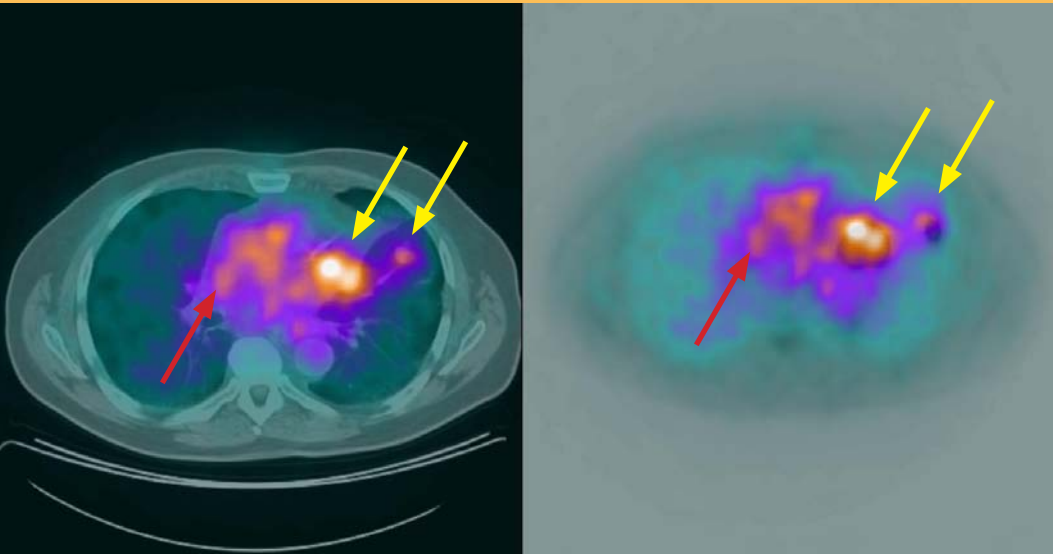




LUDWIG  
INSTITUTE  
FOR  
CANCER  
RESEARCH

## Antibody Targeting Program

Integrated program of laboratory and clinical research to establish the principles of selective tumor targeting and maximize the effectiveness of antibody therapies.



*Targeting by LICR humanized monoclonal antibody (mAb) 3S193 in a patient with small cell lung cancer detected by single photon emission computed tomography. The yellow arrows show the radiolabeled mAb targeting tumors in the lung. The red arrow indicates the blood pool in the heart. The white color shows that much more antibody is concentrated in the tumor than is circulating in the blood.*

# Targeted Antibodies

An antibody (a protein produced by the immune system) that targets a defined cancer antigen (a molecule recognized by the immune system) can be designed and produced in large quantities for cancer therapy. These antibodies are also called 'monoclonal antibodies' (mAbs).

Antibody-based therapies against cancer antigens or signalling molecules involved in the promotion of cancer cell growth currently represent one of the most promising areas in the development of new treatments for cancer.

## Targeted antibodies can be employed in several ways

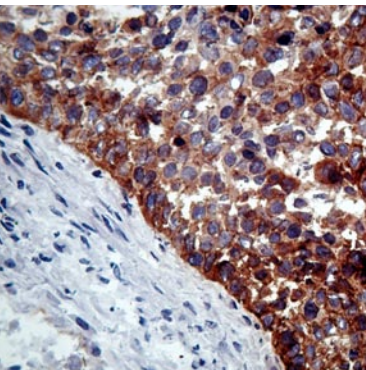
- Bind to antigen and thereby attract other components of the immune system to recognize and destroy mAb-covered tumor cells through antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), or phagocytosis (digestion by specialized cells);
- Bind to antigen and deliver cytotoxic ('cell-killing') molecules such as toxins or radioisotopes (radioactive molecules) for radioimmunotherapy (RIT);
- Bind to antigen to block vital cell functions (signal transduction antagonism) and/or induce apoptosis (programmed cell death);
- Bind to antigen to allow visualization of the primary tumor and metastatic spread for diagnostic and prognostic purposes. This approach utilizes radioactively labelled (radiolabeled) mAb and positron emission tomography (PET) or single photon emission computed tomography (SPECT) scanning to provide images of tumor localization.

# Antibody Generation & Preclinical Analysis

Antibodies in the LICR *Antibody Targeting Program* may be generated in several ways. One strategy is to use an immunogen (the entity that stimulates production, and determines specificity of a mAb) corresponding to a cancer antigen identified by the LICR's *Cancer Antigen Discovery Program*. Whole cancer cells may also be used as the immunogen as an alternative to first identifying specific antigens. The latter approach maximizes the chances of generating a mAb that is able to recognize subtle conformational differences between a molecule on a cancer cell and the same molecule on a normal cell.

Once the mAbs have been generated, they are tested for their ability to bind to tumor and normal tissues using an antibody-staining technique called immunohistochemistry. Those mAbs found to have good specificity for cancer cells are then comprehensively analyzed to determine their fine specificity, immunological effector function, and biochemical characteristics.

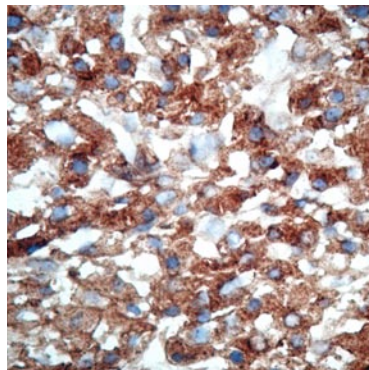
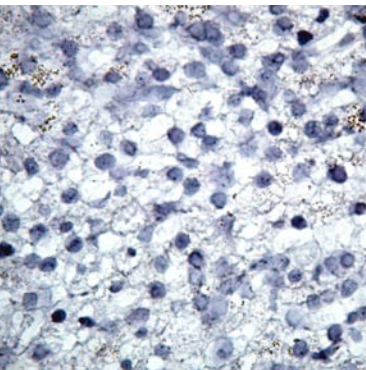
In addition to the six mAbs in clinical trials, the LICR *Antibody Targeting Program* has a portfolio of over 15 mAbs in the development pipeline.



*Left: Immunohistochemistry of a squamous cell carcinoma of the lung using LICR mAb 806 shows staining (brown color) of cells that overexpress (produce more copies than normal of) the epidermal growth factor receptor (EGFR). Non-cancerous cells that have normal levels or no EGFR are white or pale blue. The dark blue dots and ovals are the nuclei of the cells.*

*Right: The advantage of 806 over other anti-EGFR mAbs is clear from immunohistochemistry on normal liver samples: 806 does not target normal cells (near right), whereas other anti-EGFR mAbs do (far right).*

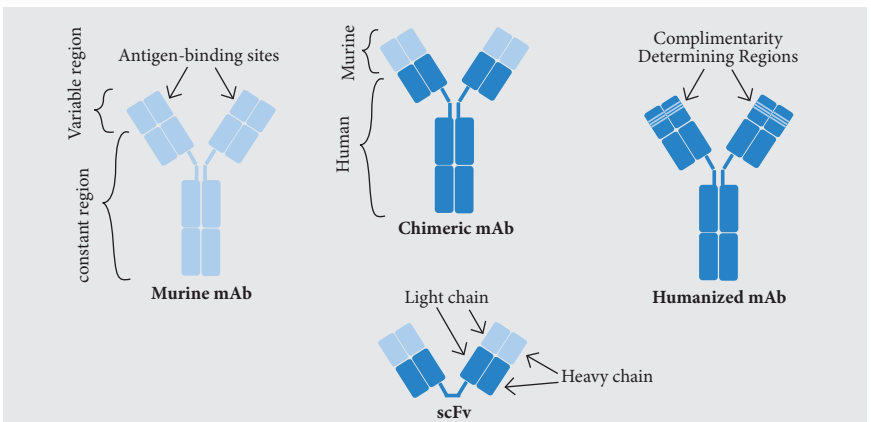
With the sequencing of the human genome, we now have the opportunity to construct a comprehensive picture of the cell surface antigens of normal and malignant cells and thus select the most promising targets for antibody-based therapies. To this end, the recently established *James R. Kerr Program* (which aims to foster interactions with investigators in countries that have had little opportunity for international collaboration in advanced cancer research) is adding great strength to LICR's existing target discovery efforts. Collaborations with investigators at the Institute of Molecular Biology and Genetics in Kyiv, Ukraine, and the Fourth Military Medical University in Xi'an, China are now accelerating our search for effective anti-cancer antibodies.



# Antibody Engineering

Because mAbs are frequently generated in a mouse cellular system, the basic protein structure of the antibody has a mouse sequence. Clinical investigation has taught us that these mAbs are immunogenic in humans, i.e. the patient's immune system produces secondary 'human anti-mouse antibodies' (HAMA) that bind and neutralize the injected mAb. Thus LICR mAbs that pass the first pre-clinical analyses for desired specificity must be re-engineered to produce a 'chimeric' mAb (part-human and part-mouse sequence) or a 'humanized' mAb (a mostly human variation of the original mouse mAb). In addition, mAbs with a fully human sequence can be produced using bacteriophage libraries or mice that have 'human' antibody genes.

The LICR *Antibody Targeting Program* engineers and produces several different antibody forms, including 'anti-idiotypic' mAbs (which recognize and bind to the targeted antibody for the validation of pharmacokinetic and immunogenicity assays), antibody fused to effector proteins ('fusion proteins'), and small 'single-chain' (scFv) antibody constructs (see 'Clinical Development').



# Antibody Production

LICR is one of very few academic organizations to have biological production facilities (BPFs) to produce mAbs and antibody fragments of sufficient quality and quantity to be given to patients. The BPF at the LICR Melbourne Branch utilizes mammalian (mouse and human cell) systems, while the LICR/Cornell University BPF utilizes non-mammalian (bacteria and yeast cell) systems. LICR has produced several 'current Good Manufacturing Practice' (cGMP) quality mAbs that are currently in clinical trials in Australia, Europe, Japan and the USA (see next pages). Two radiochemistry and chelation laboratory programs have also been established to provide clinical reagents for LICR mAb trials.



# LICR First in Man Antibody Investigation

LICR utilizes its own clinical investigation model to efficiently and comprehensively evaluate the potential of a mAb for clinical development. The objective is to acquire as much information as possible in a single clinical trial about the mAb's safety, immunogenicity, targeting, pharmacokinetics and anti-tumor activity.

## Safety

The health and welfare of patients following antibody transfusions are key concerns of all clinical trials. All adverse events experienced by patients while on study are reported to the central LICR Office of Clinical Trials Management and assessed for relationship to the therapy.

## Immunogenicity

Blood samples are tested for the presence of secondary antibodies - human anti-human antibodies (HAHA), or human anti-chimeric antibodies (HACA) - that indicate the mAb is immunogenic. HAHA and HACA build up over time, and are thus most effectively monitored using a multiple infusion and testing strategy such as that employed by LICR.

## Targeting

Utilization of a trace-labeled mAb enables the visualization of tumor targeting and distribution to normal tissues, and the duration of mAb binding (see figure at right). In addition, the rate of clearance of a second injection of trace-labelled mAb after completing a course of antibody treatment provides another way to assess immunogenicity. More rapid clearance indicates that the mAb has elicited an immune reaction and is therefore unlikely to be of clinical value.

## Pharmacokinetics

The mAb protein concentration in blood is used to calculate half-life, body clearance, volume of distribution and other pharmacokinetic parameters, which will be used to establish dose and delivery.

## Tumor Response

The tumor response to mAb treatment is evaluated in accordance with the accepted standards of clinical practice and patient care.



**Whole body image following infusion of radiolabeled humanized mAb A33.** Targeting of a metastatic lesion in the liver by mAb A33 is clearly seen (black arrow), with minor bowel uptake (red arrow) also visible.

# Clinical Development

To optimize targeting and therapeutic efficiency, preclinical and clinical investigations are addressing the many variables involved in evolving successful antibody-based therapies:

## Antibody Variants

The LICR *Antibody Targeting Program* is assessing the therapeutic potential of chimeric, humanized, and scFv antibodies. The smaller scFv constructs may be better suited to the delivery of particular isotopes/toxins to certain tumors. In addition, antibody-cytokine fusion proteins have been constructed as a way to deliver inflammatory or immunomodulatory cytokines, eg tumor necrosis factor (TNF) to the tumor site.

## Isotopes/Toxins

LICR is assessing several different radioisotopes for their potential for RIT, or for diagnostic imaging using PET and SPECT. Different isotopes have different physical properties making them better suited to therapy or diagnostic imaging depending on the half-life of the mAb and the radioisotope. The therapeutic potential of selected drugs and toxins is also being investigated.

## Antibody Fate

The first objective of LICR's *Antibody Targeting Program* is to identify antibodies with high selectivity for tumor cells, no immunogenicity, and excellent tumor targeting characteristics in humans. Much attention is now being focused on the fate of the different antibodies in the LICR portfolio after they bind to the tumor cell surface. Some

antibodies remain on the cell surface for extended periods, others are promptly taken up by the cell, in a process called internalization, and directed to various cellular compartments. Understanding the fate of the antibody after localization to the tumor will help define the optimal therapeutic strategies for different antibodies and antibody constructs.

	<b>Tumor Types</b>	<b>Clinical Trials (Active and Planned)</b>
<b>Humanized A33</b> targets differentiation antigen on colonocytes	Colorectal	mAb alone mAb + chemotherapy RIT RIT + chemotherapy PET imaging
<b>Chimeric G250</b> targets carbonic anhydrase	Renal	mAb alone mAb + chemotherapy RIT Ab-cytokine fusion protein PET imaging
<b>Humanized F19</b> targets FAP $\alpha$ in stroma, the connective tissue around tumor cells	Breast Colon Non-small cell lung Head and neck	mAb alone RIT Combined stromal mAb + cancer cell mAb
<b>Chimeric KM871</b> targets GD3 ganglioside	Melanoma	mAb alone mAb + chemotherapy mAb + interferon
<b>Humanized 3S193</b> targets Le <sup>y</sup> blood group antigen	Breast Colon Small cell lung Ovarian	mAb alone mAb + chemotherapy RIT PET imaging
<b>Humanized LK26</b> targets folate binding protein	Ovarian	mAb alone
<b>Chimeric 806</b> targets overexpressed EGFR and $\Delta 2-7$ EGFR	SCC (head and neck, lung, esophageal) Glioma	mAb alone mAb + EGFR inhibitors

**Legend:** mAb = monoclonal antibody; RIT = radio immunotherapy; PET = positron emission tomography; EGFR = epidermal growth factor receptor; SCC = squamous cell carcinoma

# Antibody Targeting Program Centers

## Asia

- Drs. Junichi Sakamoto, Kyoto University, and Keigo Endo, Gunma University, Gunma, Japan
- Dr. Bo-Quan Jin, Fourth Military Medical University, Xi'an, China

## Australasia

- Drs. Andrew Scott, F.T. Lee, Terrance Johns and Antony Burgess, LICR Melbourne Branch for Tumour Biology, Melbourne, Australia
- Drs. David Macfarlane and David Wyld, Royal Brisbane and Women's Hospital, Brisbane, Australia

## Europe

- Dr. Elke Jäger, Krankenhaus Nordwest, Frankfurt, Germany
- Drs. Christoph Renner and Michael Pfreundschuh, Saarland University, Homburg, Germany
- Drs. Ivan Gout and Valeriy Filonenko, Institute of Molecular Biology and Genetics, Kyiv, Ukraine
- Dr. Aaron Goldhirsch, European Institute of Oncology, Milan, Italy
- Drs. Egbert Oosterwijk, Wim Oyen and Franz Corstens, University Hospital, Nijmegen, Netherlands
- Drs. Alexander Knuth and Dirk Jäger, University Hospital Zürich, Zürich, Switzerland

## North America

- Drs. Gerd Ritter, Achim Jungbluth and Lloyd Old, LICR New York Branch for Cancer Immunology at Memorial Sloan-Kettering Cancer Center, New York, USA
- Drs. Chaitanya Divgi, Steven Larson, Lee Krug, Peter Smith-Jones, Joseph O'Donohoe, Ron Finn, and John Humm, Memorial Sloan-Kettering Cancer Center, New York, USA
- Dr. Carl Batt, Cornell University, Ithaca, USA
- Dr. Ira Mellman, Yale University, New Haven, USA
- Drs. Frank Furnari and Webster K. Cavenee, LICR San Diego Branch of Cancer Genetics, San Diego, USA



### **Program Director**

Lloyd J. Old, M.D.

### **Associate Directors**

Chaitanya Divgi, M.D., Gerd Ritter, Ph.D., Andrew M. Scott, M.D.

### **Director, LICR Office of Clinical Trials Management**

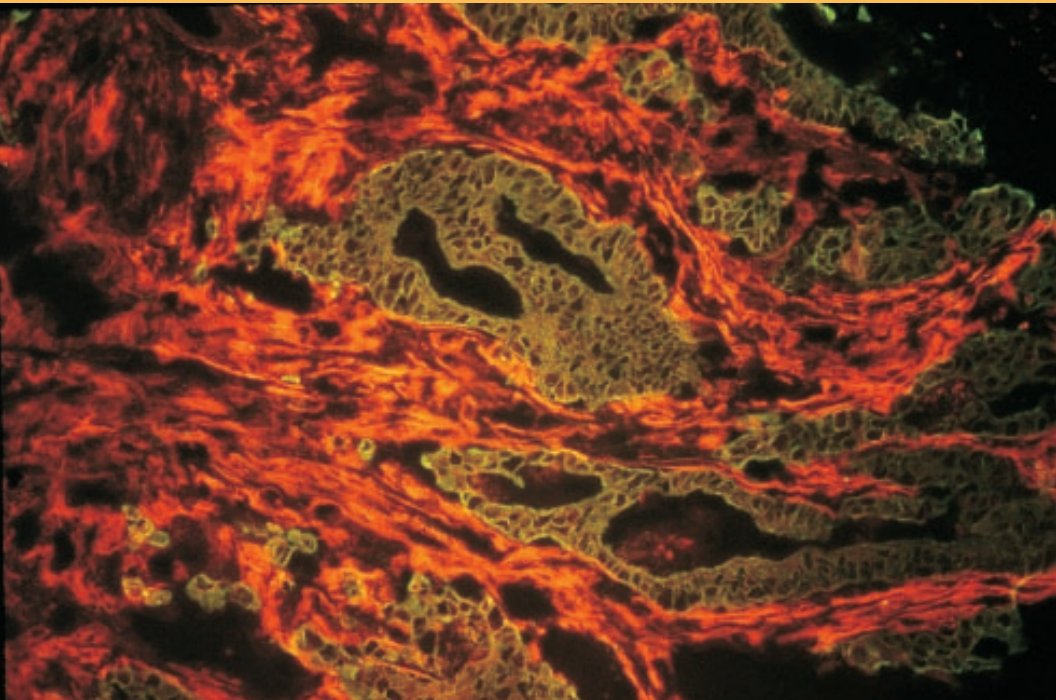
Eric W. Hoffman, Pharm.D.

### **Director, LICR Office of Protocol Review**

Herbert F. Oettgen, M.D.

# LICR Firsts/Successes

- Six active LICR investigational new drug applications (IND) for mAbs
- Two in-house BPFs for mAbs used in clinical studies
- Radiochemistry and chelation laboratory programs for labeling of mAbs for clinical trials
- In-house *in vitro* and animal model preclinical testing
- Six first in man mAb studies
- Single and fractionated dose RIT
- Clinical study of mAb targeting demonstrated by PET scanning and confirmed by biopsy
- Integration of tumor metabolism by PET imaging with mAb targeting
- Combination treatment of mAb with other therapies
- Demonstration of specific targeting of stroma by a mAb in epithelial cancers
- Multiple collaborations with other academic organizations and with pharmaceutical and biotechnology companies.



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



**New York Office**

605 Third Ave

New York, NY 10158

Tel: +1 212 450 1500

Fax: +1 212 450 1555

[www.licr.org](http://www.licr.org)