of the IFN-γ receptor. During the last year, we extended these initial observations to other tumor cell lines. Specifically, we assessed IFN responsiveness in prostate cancer cell lines since these types of tumors were known to display high immunoevasive potential. One heavily studied prostate tumor cell line, LNCaP, was found to be completely insensitive to both IFN-γ and IFN-α. We determined that the functional lesion in these cells was caused by the absence of JAK1, an obligate component of the receptors for either IFN-γ or IFN-α. JAK1 was undetectable in LNCaP cells at both the protein and message levels. Enforced expression of JAK1 in LNCaP cells restored their ability to signal through both the IFN-γ and IFN-α receptors and complemented the cell line's ability to manifest IFN-dependent biologic responses. Treatment of LNCaP cells with a combination of inhibitors of DNA methyltransferases and histone deacetylases induced expression of JAK1 message. This study therefore not only identifies the molecular basis for IFN insensitivity in the LNCaP cell line but also suggests that epigenetic silencing of key immunologic signaling components may be one mechanism by which tumor cells evade immune detection and elimination.

In a second study we asked whether type I IFN (IFN- 2/β) played an important role in the cancer immunoediting process and whether it acted like IFN-γ to regulate tumor cell immunogenicity. We found that in immunocompetent mice, endogenously produced IFN-α/β is required for rejection of highly immunogenic methylcholanthrene (MCA)-induced sarcomas and also prevents the outgrowth of primary MCA-induced tumors. We also found that whereas several IFN-α/β-insensitive MCA sarcoma cell lines derived from mice lacking the type I IFN

receptor (IFNAR1$^{-/-}$ mice) were rejected when transplanted into wild type mice in a lymphocyte dependent manner, tumor cells generated in wild type mice invariably grew when transplanted into wild type recipients. This result revealed that tumors from IFN-α/β unresponsive mice are more immunogenic as a group than tumors arising in immunocompetent hosts. Comparative analysis of the in vivo growth of matched sets of IFN-α/β versus IFN-γ responsive and unresponsive tumor cells showed that although tumor cells were critical targets of IFN-γ's actions, they were not physiologically relevant targets of endogenously produced type I IFN during the development of host protective anti-tumor responses. Rather, hematopoietic cells of the host were the functionally important IFN-α/β targets that resulted in development of anti-tumor immunity. Thus, these data are not only some of the first to unequivocally establish the importance of endogenously produced IFN-α/β in cancer immunoediting but also demonstrate that type I IFN functions the cancer immunoediting process in a manner that does not completely overlap with that of IFN-γ.

Publications

Primary Research Articles

1. Maldonado R.A., Irvine D.J., Schreiber R., Glimcher L.H. A role for the immunological synapse in lineage commitment of CD4 lymphocytes. Nature (2004) 431:527-532.

Reviews / Commentaries / Book Chapters

1. Schreiber R.D., Old L.J., Hayday A.C., Smyth M.J. A cancer immunosurveillance controversy. Nature Immunology (2004) 5:4-5.
2. Dunn G.P., Old L.J., Schreiber, R.D. The Three Es of Cancer Immunoediting. In: Annual Review of Immunology, edited by W. E. Paul, 2004, Vol. 22, pp. 329-360.
3. Dunn G.P., Old L.J., Schreiber R.D. The immunobiology of cancer immunosurveillance and immunoediting. Immunity (2004) 21:137-148.

Kohei Miyazono, M.D.

Department of Biochemistry
The Cancer Institute of the Japanese Foundation for Cancer Research (JFCR)
Tokyo, Japan, and
Department of Molecular Pathology
Graduate School of Medicine, University of Tokyo
Tokyo, Japan

Research Report

Our major research interest is to elucidate how Smad signaling is regulated by various molecules, including inhibitory Smads (Smad6 and Smad7) and transcriptional co-repressors (c-Ski and SnoN). We have previously found that the E3 ubiquitin ligase Smurf1 induces degradation of the Smad7-transforming growth factor β (TGF-β) receptor complexes, and represses TGF-β signaling. In contrast, the E3 ligase Arkadia enhances TGF-β signaling through degradation of Smad7. We have recently found that E3 ubiquitin ligases WWP1 and NEDD4-2 also induce degradation of the Smad7-TGF-β receptor complexes and repress TGF-β signaling, similar to Smurf1. Thus, TGF-β signaling is fine-tuned by various ubiquitin ligases through interacting with Smad7. We have also found that Smad7 and c-Ski prevent metastasis of breast cancer in mouse. In addition, we have found that bone morphogenetic protein-4 (BMP-4) induces apoptosis of B-cell hybridoma and multiple myeloma cells in p53- and endoplasmic reticulum (ER) stress-dependent manners. These findings suggest that molecules that regulate TGF-β superfamily signaling can be used for treatment of certain cancers.

Smad7 inhibits metastasis of mouse breast cancer by direct action on cancer cells

TGF-β facilitates metastasis in the advanced stages of cancer. We have examined whether systemic gene therapy using adenovirus vectors containing inhibitory Smads, Smad6 or Smad7, has effects on metastasis of the murine breast cancer cell line, JygMC(A). JygMC(A) spontaneously metastasized to lung and liver within 50 days after inoculated subcutaneously in nude mice. Systemic administration of Smad7 adenovirus to mice demonstrated a dramatic decrease in metastasis and an increase in the mean survival time. c-Ski, a transcriptional co-repressor of TGF-β superfamily signaling, also prevented metastasis. In contrast, systemic gene transfer of Smad6 affected neither the development of metastasis, nor the mean survival time. Moreover, JygMC(A) cells stably expressing Smad7 developed significantly less extensive metastasis in nude mice compared to the mock- and Smad6-transfected cells, indicating that Smad7 acted directly on the cancer cells to prevent metastasis. Major components of adherens and tight junctions (e.g. E-cadherin) and a mesenchymal marker, N-cadherin, were up-regulated and down-regulated by Smad7, respectively. *In vitro* assays demonstrated that Smad7 changed morphology and decreased the migratory and invasive abilities of the JygMC(A) cells. The blocking of TGF-β superfamily signaling by Smad7 may thus provide new therapeutic possibilities to prevent metastasis in patients with advanced cancers.

BMP-4-induced apoptosis by ER dysfunction in myeloma and B-cell hybridoma cells

BMP-4 inhibits proliferation and induces the apoptosis of myeloma cells. We investigated the roles of p53 and the ER in BMP-4-induced apoptosis of mouse hybridoma HS-72 cells. We found that 3 ng/mL of BMP-4 is sufficient to induce the expression of BH3-only proteins, Puma and Bax, in a p53-dependent manner, and facilitate Ca^{2+} release from the ER to the cytosol, resulting in the activation of caspase-12 and ER dysfunction. Multiple myeloma cells with wild-type p53 gene showed much higher sensitivity to BMP-4-induced apoptosis than cells without wild-type p53 gene, suggesting that wild-type p53 is required for dysfunction of the ER during BMP-4-induced

apoptosis in ER-enriched cells, such as hybridoma and myeloma cells. An ER stress-inducing agent, thapsigargin, inhibits the viability of multiple myeloma cells, irrespective of the p53 status. These findings demonstrate that the presence of wild-type p53 gene and enrichment of the ER determine the sensitivity to effective apoptosis by BMP-4, and suggest that ER stress-inducing agents would be valuable for the treatment of multiple myeloma.

Publications

Primary Research Articles

1. Maeda, S., Hayashi, M., Komiya, S., Imamura, T., Miyazono, K. Endogenous TGF-β signaling suppresses maturation of osteoblastic mesenchymal cells. EMBO Journal (2004) 23:552-563.
2. Kahata, K., Hayashi, M., Asaka, M., Hellman, U., Kitagawa, H., Yanagisawa, J., Kato, S., Imamura, T., Miyazono, K. Regulation of transforming growth factor-β and bone morphogenetic protein signaling by transcriptional co-activator GCN5. Genes to Cells (2004) 9:143-151.
3. Takeda, M., Mizuide, M., Oka, M., Watabe, T., Inoue, H., Suzuki, H., Fujita, T., Imamura, T., Miyazono, K., Miyazawa, K. Interaction with Smad4 is indispensable for suppression of BMP signaling by c-Ski. Molecular Biology of the Cell (2004) 15:963-972.
4. Ijichi, H., Otsuka, M., Tateishi, K., Ikenoue, T., Kawakami, T., Kanai, F., Arakawa, Y., Seki, N., Shimizu, K., Miyazono, K., Kawabe, T., Omata, M. Smad4-independent regulation of p21/WAF1 by transforming growth factor-β. Oncogene (2004) 23:1043-1051
5. Kondo, M., Suzuki, H., Takehara, K., Miyazono, K., Kato, M. Transforming growth factor-β signaling is differentially inhibited by Smad2D450E and Smad3D407E. Cancer Science (2004) 95:12-17.
6. Yanagita, M., Oka, M., Watabe, T., Iguchi, H., Niida, A., Takahashi, S., Akiyama, T., Miyazono, K., Yanagisawa, M., Sakurai, T. USAG-1: a bone morphogenetic protein antagonist abundantly expressed in the kidney. Biochemical and Biophysical Research Communications (2004) 316:490-500.
7. Usui, M., Yoshida, Y., Tsuji, K., Oikawa, K., Miyazono, K., Ishikawa, I., Yamamoto, T., Nifuji, A., Noda, M. Tob-deficiency super-enhances osteoblastic activity after ovariectomy to block estrogen deficiency-induced osteoporosis. Proceedings of the National Academy of Sciences USA (2004) 101:6653-6658.
8. Horiki, M., Imamura, T., Okamoto, M., Hayashi, M., Murai, J., Myoui, A., Ochi, T., Miyazono, K., Yoshikawa, H., Tsumaki, N. Smad6/Smurf1 overexpression in cartilage delays chondrocyte hypertrophy and causes dwarfism with osteopenia. Journal of Cell Biology (2004) 165:433-445.
9. Mochizuki, T., Miyazaki, H., Hara, T., Furuya, T., Imamura, T., Watabe, T., Miyazono, K. Roles for the MH2 domain of Smad7 in the specific inhibition of transforming growth factor-β superfamily signaling. Journal of Biological Chemistry (2004) 279:31568-31574.
10. Kondo, M., Cubillo, E., Tobiume, K., Fukuda, N., Suzuki, H., Takehara, K., Cano, A., Saitoh, M., Miyazono, K. A role for Id in the regulation of TGF-β-induced epithelial-mesenchymal transdifferentiation. Cell Death and Differentiation (2004) 11:1092-1101.
11. Suzuki, H., Yagi, K., Kondo, M., Kato, M., Miyazono, K., Miyazawa, K. c-Ski inhibits the TGF-β signaling pathway through stabilization of inactive Smad complexes on Smad-binding elements. Oncogene (2004) 23:5068-5076.

12. Komuro, A., Imamura, T., Saitoh, M., Yoshida, Y., Yamori, T., Miyazono, K., Miyazawa, K. Negative regulation of transforming growth factor-β (TGF-β) signaling by WW domain-containing protein 1 (WWP1). Oncogene (2004) 23:6914-6923.

13. Brederlau, A., Faigle, R., Elmi, M., Zarebski, A., Sjoberg, S., Fujii, M., Miyazono, K., Funa, K. The bone morphogenetic protein type Ib receptor is a major mediator of glial differentiation and cell survival in adult hippocampal progenitor cell culture. Molecular Biology of the Cell (2004) 15:3863-3875.

14. Ikeda, Y., Imai, Y., Kumagai, H., Nosaka, T., Morikawa, Y., Hisaoka, T., Manabe, I., Maemura, K., Nakaoka, T., Imamura, T., Miyazono, K., Komuro, I., Nagai, R., Kitamura, T. Vasorin, a transforming growth factor β-binding protein expressed in vascular smooth muscle cells, modulates the arterial response to injury in vivo. Proceedings of the National Academy of Sciences USA (2004) 101:10732-10737.

15. Miyazaki, H., Watabe, T., Kitamura, T., Miyazono, K. BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells. Oncogene (2004) 23, 9326-9335.

16. Beppu, H., Ichinose, F., Kawai, N., Jones, R.C., Yu, P.B., Zapol, W.M., Miyazono, K. Li, E., Bloch, K.D. BMPR-II heterozygous mice have mild pulmonary hypertension and an impaired pulmonary vascular remodeling response to prolonged hypoxia. American Journal of Physiology-Lung Cellular and Molecular Physiology (2004) 287:L1241-1247.

Reviews / Commentaries / Book Chapters

1. Miyazono, K., Ishikawa, F., Winterhager, E., Deppert, W., Rajewsky, M.F. Ninth Japanese-German workshop on molecular and cellular aspects of carcinogenesis, essen, Germany, 18-20 September, 2003. Cancer Science (2004) 95:276-281.

2. Miyazono K., Maeda S., Imamura T. Coordinate regulation of cell growth and differentiation by TGF-beta superfamily and Runx proteins. Oncogene (2004) 23:4232-4237.

LUDWIG INSTITUTE FOR CANCER RESEARCH

James R. Kerr Program

Research Report

Publications

James R. Kerr Program

The James R. Kerr Program was initiated to foster scientific collaborations with talented scientists from countries that have had limited opportunities for funding cancer research studies. The LICR has established collaborative research projects at leading academic centers in China, Ukraine, Russia, South Africa, Turkey and Brazil. Scientists of the Program interact with researchers throughout the global Institute in collaborations that form integrative networks of scientific investigation and discovery in cancer research.

A novel initiative in 2004 was the inauguration of a bioinformatics collaborative, BRAIN (Bioinformatics Resource for the Analysis of Information on Neoplasia) aimed at providing a tool to efficiently access the high-volume data that is generated in cancer research today. James R. Kerr Program participants, Dr. Mikhail Gelfand of Russia and Dr. Winston Hide of South Africa are bioinformaticians working on this initiative together with LICR staff members.

Dr. Hide, and coworkers at the South African National Bioinformatics Institute, have developed and implemented a gene expression vocabulary for data exchange between gene expression systems, delivered a list of mouse/human/other species orthologs and developed a standardized gene list structure that has been incorporated into the BRAIN system. They are in the early stages of development of appropriate approaches to predict expression profiles of relevant genes in collaboration with Dr. Marketa Zvelebil of the LICR London University College Branch. This past year, the Hide Group has delivered data exchange for gene expression vocabularies, in particular specific annotation, more highly detailed than Genbank submitted data, has been incorporated into eVOC expression vocabularies for ORESTES data. The vocabularies are under consistent development and monthly release and are now available from www.evocontology.org. In addition mouse/human orthologs for cancer testis (CT) genes were studied by the Group, with a set of orthologous genes between human and other species being developed. This work has characterized CT genes on the basis of their presence in mammals (chimp, mouse, rat, and dog), birds, amphibia, fish, insects, worms and plants. The BAGE family and TPTE appear to be found only in humans. Of 83 CT genes, 77% could only be found in eutherian organisms with 51 of the 66 eutherian restricted CT genes being X-linked. At present, an effort is underway to define areas of comparative genomic study examining the phylogenetic distribution of the CT genes. The genomic distribution of the known 83 CT genes is biased towards the X-chromosome with 66% residing on the X chromosome. Curation of CT genes belonging to multi-gene families that have arisen by duplication indicate 50% of the 44 'founding' CT gene family members are X-linked, as opposed to one or two CT genes that would be predicted by a random genomic distribution model.

The current studies of Dr. Mikhail Gelfand, a bioinformatician at the Department of Bioengineering and Bioinformatics at Moscow State University, include an analysis of the evolution of CT genes. Recently the PAGE family has been analyzed. Future plans include analyzing the MAGE-A family in mouse (where an independent multiplication of genes occurred) and also to look at the MAGE-B family with its complicated pattern of duplications: some human-specific, some mouse-specific, and some seemingly occurring in the common ancestor. The study will begin with these two genomes and then try to look at other mammalian genomes, chimpanzee, rat, dog etc. as they become available for this specific type of analysis. Overall, the aim is to analyze the evolution of alternative splicing in CT antigen families. The analysis of signals regulating alternative splicing is also being pursued. The analysis of micro RNA (miRNA) targets in alternatively spliced genes is undergoing analysis as well. The aim is to determine whether there is a link between miRNA-based regulation and alternative splicing.

The Group has developed a technique for enumeration of all isoforms of genes with multiple alternative regions and is currently applying it to various groups of proteins. Also in development is the application of algorithms for identification of residues determining functional specificity in members of large protein families. Dr. Gelfand contributes to two databases on alternative splicing (AS); EDAS, based on mapping ESTs to the human genome; and ASDB, alternative splicing from the literature. An initial version of the former can be found at http://www.belozersky.msu.ru/edas/. The latter is at the preliminary stage, with the goal being to collect all data about regulation of AS of CT antigens and genes having AS specific to testis, ovary and placenta, as well as AS of transcription factors and surface receptors.

Dr. Alexandre Alexeevski of the GeneBee Group of the Belozersky Institute, Moscow State University, continues his work in the area of bioinformatics and new algorithms and software for protein annotation including the on-line program 'Cluster Detector' (http://math.belozersky.msu.ru/~mlt/HF_page.html) for detecting hydrophobic clusters in 3D structures of proteins and multi-molecular complexes. A 3D program (http://dpidb.belozersky.msu.ru:8080/CoreProc/index.htm) permits the measure and localizes the similarity between 3D structures of related proteins or protein-nucleic acid complexes, and to classify structures of a given family on the basis of the 3D similarity. A new method of high-quality automated annotation of protein sequences has been developed and is available at the GeneBee server: http://www.genebee.msu.su/services/annot/basic.html.

A new method of computing high quality multiple alignments has been developed by the group and tested. The method was implemented as the program AliBee available at the GeneBee server: http://www.genebee.msu.su/services/malign_reduced.html. Program tools include data mining from multiple alignment databases, profile calculation, sequence database search with the constructed profile, and plausibility checking of new sequences and multiple alignments construction for next iteration. The main utility of the developed tools is to facilitate the work of profile developers. DotHelix, (http://www.genebee.msu.su/services/dhm/advanced.html), a program for sensitive and fast calculation of pair motifs and alignments, has also been adapted and provided to the LICR. The on-line database of human endogenous elements (https://math.genebee.msu.ru/genome/forms/main.php) has been created that contains all long terminal repeats of class LTR5. The database is equipped with a synchronization program, which weekly synchronizes data with current release of EnsEMBL.

LICR has established a Human Lung Cancer Initiative to integrate study of this disease and for the development of novel therapies. Members of the James R. Kerr Program are part of this initiative include Dr. Pavel Philippov of the Belozersky Institute of Moscow State University and Dr. Ugur Ozbek of the Genetics Department of Istanbul University, Institute for Experimental Medicine (DETAE). Dr. Philippov continues to study the calcium-binding protein recoverin as a paraneoplastic antigen in small cell lung cancer (SCLC). Antibodies to the protein recoverin may serve as markers of SCLC in patients. To test this hypothesis, serial sera have been screened in patients with SCLC and non-small cell lung cancer (NSCLC) for the presence of anti-recoverin antibodies. The study includes a total of 279 serum samples from SCLC (99 patients), NSCLC (44 patients), non-malignant pulmonary disorders (86 patients), and sera from 50 healthy donors. Anti-recoverin antibodies were detected in the sera from 15 patients with SCLC and from nine patients with NSCLC (15% and ~20%, respectively). Only two anti-recoverin positive cases (~2%) were detected in patients with non-malignant pulmonary disorders, while no antibodies were detected in healthy individuals. Antibodies to recoverin were also found in the sera of patients with 17 different cancer types. The frequency of occurrence did not exceed 10%. Anti-Recoverin antibodies are able to initiate apoptotic degeneration of recoverin-containing retinal cells, but do not act upon cells devoid of recoverin. The role of anti-recoverin antibodies to potentiate apoptotic death upon recoverin-positive cancer cells is being investigated.

Immunohistochemical analysis was performed on 30 SCLC and 34 NSCLC recoverin-positive sections. Expression of recoverin in SCLC and NSCLC tumors was 68% and 85% in these studies. These findings identify recoverin as a potential target for immunotherapy of lung cancer.

In collaboration with the LICR Brussels Branch, Dr. Philippov's Group has demonstrated that in normal tissues, a high level of methylation of the recoverin gene promoter correlates with a low level of expression of mRNA for recoverin. This observation suggests that in normal tissues, methylation of the recoverin gene promoter is involved in gene silencing. In cancer tissues, methylation of the recoverin gene promoter is decreased in comparison with that in normal tissues. Thus, promoter methylation might control expression of mRNA for recoverin and could be part of the mechanism underlying the aberrant expression of recoverin in cancer cells.

In parallel to the investigation of recoverin as a paraneoplastic antigen, Dr. Philippov's Group continues to study recoverin and its role in vision, which may lead to a better understanding of the role of this antigen in cancer. These studies are ongoing in a collaborative project with the LICR Uppsala Branch.

Dr. Ugur Ozbek of the Genetics Department of Istanbul University, Institute for Experimental Medicine (DETAE) in Turkey in collaboration with Dr. Ali Gure of the LICR New York Branch, has continued to work on analyzing seroreactivity to the neuro-embryonic antigens, SOX Group B (1, 2 and 3) and ZIC2 in patients with SCLC. Initial studies indicate that prospective screening for neuro-ectodermal antibodies might be useful in the early detection of SCLC in high-risk populations. Further studies to clarify the nature of the immune responses and their clinical significance are warranted. Dr. Ozbek, in collaboration with Dr. Gure, plans to collect further data on these patients and extend the study with greater numbers of patients.

Dr. Vladimir Skulachev, Belozersky Institute of Physico-Chemical Biology, Moscow State University in Moscow, continued his study of apoptosis within the framework of the research project "Mitochondria in TNF-induced apoptosis" with HeLa cell lines. A combination of electron microscopic, confocal miscroscopic and polarographic methods revealed the following chain of mitochondrial changes during TNF initiated apoptosis: Long thread-like mitochondria → beads-like mitochondria → small spherical mitochondria → swollen spherical mitochondria → very small super-condensed mitochondria. The first step has a lag-phase of several hours but when initiated progresses rapidly and is completed in one to three minutes. Membrane potential collapse and cytochrome c release from mitochondria occurs later than decomposition of the long mitochondria. Formation of super-condensed mitochondria is accompanied by strong inhibition of mitochondrial respiration. This effect is followed by absorption of mitochondria by autophagosomes. MitoQ and its derivatives are being investigated as treatments in apoptosis-linked pathologies such as heart attack, stroke, etc. The role of cytochrome c, a proapoptotic protein operating in the TNF cascade downstream of mitochondrial thread-grain transition and PTP opening, is being investigated in cooperation with the laboratory of Dr. M.P. Kirpichnikov. It was found that the K72W mutant of cytochrome c is competent in the respiratory electron transfer and O_2-oxidation but is completely inactive in caspase activation. On the other hand, the K72A mutant shows 100% caspase activation but at a concentration about 10 times higher than the wild type protein.

A novel mechanism of mitoptosis, clearly differing from that induced by TNF, has been discovered. Incubation of HeLa cells with an uncoupler and a respiratory inhibitor results in thread-grain transition of mitochondria, their concentration near nucleus, segregation by a single membrane from the cytoplasm and exocytosis of such mitochondria-containing vesicles (called "mitoptotic bodies") from the cell.

Dr. Andrey B. Vartapetian of the Belozersky Institute of Physico-Chemical Biology, Moscow State University in Moscow is examining the role of prothymosin α (ProTα) an essential human

proliferation-related nuclear protein, and its relation to cancer development, apoptosis, and the immune response. ProTα protects human cells from oxidative and chemical stress. Defense against oxidative stress and electrophilic attack is essential for cancer prevention. Animals counteract such insults through coordinated expression of a set of detoxifying and antioxidant enzyme genes mediated by transcription factor Nrf2. The laboratory of Dr. Vartapetian has demonstrated that ProTα participates in the oxidative stress response through its interaction with Keap1, a protein inhibitor of the Nrf2 transcription factor and showed that ProTα competes with Nrf2 for binding to Keap1, and that ProTα contributes to the Nrf2-dependent gene expression *in vivo*. These results attribute the role of ProTα as an intra-nuclear dissociator of the Nrf2-Keap1 complex that releases Nrf2 from inhibition and thus induces expression of the protective genes.

The Vartapetian Laboratory demonstrated nuclear-cytoplasmic shuttling of the Nrf2-Keap1 complex mediated by the nuclear export signal in Keap1. This unexpected finding added a new dimension to the molecular mechanisms underlying expression of oxidative stress-protecting genes. A role of ProTα in apoptosis was elucidated; Dr. Vartapetian's Laboratory demonstrated that ProTα is an anti-apoptotic protein conferring enhanced cell resistance to apoptosis-inducing signals. If however cellular defense is surmounted and caspase activation occurs, ProTα is subjected to caspase-3-mediated fragmentation resulting in the detachment of ProTα's nuclear localization signal. Such a truncation was shown to abrogate nuclear uptake of ProTα. Furthermore, caspase cleavage triggers externalization of ProTα from apoptotic cells. Studies demonstrate specific surface marking of apoptotic, but not of healthy, cells with ProTα, which might contribute to recognition and elimination of the dying cells.

Caspase-mediated protein fragmentation performs a pivotal role in the implementation of apoptosis. A bioinformatics approach for the identification of novel nuclear proteins subject to caspase cleavage has been designed. Several dozens of candidate animal proteins were identified, and random experimental confirmation of caspase fragmentation was performed. An unexpected outcome of this bioinformatics strategy was the identification of a candidate plant caspase target, the VirD2 protein. Of note, although programmed cell death in animal and plant kingdoms exhibits many common features, all previous attempts to identify plant caspases and their possible targets by other means were unsuccessful. By using the VirD2 protein as a target, Dr. Vartapetian's Laboratory detected, purified, and characterized an elusive plant caspase. Experiments provide evidence that in plants, like in animals, caspase is activated in the course of apoptosis, it has a substrate specificity similar to that of human caspase-3, and it is essential for the implementation of programmed cell death in plants. The anti-apoptotic properties of ProTα were analyzed in collaboration with Professor Ralf Pettersson's Group at the LICR Stockholm Branch.

The main focus of James R. Kerr Program-sponsored research in Dr. Sergei Nedospasov's Laboratory at the Belozersky Institute of Moscow State University is the discovery and evaluation of new SEREX antigens for colon, breast, ovarian, renal and lung cancers. Colon cancer has been the focus of study in the past year. A new antigen, TES-391, identified by the screening of a normal testis expression library with sera from colon cancer patients was molecularly characterized. Findings suggest that the cancer association is due to a splice variant of a transcript from a gene, homologous to the murine *hydin* gene, which is linked to congenital hydrocephalus in mice. The laboratory has established that human hydin is a true ortholog of mouse *hydin*, expressed in most tissues as a single 15 kb transcript. Tes-391 is a *hydin* splice variant with predominant expression in testis and elevated immunogenicity due to short unique C-terminal amino acid sequence. Colon cancer patient tissue samples and sera samples continue to accrue in the tissue bank created for the development of finding new antigens to be used as therapeutic vaccine targets for colon cancer patients.

Assay development has also been a focus of the laboratory in the past year. Patient sera are routinely tested in the laboratory for response to particular antigens. A new recombinant assay

has been developed using miniarrays. Preliminary results suggest that this method is more sensitive and more reproducible compared with the previous recombinant phage format. This observation may explain discrepancies in serology results between several groups utilizing SEREX technology. It is envisioned that the developed assay using miniarrays will be helpful for serological monitoring of patients during cancer vaccine trials.

Drs. Ivan Gout and Valeriy Filonenko of the Institute of Molecular Biology and Genetics (IMBG), of the National Academy of Science of Ukraine have collaborated in several projects involving therapeutic targets during 2004. The Group at IMBG has been actively involved in a multi-laboratory consortium, focusing on the molecular cloning of MX35, an antigen that is the focus of a monoclonal antibody with specificity for ovarian tumors. An ovarian cancer cell line library has been used to identify cDNA encoding MX35 using the MX35 monoclonal antibody reagent and one positive clone has been isolated.

A33, a therapeutic target in colon cancer, is being investigated in several laboratories throughout LICR, and IMBG has initiated a search for A33 binding partners using a yeast two-hybrid approach. Extensive screening of HeLa and mouse embryo libraries, with a bait construct with the C-terminal and the full length of A33, allowed the isolation of a panel of potential A33 binding partners. The specificity of interaction and functional importance of identified interactions is currently under investigation.

In collaborative studies with Dr. Marco Zago of Brazil, cDNA encoding the PRAME antigen was subcloned into a pET24a vector and expressed recombinant protein in BL21(DE3) cells as His-tagged fusion protein and purified by Ni-NTA chromatography. The purified peptide was used to generate a panel of monoclonal antibodies specific for PRAME. Specific IgG corresponding to two selected hybridomas were produced in sufficient quantities for immunohistochemical and biochemical studies. Immunohistochemical analysis is currently being performed by Dr. Achim Jungbluth at the LICR New York Branch on a large panel of human tissue. PRAME is a potential target for several different cancer types. Several reagents have been created in collaboration with Dr. Alexander Knuth (LICR Zürich Affiliate Center) for the analysis of NY-BR-1 and RAb38, cancer vaccine targets. Various fragments of the NY-BR-1 and Rab38 antigens have been expressed in bacterial systems. Affinity purified recombinant proteins were used to produce monoclonal antibodies, recognizing both antigens. Anti-NY-BR1 Mabs were found to be excellent tools for immunohistochemical studies and the analysis of Rab 38 Mabs is currently in progress. As part of the LICR Antibody Targeting Program the IMBG is working collaboratively with Dr Peter ten Dijke (LICR Amsterdam Affiliate Center) towards developing monoclonal antibody reagents against the cancer target ALK-1.

Dr. Wei-Feng Chen, from Peking University Health Science Center in Beijing, China, has been working on several major projects with the LICR. First and foremost is the planning of the first LICR sponsored clinical trial in China. An application has been submitted to the CSFDA (Chinese regulatory authority) for a Phase I clinical trial of NY-ESO-1b vaccine in hepatocellular carcinoma (HCC) patients. In vitro testing of the vaccine is ongoing.

A major area of interest at this center is tumor antigen targeting. A novel CT antigen, TSPY, was cloned and identified by cDNA microarray from HCC tissues. TSPY was selectively expressed in normal testis, in 50% (16/32) of well-and moderately differentiated HCC samples, in 16% (4/25) of poorly differentiated HCC samples, and in 5% (1/19) of renal cell cancer samples. TSPY protein was localized mainly in the cytoplasm and 6.6% (7/106) of HCC patients had anti-TSPY antibody response. The CT antigen CAGE was expressed in 39.4% (13/33), 73.3% (11/15) and 30.8% (4/13) of HCC, gastric cancer, and colorectal cancer patients, respectively. Seroreactivity to CAGE protein was detected in the range of 5-8 % in these cancer patients. Transgelin 2 was shown by cDNA microarray to be over-expressed in 69% of HCC, and thus may be a potential diagnostic marker for HCC.

Tumor antigen epitope identification was evaluated using tissue samples from an HCC patient. Using proteomic analysis, both MAGE-3 p271-279 and MAGE-1 p294-302 were identified by acid elution from HCC cells separated from a resected tissue sample of an HCC patient. The MAGE-3 p271-279/MHC tetramer was found to be positive (0.06%) in this patient; illustrating, that these two peptides can be naturally processed and presented on HCC cell surface. T cell responses to tumor antigens were measured in HCC patients. Three CT antigens were tested for elicitation of spontaneous CD8+T cell responses in HLA-A2+HCC patients with the HCC expressing respective CT antigens. The CD8+T cell response was 35.7% (10/28) to NY-ESO-1b; 30.8% (8/26) to MAGE-3 p271-279; and in two of four patients tested there was a response detected to HCA587. Additional testing is planned. One CT antigen HCA661 was demonstrated to elicit CD8+T cell response in healthy donors.

The biological activity of the CT antigen HCA661 was studied. The CT antigen HCA661 is a novel member of the TFDP transcription factor family and is designated TFDP-3. TFDP-3 interacts and forms heterodimer with E2Fs. However, the formed TFDP-3/E2Fs heterodimers failed to bind to the consensus E2F DNA recognition sequence and dramatically inhibited the endogenous E2F- as well as E2F/DP-1/-2-mediated transcriptional activation. Thus, TFDP3 functions as negative regulator of the activity of E2F/TFDP complexes.

The LICR Monoclonal Antibody Facility at the Fourth Military Medical University in Xi'an, China has expanded under the direction of Dr. Boquan Jin. In 2004, hundreds of fusions have been performed in order to produce stable hybridoma cell lines. Monoclonal antibodies have been generated to 45 different proteins or peptides. On average 11 clones are generated against each immunogen. Clones are routinely screened in Xi'an using ELISA and western blot analyses. In addition, the facility in Xi'an has expanded its screening capability. Selected clones can now be pre-screened on tissues before these reagents are forwarded to researchers for more extensive testing and characterization. A pathologist has been identified from the University who will work to pre-screen selected reagents. The Xi'an Laboratory works closely with individual researchers on collaborative projects initiated through the generation of monoclonal antibodies. An important aspect of the development of the LICR Antibody Targeting Program is the characterization of reagents as they are produced. This past year, Dr. Luciano Neder, an immunopathologist from Brazil, joined the James R. Kerr Program and began immunohistochemical analyses of LICR reagents. This will become a part of the development of new cancer therapies in the future.

The aim for the future is to continue to integrate the work of the James R. Kerr Program within the programmatic work of LICR,and to strengthen the growing links between the Program and the LICR Branches.

Publications

Primary Research Papers

1. Artamonova I.I., Gelfand M.S. Evolution of the exon-intron structure and alternative splicing of the MAGEA family of cancer/testis antigens. Journal of Molecular Evolution (2004). 69(5):620-631.
2. Bazhin A.V., Savchenko M.S., Belousov E.V., Jaques G., Philippov P.P. Stimulation of the aberrant expression of a paraneoplastic antigen, recoverin, in small cell lung cancer cell lines. Lung Cancer (2004) 45:299-305.

3. Bazhin AV, Savchenko MS, Shifrina ON, Demoura SA, Chikina SYu, Jaques G, Kogan EA, Chuchalin AG, Philippov PP. Recoverin as a paraneoplastic antigen in lung cancer: the occurrence of anti-recoverin autoantibodies in sera and recoverin in tumors. Lung Cancer (2004) 44:193-8.

4. Bergamaschi D., Samuels Y., Jin B., Duraisingham S., Crook T., Lu X. ASPP1 and ASPP2: Common activators of p53 family members. Molecular Cell Biology (2004) 24(3):1341-1350.

5. Chan R.C-F., Pang X-W., Wang Y-D., Chen W-F., Xie Y. Transduction of dendritic cells with recombinant adenovirus encoding 661 activates autologous cytotoxic T lymphocytes to target hepatoma cells. British Journal of Cancer (2004) 90(8):1636-1643.

6. Chen L., Liu X., Zhu Y., Cao Y., Sun L., Jin B. Localization and variation of TRAIL and its receptors in human placenta during gestation. Life Sciences (2004) 74:1479-1486.

7. Chichkova N.V., Kim S.H., Titova E.S., Kalkum M., Morozov V.S., Rubtsov Y.P., Kalinina N.O., Taliansky M.E., and Vartapetian A.B. A plant caspase-like protease activated during the hypersensitive response. Plant Cell (2004) 16:157-171.

8. Domnina L.V., Ivanova O.Y., Pletjushkina O.Y., Fetisova E.K., Chernyak B.V., Skulachev V.P., Vasiliev J.M. Marginal blebbing during the early stages of TNF-induced apoptosis indicates alteration in actomyosin contractility. Cell Biology International (2004) 28:471-475.

9. Dong X-Y., Li Y-Y., Yang X-A., Chen W-F. BJ-HCC-20 A Novel Member of the Human Cancer-Testis Antigens. Biochemistry and Cell Biology (2004) 82:577-582.

10. Dong X-Y., Pang X-W., Yu S-T., Su Y-R., Wang H-C., Yin Y-H, Wang Y-D., Chen W-F. Identification of Genes Differentially Expressed in Human Hepatocellular Carcinoma by a Modified Suppression Subtractive Hybridization. International Journal of Cancer (2004) 112:239-48.

11. Dong X-Y., Yang X-A., Wang Y-D., Chen W-F. Zinc-finger protein ZNF165 is a novel cancer-testis antigen capable of eliciting antibody response in hepatocellular carcinoma patients. British Journal of Cancer (2004) 91(8):1566-70.

12. Gorbenko O., Kuznetsov V., Kukharenko O., Zhyvoloup A., Panasyuk G., Nemazanyy I., Filonenko V. Gout I. Identification of novel binding partners for tumor suppressor PTEN by a yeast two-hybrid approach. Eksperimentalnaia Onkologiia (2004) 26:15-9.

13. Imanishi T., Itoh T., Suzuki Y., O'Donovan C., Fukuchi S., Koyanagi K.O., Barrero R.A., Tamura T., Yamaguchi-Kabata Y., Tanino M., Yura K., Miyazaki S., Ikeo K., Homma K., Kasprzyk A., Nishikawa T., Hirakawa M., Thierry-Mieg J., Thierry-Mieg D., Ashurst J., Jia L., Nakao M., Thomas M.A., Mulder N., Karavidopoulou Y., Jin L., Kim S., Yasuda T., Lenhard B., Eveno E., Suzuki Y., Yamasaki C., Takeda J., Gough C., Hilton P., Fujii Y., Sakai H., Tanaka S., Amid C., Bellgard M., Bonaldo F., Bono H., Bromberg S.K., Brookes A.J., Bruford E., Carninci P., Chelala C., Couillault C., de Souza S.J., Debily M.A., Devignes M.D., Dubchak I., Endo T., Estreicher A., Eyras E., Fukami-Kobayashi K., Gopinath G.R., Graudens E., Hahn Y., Han M., Han Z.G., Hanada K., Hanaoka H., Harada E., Hashimoto K., Hinz U., Hirai M., Hishiki T., Hopkinson I., Imbeaud S., Inoko H., Kanapin A., Kaneko Y., Kasukawa T., Kelso J., Kersey P., Kikuno R., Kimura K., Korn B., Kuryshev V., Makalowska I. Makino T., Mano S., Mariage-Samson R., Mashima J., Matsuda H., Mewes H.W., Minoshima S., Nagai K., Nagasaki H., Nagata N., Nigam R., Ogasawara O., Ohara O., Ohtsubo M., Okada N., Okido T., Oota S., Ota M., Ota T., Otsuki T., Piatier-Tonneau D., Poustka A., Ren S.X., Saitou N., Sakai K., Sakamoto S., Sakate R., Schupp I., Servant F., Sherry S., Shiba R., Shimizu

N., Shimoyama M., Simpson A.J., Soares B., Steward C., Suwa M., Suzuki M., Takahashi A., Tamiya G., Tanaka H., Taylor T., Terwilliger J.D., Unneberg P., Veeramachaneni V., Watanabe S., Wilming L., Yasuda N., Yoo H.S., Stodolsky M., Makalowski W., Go M., Nakai K., Takagi T., Kanehisa M., Sakaki Y., Quackenbush J., Okazaki Y., Hayashizaki Y., Hide W., Chakraborty R., Nishikawa K., Sugawara H., Tateno Y., Chen Z., Oishi M., Tonellato P., Apweiler R., Okubo K., Wagner L., Wiemann S., Strausberg .RL., Isogai T., Auffray C., Nomura N., Gojobori T., Sugano S. Integrative Annotation of 21,037 Human Genes Validated by Full-Length cDNA Clones. Public Library of Science Biology (2004) 6:e162.

14. Izyumov D.S., Avetisyan A.V., Pletjushkina O.Y., Sakharov D.V., Wirtz K.W., Chernyak B.V., Skulachev V.P. "Wages of fear": transient threefold decrease in intracellular ATP level imposes apoptosis. Biochimica et Biophysica Acta (2004) 1658, 141-147.

15. Kalinina O.V., Mironov A.A., Gelfand M.S., Rakhmaninova A.B.. Automated selection of positions determining functional specificity of proteins by comparative analysis of orthologous groups in protein families. Protein Science (2004) 13(2):443-456.

16. Kalinina O.V., Novichkov P.S., Mironov A.A., Gelfand M.S., Rakhmaninova A.B.. SDPpred: a tool for prediction of amino acid residues that determine differences in functional specificity of homologous proteins. Nucleic Acids Research (2004) 32(Web server issue): W424-W428.

17. Li B., He X-Y., Pang X-W, Zhang H-G., Chen J., Chen W-F. Elicitation of both CD4 and CD8 T-cell-mediated specific immune responses to HCA587 protein by autologous dendritic cells. Scandinavian Journal of Immunology (2004) 60(5):506-13.

18. Lyamzaev K.G., Pletjushkina O.Y., Saprunova V.B., Bakeeva L.E., Chernyak B.V., Skulachev V.P. Selective elimination of mitochondria from living cells induced by inhibitors of bioenergetic functions. Biochemical Society Transactions (2004) 32:1070-1071.

19. Lyzogubov V.V., Lytvyn D.I., Dudchenko T.M., Lubchenko N.V., Pogrybniy P.V., Nespryadko S.V., Vinnitska A.B., Usenko V.S, Gout I.T., Filonenko V.V. Immunohistochemical analysis of S6K1 and S6K2 expression in endometrial adenocarcinomas. Experimental Oncology (2004) 4:287-93.

20. Mironov A.A., Gelfand M.S.Prediction and Computer Analysis of the Exon–Intron Structure of Human Genes. Molecula Biology (2004) 38(1):70-77.

21. Nemazanyy I., Panasyuk G., Zhyvoloup A., Panayotou G., Gout I.T., Filonenko V. Specific interaction between S6K1 and CoA synthase: a potential link between the mTOR/S6K pathway, CoA biosynthesis and energy metabolism. FEBS Letters (2004) 578:357-62.

22. Nomura S., Baxter T., Yamaguchi H., Leys C., Vartapetian A.B., Fox J.G., Lee J.R., Wang T.C., and Goldenring J.R. Spasmolytic polypeptide expressing metaplasia to preneoplasia in H.felis-infected mice. Gastroenterology (2004) 127:582-594.

23. Offman M., Nurtdinov R.N., Gelfand M.S., Frishman D. No statistical support for correlation between the positions of protein interaction sites and alternatively spliced regions. BMC Bioinformatics (2004) 5(1):41.

24. Ouyang W., Xue J., Liu J., Jia W., Li Z., Xie X., Liu X., Jian J., Li Q., Zhu Y., Yang A., Jin B. Establishment of an ELISA system for determining soluble LAIR-1 levels in sera of patients with HFRS and kidney transplant. Journal of Immunological Methods (2004) 292:109-117.

25. Panasyuk G., Nemazanyy I., Filonenko V., Zhyvoloup A. Large-scale yeast transformation in low-percentage agarose medium. Biotechniques (2004) 36:40-2, 44.

26. Ralston K.J., Hird S.L., Zhang X., Scott J.L., Jin B., Thorne R.F., Berndt M.C., Boyd A.W., Burns G.F. The LFA-1-associated molecule PTA1 (CD226) on T cells forms a dynamic molecular complex with protein 4.1 G and human discs large. Journal of Biological Chemistry (2004) 279(32):33816-28.

27. Savinska LO, Lyzogubov VV, Usenko VS, Ovcharenko GV, Gorbenko ON, Rodnin MV, Vudmaska MI, Pogribniy PV, Kyyamova RG, Panasyuk GG, Nemazanyy IO, Malets MS, Palchevskyy SS, Gout IT, Filonenko VV. Immunohistochemical analysis of S6K1 and S6K2 expression in human breast tumors. Eksperimentalnaia Onkologiia (2004) 26:24-30.

28. Senin I.I., Höppner-Heitmann D., Polkovnikova O.O., Churumova V.A., Tikhomirova N.K., Philippov P.P., Koch K-W. Recoverin and rhodopsin kinase activity in detergent-resistant membrane rafts from rod outer segments. Journal of Biological Chemistry (2004) 47:48647-53.

29. Shang X-Y., Chen H-S., Zhang H-G., Pang X-W., Qiao H., Peng J-R., Qin L-L., Fei R. , Mei M-H, Leng X-S., Gnjatic S., Ritter G., Simpson A.J.G., Old L.J., Wei-Feng Chen W-F. The Spontaneous CD8+ T Cell Response to HLA-A2 Restricted NY-ESO-1b peptide in Hepatocellular Carcinoma Patients. Clinical Cancer Research (2004) 10:6946-55.

30. Skulachev, V.P., Bakeeva L.E., Chernyak B.V., Domnina L.V., Minin A.A., Pletjushkina O.Y., Saprunova V.B., Skulachev I.V., Tsyplenkova V.G., Vasiliev J.M., Yaguzhinsky L.S., Zorov D.B. Thread-grain transition of mitochondrial reticulum as a step of mitoptosis and apoptosis. Molecular and Cellular Biochemistry (2004) 256/257, 341-358.

31. Slee, E.A., Gillotin S., Bergamaschi D., Royer C., Llanos S., Ali S., Jin B., Trigiante G., Lu X. The N-terminus of a novel isoform of human iASPP is required for its cytoplasmic localisation. Oncogene (2004) 23(56):9007-16.

32. Song C.J., Liu X., Zhu Y., Chen L., Jia W., Li Y., Cao Y., Xie X., Zhuang R., Zhu C., Jin B. Expression of TRAIL, DR4, and DR5 in Kidney and Serum From Patients Receiving Renal Transplantation. Transplantation Proceedings (2004) 36:1340-1343.

33. Wang H-C., Su Y-R., Han K-J., Pang X-W., Peng J-R., Liang B., Wang S., and Chen W-F. Multiple variants and a differential splicing pattern of kinectin in human hepatocellular carcinoma. Biochemistry and Cell Biology (2004) 82(2):321-327.

34. Xu, Y.M., Wang L-F., Jia L-T., Qiu X-C., Zhao J., Yu C-J., Zhang R., Zhu F., Wang C-J., Jin B., Chen S-Y., Yang A-G. A Caspase-6 and Anti-Human Epidermal Growth Factor Receptor-2 (HER2) Antibody Chimeric Molecule Suppresses the Growth of HER2-Overexpressing Tumors. The Journal of Immunology (2004) 173:61-67.

35. Yang C.H., Murti A., Baker S., Frangou-Lazaridis M., Vartapetian A.B., Murti K.G., Pfeffer L.M. Interferon induces the interaction of prothymosin α with STAT3 and results in the nuclear translocation of the complex. Experimental Cell Research (2004) 298:197-206.

36. Yang X-A., Dong X-Y., Li Y., Wang Y-D., Chen W-F. Purification and refolding of novel cancer/testis antigen BJ-HCC-2 expressed in the inclusion bodies of Escherichia coli. Protein Expression and Purification. (2004) 33(2):332-338.

Reviews / Commentaries / Book Chapters

1. Gelfand M.S., Kriventseva E.V. Alternative splicing: conservation and function. In: Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics (Wiley). Volume 2 "Genomics" (P.F.R.Little, volume ed.). Section 3 "The Human Genome" (R.K.Wilson, section ed.)
2. Gelfand M.S. Computational analysis of alternative splicing. In: Handbook on Computational Molecular Biology (CRC Press). S.Alluru, ed.; S.Sahni (series ed.)
3. Neverov A.D., Mironov A.A., Gelfand M.S. Similarity-based gene recognition. In: Handbook on Computational Molecular Biology (CRC Press). S.Alluru, ed.; S.Sahni (series ed.)

LUDWIG INSTITUTE FOR CANCER RESEARCH

Clinical Trials Program

Office of Clinical Trials Management

Director's Report

Affiliates

Cancer Vaccines

Targeted Antibodies

Directors Report

The Office of Clinical Trials Management, which celebrated its eighth birthday in 2004, is currently managing 42 active protocols (see following pages), and a further 31 protocols that are closed for patient accrual but are still being monitored or awaiting final reports. Efforts are well underway to reduce this workload by closing trials, and identifying trials that can be truncated in terms of closure/data management, analysis, and writing of study reports and manuscripts.

The Melbourne Biological Production Facility (see Melbourne Branch report) began a limited upgrade to increase its versatility. It remains a vital link for the clinical trials program as a documentation depot with full responsibility for storage of clinical trial materials, stability testing, and worldwide distribution. The development in reagent production at the LICR Bioprocess Production Facility at Cornell University has been promising (see report by Dr. Carl Batt, Ithaca Affiliate Center), and the Facility should provide the Institute with GMP NY-ESO-1 protein, by Q3, 2005.

Notable milestones during 2004 included:

1. Completion of Ludwig Electronic Advancement Program (LEAP) implementation. Thus far, study data is being entered remotely into the data base for 15 clinical studies. The data base has already demonstrated its utility by supplying data from ongoing trials to investigators preparing for interim assessments, presentations; etc.;
2. Four abstracts were accepted for presentation at the 2004 American Society of Clinical Oncology annual meeting;
3. The first patients were accrued into a U.S. NY-ESO-1 protocol using vaccinia and fowlpox vectors (in collaboration with Therion Biologics);
4. The first patients were accrued into a U.S. NY-ESO-1 protocol of NY-ESO-1 DNA (in collaboration with PowderMed);
5. The first LICR positron emission tomography (PET) imaging clinical protocol using ^{124}I-huA33 was initiated at Memorial Sloan Kettering Cancer Center;
6. LICR approved a randomized protocol entitled, *"Randomized, double-blind phase II trial of NY-ESO-1 ISCOMATRIXR® vaccine and ISCOMATRIX® adjuvant alone in patients with resected stage IIc, III or IV malignant melanoma"*. This study will be conducted at 19 sites in Australia, New Zealand, and the United Kingdom;
7. A trial of the ch806 monoclonal antibody, which has been shown to target epidermal growth factor receptor (EGFR) over-expression was initiated;
8. Clinical project management was decentralized with three clinical project managers each being physically located in Australia, Europe, and the USA;
10. A collaborative agreement between LICR and the European Institute of Oncology in Milan was executed.

Eric W. Hoffman

Kunle Odunsi, M.D., Ph.D.

Roswell Park Cancer Institute
Buffalo, New York, USA

Research Report

These research experiments continued to be performed in conjunction with the New York Branch of the Ludwig Institute for Cancer Research as part of the LICR Cancer Vaccine Program

1. Prognostic significance of tumor infiltrating lymphocytes in ovarian cancer

We performed immunohistochemical analysis for $CD3^+$, $CD4^+$, $CD8^+$, $CD20^+$ tumor infiltrating lymphocytes (TIL) in 117 epithelial ovarian cancer cases (EOC). The inter-relationship between subpopulations of TIL and clinical outcome was investigated. We found that patients with higher frequencies of intraepithelial $CD8^+$ T cell had a median survival of 55 months while patients with the lowest frequency had a median survival of 26 months (Hazard ratio = 0.36, CI 0.20-0.65, p=0.001). In addition, the subgroup with the highest intraepithelial $CD8^+/CD4^+$ TIL ratio showed a significantly improved median survival of 74 months compared with 25 months for the remaining patients (Hazard ratio = 0.31; CI 0.16-0.60; p=0.001). These results indicate that $CD4^+$ TIL influence the prognostic significance of $CD8^+$ TIL, possibly relating to $CD4^+CD25^+$ regulatory T cells (Tregs). We are currently testing the mechanisms by which Tregs interact with antigen-specific T cells in the ovarian tumor microenvironment.

2. Assessment of spontaneous CD4+ Th1/Th2 response to NY ESO-1 in ovarian cancer patients

The NY-ESO-1 antigen is expressed in a significant proportion of patients with EOC and appears to be an ideal target for immunotherapy. In order to elucidate the nature of the HLA-DPB1*0401/0402 ($DP4^+$) restricted $CD4^+$ immune response in patients with NY-ESO-1 expressing EOC, peripheral blood $CD4^+$ T cells from HLA-DP4+ patients were stimulated with the NY-ESO-1 epitope 157-170 and tested for the release of type 1 (IFN-gamma) and type 2 (IL-5) cytokines in enzyme-linked immunospot assays. Among 14 $DP4^+$ EOC patients that tested seronegative for NY-ESO-1, three patients had detectable $CD4^+$ T cell response to NY-ESO-1 epitope 157-170 by IFN-gamma ELISPOT assay. In contrast, 6 of 10 $DP4^+$ EOC patients with serum antibodies to NY-ESO-1 had $CD4^+$ T cell responses to NY-ESO-1 epitope 157-170 by IFN-gamma assay. Overall, six patients had mixed Th1/Th2 $CD4^+$ T cell responses to NY-ESO-1 epitope 157-170, regardless their antibody response to NY-ESO-1. The remaining four EOC patients had Th1 cells expressing IFN-gamma, but not IL-5. These results suggest that the NY-ESO-1 epitope 157-170 stimulates both Th1- and Th2-type $CD4^+$ T cell responses in EOC patients and support the relevance of cancer vaccine trials with the NY-ESO-1 epitope 157-170 in HLA-$DP4^+$ EOC patients with NY-ESO-1 - expressing tumors.

3. Expression analysis of additional CT antigens in epithelial ovarian cancer

In an effort to identify additional candidates for antigen-specific immunotherapy of EOC, we have undertaken a comprehensive analysis of 22 CT antigens. These studies were conducted using RT-PCR and/or immunohistochemistry. The results indicate a variable frequency of expression of the CT antigens in EOC. The CT antigens that are co-expressed with NY-ESO-1 could be potential candidates for multi-antigen vaccine strategies.

4. ^{1}H-NMR-based metabonomics for the detection of epithelial ovarian cancer

Metabonomics, the study of metabolic processes in biological systems, is based on the use of ^{1}H-NMR spectroscopy and multivariate statistics for biochemical data generation and interpretation, and may provide a characteristic fingerprint in disease. In an effort to examine the utility of the metabonomic approach for the identification of additional targets in EOC, we

performed ^{1}H-NMR spectroscopic analysis on pre-operative serum specimens obtained from 38 patients with EOC, 12 patients with benign ovarian cysts and 53 healthy women. Following data reduction, we applied both unsupervised Principal Component Analysis (PCA) and supervised Soft Independent Modeling of Class Analogy (SIMCA) for pattern recognition. The sensitivity and specificity trade-offs were summarized for each variable using the area under the receiver-operating characteristic (ROC) curve. In addition, we analyzed the regions of NMR spectra that most strongly influence separation of sera of EOC patients from healthy controls. PCA analysis allowed correct separation of all serum specimens from 38 patients with EOC (100%) from all of the 21 pre-menopausal normal samples (100%), and from all the sera from patients with benign ovarian disease (100%). In addition, it was possible to correctly separate 37 of 38 (97.4%) cancer specimens from 31 of 32 (97%) postmenopausal control sera. SIMCA analysis using the Cooman's plot demonstrated that sera classes from patients with EOC, benign ovarian cysts and the postmenopausal healthy controls did not share multivariate space, providing validation for the class separation. ROC analysis indicated that the sera from patients with and without disease could be identified with 100% sensitivity and specificity at the ^{1}H-NMR regions 2.77ppm and 2.04ppm from the origin (AUC of ROC curve=1.0). In addition, the regression coefficients most influential for the EOC samples compared with postmenopausal controls lie around δ3.7ppm (due mainly to sugar hydrogens). Other loadings most influential for the EOC samples lie around δ2.25ppm and δ1.18ppm. These findings indicate that ^{1}H-NMR metabonomic analysis of serum achieves complete separation of EOC patients from healthy controls. The metabonomic approach deserves further evaluation as a potential novel strategy for the early detection of EOC, and as a method for identifying targets for therapeutic intervention.

5. Clinical Trials Activity

Two clinical immunotherapy studies at Roswell Park Cancer Institute recruited patients during the year 2004.

LUD02-011: *A pilot clinical trial of NY-ESO-1DP4 p157-170 (NY-ESO-1DP4), a peptide of potentially dual MHC class I and class II specificities, in patients with epithelial ovarian, fallopian tube or primary peritoneal carcinoma whose tumors express NY-ESO-1 or LAGE-1*

The objectives of this study were: (i) To evaluate the safety of NY-ESO-1DP4 when given concurrently with Montanide ISA™51 (Incomplete Freund's Adjuvant) in patients with NY-ESO-1 or LAGE-1 expressing epithelial ovarian, fallopian tube or primary peritoneal carcinoma; (ii) To determine whether vaccination with NY-ESO-1DP4 peptide and Montanide ISA™51 induces or augments a NY-ESO-1 specific immune response in patients with epithelial ovarian, fallopian tube or primary peritoneal carcinoma with NY-ESO-1 or LAGE-1 expressing tumors.

Eighteen HLA-DP4-positive EOC patients who had no evidence of disease, or only minimal residual disease, following completion of chemotherapy for primary or recurrent disease were enrolled. Tumor expression of NY-ESO-1 was determined by RT-PCR and immunohistochemistry. Patients were immunized with 100 μg of ESO:157-170, mixed with Montanide, every three weeks for a minimum of five injections, or up to 15 injections, or until disease progression. Immune responses were measured by delayed-type hypersensitivity (DTH) test, ELISA, HLA-A2 and A24 tetramers, and interferon γ (IFN-γ) ELISPOT assays. 16/18 patients were NY-ESO-1 serum antibody negative, and 2/18 patients were NY-ESO-1 serum antibody positive at the outset of the study. So far, 12 patients have been analyzed for immune responses. Primary peptide-specific $CD4^{+}$ IFN-γ T-cell reactivity was generated in eight out of ten (80%) NY-ESO-1 antibody-negative patients. DTH responses were generated in three of the eight patients, and antibody response in one patient. In one patient, induction of a specific CD4+ T-cell response to NY-ESO-1 peptide immunization was associated with complete regression of a single metastasis. One of the two NY-ESO-1 antibody-positive patients developed a significant increase NY-ESO-1-specific $CD4^{+}$T-

cell reactivity. Two of nine (22%) HLA-A2 and/or HLA-A24 patients demonstrated simultaneous peptide specific $CD8^+$ T cell reactivity. Our results indicate that primary NY-ESO-1-specific antibody, $CD4^+$ and $CD8^+$ T-cell responses can be induced by immunization with the NY-ESO-1 epitope 157-170. The peptide vaccine was found to be safe and well tolerated. A phase II study design utilizing this epitope, or the full length protein, is warranted in patients with EOC.

LUD02-012: *Phase II study of recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1) in patients with epithelial ovarian, fallopian tube or primary peritoneal carcinoma whose tumors express NY-ESO-1 or LAGE-1 antigen*

The objectives of this study were: (i) To establish that the time to failure (ttf) for the proposed therapy is greater than the ttf for standard therapy. The ttf is defined to be the time from when a patient is declared to be in remission until it is necessary to initiate additional hormonal or cytotoxic chemotherapy; (ii) To evaluate NY-ESO-1 specific cellular and humoral immunity by determination of NY-ESO-1 specific antibody and CD8+ T cells induced by rV-NY-ESO-1 and rF-NY-ESO-1 after sequential administration to patients with NY-ESO-1 or LAGE-1 expressing epithelial ovarian, fallopian tube or primary peritoneal carcinoma; (iii) To assess the degree of the association between ttf and rV-NY-ESO-1 and rF-NY-ESO-1 induced NY-ESO-1 antigen specific cellular immunity; (iv) To assess the degree of the association between ttf and rV-NY-ESO-1 and rF-NY-ESO-1 induced NY-ESO-1 antigen specific humoral immunity; (v) To determine the safety of rV-NY-ESO-1 and rF-NY-ESO-1 in patients with NY-ESO-1 or LAGE-1 expressing epithelial ovarian, fallopian tube or primary peritoneal carcinoma.

Twenty-two (22) patients will receive one intradermal injection of rV-NY-ESO-1, 3.1 x 107 plaque-forming units (PFU), followed by monthly subcutaneous injections of rF-NY-ESO-1, 7.41 x 107 PFU for six months. Doses will be administered during a 28-week evaluation period. Toxicity and immunological assessments will be made after each dose. So far, three patients have been enrolled on this trial. No adverse events related to the vaccine have been noted, and the assessment of immune responses has not yet been performed.

Publications

Primary Research Articles

1. Tammela J., Jungbluth A.A., Qian F., Santiago D., Scanlan M., Keitz B., Driscoll D., Rodabaugh K., Lele S., Old L.J., Odunsi K. SCP-1 "Cancer/Testis" antigen is a prognostic indicator and a candidate target for immunotherapy in epithelial ovarian cancer. Cancer Immunity (2004) 4:10.
2. Qian F., Gnjatic S., Jager E., Santiago D., Jungbluth A., Grande C., Schneider S., Keitz B., Ritter G., Lele S., Sood A., Old L.J., Odunsi K. T Helper Type1 (Th1)/Th2 $CD4^+$ T cell responses against NY-ESO-1 in HLA-DPB1*0401/0402 patients with epithelial ovarian cancer. Cancer Immunity (2004) 4:12.

Elke Jäger, M.D.

Krankenhaus Nordwest
Frankfurt, Germany

Research Report

1. Identification of new NY-BR-1 epitopes as targets for CD4 and CD8 T cell responses in cancer patients

NY-BR-1 specific CD4 and CD8 T cell responses were assessed using synthetic NY-BR-1 18-mer peptides covering amino acids (aa) 1004 – 1397 of the entire NY-BR-1 gene product. This region of the NY-BR-1 protein was chosen because it was predicted, by several software programs used for the analysis of peptide binding motifs, to contain the highest rate of potential MHC class I and class II binding epitopes. Thirty-nine NY-BR-1 18-mer peptides that overlap by eight amino acids were synthesized.

Twenty-three patients and one healthy individual were chosen for the assessment of NY-BR-1 specific CD4 and CD8 T cell reactivity. Twenty patients had NY-BR-1 positive cancers (18 breast and two prostate). CD4 and CD8 T cells were separated from peripheral blood lymphocytes (PBL) and used for stimulation assays. T cells were presensitized using the whole panel of 39 NY-BR-1 peptides in separate wells, or by pools containing five NY-BR-1 peptides in each well. After five to six days of presensitization, CD4 or CD8 T cells were tested in ELISPOT assays to determine the effector reactivity against the whole panel of 39 single NY-BR-1 peptides, which were loaded onto autologous EBV-B cells as antigen-presenting cells (APC). In case CD4 or CD8 T cells reactive with specific NY-BR-1 peptides were detected, ELISPOT assays were repeated using single peptides to confirm the specificity of the reaction at the level of presensitization and target recognition.

CD4 T cell responses were detected in eight patients with NY-BR-1 positive cancers (seven breast and one prostate). CD4 T cells from all eight patients reacted with a total of 12 NY-BR-1 18-mer peptides in ELISPOT assays. The results were confirmed in at least one independent experiment. One of these eight patients also showed CD8 T cell reactivity against four different NY-BR-1 peptides. CD4 T cell reactivity was also detected in one healthy individual, who is NY-BR-1 antibody negative and does not show NY-BR-1 specific CD8 T cell reactivity. We established CD4 T cell clones of two patients which were specific for two different peptides. The restriction elements for these peptides are human leukocyte antigen (HLA)-DQ B1 05 and HLA-DR B1 13, respectively. Restriction analysis was done by use of allogeneic EBV as APC in ELISPOT assays and antibody blocking tests.

CD8 T cell responses were generally difficult to detect following the approach described above. It is assumed that presensitization with 18-mer peptides has low efficacy due to the length of the reagent that probably provides multiple competing binding sites to different MHC class I and class II alleles of the APC. Nevertheless, CD8 T cell reactivity was detected in two patients, against five different 18-mer NY-BR-1 peptides. The analysis of the MHC class I restriction elements is ongoing using allogeneic EBV-B cell lines which share only one single MHC class I allele with the peptide-reactive effector T cells as APC. One 18-mer peptide that stimulated CD8 T cells of one patient was divided into 9-mer peptides overlapping by eight amino acids. CD8 T cells of 16 NY-BR-1 positive patients were presensitized with these peptides and tested in ELISPOT assays. Five of these showed reactivity for one 9-mer peptide. Since all five patients are HLA A2 positive and the peptide is recognized when presented on T2 cells, the restriction element for this peptide is probably HLA A2.

Once the MHC classI/II restriction elements have been identified, NY-BR-1 peptide-reactive CD4 and CD8 T cells will be tested for recognition of -HLA)--matched NY-BR-1 positive tumor cell lines. The Group recently succeeded in establishing two new breast cancer cell lines that will be evaluated in this context. Based on the findings of NY-BR-1 expression in ovarian cancer, it may be possible to analyze a much larger panel of NY-BR-1+ tumor cell lines in the near future, because ovarian cancer is significantly easier to culture.

2. Identification of new NY-ESO-1 epitopes as targets for CD4 and CD8 T cell responses in patients pre and post NY-ESO-1–specific vaccination

Patients with NY-ESO-1 positive cancer were immunized with synthetic NY-ESO-1 peptides p157-167 and p157-165 (LUD2000-009), and/or with recombinant NY-ESO-1 vaccinia- or fowlpox viral constructs (LUD2000-014) to generate NY-ESO-1 peptide-specific CD8 T cell responses (LUD2000-009) or NY-ESO-1-specific CD4 and CD8 T cell responses (LUD2000-014) *in vivo*. Since the studies are ongoing, this is an incomplete reporting on new NY-ESO-1 epitopes found to be recognized by post-vaccine CD4 or CD8 T cells. Non-vaccinated patients with detectable NY-ESO-1 antibody were included in the analysis.

CD4 T cell reactivity was assessed using NY-ESO-1 overlapping 18-mer peptides spanning the entire NY-ESO-1 gene product (total number of synthetic peptides was 28). CD4 effector T cells were presensitized for 10-12 days with each peptide in single wells and tested for recognition of the stimulating peptide in ELISPOT assays using autologous EBV-B-cells or PHA-blasts as APC. Post-vaccine CD4 T cell responses have been evaluated in eight patients so far. Four of these had detectable CD4 T cell responses against 16/28 NY-ESO-1 peptides. Three of 16 peptides represent known target epitopes for CD4 T cell recognition in the context of HLA-DRB4*-0101-0104, but13 peptides represent new target epitopes. Using a set of partially histocompatible EBV-B-cell lines and MHC class II specific antibodies, we identified two new NY-ESO-1 CD4 epitopes (p49-66 and p55-72) presented by HLA-DQ B1 03011 (DQ7). We demonstrated that these new epitopes were naturally processed and presented by recognition of dentritic cells exogenously loaded with NY-ESO-1 protein or infected with recombinant NY-ESO-1 adenoviral construct and by recognition of a HLA-DQ B1 03011/NY-ESO-1 expressing lymphoma cell line. NY-ESO-1p49-66 was found in three patients, p55-72 was found in two patients. Studies to identify more MHC class II restriction elements are ongoing using allogeneic EBV-B cell lines matched for 1 single MHC class II allele with effector cells as APC in ELISPOT assays. Once the MHC restriction has been defined for each epitope, effector CD4 T cells will be tested for recognition of MHC class II matched NY-ESO-1 positive tumor cell lines.

NY-ESO-1 specific CD8 T cell responses in non HLA A2 patients were assessed after presensitization of effector cells with recombinant adeno-ESO viral constructs against a panel of synthetic NY-ESO-1 overlapping 18-mer peptides separately pulsed onto autologous dentritic cells, EBV-B-cells or PHA blasts as APC. One new NY-ESO-1 epitope (p55-72) was identified that is probably presented by HLA-A3 (confirmatory studies are ongoing). NY-ESO-1 p55-72 was recognized by CD8 T cells of four different patients with detectable NY-ESO-1 serum antibody. NY-ESO-1 epitope p91-110 was found in five patients after vaccination with recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1). Studies to identify the MHC class I restriction element for this epitope are ongoing using allogeneic EBV-B cell lines matched for 1 single MHC class I allele as APC in ELISPOT assays. To further identify the exact epitope in the sequence of p91-110 necessary for recognition by CD8+ T cells, we will synthesize and use 9-mer/10-mer peptides pulsed on autologous APC in ELISPOT.

3. Clinical Trials Center Activity

Six different clinical immunotherapy studies being performed at Krankenhaus Nordwest recruited patients during the year 2004.

LUD2000-009: *Phase 1 study of intensive course immunization with NY-ESO-1 derived peptides presented by MHC class I molecules alone and in combination with GM-CSF in patients with advanced NY-ESO-1 or LAGE expressing tumors*

Twenty patients with different types of cancer have been entered in this study; nine patients were NY-ESO-1 antibody negative, 11 were NY-ESO-1 antibody positive. Nine patients have completed three cycles of immunization, 11 discontinued the treatment because of rapid tumor progression or at their own discretion. Five patients achieved disease stabilization over a 3-12 month period. In two patients, no evidence of disease is observed for 26+ and 33+ months. There was no significant toxicity observed. NY-ESO-1 specific T cell responses of high magnitude and early onset were observed in the majority of patients who completed three cycles of immunization. Peptide specific DTH reactions of the inflammatory type correlated with high magnitude CD8+ T cell responses detectable in the peripheral blood. The rapid induction of CD8+ T cell responses is believed to be of therapeutic significance, potentially preventing the development of immune escape mechanisms in vivo. Spontaneous NY-ESO-1b specific CD8+ T cells were found in 4/6 evaluable NY-ESO-1 seropositive patients. There was a loss of NY-ESO-1 antibody in one patient. No detectable NY-ESO-1b specific CD8+ T cells were induced in 3 NY-ESO-1 seronegative patients who received NY-ESO-1b only.

LUD2000-014: *Phase 1 study of recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1) in patients with NY-ESO-1 positive cancers*

Thirty-five patients with different types of cancer have been entered in this study, 24 patients have completed at least four immunizations, and 11 patients have discontinued treatment earlier for rapid disease progression or on their own discretion.

Patients were assigned to five Cohorts: In part 1, vaccination was administered 4x at four week intervals. In Cohort 1, patients received $3.1x10^7$ plaque forming units (PFU) rV-NY-ESO-1 intradermally (id), and in Cohort 3 patients received $3.1x10^8$ PFU rV-NY-ESO-1. In Cohort 2, patients received $7.41x10^7$ PFU rF-NY-ESO-1 subcutaneously, and in Cohort 4 patients received rF-NY-ESO-1 $7.41x10^8$ PFU. Patients enrolled in part 2 received two injections of $3.1x10^7$ rV-NY-ESO-1 PFU id, followed by multiple injections of $7.41x10^7$ PFU rF-NY-ESO-1 at monthly intervals in case there was no evidence of disease progression during vaccination.

There was no significant toxicity other than skin reactions at the immunization sites observed. Twenty-one patients were HLA-A2 positive, and T cell responses were monitored against the HLA-A2 restricted NY-ESO-1 peptides p157-165 and 157-167. The majority of HLA-A2 positive patients who completed at least four immunizations have developed NY-ESO-1 specific CD8+ T cell responses within two to four months after the start of immunization. Five of 30 NY-ESO-1 antibody negative patients showed a seroconversion toward a detectable NY-ESO-1 serum antibody during vaccination. One patient developed a partial remission of subcutaneous and peritoneal melanoma metastases during the first four months of immunization, lasting for 18+ months, along with a strong NY-ESO-1 specific CD8+ T cell response. One minor response over a 12 month period was observed. Five patients achieved disease stabilization with 3-17+ months until disease progression. No evidence of disease was seen in five patients. Ten patients had progressive disease. One patient is too early to evaluate.

Vaccination with rV- and rF-NY-ESO-1 is safe at both dose levels. NY-ESO-1 seroconversion was observed in 5/30 NY-ESO-1 seronegative patients. In single patients, DTH reactions were observed, but a dose dependency remains to be determined. NY-ESO-1b specific CD8+ T cell responses were induced in 10/18 NY-ESO-1 seronegative, HLA A2+ patients and in 6/10 HLA A2- patients. NY-ESO-1 specific CD4+ T cells were induced in 9/28 patients.

These finding suggest that immunization with recombinant NY-ESO-1 vaccinia and fowlpox constructs can induce NY-ESO-1 specific humoral and cellular immune responses in vivo that may have therapeutic effects in patients with NY-ESO-1 expressing cancer.

LUD2000-026: *Phase 1 study of immunization with NY-ESO-1 derived peptides presented by MHC class I molecules alone or combined with Poly Arginine in patients with advanced NY-ESO-1 or LAGE expressing tumors*

Nine HLA-A2 positive patients with different types of cancer have been entered on this protocol. Four patients received polyarginine as an adjuvant. There was no significant toxicity observed. Patients who received polyarginine did not develop stronger skin reactions or immune responses to the vaccine. Three patients are continuing the protocol for extended progression-free intervals during immunization.CD8+ T cell responses against the immunizing peptides were observed in five patients that correlated with detectable DTH reactions at the sites of peptide inoculation. The best clinical response observed in this study is stabilization of disease of extended duration and no evidence of disease over a 12+ - 29+months period in four patients.

LUD2001-014: *Phase 1/2 study of chimeric monoclonal antibody G250 (cG250) in combination with vinblastine administered weekly by intravenous infusion to patients with advanced renal cell carcinoma*

Twenty-six patients with advanced renal cell carcinoma progressive before study entry have been included in this protocol in Frankfurt. Twenty-two patients have completed at least one cycle consisting of eight weekly treatments. No cG250 antibody-related toxicity was observed in this study. There was no indication of HACA development in any of the patients treated. The best clinical response observed is partial response in two patients, minor response in one patient, with extended progression-free intervals observed in these patients of 17.6, 19.7 and 20.9 months, respectively. The immunological monitoring of this study will include the assessment of G250 specific CD1d-restricted T cell responses in collaboration with Drs. Gerd Ritter (LICR New York Branch) and Vincenzo Cerundolo (Oxford Affiliate Center, UK).

LUD2002-007: *Phase 1/2 study of NY-ESO-1b peptide plus CpG 7909 and Montanide®ISA-51 in patients with cancer expressing NY-ESO-1 or LAGE-1.*

Nine HLA A2+ patients with advanced NY-ESO-1 or LAGE positive tumors or NY-ESO-1 seropositivity have been included on this protocol since June 2004. Three patients were withdrawn before completion of four vaccinations because of rapid disease progression. Three patients are still on protocol: Two patients achieved disease stabilization and one patient has no evidence of disease. One patient had disease progression. Two patients are too early to evaluate. Five of nine patients experienced fever and flu like symptoms CTC grade 1. Eight of nine patients had injection site reactions of 8-10 cm reddening in diameters, local pruritus CTC grade 1, local pain CTC grade 1 and local swelling CTC grade 1.

Vaccination with NY-ESO-1b peptide combined with CpG 7909 and Montanide is safe. Moderate side effects are most likely related to the adjuvants. DTH reactions have rarely been observed in one patient. NY-ESO-1 specific CD8+ T cell responses were seen in 2/4 NY-ESO-1 seronegative and in 1/1 seropositive patients. The antibody status of initially 3 AB+ patients and 6 AB- patients remained unchanged.

LUD2003-024 *Phase 1 study of immunization with NY-ESO-1 protein combined with CpG 7909 in patients with high-risk stage D1 or advanced prostate cancer*

Seven patients with advanced or resected high risk prostate cancer have been included on this protocol in Frankfurt. One patient was withdrawn after two vaccinations for disease progression. One patient achieved disease stabilization after 4 immunizations and remains on study. Five

patients are too early to evaluate. Three of nine patients were HLA A2 positive and all patients were HLA DP4 positive. All patients were NY-ESO-1 seronegative at baseline. In 6/7 patients, fever and flu like symptoms CTC grade 1 were observed. In all patients reddening of 20cm in diameters, local pruritus, pain and swelling CTC grade 2 was seen at the injection site. DTH Testing of NY-ESO-1b peptide was negative in 3/3 patients. One of seven patients had a positive DTH to NY-ESO-DP4 peptide on week 10. Vaccination with NY-ESO-1 protein combined with CpG 7909 is safe. Moderate side effects are most likely related to CpG. NY-ESO-1 seroconversion was observed in 6/6 patients. As far as this can be evaluated at this point of the study, NY-ESO-1 specific CD8+ T cell responses could not be found in any of the three HLA A2+ patients tested. Decrease in prostate specific antigen (PSA) levels during vaccination was seen in 3/3 evaluable patients.

Publications

Primary Research Articles

1. Al-Batran S.E., Atmaca A., Hegewisch-Becker S., Jäger D., Hahnfeld S., Rummel M.J., Seipelt G., Rost A., Orth J., Knuth A., Jäger E. Phase II trial of biweekly infusional fluorouracil, folinic acid, and oxaliplatin in patients with advanced gastric cancer. Journal of Clinical Oncology (2004) 22:658-63.
2. Al-Batran S.E., Atmaca A., Bert F., Jager D., Frisch C., Neumann A., Orth J., Knuth A. Jäger E. Dose escalation study for defining the maximum tolerated dose of continuous oral trofosfamide in pretreated patients with metastatic lung cancer. Onkologie (2004) 27(6):534-8.
3. Hartmann J.T., Oechsle K., Jäger E., Reis H.E., Haag C., Niederle N., Wilke H.J., Pfluger K.H., Al-Batran S.A., Buchele T., Hofheinz R.D., Kanz L., Bokemeyer C. Prospective multicenter phase II study of irinotecan as third-line therapy in metastatic colorectal cancer and progression after bolus and infusional 5-fluorouracil. Anticancer Drugs (2004) 15:473-477.

Carl Batt, Ph.D.

Cornell University
Ithaca, USA

Research Report

In 2003, the Cornell University/Ludwig Institute for Cancer Research Partnership inaugurated a 1200 square foot clean room complete with all of the necessary equipment and infrastructure for the Good Manufacturing Practice (GMP) production of recombinant proteins. Two primary expression systems have been pursued almost in parallel, *Escherichia coli* and *Pichia pastoris*. Each offers its own advantages and challenges. Recently, another expression system based on non-pathogenic *Salmonella typhimurium* has been added. This strain was engineered by Dr. Jorge Galan (Yale University School of Medicine) to express significant amounts of recombinant protein as secreted fusion proteins. To support the GMP effort, a quality control laboratory has been established to ensure that the product is consistent by a number of analytical measures. This GMP facility, in conjunction with the bioprocess development laboratory, enables the Partnership to offer LICR a core competence in the area of recombinant protein production under conditions that allow these therapeutic agents to be used in Phase I clinical trials.

To date, the Partnership has focused on two broad areas of targets; single chain antibodies and cancer testis (CT) antigens. The current list of targets has been formulated with input from a wide representation of the LICR, and prioritized with respect to needs and technical progress against the goals of the program. We envision a minimum of four targets being produced by the GMP in any 12 month period with a much greater number in development at any one time.

We have expressed the single chain antibody that recognizes the A33 antigen. This scFv was developed by Dr. Christoph Rader at The Scripps Research Institute (California) and was transferred to the Partnership under the auspices of the LICR. The ScFv was expressed in *Pichia pastoris* and served as a model for the development of unique methanol feeding strategies that allow for optimization of protein production. The expression of the scFv through modeling, assembly and implementation of a PID controller was optimized to yield a final production level of more than 4.8 g/L of fermentation broth. The fermentation and purification process has been transferred to the GMP, and the production of the A33scFv represents the inaugural product produced in the GMP. Further, a 6xhistidine-tagged A33scFv has been developed to label quantum dots that will eventually be used to study protein trafficking inside tumor cell lines.

A series of other targets, mainly CT antigens, are currently under development in the Partnership bioprocess development laboratory including NY-ESO-1, MAGE A3, Melan-A SSX2, NY-BR-1 and LAGE-1. The expression of these targets is being pursued in *E. coli* and *P. pastoris* in parallel, with priority to be given to the system that is most likely to succeed in the GMP. A high level expression, high cell density fermentation protocol has been developed for *E. coli* that produces over 300 g/l wet weight cells. The use of the strain C41(DE3) (Avidis SA, Saint-Beauzire, France) and rare tRNA encoding plasmid pRARE (Novagen, Madison, WI) have greatly increased the expression all the antigens tested so far. NY-ESO-1, MAGE A3, SSX2 and Melan-A have all been successfully expressed in *E. coli* at the 2.5 liter scale or higher and their purification protocols are currently at various stages of development. In particular, MAGE A3 can be expressed as a soluble protein at low fermentation temperature, and this has facilitated the purification process. SSX2 has been show to be secreted as a soluble protein into the periplasm via a TAT-signal peptide sequence using the twin arginine secretion pathway. This approach has been in collaboration with Dr. Mathew DeLisa (Cornell University, Dept. of Chemical Engineering) and is being applied to many of the other CT antigens in order to produce soluble protein. However, many of these

antigens will form insoluble inclusion bodies in *E. coli* which can be solubilized in guanidine or urea, although this complicates their purification. Therefore *P. pastoris* is the system of choice when secretion of the target is observed and/or a catalytically functional protein is needed.

NY-ESO-1 has been expressed in *E. coli* both as a native protein and as a 6xhistidine-tagged protein, which facilitates subsequent purification. While NY-ESO-1 forms inclusion bodies, subsequent purification protocols have been developed and optimized that result in a moderate degree of purification. The resulting product appears to be as pure as reagents currently used in clinical trials and this process has been transferred to the GMP. Expression of NY-ESO-1 is also being simultaneously pursued in *P. pastoris* with some success. In commercially available strains of *P. pastoris*, NY-ESO-1 appears to be secreted as a soluble protein, but the N-terminal signal sequence is not being processed and remains on the protein found in the culture medium. Cell lysis has been largely eliminated as an explanation and intracellular analysis shows that most of this unprocessed form of NY-ESO-1 is retained in the endoplasmic reticulum (ER). Similar results with SSX2 have also been observed with the soluble protein being retained in either the ER or Golgi apparatus. Recently, we have begun engineering strains of *P. pastoris* to increase expression of key chaperones found in the ER to promote improved protein processing and secretion. Co-expression of these chaperones with NY-ESO-1 has shown positive results with an increase in correct processing of secreted NY-ESO-1. Since we have been successful in expressing secreted A33scFv in *P. pastoris*, this protein is being used as the expression/secretion control in our chaperone co-expression experiments, which has also shown improved expression/secretion levels.

Publications

Primary Research Articles

1. Damasceno L.M., Pla I., Chang H.J., Cohen L., Ritter G., Old L.J., Batt C.A. An optimized fermentation process for high-level production of a single-chain Fv antibody fragment in *Pichia pastoris*. Protein Expression and Purification (2004) 37(1):18-26.

Ferdy J. Lejeune, M.D., Ph.D.

Centre Hospitalier Universitaire Vaudois
Lausanne, Switzerland

Research Report

Our research activity related to LICR concerns two distinct fields :

- Melanoma immunology and immunotherapy
- Anti-angiogenesis.

Dr. Olivier Michelin who received his MD/PhD degree in 2002, during his joint appointment with LICR and CePO, continued his training in clinical oncology at Centre Pluridisciplinaire d'Oncologie (CePO) together with his research at the LICR and Swiss Institute of Bioinformatics. His in silico studies focus on melanoma antigens and TCRs. It is expected that this approach will help defining optimal epitopes and effectors. Dr. Michelin also initiated a collaboration with Dr. Curzio Rüegg of CePO on in silico design of anti-integrins agents, in order to design effective antiangiogenic agents.

Melanoma immunology and immunotherapy

The Melanoma Immunology and Immunotherapy program was initiated in 1992 as a joint venture with the division of Immuno-Oncology of the LICR. This program continued in 2003 with completion of ongoing studies and new protocols. The publications focused on cytotoxic lymphocyte (CTL) phenotype in relation to melanoma immunity and vaccination.

The implementation of two new vaccination protocols at CePO:

1) "Vaccination of patients with stage III of IV Malignant Melanoma with Melanoma Antigen Peptides [Melan-A analog (ELA), NY-ESO-1b(A) and MAGE-A10] and montanide adjuvant". Principal investigator : Dr. D. Liénard.

In comparison with previous protocols, this study uses a multipeptide strategy. It was accepted by the scientific review board of the Centre Pluridisciplinaire d'Oncologie on April 24th 2003 and accepted by the Ethics Committee.

2) "Phase I study of in vivo expansion of Melan-A/MART-1 antigen-specific CD8 T lymphocytes following transient immunosuppression in patients with advanced melanoma." Principal investigator : Dr. S. Leyvraz from CePO.

This is the first protocol of adoptive immunotherapy ever initiated in Switzerland. It results from the synergy between LICR, CePO and NCCR (National Center for Competence in Research). This protocol was accepted by the scientific review board on November 20th 2003 and is being submitted to the Ethics Committee.

Since LICR studies always require blood and tumor samples from patients, we have initiated the preparation of a master protocol for blood and tissue donation. It should concern most patients who could become eligible for vaccination studies or immunology explorations. The draft is being examined by the Ethics Committee.

Antiangiogenesis

With Dr. Rüegg (a member of the LICR angiogenesis group), a large translational research program has been initiated aiming at defining cell and soluble markers of angiogenesis in cancer patients, as well as evaluating tumor vascularisation (collaboration with Dr. Meuwly, ultrasound specialist CHUV). This program is part of the NCCR in collaboration with the Swiss Institute of Experimental Cancer Research (ISREC).

Publications

Primary Research Articles

1. Ayyoub, M., Zippelius A., Pittet M.J., Rimoldi D., Valmori D., Cerottini J.C., Romero P., Lejeune F., Lienard D., Speiser D.E. Activation of Human Melanoma Reactive CD8+ T Cells by Vaccination with an Immunogenic Peptide Analog Derived from Melan-A/Melanoma Antigen Recognized by T Cells-1. Clinical Cancer Research (2003) 9(2):669-77.
2. Rimoldi, D., Lemoine R., Kurt A.M., Salvi S., Berset M., Matter M., Roche B., Cerottini J.C., Guggisberg D., Krischer J., Braun R., Willi J.P., Antonescu C., Slosman D., Lejeune F.J., Lienard D. Detection of micrometastases in sentinel lymph nodes from melanoma patients: direct comparison of multimarker molecular and immunopathological methods. Melanoma Research (2003) 13(5):511-520.
3. Rimoldi, D., Salvi S., Lienard D., Lejeune F.J., Speiser D., Zografos L., Cerottini J.C. Lack of BRAF mutations in uveal melanoma. Cancer Res. (2003) 63(18): 5712-5.
4. Speiser, D.E., Rimoldi D., Batard P., Lienard D., Lejeune F., Cerottini J.C., Romero P. Disease-driven T cell activation predicts immune responses to vaccination against melanoma. Cancer Immunity (2003) 3:12.
5. Valmori, D., Dutoit V., Ayyoub M., Rimoldi D., Guillaume P., Lienard D., Lejeune F., Cerottini J.C., Romero P., Speiser D.E. Simultaneous CD8+ T cell responses to multiple tumor antigen epitopes in a multipeptide melanoma vaccine. Cancer Immunity (2003) 3:15.

Hiroshi Shiku, M.D.

Second Department of Internal Medicine
Mie University School of Medicine
Mie, Japan

Research Report

CHP-HER2 cancer vaccine

Cholesteryl hydrophobized polysacharide (CHP), a novel formula of hydrophobized polysaccharide nanoparticles, was designed to deliver HER2 protein to the MHC class I and class II pathway. Pullulan, cholesteryl group-bearing polysaccharide (molecular weight: 108,000) is substituted with 0.9 cholesteryl moieties per 100 glucose units. CHP is a protein delivery system, and is complexed with the truncated HER2 protein containing the 146 N-terminal amino acids, forming a CHP-HER2 complex (CHP:HER2 protein = 20:1), and its diameter is 20 to 30 nm.

Phase I clinical trial

Phase I clinical trial of CHP-HER2 vaccine (LUD2001-016) was conducted for HLA-A2402-positive patients with HER2-expressing refractory cancers. CHP-HER2 vaccine was given by subcutaneous injection with the interval of two weeks with the dose of 300 μg of HER2 protein. Endpoints were safety and the immune responses after CHP-HER2 vaccination. The adverse events were transient skin reactions at the injection sites (Grade 1) in all nine patients. The others included Grade 1 of general fatigue, diarrhea and dry skin symptom in one patient. In the long-term follow-up, two patients developed disease progression and died. Seven other patients were followed-up ranging from four to 15 months, with the median period being seven months.

Immunomonitoring

CD8+ T cells or CD4+ T cells obtained from peripheral blood mononuclear cells (PBMCs) were sensitized *in vitro* with autologous 146HER2mRNA-electroporated T-antigen presenting cells (T-APCs) (PHA stimulated CD4+ T cells). Their activity was measured by ELISPOT assay using autologous T-APCs transduced with 146HER2 mRNA or control mRNA as targets. HER2 specific CD8+ and CD4+ T cells became detectable after four to eight vaccinations. HER2-specific cellular immune responses became detectable in five out of the eight vaccinated patients. In all patients with more than three to six vaccinations, IgG antibodies specific for truncated HER2 protein became detectable, with the IgG1 subtype being dominant.

In conclusion, CHP-HER2 is a novel antigen delivery system suitable for a polyvalent protein based cancer vaccine, which can elicit both specific cellular and humoral responses, involving MHC class I as well as MHC class II pathways.

Publications

1. Greiner J., Ringhoffer M., Taniguchi M., Li L., Schmitt A., Shiku H., Dohner H., Schmitt M. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. International Journal of Cancer (2004) 108(5):704-711.
2. Suzuki H., Katayama N., Ikuta Y., Mukai K., Fujieda A., Mitani H., Araki H., Miyashita H., Hoshino N., Nishikawa H., Nishii K., Minami N., Shiku H. Activities of granulocyte-macrophage colony-stimulating factor and interleukin-3 on monocytes. American Journal of Hematology (2004) 75(4):179-189.

3. Ogawa S., Yamaguchi M., Oka K., Taniguchi M., Ito M., Nishii K., Nakase K., Ohno T., Kita K., Kobayashi T., Shiku H. CD21S antigen expression in tumour cells of diffuse large B-cell lymphomas is an independent prognostic factor indicating better overall survival. British Journal of Haematology (2004) 125(2):180-186.

4. Araki H., Katayama N., Yamashita Y., Mano H., Fujieda A., Usui E., Mitani H., Ohishi K., Nishii K., Masuya M., Minami N., Nobori T., Shiku H. Reprogramming of human postmitotic neutrophils into macrophages by growth factors. Blood (2004) 103(8):2973-2980.

5. Monma F., Nishii K., Yamamori S., Hosokai N., Nakazaki T., Lorenzo F. 5th, Usui E., Sakakura M., Miyashita H., Fujieda A., Ohishi K., Katayama N., Shiku H. Fluorescent in situ hybridization analysis of Philadelphia chromosome-negative chronic myeloid leukemia with the bcr/abl fusion gene. International Journal of Hematology (2004) 80(2):155-158.

Chaitanya Divgi, M.D.

Memorial Sloan-Kettering Cancer Center
New York, USA

Research Report

There are three antibody systems under investigation at the LICR Affiliate Center at Memorial Sloan-Kettering Cancer Center (MSKCC): a) cG250, a chimeric antibody against the carbonic anhydrase-IX antigen overexpressed in the vast majority of clear cell renal cancers; b) hu3S193, a humanized antibody against the Lewis^{y} antigen, overexpressed in a variety of epithelial cancers; and c) huA33, a humanized antibody against the A33 antigen, overexpressed in virtually all colon cancers. These three antibodies are being studied in diagnostic and therapeutic clinical trials as part of the LICR Antibody Targeting and Clinical Trials Programs.

a) cG250

(i) Radioimmunotherapy (RIT)

A fractionated RIT trial with ^{131}Iodine (^{131}I)-cG250 in metastatic clear cell renal cancer was completed, and the results published. These results, along with a comparable Phase I study carried out by our colleagues at the LICR Nijmegen Affiliate Center, suggested that the tumor radiation absorbed dose from non-myeloablative amounts of ^{131}I-cG250 was inadequate. Further, the Nijmegen Center carried out animal and preliminary clinical studies suggesting that radiometal-labeled cG250 may have greater therapeutic potential.

Accordingly, we conjugated cG250 with DOTA, a chelate that would allow stable attachment of radiometals including ^{111}Indium (^{111}In), ^{90}Yttrium (^{90}Y) and ^{177}Lutesium (^{177}Lu). This was carried out in the Manufacturing Pharmacy at MSKCC under FDA guidelines; an application has been filed with the FDA, by the LICR Office of Clinical Trials Management, for permission to use the material in a radiometal-labeled Phase I study in metastatic renal cancer. Parallel Phase I trials will be carried out: the MSKCC Center will study ^{90}Y-DOTA-cG250 while the Nijmegen Center will study ^{177}Lu-DOTA-cG250. For these studies, all DOTA-cG250 will be manufactured at MSKCC and provided to each of the centers for clinical use.

(ii)Positron Emission Tomography (PET)

The ability of PET to provide truly quantitative *in vivo* data, and the generation of long-lived positron-emitters such as ^{124}I at MSKCC, stimulated us to explore the utility of immunoPET in the quantification of antigen distribution in humans. The increasing use of minimally invasive surgery for renal masses has underscored the need for thorough pre-operative characterization of renal masses, and we believe that the specificity of cG250 for clear cell renal cancer will enable pre-operative characterization of renal masses by immunoPET imaging with ^{124}I-cG250. Accordingly, we have initiated planning for a study in pre-operative patients with renal masses.

An immunoPET study with ^{124}I-cG250 was initiated early in 2004, in patients with biliary cancers. This followed immunohistochemistry observations that the antigen was expressed in such cancers. Since the antigen is also present on normal bile duct cells, it was imperative to determine whether there was preferential targeting to tumor. However, no patients have been entered into this study, which has been open for almost a year; pending discussions with the Hepatobiliary team at MSKCC, this study will likely be closed. (The Nijmegen Center carried out a similar study with ^{131}I-cG250 and found no evidence of preferential targeting to biliary cancers.)

b) Hu3S193

(i) Intraperitoneal RIT

Ovarian cancer is limited to the peritoneal cavity for much of its natural course, and the peritoneal cavity is also rich in immune effector cells. It was therefore postulated that intraperitoneal RIT using an immunologically active antibody would have therapeutic potential. We have accordingly begun a Phase 1 intraperitoneal RIT study with ^{90}Y-CHX(A)DTPA-hu3S193 trial in patients with Stage 3 ovarian cancer. The mass amount of hu3S193, 10 mg, was chosen as capable of inducing ADCC intraperitoneally. Initially, this trial accrued only patients with minimal residual disease; expanding the eligible patient population to all patients with Stage 3 disease has improved patient accrual, and eight patients have completed therapy without acute adverse events. The first two dose levels (10 mCi and 15 mCi ^{90}Y) have been completed without evidence of toxicity. The ^{90}Y-CHX(A)DTPA-hu3S193 is co-administered with ^{111}In-CHX(A)DTPA-hu3S193 to permit evaluation of biodistribution and imaging.

(ii) Immunotherapy

Hu3S193 is capable of evoking significant antibody-dependent cell-mediated cytotoxicity (ADCC). Since the Lewisy antigen is present in the majority of neuroendocrine tumors, particularly small cell lung cancer (SCLC, a disease for which few therapeutic options exist), a pilot study was initiated at MSKCC in patients with measurable advanced SCLC (Principal Investigator, Dr. Lee Krug). Five patients have thus far received four weekly infusions of 10 mg/m^2 hu3S193, with the first and last doses being trace-labeled with ^{111}In (as above). Targeting to disease has been excellent.

The safety of a higher dose – 20 mg/m^2 – of hu3S193 demonstrated by our collaborators at the LICR Melbourne Branch has resulted in our amending the protocol to increase the weekly treatment dose to that amount. The amended protocol has recently received regulatory approval.

c) huA33

(i) ImmunoPET with ^{124}I-huA33

As stated above, immunoPET holds great potential for *in vivo* immunohistology. We elected to validate this principle in a study (Principal Investigator, Dr. Steven Larsen) with patients scheduled for surgery (radical colectomy) as this enables us to obtain immunoPET immediately prior to surgery, at which point we obtain surgical specimens for *in vitro* analysis. Five patients have so far been studied. Serial immunoPET was shown to distinguish between early (vascular) and late (antigen-binding) distribution of antibody, and also provided insights into the potential of this method to distinguish between carbohydrate metabolism, vascularity and antigen distribution. Concurrent use of computer tomography imaging has allowed accurate co-registration of anatomy and pathophysiology *in vivo*.

Preliminary data comparing *in vivo* and *in vitro* quantitation of radioactivity as well as *in vivo* estimates of antigen density with *in vitro* measurement by binding assays in tumor and normal colon (which also expresses antigen) have shown strong correlation. We are also collaborating closely with Dr. Ira Mellman and his colleagues at the LICR New Haven Affiliate Center (at Yale University) to attempt to determine the trafficking of the antibody-antigen complex: whether it is internalized into macropinosomes, as early results from our group suggested, or whether the antigen resides in tight junctions, as the Yale data using cell lines have suggested. Quantitative autoradiography has demonstrated that antigen distribution is limited to cancer cells, which constitute a fraction of gross tumor that is measured.

(ii) scFv A33

In collaboration with the Cornell University/LICR Partnership (Ithaca Affiliate Center), we have characterized single chain A33 (grown in *Pichia pastoris* under Good Manufacturing Practice (GMP) conditions at the Biologic Production Facility in Ithaca) labeled with radioiodine and ^{111}In. Results with iodinated scFv A33 were similar to unpublished observations with *E. coli*-produced scFv A33, confirming that the presence of tyrosines near and in the A33 CDR precluded iodination of this novel construct. Dr. Peter Smith-Jones at MSKCC has therefore labeled ^{111}In to the construct using DOTA. Preliminary results have shown that the radioimmunoconjugate is indeed retained in tumor far more than its iodinated counterpart.

Publications

Primary Research Articles

1. Divgi C.R., O'Donoghue J.A., Welt S., O'Neel J., Finn R., Motzer R.J., Jungbluth A., Hoffman E., Ritter G., Larson S.M., Old L.J. Phase I clinical trial with fractionated radioimmunotherapy using ^{131}I-labeled chimeric G250 in metastatic renal cancer. Journal of Nuclear Medicine (2004) 45:1412-21.

Egbert Oosterwijk, Ph.D.

Radboud University Nijmegen Medical Center
Nijmegen, The Netherlands

Research Report

In 2004, various preclinical and clinical studies were performed, as part of the LICR Antibody Targeting Program, with chimeric monoclonal antibody G250 (cG250). The cG250 antibody recognizes carbonic anhydrase IX (CAIX), a renal cell carcinoma (RCC)-associated antigen that is most abundant on RCC of the clear cell type. Preclinical studies were aimed at optimization of radioimmunotherapy (RIT). Labeling technology was developed, and stability, biodistribution, and therapeutic efficacy of 131-/Iodine, 88/90-Yttrium, 177-Lutetium, and 186-Rhenium labeled cG250 were studied in nude mice grafted with subcutaneous RCC tumors. Stable radioimmunoconjugates were produced, and RIT studies showed that the therapeutic efficacy of cG250 labeled with ^{177}Lu-, ^{90}Y-, or ^{186}Re- was superior to that of ^{131}I-cG250. The residualizing radionuclides ^{177}Lu and ^{90}Y led to higher radiation doses to the tumor and we concluded that these are most likely better candidates than conventionally radiolabeled ^{131}I for RIT with cG250 in patients with RCC. Based on this evidence, a Phase I ^{177}Lu dose escalation study has been designed which will be paralleled by an identical study with ^{90}Y labeled cG250 performed under the guidance of Dr. Chaitanya Divgi at the LICR New York (Memorial Sloan-Kettering Cancer Center, MSKCC) Affiliate Center. The LICR Protocol Review Committee and Internal Review Boards of MSKCC and Radboud University Nijmegen Medical Center have approved both studies, and inclusion of participating patients is commencing.

Positron emission tomography (PET) cG250 radioimmunoscintigraphy was studied in a preclinical nude rat model. There is considerable interest in using cG250 as an imaging reagent, and PET imaging would be desirable. We developed stable ^{89}Zr-cG250, while preserving the immunoreactivity of cG250. In this rat model, the *in vivo* biodistribution of ^{89}Zr-cG250 was identical to that of ^{111}In-DTPA-cG250, demonstrating the feasibility of immuno-PET of RCC.

In a preclinical and clinical study with cG250 F(ab')2 antibody fragments, we investigated whether F(ab')2 fragments were more suited for RIT than intact IgG. In mice, the tissue-to-blood ratios were similar for intact IgG and the ^{131}I-cG250-F(ab')2 fragments for most tissues and at most time points, although absolute uptake in all tissues was, as expected, considerably lower for the F(ab')2 fragments. Surprisingly, in patients with primary RCC, RCC tissue was only faintly visualized with ^{131}I-cG250-F(ab')2 fragments, i.e. the intact IgG form of cG250 appeared to be more suitable than cG250-F(ab')2 fragments for targeting clear-cell RCC.

Finally, we reported on a multicenter Phase II study evaluating the safety and efficacy of cGG250 in metastatic RCC (mRCC) patients. In all, 36 patients with mRCC were included. cG250 was given weekly by intravenous infusion for 12 weeks. None of the 36 enrolled patients developed any drug-related grade III or IV toxicity. Ten patients experienced stabilization of disease and received extended treatment. One patient showed a complete response and significant disease regression was observed during the follow-up of the treatment in another patient. Five patients with progressive disease at study entry were stable for more than six months after study entry. The median survival after treatment start was 15 months. The weekly schedule of cG250 was well tolerated and with a median survival of 15 months after the start of this treatment and two late clinical responses, cG250 appeared to be able to modulate mRCC. To improve the activity of cG250-specific antibody-dependent cellular cytotoxicity and the clinical response rate, a multicenter phase II study evaluating cG250 combined with IL-2 was initiated and finalized. The results of this trial are currently being evaluated.

To understand the mechanism ruling the specific upregulation of CAIX in clear cell RCC, we also studied the mechanism of transcriptional regulation of the CAIXG250 gene in RCC. Previous studies identified hypoxia-inducible factor (HIF) as one of the regulatory transcription factors of G250 in various non-RCC backgrounds. However, G250 regulation in RCC had not been studied and might be differently regulated in view of the HIF accumulation under normoxic conditions due to Von Hippel Lindau mutations. Transient transfection of different G250 promoter constructs revealed strong promoter activity in G250-positive RCC cell lines, but no activity in G250-negative cell lines. DNase-I footprint and band-shift analysis demonstrated that Sp1 and HIF-1alpha proteins in nuclear extracts of RCC cells bind to the CAIX promoter. Mutations in the most proximal Sp1 binding element or HIF binding element completely abolished CAIX promoter activity, indicating their critical importance for the activation of G250 expression in RCC. A close correlation between HIF-1alpha expression and G250 expression was observed. In contrast, no relationship between HIF-2alpha expression and G250 was seen. The participation of cofactor CBP/p300 in the regulation of G250 transcription was shown. In conclusion, the studies showed that HIF-1alpha and Sp1, in combination with CBP/p300, are crucial elements for G250 expression in ccRCC, and CAIXG250 can be regarded as a unique HIF-1alpha target gene in ccRCC.

In view of the close correlation between CAIX and hypoxia, CAIX is becoming viewed as an endogenous marker for tumor hypoxia, contributing to the pH regulation of tumor cells. Possibly, CAIX might allow tumors to acclimate to a hypoxic microenvironment, promoting tumor cell proliferation. In a study in early stage non small-cell lung cancer (NSCLC) we concluded that CAIX expression of tumor cells might be an indicator for poor disease-free survival in early-stage NSCLC. Additional studies investigating the co-localization of CAIX and cell proliferation and patient survival are in patients with head and neck squamous cell carcinoma are in progress.

In vivo experiments with conditional replicating Adenovirus (CRAd) under control of the minimal G250-promoter region were performed to test whether selective infection and replication in G250-positive cells could be achieved. Intratumoral injections lead to selective AdV replication, but intravenous injections did not achieve the desired, tumor-specific AdV replication. New AdV vectors, including vectors producing bispecific anti-AdV X G250 antibody have been constructed and showed exquisite selectivity for G250-positive cells *in vitro*.

Publications

Primary Research Articles

1. Brouwers A.H., van Eerd J.E., Frielink C., Oosterwijk E., Oyen W.J., Corstens F.H., Boerman O.C.Optimization of radioimmunotherapy of renal cell carcinoma: labeling of monoclonal antibody cG250 with 131I, 90Y, 177Lu, or 186Re. Journal of Nuclear Medicine (2004) 45:327-37.
2. Bleumer I., Knuth A., Oosterwijk E., Hofmann R., Varga Z., Lamers C., Kruit W., Melchior S., Mala C., Ullrich S., De Mulder P., Mulders P.F., Beck J. A phase II trial of chimeric monoclonal antibody G250 for advanced renal cell carcinoma patients. British Journal of Cancer (2004) 90:985-90.
3. Mulders P., Bleumer I., Debruyne F., Oosterwijk E. Specific monoclonal antibody-based immunotherapy by targeting the RCC-associated antigen carbonic anhydrase-IX (G250/MN) Urologe Ausgabe A 43(S3):146-7.

4. Grabmaier K., A de Weijert M.C., Verhaegh G.W., Schalken J.A., Oosterwijk E. Strict regulation of CAIX(G250/MN) by HIF-1alpha in clear cell renal cell carcinoma. Oncogene (2004) 23:5624-5631.

5. Brouwers A., Verel I., Van Eerd J., Visser G., Steffens M., Oosterwijk E., Corstens F., Oyen W., Van Dongen G., Boerman O. PET radioimmunoscintigraphy of renal cell cancer using 89Zr-labeled cG250 monoclonal antibody in nude rats. Cancer Biotherapy Radiopharmaceuticals (2004) 19:155-63.

6. Brouwers A., Mulders P., Oosterwijk E., Buijs W., Corstens F., Boerman O., Oyen W. Pharmacokinetics and tumor targeting of 131I-labeled F(ab')2 fragments of the chimeric monoclonal antibody G250: preclinical and clinical pilot studies. Cancer Biotherapy Radiopharmaceuticals (2004) 19:466-77.

7. Kim S.J., Rabbani Z.N., Vollmer R.T., Schreiber E.G., Oosterwijk E., Dewhirst M.W., Vujaskovic Z., Kelley M.J. Carbonic Anhydrase IX in Early-Stage Non-Small Cell Lung Cancer. Clinical Cancer Research (2004) 10:7925-33.

Vincenzo Cerundolo, M.A., M.D., Ph.D., F.R.C.Path., F.Med.Sci.

Institute of Molecular Medicine
Oxford, UK

Research Report

Over the last year we have carried out three complementary lines of research:

1) We have investigated the structural and kinetic features of T cell recognition (TCR) recognition of HLA-A2 (A2) loaded with the wild type NY-ESO-$1_{157\text{-}165}$ peptide (SLLMWITQC; hereafter referred to as "ESO 9C peptide") and produced a soluble version of a NY-ESO-$1_{157\text{-}165}$ specific TCR. We demonstrated that TCR interactions with the NY-ESO-$1_{157\text{-}165}$ native epitope are dominated by two highly exposed residues, methionine and tryptophan, whose side chains protrude from the central part of the peptide (at positions four and five). The presence of this prominent hydrophobic feature suggests that for this system TCR binding in general will focus on the central region of the peptide and be relatively tolerant of changes in the pMHC surface near the peptide carboxyl terminus. To test this hypothesis and its implications for the immunogenic characteristics of the system, we carried out a series of studies to provide structural, solution binding, *in vitro* functional and *in vivo* immunogenic data. Since we had previously demonstrated that substitution of the cysteine to valine at position 165 enhances the ability of the epitope to be recognised *in vitro* by NY-ESO-$1_{157\text{-}165}$ specific cytotoxic T lymphocytes (CTL), we focused on this peptide analogue (SLLMWITQV; hereafter referred to as "ESO 9V peptide"). Our results have provided insights into the mechanisms controlling the enhanced immunogenicity of superagonist peptides by demonstrating a correlation between the stability of the peptide: MHC-TCR complex, role of CD8 in killing of target cells, ability to stimulate a faster formation of conjugates, resulting in polarisation of lytic granules and *in vivo* T cell proliferation.

2) We have demonstrated that stimulating *i*NKT cells *in vivo* with the specific synthetic ligand α-GalCer served to significantly enhance immune responses to protein-based vaccines. Of significance was the finding that the adjuvancy of *i*NKT cell ligands was observed to vaccines administered orally, which for practical reasons, is seen as a preferred administration option in terms of likely patient uptake and ease of administration. We also described the impact of combining injection of protein vaccines with a combination of *i*NKT cell-agonist α-GalCer and MPL, a detoxified version of LPS that signals through TLR4. This combination treatment stimulates antigen-specific T cell responses that are greater than those elicited with α-GalCer or MPL alone. In fact, a synergy was observed that resulted in a 60-fold increase in antigen-specific $CD8^+$ T cells over injection of protein antigen alone. We are now in the position to study this interaction further by analysing the impact of using combinations of *i*NKT cell ligands and pathogen-derived components that signal through Toll-like receptors (TLRs) to shape immune responses, with specific emphasis on changing the quality of T cell- and antibody responses.

3) An important aspect of my future work will continue to be a translational research programme in cancer patients based on the application of protocols derived from the above pre-clinical studies. We have recently completed a Phase I clinical trial in melanoma patients vaccinated with plasmid DNA followed by defective pox-virus (MVA) encoding a string of melanoma epitopes. The results of this trial demonstrated that 50% of vaccinated patients (seven out of 14 vaccinated patients) mounted a response specific for the recombinant melanoma antigens after MVA injection. However, responses against MVA proteins were out-competed by T cell responses specific for the recombinant melanoma antigens, possibly due to the failure of

plasmid DNA to prime melanoma specific T cells. The results of this trial were very informative to assess the kinetic of T cell expansion and interplay between T cell responses.

I have recently received approval from the Medicine Control Agency and Gene Therapy Advisory Committee (GTAC) to carry out a Phase II clinical trial (which should start in May 2005) in 40 melanoma and prostate cancer patients with NY-ESO-1 positive tumor. Eligible patients will be divided in two groups and will receive either three injections of the NY-ESO-1/ISCOMATRIX® followed by three injections of recombinant Fowlpox-NY-ESO-1 or 6 injections of NY-ESO-1/ISCOMATRIX®. This clinical trial is designed to confirm the results of a recently published Phase I trial from the LICR Melbourne Branch and to extend them by studying the effect of a prime-boost protocol on the frequency and phenotype of NY-ESO-1 specific responses.

Publications

Primary Research Articles

1. Barnes E., Salio M., Cerundolo V, Medlin J., Murphy S., Dusheiko G., Klenerman P. Impact ofalpha interferon and ribavirin on the function of maturing dendritic cells. Antimicrobial Agents in Chemotherapy (2004) 48(9):3382-9.
2. Batuwangala T., Shepherd D., Gadola S., Gibson K., Zaccai N., Fersht A., Besra G.S., Cerundolo V., Jones Y. The crystal structure of CD1b with a bound bacterial glycolipid. Journal of Immunology (2004) 172(4):2382-2388.
3. Hermans I.F., Silk J.D., Yang J., Palmowski, M.J., Gileadi U., McCarthy C., Salio M., Ronchese F., Cerundolo V. The VITAL assay: A versatile fluorometric technique for assessing CTL- and NKT-mediated cytotoxicity against multiple targets in vitro and in vivo. Journal of Immunological Methods (2004) 285(1):25-40.
4. Markel G., Achdout H., Katz G., Ling K-L., Salio M., Gruda R., Gazit R., Mizrahi S., Hanna J., Gonen-Gross T., Arnon T., Lieberman N., Stren N., Nachmias B., Blumberg R., Steuer G., Blau H., Cerundolo V., Mussafi H., Mandelboim O. Biological function of the soluble CEACAM1 and implications in TAP2 deficient patients. European Journal of Immunology 34(8):2138-48.
5. Palmowski M.J., Lopes L., Ikeda Y., Salio M., Cerundolo V., Collins M.K. Intravenous Injection of Lentiviral Vector Encoding NY-ESO-1 induces effective CTL responses. Journal of Immunology (2004) 172(3): 1582-7.
6. Papagno L., Spina C.A., Marchant A., Salio M., Rufer N., Little S., Dong T., Chesney G., Waters A., Easterbrook P., Dunbar P.R., Shepherd D., Cerundolo V., Emery V., Griffiths P., Conlon C., McMichael A.J., Richman D.D., Rowland-Jones S.L., Appay V. Immune Activation and CD8(+) T-Cell Differentiation towards Senescence in HIV-1 Infection. Public Library of Science - Biology (2004) 2(2):E20.
7. Salio M., Palmowski M.J., Atzberger A., Hermans I.F., Cerundolo V. CpG-matured murine Plasmacytoid Dendritic Cells: professional antigen presenting cells capable of in vivo priming functional antigen-specific CD8 T cell responses to endogenous but not exogenous antigens. Journal of Experimental Medicine (2004) 199(4):567-579.
8. Silk J., Hermans I., Gileadi U., Chong W., Shepherd D., Salio M., Mathew B., Schmidt R.R., Lunt S., Williams K., Stratford I., Harris A. Cerundolo V. Utilizing the adjuvant properties of CD1d-dependent NKT cells in T cell-mediated immunotherapy. Journal of Clinical Investigation (2004) 114(12):1800-11.

9. Smith D.C., Salio M.J., Lord M., Roberts L.M., Cerundolo V. Lack of dendritic cell maturation by the plant toxin ricin. European Journal of Immunology (2004) 34(8):2149-57.

Reviews / Commentaries / Book Chapters

1. Cerundolo V., Hermans I,. Salio M. Dendritic cells: a journey from the laboratory to the clinic. Nature Immunology (2004) 5(1):7-10.
2. Collins M.K., Cerundolo V. Gene therapy meets vaccine development. Trends Biotechnology (2004) 22(12):623-6.

Svetomir N. Markovic, M.D., Ph.D.

Mayo Clinic
Rochester, MN, USA

Research Report

As an affiliate member of LICR our group has been awarded the opportunity and privilege to collaborate with world leaders in cancer immunotherapy. The interactions that have been made possible by our membership to LICR have allowed our efforts to grow and slowly develop into a comprehensive program for translational cancer immunotherapy at the Mayo Clinic. Our group efforts are divided into two major projects: melanoma immunotherapy; and post-autologous stem cell transplant immune recovery and immunomodulation. Herein is a brief summary of our activities in both projects in 2003:

Melanoma Immunotherapy

- **Peptide vaccines:** In continuation of our efforts to improve the efficacy of peptide based vaccines for patients with melanoma, we completed a randomized phase II clinical trial evaluating the role of low dose GM-CSF as immune adjuvant co-emulsified with melanoma peptide vaccines (MART-1, tyrosinase and gp100) in Montanide ISA 51. The doses of GM-CSF used in this study were 0, 10 and 50µg. The study did not demonstrate improved efficacy of immunization using the highest dose of GM-CSF. In view of our pre-clinical data suggesting that further dose escalation of GM-CSF would be warranted, we proceeded to a follow-up trial using GM-CSF at higher doses (75 and 100µg) as well as a gp100 peptide that contained overlapping HLA class I and class II binding sites. This study is currently active and anticipated to complete accrual by late 2004.
- **Aerosol vaccines:** In close collaboration with our colleagues at the LICR New York Branch, we have developed a Phase I study evaluating the safety and immunological efficacy of aerosol NY-ESO-1 protein immunization. Pre-clinical studies demonstrate effective immunization of mice using aerosol delivery of peptide antigens. The clinical trial is an extension of our pre-clinical work to improve immunization efficacy of a cancer vaccine using aerosol delivery. The clinical trial is approaching final stages of development and we anticipate study activation by mid to late 2004.
- **Cytokine therapy:** Two projects have been in ongoing development using cytokines for the treatment of advanced stage malignant melanoma. The first project evaluates the utility of aerosolized GM-CSF delivered by inhalation to patients with metastatic melanoma in the lung. The current study (N0071) is a dose escalating trial evaluating the ability of increasing doses of aerosolized GM-CSF to induce melanoma specific cytotoxic T lymphocyte responses. The study is ongoing and preliminary evidence suggests development of systemic anti-melanoma immunity at a dose of 1250µg of GM-CSF administered twice/day for 14 days. The metastatic tumor in the lung is the source of the antigens.

  The second project is focused on the use of natural alpha interferon (Alferon N) and its ability to up-regulate NK cell cytotoxicity pre-operatively in the setting of resected stage IV melanoma. Based on our pre-clinical data we have demonstrated that effective pre-surgical resection up-regulation of NK cytotoxicity is effective in preventing systemic tumor dissemination in 70% of treated animals. The current clinical trial is attempting to translate these observations into patients that are treated with interferon prior to resection of metastatic melanoma. The first phase of the study, identifying the dose/regimen of interferon

necessary to up-regulate NK cytotoxicity is complete. The phase II component, evaluating the clinical impact of this intervention, is currently awaiting activation.

Post-autologous stem cell transplant (post-ASCT) immune reconstitution/immunotherapy

In 2003, we were able to further our understanding of the role of early lymphocyte reconstitution and its impact on clinical outcomes in patient undergoing ASCT for the treatment of hematological malignancies and breast cancer. We have been able to identify NK cells as the key element influencing the ≥80% 10 year survival of patients with non-Hodgking's lymphoma that reconstitute their lymphocyte counts by day 15 post transplant. We have also identified the autograft as the source of reconstituting NK cells having direct impact on survival. Our first clinical trial attempting to "engineer" the autograft in such a way that it would be enriched for NK cells is currently under way, and is expected to be completed by mid 2004.

Publications

Primary Research Articles

1. McWilliams R.R., Brown P.D., Buckner J.C., Link M.J., Markovic S.N. Treatment of brain metastases from melanoma. Mayo Clinic Proceedings (2003) 78(12):1529-1536.
2. Rao R.D., Anderson P.M., Arndt C.A., Wettstein P.J., Markovic S.N. Aerosolized granulocyte macrophage colony-stimulating factor (GM-CSF) therapy in metastatic cancer. American Journal of Clinical Oncology (2003) 26(5):493-498.
3. Rao R.D., Markovic S.N., Anderson P.M. Aerosol therapy for malignancy involving the lungs. Current Cancer Drug Targets (2003) 3(4):239-250.
4. Porrata L.F., Gastineau D.A., Padley D., Bundy K., Markovic S.N.. Re-infused autologous graft natural killer cells correlates with absolute lymphocyte count recovery after autologous stem cell transplantation. Leukemia & Lymphoma (2003) 44(6):997-1000.
5. Pockaj B.A., Jaroszewski D.E., DiCaudo D.J., Hentz J.G., Buchel E.W., Gray R.J., Markovic S.N., and Bite U. Changing surgical therapy for melanoma of the external ear. Annals of Surgical Oncology (2003) 10(6):689-696.

Dirk Jäger M.D., Alfred Zippelius M.D., Alexander Knuth M.D.

Universitätsspital Zürich, Switzerland
Zürich, Switzerland

Research Report

After setting up the technical and administrative infrastructure for the participation in the LICR Cancer Antigen Discovery, Cancer Vacccine and Clinical Trial Programs, our goal was to install a system where patients with malignant diseases treated at different departments at UniversitätsSpital Zurich (USZ) are typed for tumor antigen expression on a routine basis. A close collaboration with the department of pathology and clinical collaborations with the departments of gynecology, surgery, neurosurgery, dermatology, urology, and ear, nose and throat (ENT) were built. Tumor tissue is being typed for tumor antigen expression on a routine basis by immunohistochemistry (IHC) for NY-ESO-1 and MAGE. Other antibody reagents available at the LICR New York Branch will be provided (by Dr. Achim Jungbluth) to broaden the antigen spectrum routinely assessed by IHC. In addition, reverse transcription (RT)-PCR is performed in our laboratory for all relevant tumor antigens. All patient sera are analyzed for antibody directed against relevant tumor antigens, e.g. NY-ESO-1, NY-BR-1, SSX2, RAB38. All data are deposited into an ACCESS-based database allowing the systematic analysis of antigen expression in different tumor types as well as spontaneous immune responses in correlation to the clinical development of the individual patient. This systems allows us to select patients qualifying for clinical immunotherapy protocols.

1.) SEREX- based Antigen Discovery

In an autologous SEREX screening of an undifferentiated adenocarcinoma, we identified SPANX-a as a cancer-testis (CT) antigen. By RT-PCR, SPANX-a is expresssed in about 30% of colorectal cancers, and less frequently in breast cancers and melanomas. To date, immune responses against SPANX-a have not been reported, whereas SPANX-b was reported to induce frequent humoral immune responses in patients with myeloma and chronic lymphatic leukemia. We produced recombinant SPANX-a and SPANX-b proteins and are now testing for antigen specific humoral immune responses against both antigens in cancer patients.

2) Characterization of new tumor antigens

a) RAB38:

RAB38 was identified as a new melanocyte differentiation antigen in an autologous melanoma SEREX screening. We analyzed RAB38 mRNA expression and protein expression in melanoma patients and found RAB38 to be expressed in 80% of melanomas. We generated the recombinant RAB38 protein to analyze spontaneous humoral immune responses directed against RAB38. Interestingly, 23% of melanoma patients (12/52) had detectable RAB38 specific antibody whereas there was no detectable humoral immunity in patients with other cancers, vitiligo patients or healthy individuals. Using overlapping peptides (18-mers) covering the entire RAB38 protein sequence we identified different antigenic sites recognized by antibody positive sera.

In an ongoing project in collaboration with Dr. Reinhard Dummer (Department of Dermatology, USZ) we are assessing spontaneous humoral immune responses against 13 different tumor-associated antigens (CT antigens and differentiation antigens including RAB38) in 400

melanoma patients (serum bank at Dermatology Department and Oncology Department, USZ) and correlating humoral immunity to stage of the disease and the clinical development of individual patients. If available, we will also test urine samples for antigen-specific antibodies.

b) NY-BR-1:

NY-BR-1 is a breast differentiation antigen identified in two previous autologous SEREX screenings. We evaluated mRNA expression in a series of 60 primary breast cancers and found 43 positives (72%). In collaboration with Dr. Valeriy Filonenko (James R. Kerr Program at the Institute of Molecular Biology and Genetics, Kyiv) we generated a monoclonal NY-BR-1 specific antibody. We demonstrated its specificity in western blot stainings of NY-BR-1 positive and negative cell line lysates. The monoclonal antibody works in paraffin embedded tissue, and we are now assessing NY-BR-1 expression in a series of 1000 breast cancer specimens using a breast cancer tissue array (with Dr. Moch, Department of Pathology, USZ).

Confocal microscopy of NY-BR-1-GFP transfected 293T cells showed cell membrane localization suggesting NY-BR-1 as a potential target antigen for antibody based therapy. Cell surface localization was confirmed by two independent assay systems: We demonstrated that NY-BR-1 can be detected in the biotinylated cell fraction using biotinylation assays of NY-BR-1 transfected 293T cells where biotin binds covalently to all cell-surface proteins. Furthermore we could stain NY-BR-1 transfected non permeabelized 293T cells by flow cytometry using the monoclonal NY-BR-1 antibody. These data strongly support the evaluation of NY-BR-1 as a target antigen for antibody based therapy in breast cancer (manuscript in preparation).

3) Characterization of Anti-tumor T cell responses

a) Identification of HLA-A2 restricted CD8 T cell epitopes for RAB38:

To determine HLA-A*0201 restricted epitopes, CD8 T cells of HLA-A*0201 positive melanoma patients were stimulated with both overlapping peptides covering the entire amino acid sequence and RAB38 deca- and nonapeptides with strong affinity to HLA-A*0201. Frequency of peptide-specific T cells was determined by ELISPOT and IFN-γ secretion assay. In addition, the latter was used to generate poly- and monoclonal T cell populations from melanoma patients which were further tested for functional avidity and tumor cell recognition. Those populations showed specific lysis of TAP-deficient antigen-presenting cells pulsed with the nonameric RAB38 peptide and showed intermediate to high avidity. Importantly, the populations lysed melanoma cell lines that expressed both HLA-A2 and RAB38. Specific lysis was not increased when the peptide was pulsed exogenously, and could be blocked by a pan-MHC class I antibody. No lysis was observed when either HLA-A2 or RAB38 was not expressed. This demonstrates that the nonameric HLA-A*0201-restricted RAB38 peptide is naturally processed and presented by melanoma cells. RAB38-specific CD8 T cells detectable *ex vivo* were identified in melanoma patients, while healthy donors were negative in multimer analysis. In contrast, after specific stimulation one of four melanoma patients, and three of six healthy donors exhibited RAB38-specific CD8 T cells. Altogether, we identified the first HLA-A*0201 restricted CD8 T cell epitope of the melanocyte differentiation antigen RAB38 that may be involved in anti-melanoma T cell responses. These data form the basis to initiate immunotherapy trials in melanoma patients targeting RAB38.

b) Identification of CD4 T cell epitopes for RAB38:

The optimal experimental conditions to stimulate and clone antigen-specific CD4 T cells were established by generating T cells specific for an HLA-DPB*0401 restricted tetanus toxoid peptide previously vaccinated with the recombinant protein. Similarly, to elucidate RAB38-specific CD4 T cell responses, CD4 T cells melanoma patients were stimulated with overlapping peptides covering the entire amino acid sequence of RAB38. Frequency of RAB38 peptide-specific T cells

were determined by ELISPOT assays. This analysis revealed a broad CD4 T cell response against multiple peptides. Further sorting applying the IFN-γ secretion assay allowed us to generate monoclonal, RAB38-specific CD4 T cell populations. Experiments to determine the HLA restriction element and the optimally recognized RAB38-derived peptides are currently ongoing.

c) Identification of HLA-A2 restricted CD8 T cell epitopes for NY-BR-1:

Applying the "reverse immunology" approach (www.syfpeithi.de) we synthesized 35 NY-BR-1 peptides to subsequently generate cytotoxic T lymphocytes (CTLs) against the predicted peptides from HLA-A*0201 patients with NY-BR-1 expressing breast cancer. We initially concentrated on two peptides that induced the highest T cell response in in vitro stimulations. As the expression of NY-BR-1 in breast cancer cell lines is low to absent, we initially tested T cell recognition of two T cell populations that specifically recognized both peptides using COS cells transfected with the NY-BR-1 sequence. Both populations efficiently recognized transfected COS cells. To further document the processing of those peptides by the proteasome, we performed an *in vitro* proteasomal degradation assay in collaboration with Dr. Frédéric Levy (LICR Lausanne Branch). For this purpose, 20 amino acid long peptides containing the sequence of both NY-BR-1 peptides were incubated with standard proteasomes purified from human erythrocytes, and the digested products were subsequently analyzed by mass spectometry. Interestingly, however, the predicted nonamer for only for one peptide is likely to be generated intracellularly by proteasomal degradation. Our current experiments are focused on permanently transfect tumor cell lines with NY-BR-1 to prove the natural processing and presentation of this peptide. Additionally, other potential HLA-A2 restricted peptides derived from NY-BR-1 are currently evaluated.

4) Clinical Trials Center Activity

LUD01-014: Phase 1/2 study of chimeric monoclonal antibody G250 (cG250) in combination with vinblastine administered weekly by intravenous infusion to patients with advanced renal cell carcinoma.

Approved by local ethic committee and Swissmedic. Three patients were entered from our site.

LUD 2003-024: Phase 1 study of immunization with NY-ESO-1 protein combined with CpG 7909 and Montanide in patients with high-risk stage D1 or advanced prostate cancer.

Approved by local ethic committee and Swissmedic. One patient entered.

Publications

Primary Research Articles

1. Al-Batran S.E., Atmaca A., Bert F., Jager D., Frisch C., Neumann A., Orth J., Knuth A., Jager E. Dose escalation study for defining the maximum tolerated dose of continuous oral trofosfamide in pretreated patients with metastatic lung cancer. Onkologie (2004) 27(6):534-8.
2. Al-Batran S.E., Atmaca A., Hegewisch-Becker S., Jaeger D., Hahnfeld S., Rummel M.J., Seipelt G., Rost A., Orth J., Knuth A., Jaeger E. Phase II trial of biweekly infusional fluorouracil, folinic acid, and oxaliplatin in patients with advanced gastric cancer. Journal of Clinical Oncology (2004) 22(4):658-63.
3. Hofmann W.K., Heil G., Zander C., Wiebe S., Ottmann O.G., Bergmann L., Hoeffken K., Fischer J.T., Knuth A., Kolbe K., Schmoll H.J., Langer W., Westerhausen M., Koelbel C.B., Hoelzer D., Ganser A. Intensive chemotherapy with idarubicin, cytarabine, etoposide, and G-CSF priming in patients with advanced myelodysplastic syndrome and high-risk acute myeloid leukemia. Annals of Hematology (2004) 83(8):498-503.

4. Huarte E., Karbach J., Gnjatic S., Bender A., Jager D., Arand M., Atanackovic D., Skipper J., Ritter G., Chen Y.T., Old L.J., Knuth A., Jager E. HLA-DP4 expression and immunity to NY-ESO-1: correlation and characterization of cytotoxic CD4+ CD25- CD8- T cell clones. Cancer Immunity (2004) 4:15.

5. Laack E., Dickgreber N., Muller T., Knuth A., Benk J., Lorenz C., Gieseler F., Durk H., Engel-Riedel W., Dalhoff K., Kortsik C., Graeven U., Burk M., Dierlamm T., Welte T., Burkholder I., Edler L., Hossfeld D.K. Randomized phase III study of gemcitabine and vinorelbine versus gemcitabine, vinorelbine, and cisplatin in the treatment of advanced non-small-cell lung cancer: from the German and Swiss Lung Cancer Study Group. Journal of Clinical Oncology (2004) 22(12):2348-56.

6. Lienard D., Rimoldi D., Marchand M., Dietrich P.Y., van Baren N., Geldhof C., Batard P., Guillaume P., Ayyoub M., Pittet M.J., Zippelius A., Fleischhauer K., Lejeune F., Cerottini J.C., Romero P., Speiser D.E. Ex vivo detectable activation of Melan-A-specific T cells correlating with inflammatory skin reactions in melanoma patients vaccinated with peptides in IFA. Cancer Immunity (2004) 4:4.

7. Meidenbauer N., Zippelius A., Pittet M.J., Laumer M., Vogl S., Heymann J., Rehli M., Seliger B., Schwarz S., Le Gal F.A., Dietrich P.Y., Andreesen R., Romero P., Mackensen A. High frequency of functionally active Melan-a-specific T cells in a patient with progressive immunoproteasome-deficient melanoma. Cancer Research (2004) 64(17):6319-26.

8. Zippelius A., Batard P., Rubio-Godoy V., Bioley G., Lienard D., Lejeune F. Rimoldi D., Guillaume P., Meidenbauer N., Mackensen A., Rufer N., Lubenow N., Speiser D., Cerottini J.C., Romero P., Pittet M.J. Effector function of human tumor-specific CD8 T cells in melanoma lesions: a state of local functional tolerance. Cancer Research (2004) 64(8):2865-73.

9. Zippelius A., Bioley G., Le Gal F.A., Rufer N., Brandes M., Batard P., De Smedt M., Plum J., Speiser D.E., Cerottini J.C., Dietrich P.Y., Romero P., Pittet M.J. Human thymus exports naive CD8 T cells that can home to nonlymphoid tissues. Journal of Immunology (2004) 172(5):2773-7.

Reviews / Commentaries / Book Chapters

1. Jager D., Zippelius A., Knuth A. Identification of tumor antigens: strategies and perspectives. Schweiz Rundsch Med Prax (2004) 93(39):1584-8.

2. Jager D., Taverna C., Zippelius A., Knuth A. Identification of tumor antigens as potential target antigens for immunotherapy by serological expression cloning. Cancer Immunology and Immunotherapy (2004) 53(3):144-7.

3. Pestalozzi B.C., Knuth A. [New antibodies in cancer treatment]. Schweiz Rundsch Med Prax (2004) 93(39):1589-92.

4. Pestalozzi B.C., Jager D., Knuth A. [New drugs for colorectal cancer]. Schweiz Rundsch Med Prax (2004) 93(36):1455-9.

Cancer Vaccines

Clinical Trials - January 1st 2004 to December 31st 2004

HER-2

Trial: LUD1998-014

Title: Phase 1 Trial of Immunization with HER2-Derived Peptides in HLA-A2 Pateints with HER2-Expressing Cancers of the Breast, Ovary, Stomach or Lung

Principal Investigator: Drs. H. Shiku and Y. Nagata

Principal Sites: Mie University School of Medicine (LICR Mie Clinical Trials Center), and Nagasaki University Graduate School of Biomedical Sciences

Trial: LUD2001-016

Title: Immunization with cholesteryl hydrophobized polysaccharide-truncated HER2 protein 1-146 (CHP-HER2) complex in HLA-A24-positive patients with HER2-overexpressing cancers

Principal Investigator: Dr. H. Shiku

Principal Site: Mie University School of Medicine (LICR Mie Clinical Trials Center)

MAGE

Trial: LUD1995-004 (Closed for accrual)

Title: Pilot study of immunization with the MAGE-3.A2 Peptide in patients with malignant melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, esophageal squamous cell carcinoma or bladder carcinoma

Principal Investigator: Dr. M. Marchand

Principal Site: Clinique Universitaires Saint-Luc (LICR Brussels Branch), Belgium

Trial: LUD1997-004 (Closed for accrual)

Title: Phase I/II Study of High-Frequency and Prolonged Immunization with the MAGE-3.A1 and Mage-3.DP4 Peptides in Stage III/IV Patients with Malignant Melanoma

Principal Investigators: Drs. N. Van Baren, J. Cebon, A. Knuth and E. Jäger

Principal Sites: Clinique Universitaires Saint-Luc (LICR Brussels Branch), Austin Health (LICR Melbourne Branch), and Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Trial: LUD1999-003 (Closed for accrual)

Title: Ph. I/II study of i.d./s.c immunization with recombinant MAGE-3 protein in pts. with MAGE-3 positive, measurable non-visceral metastatic melanoma followed in selected patients by immunization with the Mage-3 protein combined with antigenic peptides

Principal Investigators: Drs. N. Van Baren and M. Marchand

Principal Site: Clinique Universitaires Saint-Luc (LICR Brussels Branch)

Trial: LUD1999-010 (Closed for accrual)

Title: Phase 2 (Immunological Efficacy) Trial of Immunization with ProteinD MAGE-3/His alone or combined with Immunological Adjuvant AS02B in Patients with Non-Small Cell Lung Cancer

Principal Investigator: Dr. N. Altorki (Principal Investigator; Cornell)

Principal Site: Weill Medical College of Cornell University, (LICR New York (Cornell) Clinical Trials Center)

Trial: LUD2002-001

Title: Phase 1/2 study of immunization with MAGE-3.A1 peptide associated with CpG 7909 used as immunological adjuvant in patients with measurable metastatic melanoma

Principal Investigator: Dr. N. Van Baren

Principal Site: Clinique Universitaires Saint-Luc (LICR Brussels Branch), Belgium

Trial: LUD2002-002 (Closed for accrual)

Title: Phase I/II study of i.m. immunization with the recombinant MAGE-3 protein mixed with an immunological adjuvant containing CpG/QS21/MPL (AS15) and combined with peptides, in patients with MAGE-3 positive, measurable metastatic cutaneous melanoma.

Principal Investigator: Dr. M. Marchand

Principal Site: Clinique Universitaires Saint-Luc (LICR Brussels Branch), Belgium

Trial: LUD2003-007

Title: Phase I/II study of immunization with multiple peptides associated with CpG7909 used as an immunological adjuvant in HLA-A2 patients with MAGE and/or LAGE positive evaluable metastatic cutaneous melanoma.

Principal Investigator: Dr. M. Marchand

Principal Site: Clinique Universitaire Saint-Luc (LICR Brussels Branch)

Melan-A/MART-1

Trial: LUD1996-010 (Closed for accrual)

Title: Phase I Study of Immunization with Melan-A Peptide, Influenza Matrix Peptide and Adjuvant SB AS-2 in HLA-A2 + Patients with High Risk Stage III or Stage IV Malignant Melanoma

Principal Investigators: Drs. M. Marchand and D. Liénard

Principal Sites: Clinique Universitaires Saint-Luc (LICR Brussels Branch), and Centre Hospitalier Universitaire Vaudois (LICR Lausanne Branch)

Trial: LUD2000-018

Title: Immunotherapy of HLA-A2 positive stage III / IV melanoma patients with CpG and Melan-A peptide (phase I study)

Principal Investigators: Drs. D. Speiser and D. Liénard

Principal Site: Centre Hospitalier Universitaire Vaudois (LICR Lausanne Branch)

Multiple Antigens

Trial: LUD2000-005 (Closed for accrual)

Title: A Phase I Study of Immunization with melanoma antigen in conjunction with GM-CSF in patients with fully resected stage 2, 3 or 4 malignant melanom

Principal Investigators: Drs. J. Cebon and I. Davis

Principal Site: Austin Health (LICR Melbourne Branch)

Trial: LUD2000-025 (Closed for accrual)

Title: Phase I Study of Peptide-based Vaccine Therapy in Patients with High Risk Metastatic Melanoma.

Principal Investigators: Dr. C. Hesdorffer

Principal Site: Columbia-Presbyterian Medical Center (LICR New York (Columbia) Affiliate Center)

Trial: LUD2001-003

Title: Vaccination of Patients with Stage III or IV Malignant Melanoma with Melanoma Antigen Peptides [Melan-A analog (ELA), NY-ESO-1b(A) and MAGE-A10] and Montanide adjuvant.

Principal Investigators: Drs. D. Speiser and D. Liénard

Principal Site: Centre Hospitalier Universitaire Vaudois (LICR Lausanne Branch)

Trial: LUD2003-003

Title: Pilot study of immunization with peptides of melanoma antigens following application of imiquimod cream in patients with resected stage II, III or IV malignant melanoma

Principal Investigator: Dr. I. Davis

Principal Site: Austin Health (LICR Melbourne Branch)

NY-ESO-1

Trial: LUD2001-017 (Closed for accrual)

Title: Evaluation of NY-ESO-1 immunity in patients who have previously been vaccinated with NY-ESO-1 protein

Principal Investigators: Drs. J. Cebon and I. Davis

Principal Site: Austin Health (LICR Melbourne Branch)

Trial: LUD2002-007

Title: A pilot study of NY-ESO-1b peptide plus CpG 7909 and Montanide ISA-51 in patients with cancer expressing NY-ESO-1 or LAGE-1.

Principal Investigators: Drs. N. Altorki and E. Jäger

Principal Sites: Weill Medical College of Cornell University, (LICR Cornell Clinical Trials Center) and Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Trial: LUD2000-009 (Closed for accrual)

Title: Phase 1 study of intensive course immunization with NY-ESO-1 derived peptides presented by MHC class I molecules alone and combined with GM-CSF in patients with advanced NY-ESO-1- or LAGE-expressing cancers

Principal Investigator: Dr. E. Jäger

Principal Site: Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Trial: LUD2000-014

Title: Phase 1 study of recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1) in patients with NY-ESO-1 or LAGE positive cancers

Principal Investigator: Dr. E. Jäger

Principal Site: Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Trial: LUD2000-024 (Closed for accrual)

Title: NY-ESO-1 peptide immunization of patients with cancer expressing NY-ESO-1 or LAGE antigen.

Principal Investigator: Dr. C. Hesdorffer

Principal Site: Columbia-Presbyterian Medical Center

Trial: LUD2000-026

Title: Phase 1 study of immunization with NY-ESO-1 derived peptides presented by MHC class I molecules administered alone or combined with polyarginine in patients with advanced NY-ESO-1- or LAGE-expressing cancers

Principal Investigator: Dr. E. Jäger

Principal Site: Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Trial: LUD2002-004

Title: NY-ESO-1 protein immunization of post-cystectomy patients with transitional cell carcinomas (TCC).

Principal Investigator: Dr. D. Bajorin

Principal Site: Memorial Sloan-Kettering Cancer Center (LICR New York Affiliate Center)

Trial: LUD2002-005

Title: Immunization of patients with tumors expressing NY-ESO-1 or LAGE antigen with a complex of NY-ESO-1 protein and cholesterol-bearing hydrophobized pullulan (CHP)

Principal Investigators: Drs. E. Nakayama and M. Monden

Principal Sites: Okayama University Medical School and Osaka University Graduate School of Medicine

Trial: LUD2002-006

Title: Safety and immunological evaluation of NY-ESO-1 Plasmid DNA (pPJV7611) Cancer Vaccine given by particle mediated epidermal delivery (PMED) in patients with NY-ESO-1 or LAGE-1 expressing non-small cell lung cancer.

Principal Investigator: Dr. N. Altorki (Principal Investigator; Cornell)

Principal Site: Weill Medical College of Cornell University, (LICR New York (Cornell) Clinical Trials Center)

Trial: LUD2002-011

Title: Pilot clinical trial of NY-ESO-1DP4 p157-170 (NY-ESO-1DP4), a peptide of potentially dual MHC class I and class II specificities, in pts. with epithelial ovarian, fallopian tube or primary peritoneal carcinoma whose tumors express NY-ESO-1 or LAGE-1

Principal Investigator: Dr. K. Odunsi

Principal Site: Roswell Park Cancer Institute (LICR Buffalo Affiliate Center)

Trial: LUD2002-012

Title: Phase 1 study of recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1) in patients with epithelial ovarian, fallopian tube or primary peritoneal carcinoma whose tumors express NY-ESO-1 or LAGE-1 antigen.

Principal Investigator: Dr. K. Odunsi

Principal Site: Roswell Park Cancer Institute (LICR Buffalo Affiliate Center)

Trial: LUD2002-013

Title: Clinical and immunological effects of NY-ESO-1 ISCOM® vaccine in patients with measurable stage III and IV malignant melanoma

Principal Investigator: Drs. J. Cebon and I. Davis

Principal Site: Austin Health (LICR Melbourne Branch)

Trial: LUD2002-014

Title: A Phase 1 study of NY-ESO-1b peptide plus Incomplete Freund's Adjuvant in patients with ovarian, primary peritoneal, or fallopian tube cancer expressing NY-ESO-1

Principal Investigator: Dr. J. Dupont

Principal Site: Memorial Sloan-Kettering Cancer Center (LICR New York Affiliate Center)

Trial: LUD2003-024

Title: Phase 1 study of immunization with NY-ESO-1 protein combined with CpG 7909 in patients with high-risk stage D1 or advanced prostate cancer expressing NY-ESO-1 and/or LAGE-1

Principal Investigators: Drs. A. Knuth and E. Jäger

Principal Sites: University Hospital Zürich (LICR Zürich Affiliate Center) and Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Miscellaneous

Trial: LUD1998-009

Title: Specific Immunotherapy of Skin Melanoma Patients with Antigenic Peptides and Immunological Analysis of the Vaccine Site Sentinel Lymph Node

Principal Investigator: Dr. D. Speiser

Principal Site: Centre Hospitalier Universitaire Vaudois (LICR Lausanne Branch)

Trial: LUD2000-002 (Closed for accrual)

Title: Phase II study of CpG Oligodeoxynucleotide 7909 in Patients with Advanced Renal Cell Carcinoma

Principal Investigators: Drs. J. Cebon and I. Davis

Principal Site: Austin Health (LICR Melbourne Branch)

Targeted Antibodies

3S193

Trial: LUD1997-010 (Closed for accrual)

Title: Phase I Trial of hu3S193 in Patients with Metastatic Breast Carcinoma

Principal Investigator: Dr. A. Scott

Principal Site: Austin Health (LICR Melbourne Branch)

Trial: LUD2001-018

Title: Single-dose, cohort study of increasing doses of yttrium-90 conjugated to humanized monoclonal antibody 3S193 (90Y-hu3S193) in patients with advanced ovarian cancer

Principal Investigator: Dr. C. Divgi

Principal Site: Memorial Sloan-Kettering Cancer Center (LICR New York Affiliate Center)

Trial: LUD2002-015

Title: Pilot study of hu3S193 in patients with small cell lung cancer espressing LeY antigen

Principal Investigator: Dr. L. Krug

Principal Site: Memorial Sloan-Kettering Cancer Center (LICR New York Affiliate Center)

806

Trial: LUD2004-001

Title: A phase 1 single dose escalation trial of ch806 in patients with advanced tumors expressing the 806 antigen.

Principal Investigator: Dr. A. Scott

Principal Site: Austin Health (LICR Melbourne Branch)

A33

Trial: LUD2001-011

Title: A Pilot Evaluation of Biology and Radioimmunodetection of 124Iodine-Labeled Humanized A33 Antibody in Patients with Colorectal Cancer

Principal Investigator: Dr. C. Divgi

Principal Site: Memorial Sloan-Kettering Cancer Center (LICR New York Affiliate Center)

Trial: LUD2002-017

Title: Phase 1 trial of oral capecitabine combined with 131I-huA33 in patients with metastic colorectal cancer

Principal Investigator: Dr. A. Scott

Principal Site: Austin Health (LICR Melbourne Branch)

G250

Trial: LUD2000-010 (Closed for accrual)

Title: A pilot study of G250 and low dose SC IL-2 in patients with advanced renal cancer

Principal Investigator: Dr. A. Scott

Principal Site: Austin Health (LICR Melbourne Branch)

Trial: LUD2001-010

Title: Biopsy based radioimmunotargeting study with iodine-124 labeled chimeric monoclonal antibody G250 (124I-cG250) in patients with biliary cancers

Principal Investigator: Dr. C. Divgi

Principal Site: Memorial Sloan-Kettering Cancer Center (LICR New York Affiliate Center)

Trial: LUD2001-014

Title: Phase 1/2 study of chimeric monoclonal antibody G250 (cG250) in combination with vinblastine administered weekly by intravenous infusion to patients with advanced renal cell carcinoma

Principal Investigators: Drs. A. Knuth and E. Jäger

Principal Sites: University Hospital Zürich (LICR Zürich Affiliate Center) and Krankenhaus Nordwest (LICR Frankfurt Affiliate Center)

Trial: LUD2003-006

Title: Phase 1 trial of 177Lu-DOTA-cG250 in patients with advanced renal cancer

Principal Investigator: Dr. W.J.G. Oyen

Principal Site: University Hospital Nijmegen

Dendritic Cell

Trial: LUD2003-013

Title: A Pilot Study of Peripheral Blood Dendritic Cells Pulsed with NY-ESO-1 ISCOM® in Patients with Treated Cancer and Minimal Residual Disease at High Risk of Relapse

Clinical Status: Open/Active

Principal Investigator: Dr. I. Davis

Principal Site: Austin Health (LICR Melbourne Branch)