International Society for the Cell & Gene Therapy of Cancer

Official Meeting Program

Annual Meeting · September 25th - 27th 2014

Amsterdam · The Netherlands
<table>
<thead>
<tr>
<th>Page</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>Travel Directions</td>
</tr>
<tr>
<td>8-9</td>
<td>Keynote Speakers &amp; Specials Guests</td>
</tr>
<tr>
<td>10</td>
<td>Program Guide</td>
</tr>
<tr>
<td>16</td>
<td>Abstracts</td>
</tr>
<tr>
<td>38</td>
<td>Sponsors</td>
</tr>
<tr>
<td>40</td>
<td>Directory</td>
</tr>
</tbody>
</table>
Welcome to the 2014 ISCGT Annual Meeting

We invite you to participate in the Annual Meeting of the International Society for the Cell and Gene Therapy of Cancer (ISCGT) in Amsterdam over September 25th-27th, 2014.

We will highlight at our meeting the most cutting edge discoveries in translational development of molecular therapeutics and diagnostics for cancer. As always, our unique focus on bench-to-bed development will allow us to highlight the most exciting high-impact findings.

We are especially pleased to enjoy this year support from two major cancer centers who are partnering to sponsor our meeting—the Siteman Cancer Center of the Washington University in St. Louis School of Medicine and the VU University Medical Center—Cancer Center Amsterdam.

We are also most excited to confirm the participation of Drs. Hans Clevers and Ton Schumacher as our Keynote Speakers.

The Dutch nickname for Amsterdam, “Mokum”, derives from the Yiddish word describing “the place or the center”. Amsterdam’s locus as the hub of advanced translational therapeutics in cancer and entrepreneurship will provide an optimal locale for participants to network and enjoy maximized impact of their presented findings.

Welcome to Amsterdam!

David T. Curiel, MD, PhD
Chair, ISCGT Amsterdam 2014
Welcome to the ISCGT

Our mission is to become a truly International Society for clinicians and scientists alike. The main focus of the Society is to foster camaraderie and scientific collaboration between scientists and clinicians around the world in cell and gene therapies of cancer.

The ISCGT is totally unique as it works closely with national societies and organisations, in close collaboration with local clinicians, to promote cell and gene therapies for cancer.

The ISCGT has now held numerous meetings in the USA and UK, as well as conferences in France, Germany, Italy, Egypt and Singapore.

ISCGT
CORE VALUES

• Be International: Educate Globally
• President comes from USA, EU and Asia
• Conferences are in USA, EU and Asia
• Membership is inclusive not exclusive

History of ISCGT & Annual Meeting Milestones

1995
• Inaugural Cancer Gene Therapy Conference held at the iconic Del Coronado Hotel in San Diego, CA

1995-1997
• With a shared vision of a more personal, informal conference at the beach, the conference thrives, bringing new technologies to the gene therapy field and enhancing collaborations.

1998
• Cancer Gene Therapy Conferences evolves into the International Society for the Cell and Gene Therapy of Cancer (ISCGT); Manfred Deitel is elected as the first President of ISCGT.

2003
• ISCGT Annual Meeting in Egypt over 22-24 October
• ISCGT Annual Meeting in San Diego, California over 11-13 December

2004
• ISCGT Annual Meeting in Singapore over 20-22 February

2005
• ISCGT/American Society for Gene Therapy (ASGT) Joint Workshop in St. Louis, MO over 1-5 June
• British Society for Gene Therapy (BSGT) in Manchester over 3-5 April
• ASCO/ISCGT Joint Symposium in Orlando, Florida over 13-17 May
• ISCGT Annual Meeting in Shenzhen, China over 9-11 December

2006
• ISCGT/Sidney Kimmel Cancer Center Meeting, San Diego, California, over 27 February -1 March
• ISCGT/UK-Japan Gene Therapy Workshop/Japan Society of Gene Therapy Joint Conference in Chiba, Japan over 13-15 October

2007
• ISCGT Annual Meeting in Mumbai, India over 16-18 November

2008
• ISGCT Annual Meeting in Cork, Ireland over 15-16 May
• ISCGT Annual Meeting in Shijiazhuang China over 19-22 September

2009
• ISCGT Annual Meeting in Cork, Ireland over 3-4 September

2012
• ISCGT Annual Meeting in Singapore over 4-6 October

2013
• ISCGT Annual Meeting in Shijiazhuang, China over 13-16th September

2014
• ISCGT Annual Meeting in Amsterdam, The Netherlands over 25-27 September
Dear colleagues,

On behalf of the VUmc Cancer Center Amsterdam (VUmc CCA), I am honored to welcome you to the 2014 annual meeting of the International Society for Cell and Gene Therapy of Cancer (ISCGT).

I also welcome you to our great city and institute. The VUmc CCA is the cancer expertise center of the VU University medical center in Amsterdam. VUmc CCA aims to provide the best possible care and treatment to patients with cancer. To this end, VUmc CCA integrates and strengthens oncology expertise at the VUmc, stimulating translation of basic research programs into clinical useful tools and methods. VUmc CCA participates in two research and graduate schools accredited by the Royal Netherlands Academy of Arts and Sciences, i.e., the Oncology Graduate School Amsterdam and the Amsterdam-Leiden Institute for Immunology. Since 2006, most basic and translational oncology research conducted at VUmc is clustered in the landmark CCA research building, providing core facilities for genomics, proteomics and functional genomics research.

In 1997, Professor Bob Pinedo, founder of the VUmc CCA and at that time head of the department of Medical Oncology, received the prestigious Spinoza Award, often referred to as the Dutch Nobel Prize for Science. He decided to invest the prize money to establish two new independent research lines in his department, Cancer Gene Therapy and Cancer Immunotherapy. This laid the foundation for innovative tumor therapies being developed in the VUmc CCA today. As Bob Pinedo had foreseen, Cancer Gene and Immunotherapy research, including therapeutic target discovery, gene modulation, cancer stem cells, vaccination and virus therapy, are now the center of attention for finding new ways to fight cancer – in our institute as well as worldwide.

It is therefore with great pleasure that VUmc CCA hosts the 2014 annual meeting of the ISCGT, the prime society fostering international cooperation in this exciting research area. This meeting provides an excellent forum for exchange of the most recent findings in the laboratory and clinic. I particularly embrace the goal of the ISCGT to promote interactions between scientists and clinicians. This fits very well with VUmc CCA’s mission to contribute to the development of the best possible treatment to future cancer patients by stimulating multidisciplinary translational research today. The meeting will be of particular interest to our young investigators and trainees, as renowned international leaders in the field will update them on the most recent developments in bringing novel anticancer therapies from the bench to the bedside. This will undoubtedly fuel their enthusiasm for innovative cancer research.

As proud host of this meeting, VUmc CCA will do its best to offer a convenient venue for all participants to converge. I wish you, scientists and clinicians from around the globe, a fruitful meeting and thank you all for sharing your expertise and time to make this a truly unique event.

Prof. dr. Geert Kazemier
Director, Stichting VUmc CCA
**About DNAtrix:**

DNAtrix is a privately held biotech company focusing on the development of oncolytic virotherapy for cancer. The company’s initial focus is glioblastoma (GBM), a devastating brain tumor that is currently incurable. DNAtrix’s lead product, DNX-2401, represents a platform technology that is the culmination of more than a decade of scientific and clinical research. Its excellent safety profile coupled with unique potency make it one of the most effective oncolytic viruses ever delivered to human tumors. The company has offices located in Houston, Texas and San Diego, California.

**Science & Technology**

DNX-2401 (Delta-24-RGD) is a conditionally-replicative oncolytic adenovirus in clinical development that has been engineered to be specific for tumor cells with defects in the Rb pathway. Its potency is also enhanced by the addition of an RGD motif decorating the fiber protein that allows the virus to efficiently infect a wide range of tumor cells.

Because virtually all tumor cells, including GBM, are defective in Rb function and already in the cell cycle, DNX-2401 replicates in and kills these tumor cells selectively and efficiently. DNX-2401 is vastly superior to wild type adenovirus with respect to killing rapidly growing tumor cells, an unprecedented property for an oncolytic virus.

While DNX-2401 should be effective against multiple tumor types, the initial focus is on high-grade gliomas for three reasons: (1) these tumors remain confined to the brain, so patient assessment is not complicated by the presence of metastatic disease, (2) tumor responses can be accurately documented (3) a single intratumoral dose has been shown to be effective.

**Fast-Track Clinical Trials**

More than 75 subjects with recurrent glioblastoma (GBM) have received a single injection of DNX-2401 at a variety of doses in four clinical studies. In certain subjects, DNX-2401 appears to trigger antitumor immunity that can lead to durable tumor eradication. DNAtrix is currently testing sequential therapy with immunomodulatory drugs to promote this outcome. A phase 2 clinical study is planned for early next year.
Getting Around Amsterdam

CONFERENCE VENUES
VU Medical Center - Amstelzaal
De Boelelaan 1117
1081 HV Amsterdam, The Netherlands

Conservatorium Amsterdam
Paulus Potterstraat 50, 1071 DB Amsterdam, The Netherlands
Tel: +31.20.5700036 • http://www.conservatoriumhotel.com/reservations/

The Conservatorium is ideally located in the heart of the city’s major museum square (Museumplein) and the Royal Concertgebouw. It is close to the designer-fashion district of P.C. Hooftstraat and Van Baerlestraat, with unrivalled access to all of the capital’s cultural destinations including the Rijksmuseum, the Stedelijk Museum and the Van Gogh Museum.

*site of opening reception and closing gala

With 16 tram routes, around 50 bus routes, 4 metro lines and 5 ferry links, public transportation is a safe, easy and reliable way to navigate the city.

https://shop.gvb.nl/en/webshop/product/index/locale/nl
http://en.gvb.nl/pages/home.aspx
Travel Directions

Travel to VU University Medical Center - take tram 16 or 24, both trams terminate at 'VU medisch centrum', leave the tram at stop 'De Boelelaan'.

Travel to the Boat trip departure - take tram 5 from VU University, this tram terminates at 'Centraal Station', leave the tram at stop 'Rijksmuseum'.

Walk the last few minutes to the boat departure.
Travel Directions

Travel to your hotel at Museum Square - take tram 16 or 24, both trams terminate at 'Centraal Station'.

For the Conservatorium Hotel, leave at stop 'Museumplein (Gabriel Metsusstraat)'.

For the NH Museum Square, leave at stop 'Ruysdaelstraat'.

Travel to the Boat trip departure - take tram 5 from VU University, this tram terminates at 'Centraal Station', leave the tram at stop 'Rijksmuseum'. Walk the last few minutes to the boat departure.
Hans Clevers, MD, PhD

Hans Clevers obtained his MD degree in 1984 and his PhD degree in 1985 from the University Utrecht, the Netherlands. His postdoctoral work (1986-1989) was done with Cox Terhorst at the Dana-Farber Cancer Institute of the Harvard University, Boston, USA.

From 1991-2002 Hans Clevers was Professor in Immunology at the University Utrecht and, since 2002, Professor in Molecular Genetics. From 2002-2012 he was director of the Hubrecht Institute in Utrecht. Since 2012 he is President of the Royal Netherlands Academy of Arts and Sciences (KNAW).

Hans Clevers has been a member of the Royal Netherlands Academy of Arts and Sciences since 2000 and a member of the American Academy of Arts and Sciences since 2012. He is the recipient of several awards, including the Dutch Spinoza Award in 2001, the Swiss Louis Jeantet Prize in 2004, the Memorial Sloan-Kettering Katharine Berkan Judd Award in 2005, the Israeli Rabbi Shai Shacknai Memorial Prize in 2006, the Dutch Josephine Nefkens Prize for Cancer Research and the German Meyenburg Cancer Research Award in 2008, the Dutch Cancer Society Award in 2009, the United European Gastroenterology Federation (UEGF) Research Prize in 2010, the German Ernst Jung-Preis für Medizin in 2011, the French Association pour la Recherche sur le Cancer (ARC) Léopold Griffuel Prize and the Heineken Prize in 2012 and the Breakthrough Prize in Life Sciences in 2013. He obtained an ERC Advanced Investigator grant in 2008. He is Chevalier de la Legion d’Honneur since 2005 and Knight in the Order of the Netherlands Lion since 2012.

Ton Schumacher, MSc, PhD

Ton Schumacher received his M.Sc. degree in 1988 from the University of Amsterdam and his PhD degree in 1992 from the Free University of Amsterdam. His postdoctoral work was completed at the Center for Cancer Research, MIT, Cambridge, USA (1992-1994), and The Whitehead Institute for Biomedical Research, MIT, Cambridge, USA (1994-1996).

From 1996-2001 Ton Schumacher was an Assistant Member in the Department of Immunology at The Netherlands Cancer Institute in Amsterdam and became an Associate Member in 2001-2002. From 2002 to present he has been a Full Member in the Department of Immunology at The Netherlands Cancer Institute in Amsterdam, Professor of Immunotechnology at Leiden University Medical Center, and Deputy Director of The Netherlands Cancer Center.

Ton Schumacher of the Netherlands Cancer Institute was the recipient of the Koningin Wilhelmina Onderzoeksprijs (KWO prize) in 2014. The KWO prize is the largest grant for cancer research that is given in The Netherlands, and is awarded once a year by the Dutch Cancer Society. He is also the recipient of several awards, including the San Salvatore Award in 2014, the Scientific Advisory Council in 2013, SU2C Immunotherapy Dream Team Member in 2012, ERC AdG recipient in 2011, EMBO Member and Amsterdam Inventor Award in 2010. From 1994-1996, he was a Howard Hughes Medical Institute Fellow at The Life Sciences Research Foundation.

In addition, Dr. Schumacher founded Impact Biotechnology in 2003 and Amsterdam Biotherapeutics Unit in 2006.
Timothy Broas was nominated by President Barack Obama to be the U.S. Ambassador to the Kingdom of the Netherlands on January 6, 2014. He was confirmed by the U.S. Senate on March 13, 2014. Ambassador Broas presented his credentials to His Majesty King Willem-Alexander on March 19, 2014, and has officially taken up his duties as Ambassador Extraordinary and Plenipotentiary of the United States of America to the Kingdom of the Netherlands.

Mr. Broas was a partner in the litigation department of the Washington, DC office of Winston & Strawn, LLP. He was named in the 2010, 2011, 2012, 2013, and 2014 editions of Best Lawyers in America.

In 2010, President Obama appointed Mr. Broas to the Board of Trustees of the Woodrow Wilson International Center for Scholars. The Wilson Center, created by Congress in 1968, is a nonpartisan institute for advanced study and a neutral forum for open, serious, and informed dialogue among preeminent thinkers and policymakers.

Mr. Broas was named to the Board of Trustees of Partners in Health in 2012. He was appointed by Maryland Governor Martin O’Malley to the Board of Trustees of St. Mary’s College of Maryland in 2011 and was selected as a Fellow of the Litigation Counsel of America in 2010. In 2005, Mr. Broas was appointed to the Board of Visitors of Mount Vernon by Virginia Governor Mark Warner and was reappointed in 2009 by Governor Tim Kaine.

A native of Maryland, Mr. Broas received an A.B., magna cum laude, in Economics and History from Boston College in 1976 and a J.D. from the College of William and Mary in 1979.

Mr. Broas is married with three grown daughters.

Tracy Metz, a native of California, is a journalist and author based in the Netherlands. She is the director of the John Adams Institute, an independent foundation that brings the best and brightest of American culture to the Netherlands. She writes about urban issues for the quality national newspaper NRC Handelsblad and the weekly magazine De Groene Amsterdammer. Metz has a monthly live talkshow called Stadsleven (‘City Life’) and a monthly column on the respected television program Buitenhof.

She is also an international correspondent for the American magazine Architectural Record and a visiting fellow at Harvard, subsequent to her Loeb Fellowship (’06 - ’07) at the Graduate School of Design there. She is the author of a number of books, most recently Sweet&Salt: Water and the Dutch, about the ‘extreme makeover’ of the Dutch landscape to accommodate a new, more natural relationship to water in times of climate change.
PROGRAM GUIDE
Conservatorium Hotel
Paulus Potterstraat 50, 1071 DB Amsterdam, The Netherlands

19.30 - 21.30  Opening reception with remarks by:

- **David T. Curiel**, MD, PhD (Chair, ISCGT 2014),
- **Ambassador Timothy Broas** (Embassy of the United States; The Hague, the Netherlands),
- **Tracy Metz** (Executive Director, John Adams Institute) and
- **Geert Kazemier**, MD, PhD (Director, VUmc CCA Foundation)
Friday, September 26th 2014

8.30 - 9.00  Registration, poster set-up, coffee

9.00 - 9.15  Opening remarks and welcome:
David T Curiel, MD, PhD (Washington University in St. Louis School of Medicine - USA)
Henk M. Verheul, MD (VUmc Cancer Center - The Netherlands)

9.15 - 10.55  Session I

Chairs:
Dennis E. Hallahan, MD (Washington University in St. Louis School of Medicine - USA)
Victor van Beusechem, PhD (VUmc Cancer Center - The Netherlands)

Speakers:
9.15 - 9.35  Dennis E. Hallahan, MD (Washington University in St. Louis School of Medicine - USA)
9.35 - 9.40  Q & A

9.40 - 10.00  Stephen J. Russell, MD, PhD (Mayo Clinic - USA)
10.00 - 10.05  Q & A

10.05 - 10.25  Manfred Dietel, MD, PhD (University Hospital Charité - Germany)
10.25 - 10.30  Q & A

10.30 - 10.50  Jeffrey Arbeit, MD (Washington University in St. Louis School of Medicine - USA)
10.50 - 10.55  Q & A

10.55 - 11.15  Coffee

11.15 - 13.15  Session II

Chairs:
Ton Schumacher, PhD (The Netherlands Cancer Institute - The Netherlands)
Carl Figdor, PhD (Radboud Institute for Molecular Life Sciences - The Netherlands)

Keynote speaker:
11.15 - 11.50  Ton Schumacher, PhD (The Netherlands Cancer Institute - The Netherlands)
11.50 - 12.00  Q & A

Speakers:
12.00 - 12.20  Carl Figdor, PhD (Radboud Institute for Molecular Life Sciences - The Netherlands)
12.20 - 12.25  Q & A
Friday, VUmc Amstelzaal and Foyer

12.25 - 12.45  Barbara Guinn, PhD (University of Bedfordshire)
12.45 - 12.50  Q & A
12.50 - 13.10  Tanja de Gruijl, PhD (VUmc Cancer Center - The Netherlands)
13.10 - 13.15  Q & A
13.15 - 14.15  Lunch and poster viewing
14.15 - 15.30  **Session III**

*Chairs:*
Farzin Farzaneh, D.Phil. FRCPPath. FSB (King’s College London - UK)
Albert Deisseroth, MD, PhD (United States Food and Drug Administration - USA)

*Speakers:*
14.15 - 14.35  Albert Deisseroth, MD, PhD (United States Food and Drug Administration - USA)
14.35 - 14.40  Q & A
14.40 - 15.00  Toos Daemen, PhD (University of Groningen, The Netherlands)
15.00 - 15.05  Q & A
15.05 - 15.25  Farzin Farzaneh, D.Phil. FRCPPath. FSB (King’s College London - UK)
15.25 - 15.30  Q & A

15.30 - 16.15  **Session IV**

*Proffered abstracts I*

*Chairs:*
Manfred Dietel, MD, PhD (University Hospital Charité - Germany)
Toos Daemen, PhD (University of Groningen, The Netherlands)

*Speakers:*
15.30 - 15.45  Bruce F. Smith, V.M.D., Ph.D. (Auburn University)
15.45 - 16.00  Elisabeth van Erp (Washington University in St. Louis, School of Medicine, USA; Utrecht University, The Netherlands)
16.00 - 16.15  Nenad Petrovic, PhD (University of South Australia, Australia)
17.45  Check-in at Pure Amsterdam for Canal Cruise
18.00  Boat tour departs
19.15  Boat tour returns
Saturday, September 27th 2014

8.30  Coffee/Check-in

9.00 - 10.40  Session V
  Chairs:
  Mark Tangney, PhD (Cork Cancer Research Centre - Ireland)
  Chae-Ok Yun, PhD (Hanyang University - South Korea)

Speakers:
  9.00 - 9.20  Mark Tangney, PhD (Cork Cancer Research Centre - Ireland)
  9.20 - 9.25  Q & A
  9.25 - 9.45  Nagy Habib, PhD (Imperial College London - UK)
  9.45 - 9.50  Q & A
  9.50 - 10.10  David Klatzmann, MD (Hôpital Pitié-Salpêtrière - France)
  10.10 - 10.15  Q & A
  10.15 - 10.35  Chae-Ok Yun, PhD (Hanyang University - South Korea)
  10.35 - 10.40  Q & A
  10.40 - 11.00  Coffee

11.00 - 12.15  Session VI
  Chairs:
  Nori Kasahara, MD, PhD (University of Miami - USA)
  Clemens M. F. Dirven, MD, PhD (Erasmus University & Academic Hospital Rotterdam - The Netherlands)

Speakers:
  11.00 - 11.20  Clemens M. F. Dirven, MD, PhD (Erasmus University & Academic Hospital Rotterdam - The Netherlands)
  11.20 - 11.25  Q & A
  11.25 - 11.45  Nori Kasahara, MD, PhD (University of Miami - USA)
  11.45 - 11.50  Q & A

12.15 - 13.15  Lunch and poster sessions

13.15 - 14.15  Session VII
  Proffered abstracts II
  Chair:
  Len Seymour, PhD (University of Oxford - UK)
Speakers:
13.15 - 13.30  Renata Stripecke, PhD (Hannover Medical School, Germany)
13.30 - 13.45  Zsolt Sebestyen, PhD (UMC Utrecht, The Netherlands)
13.45 - 14.00  Michelle Cronin, PhD (Cork Cancer Research Center, Ireland)

14.00 - 15.50  Session VIII
Chairs:
Hans Clevers, MD, PhD (Hubrecht Institute - The Netherlands)
David Curiel, MD, PhD (Washington University in St. Louis School of Medicine - USA)

Keynote speaker:
14.15 - 14.50  Hans Clevers, MD, PhD (Hubrecht Institute - The Netherlands)
14.50 - 15.00  Q & A

Speakers:
15.00 - 15.20  Rob Coppes, PhD (University Medical Center Groningen - The Netherlands)
15.20 - 15.25  Q & A
15.25 - 15.45  Carolyn J. Henry, DVM, MS (University of Missouri - USA)
15.45 - 15.50  Q & A
15.50 - 16.00  Refreshments

16.00 - 18.15  Session IX
Chairs:
Victor van Beusechem, PhD (VUMC Cancer Center - The Netherlands)
Rob Hoeben, PhD (Leiden University Medical Center - The Netherlands)

Speakers:
16.00 - 16.20  Victor van Beusechem, PhD (VUMC Cancer Center - The Netherlands)
16.20 - 16.25  Q & A
16.25 - 16.45  Rob Hoeben, PhD (Leiden University Medical Center - The Netherlands)
16.45 - 16.50  Q & A
16.50 - 17.10  Len Seymour, PhD (University of Oxford - UK)
17.10 - 17.15  Q & A
17.15 - 17.35  Frank Tufaro, PhD (DNAtrix - USA)
17.35 - 17.40  Q & A
17.40 - 18.00  David Kirn, MD (SillaJen, Inc. - USA/South Korea)
18.00 - 18.05  Q & A
18.05 - 18.15  Closing remarks by Victor van Beusechem and Tanja de Gruijl (ISCGT Amsterdam 2014, Organizing Committee).

Saturday, VUmc Amstelzaal and Foyer

Saturday night, Conservatorium Hotel
19.30 - 21.30  Closing Gala with remarks by Nori Kashahara, MD, PhD (Past President, ISCGT) and Frank Tufaro, PhD (Chief Executive Officer, DNAtrix).
JEFFREY ARBEIT [Speaker]

Creation of endothelial-targeted adenoviral vectors for genetic engineering of the metastatic tumor microenvironment.

Zhi Hong Lu1, Sergey Kaliberov2, Lyudmila Kaliberova2, Rebecca E. Sohn1, Yingqui Du1, David T. Curiel2,3, Jeffrey M. Arbeit1,3. 1Urology Division, Department of Surgery. 2Division of Cancer Biology, Biological Therapeutics Center, Department of Radiation Oncology. 3Siteman Cancer Center, Washington University in St. Louis School of Medicine.

The endothelium is an attractive gene therapy target for metastatic cancer, providing access to systemic tumors. However, obtaining widespread endothelial vector expression within tumors has been an obstacle due to viral particle liver sequestration and host preformed immunity. While previous studies demonstrated endothelial targeting, the tumor wide extent and transgene expression level quantification have been uncertain. We developed a set of adenoviral vectors, containing 3 kb of the human ROBO4 enhancer/promoter, that are expressed at high levels throughout tumor vasculature. We now have four vectors representing four tiers of genetic modification. Transgenes encoding fluorescent proteins allowed us to quantify endothelial expression frequency by image analysis, and expression level using whole tissue Western blotting. Our first vector was Ad5.ROBO4. Compared with Ad5.CMV, Ad5.ROBO4 evidenced complete retargeting from hepatocytes to low-level expression in host organ and tumor endothelial cells. Warfarin-mediated hepatocyte detargeting produced a striking increase in tumor and bone marrow sinusoidal endothelial expression, without change in other host organs. Our next vector was Ad.MBP.ROBO4. This vector evidenced both liver detargeting and tumor vascular induction. Most strikingly, this vector is not expressed in bone. Our third vector is Ad.RGD.H5/H3.ROBO4. This vector produces warfarin-independent, high level pan-intratumoral vascular expression particularly in cancers with markedly elevated VEGF production, such as renal cell carcinoma. This vector is also expressed throughout the bone marrow sinusoidal endothelium and in prostate cancer bone, brain, and liver metastases. Our fourth vector contains a polycistronic array and produces triple transgene expression within the vasculature. Thus, we have panel of endothelial-targeted vectors with distinctive vascular tropism for use in cancers metastatic to individual host organs. These vectors enable expression of a palette of transgenes that can manipulate the tumor microenvironment to achieve metastatic growth inhibition alone, or as “staggered” with chemo- or irradiation therapies.

CHRIS BANGMA [Speaker]

Oncolytic adenoviral therapy as a neoadjuvant in a Phase 1 prostate cancer trial: a progress report

Chris H Bangma1, Magnus Essand2, Anna de Goede3, Stacy Crow3, Arnold Vulto3, René Debets4, Arno van Leenders5, Bart Haagmans6, Ebelien Mulder1, Wilma Teubel1, Robert Kraaij1, Norman Maitland7, Ellen Schenk1

1 Department of Urology, Erasmus MC, Rotterdam, the Netherlands. 2 Department of Immunology, Genetics, Pathology, Uppsala University, Rudbeck Laboratory, Uppsala, Sweden. 3 Hospital Pharmacy, Erasmus MC, Rotterdam, the Netherlands. 4 Department of Medical Oncology, Erasmus MC, Rotterdam, the Netherlands. 5 Department of Pathology, Erasmus MC, Rotterdam, the Netherlands. 6 Department of Virology, Erasmus MC, Rotterdam, the Netherlands. 7 YCR Cancer Research Unit, Department of Biology, University of York, Heslington, York, United Kingdom.

Prostate cancer is the most common malignancy in the Western world. Patients can only be cured when the tumour is not metastasized outside the prostate. However, treatment with curative intent fails in a significant number of men, often resulting in untreatable progressive disease with a fatal outcome. Oncolytic adenovirus therapy might be a promising adjuvant treatment to reduce local failure or the outgrowth of micrometastatic disease, in this way enhancing the cure rate of patients with localised prostate cancer. Within the European gene therapy consortium GIANT (2004-2009), we have developed a novel prostate-specific oncolytic adenovirus named Ad[I/PPT-E1A] that specifically kills prostate cells via prostate-specific replication. We here report on the initiation of a Phase 1 clinical dose-escalating trial in which Ad[I/PPT-E1A] is used as a neoadjuvant therapy.
just before radical prostatectomy in patients with clinically localized prostate cancer. This trial assesses the safety as well as the immunological effects of Ad[I/PPT-E1A]. We highlight the pre-clinical safety assessment of this novel virus.

LAURA BIES [Poster]

Intratumoral CD4+ T cell reactivity against mutated antigens is commonly observed in human melanoma

Laura Bies1, Carsten Linnemann1, Marit M. van Buuren1, Els M. Verdegaal3, Remko Schotte4, Jorg J.A. Calis1, Sam Behjati3, Arno Velds3, Henk Hilkmann3, Dris el Atmioui4, Marten Visser4, Michael R. Stratton7, John B.A.G. Haanen1,2, Hergen Spits6, Sjoerd H. van der Burg4 and Ton N.M. Schumacher1

1Division of Immunology, 2Division of Medical Oncology, 3Central Genomics Facility and 4Peptide Synthesis Facility, The Netherlands Cancer Institute, Amsterdam, The Netherlands. 5Department of Clinical Oncology, Leiden University Medical Center, Leiden, The Netherlands. 6AIMM Therapeutics B.V., Amsterdam, The Netherlands. 7Cancer Genome Project, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom.

Tumor-specific neo-antigens that arise as a consequence of mutations are thought to be important for the efficacy of tumor-infiltrating lymphocyte (TIL) therapy and other clinically used cancer immunotherapies. Accumulating evidence suggests that neo-antigens may be commonly recognized by intratumoral CD8+ T cells, however it is unclear whether neo-antigen specific CD4+ T cells also frequently reside within human tumors. In view of the accepted role of tumor-specific CD4+ T cell responses in tumor control, it will be important to address whether neo-antigen specific CD4+ T cell reactivity is a common or rare property in human cancers. Here, we exploit oncogene-immortalized autologous B cells to measure the occurrence of CD4+ T cell responses against putative neo-epitopes that are identified by tumor exome-sequencing. Using this approach, we show the presence of neo-antigen reactive CD4+ T cells in 4 out of 5 melanoma patients analyzed, including melanoma patients who demonstrate a clinical response after adoptive T cell therapy. Furthermore, we provide evidence that neo-epitope specific CD4+ T cells can both persist long-term after adoptive T cell therapy and can directly recognize the neo-epitope on autologous tumor cells. These data reveal that recognition of neo-antigens by CD4+ T cells is a common phenomenon in melanoma. Furthermore, based on the mutational landscape in human tumors, we conclude that neo-epitopes that can be recognized by CD4+ T cells can also be expected in a substantial fraction of patients with other common cancers.

HANS CLEVERS [Keynote Speaker]

Wnt signaling, Lgr5 stem cells and cancer

Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences & University Medical Centre Utrecht, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands.

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using lineage tracing experiments in adult mice, we found that these Lgr5+ crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. Lgr5 was subsequently found to represent an exquisitely specific and almost `generic’ marker for stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear tongue and stomach epithelium. Single sorted Lgr5+ stem cells can initiate ever-expanding crypt-villus organoids, or so called ‘mini-guts’ in 3D culture. The technology is based on the observation that Lgr5 is the receptor for a potent stem cell growth factor, R-spondin. Similar 3D cultures systems have been developed for the Lgr5+ stem cells of stomach, liver, pancreas and kidney. Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the “cancer stem cell”-concept.

ROBERT P. COPPES [Speaker]

A Cell Therapy for Radiation-Induced Xerostomia

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Hyposalivation and its consequence xerostomia are sequelae of salivary gland (SG) functional ablation, commonly caused by radiotherapy (RT) treatment for head and neck cancers. 40% of the patients treated for head and neck cancer suffer from oral dryness leading to impaired speech, chewing, taste and swallowing, higher susceptibility for infections, and caries. These sequelae severely affect the patients’ wellbeing and quality of life. A lack of viable stem cells able to maintain glandular homeostasis underlies age and radiotherapy induced SG dysfunction. Therefore, stem cell therapy could
ameliorate xerostomia. Indeed, recently we showed that transplantation of mouse stem cells can rescue murine SG from radiation damage. Currently, we are translating our findings in mice to the human situation. Using human submandibular and parotid biopsies material we are able to culture human primary salispheres (hS) from which single cells could be obtained that were able to self-renew for ≥ 5 passages in vitro. Moreover, single hS cell derived salispheres could be stimulated to develop into an organoid with cells differentiating in ductal and acinar lineages as indicated by the expression of cytokeratins (Cyt+) and aquaporin-5 (AQP5+), respectively. These results indicate that in vitro we can obtain cells that are able to self-renew and differentiate into salivary gland lineages, two prerequisites of tissue stem cells. When xeno-transplanted into a mouse model of radiation-induced hyposalivation, hS cells proliferate extensively and were found to differentiate into human salivary gland structures within the mouse. Transplanted cells restored saliva production and improved regenerative potential of irradiated SGs. Tissue regeneration was elicited through different mechanisms, which will be discussed during the presentation. In conclusion, from human salivary glands a population of cells can be obtained that contain stem cell capable of self-renewal and differentiation and rescuing saliva production. Therefore, stem cell therapy may be a viable therapeutic option in the near future for the treatment of radiation-induced xerostomia.

**MICHELLE CRONIN [Speaker]**

Bacterial-mediated knockdown of tumour resistance to an oncolytic virus enhances therapy

Michelle Cronin¹, Fabrice Le Boeuf², Carola Murphy¹, Theresa Falls², John C Bell², Mark Tangney³

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Oncolytic viruses (OV) and bacteria share the property of tumour-selective replication following systemic administration. In the case of non-pathogenic bacteria, tumour selectivity relates to their ability to grow extracellularly within tumour stroma, and are therefore ideally suited to restricting the production of bacterially produced therapeutic agents to tumours. We have previously shown the ability of the type 1 interferon (IFN) antagonist B18R to enhance the replication and spread of Vesicular Stomatitis Virus (VSV) by overcoming related cellular innate immunity. In this study, we utilised non-pathogenic bacteria (E. coli) expressing B18R to facilitate tumour-specific production of B18R, resulting in a microenvironment depleted of bioactive antiviral cytokine, thus ‘pre-conditioning’ the tumour to enhance subsequent tumour destruction by the OV. Both in vitro and in vivo infection by VSVΔ51 was greatly enhanced by B18R produced from E. coli. Moreover, a significant increase in therapeutic efficacy resulted from intravenous (IV) injection of bacteria to tumour-bearing mice five days prior to IV VSVΔ51 administration, as evidenced by a significant reduction in tumour growth and increased survival in mice. Our strategy is the first example where two such diverse microorganisms are rationally combined and demonstrates the feasibility of combining complementary microorganisms to improve therapeutic outcome.

**TOOS DAEMEN [Speaker]**

Efficacy and PET monitoring of rationally designed treatments with an alphavirus-based cancer vaccine, sunitinib and low-dose tumor irradiation

Oana Draghiciu¹, S.V.Hartimath³, Annemarie Boerma¹, Baukje Nynke Hoogeboom¹, Erik F.J. de Vries², Hans W. Nijman³, Toos Daemen¹

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The clinical efficacy of therapeutic cancer vaccines is currently still limited. For effective immunotherapeutic responses multimodal approaches that can induce antitumor immune responses and bypass tumor-mediated immune escape seem essential. Here, we report on a combination of sunitinib, single low-dose tumor irradiation and immunization with a therapeutic cancer vaccine based on a Semliki Forest virus vector encoding the oncoproteins E6 and E7 of human papillomavirus (SFVeE6,7).

We demonstrate using a murine tumor model that low-dose irradiation and sunitinib in single combination with SFVeE6,7 immunizations enhance the ratio of antitumor effector cells to myeloid-derived suppressor cells (MDSCs) in the tumor. Based on these results we next designed a regime involving a triple treatment combination. The combination of sunitinib, low-dose tumor irradiation and SFVeE6,7 immunization dramatically changed the intratumoral immune compartment. Whereas in control mice, tumors contained 0.02 E7-specific CD8+ T cells per MDSC, triple treatment
tumors contained more than 200 E7-specific CD8+ T cells per MDSC; a 10,000-fold increased effector-to-suppressor ratio. As a result, the triple treatment strongly enhanced the immunotherapeutic antitumor effect, blocking tumor development altogether and leading to 100% tumor-free survival.

To allow the in vivo visualization of tumor infiltration of immune cells we developed a novel PET tracer [18F]FB-IL2 that specifically binds to IL-2 receptors overexpressed on activated T lymphocytes. PET imaging with this novel tracer showed a 9-fold and 22-fold higher [18F]FB-IL2 uptake in the tumor of mice receiving local tumor irradiation alone or local tumor irradiation followed by SFVeE6,7 immunization, respectively.

This study demonstrates that this multimodal approach, which targets the activation and recruitment of immune effectors and at the same time depletes immune suppressors, elicits superior antitumor effects and should be considered for clinical application. Moreover, we here present a novel method to visualize tumor infiltration of activated T cells by PET monitoring.

**TANJA D. DE GRUIJL** [Speaker]

Myeloid plasticity in tumor-conditioned tissues: consequences for dendritic cell-targeted therapeutic gene delivery

Rieneke van de Ven and Tanja D. de Gruijl

Dept Medical Oncology, Immunotherapy Lab, VU University medical center-Cancer Center Amsterdam, Amsterdam, The Netherlands.

A large number of studies attest to the remarkable phenotypic plasticity of the myeloid lineage. Tumors abuse this plasticity to re-direct myeloid differentiation from T cell stimulatory subsets to the development of immune suppressive subsets that can interfere with antitumor immunity. Both monocytes and dendritic cells (DC) can be recruited to the tumor microenvironment and are exposed to immunosuppressive factors which convert them to tumor-promoting suppressor and macrophage-like cells. As tumors progress these suppressive effects can become systemic and hamper DC development and activation at distant sites. We and others have been investigating the possibility of in vivo DC-targeted tumor immunotherapy, e.g. through adenoviral delivery of immune stimulatory genes and/or genes encoding tumor antigens. In order for these DC-targeted therapies to be effective they will have to overcome any tumor associated suppression imposed on the DC in situ. We have therefore been focusing on functional aspects of DC subsets in human skin, melanoma tumors and tumor-draining lymph nodes (TDLN) and how these may be affected by tumor-derived suppressive factors. Delineation of the tumor-derived factors and down-stream signalling pathways involved in DC suppression as well as phenotypic and functional characterization of DC subsets in human tissues will allow for the more rational design of targeted gene therapies and oncolytic virotherapies aiming to kick-start an effective antitumor immune response in vivo.

**SOFIEKE DE WILDE** [Poster]

Implementation of academic Advanced Therapy Medicinal Products in clinical practice in The Netherlands

Sofieke de Wilde1*, Louise Veltrop-Duits*1, Merel Hoozemans-Strik2, Janine Blom2, Henk Jan Guchelaar1, Maarten Zandvliet1, Pauline Meij1

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Advanced Therapy Medicinal Products (ATMPs), including gene therapy, somatic cell therapy and tissue engineered products, can only be applied under the European regulatory framework, (1) within a clinical study protocol, (2) within the hospital exemption and (3) with an (European Medicines Agency) EMA marketing Authorization. Until now only four ATMPs (Chondrocelect®, Glybera®, MACI® and Provenge®) succeeded to achieve EMA approval, despite the fact that a lot of ATMPs were tested in clinical trials. Most ATMPs developed in academia, which appear to be safe and effective, remain in developmental phase and are not available for standard clinical care. This project aims to identify how academic ATMPs can efficiently become available for regular clinical care.

64 projects of ATMP development** were aiming for clinical implementation in The Netherlands in the period of 2005 until 2013. An inventory of these ATMPs in different academic centers was made, focussing on the clinical development route and the approach towards implementation in standard patient care. Variables (e.g. ATMP type, phase of study, cooperation with partners including companies, financial support) that could have impact on either the development or the implementation of the product are analysed using interviews with research groups and stakeholders. The association between these variables and success or failure in development and the route towards implementation in standard care will be analysed. Furthermore, the analyses will be repeated for conventional medicinal products, to allow a direct comparison.
Based on the results, optimal routes for academic development and implementation of ATMPs will be designed and communicated to the academic field.

ALBERT DEISSEROTH [Speaker]

Adenoviral cancer vaccine encoding chimeras of the target associated antigen with CD40L

Albert Deisseroth, Yucheng Tang, Lixin Zhang, Hakan Akbulut and Nagy Habib.

Phase I testing of the Ad-sig-hMUC1/ecdCD40L cancer vaccine has begun under the direction of Dr. Han Chong Toh at the National Cancer Center of Singapore in patients recently relapsed from first salvage therapy for cancers of the breast, prostate, colon, lung and ovary. The goals of the trial are to characterize side effects of the vaccine and to monitor the changes in the cellular and humoral response to the cancer cells as measured in the peripheral blood mononuclear cells collected from study participants before and after vaccination. In this vaccine platform, a fragment of the target associated antigen (TAA) is attached to the extracellular domain (ecd) of the potent immunostimulatory ligand CD40L, thereby creating the TAA/ecdCD40L vaccine. Fragments of the TAA are chosen on the basis of the functional domain to which they belong in the parent target protein, the presence of more than one target epitope, and the presence in each epitope of peptide stretches that will bind to both Class I as well as Class II MHC. Attachment of the TAA to the CD40L accomplishes the following:

1. Promotes Class I and Class II MHC presentation of target associated antigens (TAAs) on dendritic cells thereby resulting in the expansion of TAA specific T cells, and increased levels of TAA specific antibodies leading to the induction of an adaptive immune response;
2. Promotes activation of dendritic cells and CD4 helper T cells;
3. Promotes induction of a TAA specific memory response; and,
4. Overcomes pre-existing anergy arising from chronic disease, advanced chronological age and cancer as well as other acquired functional defects of the immune response.

Also, the attachment of TAAs which are weak antigens to the ecdCD40L, converts them into more potent immunogens to induce very high levels of anti-TAA antibodies and TAA specific CD8 effector T cells.

MANFRED DIETEL [Speaker]

NGS in tissue based diagnostics and personalized medicine

Institute of Pathology, Charité, Humboldt University Berlin, Germany

Applications of new immunological and molecular techniques play an increasing role in the routine process of tissue-based diagnostics of infectious and neoplastic diseases as well as in translational cancer research. The major up-coming challenges are:

- to directly detect a great spectrum of microorganisms in surgical specimens,
- to define the individual prognosis of an actual cancer patient as precise as possible,
- to reproducibly predict the biological behaviour of malignant tumors by genetic profiling,
- to assess the probability of metastases, e.g. in case of clinical state M0 at time of tumor diagnosis,
- to predict response/resistance of each individual tumor against conventional or targeted anticancer drugs,
- to define new biomarker as predictors of drug efficiency and
- to establish internationally accepted therapeutic algorithms based on molecular assays.

Due to continuous technical developments in immunohistochemistry (IHC) and in-situ hybridization (ISH) assisted by different molecular and computational techniques the power of tissue-based diagnostic histopathology increased dramatically during the last decade. The most recent step is to perform tissue-adapted next generation sequencing, i.e. amplicon sequencing, whole exome or whole genome sequencing using FFPE tissue. This opens the door to broad molecular profiling of patient’s tumors obtained by taking biopsies and surgical measures.

These approaches all have to be performed under standard operating procedures in order to guarantee reliable results for the patients. Combined application of the different technologies will further improve the importance of tissue-based diagnoses and their predictive accuracy and all further efforts should be directed to improve the tissue-based diagnosis and predictive relevance of surgical pathology and to provide the clinicians with all those information needed for optimal treatment.

CLEMENS DIRVEN [Speaker]

Preliminary results of the phase 1 trial with the Delta24RGD oncolytic adenovirus, administered by CED in patients with
This phase 1 trial started accrual in 2010 and since 20 patients have been treated with escalating doses of the oncolytic adenovirus Delta24 RGD. This is a double mutated serotype 5 adenovirus, with a deletion of 24 bp prohibiting cell cycle initiation via E2F and with an expanded tropism by insertion of RGD in the fiber knob, enabling binding to integrins, as well as to the native receptor CAR.

This virus is administered in patients with recurrent Glioblastoma after failure of standard treatment and often second line treatments. The delivery method is “Convection Enhanced Delivery” (CED) which consists of prolonged microinfusion through up to 4 catheters in and around the tumor over a period of 2 to 3 days.

Doses of 1 x 10e7 to 1 x 10e11 were scheduled in 6 cohorts of 3 patients each. Data on toxicity, adverse events, and survival were collected. The MTD was reached at a dose of 1 x 10e10 viral particles, due to environmental risk issues and not because of patient toxicity. Dose limiting toxicities with respect to patient safety were not encountered. Viral titers in CSF support the occurrence of prolonged viral replication in the tumor.

ORCA-010, a novel potency enhanced oncolytic adenovirus, exerts strong antitumor activity in preclinical models

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Oncolytic adenoviruses represent a novel class of anticancer agents, designed to selectively replicate in and destroy tumor cells by lysis. We have investigated various strategies to enhance the cancer cell killing potency. One particularly successful strategy is inclusion of the potency-enhancing T1 mutation in the viral E3-gp19K gene. Thus, we have developed a new oncolytic adenovirus, ORCA-010 that contains the E1A-Delta24 mutation for cancer-selective replication, the fiber-RGD mutation for efficient infection and the T1 mutation to promote cell lysis and progeny virus spread.

The oncolytic potency of ORCA-010 was evaluated in vitro cytotoxicity assays using a panel of 15 cancer cell lines derived from a variety of tissue origins. These assays indicated that ORCA-010 is on average 10,000-fold more potent than the first generation oncolytic adenovirus ONYX-015 and 7-fold more potent than Ad5-Δ24RGD, a more recent oncolytic adenovirus that is currently in clinical trials. Increased potency was observed on all cancer cell lines tested.

As ORCA-010 will initially be developed for the treatment of prostate cancer, selectivity experiments were performed using primary human prostate cells. In both primary prostate fibroblasts and epithelial cells ORCA-010 was shown to be as safe as Ad5-Δ24RGD.

In vivo studies using human tumor-bearing nude mice showed that intra-tumoral injection of ORCA-010 significantly inhibited the growth of subcutaneous pre-established PC-3 prostate (p=0.015), A549 lung (p<0.001) and OVCAR-3 ovarian (p<0.001) tumors. Furthermore, PC-3 tumor analysis showed a 200-fold increase in infectious ORCA-010 viral particles over the inoculum at 14 days post injection. Increased levels of ORCA-010 virus were found up to the last time-point tested, i.e., 4 weeks post injection. Immunohistochemical analysis showed that intratumoral virus replication was associated with necrosis and fibrosis. These promising preclinical data support further development of ORCA-010 for clinical testing in a Phase I/II clinical trial in patients with prostate cancer.

Targeted adenoviral vectors for radioprotection

William H. Everett1, Zhi Hong Lu2, Jeffrey M. Arbeit2,3, and David T. Curiel1,4

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Radiation therapy, a powerful and widely used modality in the treatment of cancer, is limited by the generation of off-target effects due to normal tissue damage, especially in the bone marrow and intestines which are particularly vulnerable. To avoid these side effects, methods have been advanced to develop radioprotectors, agents capable of reducing damage to normal tissue without affecting the potency of treatment against tumor cells. Though early research focused on small molecule agents, the flexibility of gene therapy to achieve augmentation or ablation of a variety of genes has lead to
a growing interest in this technology for radioprotection. Despite promising early results, attempts to achieve radioprotection via gene therapy have been severely limited by current vectors, which cannot achieve the in vivo specificity and efficiency of gene delivery to vulnerable tissues required for radioprotection. To improve gene delivery, we have developed targeted adenoviral vectors using transductional and transcriptional approaches that utilize the endothelium of vulnerable tissues for in situ production of radioprotective agents. Vectors incorporating a myeloid-binding peptide or an RGD integrin-binding motif into the fiber of the adenovirus transduce endothelial beds throughout or are specific for activated endothelium, respectively. The addition of an endothelial-specific promoter, Roundabout 4, reduces off-target expression in the liver and enhances expression within bone marrow endothelium. These targeting gains have been supported by our preliminary data using vectors expressing a GFP reporter that show that these vectors achieve high levels of transduction in the endothelium of the bone marrow and small bowel. We are currently constructing adenoviral vectors utilizing these targeting strategies that express cytokines, growth factors, and transcription factors known to improve the resistance of these normal tissues to radiation. These improvements to vector targeting should increase the effectiveness of gene therapy for radioprotection.

FARZIN FARZANEH [Speaker]

Clinical immune responses to therapeutic vaccination with an autologous CD80/IL-2 expressing leukaemia cell vaccine

Lucas Chan, Yuqian Ma, Sabine Domning, Giulia Giunti, Kyriaki Ioannou, Ruby Quartey-Papafio, Linda Barber, Ghulam Mufti and Farzin Farzaneh. Department of Haematological Medicine, King’s College London

In pre-clinical studies we have demonstrated that tumour cells expressing immune co-stimulatory molecules and the appropriate Th1 cytokines can induce immune mediated rejection of previously established tumours. We are now assessing this strategy in a Phase-I clinical study of relapsed poor prognosis acute myeloid leukaemia (AML). In this study patients in temporary chemotherapy induced remission, are vaccinated with autologous AML cells that are genetically modified to express CD80 and IL-2. Early results indicate, feasibility, safety and stimulation of immunological responses against the unmodified AML blasts. We have also developed a new vaccination strategy based on a combination of adjuvants for synergistic activation of cellular immunity (CASAC). Pre-clinical studies show that subcutaneous vaccination with CASAC and target peptides is able to break tolerance to chronically experienced antigens that are associated with cancer (e.g. TRP2, WT1, Glypican-3) or with chronic infections (e.g. HBV surface antigen). This vaccination induces antigen-specific immunity, including in vivo cytolytic activity and lysis of antigen positive cells, even in immune senescent aged mice with previously established tolerance. This strategy is now being developed for clinical evaluation.

CARL FIGDOR [Speaker]

Dendritic cell based cancer-immunotherapy ‘Mapping the way’

Carl G. Figdor, Gerty Schreibelt, Harm Westdorp, Kalijin Bol, Sonja Buschow Winald Gerritsen and Jolanda de Vries Department of Tumor Immunology. Radboud University Medical Center, The Netherlands

To date, Dendritic Cell (DC)-based immunotherapy is explored worldwide in clinical vaccination trials with cancer patients, So far, predominantly ex vivo-cultured monocyte- or CD34+ derived DCs have been used. Although during the past 15 years the concept of DC vaccination has been clearly proven and found safe, the number of patients that have long-term benefit is limited. Instead of monocyte derived DC, we recently performed studies with two major types of naturally occurring DCs: myeloid DCs (mDCs) and plasmacytoid (pDCs). Despite their low abundance, in the peripheral blood, the first results indicate that these cells are extremely potent in initiating immune responses in cancer patients. I will discuss the future perspective of DC based cancer immunotherapy, also in view of the developments on biomarkers that may help to select patients might benefit from immunotherapy.

BARBARA-ANN GUINN [Poster]

SSX2IP and GKT-AML5 are frequently expressed in adult B-cell acute lymphocytic leukaemia

Payalben Savaliya†, Stephanie Bonney‡, Laurence Orchard‡, Kim Orchard‡, Barbara-ann Guinn†

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‡: Contributed equally to the work

Background: Acute lymphoblastic leukaemia (ALL) is a form of leukaemia characterized by excess lymphoblasts. If untreated the disease progresses rapidly and can be fatal within weeks to months. Adult patients with ALL who have an allogeneic
stem cell transplant (allo-SCT) have an improved overall survival rate of 27-65% compared with 15-45% in patients receiving chemotherapy only (1-3). The benefits of allogeneic transplantation may in part be due to a ‘graft-versus-leukaemia’ response however in ALL few disease related target antigens have been described. The identification of ‘ALL-associated antigens’ has the potential to identify new targets for immunotherapy during first remission when tumour loads are low, or post-transplant when healthy donor T cells persist and the occurrence of graft versus leukaemia responses can crucially improve outcome.

Methods: We have performed specific antibody profiling on 13 sera samples from nine ALL patients, and nine age and sex-matched healthy donor controls. Signals from 9,000 peptides were analysed on the ProScanArray using ProtoArray® Prospector v5.2 software. The mean value and standard deviation of each signal was calculated to produce a z-score and the five most promising antigens were identified. Using RT-PCR we analysed the expression of a total of 23 known antigens, including the five identified by antibody specific-profiling, in 20 human cell lines derived from various solid and haematological malignancies, eight presentation adult B-ALL patient samples and eight healthy volunteer peripheral blood mononuclear cell samples.

Results: We found that a novel antigen called GKT-AML5, identified through the immunoscreening of an AML cDNA library with autologous sera (4) and SSX2IP were the most frequently expressed in patient samples but not found in healthy donor peripheral blood samples.

Conclusion: Further analysis of ALL patient samples and CD34+ healthy donor leucocytes will determine whether these antigens remain the most promising for the immunotherapy of adult B-ALL.

BARBARA-ANN GUINN [Speaker]
A disease specific biomarker for the early detection of ovarian cancer

Ghazala Khan1, Andrew Mead1, Suzanne Brooks2, Barbara-ann Guinn1

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Background: Only 30% of all patients diagnosed with ovarian cancer are at stage I disease when survival rates are 90%. Currently diagnosis is made by a pelvic examination, transvaginal ultrasonography and detection of carbohydrate antigen 125 (CA125). However CA125 has variable expression between patients with some expression in endometrial tissue. Human epididymis secretory protein 4 (HE4) shows promise with overexpression in ovarian cancers however it has also been found in endometrial adenocarcinomas.

Methods: We have immunolabelled samples on paraffin-embedded tissue microarrays from 195 ovarian cancer samples, 12 healthy ovarian tissues and one skin cancer sample, 25 cases of endometrial adenocarcinoma, five uterine squamous cell carcinoma, six endometrial metastatic carcinoma, three polyps, 20 endometrial hyperplasia, five endometrial inflammation, 10 adjacent normal tissue and six normal tissues of uterus (all Biomax). Arrays were de-waxed and prepared for antigen retrieval. Actin was used as a positive control and isotype controls to detect non-specific staining. Immunolabelling for each of the antigens was performed using anti-human primary antibodies and HRP conjugated secondary antibodies (all Abcam, U.K.), visualised using an Envision-HRP labelling kit (Dako, U.K.).

Results: Expression of a novel Ovarian Cancer Protein (OCP) was detected in 21% of ovarian cancer samples. The levels and frequency of expression exceeded that of CA125 in sections from the same tissues (12%) and was limited to ovarian cancer tissues predominantly at stage I and II of disease.

Conclusion: Early stage ovarian cancer samples (predominantly stage I) expressed OCP at higher intensities than CA125 and HE4. Expression was restricted to samples from ovarian cancer patients. The possible detection of this small protein is now being investigated in urine samples from healthy age and sex-matched volunteers and ovarian cancer patients as part of the development of an early diagnosis lateral flow assay to screen for ovarian cancer.

NAGY HABIB [Speaker]
Gene activation by non-coding RNA. A targeted approach for novel therapy in liver regeneration

Nagy Habib1, V. Reebye1, K.A. Czysz1 P. Sætrom2,3, P.J. Mintz1, N. Kasahara4, J.P. Nicholls1, J.J. Rossi5
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Positive regulation of gene expression by non-coding RNAs (ncRNA) is fast becoming a new turning point in biology. Although RNA mediated gene silencing is an extensively studied area, the roles of ncRNA in activating gene expression is an exciting field with enormous therapeutic implications. We have previously demonstrated that a synthetically derived ncRNA duplex (we term here as short-activating RNA (saRNA)) designed to activate the liver enriched transcription factor C/EBPA significantly reduced liver tumour burden and improved liver function in a cirrhotic/HCC rat model. From this exciting work, which has now entered the early stages of a clinical study, we now extend our investigations into the use of activating ncRNA molecules for improving lipid metabolism and hepatocyte function.

Using an iterative approach to identify putative saRNA strands that transcriptionally activate target genes, we demonstrate by chromatin-immunoprecipitation assay that saRNA duplexes can directly interact within transcription start sites of the intended target genes. Although the mechanism of saRNA action is still being defined, our data suggests that these double stranded ncRNAs are present as part of a transcriptionally activate module. More recently we have shown that exogenously introduced saRNA targeting C/EBPA also favourably regulates lipid metabolism in obese rats. Preliminary data here suggests a reduction in genes involved in fatty acid transport (CD36), triglyceride synthesis (DGAT2), fatty acid biosynthesis (FASN) and genes known to be involved in insulin resistance (SREBP and CETP). To extend the concept of identifying and synthesising ncRNAs that can improve hepatocyte function we show that saRNA activation of HNF4a in HepG2 cells greatly enhances regulation of albumin expression and rifampicin induced activation of cytochrome P450 activity. As we and others unravel the complexity of gene activation by ncRNAs, this new field of biology will undoubtedly lead to the development of many new therapeutic avenues for liver regeneration.

Disclosure: This work was funded by MiNA Therapeutics Ltd. MiNA Therapeutics holds licences for intellectual property related to saRNA technology.

DENNIS HALLAHAN [Speaker]
Retargeting Ad to radiation inducible receptors in cancer

Dennis E. Hallahan MD, David T. Curiel MD, PhD, Heping Yan MD, Lyudmila Kaliberova MD, Sergey Kaliberov MD, PhD.

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Gene Therapy

Positive regulation of gene expression by non-coding RNAs (ncRNA) is fast becoming a new turning point in biology. Although RNA mediated gene silencing is an extensively studied area, the roles of ncRNA in activating gene expression is an exciting field with enormous therapeutic implications. We have previously demonstrated that a synthetically derived ncRNA duplex (we term here as short-activating RNA (saRNA)) designed to activate the liver enriched transcription factor C/EBPA significantly reduced liver tumour burden and improved liver function in a cirrhotic/HCC rat model. From this exciting work, which has now entered the early stages of a clinical study, we now extend our investigations into the use of activating ncRNA molecules for improving lipid metabolism and hepatocyte function.

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ROB HOEBEN [Speaker]
iLOV reoviruses: Replication-competent, expanded-tropism reoviruses carrying a reporter transgene

Diana van den Wollenberg, Iris Dautzenberg, Sanne van den Hengel, Vera Kemp, Willeke Ros, Rob Hoeben. Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands
The reovirus Type 3 Dearing (T3D) is a mammalian orthoreovirus which is being evaluated as oncolytic agent in various clinical trials. T3D infection is strongly cytolitic with a marked preference for tumor cells. In humans, reovirus infection has no associated pathology. The segmented dsRNA genome of reoviruses thwarted genetic modification but recently systems for their genetic modification have been described. In our studies, we have combined forward-and reverse-genetics strategies to generate expanded-tropism, replication-competent reoviruses carrying a heterologous transgene.

Infection of reovirus T3D is dependent on expression of the reovirus receptor JAM-A or, in CNS neurons, NgR1. We used bioselection to select T3D reoviruses that can infect tumor cells lacking expression of these receptors. The genomes of T3D three JAM-independent jin mutants were found to harbor mutations near the codons for the sialic acid-binding region in the shaft of the spike protein S1. The functional significance of these mutations was evident by demonstrating that the jin mutants rely on sialic acids for infection of JAM-Abearing cells.

With the aid of a plasmid-based system we have modified the spike encoding S1 segment of T3D. The region encoding the JAM-A binding head domain was replaced by a synthetic gene encoding iLOV, a small green-fluorescent protein. In the modified reovirus we utilized the spike's shaft-encoding region from a jin mutant. The resulting iLOV-encoding reoviruses could be passaged helper-free and in absence of heterologous helper functions. In addition, these recombinant viruses could infect JAM-A deficient cells. This demonstrates that head-replacement can be used as a strategy for generating small-transgene containing reoviruses. This technology may facilitate the generation of new enhanced potency armed reoviruses for oncolytic virus therapy.

**AI JUN [Poster]**

Expression of the stem cell factor OCT4 and EMT-associated factor in invasive breast cancer and its relationship with clinicopathological features and prognosis

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Objective: This experiment mainly study was the expression of EMT related factor E-cadherin, vimentin and stem cells factor OCT4 in breast cancer in order to investigate the correlation between EMT and cancer stem cells. Method: Quantitative real-time PCR and immunohistochemical staining were used to detect the mRNA and protein expression of OCT4, E-cadherin and vimentin in invasive breast cancer tissue and the corresponding adjacent tissues. OCT4, E-cadherin and vimentin in invasive breast cancer tissue the clinical/biological factors of breast cancer were analyzed. And further analysis of the correlation between OCT4 and EMT-related proteins, the relationship between them and survival of breast cancer patients. Result: In invasive breast cancer tissues, the expression rate of OCT4, E-cadherin and vimentin respectively were 30%(12/40), 55%(22/40), 65%(26/40). OCT4 gene expression was correlated with the age of breast cancer patients, histological grade, lymph node metastasis and the expression of Her-2. E-cadherin gene expression was correlated with the lymph node metastasis. Vimentin gene expression was correlated with the histological grade and lymph node metastasis. The expression of OCT4 mRNA and protein negatively correlated with the expression of E-cadherin mRNA and protein, positively correlated with the expression of vimentin mRNA and protein. And the expression of E-cadherin mRNA and protein correlated negatively with the expression of vimentin mRNA and protein. The survival rate with OCT4 positive expression breast cancer patients was significantly lower than patients with OCT4 negative expression. Conclusion: Stem cells factor OCT4 may promote the metastasis of breast cancer cells via EMT. OCT4 gene expression is an independent prognostic factor for breast cancer.

**LYUDMILA KALIBEROVA [Poster]**

Development of adenoviral vectors for radiation-guided gene therapy

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Radiotherapy has improved the cure rates in many malignant neoplasms. However, this therapeutic approach is limited by development of tumor cell radioresistance and normal tissue toxicity. The combination of radiation treatment with specific radiosensitizing gene delivery should provide a major advantage to achieve a higher therapeutic result. In this regard, it has been firmly established that x-rays can induce significant molecular changes in target tissues. Recently, phage display technology was used to isolate novel peptides GIRLRG and HVGGSSV that specifically bind to the 78-kDa glucose-regulated protein (GRP78) and the Tax-interacting protein (TIP-1), respectively. It was shown that these proteins are overexpressed on cell membranes of irradiated cancer and tumor-associated microvascular endothelial cells. The goal of our studies was to achieve tumor-specific adenoviral (Ad) mediated gene delivery by using these radiation-
inducible neoantigen targeting peptides. To this end, we employed the Ad fiber replacement approach to endeavor study of the targeting utility of GIRLRG and HVGGSSV peptides. For these studies we have developed a panel of fiber-modified Ad vectors containing a gene encoding GIRLRG or HVGGSSV peptides inserted into the C-terminus of a de-knobbed fiber-fibrinogen protein. We have evaluated reporter gene expression of fiber modified Ads in vitro and in vivo using a panel of glioma and lung cancer cells. Obtained results demonstrated that employment of GRP78 and TIP-1 binding peptides resulted in increased gene expression in irradiated tumor cells following infection with fiber-modified Ads. These studies clearly demonstrate the feasibility of Ad retargeting using GIRLRG and HVGGSSV peptides that selectively recognize tumor cells responding to radiation treatment. The results generated in this project provide the rationale to continue these investigations using fiber-modified Ads for radiation-guided therapeutic gene delivery in combination with radiotherapy.

WON JONG KIM [Poster]
Polymeric gene and drug delivery for anticancer therapy

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A siRNA has emerged as a new strategy of nucleic acid-mediated gene therapy owing to its target specific gene silencing capability. In this research, siRNAs were utilized for two functions: therapeutic agent and cross-linking building block for nanoconstruct. We developed a novel nanoconstruct (NC) which was composed of siRNA-conjugated natural polymers through spontaneous hybridization of sense- and antisense strand siRNAs conjugated to dextran polymer. Furthermore, disulfide bonds were introduced between siRNA and polymer to allow disintegration of NC and simultaneously releasing of siRNA by disulfide exchange reaction in reductive intracellular condition. In addition, peptamers (peptide aptamers) were introduced on the NC for prostate cancer targeted siRNA delivery. This siRNA mediated cross-linked NC is a condensed nano-sized particle through gathering each polymer chains by siRNA double helix formation. Increasing the concentration of NC inhibited gene expression more efficiently up to about 40%. Paclitaxel (PTX) is one of the most effective chemotherapeutic drugs used in breast, ovarian, lung, head and neck cancers. However PTX has very low solubility in water and physiological conditions. To enhance its solubility, PTX would be formed inclusion complex with β-cyclodextrin (β-CD). β-CD is a cup-shaped molecules that consists of 7 glucopyranoside units and has hydrophobic cavity and hydrophilic exterior. It has long-term biocompatibility, low toxicity and do not elicits any immune response. It has also well-known host-guest interactions with many small molecules and portions of large compounds, such as benzoate moiety of PTX. Herein, we designed self-assembled nanoparticles for PTX delivery toward tumor cell. Self-assembled nanoparticles were constructed through host-guest chemistry between PTX and CD. CD and PTX are covalently conjugated with poly maleic anhydrides that provide higher solubility of nanoparticles. This inclusion complex showed enhanced antitumor effect than PTX.

DAVID KIRN [Speaker]
Targeted oncolytic and immunotherapeutic viruses: Emerging multi-mechanistic biologics for cancer

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Targeted oncolytic and immunotherapeutic viruses are an emerging multi-mechanistic therapeutic platform designed to induce both acute tumor debulking as well as chronic suppression of tumor outgrowth. Cancer-specific viral replication and immunostimulatory transgene expression (e.g. GM-CSF) result in direct cytolysis followed by tumor-specific humoral and cellular immunity. Product activation is driven by commonly activated genetic pathways in cancer. JX-594 is an oncolytic vaccinia virus derived from the Wyeth vaccine strain and has been engineered for 1) enhanced cancer targeting by TK disruption and 2) has been “armed” with the transgene of granulocyte-macrophage colony stimulating factor (GM-CSF) to augment oncolysis-induced anti-tumoral immunity (Nature Rev Cancer 2009). JX-594 replication within tumors, coupled with tumor-specific expression of GM-CSF, creates a pro-inflammatory microenvironment and exposes tumor antigens resulting in immune response induction to the patient’s endogenous tumor antigens. Recent clinical results demonstrate convincingly that products from this therapeutic class can achieve highly selective and potent cancer destruction systemically through a multi-pronged MOA, including tumor-specific immunity (Nature 2011; Nature Med 2013; Science Translat Med 2013). Recent preclinical
and clinical trial results demonstrated potential synergy with checkpoint inhibitor antibodies. “Directed Evolution” vector discovery principles can be applied to diverse viral species to create and isolate optimized, proprietary vectors targeting specific cancer cells or immune cells (Nature Reviews Genetics 2014). Given recent clinical validation, we expect this therapeutic class of “personalized” yet “off-the-shelf” active immunotherapeutics to expand rapidly.

DAVID KLATZMANN  [Speaker]

Using systems immunology to study tumor microenvironment: transcriptomics reveal an early immunological storm induced by tumor cells and identify potential therapeutic targets

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*Equal contribution

Anti-tumoral immune responses are usually studied at relatively late stages after tumor emergence. Our previous studies analyzed immune responses occurring in the first 2–6 days after tumor implantation in mice. We showed that an early recruitment of Tregs imprints a dominant tolerant tumor microenvironment\(^1\). These Tregs are specific for tumor expressed self-antigens\(^1\), as later confirmed\(^2\).

To identify molecules and mechanisms implicated in tolerance imprinting, we analyzed the early changes in the tumor microenvironment through transcriptomic studies.

We determined the whole transcriptome of the tumor microenvironment and of the tumor draining and non-draining lymph nodes at 2, 4, and 14 days after tumor cell injection. We analyzed results using simple differential gene expression as well as more complex unsupervised methods\(^3\).

In BALB/c mice inoculated with AB1 tumors, we identified a very early immunological “storm”, with up- and down-regulation of numerous immune-related genes and pathways. These early up-regulations notably concerned transcripts associated with antigen presentation and immune suppression. Of the 2 most discriminant molecular signatures that were up-regulated early, one included the key target genes of the transcription factor Foxp3. We also identified VEGF and TGFβ as 2 up-regulated pathways that could be functionally involved in tolerance imprinting.

We then similarly analyzed the transcriptome of the tumor microenvironment in C57BL/6 mice challenged with wild-type B16 tumor cells or tumors silenced for or tumors silenced for VEGF and TGFβ expression. Compared to the BALB/c setting, we observed a similar early regulation of immune related genes and pathways in the tumor microenvironment of wild-type B16 cells. Dramatic modifications of the immune-related signatures were seen in silenced tumors, which shifted from a suppressive profile in wild-type tumors to an effector profile in silenced one. This correlated with a dramatic tumor growth delay of the silenced tumors in immunocompetent, but not in immunodeficient mice.

Our results identified immune related genes and pathways that are early affected during tumor growth and could be molecular targets for immunotherapy.


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CARA LUNN SHIRAI  [Poster]

Mutant U2AF1 alters hematopoiesis and pre-mRNA splicing in transgenic mice

Cara Lunn Shirai,\(^1\) James N. Ley,\(^1\) Brian White,\(^1\) Justin Tibbitts,\(^1\) Jin Shao,\(^1\) Matthew Ndonwi,\(^1\) Sanghyun Kim,\(^1\) Theresa Okeyo-Owuor,\(^1\) Timothy A. Graubert\(^2\) and Matthew J. Walter\(^1\)

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Myelodysplastic syndromes (MDS) are hematopoietic cancers common in the elderly. Our group and others discovered recurrent heterozygous missense mutations in the splicing factor gene U2AF1 in 11% of patients with MDS, suggesting that perturbations in pre-mRNA splicing play a role in disease pathogenesis. To study the effects of expression of U2AF1(S34F), the most common U2AF1 mutation, on hematopoiesis and pre-mRNA splicing in vivo, we created site-specific, single-copy, doxycycline-inducible U2AF1(S34F) and U2AF1(WT) transgenic mice.

Following four weeks of transgene induction, U2AF1(S34F) mice have reduced total WBCs in their peripheral blood compared to U2AF1(WT) and rtTA only controls (p≤0.01, n=9-11). U2AF1(S34F) mice have a perturbed mature cell lineage distribution by flow cytometry, including reduced monocytes and B cells in both peripheral blood (p≤0.05) and bone marrow (p≤0.01), and increased numbers of hematopoietic progenitors and stem cells in both bone marrow and spleen by colony forming assays and flow cytometry when compared to controls (p<0.05, n=5-11). The increase in bone marrow progenitor cell number in U2AF1(S34F) mice is associated with increased intracellular Ki67 (a marker of cell proliferation) compared to controls (p<0.05, n=8-13).
Next, we performed unbiased RNA sequencing on purified progenitors from bone marrow of mice following transgene induction. We identified 460 differentially-expressed splicing junctions in U2AF1(S34F) samples compared to U2AF1(WT) (FDR <5%). We also observed a preference of the mutant U2AF1(S34F) to skip exons (n=72) and alternative splice sites (n=45) with a T in the -3 position relative to the AG splice acceptor site (p value). We intersected our junction results with RNA sequencing from leukemia patient samples with U2AF1 mutations and human CD34+ cells transiently expressing U2AF1(S34F) and identified homologous dysregulated junctions in all 3 datasets. Together, these results suggest that U2AF1(S34F) expression contributes to the altered pre-mRNA splicing and hematopoiesis observed in patients with U2AF1 mutations.

**NENAD PETROVIC** [Speaker]

Prostanoids activate angiogenesis acting on IP and EP4 receptors

Hoang Khuyen Gia and Nenad Petrovic

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Tumorigenesis and metastasis depend on new vascular growth (angiogenesis) since proliferation and spread of cancer cells is conditional on adequate blood supply. Angiogenesis progression is regulated by numerous activators and inhibitors and is directly correlated with tumor aggressiveness. Important activators of angiogenesis include large family of specific lipid mediators called prostanoids. Although numerous studies have dissected the role of prostanoids in inflammation, their identity and function in angiogenesis is poorly understood. In this work we have compared effects of two prostanoids relevant to vasculature, prostaglandin E2 (PGE2), overproduced in many tumors and prostacyclin (PGI2) on angiogenic processes in vitro. PGE2 is thought to be the most important prostanoid activator of angiogenesis and prostacyclin receptors are mostly found in endothelial cells. Both of those prostanoids activate corresponding G-protein coupled receptors. Four of them are activated by PGE2 (EP1, EP2, EP3 and EP4) and one by prostacyclin (IP). In our experiments we have used Human Umbilical Vein Endothelial Cells (HUVEC) and characterized two important angiogenic processes: cell migration (with original method developed in our laboratory) and HUVEC “tube formation” (widely accepted method of assessing formation of blood vessel precursors). We detected efficient suppression (of about 80%) of both cell migration and tube formation with specific IP antagonist CAY10441 compared to specific EP4 antagonist L-161,982 which inhibited those processes by approximately 20%. AH6809, a specific antagonist of EP1, EP2 and EP3 receptors did not significantly suppress angiogenesis in our system. Our data were confirmed by measuring receptor activation coupled with rapid desensitization of both EP4 and IP receptors occurring at high agonist concentration (a well-documented characteristic of these receptors). Our results, taken together, suggest that IP receptors play the major role in regulation of angiogenesis (with EP4 having a minor role) and that IP antagonists could be potentially used to inhibit cancer angiogenesis.

**TON SCHUMACHER**
(Keynote Speaker)

What T cells see on human cancer

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With the aim to develop more specific and effective approaches to adoptive T cell therapy and other cancer immunotherapies, we have set out to explore how the T cell-based immune system of individual patients can recognize autologous tumor cells. Most human tumors contain large numbers of mutations, of which hundreds can be present within expressed genes. As the resulting altered protein sequences are foreign to the immune system, T cell recognition of these ‘neo-antigens’ is likely to be of importance. However, the vast majority of the mutations in human cancers are unique to individual patients and, because of this, broadly applicable approaches to link the consequences of DNA damage to tumor-specific T cell activity have long been lacking. Using in-house developed technologies, we have recently demonstrated the feasibility of cancer exome-driven analysis of tumor-specific CD8+ T cell reactivity. The data obtained demonstrate that CD8+ T cell recognition of neo-antigens is a common feature in human melanoma. Furthermore, based on the distribution of mutation loads in other major human cancer types, we propose that also in many other tumors, the repertoire of neo-antigens should suffice to allow CD8+ T cell recognition. In ongoing projects, we are exploring 1) whether the development of neo-antigen specific CD4+ T cell reactivity is also common in melanoma; 2) whether neo-antigen specific CD8+ T cell reactivity is observed in other tumor types; and 3) whether T cell responses against neo-antigens are also qualitatively superior as compared to T cell responses against non-mutant antigens. Based on the data obtained it is apparent that the mutations
that on the one hand form the driver behind tumor outgrowth, also form a handle for the T cell-based immune system to recognize tumors as foreign. As such, the development of ‘personalized immunotherapies’ that exploit cancer genome information to target patient-specific antigens should be explored.

ZSOLT SEBESTYÉN [Speaker]
Tumor cell recognition by g9d2TCR T cells is dictated via Small Rho GTPase by linking mevalonate pathway to BTN3A1 (CD277)

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Recent advances in g9d2 T-cell biology have pointed towards BTN3A1 to play a major role in tumor cell recognition. However, given the ubiquitous expression of BTN3A1 on both healthy and diseased cells, the molecular mechanisms linking the disregulated mevalonate pathway to BTN3A1 in g9d2 T-cell target recognition remain unclear.

To identify these yet unknown mechanisms, we developed a novel approach: SAPPHIRE (SNP-Associated-comPutional-Protein-Hunt-Including-RNAi-Evaluation) based on the assumption that distinct genetic variations among target cells determine recognition by g9d2TCR T cells. Consequently, we hypothesized that single nucleotide polymorphisms (SNPs) could serve as a surrogate marker for variation loci involved in determining g9d2TCR T-cell reactivity. EBV-LCLs from the CEPH library, genotyped for millions of SNPs, were used as target cells for abT-cells engineered to express a defined g9d2TCR in T cell functional assays. We then correlated the recognition of EBV-LCLs with SNP genotypes using genetic linkage analysis. Next, we used RNAi to evaluate functional relevance of genes that were marked by 100% correlating SNPs. Knock-down of only a small Rho-GTPase resulted in reduced target cell recognition by g9d2TCR T-cells. Importantly, Rho-GTPase is directly prenylated by phospho-intermediates of the mevalonate pathway, and inhibition of prenylation led to reduced target recognition. Rho-GTPase activity and subcellular localization, but not expression levels, associated with differential recognition of CEPH LCLs but also additional tumor cells. Crucially, using FRET and Duolink techniques we showed that Rho-GTPase directly interacts with BTN3A1 only in recognized tumor cells. Importantly, we show that this mechanism is also active in AML stem cells, which makes them a feasible g9d2TCR tumor target.

In summary, using our genome-wide semi-high throughput approach, we identified Rho-GTPase as an important determinant/mediator of target cell recognition, providing a potential “missing link” between the mevalonate pathway and BTN3A1. This better understanding of g9d2TCR T-cell tumor specificity paves the way to safer clinical application of g9d2TCR T-cells. Moreover, Rho-GTPase localization could be used as a prognostic marker for patient eligibility for g9d2TCR immunotherapy.

ZSOLT SEBESTYÉN [Poster]
Towards the development of engineered immune cells broadly applicable to cancer patients

Trudy Straetemans1, Cordula Gründer1, Sabine Heijhuurs1, Samantha Hol1, Kirsten Scholten1, Ineke Slaper2, Halvard Bönig1, Zsolt Sebestyen1 and Jürgen Kuball1

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Engineering αβT cells with receptors to re-direct the immune system against cancer has most recently been described as one of the scientific breakthroughs. However, a more careful clinical translation is clearly needed given the many challenges and pitfalls during the implementation resulting frequently in an inefficient clinical product or even failure of clinical efforts. Therefore, we took advantage of selected highly tumor-reactive γδTCR genes, recently proposed as a very intriguing alternative strategy to CARs and αβTCRs, developed a novel stepwise evaluation process and assessed efficacy of the GMP-grade cell product. By studying the optimal expression cassette we found transgene expression is depending on TCR-chain orientation and different 2A elements. In addition, suicide mechanisms strongly suppressed expression of introduced receptors and the introduced suicide gene itself, questioning safety and clinical applicability of suicide cassettes in the context of engineered immune cells. Most importantly, we developed a novel selection method by depleting non and poorly engineered cells with clinical-grade anti-αβTCR-beads. This selection method translated into highly purified, but untouched engineered immune cells with strong anti-tumor reactivity both in vitro but also in two different humanized mouse models. Furthermore, this strategy eliminated residual allo-
reactivity. All together, we generated strong evidence for the need of a careful and step-wise evaluation of newly designed engineered immune cells. Finally, we provide for the first time a GMP-grade enrichment procedure for the generation of untouched engineered immune cells with improved antitumor and reduced allo-reactivity suitable for the application in both an autologous and allogeneic clinical scenario.

LEN SEYMOUR [Speaker]

Enadenotucirev (ColoAd1) a group B oncolytic adenovirus: pre-clinical assessment of potency, safety and selectivity.

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Enadenotucirev (EnAd; formerly called ColoAd1) is a chimeric Ad11p/Ad3 adenovirus, discovered by bio-selection from a library of chimeric adenoviruses for the ability to replicate and exit rapidly from tumour cells. The virus is active against a broad range of cancer cell lines demonstrating a shorter time-to-lysis than either wild type Ad11p, Ad3 or Ad5. In normal cells, EnAd is attenuated and shows little or no activity by either cytotoxicity or by quantitative PCR. The mechanism of tumour cell lysis is independent of apoptosis pathways and EnAd readily kills drug resistant cells. In vivo, EnAd shows efficacy in a range of subcutaneous and orthotopic metastatic tumour models following intratumoral, intravenous and intraperitoneal injection. When subpopulations of cells are isolated from tumour samples, sphere forming cells (with a self-renewing phenotype) were shown to be disproportionately killed by ColoAd1. The virus capsid is entirely derived from Ad11p for which there are limited circulating levels of neutralising antibodies in the general population. EnAd associates with blood cells reversibly in the cytoplasm and nucleus of dysplasia and cancer cells. Immunohistochemically, β-catenin was positively expressed in the cytoplasm and nucleus of dysplasia and cancer cells. The expression of β-catenin in esophageal tissues from week 24, the rest mice were euthanized. HE staining is used to observe the pathological changes of esophageal tissues. The effects of "Lianhua Shenjia formula" on canonical Wnt/β-catenin signaling pathway in 4NQO-induced mouse esophageal precancerous lesions

BAO-EN SHAN [Poster]

The effects of “Lianhua Shenjia formula” on canonical Wnt/β-catenin signaling pathway in 4NQO-induced mouse esophageal precancerous lesions

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Objective: To investigate the reversal effect of “Lianhua Shenjia formula” on differentiation of esophageal precancerous lesions cell and its mechanism. Methods: 300 C57BL/6 mice were randomly divided into six experimental groups, (1) normal control group, (2) MC control group: each one was fed with 0.1 ml Methyl cellulose solution three times per week, (3) 4NQO control group: 60 mice fed with the carcinogen 4NQO solution diluted in the drinking water at 100μg/ml for 15 weeks, (4) TCM treatment group: the supply of 100μg/ml 4NQO stopped at week 15. After that, the 60 mice received 0.1 ml solution of the Lianhua Shenjia formula three times a week, (5) TCM prevention group: the 60 mice received 100μg/ml 4NQO plus 0.1 ml solution of the Lianhua Shenjia formula three times a week. The 4NQO only lasted for the first 15th week, (6) ATRA treatment group: 60 mice were fed with 100μg/ml 4NQO for the first 15th week. After that, the mice received 0.1 ml solution of ATRA three times a week. At week 12 and 15, 5 mice from the normal control group, MC control group, 4NQO control group and 4NQO prevention group were euthanized. At week 18 and 21, 5 mice from the normal control group and MC control group were euthanized. At week 24, the rest mice were euthanized. HE staining is used to observe the pathological changes of esophageal tissues. The expression of β-catenin in esophageal tissues from mice in each group was detected by immunohischemistry. Results: At week 18, 21 and 24, the incidence of esophageal carcinoma in 4NQO control group was 13.3%, 26.7% and 68.7% respectively. Compared to 4NQO control group, there was a decrease in the ratio of esophageal carcinoma in TCM treatment group, TCM prevention group and ATRA treatment group at week 18 and 21, yet the decrease is not significant (P>0.05). However, there were a markedly decrease for these three groups at week 24 (TCM treatment group: 17.9%; TCM prevention group: 26.7%; ATRA treatment group: 10.3%), as compared with 4NQO control group (68.7%)(P<0.05). Immunohistochemically, β-catenin was positively expressed in the cytoplasm and nucleus of dysplasia and cancer cells. The ectopic expression rates of β-catenin in 4NQO control group increased significantly with the severity
Bruce F. Smith [Speaker]

A targeted oncolytic adenovirus for canine osteosarcoma: a translational model

Bruce F. Smith1,2, Payal Agarwal2, David Curiel3, Igor Dmitriev3, Annette Smith4

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Oncolytic viruses represent an opportunity to specifically target tumor cells through the use of tumor specific replication. However, most replication competent viruses are replication incompetent outside of their host species. For this reason, we have created a fully syngeneic oncolytic virus system to use in evaluating the efficacy of oncolytic viruses. Canine adenovirus type 2 (CAV2) is a respiratory virus of dogs used to immunize against CAV1, the causative agent of canine infectious hepatitis. CAV2 was modified so that the E1 gene was placed under the control of the osteocalcin promoter. The virus was evaluated for replication specificity in cell culture and in xenogeneic canine osteosarcoma transplants. In these transplants, the oncolytic virus abrogated tumor growth. Subsequently, the virus was administered to normal dogs to evaluate distribution and safety. After demonstrating circulation of the virus for up to 5 days, and no significant adverse events, the virus was tested in a small clinical trial in client-owned dogs with osteosarcoma and is currently in a larger clinical trial. Preliminary findings from these trials will be presented. These studies indicate that the canine model has outstanding potential to evaluate the mechanism of action and potential success of oncolytic viral therapies.

Richard Michael Stanton [Poster]

Development of tumour therapy and imaging strategies utilising bacteria

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The ability of systemically administered bacteria to target and replicate to high numbers within solid tumours is well established. Localisation of bacteria to a tumour site allows for the targeted activation of therapeutics and the confinement of therapeutic effect to the tumour site, for example, bacteria directed activation of a prodrug within a solid tumour. In order to monitor therapeutic activity there is a requirement for the development of non-invasive imaging strategies to track bacteria within a live host. Optical imaging is the most commonly utilised preclinical imaging system. To date, the vast majority of non-invasive in vivo imaging systems for bacteria have relied on the use of bacteria that have been genetically modified or harbour genetic expression constructs. However, the use of engineered bacteria is often not experimentally appropriate or technologically feasible as engineering tools are not available for a number of bacterial strains. Therefore, there is a need for the development of optical imaging strategies using unmodified bacteria. The current work aims to develop novel therapeutic and non-invasive imaging strategies based on the endogenous enzymatic activity of tumour targeting bacteria. Nitroreductases are a family of bacteria specific enzymes that are capable of activating the fluorescent probe CytoCy5S as well as the cytotoxic prodrug CB1954. In vitro and in vivo data demonstrate that endogenous levels of these enzymes are sufficient for probe activation and elicitation of a therapeutic effect in BALB/c mice bearing subcutaneous CT26 tumours. This study introduces the concept of utilising endogenous nitroreductases for therapeutic effect and as a reporter for wild-type bacteria. The system may be adapted for use with other imaging modalities or for other research fields such as infectious disease.

Calvin J. Stephens [Poster]

Targeted adenoviral delivery of a secrétable Notch1 decoy

Calvin J. Stephens1, Zhi Hong Lu2, Jeffrey M. Arbeid2,3, and David T. Curiel1,4

1Department of Radiation Oncology, 2Urology Division, 3Cell Biology Department, and 4Biological Therapeutics Center,
Notch signaling is instrumental in normal organogenesis, development, and the maintenance of adult tissues. Perturbation in this signaling cascade has been identified as a key hallmark of several aggressive cancers including solid tumors, breast cancer, bone metastases, and renal cell carcinomas. The importance of Notch signaling in these cancers has been highlighted in studies that show over-activation of Notch signaling leads to increased tumor invasiveness, angiogenesis, and proliferation; additional studies have identified the cancer microenvironment as a contributor to Notch-mediated cancer proliferation. Corroborating evidence shows inhibition of Notch signaling, using secretable Notch decoys that mimic Notch receptors, antibodies or inhibitory drugs, reduces tumor metastatic ability, angiogenesis, and size in various cancer types. Although the therapeutic effect of Notch inhibition is being explored in clinical trials, current strategies to inhibit Notch signaling, such as -secretase inhibitors, have been associated with off target effects such as reduced thymic weight and severe gastrointestinal disturbances. In this regard, localized delivery of an anti-Notch decoy may provide a strategy to accrue their anti-tumor effects while circumventing systemic toxicity. We are addressing this pharmacological mandate via adenoviral vectors, which may be ideally suited for this purpose owing to the fact that current vector targeting methods can achieve cancer cell and cancer microenvironment specificity. Using our adenoviral vector re-targeting strategies, such as fiber modification and transcriptionally regulated expression, we will deliver a secretable mouse Notch1 decoy, containing a truncated extracellular EGF repeat domain, to tumor vascular niches and metastatic prostate cancer within bone tissue. We expect a reduced proliferative and angiogenic phenotype in the cancer cells resulting from inhibition of Notch-mediated signaling due to competitive binding of the decoy to Notch receptor ligands. Such genetic targeting may be key to harnessing the therapeutic utility of Notch axis inhibition by reducing dose limiting toxicity.

RENA TAB STRIPECKE [Speaker]

Reprogrammed dendritic cells accelerate de novo functional adaptive responses after stem cell transplantation in humanized mice

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Justification: More than 50,000 hematopoietic stem cell transplantsations (HSCT) are performed worldwide per year. Although for some types of hematologic malignancies HSCT is the only curative therapeutic option, it is a very aggressive procedure and the mortality, morbidity and risk of infections are high. There is a need for animal models to carry out in vivo HSCT studies to test novel cellular and molecular therapies without putting individuals at risk. Predictive mouse models of HSCT to pinpoint experimentally the spatio-temporal events during human adaptive immune reconstitution in vivo are still lacking. HSCT modelling in humanized mouse models is currently limited due to incomplete maturation of T and B lymphocytes and lack of lymph nodes (LN) and normal lymphatic flow. Conventional dendritic cells (cDC) are capable of potently stimulating naïve T cells and with vast potential for immunotherapeutic applications. However, the manufacture of clinical-grade cDC is complex. Clinical trials showed poor trafficking of cDC from subcutaneous injection sites to LN, where DC can optimally stimulate naïve lymphocytes for long-lasting memory responses. We demonstrated that a single overnight manipulation of monocytes with integrase defective lentiviral vector (IDLV) for production of complementary cytokines (GM-CSF/IFN-a) and a viral immunodominant antigen (pp65) was capable to induce autonomous self-differentiation of antigen-loaded DC (SmyleDCpp65) in vitro and in vivo. Nod-Rag-/-IL2gc-/- mice transplanted with CD34+ human stem cells and immunized with autologous SmyleDCpp65 developed lymph node-like structures (LN-LS), showed functional expansion of memory T helper and T cytotoxic cells reactive against pp65 and production human antibodies (IgM and IgG) against pp65. Comparative studies of SmyleDCpp65 immunization were performed with CD34+ HSC obtained from adult G-CSF mobilized peripheral blood (PB) and neonate cord blood (CB). Whereas the expansion of human T cells derived from PB-HSCT immune reconstitution was preferentially localized in secondary lymphatic tissues, T cells that developed from CB-HSCT showed initially an enhanced expansion in the thymus. The clinical translation of SmyleDCpp65 for HSCT and its practical uses for humanized animal experimental models to address potency of vaccines, gene therapy and immune therapeutic approaches will be discussed.

MASATOSHI TAGAWA [Poster]

Restoration of p53 functions is a key element in gene therapy of malignant mesotheliomas

Masatoshi Tagawa1,2, Kiyoko Kawamura1, Shinya Okamoto1,3, Masato Shingyohji4, Ikuo Sekine5, Yuichi Takiguchi6, Yuji Tada7, Koichiro Tatsumi3, Hideaki Shimada6, Kenzo Hiroshima7
Malignant mesothelioma develops in the thoracic cavity and is often resistant to chemotherapy. Genetic characterization of the clinical specimens revealed that the majority was defective of the INK4A/ARF locus containing the p14ARF and p16INK4A genes but possessed the wild-type p53. Since p14ARF blocks MDM2-mediated p53 degradation and p16INK4A inhibits cyclin-dependent kinases, the deletion results in loss of p53 functions and pRb hyper-phosphorylation, which subsequently leads to uncontrolled cell proliferation with enhanced resistance to apoptotic stimuli. We thereby examined a possible therapeutic strategy by restoring the p53 functions. Transduction of the p53 wild-type mesothelioma with adenoviruses expressing wild-type p53 (Ad-p53) up-regulated p53 expression levels with the phosphorylation, dephosphorylated pRb and induced G1 arrest followed by increased sub-G1 fractions with a preferential activation of the extrinsic apoptosis pathways. A combinatory use of Ad-p53 with cisplatin or pemetrexed, the first line agents for mesothelioma, produced synergistic cytotoxic effects. Expression of p53 is modulated by a number of molecules. MDM2 promotes p53 degradation through an ubiquitin-proteasome pathway, whereas MDM4 inhibits a transcriptional activity of p53. Nutlin-3a, an inhibitor of p53-MDM2 interactions, increased expression levels of p53 and subsequently produced apoptosis-mediated cytotoxicity. Inhibitors for heat shock protein 90 (Hsp90) suppressed MDM4 expression and inhibited viability of mesothelioma cells. These agents in combination augmented stability of endogenous and transduced p53 molecules, which resulted in enhanced cytotoxicity. Bisphosphonates, an agent for osteoporosis, produced cytotoxic effects on mesothelioma. Zoledronic acid (ZOL), the third generation of bisphosphonates, up-regulated p53 expression levels in mesothelioma and induced the p53 phosphorylation although the main function was suppression of small G proteins' functions by inhibiting the prenylation processes. We demonstrated the combination of ZOL and Ad-p53 or cisplatin produced synergistic cytotoxic effects. There date collectively indicate that restoration of p53 functions is a key element for mesothelioma treatments.

**MARK TANGNEY** [Speaker]

Bacterial-based tumour modification technology

Bacteria present an attractive class of gene vector for cancer treatment, possessing a natural ability to grow specifically within tumours following systemic administration. We describe a range of strategies under investigation by our group, designed to exploit tumour-specific bacterial location, utilising non-pathogenic bacterial vectors. We have engineered a number of replication-competent non-pathogenic probiotic bacteria to express heterologous genes and mediate long-term production of agents within tumour masses (external to tumour cells) following systemic administration. We have demonstrated that various bacterial vectors can be exploited in a wide range of therapeutic strategies and cancer indications, in isolation or in combination with other therapies, presenting a powerful and safe approach to restricting the activity of agents to tumours.

**PAUL W. TETTEH** [Poster]

Differentiated colonic epithelial cells as cells of origin of colon cancer

Paul W. Tetteh, Harry Begthel, Maaike van den Born, Jeroen Korving, Henner F. Farin, Johan van Es and Hans Clevers

The progression of sporadic colorectal cancer, the second leading cause of cancer-related death in the Western world, involves initiation of activating mutations in the Wnt signalling pathway in colonic epithelial cells followed by a series of activating and inactivating mutations in oncogenes and tumor suppressor genes respectively in other signalling pathways. Animal models for human colon cancer can be useful for studying the mechanism of colon cancer development and for testing cancer prevention and treatment approaches. Current mouse models often significantly differ from human colon cancer, e.g. in intestinal location. We aimed to develop a colon-specific inducible mouse model which can faithfully recapitulate human colon cancer initiation and progression. Carbonic anhydrase I (Car1) is a gene expressed in colonic epithelial cells. We generated a novel colon specific inducible Cre Knock-In mouse (Car1-Creert2 KI). Characterization of Cre activity by crossing these mice to the Rosa26 LacZ reporter line showed that Car1 gene and Cre expression is restricted to short lived differentiated epithelial cells in mid crypt to luminal regions of the proximal colon however LacZ activity was not detected in crypt bottom stem cells. Deletion of either Apc tumor suppressor gene or activation of Kras oncogene using the Car1-Creert2 KI did not yield adenomas. However combined...
mutation of both Apc and Kras yielded microadenomas which progressed to macroadenomas with significant morbidity. Importantly no adenomas were observed in the small intestine. Tumor cells were highly proliferative, poorly differentiated and expressed putative marker genes for both normal and cancer stem cells.

Our results indicate that differentiated colonic epithelial cells require mutations in both Wnt and KRas signalling to transform them into tumor initiating cancer stem cell-like cells and have important implications on our understanding of cell plasticity and cancer.

FRANK TUFARO [Speaker]

Current progress in the clinical development of adenovirus DNX-2401 for malignant glioma

Frank Tufaro, Ph.D., DNAtrix, Inc. Houston, TX

DNX-2401 (Delta-24-RGD) is a conditionally-replicative oncolytic adenovirus in clinical development that has been engineered to be specific for tumor cells with defects in the Rb pathway. Its potency is also enhanced by the addition of an RGD motif decorating the fiber protein that allows the virus to efficiently infect a wide range of tumor cells. More than 75 subjects with recurrent glioblastoma (GBM) have been administered single-dose DNX-2401 at a variety of doses in five clinical studies. Patients at first or second recurrence of a GBM after standard treatment with surgery, radiotherapy and temozolomide undergo biopsy to confirm recurrence. In two study arms, patients with resectable lesions and significant mass effect undergo craniotomy and tumor resection followed by intramural injection of up to 3e10 vp DNX-2401; in patients who cannot undergo a gross total resection of the recurrent tumor, DNX-2401 is injected intratumorally. To date, there is no toxicity associated with the administration of DNX-2401 and it is well tolerated. Perhaps more importantly, antitumor activity and durable responses to therapy have been observed following a single injection of DNX-2401. The latest clinical data from these studies will be presented.

VICTOR W. VAN BEUSECHEM [Speaker]

Functional genomics screening identifies orphan G-protein coupled receptor 27 as radiation susceptibility gene in prostate cancer

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Prostate cancer (PCA) is the most commonly diagnosed cancer and the second leading cause of cancer death in Western world males. Treatment options for patients with local disease include radiotherapy and surgery. However, local tumor control rates after radiotherapy are highly variable. Approximately 40% of treated patients will experience disease recurrence and progression. Advanced prostate cancer has a very poor prognosis, emphasizing the need for more effective early local treatment, preventing advanced disease. Therefore, we set out to identify molecular targets of radiation susceptibility in PCA cells as a starting-point to increase the therapeutic index of radiotherapy.

We conducted genome-wide siRNA library screens with and without irradiation in PC-3 cells, using a cell viability assay based on high-throughput cell counting. We selected 45 candidate target genes exhibiting a strong combined effect of radiation and gene silencing on cell viability for further research. For 17 of these, multiple independent siRNAs induced ≥2-fold more cell death upon 4Gy irradiation. Sixteen confirmed hits were validated independently using the colony formation assay. Importantly, silencing of 8 genes sensitized PC-3 cells to radiation more effectively than did silencing of the known radiation susceptibility gene PRKDC. Seven of these were also validated using stable lentiviral shRNA-mediated knockdown.

Silencing G-protein coupled receptor 27 (GPR27) was particularly effective, resulting in a spectacular decrease in surviving fraction after 2Gy irradiation from 70% in controls to 23% in knockdown cells. GPR27 knockdown also sensitized PC-3 and DU145 PCA cells to a fractionated low dose irradiation scheme. GPR27 knockdown delayed DNA repair and recovery of cell cycle upon irradiation. Expression of a silencing-resistant GPR27-variant gene nullified the radiosensitizing effect of siRNA against GPR27, demonstrating that radioresistance was truly GPR27-dependent.

Our findings warrant testing of GPR27 inhibition to enhance the efficacy of radiotherapy in prostate cancer. This could be achieved by gene silencing as was done here or by employing a small molecule inhibitor. In this respect, it is of note that G-protein coupled receptors are highly druggable. Combined irradiation and GPR27 inhibition could contribute significantly to improving overall success of prostate cancer intervention.
RIENEKE VAN DE VEN [Poster]
Effective induction of anti-melanoma T cells by targeting human DC with a CD80/CD86-targeted fiber-modified Adenovirus-5/3

Dafni Chondronasiou, Anita G.M. Stam, Qiana L. Matthews, David T. Curiel, Tanja D. de Gruijl and Rieneke van de Ven

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In vivo targeted delivery of tumour-associated antigens to DCs, relying on the natural functions of primary DC in situ, represents an attractive vaccination strategy to circumvent ex vivo DC-based vaccine limitations. In this study we made use of a full-length MART-1 expressing C/B-chimeric adenoviral vector (Ad5/3), consisting of the Ad5 capsid and the Ad3 knob, to target DC through CD80 and CD86. We previously showed that Ad5/3 viruses selectively targeted mature myeloid DC from both human skin and skin-draining lymph nodes (LN). Here, we show that in vitro transduction of monocyte-derived DC (MoDC) by Ad5/3-MART-1 enhanced their MART-1-specific CD8+ T cell priming efficiency as compared to Ad5-MART-1. The primed T cells recognized endogenously processed MART-1 epitopes and were cytotoxic to MART-1 expressing melanoma tumor cells. Functional avidity of the CTL primed by Ad5/3 targeted DC was high and similar to those induced by peptide pulsed DC. We also used Ad5/3-MART-1 to target DC within cell suspensions from melanoma-draining sentinel lymph nodes, which resulted in an increased number of expanding and MART-1 recognizing CTL as compared to the use of a wild-type Ad5-MART-1 vector. Collectively, we demonstrate that chimeric Ad5/3 adenoviral vectors encoding tumor antigens could be used to target DC and efficiently induce expansion of functional tumor-specific CTL in vivo. These data support the use of Ad3-knob containing viruses as vaccine vehicle in prime-boost protocols in order to circumvent pre-existing Ad5 immunity. “Off-the-shelf” DC-targeted Ad vaccines encoding tumor-associated antigens could clearly benefit future immunotherapeutic approaches.

ELISABETH VAN ERP [Speaker]
Single domain antibody-targeting allows CRAd to infect and replicate in tumor cells

E.A. van Erp, L.N. Kaliberova, S.A. Kaliberov, E.J. Wiertz, D.T. Curiel

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Conditionally replicative adenoviruses (CRAds) are promising agents for the treatment of tumors. Transcriptional activation of replication and oncolysis by CRAds depends on an incorporated tumor specific promoter (TSP). The specificity of the TSP is the main dictate of tumor selectivity in this CRAd design. Even though incorporation of a TSP enables tumor specific replication, CRAd infection is still mediated by binding of the Ad5 fiber to CAR, a receptor which is lost on most cancer cells. Various approaches have been attempted to retarget Ad5 to tumor specific antigens to circumvent CAR deficiency and to provide an additional level of tumor specificity. In this regard, antibodies are highly specific targeting molecules and will potentially retarget the CRAd if functionally incorporated into the viral capsid. However, conventional antibodies are not compatible with Ad capsid formation. Our group has proven the utility of single variable domains derived from camelid antibodies as an effective way to retarget Ads. These single domain antibodies (sdAbs) are highly stable in the cytoplasmic environment and can be incorporated in a fiber-fibritin-based Ad5 fiber. We have shown high tumor specificity with sdAb-targeted Ads against hCEA and abolished CAR mediated transduction, reducing the off-target effect. In the present study we have combined transcriptional targeting through tumor specific promoter CXCR4, with sdAb-mediated transductional targeting against hCEA. The objective of this study is to show the enhanced antitumor selectivity and therapeutic potential of our sdAb-targeted CRAd. Of note in this regard Ad.CXCR4E1.B2 was highly tumor specific, showed efficient replication in tumor cells and caused subsequent cytotoxicity. To our knowledge this is the first time that infection and replication of an sdAb-targeted CRAd is shown. The double targeting, both transcriptional through the CXCR4 promoter and transductional through the sdAb, is a promising means to improve the therapeutic index for these advanced generation CRAds.

JOHN P. VELUCHAMY [Poster]
A novel combinatorial therapy using cytolytic NK cells and anti-EGFR mAb to improve the treatment of EGFR expressing solid tumours

Veluchamy JP, Spanholtz, Stam AG, Tordoir M, Bohme F
The ability of Natural Killer (NK) cells to kill tumour targets has been explored in various haematological malignancies. However, NK cell therapy directed against solid tumors is still in its infancy. Epidermal growth factor receptor (EGFR) targeted therapies using monoclonal antibodies (mAbs) such as Cetuximab (Cet) and Panitumumab (Pan) are widely used for the treatment of colorectal cancer and head and neck squamous cell carcinoma. The clinical efficacy of this approach has been hampered by several factors, including activating mutations in RAS. Colon cancer subtype 3 (CCS3) comprising a heterogeneous population of KRAS and BRAF mutations are less susceptible to anti-EGFR mAb therapy. It is well established that NK cells can kill tumor cells by natural cytotoxicity and NK cells can also be activated upon binding of anti-EGFR mAbs through Fc receptors (FcR) mediating effective antibody dependent cellular cytotoxicity (ADCC) against the tumor. In the current setting we aim to combine this approach with isolated and activated Peripheral blood NK cells (PBNK) and anti-EGFR mAbs to increase killing of EGFR+ tumours, including those with RAS mutations. In vitro 4h cytotoxicity experiments on CCS3 cell lines, using a low E:T ratio of 1:1, demonstrated that PBNK cells kill RAS wild type (COL320, 22%, n=7), RAS mutant (SW480, 12%, n=9) tumor cells irrespective of their EGFR expression levels. Furthermore, the target cell killing was even higher when combined with Cet for RAS mutant (SW480, 17%, n=9). Cet showed more potent anti-tumour activity compared to Pan on RAS mutant and wild type cells when used alone or in combination with NK cells. Overall our studies provide a clear rationale to further develop the NK cell platform in combination with Cet in patients with EGFR+ solid tumors.

HAN VERHAGEN [Poster]

The potential of IGFBP7 to specifically eradicate leukemic stem cells in acute myeloid leukemia


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In 70-80% of acute myeloid leukemia (AML) patients, treatment with chemotherapy results in complete remission, however, only 30-40% of patients (< 60 years) survive five years after diagnosis. This poor prognosis is mainly due to relapse, caused by the insufficient eradication of a subpopulation of chemotherapy resistant leukemic cells with stem cell-like features, named leukemic stem cells (LSC). LSC co-exist with normal hematopoietic stem cells (HSC) in the AML bone marrow and to improve AML outcome, it will be crucial to develop alternative therapies that specifically target LSC while sparing HSC. To develop these anti-LSC therapies, the identification of genes differentially expressed between LSC, HSC and the AML bulk are of critical importance. The Identification of target genes is most relevant in cell fractions obtained from the same AML bone marrow, taking into account effects of the leukemic microenvironment.

We have identified markers, including CLL-1, CD34 and scatter properties to discriminate LSC from HSC. Using these markers we purified LSC, HSC and progenitors (LP) from AML bone marrows and identified insulin-like growth factor binding-protein-7 (IGFBP7) as differentially expressed between LSC and HSC and between LSC and the AML bulk. IGFBP7 is a potential tumor suppressor and based on our gene expression data we hypothesize that IGFBP7 might eradicate LSC while sparing HSC. To that end, we have generated recombinant human IGFBP7 and showed that both IGFBP7 overexpression and the addition of rhIGFBP7 induce apoptosis and cooperate with chemotherapy to induce AML cell death. Most importantly, IGFBP7 reduces the survival of AML progenitor and stem cells from a number of AML bone marrows and reduces leukemic engraftment in immune-deficient mice. Altogether, our results suggest that AML patients might benefit from a combination of chemotherapy and IGFBP7 and adding IGFBP7 might potentially overcome LSC initiated chemotherapy resistance and thereby improve AML outcome.

CHAE-OK YUN [Speaker]

In situ gel systems as ‘smart’ carriers for sustained delivery of oncolytic adenovirus

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Adenoviruses (Ad) have been investigated for their efficacy in reducing primary tumors after local intratumoral administration. Despite high Ad concentrations and repetitive administration, the therapeutic efficacy of Ad has been limited because of rapid dissemination of the Ad into the surrounding normal tissues and short maintenance of Ad biological activity in vivo. To maximize the therapeutic...
potential of Ad-mediated gene therapeutics, we investigated the efficacy of local, sustained Ad delivery, using an injectable in situ gel matrix system. The biological activity of Ad loaded in gel was prolonged compared with naked Ad, as evidenced by the high green fluorescent protein gene transduction efficiency over an extended time period. Moreover, oncolytic Ad encapsulated in hydrogel elicited 1.9- to 2.4-fold greater antitumor activity than naked Ad in both C33A and U343 human tumor xenograft models. Histological and quantitative PCR analysis confirmed that the oncolytic Ad/hydrogel matrix system significantly increased preferential replication and dissemination of oncolytic Ad in a larger area of tumor tissue in vivo. Taken together, these results show that local sustained delivery of oncolytic Ad in hydrogel augments therapeutic effect through selective infection of tumor cells, sustained release and prolonged maintenance of Ad activity.

ZHENG-MAO ZHANG [Poster]
Reversing drug resistance of cisplatin by Hsp90 inhibitors in human ovarian cancer cells

Zhang Zheng-mao¹, Xie Zhen¹, Li Jia¹, Yang Ping-fang¹, Yang Hong-fang¹, Zhang Chao², Zhang Feng-hua³, Liu Yang¹, Qiu Hong-bing¹, Shan Bao-en²

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Objective: To investigate the mechanisms for reversing drug resistance of cisplatin (DDP) by Hsp90 inhibitors (geldanamycin (GA), 17-AAG, 17-DMAG) in human ovarian cancer.
Methods: Cell proliferation rate in DDP resistant human ovarian cancer cell line SKOV3/DDP and its parent cell line SKOV3 after treatment with Hsp90 inhibitors and/or DDP were tested by MTT assay, and the reversing fold (RF) of DDP by Hsp90 inhibitors was calculated. Cell cycle and cell apoptosis status after treatment were analyzed by flow cytometry. The expression of multiple drug resistance related genes was analyzed by RT-PCR and Western-blot.
Results: All three tested Hsp90 inhibitors synergistically inhibited the cell proliferation of SKOV3 with DDP and enhanced the sensitivity of SKOV3/DDP cells to DDP. The RF of DDP by Hsp90 inhibitors were all more than two fold. GA caused cell cycle arrest in G2/M phase in SKOV3 cells. 17-DMAG and 17-AAG increased cell apoptosis but did not change cell cycle in SKOV3/DDP cells. The mRNA and protein expression levels of various drug resistant related genes including LRP, GST-π, p53, bcl-2, survivin, ERCC1, XRCC1, BRCA1 and BRCA2 were more dramatically altered by Hsp90 inhibitors and DDP in combination compared to Hsp90 inhibitors or DDP treatment alone.
Conclusions: Exposure of SKOV3/DDP cells to Hsp90 inhibitors and DDP in combination results in synergistic cytotoxic and pro-apoptotic effects. Hsp90 inhibitors reverse the drug resistance of SKOV3/DDP cells to DDP by modifying the expression of multiple drug resistance related genes.
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Dr. Curiel is the Director of the Biologic Therapeutics Center, the Biologic Therapy Core Facility and the Cancer Biology Division within the Department of Radiation Oncology at Washington University and the ISCGT’s Conference Organizer.

The focus of Dr. Curiel’s research program is to exploit adenoviral agents for applications to the applied contexts of cancer therapeutics. These efforts embody basic molecular virology to modify adenoviral tropism towards the goal of targeted delivery.

He is a long-standing member of the ISCGT.

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Dr. de Grujil is Professor Translational Tumor Immunology and Head of the Immunotherapy Laboratory at the VU University Medical Center, Dept of Medical Oncology in Amsterdam. She specializes in translational tumor immunology, from preclinical research to immune-monitoring of Phase I-III clinical trials.

She is an active member of both the Dutch Society for Immunology (NVVI), current chair of the Dutch Tumor Immunology Working Party and a member of the International Society for Dendritic Cell and Vaccine Science.

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Dr. van Beusechem is associate professor and head of the Oncogenomics Laboratory of the department of Medical Oncology at Vumc and director of the RIFOL, a VUmc Cancer Center Amsterdam core facility for whole-genome siRNA library screening. He is CSO of ORCA Therapeutics, a biopharmaceutical company focusing on the development of new anti-cancer treatments using oncolytic viruses.

Dr. van Beusechem is board member and chair of the science committee of the Netherlands Society of Gene and Cell Therapy and active member of the ASCGT, the AACR and the RNAi Global Initiative. He serves as member of the Netherlands Medicine Evaluation Board expert group on Advanced Therapy Medicinal Products.

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Thank you! Dank u wel!