We have 80,000 R&D centres like this one.

Roche develops and produces highly effective treatments and diagnostic tools for diseases such as cancer, hepatitis, diabetes and rheumatoid arthritis.

Our innovations help millions of people by alleviating their suffering and improving their quality of life. We give them hope.
Table of Contents

**Roche/Nature Medicine 2011 Symposium on Cancer Immunology and Immunotherapy**

Roche, Nutley, New Jersey, USA

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome</td>
<td>2</td>
</tr>
<tr>
<td>Scientific Committee</td>
<td>3</td>
</tr>
<tr>
<td>Sponsors</td>
<td>3</td>
</tr>
<tr>
<td>Awards</td>
<td>4</td>
</tr>
<tr>
<td>Meeting Program</td>
<td>5</td>
</tr>
<tr>
<td>Speaker Biographies</td>
<td>7</td>
</tr>
<tr>
<td>Speaker Abstracts</td>
<td>15</td>
</tr>
<tr>
<td>Posters, Presenting Authors and Abstracts</td>
<td>33</td>
</tr>
<tr>
<td>Attendees</td>
<td>81</td>
</tr>
<tr>
<td>Roche’s Commitment to the Future</td>
<td>90</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>92</td>
</tr>
<tr>
<td>Notes</td>
<td>93</td>
</tr>
</tbody>
</table>
Welcome

Dear Colleagues,

On behalf of Roche’s Pharma Research and Early Development organization and Nature Medicine, it is our genuine pleasure to welcome you to Roche Nutley for the first Roche/Nature Medicine Symposium on Cancer Immunology and Immunotherapy.

This symposium brings together internationally renowned experts to present recent advances in cancer immunotherapy that are making tremendous strides in the clinic, as well as groundbreaking results in tumor immunology that will pave the way to new cancer treatments. We will discuss the tremendous progress in the development of molecular and cellular cancer immunotherapies, as well as the role of innate and adaptive immunity in both promoting and arresting cancer, with the aim of identifying new avenues for future clinical gains.

During the symposium we will also present the first Roche Award for Cancer Immunology and Immunotherapy to recognize the contributions of an outstanding scientist in this field. The award will honor a scientist whose research has generated seminal insights into tumor immunology and immunotherapy that are expected to lead to clinical advances and therapeutic benefit to cancer patients. We will also be presenting awards for posters that are considered to have had the largest impact within the theme of this symposium.

We hope that this symposium offers you a rewarding forum for the exchange of knowledge and ideas. We urge you to share your perspectives and challenge those of others; to embrace a Socratic approach to further illuminate our way forward in this exciting field of research. We also encourage you to take advantage of this opportunity to forge new collaborations and renew old acquaintances. In addition, please enjoy the hospitality of our Roche and Nature Medicine hosts.

We look forward to meeting you personally,

Sincerely,

Jacques Banchereau
Global Head for Inflammation and Virology Discovery & Translational Areas and Chief Scientific Officer, Nutley, Pharma Research and Early Development (pRED), Roche

Mike Burgess
Global Head for Oncology Discovery & Translational Area and Head of Large Molecule Research, Pharma Research and Early Development (pRED), Roche

Alison Farrell
Senior Editor
Nature Medicine

Juan-Carlos Lopez
Chief Editor
Nature Medicine
Scientific Committee

Jacques Banchereau  
Mike Burgess  
Alison Farrell  
Juan-Carlos Lopez

Sponsors

Headquartered in Basel, Switzerland, Roche is a leader in research-focused healthcare with combined strengths in pharmaceuticals and diagnostics. Roche is the world’s largest biotech company with truly differentiated medicines in oncology, virology, inflammation, metabolism and CNS. Roche is also the world leader in in-vitro diagnostics, tissue-based cancer diagnostics and a pioneer in diabetes management. Roche’s personalised healthcare strategy aims at providing medicines and diagnostic tools that enable tangible improvements in the health, quality of life and survival of patients. In 2010, Roche had over 80,000 employees worldwide and invested over 9 billion Swiss francs in R&D. The Group posted sales of 47.5 billion Swiss francs. Genentech, United States, is a wholly owned member of the Roche Group. Roche has a majority stake in Chugai Pharmaceutical, Japan. For more information: www.roche.com.

*Nature Medicine* is a biomedical research journal devoted to publishing the most exciting advances in biomedical research for scientists and physicians. Articles cover fields such as cancer biology, cardiovascular research, gene therapy, immunology, vaccine development, and neuroscience, aiming to keep PhD and MD readers informed of a wide range of biomedical research findings. Original research articles published in *Nature Medicine* range from basic findings that have clear implications for disease pathogenesis and therapy to the earliest phases of human investigation. Additional information is available at www.nature.com/nm
The Roche Award for Cancer Immunology and Immunotherapy

The Roche Award for Cancer Immunology and Immunotherapy recognizes an outstanding scientist who has contributed innovative and groundbreaking research in the field of cancer immunology and immunotherapy leading to therapeutic benefit to patients.

The recipient will be an established investigator:

• Who is internationally recognized in the field of cancer immunology and immunotherapy and who has published groundbreaking research in the areas of immunology, cancer immunotherapy and in the translation of preclinical experimental models to the clinic.

• Who is a major contributor to developing or applying novel immunotherapeutics that have or are expected to enhance the treatment of cancer patients.

• Whose contributions are expected to have a major influence on the understanding of the role of the immune system in the development, maintenance and possible treatment of cancer.

The winner of this award receives a $25,000 prize from Roche.

Note that the recipient is selected by the Scientific Committee of the Roche/Nature Medicine Symposium on Cancer Immunology and Immunotherapy.

Symposium Poster Award

We will also recognize three posters at the symposium that will be considered to have had the largest impact within the theme of the Roche/Nature Medicine Symposium on Cancer Immunology and Immunotherapy.

Prizes of $2,500, $1,000 and $500 will be awarded for the three posters selected by a panel of the Symposium speaker faculty.
# Meeting Program

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>Sunday</td>
<td>4:00</td>
<td>Conference Registration at The W Hoboken</td>
</tr>
<tr>
<td></td>
<td>5:30</td>
<td>Welcome Reception at The W Hoboken</td>
</tr>
<tr>
<td>Day One</td>
<td></td>
<td>Buses load at The W Hoboken. Depart for Nutley</td>
</tr>
<tr>
<td>Monday</td>
<td>7:30</td>
<td>Registration and Breakfast</td>
</tr>
<tr>
<td></td>
<td>8:00</td>
<td>Welcome</td>
</tr>
<tr>
<td></td>
<td>9:15</td>
<td>Keynote Lecture</td>
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<tr>
<td></td>
<td>9:30</td>
<td>In the footsteps of Virchow: Lymphocyte-produced cytokines in tumor cell development and responses to therapy</td>
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<td></td>
<td></td>
<td>Michael Karin, University of California, San Diego</td>
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<td></td>
<td>10:20</td>
<td>Session 1: Role of Innate Immunity in Cancer</td>
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<tr>
<td></td>
<td>10:20</td>
<td>The yin-yang of cancer related inflammation</td>
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<tr>
<td></td>
<td>10:20</td>
<td>Alberto Mantovani, University of Milan</td>
</tr>
<tr>
<td></td>
<td>10:55</td>
<td>Coffee Break</td>
</tr>
<tr>
<td></td>
<td>11:20</td>
<td>Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells</td>
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<tr>
<td></td>
<td>11:20</td>
<td>Dmitry Gabrilovich, H. Lee Moffitt Cancer Center</td>
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<tr>
<td></td>
<td>11:55</td>
<td>Monocyte and macrophage diversity promotes tumor progression and metastasis</td>
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<tr>
<td></td>
<td>11:55</td>
<td>Jeffrey Pollard, Albert Einstein College of Medicine</td>
</tr>
<tr>
<td></td>
<td>12:30</td>
<td>Alternately spliced 1CF/NKp30/NCR3 isoforms dictate the prognosis of localized and metastatic gastrointestinal sarcoma (GIST)</td>
</tr>
<tr>
<td></td>
<td>12:30</td>
<td>Laurence Zitvogel, Institute Gustave Roussy</td>
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<tr>
<td></td>
<td>1:05</td>
<td>Lunch</td>
</tr>
<tr>
<td></td>
<td>2:20</td>
<td>Session 2: Molecular Advances in Immunotherapy</td>
</tr>
<tr>
<td></td>
<td>2:20</td>
<td>Immune checkpoint blockade in cancer therapy: New insights and opportunities</td>
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<tr>
<td></td>
<td>2:20</td>
<td>James Allison, Howard Hughes Medical Institute / Memorial Sloan-Kettering Cancer Center</td>
</tr>
<tr>
<td></td>
<td>2:55</td>
<td>Blockade of immunologic checkpoints in cancer therapy: The PD/1-B7 – H1 axis</td>
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<tr>
<td></td>
<td>2:55</td>
<td>Suzanne Topalian, Johns Hopkins University School of Medicine</td>
</tr>
<tr>
<td></td>
<td>3:30</td>
<td>Coffee Break</td>
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<tr>
<td></td>
<td>4:00</td>
<td>Engineered anti cancer antibodies with enhanced immune effector functions</td>
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<td></td>
<td>4:00</td>
<td>Pablo Umaña, Pharma Research and Early Development, Roche Glycart</td>
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<tr>
<td></td>
<td>4:35</td>
<td>Genetically engineered receptors and adoptive cell therapies</td>
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<tr>
<td></td>
<td>4:35</td>
<td>Carl June, University of Pennsylvania</td>
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<tr>
<td></td>
<td>5:10</td>
<td>Poster Session and Reception</td>
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<tr>
<td></td>
<td>6:30</td>
<td>Dinner</td>
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<tr>
<td></td>
<td>7:30</td>
<td>Transport from Nutley to The W Hoboken</td>
</tr>
</tbody>
</table>
# Meeting Program

## Day Two

**Tuesday**

**September 13**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:45</td>
<td>Buses load at The W Hoboken. Depart for Nutley</td>
</tr>
<tr>
<td>8:30</td>
<td><strong>Breakfast</strong></td>
</tr>
<tr>
<td>9:30</td>
<td><strong>Session 3: Pro-Versus Antitumorigenic Actions of the Immune System</strong></td>
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<tr>
<td></td>
<td>Chair: Alison Farrell, <em>Nature Medicine</em></td>
</tr>
<tr>
<td>9:30</td>
<td><strong>Deconstructing cancer immunoediting</strong></td>
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<tr>
<td></td>
<td>Robert Schreiber, <em>Washington University School of Medicine</em></td>
</tr>
<tr>
<td>10:05</td>
<td><strong>Immune responses to newly identified leukemia antigens in relation to clinical responses to a therapeuti</strong></td>
</tr>
<tr>
<td></td>
<td>c vaccine for chronic myelogenous leukemia (CML)**</td>
</tr>
<tr>
<td></td>
<td>Hy Levitsky, Pharma Research and Early Development, <em>Roche</em></td>
</tr>
<tr>
<td>10:40</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>11:10</td>
<td><strong>CD40 and immunosurveillance in pancreatic carcinoma in mice and humans</strong></td>
</tr>
<tr>
<td></td>
<td>Robert Vonderheide, <em>Abramson Family Cancer Research Institute at the University of Pennsylvania</em></td>
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<tr>
<td>11:15</td>
<td><strong>Interleukin-15: A new oncogene?</strong></td>
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<tr>
<td></td>
<td>Michael Caligiuri, <em>Ohio State University Comprehensive Cancer Center</em></td>
</tr>
<tr>
<td>12:20</td>
<td><strong>Lunch</strong></td>
</tr>
<tr>
<td>1:30</td>
<td><strong>Session 4: Cellular Advances in Immunotherapy</strong></td>
</tr>
<tr>
<td></td>
<td>Chair: Jim Cassidy, <em>Roche</em></td>
</tr>
<tr>
<td>1:30</td>
<td><strong>Reprogramming the immune environment in cancer via dendritic cells</strong></td>
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<tr>
<td></td>
<td>Karolina Palucka, <em>Baylor Institute for Immunology Research</em></td>
</tr>
<tr>
<td>2:05</td>
<td><strong>A human memory T-cell subset with stem cell-like properties</strong></td>
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<tr>
<td></td>
<td>Nick Restifo, <em>National Cancer Institute, National Institutes of Health</em></td>
</tr>
<tr>
<td>2:40</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>3:10</td>
<td><strong>The interplay of inflammation and tumor immunity</strong></td>
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<tr>
<td></td>
<td>Glenn Dranoff, <em>Dana-Farber Cancer Institute</em></td>
</tr>
<tr>
<td>3:45</td>
<td><strong>Award Presentations</strong></td>
</tr>
<tr>
<td>4:00</td>
<td><strong>Closing Remarks</strong></td>
</tr>
<tr>
<td>4:15</td>
<td>Buses load and depart for The W Hoboken</td>
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</tbody>
</table>
Speaker Biographies

**Jim Allison** studies the regulation of immune responses by a type of lymphocyte called T-cells, and the development of novel strategies of manipulating those responses to attack tumor cells. His work over the last several years has shown that the process is considerably more complex than originally thought, and involves integration of at least three signals: activating signals generated by recognition of specific antigens by the antigen receptor; costimulatory signals generated by engagement of CD28 on the T-cell surface, and competing inhibitory signals mediated by the CD28 homologue CTLA-4. He has shown that blockade of the inhibitory signals of CTLA-4 can greatly enhance immune responses, including those directed against tumors. CTLA-4 blockade is currently in clinical trials and objective responses have been observed in melanoma as well as prostate, ovarian, renal, and lung cancer. A recently published trial of CTLA-4 blockade as second line therapy in metastatic melanoma showed a significant survival benefit, the first drug of any type to do so in a randomized phase-III trial. The FDA recently approved this drug (Ipilimumab) for the treatment of late-stage melanoma. This work has established immune checkpoint blockade of CTLA-4 and other inhibitory molecules as a promising strategy for the treatment of cancer.

Allison received his PhD from the University of Texas, Austin, and after postdoctoral training at Scripps Clinic in La Jolla, California, he returned to Texas and joined the faculty of the University of Texas System Cancer Center, Science Park, in Smithville. He then moved to the University of California, Berkeley, where he remained for 20 years, holding the positions of Director of the Cancer Research Laboratory, Director of the Immunology Program, and Co-Chair of the Department of Immunology. In 2004 he moved to Memorial Sloan-Kettering Cancer Center where he is the Chair of the Immunology Program and Director of the Ludwig Center for Cancer Immunotherapy.

Allison is a member of the National Academy of Sciences and the Institute of Medicine.

**Michael A. Caligiuri**, MD, is the director of The Ohio State University Comprehensive Cancer Center, chief executive officer of the James Cancer Hospital and Solove Research Institute, and holds the John L. Marakas Nationwide Insurance Enterprise Foundation Chair in Cancer Research. He is a professor in the departments of Internal Medicine and Molecular Virology, Immunology and Medical Genetics.

Dr. Caligiuri’s research focuses on the molecular biology of leukemia, vaccine development to prevent lymphoma, and natural killer cell biology. More than 1,000 patients have been entered in his clinical trials in leukemia and lymphoma. He has authored more than 225 original scientific publications, holds numerous patents, and has trained over 100 students in his laboratory. He is an elected member of the American Association for Clinical Investigation and the American Association of Physicians, and he is an elected Fellow in the American Association for the Advancement of Science. In 2009, Dr. Caligiuri began serving a two-year term as president of the Association for Cancer Research Institutes. He is the chair of the AACR Publications Committee, an associate editor of the journal *Blood,* and he sits on the editorial boards of *Clinical Cancer Research* and *Molecular Cancer Therapeutics.* Dr. Caligiuri is on numerous national committees, including the National Cancer Institute’s Board of Scientific Advisors. In 2010, Dr. Caligiuri was one of four individuals in the country to receive a MERIT award from the National Cancer Institute for his work on immunity and cancer.
Speaker Biographies

**Dmitry Gabrilovich**, MD PhD, is a Senior Faculty Member at the Moffitt Cancer Center. He holds the Robert Rothman Endowed Chair in Cancer Research and is the Head for the Section of Dendritic Cell Biology. Dr. Gabrilovich received his medical degree from Kabardino-Balkarian State University Medical School, Nalchik, Russia. He received his doctorate in Immunology from the Central Institute for Epidemiology in Moscow, Russia. He completed post-doctoral training at Imperial College, London, and at the University of Texas Southwestern Medical Center, Dallas. Dr. Gabrilovich has published extensively in the field of tumor immunology and dendritic cell biology. Dr. Gabrilovich and his colleagues are focused on understanding the cellular and molecular mechanisms of tumor-associated immunosuppression, the role of myeloid cells in cancer, and on the development of new and effective cancer vaccines. Several of their findings are currently testing in clinical trials. Dr. Gabrilovich has trained numerous postdoctoral fellows throughout his career.

**Glenn Dranoff**, MD, received his AB in chemistry from Duke University and his MD from Duke University Medical School, where he studied cancer chemotherapy with Drs. Gertrude Elion and Darell Bigner. He served as intern and resident in Internal Medicine at the Massachusetts General Hospital and was a Clinical Fellow in Medical Oncology at the Dana-Farber Cancer Institute. He was a post-doctoral fellow in genetics with Dr. Richard C. Mulligan at the Whitehead Institute for Biomedical Research.

Dr. Dranoff is Professor of Medicine at Dana-Farber Cancer Institute and Harvard Medical School. He is the Leader of the Dana-Farber/Harvard Cancer Center Program in Cancer Immunology, a Co-Leader of the Dana-Farber Cancer Vaccine Center, and the Director of the Human Gene Transfer Laboratory at Dana-Farber. Work in his laboratory has given rise to multiple clinical protocols that have defined the biologic activity of several different cancer immunotherapies in patients with solid or hematologic malignancies.
Speaker Biographies

**Carl June**
Professor, Department of Pathology and Laboratory Medicine  
Director, Translational Research Programs, Abramson Cancer Center  
University of Pennsylvania School of Medicine

Dr. June is a 1975 graduate of the Naval Academy in Annapolis, and a graduate of Baylor College of Medicine in Houston, 1979. He had graduate training in Immunology at the World Health Organization, Geneva, Switzerland from 1978-79, and post-doctoral training in transplantation biology at the Fred Hutchinson Cancer Research Center in Seattle from 1983 – 1986. He is board certified in Internal Medicine and Medical Oncology. He was a faculty member in the Departments of Medicine and Cell and Molecular Biology at the Uniformed Services University for the Health Sciences in Bethesda from 1987 to 1998. He is a member of the American Academy of Physicians, and a recipient of the Bristol-Myers Squibb Freedom to Discover Award. Since moving to the University of Pennsylvania in 1999 as a tenured Professor of Pathology and Laboratory Medicine, he has established a facility to produce experimental cell based therapeutics. Currently, Dr. June is involved with several clinical trials that are testing various forms of cell-based therapies for cancer and HIV infection.

**Michael Karin** received his BSc in Biology in 1975 at Tel Aviv University, Tel Aviv, Israel and his PhD in Molecular Biology in 1979, at the University of California, Los Angeles. Dr. Karin is currently a Distinguished Professor of Pharmacology and Pathology at the School of Medicine, University of California, San Diego, where has been on the faculty since 1987. He was a cofounder of Signal Pharmaceutical (currently Celgene) and had served as a member of its Scientific Advisory Board. Dr. Karin also served as a member of the National Advisory Council for Environmental Health Sciences and has been an American Cancer Society Research Professor since 1999. Dr. Karin was elected as a member of the US National Academy of Sciences in 2005 and as an associate member of the European Molecular Biology Association in 2007. He is a leading world authority on signal transduction pathways that regulate gene expression in response to extracellular stimuli, infection, inflammation and stress. Key achievements include the definition of cis elements that mediate gene induction by hormones, cytokines and stress, identification and characterization of the transcription factors that recognize these elements (members of the AP-1/ATF family) and the protein kinase cascades that regulate their activities, including the Jun kinases (JNK) and I B kinases (IKK). Much of Dr. Karin’s current activity is focused on understanding the link between inflammation, cancer and metabolic disease as well as on understanding the signaling mechanisms used by receptors involved in inflammation and innate immunity. In addition to establishing molecular links between obesity, inflammation and cancer, this work has revealed new targets for cancer prevention and therapy. Dr. Karin has published over 300 scientific articles and is an inventor on over 30 different patents or pending patent applications. In addition to numerous honors, Dr. Karin was ranked first worldwide by the Institute of Scientific Information (ISI) in a listing of most-cited molecular biology and genetic research papers published in prestigious journals.
Speaker Biographies

Hy Levitsky joined Roche in September as head of Cancer Immunology Experimental Medicine. He had been Professor of Oncology, Medicine and Urology at The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. Dr. Levitsky’s laboratory research has focused on basic studies of antigen processing and presentation, T-cell co-stimulation, T-cell priming versus tolerance, and the evolution of tumor-specific immunity during immune reconstitution. He is a co-inventor of genetically modified GM-CSF secreting tumor cell vaccines (GVAX) and has pioneered their development in the treatment of acute and chronic myeloid leukemia, and multiple myeloma. At Johns Hopkins, Dr. Levitsky was the Scientific Director of the George Santos Bone Marrow Transplant Program, where he has led the development of therapeutic cancer vaccines administered during immune reconstitution following high dose chemotherapy and stem cell transplantation. Dr. Levitsky has received numerous awards for his research, including the Stohlman Scholars Award from the Leukemia and Lymphoma Foundation of America, The Senior Research Award from the Multiple Myeloma Research Foundation, Research Awards from the CapCURE Foundation, The American Cancer Society and the National Institutes of Health. In 2001, he was elected to the American Society for Clinical Investigation. Dr. Levitsky received his undergraduate education at the University of Pennsylvania, and his medical degree at the Johns Hopkins University School of Medicine.

Alberto Mantovani was born in Milan in 1948 where he graduated in Medicine in 1973. After specializing in oncology, he has worked in England at the Chester Beatty Research Institute, London (1975-1976) and the United States, National Institutes of Health (1978-1979 and 1985-1986) and as Head of the Department of Immunology and Cell Biology of the Mario Negri Institute for Pharmacological Research in Milan (1996-2005). Professor of Pathology at the Faculty of Medicine, and Vice Dean for Research, University of Milan. Scientific Director of Istituto Clinico Humanitas since October 2005 and President of the Humanitas Foundation for Research, which supports basic and clinical research in the field of immunology and its applications in the treatment of chronic inflammatory and autoimmune diseases, oncology, gastroenterology, cardiovascular and neurological diseases.

From 2007 to 2010 he served in The Board of Global Alliance for Vaccine Immunization. He was awarded national and international prizes for his scientific contributions. He is one of or the, most quoted and /or productive Italian scientist(s). The Institute for Scientific Information (ISI) ranked him as one of the 100 most quoted immunologists in the world during the last 20 years of the 20th century. As of Spring 2011 he has had over 46,000 citations and an H index of 109 (WOS).
Speaker Biographies

**A. Karolina Palucka**, MD PhD, is an Investigator at the Baylor Institute for Immunology Research (BIIR) in Dallas. She has also been a part-time Investigator at Mount Sinai School of Medicine (MSSM) since January 2009. In April 2004, she was awarded the M. Ramsay Chair for Cancer Immunology Research. Dr. Palucka obtained her MD degree from the Warsaw Medical Academy in Poland. She obtained her PhD in tumor immunology from the Karolinska Institute in Stockholm (Sweden) and completed her postdoctoral training in dendritic-cell biology at the Immunology Laboratory of the Hospital Pitie-Salpetriere in Paris. She is an expert in dendritic cell biology, which she has been studying for more than fifteen years. Her main focus is the biology of dendritic cells in cancer and their utilization as vectors for therapeutic vaccination. She established the GMP facility for vaccine generation at BIIR and is a leading force in the design and conduct of clinical trials at BIIR. Together with Drs. Fay and Banchereau, she has launched and now leads the clinical trials program at BIIR which includes multicenter clinical trials. She, along with Dr. Miriam Merad, directs the Cancer Immune Monitoring Center and Cancer Immunotherapy Program at the Tisch Cancer Institute.

**Jeffrey W. Pollard**, PhD. I have run a continuously funded laboratory for over thirty years and have authored over 200 papers and edited five books and several journal issues. My major research interests are in women’s health with a focus upon female sex steroid hormone regulation of cell proliferation and on the tumor microenvironment in breast and other cancers. In our research on breast cancer we have pioneered studies on the tumor microenvironment and particularly upon the role of macrophages in this context. In these studies we showed that macrophages promote tumor progression and enhance metastatic rates. In acknowledgement of these studies I will be awarded the American Cancer Society (ACS) Medal of Honor in Basic Sciences in November 2010. Further, I gave the opening keynote address to the AACR Conference on the “Tumor Microenvironment” in France in 2009 and a plenary talk at the AACR meeting in 2011. My commitment to the study of women’s health has also been shown by my leadership positions at the Albert Einstein College of Medicine (Einstein). I have been in the senior leadership of the NCI-funded Einstein Cancer Center for eighteen years and been Deputy Director for the last nine years. I have also founded a Center for the Study of Reproductive Biology and Women’s Health in 2000 and this achieved NIH funding in 2009 as a “Specialized Co-operative Center in Reproduction and Infertility Research.” For many years I ran an international course in mouse genetics teaching gene targeting and transgenic technology. Currently I am Faculty Supervisor of the Transgenic Facility at Einstein. I am the Louis Goldstein Swan Chair in Women’s Cancer Research at Einstein. In addition to my positions at Einstein I have also contributed to the broader community including being Chair of two Gordon Conferences (Reproductive Tract and Mammary Gland), served on the AACR Program committee (2010), Chaired the Society for the Study of Reproduction publication committee, as well as serving on several editorial boards and NIH and other study sections. Currently I am on the Cancer Research and Prevention Research Institute of Texas review panel. In 2012 I will Chair the Keystone meeting “Role of Inflammation during carcinogenesis.” I also have served or serve on several advisory boards relevant to women’s health including the Campbell Center for Women’s Cancer Research (Dr. Tak Mak, Director) in Toronto.

Research in my laboratory has two central focuses. These are: 1) Mechanism of the action of female sex steroid hormone in the regulation of epithelial cell proliferation and on the tumor microenvironment in breast and other cancers. In our research on breast cancer we have pioneered studies on the tumor microenvironment and particularly upon the role of macrophages in this context. In these studies we showed that macrophages promote tumor progression and enhance metastatic rates. In acknowledgement of these studies I will be awarded the American Cancer Society (ACS) Medal of Honor in Basic Sciences in November 2010. Further, I gave the opening keynote address to the AACR Conference on the “Tumor Microenvironment” in France in 2009 and a plenary talk at the AACR meeting in 2011. My commitment to the study of women’s health has also been shown by my leadership positions at the Albert Einstein College of Medicine (Einstein). I have been in the senior leadership of the NCI-funded Einstein Cancer Center for eighteen years and been Deputy Director for the last nine years. I have also founded a Center for the Study of Reproductive Biology and Women’s Health in 2000 and this achieved NIH funding in 2009 as a “Specialized Co-operative Center in Reproduction and Infertility Research.” For many years I ran an international course in mouse genetics teaching gene targeting and transgenic technology. Currently I am Faculty Supervisor of the Transgenic Facility at Einstein. I am the Louis Goldstein Swan Chair in Women’s Cancer Research at Einstein. In addition to my positions at Einstein I have also contributed to the broader community including being Chair of two Gordon Conferences (Reproductive Tract and Mammary Gland), served on the AACR Program committee (2010), Chaired the Society for the Study of Reproduction publication committee, as well as serving on several editorial boards and NIH and other study sections. Currently I am on the Cancer Research and Prevention Research Institute of Texas review panel. In 2012 I will Chair the Keystone meeting “Role of Inflammation during carcinogenesis.” I also have served or serve on several advisory boards relevant to women’s health including the Campbell Center for Women’s Cancer Research (Dr. Tak Mak, Director) in Toronto.

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Speaker Biographies

**Robert D. Schreiber** received his BA degree in Chemistry in 1968 and PhD in Biochemistry in 1973 from the State University of New York at Buffalo. He obtained postdoctoral training at The Research Institute of Scripps Clinic, La Jolla, CA, and remained there attaining the rank of Tenured Associate Member. In 1985, Dr. Schreiber was recruited to the Department of Pathology, Washington University School of Medicine as Professor of Pathology and Microbiology and, since 1990, has held the rank of Alumni Endowed Professor of Pathology and Immunology and Head of the Tumor Immunology Program of the Washington University Siteman Cancer Center.

For more than 25 years, Dr. Schreiber’s work has focused on elucidating the biochemistry and molecular cell biology of cytokines and defining the role they play in promoting immune responses to cancer. He was the first to demonstrate that interferon-gamma (IFN\(\gamma\)) is the major cytokine responsible for activating macrophage anti-tumor and anti-microbial activities in mice and pioneered the \textit{in vivo} use of monoclonal antibodies against cytokines to define their physiologic roles in promoting host responses to tumors and infectious agents. Dr. Schreiber elucidated the structure of the IFN\(\gamma\) receptor, defined its mechanism of action and established the physiologic relevance of IFN\(\gamma\) receptor-dependent signaling by generating mice lacking specific components of the signaling pathway. Using IFN\(\gamma\)-unresponsive- and immunodeficient gene-targeted mice, Schreiber and colleagues demonstrated that the unmanipulated immune system can eliminate spontaneous and carcinogen-induced primary tumors, maintain occult cancer in a dormant state and ultimately facilitate cancer progression by sculpting tumor immunogenicity. These observations led Dr. Schreiber to propose the cancer immunoediting hypothesis that has led to a generalized appreciation of the profound effect of immunity on developing tumors and has contributed critical conceptual and practical support to the fields of tumor immunology and cancer immunotherapy.

Dr. Schreiber has authored more than 240 peer reviewed and invited publications. He has received many honors including the William B. Coley Award for Distinguished Research in Basic and Tumor Immunology from the Cancer Research Institute (2001), the Charles Rodolphe Brupbacher Prize for Cancer Research (2007), and membership in the American Academy of Arts and Sciences (2010).

**Nick Restifo**, a 1983 honors graduate from Johns Hopkins University, obtained his MD in 1987 from New York University. He held fellowships at the Memorial Sloan-Kettering Cancer Center and the National Cancer Institute (NCI) before becoming a Principal Investigator at the NCI in 1993, the position he still currently holds. Dr. Restifo is a mentor for research scholars for the Howard Hughes Medical Institute and has authored or co-authored more than 200 papers on cancer immunotherapy.

Dr. Restifo and his laboratory team aim to design new immunotherapies for patients with advanced cancer. Their strategy is based on the use of animal models and human \textit{in vitro} assays to test hypotheses. They then translate the most promising of these therapies into human clinical trials, which often generate new questions to be tested experimentally. The process is an iterative one that involves close collaboration with basic researchers, biotech scientists and experimental clinicians.
Suzanne L. Topalian, MD
Professor of Surgery and Oncology, Johns Hopkins University School of Medicine
Director, Melanoma Program, Sidney Kimmel Comprehensive Cancer Center

Dr. Topalian is a physician-scientist whose research focuses on cancer immunology and immunotherapy. She received a BA in English from Wellesley College and a medical degree from Tufts University School of Medicine, after which she completed a residency in general surgery at the Thomas Jefferson University Hospital in Philadelphia. Following a 21-year tenure in the Surgery Branch of the National Cancer Institute, NIH, first as a fellow and subsequently as a Senior Investigator, Dr. Topalian joined the Johns Hopkins University School of Medicine faculty in 2006 to direct the Melanoma Program in the Sidney Kimmel Comprehensive Cancer Center. Dr. Topalian has published over 100 original research articles and reviews on cancer immunology. She is internationally recognized for this work, which has provided a foundation for the translational development of immunotherapies for melanoma and other cancers, including cancer vaccines, adoptive T cell transfer, and immunomodulatory monoclonal antibodies. These efforts have opened new avenues of scientific interest and clinical investigation in cancer therapeutics.

Pablo Umaña, Head of Biologics Engineering and Discovery Oncology, Roche Glycart, pRED, Roche, Switzerland.

Dr. Umaña, obtained his PhD (Chemical Engineering and Biology) from the California Institute of Technology in 1998. Pursued post-doctoral studies in molecular medicine at the University of Manchester, U.K. He co-founded Glycart Biotechnology AG in 2001, a spin-off company based on his research at the ETH-Zurich. As Chief Scientific Officer of Glycart, he has led the discovery and preclinical development of engineered therapeutic antibodies for cancer immunotherapy, including GA101 and GA201 (currently in development by Roche in Phase III and II clinical trials, respectively). After the acquisition of Glycart by Hoffmann-La Roche AG in 2005, he continues to lead discovery research at Roche Glycart, a pRED R&D site focused on engineered antibodies and cancer immunotherapy.
Speaker Biographies

**Laurence Zitvogel**, MD (clinical oncology), PhD (tumor immunology), PU-PH Faculty Paris Sud, University Paris XI (Clinical Biology), graduated in Medical Oncology from the School of Medicine of the University of Paris in 1992.

She started her scientific career when she was at the University of Pittsburgh in the USA in Michael Lotze’s laboratory. She became Research Director at Institut National de la Santé et Recherche Médicale U1015, in a laboratory located at Institut Gustave Roussey, a large cancer Center in Villejuif/France and the Head of the Center for Clinical Investigations CICBT 507 for vaccine developments at Villejuif. Professor Zitvogel has been actively contributing to the field of cancer immunology and immunotherapy, and she brought together basic and translational research, including the design of cancer therapies through combined animal studies and Phase I/II patient trials. Her expertise is mainly dendritic cell and innate effector biology and relevance during tumour development as well as exosome-based vaccine designs.

**Robert Vonderheide** is Associate Professor of Medicine at the Perelman School of Medicine at the University of Pennsylvania and an investigator of the Abramson Family Cancer Research Institute. He graduated from the University of Notre Dame with a B.Sc. in Chemical Engineering and from Oxford University, England, as a Rhodes Scholar with a D.Phil. in immunology. After graduating from Harvard Medical School, he completed a residency in Internal Medicine at the Massachusetts General Hospital and subsequently a clinical fellowship in hematology-oncology at the Dana-Farber Cancer Institute. He joined the University of Pennsylvania faculty in 2001, and his laboratory combines efforts in both basic research and clinical investigation to advance the understanding of tumor immunology and to develop novel immunotherapies for cancer. His basic research includes deciphering the immunobiology of pancreatic carcinoma using mouse models of the disease. His translational work tests novel vaccines and antibody approaches for the treatment of pancreatic cancer, breast cancer, and other cancers. Dr. Vonderheide is the co-Leader of the Immunobiology Program of the Abramson Cancer Center.
One sometimes finds what one is not looking for.

Alexander Fleming
In the Footsteps of Virchow: Lymphocyte-Produced Cytokines in Tumor Development Progression and Responses to Therapy

Michael Karin

Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology and Pathology, UCSD School of Medicine, La Jolla, California, USA

Inflammation and immunity can intersect with tumor development in more than one way. While chronic inflammation promotes tumor development, many tumors that do not arise in the context of underlying inflammation still exhibit an inflammatory microenvironment. Furthermore, in certain cases, inflammation may act to suppress anti-tumor immunity, but it can also be used to enhance the efficacy of cancer immunotherapy. Undoubtedly, we need to learn much more about how inflammation and immunity affect tumor development. To study the pathogenic roles of tumor-elicited inflammation, we have used mouse models of prostate and breast cancers, two of the most common malignancies in men and women, respectively, which usually do not evolve in the context of underlying inflammation or infection. Yet, in both cases, we found that tumor-elicited inflammation plays a key role in promoting metastatic spread and in the case of prostate cancer, it contributes to the failure of androgen ablation therapy. Interestingly, in both types of cancer, metastatogenesis depends on the accumulation of activated \( \text{I} \kappa \B \) kinase \( \alpha \) (IKK\( \alpha \)) in the nuclei of primary cancer cells, where it acts both as an activator of chromatin modifiers that control cell cycle progression and as a repressor of an anti-metastatic gene, called maspin. In both cases, IKK\( \alpha \), whose activation has also been observed in advanced human tumors, may be activated upon production within the tumor microenvironment of two members of the TNF family of cytokines: lymphotoxin (LT)\( \alpha \):\( \beta \) and RANK ligand (RANKL). While the cells responsible for production of these cytokines during metastatic progression of prostate cancer remain to be identified, B cells were found to be a major source of LT\( \alpha \):\( \beta \) during development of castration resistant cancer. In breast cancer, however, the major culprits in metastatic progression are RANKL-producing regulatory T cells (Treg). Both in prostate and breast cancers, the recruitment of lymphocytes into the primary tumor is likely to depend on activation of myofibroblasts which produce a number of tumor promoting chemokines. LT\( \alpha \):\( \beta \), RANKL, IKK\( \alpha \) and the mechanisms responsible for myofibroblast activation, as well as the chemokines they produce provide several new opportunities for therapeutic intervention.
The Yin-Yang of Cancer-Related Inflammation

Alberto Mantovani

Istituto Clinico Humanitas IRCCS, University of Milan, Italy

As early as in the 19th century it was perceived that cancer is linked to inflammation. This perception has waned for a long time. Recent years have seen a renaissance of the inflammation-cancer connection stemming from different lines of work and leading to a generally accepted paradigm (1-4). Recent efforts have shed new light on molecular and cellular pathways linking inflammation and cancer (4). Two pathways link inflammation and cancer. In the intrinsic pathways, activation of different classes of oncogenes drives the expression of inflammation-related programmes which guide the construction of an inflammatory microenvironment. In the extrinsic pathway inflammatory conditions promote cancer development (e.g. colitis-associated cancer of the intestine). Key orchestrators at the intersection of the intrinsic and extrinsic pathway include transcription factors (e.g. NFkB) (5), cytokines (e.g. TNF) and chemokines. Thus, inflammation is a key component of the tumour microenvironment and a target for pharmacologic intervention (e.g. (6)). In a seminal contribution, Hanahan and Weinberg identify six hallmarks of cancer (7). We have surmised that CRI represents a seventh hallmark of cancer (8). Macrophages are key orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programmes. M1 macrophages which are classically activated by microbial products and interferon-g are potent effector cells which kill microorganisms and tumors. In contrast, M2 cells, tune inflammation and adaptive immunity; promote cell proliferation by producing growth factors and products of the arginase pathway (ornithine and polyamines); scavenge debris by expressing scavenger receptors; promote angiogenesis, tissue remodeling and repair. M1 and M2 cells represent simplified extremes of a continuum of functional states. Available information suggests that TAM are a prototypic M2 population. M2 polarization of phagocytes sets these cells in a tissue remodeling and repair mode and orchestrate the smouldering and polarized chronic inflammation associated to established neoplasia. Recent studies have begun to address the central issue of the relationship between genetic events causing cancer and activation of protumor inflammatory reactions. Rearrangement of the RET oncogene (RET/PTC) is a frequent, causative and sufficient event in papillary carcinoma of the thyroid. It was recently observed that RET/PTC activates a proinflammatory genetic programme in primary human thyrocytes, including in particular chemokines and chemokine receptors. These molecules are also expressed in vivo and more so in metastatic tumors. These results highlight a direct connection between an early, causative and sufficient oncogene rearrangement and activation of a proinflammatory programme in a human tumor.

Therapeutic targeting of cancer promoting inflammatory reactions is in its infancy, and its development is crucially dependent on defining the underlying cellular and molecular mechanisms in relevant systems. Chemokines are prime targets for interfering with tumor promotion by inflammatory reactions. Ongoing efforts along this line are encouraging. Evidence will be presented that targeting TAM is beneficial in patients.
Tumor-Infiltrating Myeloid Cells Induce Tumor Cell Resistance to Cytotoxic T Cells

Dmitry Gabrilovich

H. Lee Moffitt Cancer Center, Tampa, Florida, USA

Historically, the main factor limiting the success of cancer immunotherapy was felt to be the inadequate tumor-specific immune responses generated in cancer patients. In recent years, however, advances in the development of novel methods of antigen delivery and the blockade of checkpoint proteins responsible for negative signaling in the immune system as well as the generation of antigen-specific T cells ex vivo with subsequent transfer of these cells to patients after lymphoid depletion, have changed this situation. It is now possible to induce tumor-specific immune responses in most patients treated with various types of cancer immunotherapy. However, despite these successes, the proportion of patients who benefit clinically from these treatments remains small. Why has our ability to generate tumor-specific immune responses not translated into a clinical benefit? We investigated a concept that failure of cytotoxic T lymphocytes (CTLs) to eliminate tumor can be independent on inhibition of T-cell function.

Inflammation plays an important role in the development and progression of different tumors. In the context of an inflammatory response myeloid cells are the primary recruited effectors. In cancer, these cells are represented by activated macrophages (MΦs), granulocytes, and myeloid-derived suppressor cells (MDSCs). Major feature of tumor infiltrating myeloid cells is a production of large amount of peroxynitrite (PNT). In mice, myeloid-derived suppressor cells (MDSCs) were a primary source of PNT. Pre-treatment of tumor cells with PNT or with MDSC inhibited binding of processed peptides to tumor cell associated MHC, and as a result tumor cells became resistant to antigen-specific CTLs. This effect was abrogated in MDSCs with a defect in the production of reactive oxygen species or after treatment with the PNT inhibitor. As a model of tumor associated inflammation, we used Lewis lung carcinoma (LLC) cells overexpressing IL-1β (to promote myeloid cell accumulation) and ovalbumin (OVA). Adoptive transfer of antigen-specific CTLs after total body irradiation significantly reduced growth of LLC-OVA tumors. In contrast, the therapeutic effect was completely blocked in LLC-OVA-IL-1β tumor-bearing mice. Therapeutic failure was not caused by more profound suppression of T cells in IL-1β expressing tumors. Inhibition of PNT dramatically enhanced the effect of adoptive transfer in different tumor models. Our data suggest a novel concept that may contribute to our understanding of the mechanisms of tumor immune escape. The tumor-infiltrating myeloid cells can induce nitration of MHC class I molecules on tumor cells, making them unable to effectively bind and retain peptides and thus rendering the tumor cells resistant to antigen-specific CTLs. This suggests that tumors may escape immune control even if potent CTL responses against the tumor-associated antigens were generated. The escape occurs because tumor cells may not express specific peptides that were used to generate CTLs. It also suggests that this escape can be diminished by blocking the PNT production using pharmacological inhibitors of ROS or RNS.
Monocyte and Macrophage Diversity Promotes Tumor Progression and Metastasis

Jeffrey W. Pollard

Center for the Study of Reproductive Biology and Women’s Health, Albert Einstein College of Medicine, New York, NY, USA

Clinical and experimental evidence indicate that macrophages promote cancer initiation and malignant progression. Macrophages enhance malignancy at the primary tumor site by stimulating angiogenesis, inducing tumor cell migration, invasion and intravasation and by suppressing anti-tumor immunity. At metastatic sites macrophages promote tumor cell extravasation, survival and subsequent growth. Each of these activities is stimulated by a different population of macrophages (Qian and Pollard, 2010).

Lineage tracing studies show that the primary and metastatic tumors recruit different populations of monocytes partially explaining the macrophage diversity. At the metastatic site the recruitment of CCR2 expressing monocytes requires tumor synthesized CCL2, the ligand for CCR2. Inhibition of CCL2 reduces this monocyte recruitment and subsequent differentiation into macrophages and this in turn reduces tumor cell extravasation and metastatic growth (Qian and Pollard, 2011).
Alternatively Spliced 1C7/NKp30/NCR3 Isoforms Dictate the Prognosis of Localized and Metastatic Gastrointestinal Sarcoma (GIST)

Nicolas F. Delahaye, Sylvie Rusakiewicz, Guido Kroemer, Jean-Michel Coindre, Axel Le Cesne and Laurence Zitvogel

Institut Gustave Roussy (IGR), Villejuif, France

Personalized therapy of cancer, based on the pharmacological targeting of oncogenes or gene products involved in tumor progression for each individual patient is now viewed as the ideal strategy to combat malignancies. Oncogene addiction suggests that the inactivation of such oncogenes can trigger cancer cell senescence and apoptosis. Oncogenic tyrosine kinases constitute ideally "druggable" targets in that the inhibition of their catalytic activity directly impacts on the control of malignancies. The first kinase inhibitor that has been approved for clinical use is imatinib, which is mainly used to inhibit Bcr-Abl (in chronic myeloid leukemias), c-Kit (which is activated in >85% of gastrointestinal sarcoma (GIST)), and PDGFRA receptor. Imatinib resistance can be commonly explained by mutations in its pharmacological target, underscoring the strong link between its anticancer action and its capacity to inhibit oncogenic kinases. It has been postulated that the therapeutic effects of imatinib can be attributed to its cell-autonomous activity on tumor cells expressing imatinib-sensitive oncogenic kinases (residing in exon 11 of c-Kit). We have reported in the May 2011 issue of Nature Medicine, that another predictor falling in the category of "immune system" associated biomarkers was highly relevant in the pathophysiology of GIST malignancies.

Multiple gene products from the major histocompatibility complex (MHC) class IV region regulate innate immune responses, as exemplified by the natural cytotoxicity triggering receptor 3 (NCR3/1C7/CD337/NKp30) gene, which encodes a protein involved in NK cell-mediated cytotoxicity and in the cross-talk between NK cells and dendritic cells (DC). The human NCR3 gene encodes six differentially spliced transcripts which differ in their Ig domains (V versus C type), and each of the two extracellular domains can be linked to one of three different intracellular domains (that have 36, 25 and 12 amino acids) depending on which particular exon 4 they utilize.

We demonstrate that the alternative splicing of exon 4, which affects the intracellular domain of NKp30, generates three membrane-bound proteins with distinct functions. Expression cloning of the three NKp30 splice variants revealed that the NKp30 spliceoforms diverge in terms of signaling and function. Indeed, the NKp30a isoform can stimulate NK cell degranulation and Th1-type cytokine secretion, while the NKp30b isoform only signals for Th1 cytokine secretion while the NKp30c isoform transduced a delayed signal leading to IL-10 secretion. NKp30 isoforms all possess a single Ig-type domain and an arginine present in its transmembrane domain which stabilizes its association with CD3ζ chains. However, NKp30c signaling involves the rapid phosphorylation of p38 MAP kinase, which upon blockade, convert NNp30c expressing cells into IFNg producers.

Next page
In a cohort of 80 patients with gastrointestinal sarcoma (GIST), the predominant expression of NKp30c was associated with decreased NKp30-dependent TNF\(\alpha\) and CD107a release, as compared to patients with high expression of NKp30a and/or NKp30b. The predominant expression of the NKp30c isoform was an independent prognostic factor of reduced overall survival. Preferential transcription of the defective NKp30c isoform resulted at least in part, from a single nucleotide polymorphism (SNP) in position 3790 of the 3'UTR region of NCR3 (rs986475), ablating the polyadenylation signal required for optimal expression of the two functional isoforms.

While the mutational status of the KIT oncoproteins represented so far the most valuable predictive factor of clinical response to the paradigmatic KIT and PDGFR\(\alpha\) tyrosine kinase inhibitor, imatinib mesylate to date, our study reveals that the NKp30 profiling is a superior predictor of the efficacy of imatinib in metastatic GISTs, overriding exon 11 KIT mutations.

We speculate that genetically determined NK cell dysfunctions might aggravate the prognosis of some malignancies including GIST. I will also present independent work from other investigators corroborating the critical role of the immune system in the response to imatinib in GISTs. These findings have important implications for the optimal clinical management of GISTs in the future that will be discussed during the talk.
Immune Checkpoint Blockade in Cancer Therapy: New Insights and Opportunities

James P. Allison

Ludwig Center for Cancer Immunotherapy, Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

T cell intrinsic and extrinsic pathways that regulate immune responses can limit the effectiveness of active immunologic strategies for cancer therapy. We conducted extensive pre-clinical studies in mouse models which showed that blockade of the inhibitory signals mediated by CTLA-4 in T cells, either alone or in combination with a variety of immunologic and conventional therapies, led to tumor eradication and long-lived immunity. This led to the generation of antibodies to human CTLA-4 and the conduct of an extensive series of clinical trials in human cancer. Over 6,000 patients have been treated with the CTLA-4 antibody Ipilimumab (Bristol-Meyers Squibb). Objective responses have been observed in metastatic melanoma, castrate resistant prostate cancer, as well as renal, lung, and ovarian cancer. In a Phase III trial, Ipilimumab was shown to prolong survival of stage IV metastatic melanoma patients, with 25% alive 4 ½ years. This is the first drug of any type to show a survival benefit in metastatic melanoma in a placebo controlled randomized trial. In March 2011 Ipilimumab was approved by the FDA for both first and second line therapy of metastatic melanoma.

Recent studies have shown that the genetic instability inherent in cancer results in an extraordinary number of coding mutations in cancer. Many of these give rise to neoantigens, which can provide multiple avenues for attack of tumor cells. It seems logical to combine genetically “targeted” therapies with immune checkpoint blockade in order to obtain effective immune responses to these neoantigens, thereby minimizing the chances of tumor resistance and escape. We are exploring the effects of targeted therapies on immune responses and whether the combination of anti-CTLA-4 and targeted therapy in pre-clinical models. The ultimate goal is to determine whether we can take advantage of the high response rate to genetically targeted agents with the durability of immunotherapy.
Malignant cells display altered surface molecular signatures distinguishing them quantitatively and qualitatively from their normal counterparts. Such modifications may facilitate tumor progression by regulating interactions between cancer cells and immune cells in the tumor microenvironment. Central to the ability of cancer cells to evade immune destruction is the immunoglobulin-like molecule B7-H1 (PD-L1), constitutively expressed by many human cancers. Expression of cell surface B7-H1 is upregulated by proinflammatory stimuli such as interferons, and may be influenced by activated oncogenic pathways such as PI3K/AKT. B7-H1 acts as a ligand for the programmed death-1 (PD-1) co-receptor normally expressed on activated T cells, which delivers an inhibitory signal to suppress antitumor immunity. The mechanisms underlying PD-1/B7-H1-mediated immunosuppression include apoptosis induction, anergy and exhaustion of recently activated effector or memory T cells. Based on findings of B7-H1 over-expression by human cancers and the observation that tumor-infiltrating T cells can express high PD-1 levels in situ, multicenter clinical trials of fully human blocking antibodies against PD-1 or B7-H1, sponsored by Medarex/Bristol-Myers Squibb, have been undertaken. In the first-in-human trial of anti-PD-1 (MDX-1106/BMS936558), 39 patients with treatment-refractory advanced melanoma, NSCLC, RCC, colon cancer or prostate cancer were treated with intermittent (every 1-3 month) escalating doses of antibody. Three durable objective tumor regressions including one CR (colon cancer) and 2 PR’s (melanoma and RCC) were observed; 2 additional patients (melanoma and NSCLC) experienced transient lesional tumor regressions. There were no dose-limiting toxicities following a single dose of anti-PD-1, up to the highest planned dose of 10 mg/kg. Based on these results as well as correlative assays defining the PD-1/B7-H1 axis as a promising target for cancer therapy, a follow-up phase I/II trial of biweekly MDX-1106 administration, and the first-in-human trial of anti-B7-H1 (MDX-1105/BMS936559), were initiated. Early experience with biweekly MDX-1106 administration in patients with advanced metastatic solid tumors has demonstrated objective response rates of ~30% in melanoma and RCC, with generally manageable toxicities; of interest, responses have also been observed in patients with NSCLC, a “non-immunogenic” tumor. The majority of patients experiencing objective tumor regressions on MDX-1106 therapy continue in remission, with follow-up ≤ 3 years. Also of interest, early experience with anti-B7-H1 therapy has shown evidence of clinical activity in metastatic melanoma, RCC and NSCLC, further validating the PD-1/B7-H1 pathway as an important target for cancer immunotherapy. Current investigations of pharmacodynamics, tumor biomarkers and intratumoral immune events aim to explore mechanisms of action for anti-PD-1 and anti-B7-H1 in order to guide future clinical development, including synergistic combinatorial treatment strategies.
Engineered Anti-Cancer Antibodies with Enhanced Immune Effector Functions

Pablo Umaña
Roche Glycart AG, Schlieren, Zurich, Switzerland

Third generation, glycoengineered antibodies currently in clinical trials will be presented. GA101 (RG7159, obinotuzumab) is a type II anti-CD20 monoclonal antibody that is currently in PhII/III clinical trials. GA101 was engineered for increased direct cell death induction and enhanced ADCC. In preclinical models, GA101 mediates superior efficacy compared to type I CD20 antibodies. In Ph I/II clinical trials, GA101 has shown promising activity in heavily pre-treated NHL patients. GA201 (RG7160) is a glycoengineered anti-EGFR monoclonal antibody that is currently in PhII clinical trials. GA201 exhibits increased binding affinity to FcγRIIIa on immune effector cells and significantly improved immune cell-mediated killing of EGFR-overexpressing tumor cells. In preclinical models, GA201 mediates superior efficacy compared to currently marketed EGFR antibodies, cetuximab and panitumumab. Antibody engineering strategies and preclinical and clinical data, including immune-mediated mode of action studies, will be the focus of the presentation.
Genetically Engineered Receptors and Adoptive Cell Therapies

Bruce L. Levine, Michael Kalos, Adam Bagg, David Porter and Carl H. June

Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania, USA

The majority of non-Hodgkin’s lymphomas, acute lymphoblastic leukemias and chronic lymphocytic leukemias (CLL) express CD19, which is also expressed by normal B cells but not by hematopoietic stem cells or other tissues. Thus CD19 represents an attractive target for immunotherapy. Our preclinical studies show that combining robust T cell culture systems with lentiviral vector modified human T cells expressing CD19-specific Chimeric Antigen Receptor (CART-19) has potent anti-leukemic efficacy in mice bearing established leukemic xenografts. 4-1BB-containing signaling endodomains enhance this activity. In an ongoing feasibility and safety clinical trial, three patients with advanced treatment refractory CLL have been treated. CD3+CD45+ cells in leukapheresis products (range 2.3%-4.5%) were positively selected with anti-CD3/anti-CD28 magnetic beads prior to CART-19 lentiviral vector transduction and expansion. Patients were infused with a total of 0.3-5 x 10^9 total T cells, with 5%-27% of cells expressing CART-19. Two of three patients remain in complete remission beyond 8 months post infusion. The third patient had a very significant but partial response; he required corticosteroids 18 days after infusion, during an ongoing response, for symptoms presumably related to cytokine release. We observed significant in vivo expansion in two of three patients accompanied by long-term persistence in blood and migration to bone marrow, and delayed onset tumor lysis syndrome accompanied by elevated levels for a broad range of cytokines. Clinical responses were documented by normalization of blood counts, resolution of adenopathy, and clearance of MRD when assessed by flow cytometry and deep sequencing for IgH rearrangements, cytogenetics, and FISH studies in the bone marrow. On target toxicities have been observed in all patients, including cytokine release but not cytokine storm, B cell depletion, plasma cell depletion and hypogammaglobulinemia requiring replacement serotherapy. Confirmation of these observations that T cells expand and traffic to tumor sites, stimulate synergistic antitumor immune activity, and persist long term in vivo potentially offers significant therapeutic and economic advantages over existing therapies and should be confirmed in larger numbers of patients, however, the therapeutic index with potent cell based CAR therapies will depend to a large extent on the specificity of the target.
Deconstructing Cancer Immunoediting

Robert D. Schreiber
Washington University School of Medicine, St. Louis, Missouri, USA

Cancer Immunoediting is the process by which the immune system controls and shapes cancer. We originally envisaged that cancer immunoediting would occur in three phases: Elimination (also known as cancer immunosurveillance, the host protective phase of the process), Equilibrium (the phase in which tumor cells that survive immune elimination remain under immunologic growth control resulting in a state of functional tumor dormancy) and Escape (the phase where clinically apparent tumors emerge because immune sculpting of the tumor cells has produced variants that display either reduced immunogenicity or enhanced immunosuppressive activity). Strong experimental data has now been obtained using mouse models of cancer to demonstrate the existence of each phase of the cancer immunoediting process and compelling clinical data suggests that a similar process may also occur during the evolution of certain types of human cancer. Our efforts now focus on elucidating the molecular and cellular mechanisms that underlie each phase of cancer immunoediting and identifying the critical checkpoints that regulate progression from one phase of the process to the next. This approach has helped identify the nature of antigens seen by immunity in nascent developing cancers and has further shown that immunoselection is a major mechanism of immunoediting. Moreover, we have found that edited tumors can still be controlled by the immune system if natural mechanisms that prevent autoimmunity are suspended. As reported by others, we have confirmed that inhibition of CTLA-4 induces ejection of edited MCA sarcomas. However, we have also found that inhibition of PD-L1 does the same, although by perhaps different mechanisms. These differences will be discussed.
Immune Responses to Newly Identified Leukemia Antigens in Relation to Clinical Responses to a Therapeutic Vaccine for Chronic Myelogenous Leukemia (CML)

Lu Qin, Nasser Yaghi, Poornima Neela, Chris Hourigan, Hwa-Ling Tsai, Gary Rosner, Doug Smith and Hyam I. Levitsky

Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

We previously reported the results of a clinical trial testing a therapeutic cancer vaccine in patients with persistently measurable Chronic Myeloid Leukemia (CML) despite at least a year of imatinib mesylate (Gleevec) treatment. Baseline disease burden and immune status were measured during a three-month pre-vaccine monitoring period, followed by vaccination every three weeks for four with an irradiated allogeneic CML cell line (K562) engineered to express granulocyte macrophage-colony stimulating factor (GM-CSF) with or without topical imiquimod. Patients were then followed longitudinally for clinical and immunological response. Clinical response was measured by quantitative RT-PCR of bcr/abl transcript copy number, with greater than a 10-fold change from pre-vaccine baseline considered significant. Of 19 subjects enrolled, thirteen patients had a progressive decline in disease burden, 8 of whom had increasing disease burden before vaccination. Twelve patients achieved their lowest tumor burden measurements to date following vaccine, including seven subjects who became PCR-undetectable (5 durable, 2 transient). Eleven patients who either failed to respond to the initial vaccine series (5), or who had increasing disease burden after an initial decline (6) were given a second “boost” series of four vaccinations at three week intervals.

Serum samples from the above study were used to screen a phage expression library created from K562 mRNA. A total of 28 antigens were recognized by serum from clinical responders at time-points before and after vaccination. Of these, two were previously reported as leukemia associated antigens (RHAMM and PRAME). The candidate antigens included molecules reported to be involved in cell cycle regulation, resistance to apoptosis, signal transduction, and transcriptional regulation, among others. Antibodies against 12 of the 28 antigens were readily detectable in the index patient prior to vaccination, whereas responses to the remaining 16 antigens were induced or significantly amplified by vaccination. Individual clones from this set of 28 antigens were then used to characterize the presence and titer of specific antibody responses in all 19 patients enrolled in this study as well as in 19 normal volunteers. Antibodies against five of the antigens were present in only a single subject after the initial vaccine series, although additional subjects subsequently generated responses to these antigens following boosting. Nine of the 16 antigens “induced” by vaccination were recognized in two or more subjects following the primary vaccine series, with some antigens being recognized in as many as 13/19 subjects. Antigens that were identified from patient sera only after vaccination were rarely recognized in normal volunteers, whereas antibodies to antigens identified from pre-vaccine patient serum were more frequently observed in normals. Early analysis of these data suggests that those patients with the best clinical responses to vaccination (i.e. decrease in bcr/abl transcript levels) also demonstrated an induced, diverse antibody response to a number of leukemia-associated antigens in contrast to clinical non-responders in whom minimal or no response to these antigens could be detected. Data on the impact of boosting on the breadth and magnitude of the humoral response will also be presented. Initial evaluation of T cell responses to selected candidates is ongoing. These findings have relevance to future cancer vaccine strategies and may identify promising targets.
CD40 and Immunosurveillance in Pancreatic Carcinoma in Mice and Humans

Robert H. Vonderheide

Abramson Family Cancer Research Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA

Cancer-associated inflammation plays a decisive role in restraining anti-tumor immunity, particularly in pancreatic ductal adenocarcinoma (PDA). PDA remains an almost universally lethal disease with chemotherapy offering minimal benefit over best supportive care for patients who are not surgical candidates. We have demonstrated that leukocytes actively infiltrate the stromal compartment of PDA even at the earliest stages of tumor development and orchestrate an inflammatory reaction that is immunosuppressive. Because CD40 activation can reverse immune suppression and drive potent anti-tumor T cell responses, we then tested the combination of an agonist CD40 antibody with gemcitabine chemotherapy in patients with surgically incurable PDA and observed major tumor regressions. We reproduced this treatment effect in a genetically engineered mouse model of PDA and found unexpectedly that tumor regression required macrophages but not T cells or gemcitabine. CD40-activated macrophages rapidly infiltrated tumors, became tumoricidal, and facilitated the depletion of tumor stroma. Thus, productive cancer immune surveillance does not necessarily depend on T cells. Rather, our findings demonstrate a CD40-dependent mechanism for targeting cancer-associated inflammation in the treatment of cancer and support the continued development of emerging therapeutic strategies that target inflammatory cells within the tumor microenvironment.
Interleukin-15: A New Oncogene?

Michael A. Caligiuri

The Ohio State University Comprehensive Cancer Center – James Cancer Hospital, Columbus, Ohio, USA

Soluble endogenous mediators of chronic inflammation are known to be involved in the genesis of cancer, yet the mechanisms are not fully understood1,2. IL-15 is a proinflammatory cytokine that is required for the normal development, survival and growth of large granular lymphocytes (LGL)3,4. It is overexpressed in human acute LGL leukemia, a uniformly fatal disorder, and mice that overexpress IL-15 develop acute LGL leukemia5-7. Evidence that a single proinflammatory cytokine such as IL-15 can itself drive malignant transformation is lacking, yet proof of this and elucidation of the pathways involved could lead to curative or preventative therapies for cancer. Here we show that IL-15 causes acute LGL leukemia in part via the down regulation of microRNA 29b (miR-29b) which, when targeted in vivo with a novel drug, cures acute LGL leukemia. We found that IL-15 immortalizes wild-type LGL in vitro via a Myc-mediated up regulation of aurora kinases, centrosome aberrancies characteristic of chromosomal instability, and aneuploidy. Simultaneously, IL-15 mediates transcriptional repression of miR-29b via induction of Myc, NFκBp65, and Hdac-1, resulting in overexpression of Dnmt3b, DNA hypermethylation and silencing of tumor suppressor genes. Strikingly, adoptive transfer of wild-type LGL cultured with IL-15 led to malignant transformation in vivo without exogenous IL-15, and targeting miR-29b with a single novel agent cured otherwise fatal, chemotherapy resistant acute LGL leukemia without toxicity. Our results demonstrate how a proinflammatory cytokine can behave as an oncogene and how successful targeting of its pathway can produce a nontoxic and curative outcome.
Reprogramming the Immune Environment in Cancer via Dendritic Cells

A. Karolina Palucka\textsuperscript{1,2,3}, Hideki Ueno\textsuperscript{1}, Joseph W. Fay\textsuperscript{1,3} and Jacques Banchereau\textsuperscript{4}

\textsuperscript{1}Baylor Institute for Immunology Research and \textsuperscript{3}Sammons Cancer Center, Baylor University Medical Center, Dallas, TX, USA; \textsuperscript{2}Department of Oncological Sciences and Department of Medicine, Immunology Institute, Mount Sinai School of Medicine, NY, USA; \textsuperscript{4}Hoffmann-La Roche Inc, Nutley, New Jersey, USA

T cells can reject established tumors when adoptively transferred into patients, thereby demonstrating that the immune system can be harnessed for cancer therapy. Active immunotherapy with vaccines has the potential to induce tumor-specific effector and memory T cells that might control tumor outgrowth on the long term. Cancer vaccines are in a renaissance era due to recent phase III clinical trials showing some benefit to the patients. Vaccines act through dendritic cells (DCs) which induce, regulate and maintain T cell immunity. Critical to the design of improved vaccines is the concept of distinct DC subsets and distinct DC activation pathways, all contributing to the generation of unique adaptive immune responses. Rather than the quantity of IFN-\(\gamma\) secreting CD8\(^+\) T cells, we should aim at generating high quality high sensitivity poly-functional effector CD8\(^+\) T cells able to reject tumors and long-lived memory CD8\(^+\) T cells able to prevent relapse. Our pre-clinical studies actually demonstrate that Langerhans cells are superior, as compared to other DC subsets, in their capacity to prime high affinity melanoma-specific CD8\(^+\)T cells able to kill authentic tumor targets.

Strategies to overcome regulatory T cells and suppressive environment established by tumors need to be established. Solid tumors are often associated with septic inflammation. There are two types of inflammation that have opposing effects on tumors, chronic inflammation that promotes cancer cell survival, and metastasis, and acute inflammation which triggers cancer cell destruction. Chronic inflammation is often linked with the presence of type 2-polarized macrophages (M2), which are induced by Th2 cytokines, IL-4 and IL-13. Our recent studies have demonstrated the presence in breast cancer of Th2-inflammation that fosters breast cancer development. This is driven by cytokine TSLP which induces and maintains pro-tumor CD4\(^+\) T cells via OX40L-expressing dendritic cells. Thus, a better understanding of the biology of DCs within tumor environment will allow us to design novel strategies leading to efficient immunotherapy of cancer, by reprogramming DCs from inducing tumor promoting Th2-mediated chronic inflammation to inducing tumor-rejecting Th1-mediated acute inflammation and strong tumor-specific CD8\(^+\) cytotoxic T cell responses.

Thus, next generation therapeutic approaches will be based on novel cancer vaccines in combination with antibodies and/or drugs targeting tumor environment.
A Human Memory T-Cell Subset with Stem Cell-Like Properties

Nicholas P. Restifo

National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Immunological memory is thought to depend upon a stem cell-like, self-renewing population of lymphocytes capable of differentiating into effector cells in response to antigen re-exposure. Here we describe a long-lived human memory T-cell population that displays enhanced self-renewal and multipotent capacity to derive central memory, effector memory and effector T cells. These cells, specific for multiple viral and self-tumor antigens, were found within a CD45RO–, CCR7+, CD45RA+, CD62L+, CD27+, CD28+ and IL-7Rα+ T-cell compartment characteristic of naïve T cells. However, they expressed increased levels of CD95, IL-2Rβ, CXCR3, and LFA-1, and exhibited numerous functional attributes distinctive of memory cells. Compared to known memory populations, these lymphocytes displayed increased proliferative capacity, more efficiently reconstituted immunodeficient hosts and mediated superior anti-tumor responses in a humanized mouse model. The identification of a human stem cell-like memory T-cell population is of direct relevance to the design of vaccines and T-cell therapies.
The Interplay of Inflammation and Tumor Immunity

Glenn Dranoff

*Dana-Farber Cancer Institute, Boston, Massachusetts, USA*

Efficacious cancer immunotherapies will likely require combinations of strategies that enhance tumor antigen presentation and antagonize negative immune regulatory circuits. We demonstrated that vaccination with irradiated, autologous melanoma cells engineered to secrete GM-CSF followed by antibody blockade of CTLA-4 accomplishes clinically significant tumor destruction with minimal toxicity in some advanced cancer patients. The detailed analysis of patients achieving sustained clinical benefits from these therapies illustrated the importance of a coordinated cellular and humoral anti-tumor immune response. Indeed, the extent of tumor necrosis in post-treatment biopsies was linearly related to the natural logarithm of the ratio of infiltrating CD8+ effector T cells to FoxP3+ Tregs. Moreover, therapy induced a potent antibody response to several key tumor cell surface and secreted proteins; these antibodies manifest functional activity *in vitro*, antagonizing tumor cell survival, invasive potential, and angiogenesis.

Through an analysis of cytokine deficient mice, we delineated a critical role for GM-CSF in Treg homeostasis. GM-CSF is required for the expression of the phosphatidylserine binding protein MFG-E8 in antigen presenting cells, whereas the uptake of apoptotic cells by phagocyte-derived MFG-E8 maintains peripheral Treg activity. While blockade of MFG-E8 can enhance therapeutic immunity in the context of cancer vaccines, loss of Treg homeostasis with MFG-E8 deficiency can lead to chronic inflammation. Mice deficient in GM-CSF and IFN-γ develop at high frequency germinal center derived lymphomas and nonsmall cell lung carcinomas. The pathogenesis of these tumors reflects a combination of tumor-promoting inflammation and a loss of protective tumor immunity. Taken together, these findings highlight dual roles for immunity in cancer pathogenesis and therapy.
The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny...'

Isaac Asimov
## Posters and Presenting Authors (in alphabetical order)

<table>
<thead>
<tr>
<th>No.</th>
<th>Author</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adurthi, Sreenivas</td>
<td>Tumor Infiltrating Th1 and Th2 Effectors, Regulatory T Cells and Langerhans Cells in Large Early - Stage Cervical Cancer</td>
</tr>
<tr>
<td>2</td>
<td>Anwer, Khursheed</td>
<td>Clinical Development of a Novel Immunotherapeutic Agent for the Treatment of Peritoneally Disseminated Gynecological and Gastrointestinal Cancers</td>
</tr>
<tr>
<td>3</td>
<td>Bergmann, Michael</td>
<td>Increased Apoptosis by Arming Oncolytic Influenza Virus with Interleukin-24</td>
</tr>
<tr>
<td>4</td>
<td>Bottino, Dean</td>
<td>A Mathematical Modeling Framework for Antibody-Dependent Cell Mediated Cytotoxicity (ADCC)</td>
</tr>
<tr>
<td>5</td>
<td>Brazier, Helene</td>
<td>Unraveling the Contribution of Osteoclasts in Bone Metastasis Progression using an Immune-Competent Murine Model of Breast Cancer, the 4T1 Model</td>
</tr>
<tr>
<td>6</td>
<td>Brezski, Randall</td>
<td>Proteolytic Disablement of IgGs and the Restoration of Lost Cell-Killing Functions</td>
</tr>
<tr>
<td>7</td>
<td>Chae, Wook-Jin</td>
<td>Regulatory T Cells from ApcMin/+ Mice are Functionally Impaired by IL-17A</td>
</tr>
<tr>
<td>8</td>
<td>Chick, Jon</td>
<td>Phase I Open Label, Dose Escalation Study of RG7160 (GA201) in Patients with Metastatic Solid Tumors</td>
</tr>
<tr>
<td>9</td>
<td>Di Caro, Giuseppe</td>
<td>Tumor Infiltrating Macrophages as a Potential Predictive Biomarker of Response to 5-FU In Patients with Microsatellite-Stable Colorectal Cancer</td>
</tr>
<tr>
<td>10</td>
<td>Dopfer, Elaine</td>
<td>The Conformational Change at CD3 is not Necessary for Gammadeltatcr Activation</td>
</tr>
<tr>
<td>11</td>
<td>Driscoll-Carroll, Kyla</td>
<td>Targeting TGFpRII In Oncology: A Potent Immunomodulatory Antibody with Various Effects on Cancer Progression</td>
</tr>
<tr>
<td>12</td>
<td>Feng, Guo</td>
<td>A Novel Phenomenon: E. Coli Can Reduce the Expression of CD55 on Granulocytes in Lymphoma Patient’s Blood</td>
</tr>
<tr>
<td>13</td>
<td>Gal, Markel</td>
<td>Novel Immunotherapy for Malignant Melanoma with a Monoclonal Antibody that Blocks CEACAM1 Homophilic Interactions</td>
</tr>
<tr>
<td>14</td>
<td>Gerdes, Christian</td>
<td>Enhanced huFcgRIII and/or muFcgRIV Engagement on NK Cells and Macrophages Leads to a Rapid Increase in Tumoral Immune Cell Infiltration in Xenograft Tumor Models Treated with Glycoengineered Anti-EGFR Antibody GA201 (RG7160)</td>
</tr>
<tr>
<td>15</td>
<td>Gerdes, Christian</td>
<td>GA201 (RG7160), a Novel Humanised Glycoengineered Anti EGFR Antibody, with Enhanced Immune Mediated Effector Functions for the Treatment of Solid Tumors</td>
</tr>
<tr>
<td>16</td>
<td>Gerdes, Christian</td>
<td>GA201 (RG7160) Pre-Treatments and Combination Therapies Improve Efficacy Without Significantly Affecting Preclinical Anti-Tumoral ADCC</td>
</tr>
<tr>
<td>17</td>
<td>Gupta, Anukriti</td>
<td>Lymphocyte Function Antigen-1 and Leukotoxin Interaction</td>
</tr>
<tr>
<td>18</td>
<td>Hata, Ryu-Ichiro</td>
<td>NK Cell-Dependent Suppression of Tumor Growth and Metastasis in CXCK14/BRAK Transgenic Mice</td>
</tr>
<tr>
<td>19</td>
<td>Khamar, Bakulesh</td>
<td>Preclinical Evaluation of Cell Based Pancreatic Cancer Vaccine With Cadi-05 as an Adjuvant</td>
</tr>
<tr>
<td>20</td>
<td>Klinke, David</td>
<td>Tumor-Derived Wnt-Inducible Signaling Protein-1 (WISP-1) Exhibits Paracrine Immunosuppression by Inhibiting Cellular Response to Interleukin-12</td>
</tr>
<tr>
<td>21</td>
<td>Koliakos, George</td>
<td>A Metastasis Inhibiting Vaccine Based On The Laminin Peptide YIGSR</td>
</tr>
<tr>
<td>22</td>
<td>Kornbluth, Richard</td>
<td>Facile Generation of APCs using Soluble Multimeric CD40L as a B Cell Proliferation Stimulus</td>
</tr>
</tbody>
</table>
## Posters and Presenting Authors (in alphabetical order)

<table>
<thead>
<tr>
<th>No.</th>
<th>Author</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Lifke, Alexander</td>
<td>Human Adaptive Immune System In RAG2-/-γc-/- Mice: As Novel Preclinical Model for Cancer Immunology</td>
</tr>
<tr>
<td>24</td>
<td>Lopez-Toledo, Gabriela</td>
<td>L1-E2 Chimeric Capsomers Produced in Escherichia Coli as Prophylactic and Therapeutic Vaccines Against Human Papilloma Virus 16</td>
</tr>
<tr>
<td>25</td>
<td>Maisel, Daniela</td>
<td>Analysis of Tumor/Host Differences in Anti-CD44-Treated Xenografts Using Global Gene-Expression Profiling</td>
</tr>
<tr>
<td>26</td>
<td>Martin, David</td>
<td>The Ig-Likeα3 Domain of Soluble Mica can be Engineered to Target Tumor Associated Antigens and Recruit NK-Cells to Kill TAA-Expressing Cells in vitro</td>
</tr>
<tr>
<td>27</td>
<td>Martin, Sunil</td>
<td>On the Role of Foxp3 Isoforms</td>
</tr>
<tr>
<td>28</td>
<td>Martinez, Vanessa</td>
<td>Immunomodulatory Properties of Soluble Human CD5 in Mouse Models of Disease</td>
</tr>
<tr>
<td>29</td>
<td>Mejias, Raquel</td>
<td>Dimercaptosuccinic Acid-Coated Magnetite Nanoparticles as a Localized Interferon-Gamma Delivery System for Cancer Immunotherapy</td>
</tr>
<tr>
<td>30</td>
<td>Passioukov, Alexandre</td>
<td>Exploratory Biomarker Program for RG7160 (GA201) in the Phase I-II Clinical Trials</td>
</tr>
<tr>
<td>31</td>
<td>Pellegatta, Serena</td>
<td>Immunotherapy Against the Neural Stem Cell Marker Glast is Effective in a Murine Model of Malignant Glioma</td>
</tr>
<tr>
<td>32</td>
<td>Pourpe, Stephane</td>
<td>Melanoma Patients Vaccinated with Dendritic Cell Vaccines Mount Long-Lived Melanoma-Antigen Specific Polyfunctional CD8+ T Cells</td>
</tr>
<tr>
<td>33</td>
<td>Pruitt, Scott</td>
<td>Enhancement of Anti-Tumor Immunity Through Local Delivery of Anti-CTLA-4 and Anti-GITR mAbs by Dendritic Cells</td>
</tr>
<tr>
<td>34</td>
<td>Sathish, L.A.</td>
<td>Radon and Lung Cancer in Bangalore Metropolitan, India</td>
</tr>
<tr>
<td>35</td>
<td>Schulz, Thadeus</td>
<td>Breast Carcinoma Prevention and Treatment via Complement Mediated and Antibody Directed Duct Epithelial Cell Lysis Applied by Ductal Lavage</td>
</tr>
<tr>
<td>36</td>
<td>Shaked, Helena</td>
<td>The Dual Role of Epithelial NF-KB Activation in Intestinal Tumorigenesis</td>
</tr>
<tr>
<td>37</td>
<td>Sharma, Anu</td>
<td>γ-Radiation Enhances Immunogenicity of Cancer Cells by Increasing the Expression of Cancer-Testis Antigens In Vitro and In Vivo</td>
</tr>
<tr>
<td>38</td>
<td>Sigalov, Alexander</td>
<td>Multifunctional Nanoparticles for Macrophage-Targeted Breast Cancer Treatment and Imaging</td>
</tr>
<tr>
<td>39</td>
<td>Spagnoli, Giulio</td>
<td>Tumor Infiltration by FCGAMMARIII (CD16)+ Myeloid Cells is Associated with Improved Survival in Patients with Colorectal Carcinoma</td>
</tr>
<tr>
<td>40</td>
<td>Wu, Te-Chia</td>
<td>Reprogramming the Immune Environment in Breast Cancer via Dendritic Cells</td>
</tr>
<tr>
<td>41</td>
<td>Yao, Xuebiao</td>
<td>Chemokine CCL18 Promotes Breast Cancer Metastasis via an Activation of Ezrin Signaling Complex</td>
</tr>
<tr>
<td>42</td>
<td>Yin, Hao</td>
<td>S100A9 and CCT Gamma as Potential Biomarkers for Diagnosis of Cholangiocarcinoma</td>
</tr>
<tr>
<td>43</td>
<td>Zea, Arnold</td>
<td>Mechanisms of IFNα Resistance in Renal Cell Carcinoma (RCC)</td>
</tr>
<tr>
<td>44</td>
<td>Zhou, Gang</td>
<td>Cytotoxic Chemotherapy and CD4+ Effector T Cells: An Emerging Alliance for Durable Antitumor Effects</td>
</tr>
</tbody>
</table>
Tumor Infiltrating Th1 And Th2 Effectors, Regulatory T Cells and Langerhans Cells in Large Early-Stage Cervical Cancer

S. Adurthi1, M. Mahesh Kumar1, S. Ramachandran2, Geetashree Mukherjee3, H. Krishnamurthy4, S. Krishna4, U.D. Bafna5, K. Uma Devi5, R.S. Jayshree1*

1Department of Microbiology, 3Pathology, 4Gynecology, Kidwai Memorial Institute of Oncology, Bangalore; 4National Center for Biological Sciences, TIFR, Bangalore, India; 5Integrated Cancer Research Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India

An insight into the local factors contributing towards disease progression in tumors is central for developing immunotherapies. Infiltrating cells from squamous cell carcinoma of the cervix, were MACs enriched and flow sorted into CD4+CD25hi (Tregs) / CD4+CD25int (Teffs), CD1a+CD207+ (Langerhans cells) characterized phenotypically and functionally. Tissue sections were probed for IL4, γIFN, CCL17, CD1a, CD83, and CD86. Teffs constitutively expressed both γIFN and IL4 - prototypical cytokines of Th1 and Th2 respectively; were able to proliferate and secrete higher quantities of both cytokines in response to antiCD3/antiCD28 and autologous tumor lysates. Only 53% of cervical cancer Tregs were FOXP3+, elaborated TGFβ1, IL10 and were able to inhibit both Th subsets. Chemokines viz. CCL17 & or CCL22 have been shown to attract Tregs into ovarian and gastric tumors. We observed cervical tumors to be expressing CCL17. A differential expression of TLRs was seen in Langerhans Cells (LCs) isolated from cervical tumors: there was uniform presence of TLR7; TLR 8 was variably present, however, there was consistent absence of TLR9 expression. While there was increased CD83 expression in the tissues of SCC cervix compared to normal cervices, CD86 was reduced, but there was no change in CD1a expression.

Intratumoral Teffs represented functionally active subsets of both Th1 and Th2 which were not anergic but were suppressed by multiple Treg subsets against both Th subsets comprising of FOXP3+ Tregs, and Tregs secreting TGFβ1 and IL10. Also, absence of TLR 9 in LCs infiltrating cervical tumors may down regulate CD86 and hence may play a crucial role in induction of immune tolerance in cervical cancer.
Clinical Development of a Novel Immunotherapeutic Agent for the Treatment of Peritoneally Disseminated Gynecological and Gastrointestinal Cancers

Khursheed Anwer

EGEN, Inc., Huntsville, AL, USA

Clinical development of a novel immunotherapeutic, EGEN-001, for local treatment of solid tumors is described. EGEN-001 is composed of an interleukin-12 (IL-12) gene expression plasmid and a biocompatible delivery lipopolymer, PEG-PEI-Cholesterol (PPC). EGEN-001 is designed to increase the local concentration of IL-12, a potent anti-cancer cytokine that stimulates the immune system to aid in fighting cancer growth and metastases. Unlike the available therapies, EGEN-001 does not cause hematological, neurological or hepatic toxicity and can be safely given as maintenance therapy for several months in human. In comparison to IL-12 protein, EGEN-001 delivery is confined to the tumor site with minimal escape into the systemic circulation. Both preclinical and clinical studies have confirmed a peritoneal distribution of IL-12 plasmid and its downstream cytokines IFN-γ and TNF-α. The safety and biological activity of escalating doses of EGEN-001 alone or in combination with chemotherapy has been demonstrated following intraperitoneal administration in platinum-sensitive and platinum-resistant ovarian cancer patients. In both studies, the common adverse events were primarily limited to abdominal discomforts and low grade fever with no evidence of systemic toxicity. In the monotherapy study, 69% of patients had progressive disease and 31% of patients had stable disease. The average overall survival at high EGEN-001 doses was ~2 years as compared to 8-10 months anticipated for this patient population. In the combination trial with paclitaxel/docetaxel, 38% of patients had complete or partial response, 15% of patients had stable disease, and 46% of patients had progressive disease. The CA-125 levels remained below treatment levels in 61% of patients at the follow-up. A Phase II trial of EGEN-001 in platinum resistant ovarian cancer patients and a Phase I/II trial in metastatic colorectal cancer patients are in recruitment phase. A Phase I study in human glioblastoma anticipates start in 2012. EGEN-001 is produced under good manufacturing practice with a process scalable to Phase III needs and a shelf-life of more than 3 years at -20 C and -80 C. EGEN-001 has orphan drug status and orphan grants from US FDA and an active collaboration with the National Cancer Institute.
Increased Apoptosis by Arming Oncolytic Influenza Virus with Interleukin-24

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Previously we have developed a conditionally replicating oncolytic influenza A virus based on deletions in the NS1 protein (delNS1). We then developed a strategy to express foreign proteins by this prototype. Interleukin (IL)-24 could serve as a therapeutic protein in oncolysis, since it is known to induce apoptosis in tumor cells.

We have constructed a conditionally replicating influenza A NS1 deletion virus expressing interleukin-24 (delNS1-IL-24). We evaluated the stability of the virus and the expression levels of interleukin-24 by ELISA. We further determined the virus-induced induction of apoptosis by Annexin V and virus-induced activation of caspase-3 by Western blot. We further determined the effect of the chimeric virus on tumor-spheroid formation using the prostate cancer cell line DU145.

The delNS1-IL-24 virus was stable. The virus expressed around 5 ng/ml of IL-24 in the supernatant of infected cells. delNS1-IL-24 and supernatant of infected cells induced significantly higher levels of apoptosis than recombinant IL-24 or delNS1 virus. This correlated with activation of caspase-3 and significant reduced growth of tumor-spheroids.

Armed influenza A viruses expressing IL-24 might be a new therapy of solid tumor treatment.
Abstracts

**A Mathematical Modeling Framework for Antibody-Dependent Cell Mediated Cytotoxicity (ADCC)**

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**Background:** Antibody-dependent cell-mediated cytotoxicity (ADCC) is thought to augment the efficacy of IgG1 mAb therapeutics (e.g. cetuximab) via the recruitment and activation of CD16⁺ cells which in turn will mediate tumor cell killing. This observation has led to drug design efforts to intentionally exploit ADCC for enhanced anti-tumor efficacy. For example, GA201 (Roche pRED Oncology) is a humanized and glycoengineered IgG2 anti-EGFR monoclonal antibody (mAb) with enhanced ADCC currently undergoing investigation in phase 2 trials.

**Problem statement:** The dual mechanisms of action of ADCC-enhanced mAbs such as GA201 result in new and challenging drug development questions, for example:

1. Can we quantify the relative contributions of ADCC and target inhibition toward tumor shrinkage?
2. In light of (1), what markers (e.g. KRAS mutation status, NK cell function) are predicted to confer sensitivity or resistance to the mAb?
3. Given observed kinetics of CD16⁺ cell depletion and recovery following administration of the mAb, what is the optimal schedule to optimize ADCC?
4. In the case of combination therapy with an immune-enhancing treatment, what dose and schedule would provide optimal synergy with the mAb’s ADCC effect?

**Methods:** We propose a mathematical modeling framework for ADCC with the intention of addressing the questions posed above across several ADCC-relevant therapies. The proposed model would integrate clinically obtainable data sources, for example: FACS counts of (CD16⁺/56⁺) cells, NK cell function (CD107a, K562), tumor size (CT scan), mAb concentration in blood, and downstream target inhibition (e.g. pERK).

**Results:** Once qualified against clinical data, such a model could be used (1) to infer relative contributions of ADCC and target effect toward tumor size reduction, and thereby (2) to suggest which tumor molecular background and/or immune status is likely to be sensitive to the drug. The model could also be used (3) to simulate the effects of various dose schedules on ADCC and target-driven efficacy, and ultimately (4) to simulate the effects of dose and regimen of an immunomodulatory co-therapy on the ADCC activity of the mAb.

**Discussion:** We intend to share a diagram of the proposed model in order to solicit feedback from the meeting participants. We believe this modeling effort has the potential to improve decision-making in the development of enhanced ADCC compounds.
Unraveling the Contribution of Osteoclasts in Bone Metastasis Progression using an Immune-Competent Murine Model of Breast Cancer, the 4T1 Model

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Breast cancer (BrCa) is the most common malignancy in women of western countries. BrCa metastasis to the bones is detected in 70% of the patients (Kang, 2006). Research on bone metastasis (BoM) has primarily utilized xenografting of human BrCa cells into immunodeficient mice. These studies have highlighted the vicious cycle that takes place in the bone environment between BrCa cells and osteoclasts (OCL), which stimulates metastatic expansion, hyperactivation of the OCL and bone degradation (Rosa and Siegel, 2006).

We were interested in defining the contribution of the OCL to the progression of BoM in an immune-competent murine model of BrCa. To do so, we used the mouse mammary carcinoma 4T1 model. The in vitro differentiation of murine primary myeloid precursors into multi-nucleated OCL was unexpectedly inhibited by 4T1 conditioned media (4T1-CM). These 4T1-CM induced cells (4iC) did not express OCL-specific proteins, such as TRAP and CATK, and they remained mononucleated. When mature OCL were exposed to 4T1-CM, their morphology, activity and survival were not significantly affected. In vitro, 4iC expressed macrophage markers, such as CD11b and F4/80. In vivo, the analysis of femurs from mice either injected with 4T1-CM over 21 days or harboring BoM of 4T1 cells did not confirm a decrease in OCL number. The presence and the function of 4iC in these bones is currently under investigation.

As 4T1-CM promotes the differentiation of 4iC macrophage-like cells, we explored whether 4T1-CM could also drive earlier progenitors, such as hematopoietic stem cells (HSC), to the macrophage lineage. HSC were treated with 4T1-CM or co-cultured with 4iC in vitro. Preliminary results show that in both cases, more macrophage progenitors were generated by CFU-assay from these HSC compared to untreated HSC or HSC co-cultured with mature OCL.

The results of this study reveal an interesting role for 4T1 BrCa cells in stimulating the generation of macrophage-like cells in the bone environment. Most importantly, we show that 4T1-CM inhibits OCL differentiation. This challenges the commonly accepted notion that BrCa bone metastasis promotes the OCL differentiation and activity observed in immunodeficient murine models.

We are currently focused on identifying which molecule(s) in the 4T1-CM is driving the differentiation of 4iC and on understanding how 4iC macrophage-like cells affect the 4T1 BoM progression. Ultimately, this research may allow the identification of novel therapeutic targets for blocking BoM formation.
Abstracts

Proteolytic Disablement of IgGs and the Restoration of Lost Cell-Killing Functions

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For IgGs to function efficiently in eradicating pathogenic cells and microorganisms, structural components within the IgG lower hinge and adjoining Fc region must engage Fcγ receptors and/or complement. We show that a number of extracellular proteases with relevance to cancer and infectious diseases hydrolyze specific peptide bonds within a limited 5 amino acid zone in the lower hinge/CH2 region. With only a single-cleavage in one heavy chain, IgGs exhibit diminished binding to FcγRs, resulting in impaired antibody-dependent cellular cytotoxicity (ADCC) in vitro. A similar loss of complement-mediated cytotoxicity (CDC) was also observed with single-cleaved antibodies. Detection of this limited cleavage is challenging because single-cleaved antibodies retain antigen-binding capability and display the same physico-chemical properties of intact IgGs under native conditions. In an orthotopic xenograft model using human MDA-MB-231 cells implanted in SCID/Beige mice, a single clip in the lower hinge effectively eliminated the tumor suppressive effects of an anti-CD142 (tissue factor) mAb. An analogous loss of platelet clearance capability by a single-cleaved anti-platelet mAb was shown in a canine model. Interestingly, a subsequent administration of a mAb directed to the specific site of proteolytic cleavage (anti-hinge mAb) not only reversed the functional impairment of the clipped anti-platelet mAb but also achieved more rapid cell clearance than was observed with the intact anti-platelet parent mAb. These in vivo examples mirrored in vitro demonstrations of rescued cell-killing in ADCC and complement assays by anti-hinge antibodies that included mAbs as well as purified (low titer) serum-derived counterparts from human volunteers. These results suggest that a single-cleavage in the lower hinge/CH2 region not only impairs mAb-mediated cell-killing in vitro, but also suggests a potential in vivo immune evasion mechanism whereby limited proteolysis of mAbs within protease-enriched microenvironments can render mAbs silent in terms of Fc-mediated cell-killing functions.
Regulatory T Cells From Apc\textsuperscript{Min/+} Mice are Functionally Impaired by IL-17A

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A large proportion of colon cancer patients have sporadic colon cancer which has an \textit{Apc} mutation mainly in epithelial cells and yet still have increased Foxp3+ cells, developing colon cancer in their sixth or seventh decade of life. In FAP (Familial Adenomatous Polyposis) patients the onset of colon cancer is essentially 100% in the fourth decade of life. Apc\textsuperscript{Min/+} mice resemble FAP patients in their phenotype and develop spontaneous intestinal tumorigenesis. Here we show that adoptively transferred endogenous regulatory T cells (Tregs) from Apc\textsuperscript{Min/+} mice that bear a germline mutation in the \textit{Apc} gene failed to inhibit intestinal tumorigenesis. Wildtype Tregs effectively inhibited intestinal tumorigenesis while the depletion of endogenous Apc\textsuperscript{Min/+} Treg cells also significantly inhibited tumor growth. Interestingly, the ablation of IL-17A from these adoptively transferred Apc\textsuperscript{Min/+} Tregs inhibited tumor growth especially in the small intestine of Apc\textsuperscript{Min/+} mice. Treatment with IL-17A altered the suppressor function of Apc\textsuperscript{Min/+} Tregs and they express higher levels of ROR\textgamma t and less Foxp3 than wildtype Tregs. Effector CD4 T cells from Apc\textsuperscript{Min/+} mice showed increased Foxp3 expression under Th17 cell differentiation conditions, suggesting their preference to become iTregs. In addition, naive CD4 T cells from Apc\textsuperscript{Min/+} mice were more prone to generate inducible regulatory T cells (iTregs) and more resistant to differentiate to Th17 cells, suggesting intrinsic defects of Apc\textsuperscript{Min/+} CD4 T cells in Treg/Th17 cell differentiation. These intrinsic defects contribute to the minimal ability of Apc\textsuperscript{Min/+} inducible Tregs (iTregs) to inhibit intestinal tumorigenesis compared with wildtype iTreg. Taken together, our results suggest that the Apc\textsuperscript{Min/+} mutation impairs the capacity of Foxp3+ Tregs to inhibit intestinal tumorigenesis. Our findings have substantial clinical implications in that FAP patients may have intrinsic defects in CD4 effector T cells and Tregs that favor intestinal tumorigenesis.
Abstracts

Phase I Open Label, Dose Escalation Study of RG7160 (GA201) in Patients with Metastatic Solid Tumors

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Background: We conducted a Phase I dose escalation study to characterize the safety, efficacy, pharmacokinetic (PK) and pharmacodynamic properties of GA201, a humanized and glycoengineered IgG1 anti-EGFR monoclonal antibody with enhanced antibody-dependent cell-mediated cytotoxicity (L. Paz Ares, JCO, 2011 in press).

Methods: Seventy-five heavily pre-treated patients with advanced EGFR-positive solid tumors received GA201 (50 to 1,400 mg) administered qW, q2W, or q3W. Dose escalation followed a 3+3 trial design.

Results: No maximum tolerated dose was reached for any dosing schedule. Common adverse events (AE) included rash (80% of patients), infusion-related reactions (77%), and hypomagnesemia (56%). The first GA201 infusion was accompanied by a marked cytokines release (IL-6, IL-8, IL-10, TNF, IFN), which was less pronounced during the second infusion. Consistently, IRR incidence was highest with the first infusion, becoming less frequent with the second/subsequent infusion(s). Peripheral NK (CD16+/CD68+) lymphocytes reductions were detected after one treatment cycle in all regimens. In contrast, there were less marked changes in all other analyzed peripheral lymphocyte subsets (CD3+, CD4+, CD8+, CD19+ and CD14/45+). Skin rash was characterized by dose dependent infiltration of immune effector cells, which typically contained macrophages, helper T, and cytotoxic T lymphocytes. Clinical efficacy included: one confirmed complete response and two confirmed partial responses in colorectal cancer patients (including one PR in a CRC patient with KRAS mutation), and disease stabilization in 27 patients (evaluated after ≥2 months of therapy).

Conclusion: In summary, GA201 has an acceptable safety profile with manageable AEs and demonstrated promising efficacy in heavily pretreated patient cohort. Preliminary pharmacodynamics parameters analyzed are consistent with the anticipated mechanism of action of GA201.
Tumor Infiltrating Macrophages as a Potential Predictive Biomarker of Response to 5-FU in Patients with Microsatellite-Stable Colorectal Cancer

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Colorectal cancer (CRC) pathological stage predicts outcome and is used to allocate patients (pts) to chemotherapy (CHT). Microsatellite instability (MSI), the phenotype of a deficient DNA mismatch repair system, is a positive prognostic biomarker in CRC, and would predict a poor response to 5-fluoro-uracyl (5-FU) CHT. Although immune cells infiltrating CRC are promising prognostic biomarkers, potential interactions with CHT have not been explored. We explored the prognostic role of tumor infiltrating macrophage (TAM) in CRC, in relationship with cancer stage, MS-status and response to CHT.

CD68 immune-reactive area (IRA in %) was assessed by computer-assisted analysis at the CRC invasion front in 357 consecutive stage II/III CRC of known MS-status (298, 80.5%, MSS), and in 71 metastatic lymph-nodes (LN) of stage III MSS CRC.

TAM density was irrelevant to the prognosis of stage II CRC pts [n=178, p=0.38], but a high TAM density (>4.45% median IRA) was associated with a better outcome in stage III CRC pts [n=179, HR, 0.51, 95% CI 0.28-0.92; p=0.02]. TAM prognostic effect was restricted to stage III pts with MS-stable CRC [n=157, H.R. 0.42, 95% CI 0.22-0.81; p=0.009], and was not observed in MSI CRC pts [n=22; p=0.57]. Among pts with stage III MSS CRC, a high TAM density was associated with better outcome in those 5-FU treated [n=114, H.R. 0.31, 95% CI 0.14-0.71; p=0.005], not in those untreated [n=43; p=0.69]. TAM density in metastatic LN was significantly associated with that of the primary cancer (r=0.27, p=0.01), and a high TAM density in LN had a prognostic impact in stage III 5-FU treated pts [n=71, HR 0.27, 95% CI, 0.10-0.69; p=0.006].

The association of CRC TAM density with good outcome in 5-FU-treated pts with stage III MSS CRC indicates that innate immune cells can be a predictive marker of response to 5-FU. The correlation between TAM density in the primary CRC and in metastatic LN suggests that the innate immune response to CRC is not restricted to the primary cancer, pointing to a potential systemic and synergic effect between CHT and macrophages against CRC.
The Conformational Change at CD3 is not Necessary for Gammadeltatcr Activation

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Ligation of the alphabetaTCR by multivalent pMHC and antibodies induces a conformational change at the TCR CD3epsilon subunit. Induction of this conformational change leads to structural rearrangements in the CD3epsilon cytoplasmic tail and is required for full alphabeta T-cell activation. Compared to the alphabetaTCR the gammadeltaTCR has a similar complex composition but it recognizes structurally diverse antigens. In this study we investigated whether stimulation of the gammadeltaTCR induces these structural rearrangements at CD3epsilon. We show that human as well as murine gammadeltaTCRs have the intrinsic capability to undergo a conformational change at CD3epsilon stimulating with certain anti-CD3 antibodies. In contrast, when stimulated by antigen conformational change was not induced at the gammadeltaTCR. Further more the conformational change at CD3epsilon was dispensable for gammadeltaTCR activation. These findings provide an explanation for the distinctive molecular requirements for signal initiation at these two receptors.
Targeting TGFβRII in Oncology: A Potent Immunomodulatory Antibody with Various Effects on Cancer Progression

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Signaling via the TGFβ pathway plays diverse roles in tumor progression, directly and indirectly driving tumor cell growth and pathogenesis. Direct pro-tumor effects, which occur in TGFβ receptor-expressing tumor cells, include autocrine mitogen production, epithelial to mesenchymal transition (EMT), invasion, migration, and prometastatic cytokine production. Indirect pro-tumor effects, which occur in stromal cells, include immunosuppression and angiogenesis. Due to the pleiotropic effects of the TGFβ pathway in cancer, we set out to disrupt signaling using a mAb that blocks the ectodomain of TGFβRII, in order to inhibit receptor mediated signaling in target cells. Here we describe a high affinity, fully human anti-TGFβRII mAb along with its murine surrogate. We observed in vivo efficacy in various human and murine tumor models treated with anti-TGFβRII as a monotherapy. In addition, anti-TGFβRII therapy functioned at least additively with the cytotoxic cyclophosphamide. Using immune competent murine models of aggressive disease, we have shown that the major indirect mechanism of action of our antibody involves enhanced anti-tumor immunity. Specifically, using an immune–depletion strategy, we have shown that depletion of CD8+ cytotoxic T lymphocytes (CTL) eliminated the ability of anti-TGFβRII to inhibit primary, but not metastatic, tumor growth. Conversely, depletion of NK cells eliminated the ability of anti-TGFβRII to inhibit metastasis while having little effect on inhibition of primary tumor growth. Analysis of NK cells and CTL ex vivo showed that treatment with anti-TGFβRII significantly induced killing activity by these two populations of cells. In addition, the Th1 cytokine response marked by IFNγ secretion by NK cells and CTL was increased in anti-TGFβRII treated animals. Therefore, antibody treatment significantly induced anti-tumor immunity in vivo. Finally, the circulating immunosuppressive T regulatory (Treg) and MDSC populations were reduced in anti-TGFβRII treated animals. In vitro, treatment with the anti-TGFβRII antibody inhibited TGFβ-induced conversion of naive T cells into Treg cells and Treg cell mediated inhibition of T cell proliferation. Collectively, these data demonstrate that selective blockade of TGFβRII with a neutralizing antibody suppresses primary tumor growth and metastasis through both direct and indirect attenuation of TGFβ signaling.

The results of these studies provide compelling data supporting the utilization of a neutralizing anti-TGFβRII antibody as a novel therapeutic strategy for the treatment of TGFβ dependent tumors.
A Novel Phenomenon: E. Coli can Reduce the Expression of CD55 on Granulocytes in Lymphoma Patient’s Blood

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Through E. coli (vaccine) innate immune therapy model in citrate anticoagulant fresh blood, we determine that blood innate immune inflammatory response cause by biological drug. The citrate anticoagulant fresh blood is a dynamic and living whole blood cell suspension, which is a blood living system and simulation model of the blood system. In a more integrated this blood living system, blood and blood cells between soluble substances maintained the integrity of the biological information network.

A total of 11 lymphoma patient’s citrate anticoagulant fresh blood samples were tested. Fresh blood E. coli vaccine experimental group: 0.2 ml (heat inactivated) E. coli (6 × 10⁹/ml) suspension were added to 0.6 ml of fresh citrate anticoagulant whole blood; Fresh blood culture control group: 0.2 ml physiological saline were added to 0.6 ml of fresh citrate anticoagulant whole blood, mixed fully, incubated at 37º for 1 hour, then were centrifuged 2000 rounds per minute for 5 minutes. In the fresh blood system, the CD55 and CD59 expression on whole blood cells determined by flow cytometry of monoclonal antibody. The expression of mean fluorescence intensity in fresh blood E. coli vaccine experimental Group were shown: 854.30±171.21 (CD55 on GRANULOCYTES), 65.06±23.52 (CD59 on GRANULOCYTES), 49.59±10.60 (CD55 on lymphocytes), 35.40±16.79 (CD59 on lymphocytes), 15.57±2.79 (CD55 on red cells), 90.53±29.9 (CD59 on red cells). The expression of mean fluorescence intensity in fresh blood control Group were shown: 1071.77±214.3 (CD55 on GRANULOCYTES), 54.02±15.56 (CD59 on GRANULOCYTES), 46.79±16.56 (CD55 on lymphocytes), 34.50±11.35 (CD59 on lymphocytes), 13.59±3.19 (CD55 on red cells), 87.51±22.20 (CD59 on red cells). The expression levels (854.30±171.21) of CD55 on GRANULOCYTES in fresh blood E. coli vaccine experimental group were significantly lower than expression level (1071.77±214.30) of CD55 on GRANULOCYTES in Fresh blood control group (t=2.6287, P<0.05). The expression levels (90.53±29.90) of CD59 on red cells in fresh blood E. coli vaccine experimental group were slightly higher than expression levels (87.51±21.80) of CD59 on red cells in Fresh blood control group.

E. coli (Vaccine) can regulate the expression of CD55 and CD59 on whole blood cells and cytokine release of GRANULOCYTES. E. coli (Vaccine) has the role of innate immunotherapy for tumor.
Abstracts

**Novel Immunotherapy for Malignant Melanoma with a Monoclonal Antibody that Blocks CEACAM1 Homophilic Interactions**

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Ample data has highlighted CEACAM1 as a potentially important target for cancer therapy, based on mechanistic and clinical grounds. Here we present substantial evidence *in-vitro* and *in-vivo* that blocking of CEACAM1 function with a novel mAb (MRG1) is a promising strategy for cancer immunotherapy.

A murine mAb against hCEACAM1 was raised and tested for specificity and affinity. Efficacy was examined *in-vitro* in a functional reporting system and in cytotoxicity assays, and *in-vivo* in a melanoma xenograft model with adoptive lymphocyte transfer. Preliminary safety was evaluated *in-vitro* and with normal human tissue microarray.

MRG1, a CEACAM1-specific IgG1 mAb with high affinity (KD~2nM), is a potent inhibitor of CEACAM1 homophilic binding that does not induce an agonistic effect or antibody-dependent cell-cytotoxicity response. MRG1 renders melanoma cells more vulnerable to antigen-restricted T cells in a dose-dependent manner, but does not exert any direct effects on CEACAM1⁺ cells. MRG1 synergizes with adoptively transferred lymphocytes to significantly inhibit tumor growth *in-vivo*. Importantly, 49/55 metastatic melanoma specimens were CEACAM1⁺, and CEACAM1⁺ lymphocytes were present in their vicinity, implying that most patients could be amenable to MRG1-based therapy. Finally, normal human tissue microarray displayed only confined binding to some normal epithelial cells on the luminal aspect on some secretory ducts and glands, which was weaker than the broad normal cell binding of other anti-cancer antibodies in clinical use.

MRG1 provides a novel platform for tumor immunotherapy, which could be implemented in melanoma and other carcinomas with strong CEACAM1 expression, e.g. lung cancer.
Enhanced huFcgRIII and/or muFcgRIV Engagement on NK Cells and Macrophages Leads to a Rapid Increase in Tumoral Immune Cell Infiltration in Xenograft Tumor Models Treated with Glycoengineered Anti-EGFR Antibody GA201 (RG7160)

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GA201 is a humanized and glycoengineered IgG1 antibody which targets EGFR. Within its dual mode of action, a distinguishing feature of GA201 compared to currently marketed EGFR antibodies lies in its ability to bind to human FcgRIIIa on immune effector cells with a 50-fold higher affinity, a property conferred by the antibody’s afucosylated Fc glycoforms. Consequently, GA201 exhibits superior potency and efficacy in antibody dependent cell cytotoxicity (ADCC), as shown in vitro using different EGFR expressing cell lines compared to the other anti-EGFR Abs.

Here we evaluated the preclinical in vivo efficacy of GA201 vs. non-glycoengineered EGFR antibodies (cetuximab, panitumumab and non-glycoengineered-version of GA201) in a variety of human tumor orthotopic xenograft models (lung, colorectal, mammary, pancreas and kidney) in Scid-bg mice and in huFcgRIII-transgenic Scid mice. Efficacy studies with these models show very significant increase in survival for GA201 when compared to any other non glycoengineered antibody tested including cetuximab and panitumumab. Preclinical assessment of tumors revealed that only therapy with GA201 significantly increased the infiltration of immune effector cells compared to vehicle control groups (over 4-fold). Interestingly this enhanced tumoral immune infiltration occurs within hours of antibody administration, further supporting the immune effector-mediated mode of action of GA201.
GA201 (RG7160), a Novel Humanised Glycoengineered Anti EGFR Antibody, with Enhanced Immune Mediated Effector Functions for the Treatment of Solid Tumors

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The epidermal growth factor receptor (EGFR, HER1) is a clinically validated target for cancer antibody therapy. High levels of EGFR expression are a common feature of malignant phenotype in many solid human tumors, including those of lung, head and neck and colorectal.

GA201 is a novel humanized and glycoengineered IgG1 antibody which targets EGFR. Similar to other anti-EGFR antibodies, its mechanism of action involves high affinity binding to EGFR, which subsequently results in ligand binding blockade preventing receptor phosphorylation. This leads to inhibition of cell growth and tumor cell migration, VEGF secretion and resistance to apoptosis. However, a distinguishing feature of GA201 compared to its current competitors, including Cetuximab, lies in its ability to bind to human FcγRIIIa on immune effector cells with a 50-fold higher affinity, a property conferred by the antibody’s afucosylated Fc carbohydrates. Consequently, GA201 exhibits superior potency and efficacy in antibody dependent cell cytotoxicity (ADCC), as shown in vitro using different EGFR expressing cell lines compared to other anti-EGFR Abs, also translating in very significant increase in survival as assessed using lung, CRC, breast, pancreas and kidney orthotopic preclinical models.

The enhanced ADCC capability renders GA201 a promising candidate compared to the other anti-EGFR MAbs for the treatment of solid tumours and has entered now phase 2 clinical trials.
Abstracts

GA201 (RG7160) Pre-Treatments and Combination Therapies Improve Efficacy without Significantly Affecting Preclinical Anti-Tumoral ADCC

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GA201 is a humanized and glycoengineered IgG1 antibody which targets EGFR with boosted antibody dependent cell cytotoxicity (ADCC) in phase 2 clinical trials.

Pretreatment with corticosteroids and/or anti-histaminics are of normal practice for monoclonal antibody therapy to prevent potential infusion reactions. GA201 follows a premedication regimen of anti-histaminics and hydrocortisone. Due to the dual GA201 mode of action, evaluation on the impact of these drugs on ADCC is critical and was previously assessed preclinically for potential negative impacts on immune effector cells and their capacity to mediate tumor cell killing.

Combination phase II studies with GA201 + chemotherapy regimens are currently under investigation in NSCLC as first-line treatment (chemotherapy selection based on histology Cisplatin + gemcitabine for squamous and cisplatin + pemetrexed for non squamous) and in CRC as second-line treatment (FOLFIRI for KRAS-mutant CRC or cetuximab + FOLFIRI for KRAS-wildtype CRC). These combinations and others where tested prior use in the clinics to evaluate preclinical efficacy and their impact on the immune effectors in vitro, and in vivo.

Chemotherapy combos showed additive or synergistic effect in anti-tumor efficacy as assessed in orthotopic xenograft models and all pre-medications and chemotherapies tested showed no impact on ADCC supporting their combination with GA201 in the clinics.
Abstracts

Lymphocyte Function Antigen-1 and Leukotoxin Interaction

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Lymphocyte Function Antigen-1 (LFA-1) is a β-2 integrin expressed on the surface of human WBCs. LFA-1 undergoes an inducible conformational change from a non adhesive inactive to an adhesive active state, the latter mediating migration from blood vessels into tissues. Hematological malignancies are characterized by proliferation of WBCs that overexpress activated LFA-1. LFA-1 is known to be the specific target of Leukotoxin (LtxA), a major virulence factor of oral bacterium *Aggregatibacter actinomycetemcomitans* that causes aggressive periodontitis. LtxA targets primate WBCs migrated to the periodontium countering host response against the bacterium. Because of this natural specificity, LtxA was tested for its cytotoxic effect on THP-1 cells *ex vivo*, which were found to be very sensitive to LtxA. Humanized mouse models of leukemia (HL-60) showed prolonged disease free survival upon LtxA injection. Previous studies indicate preference of LtxA to activated WBCs compared to unactivated ones. Therefore, we wished to determine if this preference was due to LtxA specifically binding to active or open conformation of LFA-1. mAB24 was used as marker for active conformation of LFA-1 while CD11a (clone: HI1111) was used as a marker for inactive conformation of LFA-1. Preincubation of fixed THP-1 cells showing high levels of active LFA-1 (mAB24+) with LtxA led to decrease in mAB24 binding indicating that LtxA competes with mAB24 binding site on active conformation of LFA-1. In contrast, preincubation with LtxA had no effect on binding of CD11a mAB HI111 which recognizes only the inactive conformation of LFA-1. These results indicate specificity of LtxA for active LFA-1. The levels of active LFA-1 on healthy WBCs isolated from human blood were relatively low at baseline but increased upon stimulation with phorbol esters. This increase was more pronounced in granulocytes and monocytes. Treatment of human granulocytes and monocytes with LtxA *ex vivo* showed depletion of only active LFA-1 population.

Establishing this specificity is important to provide maximum therapeutic benefit targeting abnormal WBCS with active LFA-1 and sparing normal WBCs expressing inactive LFA-1 thereby minimizing immunosuppression.
Metastasis is responsible for most cancer deaths. We reported previously that transgenic C57BL/6 mice (Tg) over-expressing a chemokine CXCL14/BRAK, suppressed transplanted tumor cell growth. The aim of this study was to determine whether or not this Tg suppresses experimental metastasis of tumor cells and if it suppressed, natural killer (NK) cells participate in this process. Metastatic colony numbers of Lewis lung carcinoma (LLC) cells and B16 melanoma cells in the lung, when these cells were injected from tail vein of the mice, was significantly lower ($P<0.0001$) in the Tg mice than those of wild type mice (Wt). Suppression of metastasis was attenuated by the treatment with anti-NK cell antibodies, anti-asialo-GM1 antibody or anti-NK 1.1 antibody. We injected a $\beta$-Galactosylceramide, a stimulator of NKT cell activity into the Tg mice and found synergistic effect on the reduction of metastatic colony formation of the melanoma cells in the lung, indicating that NK cell activity is essential for the suppression of tumor cell growth and metastasis in these Tg mice and NKT cell further stimulated this suppressive activity. Survival rate of the mice after injection of B16 melanoma cells was significantly ($P<0.0001$) higher in the Tg mice than that of wild type C57BL/6 mice. Our data indicated that high expression level of CXCL14/BRAK is beneficial for the suppression of tumor cell growth and metastasis and increase survival rate by stimulating NK cell activity.
Preclinical Evaluation of Cell Based Pancreatic Cancer Vaccine with Cadi-05 as an Adjuvant

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Cadi-05 (Heat killed mycobacterium w) is a potent TLR 2 agonist and induces pure potent Th1 response. In advanced Non small cell lung cancer, it is found to potentiate efficacy of chemotherapy (Paclitaxel + Cisplatin) with improvement in response rate and overall survival in a randomized controlled clinical trial. It is also a potent vaccine adjuvant for prophylactic vaccines providing faster, higher and sustained antibody titers in animals and humans. The therapeutic vaccine for pancreatic cancer, with killed pancreatic cancer cells as an antigen with Cadi-05 as an adjuvant was evaluated for its ability to induce cell mediated immune responses (by elispot for IFN - γ, Il-2 and Granzyme B) and effector function (by brdu incorporation) and its effect on tumor size and survival following intradermal immunization.

The vaccine made using Mia PaCa-2 cell line induced cell mediated immune responses against the Mia PaCA-2 as well as against the other cell lines (Panc-1, ASPC-1, SW-1990). The humoral response generated was reactive (western blot) against all four cell lines. The vaccine also generated effector function of similar magnitude against all four cell lines.

For evaluating efficacy, C57BL/6 BYJ mice were injected Pan-02 cells (1x10⁵) subcutaneously for developing pancreatic cancer model. At day 10 post tumor induction tumor size achieved was ~200-225 mm³. Mice were randomized to receive vaccine or placebo (18 mice each) on day 10. No other therapy was used in the experiment.

IN Vaccine arm tumor regression was seen in 61% of animals before progression. Tumor regression was not seen in placebo arm. Tumor progression was found to be slower in vaccinated animals with mean tumor size 35% and 59% of placebo arm on day7 (633 mm³ vs. 223 mm³ ) and day 12 (740 mm³ vs. 437 mm³ ) respectively resulting in survival benefit of 36% on day 40 following immunisation.
Abstracts

Tumor-Derived Wnt-Inducible Signaling Protein-1 (Wisp-1) Exhibits Paracrine Immunosuppression by Inhibiting Cellular Response to Interleukin-12

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Interleukin-12 plays an important role in CD4+ T helper cell, CD8+ Cytotoxic T cell, and Natural Killer cell biology. Local delivery of IL12 within the tumor microenvironment enhances antitumor immunity. While the details remain unclear, immunoregulatory elements within the tumor microenvironment are thought to dampen the effect of IL12 as an adjuvant. The objective of this study was to identify biochemical mechanisms of tumor-immune cell crosstalk that locally suppress the action of IL12. A high-content in vitro assay was developed to quantify tumor-mediated suppression of the action of IL12 using a reporter cell line. Following an induction period, the melanoma cell model, B16F0, inhibited the response of an IL12-reporter cell line, in vitro that was not explained by induction of apoptosis or creation of a cytokine sink. SPARC, exosomes, and Wnt-inducible signaling protein-1 (WISP-1) were identified using a proteomics workflow and confirmed to be enriched in B16F0-conditioned media. Neutralization of WISP-1 recovered the activity of STAT4, a key transcription factor within the IL12 pathway, within the in vitro co-culture assay. Moreover, WISP-1 was observed to be expressed in vivo following intradermal challenge with B16F10 cells and exhibited dependence on tumor size.
A Metastasis Inhibiting Vaccine Based on the Laminin Peptide YIGSR

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It has been reported that metastatic melanoma cell lines selectively bind in vitro with the synthetic laminin pentapeptide tyrosyl-isoleucyl-glycyl-seryl-arginine (YIGSR). It is also known that synthetic YIGSR can bind with high affinity to a 67 kDa non integrin laminin receptor, known as “metastasis associated receptor”. Moreover, YIGSR can inhibit experimental metastasis and tumor growth. Several anti-idiotypic antibodies (anti-Id) vaccines have been used in clinical trials with promising results. In the present study we used high titer anti-YIGSR serum from immunized rabbits or pre-immune rabbit serum (control) as a vaccine to mice, in order to produce in vivo YIGSR analogous anti-Id antibodies. We have used this “vaccine” to B16 melanoma, pancreatic carcinoma (PANC) and to Lewis Lung carcinoma (LLC) bearing mice and examined spontaneous metastasis formation and survival. The data indicate that tumor bearing anti-YIGSR serum immunized mice survived at least four weeks longer than controls. Their serum contained an antibody that could inhibit the binding of ⁹⁹mTc-YIGSR to cells and could bind with the immunized mice IgG. Tumor could not be visualized by scintigraphy in these mice and in contrast to controls biopsy after sacrifice indicated no metastasis at day 75. These results indicate that the development of an anti-idiotype antimeetastatic vaccine based on peptide YIGSR may be possible.
Facile Generation of APCs Using Soluble Multimeric CD40L as a B Cell Proliferation Stimulus

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Since the initial studies of Banchereau et al., it has been recognized that human B cells proliferate massively ex vivo when treated with a CD40 stimulus plus IL-4. Such “CD40-B cells” are known to be strong antigen-presenting cells (APCs) that can replace dendritic cells (DCs) for many applications. However, the production of CD40-B cells generally requires co-culture with cell lines that express membrane CD40L, since this form of CD40L supplies a many-trimer stimulus needed to cluster CD40 receptors and thereby activate the B cells. This added level of complexity has compromised the practicality of using CD40-B cells instead of DCs for generating anti-tumor CD8+ T cells. To circumvent this problem, we tested a 4-trimer, multimeric soluble form of CD40L produced by fusing the body of surfactant protein D with the extracellular domain of CD40L to make SP-D-CD40L (UltraCD40L™). This protein was produced by CHO cells and is stable for several weeks at room temperature and > 5 months at 4°C. To grow CD40-B cells, CD19+ or CD20+ cells were immunomagnetically selected from venous blood and cultured with 10-20% CHO cell supernatant plus IL-4. About 100-fold expansion of B cells occurred in two weeks and the resulting cells were >95% positive for CD19, CD80, and CD86. These growing CD40-B cells can be cryopreserved and then thawed for further expansion many months later. To use these CD40-B cells as APCs, they were treated with mitomycin and pulsed with the NLV peptide, a prototypic antigen from CMV pp65 that is an immunodominant epitope for HLA-A2.1 donors. Using these cells as APCs for either PBMCs or purified CD8+ T cells, antigen-specific CD8+ T cells were generated that were recognized by NLV/A2.1- tetramer staining. By using UltraCD40L™, this advanced CD40-B cell system provides a facile way to generate proliferating APCs that should prove very useful for the active immunotherapy of cancer.
Abstracts

**Human Adaptive Immune System In RAG2-/-γc-/- Mice: As Novel Preclinical Model for Cancer Immunology**

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**Background:** Although a lot of biologic processes are conserved in human and mice, species-specific differences still exist, that do not allow direct translation of findings from mice to human. Research in human immunity is mostly restricted to *in vitro* experiments that lack components and complexity of a living organism. The development of humanized mice that partially or completely reconstitute the human immune system would overcome these limitations.

**Results:** Here, we report our recent results that reconstitution of newborn Rag2-/-γc-/- mice with human hematopoietic stem cells (HSC) leads to *de novo* development of major functional components of the human adaptive immune system. We found stable engraftment of human immune cells in the main immunological organs of the mice, such as spleen, bone marrow, lymph node, and blood. Development of a functional human immune system resulted in formation of splenic follicular structures in recipient mice, which were absent in non-reconstituted animals. Identification of human HSC in bone marrow of transplanted mice older than 20 weeks indicated that the engrafted HSC had successfully and stably homed to stem cell niches in the mice. As clear evidence for an intact functional interplay of all components of the engrafted human immune system, we could demonstrate stable human IgG levels in serum of reconstituted mice for at least 12 months post reconstitution.

Additionally, we tested whether these animals could be used in PD studies with xenotransplanted human cancer cell lines. Induction of tolerance was a major goal in these studies to prevent rejection of the HLA class-I mismatched transplanted cancer cells. To this end, the developing human immune system was challenged with irradiated tumor cells (Raji cell line) during the reconstitution phase. Priming of the developing immune system to the major surface antigens of irradiated Raji cells indeed prevented rejection of the same cell line placed as s.c. tumor at a later time point as indicated by the faster tumor growth kinetics of s.c. Raji tumors in reconstituted tolerized animals versus reconstituted non-tolerized mice.

Since our humanized mice mount a human B-cell response, this model is also suited for generation of antigen specific human IgGs and might have considerable promise for development of human therapeutic mAbs against e.g. cancer, autoimmune or infectious diseases.

**Conclusion:** The new humanized Rag2-/-γc-/- mouse model created by intrahepatic injection of CD34+ hematopoietic stem cells sustains long-term multi-lineage human hematopoiesis and is capable of mounting immune responses mediated by various human immune effector cells.
Abstracts

L1-E2 Chimeric Capsomers Produced In *Escherichia Coli* as Prophylactic and Therapeutic Vaccines Against Human Papilloma Virus 16

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Current prophylactic vaccines against Human Papillomavirus (HPV) types 16 and 18 do not exhibit therapeutic effects on women already infected. A combined prophylactic/therapeutic vaccine, which could prevent new infections and at the same time helps to clear current ones, is most desired. L1 is a late HPV protein used to generate humoral immune response, while E2 is an early viral protein that plays central roles during viral life cycle and, as shown, capable to induce cellular immune response. Fusion of L1-E2 as chimeric capsomers and their production in *Escherichia coli* (E. coli) could be useful for a prophylactic/therapeutic vaccine.

Generate HPV16 L1-E2 fusion proteins as chimeric capsomers able to induce humoral and cellular immune response, and antitumoral activity in murine models.

Five fragments from the HPV16 E2 gene and the complete E2 gene were cloned 3’ terminally in the GST-L1DN10+2DC29 gene in pGex 4T-2 vector. Resulting plasmids were used to express fusion proteins in *E. coli* that were purified with GST-trap columns and gel filtration. Fusion proteins were analyzed by sucrose gradient sedimentation followed by Western blot, SDS-PAGE, electron microscopy, and ELISA. On the other hand, a vector (pCDNA4/TO-E2) was constructed and transfected in TC-1 cell line to stably express the HPV16 E2 protein. The fusion proteins are used as antigens to immunize C57/BL6 mice to evaluate the induction of humoral (detected in serum by ELISA anti-L1) and cellular immune responses (detected by CD107 Degranulation Assay from splenocytes); and finally, to evaluate their capacity to reduce or inhibit tumor growth in mice.

We have generated six constructs: pL1-E2N1, pL1-E2N2, pL1-E2H, pL1-E2C, pL1-E2N2-H y pL1-E2full length. Culture conditions (bacterial strain; culture temperature; start point, length and concentration of IPTG for induction) to produce and purify the fusion proteins were standardized. The most soluble protein was L1-E2H, yielding 310 µg/L, with protein concentration of 280µg/ml, and 89% purity. Sucrose gradients sedimentation and electron microscopy showed that fusion proteins form heterogeneous aggregates, and ELISA assays indicated the presence of neutralizing epitopes. We are currently evaluating their immunogenicity in vivo, using a mouse model. In addition, the tumor growth curve for the murine model was standardized with TC-1_E2HPV16 cell line in C57BL/6 mice; the presence of E2 gene in this stably transfected cell line was corroborated by PCR and its expression by Western blot using α-E2 antibodies.

We have produced, purified and analyzed six L1-E2 fusion proteins, and found that L1-E2H is the fusion protein with the highest solubility and yield. A murine model is being implemented to test the protective effect of this construct, by using TC-1_E2HPV16 cell line and C57BL/6 mice.
Abstracts

Analysis of Tumor/Host Differences in Anti-Cd44-Treated Xenografts Using Global Gene-Expression Profiling

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CD44 is a single-chain, single-pass, transmembrane glycoprotein, which is widely expressed in physiological and pathological systems [Ponta et al., 2003]. The main ligand of CD44, hyaluronic acid (HA), binds to the constant region of CD44 [Toole, 2009]. CD44 is involved in lymphocyte activation and homing from the vascular system to secondary lymphoid organs and sites of inflammation [Heldin et al., 2008]. In addition, CD44 has been found to play a role not only in regulating cell proliferation, cell differentiation and migration but also angiogenesis, presentation of cytokines, chemokines, and growth factors to the corresponding receptors, docking of proteases at the cell membrane, and signaling for cell survival.

RO5429083 is a humanized IgG1 anti-CD44 mAb which binds to human and cynomolgus monkey, but not to murine CD44. RO5429083 binds to the constant region of CD44 in a loop that is spatially close to the reported HA binding domain and therefore potently inhibits binding of CD44 to HA. Despite broad in-vivo efficacy in a panel of cell lines and patient-derived xenograft models, the mode of action (MoA) of RO5429083 is poorly understood.

To gain a better understanding of the cellular and molecular processes involved after treatment with RO5429083, we conducted a global gene-expression profiling experiment. An MDA-MB-231 xenograft model was treated with RO5429083 samples were taken at several time points after treatment and were subjected to Affymetrix microarray analysis. Additional analyses – by mRNASEq and Luminex – were carried out on the same samples. The main rationale, separating the tumor response from the host response was followed by analyzing all samples for both human and mouse signals. Affymetrix microarray analysis was performed on both the human (HG-U133 Plus 2.0) and the mouse chip (Mouse 430 2.0). mRNASEq data was mapped to the human and the mouse genome and Luminex analyses were performed with both a human and a mouse panel of cytokines.

Both gene-expression profiling and Luminex analysis showed subtle differences between the tumor and the host response. Especially on a cytokine level a clear separation of cytokine production was observed between human and mouse cytokines. While we saw initial immune responses and signaling on the tumor side (e.g. NF B signaling, production of cytokines for macrophage induction), the host showed an up-regulation of corresponding cytokine receptors as well as production of cytokines for activating further immune responses. Combining the different data sources we got a pretty clear understanding of the processes and pathways involved in gene expression changes after treatment with RO5429083, especially in terms of the tumor/host distinction. This pilot could be a good example for future analyses on xenografts.
Abstracts

The Ig-Like α3 Domain of Soluble Mica can be Engineered to Target Tumor Associated Antigens and Recruit NK-Cells to Kill Taa-Expressing Cells In Vitro

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Membrane-bound MICA and MICB are natural ligands for the NKG2D activating receptor on NK- and certain T-cells. The expression of both MIC proteins is restricted to stressed cells such as infected or malignant cells. However, diseased cells can prevent MIC expression and thereby escape attack by NKG2D-bearing effector cells of the innate immunity system.

To overcome this escape, we intend to attach targeted, soluble MICA molecules to the exterior of specific tumor cells where they can act as adapters to recruit and activate NKG2D-bearing cells to kill the targeted cells. The α3 domain of the monomeric MIC proteins has an immunoglobulin-like structure with solvent-exposed loops. These loops can be used as targeting motifs much as antibody CDR’s. The α3 domain of MICA fused to the pIII capsid protein has been displayed on filamentous M13 phages. Peptides that bind specific integrin molecules have been grafted into 1 or into 2 of those α3 loops and direct the binding of M13 phages to the target integrins in an ELISA format.

When α3 domains with the grafted loops are integrated into soluble MICA molecules, the latter also bind the target integrins in the ELISA format. Furthermore, the targeted soluble MICA molecules can in vitro act as adapters to recruit NK-92 cells to bind MCF7 cells expressing the integrin target and kill the cells. HeLa cells, which express wild type membrane-bound MICA but do not express the target integrin, are also killed by NK-92 cells, but the addition of the targeted soluble MICA does not enhance killing of HeLa cells.

This platform using phage display of the α3 domain of MICA to create targeted, soluble MICA molecules with selected, specific immunoglobulin-like α3 domains provides an adaptive form of soluble MICA to capture the potency of MICA-NKG2D innate immunity. We are developing such targeted, soluble MICA molecules as acute immunotherapies to eliminate specific infected or malignant cells in humans.
On the Role of Foxp3 Isoforms

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Regulatory T (T-reg) cells play a key role in immune homeostasis in tumor microenvironment. The transcription factor Foxp3 is the master regulator of T-reg phenotype and function. Curiously, murine T cells express a single isoform of Foxp3, whereas human T cells express two co-dominant versions of Foxp3 of which the smaller form is missing exon 2 (Δ2). We have previously published that Foxp3Δ2 fails to up regulate the Pim 2 kinase and others have reported that members of the ROR family bind to this region of Foxp3. However, whether these isoforms differentially affect T-reg function is unclear. Here, we show that introduction of Foxp3 and Foxp3Δ2 into primary human CD4+CD45RA+CD25− T cells induced suppressive activity that was indistinguishable from natural T-regs. However, when the same vectors were introduced into CD4+CD45RA−CD25− T cells, we observed that the full-length Foxp3 construct was unable to introduce a suppressive phenotype whereas Foxp3Δ2 retained its ability to promote suppressive activity. These studies support the use of naïve T cells for strategies that transduce effector T cells with Foxp3 expressing vectors as a means to rapidly generate T-regs for use in adoptive T cell therapy. Moreover, these studies suggest that the Foxp3 isoforms expressed in humans do play distinct roles in modulating suppressive activity in T-reg subsets and alterations of the ratio of one Foxp3 isoform to another may have implications for tumor and autoimmune disease.
Abstracts

Immunomodulatory Properties of Soluble Human CD5 in Mouse Models of Disease

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CD5 is a lymphoid-specific membrane glycoprotein constitutively expressed in all T cells and a small subset of B cells, reaching its highest expression levels in T and B cells with regulatory or anergic function. CD5 is physically associated to the antigen-specific receptor in both T and B cells, negatively regulating its signaling. A soluble form of CD5 also exists which is released via proteolytic cleavage during lymphocyte activation, and whose functional relevance is unknown, in part due to the elusive nature of the CD5 ligand/s. Here, we describe the generation and characterization of a transgenic mouse expressing high circulating levels of recombinant soluble human CD5 (rshCD5), aimed at studying the relevance of conserved ligand-receptor interactions mediated by CD5. Interestingly, analysis of the transgenic (rshCD5tg) mice showed a significantly reduced proportion of lymphocyte subsets with well known regulatory properties, namely spleen Treg cells (CD4+CD25+FoxP3+), and peritoneal IL-10-producing CD5+ B cells. On the other hand, NKT cells were increased in the spleen and peritoneum of transgenic mice. All of these effects were readily reproduced in wild-type C57Bl/6 mice by prolonged infusion of exogenous rshCD5. In agreement with these phenotypical findings, rshCD5tg mice displayed more severe forms of two different experimentally induced autoimmune diseases (EAE, CIA). Finally, growth of B16 melanoma tumours was shown to be slower in rshCD5tg mice, but also in wild-type mice subjected to prolonged infusion of exogenous rshCD5 plus chemotherapy. Altogether, these results suggest that soluble forms of CD5 might be used in the treatment of cancer due to their immunomodulatory properties.
Abstracts

Dimercaptosuccinic Acid-Coated Magnetite Nanoparticles as a Localized Interferon-Gamma Delivery System for Cancer Immunotherapy

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Classical treatments for cancer present important side effects, as they affect both tumors and healthy tissue. Cancer immunotherapy attempts to specifically improve the natural immune response to tumor cells. Here we tested small, uniform dimercaptosuccinic acid (DMSA)-coated monodisperse magnetic nanoparticles as an interferon-gamma (IFN-γ) delivery system in two mouse models of cancer. We guided the IFN-γ-loaded magnetic nanoparticles to the tumor site by application of an external magnetic field, and subsequently analyzed the efficiency of nanoparticle accumulation, IFN-γ release at the area of interest, and the effects of both on tumor development. We observed a relevant cytokine dosage in the area of interest, which led to increased T cell and macrophage infiltration at the tumor site, and promoted an anti-angiogenic effect. The combined action led to a significant reduction in tumor size in both models. Our findings indicate that IFN-γ-adsorbed DMSA-coated magnetite nanoparticles can be used as an efficient in vivo drug delivery system for tumor immunotherapy.
Abstracts

Exploratory Biomarker Program for RG7160 (GA201) in the Phase I-II Clinical Trials*

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Early clinical development of GA201, a humanized and glycoengineered IgG1 anti-EGFR monoclonal antibody with enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) is accompanied by an extensive biomarker program, simultaneously addressing both downstream EGFR pathway inhibition and ADCC-related immunological components of activity.

The main GA201 biomarker hypothesis assumes the best response could be expected in EGFR-driven tumors presenting ‘immunoresponsive’ phenotype, notably a higher baseline infiltration by CD16+ cells.

An extensive bio-sampling implemented in CRC phase I / tail, H&N ’mode of action’ (MoA) and CRC and NSCLC phase II studies, comprises a baseline vs. on treatment tumor collection, often made mandatory. Apart from EGFR downstream inhibition IHC markers, single vs. duplex staining of immunocompetent cells has been done, which includes assessment of CD3, CD4, CD8, CD16, CD56, NKP46, NKP44, CD68, MHC II and perforin.

Blood samples are systematically collected at baseline and before / after every GA201 administration for a complex immunophenotyping, assessment of NKs’ functional responses, extended cytokine profiling by Luminex 30-plex, proteomics by MS as well as overall tumor antigen-specific T responses in HLA-defined subgroups.

Depending on the trial design, the anti-tumor effects of the drug are assessed using RECIST, FDG-PET, and additional IHC for Ki67 and TUNEL.

Roche Clinical Repository (RCR) samples are collected for all trials subjects to address the issue of innate host immunological characteristics.

Despite a clinically challenging bio-sampling program in GA201 trials (mandatory biopsies, time-critical blood sampling for PBMC isolation and immunophenotyping) the compliance of sites has been very high. The first biomarker analysis results for phase I / tail BO21495 will become public in late 2011, and in early 2012 for the MoA BP22350 study.

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Immunotherapy Against the Neural Stem Cell Marker Glast is Effective in a Murine Model of Malignant Glioma

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The identification of antigens preferentially expressed in glioblastoma (GB) and involved in its malignant phenotype is critical for developing immunotherapy strategies. We observed by DNA microarray analysis and confirmed by Real Time-PCR and western blot that the neural stem cell marker GLAST (Glutamate Aspartate Transporter), highly expressed in cells of the radial glia, is also expressed in the plasma membrane of murine and human Glioma Stem-like Cells (GSC).

Using murine GL261-NS, we isolated a GLAST-enriched GSC subpopulation by immunomagnetic separation and we found that survival of C57BL6N mice injected intracranially with GLAST + cells was shorter than that of mice injected with GLAST - cells (p=0.00057). To determine whether GLAST peptide-based immunotherapy protects against GL261-gliomas, we identified different peptide epitopes. The sequence of GLAST peptides was screened for high-scoring peptides containing the major histocompatibility complex (MHC) Dα peptide motif using BIMAS and SYFFEITHI softwares and we selected four peptides. Glioma-bearing mice (n=12) received sc injections at days 7, 14 and 21 after GL261 cells implantation of all four peptides in a dose of 15 microg/peptide emulsionated in Montanide ISA51VG adjuvant in a ratio of 1:1 Single peptides were injected into separate sites. Imiquimod was applied at the same site as peptides for 3 days, beginning one day before vaccination. Control mice were treated with vehicle (n=12). The survival analysis demonstrated that GLAST-peptides provide a significant protection against GL261-NS (p=0.02 vs control mice). We also found that in tumors of vaccinated mice GLAST expression was 5.2 ± 2.1 fold lower (P< 0.005), IFN-γ and TNF-α expression were 3.2 ± 0.9 (P = 0.01) and 8.3 ± 2.5 (P = 0.009) fold higher, respectively compared to controls. These data suggest that a specific anti-tumoral immune response was primed against GLAST expressing tumor cells providing a first evidence that GLAST peptide-based immunotherapy could be a promising treatment for GB patients.
Melanoma Patients Vaccinated with Dendritic Cell Vaccines Mount Long-Lived Melanoma-Antigen Specific Polyfunctional Cd8+ T Cells

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Cancer immunotherapy with dendritic cells (DCs) is based on the potential of DCs to induce CD8+ T cells tumor/antigen-specific response against cancer cells. The aim of newer generation of DC vaccines is to generate high quality high avidity and also poly-functional CD8+ effectors T cells and long-lived memory CD8+ T rather than focusing on T cell quantities. CD8+ effector T cells are expected to reject established tumors whereas memory CD8+ T cells are expected to prevent the tumor relapse on a long-term.

Patients suffering from melanoma have been treated using these vaccines and we show in our recent studies the analysis of their immune response to the disease at different time points before and after vaccination.

To illustrate the potent immunogenicity of DC vaccines, we have studied in-depth patients who experience long-term survival after DC vaccinations. Thus, PBMCs from CMR009 patient have been cultured for seven days with a mix of 4 different melanoma peptides: Mage-3, Gp100, MART-1 and Tyrosinase. The flow cytometry analysis of antigen-specific CD8+ T cells with tetramer shows 40% of antigen-specific CD8+ T cells in PBMCs taken few months after vaccination compared to the baseline, which is basically the patient blood draw taken before vaccination. This response also lasts in time as observed in the analysis of this patient blood draws 1½ years and seven years after vaccination with around 30% of melanoma tetramer positive cells. Our results demonstrate that the effectors CD8+ T cells are induced after vaccination and might prevent tumor growth. We also show that the memory CD8+ T cell response is effective and could prevent relapse in melanoma patients after vaccination even several years later. Other melanoma patients have been treated using these vaccines and around 50% of them mount a long lasting response for melanoma tetramer up to several years after vaccination.
Enhancement of Anti-Tumor Immunity Through Local Delivery of Anti-CTLA-4 and Anti-GITR mAbs by Dendritic Cells
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Immune modulatory mechanisms prevent autoimmunity but limit the effectiveness of cancer immunotherapy. Systemic administration of mAbs targeting immune modulatory receptors CTLA-4 and glucocorticoid-induced TNFR-related protein (GITR) on Tregs and effector T cells augments anti-tumor immunity both experimentally and clinically, but can induce life-threatening autoimmunity. We hypothesized that local delivery of anti-CTLA-4 and anti-GITR mAbs to the sites where T cells and tumor antigen-loaded DC vaccines interact would enhance the induction of anti-tumor immunity but avoid the induction of autoimmunity. To achieve local delivery, DCs transfected with mRNA encoding the H and L chains of anti-mouse CTLA-4 and GITR mAbs were coadministered with tumor antigen mRNA-transfected DCs. We observed enhanced induction of anti-tumor immunity and significantly improved survival in melanoma-bearing mice, without signs of autoimmunity. To translate our results to humans, we demonstrated that DCs transfected with mRNA encoding humanized anti-human CTLA-4 mAb and mRNA encoding a soluble human GITR-L fusion protein enhanced the induction of anti-tumor CTLs in response to DCs transfected with mRNAs encoding either melanoma or breast cancer antigens. Based on these results, this approach using local delivery of immune modulators to enhance vaccine-induced immunity is currently being evaluated in a Phase I clinical cancer immunotherapy trial.
Radon and Lung Cancer in Bangalore Metropolitan, India

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Radon is a radioactive gas released from the normal decay of $^{238}\text{U}$ in rocks and soil. It is an invisible, odorless, tasteless gas that seeps up through the ground and diffuses into the air. In a few areas, depending on local geology, radon dissolves into ground water and can be released into the air when the water is used. Radon gas usually exists at very low levels outdoors. However, in areas without adequate ventilation, such as underground mines, radon can accumulate to levels that substantially increase the risk of lung cancer. Radon decays quickly, giving off tiny radioactive particles. When inhaled, these radioactive particles can damage the cells that line the lung. Long-term exposure to radon can lead to lung cancer, the only cancer proven to be associated with inhaling radon.

Public interest in radon has been occasionally piqued by articles in the general press. Considerable attention has been given to the high radon levels that were uncovered in the Reading Prong region of Pennsylvania, following the discovery in late 1984 of extremely high levels in one home. Several epidemiological study programmes in different countries are in progress to estimate the population exposures due to natural radiation with a view to obtain the radiation risk coefficients at low dose rate levels. In this regard, radiation surveys in high background areas (HBRAs) can provide excellent settings for epidemiological studies relating to the effects of low doses of radiation. In view of these, a comprehensive estimate of the natural inhalation dose requires both $^{222}\text{Rn}$ and $^{220}\text{Rn}$ levels in the indoor atmosphere. In this outlook an attempt is made to investigate the $^{222}\text{Rn}$ and $^{220}\text{Rn}$ levels in dwellings of Bangalore Metropolitan, India. Three year results shows that the activity concentrations of $^{226}\text{Ra}$, $^{232}\text{Th}$, radon in ground water, the concentrations $^{222}\text{Rn}$, $^{220}\text{Rn}$ and the dose rate (mSv$\cdot$y$^{-1}$) are at alarming levels for the environment of Bangalore Metropolitan, India. The detailed experimental methodology, results and the control measures are discussed in detail.
Breast Carcinoma Prevention and Treatment via Complement Mediated and Antibody Directed Duct Epithelial Cell Lysis Applied by Ductal Lavage

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Classically it was believed that each human breast contained 15 to 20 lobes. More recently it was found that 90% of nipples contain 5-9 ductal orifices that communicate with nonanastomosing duct systems that extend to terminal duct lobular units. Ductal lavage is currently used to obtain duct epithelial cells for cytopathological microscopic analysis. Also, it is routine pathology practice to use cell type specific antibody directed immunohistochemical stains to identify epithelial derived breast carcinomas in breast biopsy specimens. Invasive breast carcinomas are generally ductal carcinoma (80%) and lobular carcinoma (5-10%). These are derived from the luminal epithelial cells of the ductal/lobular units. The less common breast medullary carcinoma (<2%) is believed to be derived from the myoepithelial cells that are next to the luminal epithelial cells. In the United States the median age at diagnosis for invasive carcinoma of the breast was 61 years of age and for breast carcinoma in situ it was 58 years of age.

The concept of the need to get rid of the breast duct epithelium in a timely fashion is not new. How to best achieve this end remains an open issue. A proposed solution is a liquid cocktail containing monoclonal antibody directed against ductal/lobular epithelium and myoepithelium. The cocktail should contain all the complement components and these could be serum derived and sterilized by irradiation and not complement inactivating heat sterilization. The cocktail can also contain monoclonal antibodies directed against complement inhibitors on the ductal epithelial cell surface such as CD59 and CD46. Such antibodies have been shown to inhibit these epithelial cell surface complement inhibitors and allow epithelial cell lysis to proceed. It could also ironically contain complement fixing monoclonal antibodies directed against the epithelial cell surface complement inhibitors and one antibody could perform the dual functions of complement inhibitor inhibition and complement fixation. Monoclonal antibodies directed against breast duct epithelial stem cell antigens such as CD44 could also be introduced. The use of humanized monoclonal antibodies can minimize antigen sensitization. Additional ingredients such as local anesthetic and antibiotic can be added. Bacteria such as Staphylococcus aureus also contain complement inhibitors and bacterial mastitis could interfere with such a ductal lavage treatment. The cocktail could be applied by ductal lavage as a minimally invasive office procedure.
The Dual Role of Epithelial NF-κB Activation in Intestinal Tumorigenesis

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NF-κB is a proinflammatory transcription factor, which is elevated in inflammatory conditions and cancer. To test the effect of chronic NF-κB activation in intestinal epithelial cells (IEC) on tumor development, we developed transgenic mice expressing a constitutively active form of the NF-κB-activating kinase IKKβ in IEC (IKKβEE<sup>IEC</sup> mice, Guma <i>et al.</i>, 2011, J Ex Med, in press). These mice exhibit constitutive NF-κB activation in IEC, but do not suffer from devastating inflammation and do not develop tumors. However, when crossed to mice with IEC-specific allelic deletion of the Apc tumor suppressor gene (Apc<sup>Δ<sub>IEC</sub></sup> mice), these mice develop by the age of 1 month, significantly more β-catenin-positive lesions throughout the whole intestine than regular Apc<sup>Δ<sub>IEC</sub></sup> mice, suggesting that NF-κB activation accelerates the loss of the wild-type (wt) Apc allele. Interestingly, by the age of 3 months, IKKβEE<sup>IEC</sup>/Apc<sup>Δ<sub>IEC</sub></sup> mice have multiple tumors in the colon and the very proximal small intestine regions, whereas most of the small bowel is spared from tumors. This is in contrast to regular Apc<sup>Δ<sub>IEC</sub></sup> mice, which display low amount of tumors evenly throughout the whole intestine at this age. Thus, NF-κB has both pro- and anti-tumorigenic effects on the intestinal epithelium, depending on the tissue.

Exploring the mode of action of NF-κB in tumorigenesis, we have detected high levels of inducible nitric oxide synthase (iNOS) in the IEC of IKKβEE<sup>IEC</sup> mice. Accordingly, the levels of the nitrosylation marker nitrotyrosine and the DNA damage marker γ-H2AX were also increased in these mice comparing to wt mice. Strikingly, treating IKKβEE<sup>IEC</sup>/Apc<sup>Δ<sub>IEC</sub></sup> mice with iNOS inhibitors during the first month of life led to significant decrease of β-catenin positive lesions, suggesting that NF-κB accelerates the loss of wt Apc allele through iNOS up-regulation.

Studying the anti-tumorigenic aspect of epithelial NF-κB activity, we have found that IKKβEE<sup>IEC</sup> small intestines display increased levels of T cell infiltration, including CD8+ cytotoxic T cells, likely through up-regulation of CCL2 chemokine expression. Mating Apc<sup>Δ<sub>IEC</sub></sup> mice with Rag1<sup>-/-</sup> mice, lacking mature B and T lymphocytes, dramatically increased the number and size of tumors in the small bowel, suggesting that T cells, recruited to intestinal epithelium by activated NF-κB, may protect the intestine from tumor development.

Taking together, our data suggest a dual role of chronic epithelial NF-κB activation in intestinal tumorigenesis: on the one hand, promoting genomic instability through iNOS upregulation and, on the other hand, recruiting cytotoxic T cells that fight tumor growth. Whether a tumor will develop depends on the balance between these two roles of NF-κB and its interaction with the local microenvironment.
Abstracts

γ-Radiation Enhances Immunogenicity of Cancer Cells by Increasing the Expression of Cancer-
Testis Antigens In Vitro and In Vivo

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γ-radiation is an effective treatment modality for cancer. There is accumulating evidence that radiotherapy supports tumor-specific immunity. It was described that irradiation induces de novo protein synthesis and enhances antigen presentation, we therefore investigated whether γ-radiation results in increased or de novo expression of cancer-testis (CT) antigens and MHC class I (MHC-I), thus allowing efficient immunological control. This is especially relevant because the expression of CT-antigens and MHC-I on tumor cells is often heterogeneous. Our results suggest that the changes induced by γ-radiation increase the immunogenicity of the tumor, which is illustrated by the increased infiltration by lymphocytes after radiotherapy.

We compared the expression of CT-antigens and MHC-I in various cancer cell lines and fresh biopsies before and after in vitro irradiation (20 Gy). Furthermore, we made a similar comparison using paired biopsies that were taken before and after radiotherapy from sarcoma patients. To investigate whether the changed expression of CT-antigens and MHC-I is specific for γ-radiation or is part of a generalized stress response, we analyzed the effect of hypoxia, hyperthermia and genotoxic stress on the expression of CT-antigens and MHC-I. We found that in vitro irradiation of cancer cell lines and of fresh tumor biopsies induced a higher or de novo expression of different CT-antigens and a higher expression of MHC-I in a time- and dose-dependent fashion. The analysis of paired biopsies taken from a cohort of sarcoma patients before and after radiotherapy confirmed our findings and, in addition showed that irradiation resulted in higher infiltration by lymphocytes. Other forms of stress did not have an impact on the expression of CT-antigens or MHC-I.

Our findings suggest that γ-radiation enhances the immunogenicity of tumors. We therefore propose that combining radiotherapy with treatments that support tumor specific immunity may result in increased therapeutic efficacy.
Abstracts

Multifunctional Nanoparticles for Macrophage-Targeted Breast Cancer Treatment and Imaging

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Breast cancer remains the second leading cause of cancer deaths among women in the United States. Current treatment of BC has multiple shortcomings including the high level of side effects and insufficient efficacy, particularly for patients with metastatic disease. Paclitaxel (PTX) is among the most active drugs for the treatment of metastatic BC. However, the strong hydrophobicity and significant toxicity of PTX drastically limit its clinical use in natural form. PTX nanoparticles show promise in overcoming these limitations. Recently, high density lipoproteins (HDL) has been used for tumor-targeted delivery of PTX mediated by the scavenger receptor B type I (SR-BI) on cancer cells. Major limitations of this approach include: 1) low specificity of PTX delivery to the tumor in vivo (SR-BI is found in numerous cell types/tissues) and 2) strong dependence on SR-BI expression level on cancer cells that may differ in various types of cancer and for patients at different stages of cancer. Further, recent findings suggest that the critical feature of PTX as an anticancer agent may not be only its microtubule-stabilizing activity, but also its ability to stimulate release of anticancer cytokines (e.g., IL-12) from tumor-associated macrophages (TAMs), the cells that are diffusely distributed throughout the tumor. Thus, TAM-targeted delivery of PTX can significantly improve its therapeutic efficacy, diminish the indiscriminate toxicity, and reduce its dose. In vivo, native HDL transports cholesterol from body cells back to liver and does not bind to macrophages. To re-route HDL to macrophages, we developed HDL-based nanoparticles, the structural protein of which, apolipoprotein (apo) A-I, contains certain modification(s). Our preliminary data suggest that: 1) modification of apo A-I does not affect size, structure, composition, stability and other properties of HDL, 2) modification of apo A-I does not affect PTX-carrying capacity of HDL, and 3) modification of apo A-I converts HDL-PTX into a substrate for macrophages and results in dramatically increased in vitro macrophage uptake of these particles. Importantly, this nanoplatform enables to deliver an iodinated computed tomography (CT) imaging agent and visualize targeted drug delivery to tumor sites. Further development of this novel integrated nanotherapeutic systems can substantially improve breast cancer treatment and diagnosis, thereby leading to a higher survival rate of the patients.
Tumor Infiltration by FCGAMMARIII (CD16)+ Myeloid Cells is Associated with Improved Survival in Patients with Colorectal Carcinoma

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The prognostic significance of macrophage and natural killer (NK) cell infiltration in colorectal carcinoma (CRC) microenvironment is unclear. We investigated the CRC innate inflammatory infiltrate in over 1600 CRC utilizing two independent tissue microarrays (TMAs) and immunohistochemistry. Survival time was assessed using the Kaplan-Meier method and Cox proportional hazards regression analysis in a multivariable setting. Spearman's rank correlation tested the association between macrophage and lymphocyte infiltration. In the Basel study including over 1400 CRCs, the level of CD16+ cell infiltration correlated with that of CD3+ and CD8+ lymphocytes but not with NK cell infiltration. Patients (pts) with high CD16+ cell infiltration (score 2) survived longer than pts with low (score 1) infiltration (p=0.001), while no survival difference between pts with score 1 or 2 for CD56+ (p=0.264) or CD57+ cell (p=0.583) infiltration was detected. CD16+ infiltrate was associated with improved survival even after adjusting for known prognostic factors including pT, pN, grade, vascular invasion, tumor growth pattern, and age [(p = 0.001: HR (95%CI) = 0.71 (0.6-0.9)]. These effects were independent from CD8+ lymphocyte infiltration [(p = 0.036: HR (95%CI) = 0.81 (0.7-0.9)] and presence of metastases [(p = 0.002: HR (95%CI) = 0.43 (0.3-0.7)]. Phenotypic studies identified CD16+ as CD45+CD33+CD11b+CD11c+ but CD64- myeloid cells. Beneficial effects of CD16+ cell infiltration were independently validated by a study carried out at the University of Athens confirming that pts with CD16 score 2 survived longer than pts with score 1 CRCs (p=0.011). Thus, CD16+ cell infiltration represents a novel favourable prognostic factor in CRC.
Reprogramming the Immune Environment in Breast Cancer via Dendritic Cells

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The human breast tumor microenvironment displays features of T helper 2 (Th2) inflammation which promotes tumor development. However, the molecular and cellular mechanisms contributing to Th2 inflammation in breast tumors remain unclear. We have shown that pro-tumor inflammation in breast cancer is driven by breast tumor derived TSLP that induce OX40L expression on mDCs. OX40L+ mDCs generate inflammatory CD4+ T cells producing TNFα and IL-13 but no IL-10. These Th2 cells promote tumor development in vivo in the humanized mouse model of breast cancer which can be inhibited by neutralization of IL-13.

We now show that β-glucan, a natural ligand for dectin-1, can block OX40L expression on tumor associated mDCs which is due to a block in STAT6 phosphorylation. The β-glucan-treated mDCs which were activated by breast tumor supernatant secrete higher levels of IL-12p70 and do not expand TNFα and IL-13-producing CD4+ T cells, suggesting the ability to inhibit the Th2 response. Thus, β-glucan can inhibit breast tumor development in humanized mice. Taken these together, our data suggest that β-glucan reprogram the function of mDCs in breast tumor microenvironment and turn tumor promoting Th2-type chronic inflammation into Th1-type acute inflammation that are able to reject tumors.
Chemokine CCL18 Promotes Breast Cancer Metastasis via an Activation of Ezrin Signaling Complex

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Tumor-associated macrophages (TAMs) can influence cancer progression and metastasis, but the mechanism remains unclear. We have recently showed that breast TAMs abundantly produce CCL18, and its expression in blood or cancer stroma is associated with metastasis and reduced patient survival. CCL18 released by breast TAMs promotes the invasiveness of cancer cells by triggering integrin clustering and enhancing their adherence to extracellular matrix (Chen et al., 2011. Cancer Cell. 19, 541-555). To delineate the molecular signaling cascade underlying CCL18-elicited breast cancer cell metastasis, we carried out a comparative proteomic analysis of phosphoproteins associated with CCL18 stimulation and revealed a series of phospho-peptides in ezrin, a membrane-cytoskeletal linker. Functional analyses using phospho-mimicking mutants of ezrin promote breast cancer cell migration and invasion in vitro and metastasis of breast cancer in animals, whereas knock-down of ezrin abrogates CCL18-elicited breast cancer cell dynamics. Real-time imaging analyses indicate that CCL18 elicits a polarized ezrin phosphorylation and signaling complex assembly. These findings suggest that ezrin orchestrates CCL18-elicited breast cancer cellular dynamics through phosphorylation-dependent protein complex assembly.
S100A9 and CCT Gamma as Potential Biomarkers for Diagnosis of Cholangiocarcinoma

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The aim is to evaluate whether the expression of S100 calcium-binding protein A9 (S100A9) and chaperonin containing TCR1, subunit 3 (gamma) (CCT gamma) have the potential to diagnosis cholangiocarcinoma.

Expression of S100A9 and CCT gamma were measured in 40 cases of cholangiocarcinoma (CCA) and 10 cases of non-tumoral tissues by tissue microarrays. The positive results were divided into four grades, *1, 1, 2, 3 staging which represented weak positive, mild positive, moderate positive and strong positive respectively.

S100A9 was not or low expressed in non-tumoral liver tissues, which were negative expressed in 7 tissue samples and low expressed in 3 cases. In CCA patients, 37 out of 40 cases had positively stained. The positive rate was significantly higher in patients with CCA (48%±35%) compared to non-tumoral control groups (5%±10%) (p<0.001). CCT gamma was also low expressed in non-tumoral liver tissues, and the positive rate was significantly higher in patients with CCA (72%±18%) compared to non-tumoral control groups (43%±22%)(p<0.001).

And the areas under the curve of the ROC curves for S100A9 and CCT gamma were 0.894 (95% CI = 0.825–0.962) and 0.860 (95% CI = 0.779–0.942), respectively.

S100A9 and CCT gamma appear to be valuable diagnostic markers in cholangiocarcinoma. Further prospective studies for serum S100A9 and CCT gamma measurements should be carried out to further investigate the potential of these molecules as biomarkers of cholangiocarcinoma.
Mechanisms of IFNα Resistance in Renal Cell Carcinoma (RCC)

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Renal cell carcinoma (RCC) remains one of the most resistant tumors to chemo-, radio-, and immunotherapy. Interferon (IFNα) has become the most common agent used to treat this tumor, but only 10%-20% of the patients respond to this treatment. The mechanisms of IFNα resistance remain to be fully elucidated, not only in RCC but also in other type of cancers. The aim of this study was to evaluate the susceptibility of three murine and five human RCC cell lines to IFNα and determine the mechanisms of resistance. Using 10 U/ml and 1,000U/ml of mouse and human-recombinant IFNα respectively, we examined the anti-proliferative effects and resistance effect of this cytokine. One of the three murine cell lines was IFNα sensitive and cell growth inhibition was due to an arrest in the G1 phase without inducing apoptosis. None of the human cell lines responded to IFNα treatment. IFNα resistance in both murine and human RCC cell lines was associated to a defective phosphorylation of Stat-1 when compared to IFNα sensitive cells. We did not observe alterations in the expression of Jak-1 and Tyk2 proteins. Expression of IFNα receptors was normal during the first 24 hours, however, a downregulation of the IFNα-receptor-1 was observed after 48 hours in culture. This downregulation appears to be associated with the depletion of L-arginine and L-glutamine in the culture supernatants, that can also play a role in IFNα resistance. The results suggest that a possible restoration of the IFNα-receptor and Stat-1 might strikingly increase the susceptibility of RCC to IFNα and should be considered as a primary target to improve the response of RCC to IFNα. A better understanding of the mechanisms that is responsible for the lack of response to IFNα would undoubtedly lead to determine what patients will benefit or not from this treatment.
Cytotoxic Chemotherapy and CD4+ Effector T Cells: An Emerging Alliance for Durable Antitumor Effects

Gang Zhou1,2 and Zhi-Chun Ding1

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Standard cytotoxic chemotherapy can initially achieve high response rates, but cancer recurrence remains a severe clinical problem. The recent discoveries that certain chemotherapeutic agents have immunostimulatory effects provide a compelling rationale for developing combined chemoimmunotherapy strategy to address the unmet medical need of preventing recurrent cancer. Current cancer immunotherapies predominantly rely on CD8+ T cells to fight against tumors. Although it is increasingly clear that pro-inflammatory CD4+ effector T cells are critical determinants of effective antitumor immune responses, the utilization of CD4+ T cells in combination with chemotherapeutic drugs to control tumor progression and recurrence has not been fully explored. Here we provide evidence that in vivo-generated highly activated, polyfunctional CD4+ effector T cells have tremendous potential in strengthening and sustaining the overall host antitumor immunity in the post-chemotherapy window.
Roche’s Commitment to the Future

*The joy of discovery is certainly the liveliest that the mind of man can ever feel.*
-- Claude Bernard (1813-78), French physiologist.

Every scientist knows the truth behind those words – the exhilarating zing of learning something for the first time truly is a joyful experience. Roche celebrates and encourages that joy of discovery, most notably with its deep commitment to promoting and shaping excellence in science education – from elementary school through the post-graduate level.

In keeping with this rich tradition, Roche Nutley is proud to fund the Science Honors Innovation Program (SHIP) at New Jersey’s Montclair State University. This two-year, advanced honors program for undergraduates is designed to be an incubator for the next generation of scientists. Supported with special mentoring and assistance, students carry out an intensive research study that yields an honors thesis and offers insight into the process of innovation and research. This intense, real-world training prepares the fledgling scientists for top PhD programs and gives them an unparalleled opportunity to build lasting relationships in the research community.

Roche Nutley is delighted to welcome two SHIP students with great scientific potential to this symposium as our special guests: Binta Jalloh and Daniel Traum.

Binta is a senior in the Health Careers program at Montclair, majoring in Biology. Since her sophomore year in high school, Binta has been doing research at institutions such as UMNDJ and the Woods Hole Marine Biological Lab. Her current research is focused on common isoforms of the RET proto-oncogene. When she graduates next year, Binta intends to enter an MD PhD program to pursue a career in clinical research.

Daniel is working on his undergraduate degree with a rigorous double major in Molecular Biology and Business, plus a minor in Chemistry. The Montclair junior, who returned to school after a hiatus working as an emergency services first responder in Vermont, is researching the inhibition of HSV1 cells by black tea theaflavins. Daniel hopes to become a physician, and wants to incorporate a focus on emergency medicine.
Roche’s Commitment to the Future

Roche Researchers Mentor Top UBC Pharmacology Interns

Five students in the B.Sc. Pharmacology program at the University of British Columbia (UBC) are applying their extensive undergraduate in vitro and in vivo training to Roche research programs during year-long internships on the Nutley campus. Under the direction of Roche mentors, four students are supporting key projects in Inflammation, while one student is involved in Virology research. Their project responsibilities are focused in the area of assay/model development and exploratory biology, and may potentially lead to inclusion in publications. The high-achieving interns, selected for the optional five-year UBC co-op program based on their scholastic achievements, are embracing the opportunity to gain hands-on insight into the world of industry scientists – including experiences such as attending this Roche/Nature Medicine symposium.

“Promoting excellence in science education has long been a major priority for Roche, and we believe this partnership with UBC will prove to be a high-value experience for both the students and the Roche Nutley scientists who will be working with them all year,” said Satwant Narula, Head of Discovery, Roche pRED Inflammation Discovery and Translation Area.

Joining Roche’s Satwant Narula (third from left) are the five University of British Columbia pharmacology interns. They are, left to right: Leena Chen, Winnie Li, Evan Woo, Robin Kim and Victoria Baronas
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Notes

Science is built up of facts, as a house is built of stones; but an accumulation of facts is no more a science than a heap of stones is a house.

Henri Poincaré
The real voyage of discovery consists not in seeking new landscapes, but in having new eyes.

Marcel Proust
Try to learn something about everything and everything about something.

Thomas H. Huxley
All men by nature desire knowledge.

Aristotle
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