cnio "LA CAIXA" FRONTIERS MEETINGS 2018

Madrid 9 — 11 July 2018

Frontiers in Immunomodulation and Cancer Therapy

Organisers

Victoria Aranda
Nature, New York, US

Nabil Djouder
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Joao Monteiro
Nature Medicine, New York, US

Marisol Soengas
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Laurence Zitvogel
Gustave Roussy Institute, Villejuif, France

Speakers

Yasmine Belkaid
The National Institute of Health (NIH), Bethesda, US

Nina Bhardwaj
Icahn School of Medicine at Mount Sinai, New York, US

Marcus W. Bosenberg
Yale Cancer Cente, New Haven, US

Peter Carmeliet
VIB-KU Leuven Center for Cancer Biology, Belgium

Thomas Gajewski
The University of Chicago, US

Carola García de Vinuesa
John Curtin School of Medical Research, The Australian National University, Australia

Nicholas W. Haining
Dana-Farber Cancer Institute & Broad Institute, US

Guido Kroemer
The Cordeliers Research Centre (CRC), Paris, France

Dan R. Littman
The Helen L. and Martin S. Kimmel Center for Stem Cell Biology, NYU Langone, US

Randy Longman
Weill Cornell Medicine, US

Alberto Mantovani
Humanitas Clinical and Research Center, Milan, Italy

Glenn Merlino
Center for Cancer Research, NCI, Bethesda, US

Graham Pawelek
The University of Tübingen, Germany

Mercedes Rincon
University of Vermont Medical Center, Burlington, US

Andrea Schietinger
Memorial Sloan Kettering Cancer Center, NY, US

Ton Schumacher
The Netherlands Cancer Institute (NIK), Amsterdam, Netherlands

Melody Swartz
Institute for Molecular Engineering, University of Chicago, US

Erwin Wagner
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Jennifer Wargo
The University of Texas MD Anderson Cancer Center, Houston, US

Laurence Zitvogel
Gustave Roussy Institute, Villejuif, France

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Frontiers in Immunomodulation and Cancer Therapy

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Frontiers in Immunomodulation and Cancer Therapy

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cnio "LA CAIXA" FRONTIERS MEETINGS 2018

Madrid 9 — 11 July 2018

Frontiers in Immunomodulation and Cancer Therapy

Organisers and speakers

cnio SPANISH NATIONAL CANCER RESEARCH CENTRE

Madrid 9 — 11 July 2018

Frontiers in Immunomodulation and Cancer Therapy

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid, Spain

Chairpersons and organizing committee:

Victoria Aranda
Nature,
New York, US

Nabil Djouder
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Joao Monteiro
Nature Medicine,
New York, US

Marisol Soengas
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Laurence Zitvogel
Gustave Roussy Institute, Villejuif, France

Rationale:

The immune system has the ability to recognize and kill tumor cells. However, tumors may invade the immune system generating an immunosuppressive tumor microenvironment making tumors resistant to immunotherapies. This conference will focus and discuss recent findings on mechanistic insights of immune escape machinery, immunotolerence and immunomodulation. We will address how tumors can escape the immune system creating an immunosuppressive tumor microenvironment and how tumours become resistant to immunothepary. This conference will also present an overview of pre-clinical and emerging clinical advances targeting the immune system for treatment of cancer or cancer-associated disorders.

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Speakers

Yasmine Belkaid
The National Institute of Health (NIH), Bethesda, US

Nina Bhardwaj
Icahn School of Medicine at Mount Sinai, New York, US

Marcus W. Bosenberg
Yale Cancer Cente,
New Haven, US

Peter Carmeliet
VIB-KU Leuven Center for Cancer Biology, Belgium

Thomas Gajewski
The University of Chicago, US

Carola García de Vinuesa
John Curtin School of Medical Research,
The Australian National University, Australia

Nicholas W. Haining
Dana-Farber Cancer Institute & Broad Institute, US

Guido Kroemer
The Cordeliers Research Centre (CRC), Paris, France

Dan R. Littman
The Helen L. and Martin S. Kimmel Center for Stem Cell Biology, NYU Langone, US

Randy Longman
Weill Cornell Medicine, US

Alberto Mantovani
Humanitas Clinical and Research Center, Milan, Italy

Glenn Merlino
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Graham Pawelec
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Andrea Schietinger
Memorial Sloan Kettering Cancer Center, NY, US

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The Netherlands Cancer Institute (NIK), Amsterdam, Netherlands

Melody Swartz
Institute for Molecular Engineering,
University of Chicago, US

Erwin Wagner
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Jennifer Wargo
The University of Texas MD Anderson Cancer Center, Houston, US

Laurence Zitvogel
Gustave Roussy Institute, Villejuif, France

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Frontiers in Immunomodulation and Cancer Therapy

Programme

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Monday July 9th, 2018

13:00 - 14:30 *Registration - welcome coffee for all participants (main hall)*

14:30 - 15:00 Welcome address **Nabil Djouder,**
Spanish National Cancer Research Centre, Madrid, Spain

15:00 - 15:45 **Keynote Lecture** *(Chair: Nabil Djouder)*
Thomas F. Gajewski,
The University of Chicago, US
"Tumor and host factors regulating anti-tumor immunity and immunotherapy efficacy"

15:45 - 19:15 S#1 Tumor-stroma-immune system crosstalk
Chair: Nabil Djouder

This session will present recent work and discuss the cross talk between stroma cells and immune system.

15:45 - 16:15 **Erwin Wagner**
Spanish National Cancer Research Centre, Madrid, Spain
"Immune signalling in Cancer-Associated Cachexia"

16:15 - 16:45 Coffee break (social room)

16:45 - 17:15 **Peter Carmeliet**
VIB-KU Leuven Center for Cancer Biology, Leuven, Belgium
"Angiogenesis revisited: role and (Therapeutic) implications of endothelial metabolism"

17:15 - 17:45 **Andrea Schietinger**
Memorial Sloan Kettering Cancer Center, New York, US
"Molecular programs defining tumor-specific T cell dysfunction"

Monday July 9th, 2018

17:45 - 18:00 **Amaia Lujambio**
Icahn School of Medicine at Mount Sinai, NY, US
"β-catenin activation promotes immune escape and resistance to anti-PD1 therapy in hepatocellular carcinoma"

SHORT TALK

18:00 - 18:15 **Nina Cortese**
Humanitas clinical and research center, Rozzano, Italy
"Exploring the interaction of macrophages and nerves in colorectal cancer"

SHORT TALK

18:15 - 18:45 **Melody Swartz**
Institute for Molecular Engineering, University of Chicago, US
"Exploiting tumor lymphangiogenesis for potentiating immunotherapy"

18:45 - 19:15 **Nicholas Haining**
Dana-Farber Cancer Institute & Broad Institute W. Nicholas Haining, Boston, US
"Genetic screens to discovery regulators of tumor immunity"

19:30 - 21:00 Welcome Cocktail with all the participants (cafeteria's terrace)

Tuesday July 10th, 2018

08:30 - 12:00 S#2 Immunomodulation and immunotolerence 1
Chair: Marisol Soengas

The two following sessions will cover the most recent mechanistic advances on immunomodulation and immunotolerance

08:30 - 09:00 **Dan Littman**
The Helen L. And Martin S. Kimmel Center For Stem Cell Biology;
New York University, US
"Microbiota influence on local and systemic immune responses"

09:00 - 09:30 **Ton Schumacher**
The Netherlands Cancer Institute (NIK), Amsterdam, Netherlands
"T cell recognition of human cancer"

09:30 - 09:45 **Oskar Fernández Capetillo**
SHORT TALK
Spanish National Cancer Research Centre, Madrid, Spain
"Pd-l1^{ATTAC}: An inducible suicidal mouse model of the immune checkpoint"

09:45 - 10:00 **Daniel Meraviglia-Crivelli**
SHORT TALK
Center for Applied Medical Research (CIMA), Pamplona, Spain
"Turning on the thermostat in cold tumors by target inhibition of Nonsense-Mediated mRNA Decay"

10:00 - 11:00 Group picture & Coffee break (CNIO main door – social room)

11:00 - 11:30 **Graham Pawelec**
University of Tübingen (UNITÜ), Germany
"Impact of immunosenescence on immunomodulatory antibody therapy?"

Tuesday July 10th, 2018

11:30 - 12:00 **Laurence Zitvogel**
Gustave Roussy Institute, Villejuif, France
"The Oncomicrobiome reality"

12:00 - 14:00 Lunch (cafeteria)

14:00 - 17:15 S#3 Immunomodulation and immunotolerence 2
Chair: Laurence Zitvogel

14:00 - 14:30 **Guido Kroemer**
The Cordeliers Research Centre (CRC), Paris, France
"Immunotherapy by tyrosne kinase inhibitors"

14:30 - 14:45 SHORT TALK **Daniela Cerezo-Wallis**
Spanish National Cancer Research Centre, Madrid, Spain
"Melanoma-secreted factor MIDKINE drives immune checkpoint blockade resistance and predicts clinical outcome"

14:45 - 15:00 SHORT TALK **María Luisa Del Río-González**
Leon University Hospital, Spain
"PD-LI/PD-l/CD80 immune check-point blockade enhances antigen-specific antitumor immunity against hematopoietic tumor cells"

15:00 - 15:30 **Alberto Mantovani**
Humanitas Clinical and Research Center, Milano, Italy
"The yin-yang of innate immunity, inflammation and cancer"

15:30 - 16:45 Poster session – Snack for all participants (social room)

Wednesday July 11th, 2018

09:30 - 12:15 **S#4 Microbiota, immune system and tumor progression**
Chair: Nina Bhardwaj

This session will present the role of microbiota and inflammation in disease development

09:30 - 10:00 **Carola García de Vinuesa**
John Curtin School of Medical Research, The Australian National University, Canberra, Australia
"Immune regulation in germinal centers"

10:00 - 10:15 SHORT TALK **Almudena Chaves**
Spanish National Cancer Research Centre, Madrid, Spain
"URI loss sensitizes to coeliac disease and intestinal tumor formation"

10:15 - 10:30 SHORT TALK **Jeffrey Hubbell**
University of Chicago, US
"Targeted antibody and cytokine cancer immunotherapies through collagen affinity"

10:30 - 11:15 *Coffee break (social room - certificates and invoices will be available at the reception desk)*

11:15 - 11:45 **Mercedes Rincon**
University of Vermont Medical Center, Burlington, US
"Regulating mitochondrial metabolism and effector function of CD8 cells through MCJ"

11:45 - 12:15 **Randy Longman,**
Weill Cornell Medicine, New York, US
"The gut microbiome in mucosal and systemic inflammatory disease"

12:15 - 14:00 *Lunch (cafeteria)*

Wednesday July 11th, 2018

14:00 - 18:45 **S#5 Targeting immune system: immunotherapy**
Chair: Joao Monteiro

This session will discuss the new therapeutic approaches to the inflammatory system. New immunomodulators in clinical trials will be discussed.

14:00 - 14:30 **Jennifer Wargo**
The University of Texas MD Anderson Cancer Center, Houston, US
"Insights in response to cancer therapy via reverse translation"

14:30 - 15:00 **Marcus Bosenberg**
Yale Cancer Center, New Haven, US
"Using mouse models to determine mechanisms of anti-cancer immune responses"

15:00 - 15:15 **Neibla Priego**
SHORT TALK
Spanish National Cancer Research Centre, Madrid, Spain
"Reactive astrocytes act as a local immunomodulatory hub in brain metastasis"

15:15 - 15:30 **Manuel Alejandro Fernández Rojo**
SHORT TALK
Institute for Advanced Studies (IMDEA) in Food, Madrid, Spain
"Targeting hepatic stellate cells to ameliorate liver inflammation"

15:30 - 16:00 Coffee break (social room)

Wednesday July 11th, 2018

16:00 - 16:30 **Glenn Merlino**
Center for Cancer Research, NCI,
Bethesda, US
"Developing a preclinical platform for the study of immunotherapies in melanoma"

16:30 - 17:00 **Nina Bhardwaj**
Icahn School of Medicine at Mount Sinai, US
"Dendritic cell targeted vaccines"

17:00 - 18:00 Closing Lecture
Yasmine Belkaid
The National Institutes of Health (NIH),
Bethesda, US

18:00 - 18:15 Closing Remarks
Marisol Soengas
Nature, New York, US

18:15 - 18:45 Wrap-up, prizes for best posters and best short talks
Marisol Soengas
Spanish National Cancer Research Centre,
Madrid, Spain

Farewell Snack for all participants (social room)

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Madrid 9 — 11 July 2018

Frontiers in Immunomodulation and Cancer Therapy

Keynote Lecture

Thomas F. Gajewski

Chairperson: ***Nabil Djouder***

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Monday July 9th, 2018

Keynote Lecture

15:00 - 15:45 **Keynote Lecture** *(Chair: Nabil Djouder)*

Thomas F. Gajewski,
The University of Chicago, US
"Tumor and host factors regulating anti-tumor immunity and immunotherapy efficacy

Tumor and host factors regulating anti-tumor immunity and immunotherapy efficacy

Thomas F. Gajewski
The University of Chicago,
Chicago, US

Two major phenotypes of human melanoma metastases have been observed based on gene expression profiling and confirmatory assays. One subgroup of patients has a T cell-inflamed phenotype that includes expression of chemokines, T cell markers, and a type I IFN signature. In contrast, the other major subset lacks this phenotype and appears to display immune "exclusion". The mechanisms of immune escape are likely distinct in these two subsets, and therefore the optimal immunotherapeutic interventions necessary to promote clinical responses may be different. The T cell-inflamed tumor microenvironment subset shows the highest expression of negative regulatory factors, including PD-L1, IDO, and FoxP3+ Tregs. Deep analysis of tumor antigen-specific T cells in the tumor microenvironment has identified additional mechanisms of immune dysfunction, including T cell apoptosis. Treatment strategies targeting several pathways have been translated back into the clinic, with a major effort focused on anti-PD-1/PD-L1 mAbs, and combination therapies are currently a major focus. In contrast to the T cell-inflamed melanomas, non-T cell-inflamed tumors are largely immunotherapy resistant with current approaches. Natural innate immune sensing of tumors appears to occur via the host STING pathway, type I IFN production, and cross-priming of T cells via Batf3-lineage DCs, and these factors are absent in non-T cell-inflamed tumors. New strategies are being developed to engage or mimic this pathway as a therapeutic endeavor, including STING agonists. The molecular mechanisms that mediate the absence of the T cell-inflamed tumor microenvironment in patients are being elucidated using parallel genomics platforms. The first oncogene pathway identified that mediates immune exclusion is the Wnt/β-catenin pathway, which argues that new pharmacologic strategies to target this pathway should be developed to restore immune access to the tumor microenvironment. Recent evidence has indicated that host factors, including the intestinal microbiota, are also critical. We recently have identified commensal bacteria in mouse models that augment spontaneous anti-tumor immunity and increase efficacy of anti-PD-L1 therapy. Similar analyses in human melanoma patients have been performed, and commensal bacteria have similarly been identified that correlate with anti-PD-1 efficacy. These results have prompted the pursuit of new probiotics that may improve spontaneous immune infiltration and expand immunotherapy efficacy in the clinic.

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Session #1

Tumor-stroma-immune system crosstalk

Chairperson: ***Nabil Djouder***

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Immune signalling in Cancer-Associated Cachexia

Erwin F. Wagner
Spanish National Cancer Research Centre,
Madrid, Spain

Erwin F. Wagner[1], Lucía Díez[1], Pia Benedikt[2], Latifa Bakiri[1], Marko Poglitsch[3], Rosa Senaris[4], Michele Petruzzelli[5] and Rudolf Zechner[2]

[1]Cancer Cell Biology Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[2]Institute of Molecular Biosciences, University of Graz, Austria
[3]Attoquant Inc. Wien
[4]CIMUS, Santiago de Compostela, Spain
[5]Department of Oncology, Cambridge, UK.

Cancer-associated cachexia (CAC) is a life threatening wasting syndrome characterized by body weight loss, muscle and white adipose tissue (WAT) atrophy. Limited therapeutic options are available and the underlying molecular mechanisms are poorly defined.

There is ample evidence that during CAC the immune system is altered, although the functional implications are not well understood. Using genetically engineered mouse models (GEMMs) of cancer and transplantation mouse models as well as patient samples, we showed that IL-6 is a key inflammatory mediator involved in a process called "browning", a phenotypic switch from WAT to brown fat (BAT) that occurs at the initial stages of CAC along with lipolysis (Petruzzelli et al., 2014). Browning is associated with increased expression of Uncoupling Protein 1 (UCP1), a protein that diverts mitochondrial respiration from ATP synthesis to thermogenesis/heat production. Treatments reducing inflammation ameliorate CAC symptoms and decrease UCP1 expression and WAT browning. Genetic experiments will be presented dissecting the changes in immune cell composition in pre-cachectic and cachectic GEMMs. Furthermore, the functional role of UCP1 expression in adipose tissue during CAC and the significance of the changes in immune cell composition, such as the neutrophil-lymphocyte ratio (NLR) occurring at early stages of CAC will be discussed.

Inflammation and lipolysis are linked to the sympathetic nervous system and the β-adrenergic system. Glucocorticoids are elevated in sera from cachectic mice and cancer patients, as well as aldosterone. Aldosterone is the final product of the Renin-Angiotensin-Aldosterone System (RAAS), which controls fluid balance, which has been linked to loss of skeletal muscle in CAC, but its involvement in lipid metabolism is unknown. Several RAAS peptides such as Angiotensin II are elevated in sera from cachectic mice and cancer patients. These results indicate that immune and endocrine systems are dysregulated in CAC and experiments dissecting the cross-talk between these systems will shed new light into the mechanisms of CAC.

Angiogenesis revisited: role and (Therapeutic) implications of endothelial metabolism

Peter Carmeliet
Laboratory of Angiogenesis and Vascular Metabolism, VIB-KU Leuven Center for Cancer Biology (CCB), Department of Oncology KU Leuven, Leuven, Belgium

The past 40 years of research in the angiogenesis field have focused on identifying genetic signals such as VEGF and Notch, which determine vessel sprouting. However, the role and therapeutic potential of targeting endothelial cell (EC) metabolism have been largely overlooked. We have recently reported that ECs are glycolysis addicted and that glycolysis importantly co-determine vessel sprouting downstream of VEGF and other pro-angiogenic signals. In addition, we documented that ECs are rather unique in utilizing fatty acidderived carbons for the de novo synthesis of deoxyribonucleotides for DNA synthesis during EC proliferation when vessels sprout. Moreover, targeting (blocking) glycolysis and fatty acid oxidation inhibit pathological angiogenesis and induce tumor vessel normalization (thereby reducing metastasis and improving chemotherapy), suggesting that these metabolic pathways are new targets for anti-angiogenic drug development without evoking systemic side effects. Furthermore, lymphatic ECs differ from other EC subtypes in their metabolic requirements for lymphangiogenesis. Since many of these metabolic targets are pharmacologically druggable, these metabolic pathways represent a new promising target for therapeutic anti-angiogenesis.

References:

B.W. Wong et al. *Nature* 542: 49-54 (2017)
S. Schoors et al. *Nature* 520: 192-97 (2015)
S. Schoors et al. *Cell Metab* 19: 37-48 (2014)
B. Ghesquière et al. *Nature* 511: 167-76 (2014)
K. De Bock et al. *Cell* 154: 651-63 (2013)
K. De Bock et al. *Cell Metab* 18: 634-47 (2013)

COI:No

Molecular programs defining tumor-specific T cell dysfunction

Andrea Schietinger
Memorial Sloan Kettering Cancer Center,
New York, US

Tumor-specific T cells in solid tumors are dysfunctional, allowing tumors to progress. We recently found that tumor-specific T cells differentiated through two discrete chromatin states of dysfunction: a plastic state from which T cells could be functionally rescued, and a fixed dysfunctional state resistant to reprogramming. We identified novel surface markers associated with each chromatin state that demarcated reprogrammable from non-reprogrammable $PD1^{hi}$ dysfunctional T cells within heterogeneous T cell populations in murine tumors. Importantly these surface markers were also expressed on human $PD1^{hi}$ tumor-infiltrating T cells (TIL) and preliminary data now reveal that these biomarkers also predict reprogrammability of human TIL. *In vivo* pharmacologic modulation of transcription factors associated with each chromatin remodeling step improved therapeutic reprogrammability of dysfunctional T cells. Our study has important implications for cancer immunotherapy by defining key transcription factors and epigenetic programs underlying T cell dysfunction and surface markers that predict therapeutic reprogrammability.

β-catenin activation promotes immune escape and resistance to anti-PD1 therapy in hepatocellular carcinoma

SHORT TALK

Marina Ruiz de Galarreta[1,2,3], Pedro Molina[1,2,3], María Casanova-Acebes[1,3], Daniela Sia[2], Verónica Miguela[1,2,3], Carlos Villacorta[2], Maxime Dhainaut[1,3], Barbara Maier[1,3], Hélène Salmon[1,3], Augusto Villanueva[2], Josep M. Llovet[2], Miriam Merad[1,3], **Amaia Lujambio[1,2,3]**

[1]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, USA.
[2]Liver Cancer Program, Division of Liver Diseases, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, USA.
[3]The Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, USA.

Hepatocellular carcinoma (HCC) represents a major health problem, causing more than 700,000 deaths annually. Recently, nivolumab, a PD1 (programmed cell death 1) immune checkpoint inhibitor, was approved for second line HCC treatment after showing unprecedented results in a phase II clinical trial. Unfortunately, not all patients are sensitive, indicating the existence of resistance to anti-PD1 therapy and highlighting the urgent need to identify biomarkers for optimal patient selection. To this end, we created a novel genetically engineered mouse model of HCC that allows interrogating how genetic alterations relevant to human disease affect immune surveillance and response to immunotherapies. The model is based on the hydrodynamic tail vein delivery of genetic elements to overexpress oncogenes (with transposon-based vectors), delete tumor suppressor genes (with CRISPRbased vectors), and modulate immunogenicity (with tumor antigens) specifically in hepatocytes. Expression of tumor antigens in the context of MYC overexpression and loss of p53 (MYC;p53-/-) led to T cell infiltration and immune surveillance, with a decrease in tumor formation and increased survival. In contrast, expression of tumor antigens in the context of MYC overexpression and β-Catenin activation (MYC;CTNNB1) led to immune escape and T cell exclusion, with an increase in tumorigenesis and decreased survival, demonstrating that CTNNB1 activation in HCC cells promotes immune escape. Accordingly, mice harboring MYC;p53-/- tumors responded to anti-PD1 therapy while mice harboring MYC;CTNNB1 tumors did not, indicating that CTNNB1 activation confers resistance to anti-PD1 therapy in HCC. We are currently validating these results in human samples and using our model to identify additional genes involved in immune escape in HCC. Together, β-catenin activation promotes immune escape and resistance to anti-PD1 therapy in our murine model of HCC and could represent a biomarker for HCC patient exclusion.

SHORT TALK

Exploring the interaction of macrophages and nerves in colorectal cancer

Nina Cortese[1], Giovanni F Castino[1], Alessandra Rigamonti[1], Diego Morone[2], Giulia Maggi[1], Federico Colombo[3], Paola Allavena[1], Alberto Mantovani[4,5], Federica Marchesi[1,6]

[1]Department of Immunology and Inflammation, Humanitas Clinical and Research Center, Rozzano, Italy
[2]Institute for Research in Biomedicine, Bellinzona, Switzerland
[3]Flow Cytometry Core, Humanitas Clinical and Research Center, Rozzano, Italy
[4]The William Harvey Research Institute, Queen Mary University of London, UK
[5]Department of Biotechnology and Translational Medicine, University of Milan, Italy

The central nervous system reflexively regulates the inflammatory response, via a direct modulation of immune cells by peripheral nerves. In the context of cancer this phenomenon is still largely unexplored. Macrophages hold a key position in neural-mediated circuits. To define whether a neural control of macrophage functions in tumors exists, we have investigated the macrophage-neural interaction in a preclinical model of colorectal cancer (AOM/DSS). Firstly, we characterized by confocal microscopy the major components (neurons and Enteric Nervous System (ENS)-associated glia) of the neural networks in the gastrointestinal wall, peculiarly organized in each layer. We have visualized the spatial interaction of the complex intestinal neural networks with macrophages through a 3D spatial distribution analysis. F4/80+ macrophages were found in closed proximity to nerve fibers throughout the intestinal wall. We found a closer association of macrophages to nerves in tumor bearing mice (P=0.016). We hypothesized that this could be due to a neural remodelling process, and in fact tumour-bearing mice showed a significantly higher number of Nestin+ cells, possibly suggesting the recruitment of neural progenitor cells. Moreover, macrophages isolated from AOM/DSS treated mice upregulated a neuro-modulatory profile. Overall, these results intimate a modification of neural-macrophage networks in colorectal cancer that could be important in the regulation of macrophage function in tumours.

Exploiting tumor lymphangiogenesis for potentiating immunotherapy

Melody A. Swartz
William B. Ogden Professor, Institute of Molecular Engineering,
University of Chicago, US

Tumor engagement or activation of surrounding lymphatic vessels is well-known to correlate with tumor progression and metastasis in melanoma and many other cancers. We and others have identified several mechanisms by which the lymphatic growth factor VEGF-C and lymphangiogenesis can promote metastasis, including (i) increasing immune suppressive cell types and factors in the tumor microenvironment both directly and indirectly, (ii) inhibiting maturation of antigen-presenting cells and T cell activation, (iii) driving changes in the stromal microenvironment that promote both cancer invasion and immune suppression. However, lymphatic activation also promotes increased antigen transport to the draining lymph node and triggers the initiation of adaptive immune responses against the tumor. Under normal conditions, the potential anti-tumor effects are rendered 'dormant' by the pro-tumor immune suppression, and the tumor progresses. However, we recently showed that lymphangiogenic tumors are exceptionally responsive to immunotherapy, implying that the anti-tumor aspects can be unleashed when the overall balance of pro- and anti-tumor immune aspects is tipped enough towards the latter (e.g., upon tumor cell killing). Here, we address two new aspects of this hypothesis. First, we explore mechanisms to show that 'lymphangiogenic potentiation' depends on tumor cell infiltration of both $CD103^+$ dendritic cells and naïve T cells, driving local T cell education post-immunotherapy and antigen spreading. Second, we have developed a novel lymphangiogenic vaccine to exploit this effect, creating broad immunity against a coimplanted tumor. Together, these studies clarify the yin and yang of tumor lymphangiogenesis in tumor immunity and highlight the exciting translational potential for cancer immunotherapy.

Genetic screens to discovery regulators of tumor immunity

W. Nicholas Haining
Dana-Farber Cancer Institute & Broad Institute W. Nicholas Haining, Boston, US

Despite the recent clinical successes of immunotherapy with checkpoint blockade, most patients with cancer still fail to respond to current immunotherapies, and the emergence of resistance to immune-based treatments is a growing concern. To discover new immunotherapy targets, we developed a pooled, loss-of-function *in vivo* genetic screening approach using CRISPR/Cas9 genome editing in mouse transplantable tumors treated with vaccination and PD-$_1$ checkpoint blockade. We tested 2,400 genes expressed by melanoma cells for those that synergize with or cause resistance to checkpoint blockade, and recovered the known immune evasion molecules, PD-L$_1$ and CD$_{47}$. Loss of function of genes required to sense interferon-γ caused resistance to immunotherapy. Deletion of *Ptpn2*, a pleiotropic protein tyrosine phosphatase, improved response to immunotherapy. Cellular, biochemical, transcriptional, and genetic epistasis experiments demonstrated that loss of function of *Ptpn2* sensitizes tumors to immunotherapy by enhancing interferon-γ-mediated effects on antigen presentation and growth suppression. We also found that that deletion of the RNA-editing enzyme Adar profoundly sensitizes tumors to immunotherapy. Genetic deletion of Adar increased the efficacy of PD-$_1$ checkpoint blockade and resulted in the spontaneous accumulation of activated immune cells in untreated tumors. Mechanistically, Adar loss reduced A-I editing in endogenous immunostimulatory dsRNA species, and initiated a positive feedback loop whereby exposure to interferons resulted in marked interferon release from tumors and tumor cell apoptosis, dependent on MAVS/MDA5 and PKR respectively. *Adar* and *Ptpn2* therefore represent a therapeutic targets that potentiate a broad range of cancer therapies which depend on tumor inflammation. More generally, our results suggest that in vivo genetic screens in tumor models can identify new immunotherapy targets in unanticipated pathways.

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Session #2

Immunomodulation and immunotolerence 1

Chairperson: ***Marisol Soengas***

Microbiota influence on local and systemic immune responses

Dan R. Littman
The Kimmel Center for Biology and Medicine of the Skirball Institute,
New York University School of Medicine, New York, NY;
Howard Hughes Medical Institute, New York, NY, US

A complex web of cellular interactions governs the ability of vertebrates to modulate physiological processes in response to myriad mutualistic microbes while compartmentalizing and tolerating them to prevent invasive growth and harmful inflammatory responses. We study how individual bacterial species induce T cells that participate in autoimmunity, resistance to pathogenic microbes, and anti-tumor responses. We have found that distinct Th17 cell programs contribute to mucosal barrier defense and autoimmune disease pathogenesis, and that effector functions are regulated by endogenous adjuvants, such as the serum amyloid A proteins. Whereas some Th17 cells contribute to protection from potentially invasive enteropathogenic microbes, others can be highly inflammatory, but are normally restrained by commensal bacteria-induced regulatory T cells. Additionally, innate lymphoid cells that receive signals from multiple cell types contribute to barrier defense and to T cell differentiation. Participation of type 3 innate lymphoid cells and their regulation by the enteric nervous system in maintenance of mucosal homeostasis will be discussed. Our studies in mice are not only relevant for human autoimmune diseases, many of which have Th17 cell involvement, but may also provide insights into how commensal microbe-specific T cell responses could be harnessed for mucosal vaccination and cancer immunotherapy.

T cell recognition in human cancer

Ton Schumacher
The Netherlands Cancer Institute (NIK),
Amsterdam, The Netherlands

The central ambition of our work is to determine which factors limit the ability of T cells to control human cancers, with the ultimate aim to overcome these barriers. In work over the past years, we have focused on the role of neo-antigens as a determinant of the activity of T cell based immunotherapies. We are now complementing this work with genetic screens and functional assays of human tumor material to achieve 2 major goals: 1). To determine whether the differentiation state of intratumoral T cells can help predict therapy outcome; 2). To identify novel regulators of the immune checkpoints that control the activity of such intratumoral T cells, and that may be used as therapeutic targets.

With respect to the former question, we have shown how CD8+ T cells that infiltrate human non-small cell lung cancer can acquire novel functional properties, and how this knowledge may be used to predict immunotherapy outcome. With respect to the latter question, through genetic screens of regulators of PD-L1 but also other immune checkpoints, we are identifying novel interaction partners for these molecules that can provide new therapeutic leads.

SHORT TALK

Pd-l1^{ATTAC}: An inducible suicidal mouse model of the immune checkpoint

Oscar Fernandez-Capetillo[1,2]

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Immunotherapy has undoubtedly become one of the most promising therapies for cancer treatment. Specifically, the immunotherapy oriented to silence the interaction between PD-1 and PD-L1 has received particular attention, and this strategy has already been approved for the treatment of several tumors. However, and despite the spectacular effects observed in the patients that do respond to this therapy, there are still key questions that remain unanswered such as: Why do some patients respond and others do not? Why do some tumors show a poor response to this therapy? What would be the consequences of killing PD-L1 expressing cells for normal and tumor tissue? Which cell types express PD-L1, when and why? To address these and other questions we have now developed a mouse model, *Pd-l1*^{ATTAC}, which will allow us to (a) identify PD-L1 expressing cells and (b) kill them selectively. We are now using these mice, and cells derived from them, to perform basic investigations on the immune checkpoint. Our early work in this area will be presented.

Turning on the thermostat in cold tumors by target inhibition of Nonsense-Mediated mRNA Decay

SHORT TALK

Daniel Meraviglia-Crivelli de Caso, Mario M Soldevilla, Javier Cebollero, Helena Villanueva and Fernando Pastor

Centro de Investigación Médica Aplicada, FIMA (Fundation for Applied Medical Research), Pamplona, Spain

Immune-checkpoint blockade immunotherapy has revolutionized cancer treatment in the last few years, but it is still far from optimal, as many patients do not respond to the treatment. One key limiting factor for a broader success of immunecheckpoint reagents, among many others, is tumor antigenicity. Tumor neoantigens can be produced by shift mutations, which often leads to premature termination codons (PTC). The translation of these type mRNAs encoding potent neoantigens can be suppressed by Nonsense-Mediated mRNA Decay (NMD). Thus, NMD inhibition in the tumor can be harvested for cancer immunotherapy. Nevertheless, an important challenge is to inhibit NMD specifically in most disseminated tumor lesions. To that end we require a tumor specific target delivery agent. AS1411 aptamer, which is currently in cancer clinical trials, binds to nucleolin and at high doses induces cell apoptosis. Nucleolin translocates from the nucleus to the membrane and is re-internalized in malignant cells. Thus, we reason that conjugating AS1411 to a SMG1 siRNA could trigger NMD disruption in the tumor busting tumor antigenicity. We have designed an aptamer-siRNA conjugate which is capable of silencing SMG1 in human and mice tumor cells. Our results show that it is able to target murine malignant cell lines and produce a significant decrease in SMG1 mRNA levels in vivo and in vitro pausing NMD function as measured by stabilization of PTC-containing transcripts. Moreover, tumor-bearing mice treated with AS1411-SMG1 conjugate display a tumor growth reduction which also encompasses a significant increase in CD8+ T-cell infiltration. Our data suggests that NMD inhibition can be a promising strategy to successfully enhance tumor antigenicity and therefore induce a more potent immune response against malignant cells. The therapeutic approach will likely turn cold tumors into hot ones paving the way for a broader use of immune-checkpoint blockade treatments in different cancer malignancies.

Impact of immunosenescence on immunomodulatory antibody therapy?

Graham Pawelec
University of Tübingen (UNITÜ),
Germany

Background: Geriatric oncology, important for the ever-increasing numbers of elderly cancer patients, has thus far focused primarily on tolerance to chemotherapy. With the advent of breakthrough immunomodulatory antibody treatments relying on the patient´s own immune system to control the tumor, the issue of immunosenescence becomes extremely important. There is increasingly a valid concern that anti-cancer immunity may be compromised in the elderly due to i) their low amounts of naïve T-cells (leading to holes in the repertoire for neoantigens) and ii) "exhaustion" of potentially tumor-specific memory T-cells. Many other physiological differences in older adults may also contribute to immune dysregulation at advanced age, eg. decreased gut barrier function, altered microbiota, increased basal inflammatory state, etc. Encouragingly, however, but only anecdotally thus far, accumulating clinical experience suggests that advanced age does not result in decreased responsiveness to immunotherapy, or increased side effects thereof. However, the fraction of patients experiencing long-term clinical benefit is generally still low, and broader and more detailed studies focusing on the age question are required.

In our own work, we have established prognostic phenotypic and functional "immune signatures" using peripheral blood from younger melanoma and breast cancer patients, which comprise phenotypic T-cell and myeloid-derived suppressor cell quantification and measurement of pro- and anti-inflammatory CD4+ and CD8+ T-cell responses to shared tumor antigens such as NY-ESO-1 and Her2 in vitro. Models including standard laboratory parameters are being including in the generation of such peripheral immune risk profiles. Thus far, older patients are indistinguishable from younger patients in this respect. Immunosenescence should therefore not be a barrier to anti-tumor immunity in elderly people, at least for responses mediated by T-cells targeting shared tumor antigens. It remains to be established whether responses to tumor neoantigens are compromised by immunosenescence. Given the current emphasis on neoantigen responsiveness, this remains a concern.

The Oncomicrobiome reality

Laurence Zitvogel
INSERM, Gustave Roussy Cancer Center, University Paris Saclay,
Paris, France

We recently highlighted the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer (chemotherapies, immunotherapy based on immune checkpoint blockers).

1/ *Context of cyclophosphamide (CTX)*: Chemotherapeutic agents, by compromising, to some extent, the intestinal integrity, facilitate the gut permeability and selective translocation of Gram positive bacteria in secondary lymphoid organs. There, anti-commensal pathogenic TH17 T cell responses are primed, facilitating the accumulation of TH1 helper T cells in tumor beds post-chemotherapy as well as tumor regression. Importantly, the redox equilibrium of myeloid cells contained in the tumor microenvironment is also influenced by the intestinal microflora, contributing to tumor responses. Hence, the anticancer efficacy of alkylating agents is compromised in germ-free mice or animals treated with antibiotics. These findings represent a paradigm shift in our understanding of the mode of action of many compounds having an impact on the host-microbe mutualism (Viaud S, *Science* 2013). These findings have been extended to platinum salts (oxaliplatine, cis-platine) as well as to a combination of anti-IL-10R mAb+CpG for Iida et al. Science Nov 2013 (Trinchieri's group at the NIH, USA).

2/ *Context of CTLA4 blockade:* The immune checkpoint blocker (ICB) anti-CTLA4 Ab is a first-in class compound approved for reinstating cancer immunosurveillance and prolonging survival in metastatic patients. However, this clinical benefit is often associated with immune –related side effects at sites exposed to commensal flora such as the large intestine. Uncoupling efficacy from toxicity is a challenging issue for the future development of ICB. Her team showed (and submitted to Science) that the antitumor effects of CTLA4 blockade, largely dependent upon Toll like receptor (TLR)2/ TLR4 receptors, markedly rely on the regulatory commensal *Bacteroides fragilis (Bf)* (in coordination with *Burkholderia cenocepacia*). Innate signaling induced by specific TLR2/TLR4 agonists failed to compensate the lack of tumoricidal activity mediated by CTLA4 blockade in germ free (GF) or antibiotics-treated mice while the IL-12-dependent cognate immunity directed against *Bf* could do so.

The Oncomicrobiome reality

Hence, anti-CTLA4 Ab elicited protective Bf-specific Th1 immune responses in specific pathogen free (SPF) mice that could be substituted, in GF animals, by oral Bf, purified *Bf*-associated polysaccharides or a *Bf*-specific adoptive T cell transfer, without triggering overt colitis. Ipilimumab could also restore *Bf*-specific Th1 immune responses in a fraction of advanced melanoma patients. This study unravels the key role of *B.fragilis* in the immunostimulatory effects of anti-CTLA4 Ab, opening up novel strategies to safely broaden its clinical efficacy (Vétizou et al. *Science Nov.* 2015). At the same time, Gajewski's group in Chicago showed that *Bifidobacteria* from the gut influence the tumor microenvironment in such a way that anti-PDL-1 Ab can induce a prominent anticancer immune responses (Sivan et al. *Science Nov*. 2015).

3/ *Setting of PD-1/PDL-1 blockade:* In September 20 2017, the demonstration of the deleterious role of antibiotics in the clinical efficacy of PD-1 blockade in lung, kidney and bladder cancer patients was brought up, highlighting the role of *Akkermansia muciniphila* as the main player in the immunomodulatory effects of pembrolizumab or nivolumab (Routy et al. Science 2017 Nov2). The mechanisms by which *A. muciniphila* restores gut dysbiosis will be discussed, involving CCR9 and IL-12.

From these findings, we infer that oncomicrobiotics and/or fecal microbial transplantation could be considered as adjuvants to the current oncological armamentarium in dysbiotic cancer bearers.

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Session #3

Immunomodulation and immunotolerence 2

Chairperson: ***Laurence Zitvogel***

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Immunotherapy by tyrosine kinase inhibitors

Guido Kroemer
University of Paris Descartes, INSERM U1138, Centre de Recherche des Cordeliers,
Hopital Européen George Pompidou, Gustave Roussy Cancer Campus, Villejuif/Paris, France
The Cordeliers Research Centre (CRC),
Paris, France

Conventional chemotherapeutics and targeted antineoplastic agents have been developed based on the simplistic notion that cancer constitutes a cell-autonomous genetic or epigenetic disease. Nonetheless, it is becoming clear that many of the available anticancer drugs that have collectively saved millions of life-years mediate therapeutic effects by eliciting de novo or reactivating pre-existing tumor-specific immune responses. I will discuss the capacity of anticancer therapies to enhance the immunogenic properties of malignant cells and to stimulate immune effector cells, either directly or by subverting the immunosuppressive circuitries that preclude antitumor immune responses in cancer patients. Accumulating evidence indicates that the therapeutic efficacy of many (if not most) antineoplastic agents relies on their capacity to influence the tumor-host interaction, tipping the balance toward the activation of an immune response specific for malignant cells. One particular way to reinstate immunosurveillance consists in the induction of immunogenic cell death (ICD), which involves elements of organelle-specific, autophagic, apoptotic and necroptotic stress signaling. ICD involves changes in the composition of the cell surface as well as the release of soluble mediators, occurring in a defined temporal sequence. Such signals operate on a series of receptors expressed by dendritic cells to stimulate the presentation of tumor antigens to T cells. We postulate that ICD constitutes a prominent pathway for the activation of the immune system against cancer, which in turn determines the long-term success of anticancer therapies. Hence, suboptimal regimens (failing to induce ICD), selective alterations in cancer cells (preventing the emission of immunogenic signals during ICD), or defects in immune effectors (abolishing the perception of ICD by the immune system) can all contribute to therapeutic failure. I will present evidence showing that a small set of tyrosine kinase inhibitors can induce ICD.

SHORT TALK

Melanoma-secreted factor MIDKINE drives immune checkpoint blockade resistance and predicts clinical outcome

Daniela Cerezo-Wallis[1], Marta Contreras-Alcalde[1], Kevin Troulé Lozano[2], Xavier Catena[1], Paula C. Pennacchi[1], Nuria Ibarz[3], Javier Muñoz Peralta[3], Javier Perales Patón[2], Héctor Tejero Franco[2], Osvaldo Graña Castro[2], Tonantzin G. Calvo[1], María González Cao[4], Sabrina A. Schindler[5], Gonzalo Gómez López[2], Fátima Al-Shahrour[2], Mitchell Levesque[5], David Olmeda[1*] and María S. Soengas[1*].

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Cutaneous melanoma is the most lethal form of skin cancer, characterized by a high metastatic potential and a remarkable ability to evade immune surveillance. Therapies aimed at the deactivation of intrinsic mechanisms of immunosuppression have improved clinical response rates, but about 40-50% of patients still succumb to metastatic disease. Primary resistance to immunotherapy is often observed in patients with either low immunogenic tumors (cold tumors), or with lesions infiltrated with immune suppressive cells, such as tumor-associated macrophages (TAMs), T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Yet, mechanisms that define tumor immunogenicity, and more importantly, biomarkers to predict clinical responses in patients, are still pending needs in the field. We have previously identified a melanoma-secreted protein, called MIDKINE (MDK), with critical roles in lymphangiogenesis and metastasis. We have now identified a new MDK-related transcriptomic gene signature with a high significant correlation to survival in melanoma and other tumor types. This MDK-associated signature was found correlated to increased immune cell infiltration, in particular, of myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs). Mechanistically, we assigned this immunomodulatory function of MDK to a secretory program acting both on tumor cells (via ALK) and on myeloid cells (associated to immunossupressive roles of STAT3). Gain-of-function assays demonstrated that MDK blunts the response to immune checkpoint blockers actively pursued in the clinic. The physiological impact of these results was further strengthened by finding that MDK expression predicts resistance to anti-PD1-based treatment in two independent cohorts of melanoma patients. These results provide insight on long-pursued mechanisms of tumor-immune evasion in melanoma, and uncovered MDK as a tractable biomarker for the response to clinically relevant immunomodulatory agents.

SHORT TALK

PD-L1/PD-1/CD80 immune check-point blockade enhances antigen-specific antitumor immunity against hematopoietic tumor cells

J.I. Rodriguez-Barbosa[1,8], Irene Carnicero Frutos[1], Maria Martin Garcia[1], Maria Carmen Ferreras-Estrada[3], Rosario Hidalgo-Arguello[4], Ana Domínguez-Berrot[2], Leo Buhler[5], Pascal Schneider[6], J.A. Perez-Simon[7] and **Maria-Luisa del Rio-Gonzalez[1,2,8]**

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Since 2013, immune checkpoint blockade and particularly the interaction PDL1/ PD-1/CD80 has created enormous expectations in the field of cancer immunotherapy based on preclinical and clinical effectiveness. Whereas the expression of PD-1 has been largely restricted to lymphocytes, PD-L1 has been broadly detected on a large variety of cells including hematopoietic (infiltrating lymphocytes and myeloid cells) and non-hematopoietic cells (tumor itself and the stromal tumor microenvironment).

To dissect the role of PD-L1 on tumor versus non-tumor cells, PD-L1 expression was targeted using CRISPR/Cas9 approach in a thymoma mouse tumor model that expresses the surrogate tumor-specific antigen chicken albumin (E.G7 transplantable tumor cells). A deletion of 14 bp within PD-L1 exon 3 generated a truncated protein due to a frameshift mutation and the formation of a premature stop codon. PD-L1 WT and PD-L1 deficient E.G7 cells were injected subcutaneously into the right flank of syngeneic C57BL/6 recipient mice. Although PD-L1 WT and PD-L1-deficient tumor cells established tumors after injection, no significant differences were found between the outgrowths of PD-L1 deficient tumor cells compared to their WT counterparts. However, an anti-tumoral protective effect was observed in recipients injected with E.G7 cells after the treatment with anti-PD-L1 (clone MIH-5), a dual blocker of PD-L1/PD-1 and PDL1/CD80 interactions when compared to isotype control-treated mice. We conclude that in this tumor model, the lack of expression of PD-L1 on tumor cells did not significantly inhibit tumor growth, but the blockade of PD-L1 on non-tumor cells contributed to the induction of the protective anti-tumoral immune response.

The yin-yang of innate immunity, inflammation and cancer

Alberto Mantovani
Humanitas Clinical and Research Center; Humanitas University, Rozzano, Milan, Italy

The tumor microenvironment (TME) is a complex network, which includes soluble factors and components of the extracellular matrix as well as stromal, endothelial and immune cells. Immune cells and, among them, myeloid cells, play important roles in cancer development and can promote or inhibit cancer initiation and progression. Among tumor-infiltrating immune cells, macrophages are well-known determinants of cancer-related inflammation and are typically characterized by their remarkable plasticity. This consists in the ability to acquire a wide spectrum of activation states in response to various signals derived from the microenvironment. Classical M1 and alternative M2 macrophages represent the paradigm of this property. Tumor-associated macrophages (TAMs) usually display a so-called "M2-like" phenotype that can foster tumor progression in different ways, namely by promoting genetic instability, angiogenesis and metastasis and by restraining anti-tumor adaptive immunity. Notably, TAMs can also play a dual role in the response to conventional anti-tumor therapies: they can enhance the anti-neoplastic effect or, in contrast, they can sustain a tumor-promoting response and so foil the anti-cancer power of these drugs. We recently identified IL-1R8, which we had cloned as TIR8 and is also known a SIGIRR, as a checkpoint in NK cells, which negatively regulates response to myeloid derived IL-18. Unleashed NK cells mediate resistance to liver carcinogenesis and metastasis at NK rich anatomical sites. Thus the organ immunological context is a key determinant of the role of innate and adaptive immunity in tumor progression.

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Session #4

Microbiota, immune system and tumor progression

Chairperson: ***Nina Bhardwaj***

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Immune regulation in germinal centers

Carola García de Vinuesa
John Curtin School of Medical Research, The Australian National University, Canberra, Australia

URI loss sensitizes to coeliac disease and intestinal tumor formation

SHORT TALK

Almudena Chaves-Pérez[1], Cristian Perna[2] and Nabil Djouder[1]

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Coeliac disease (CeD) is more and more frequent but its pathogenesis and mechanisms remain largely unknown. We show that pathogenic bacterial infection leads to downregulation of the co-chaperone unconventional prefoldin RPB5 interactor (URI) in intestinal tissues. Halving genetically URI expression in murine intestines (URI(+/Δ)Int mice) results in a number of intestinal changes including: villi atrophy, crypt hyperplasia, lymphocytic infiltration of the lamina propria and increased permeability, all recapitulating features of CeD. Consequently, gliadintreated URI(+/Δ)Int mice display increased CD4+ T cells followed by CD8+ cell activation and spontaneous intestinal tumors. Mechanistically, URI loss activates WNT/β catenin axis, causing DNA damage and intestinal atrophy. Genetic inactivation of p53 or APC in intestinal epithelium of adult URI(+/Δ)Int mice accelerates tumorigenesis. We describe here the first genetic mouse model of CeD and provide evidence that inflammatory response is secondary to intestinal morphology changes, uncovering the contribution of pathogenic microbes in CeD development.

SHORT TALK

Targeted antibody and cytokine cancer immunotherapies through collagen affinity

Jun Ishihara, Ako Ishihara1, Koichi Sasaki, Peyman Hosseinchi, Lambert Potin, Kazuto Fukunaga, John-Michael Williford, and **Jeffrey A. Hubbell**

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Immunotherapy with immune checkpoint inhibitors (CPI) and interleukin-2 is frequently accompanied with adverse events. We addressed this by targeting these immunotherapeutic molecules to tumors via conjugation or fusion to a collagenbinding domain (CBD) derived from von Willebrand factor, harnessing the exposure of tumor stroma collagen to blood components due to the leakiness of the tumor vasculature. Intravenously administered CBD protein accumulated mainly in tumors, with lesser exposure in the liver and kidney where endothelia are fenestrated. In melanoma-bearing animals, CBD conjugation or fusion decreased the systemic toxicity of both CPI and IL-2, as measured by cytokine release syndrome, liver and lung histopathology, and pulmonary edema. Both CBD-CPI and CBD-IL-2 significantly suppressed tumor growth compared to their unmodified forms in multiple murine cancer models. Both CBD-CPI and CBD-IL-2 increased tumor-infiltrating CD8+ T cells, enhanced the cytotoxic nature of CD8+ T cell phenotype, and decreased the frequency of Treg cells compared to effector CD8+ T cells. Work is under way with other cytokines, to enhance T cell infiltration into poorly inflamed tumors. Thus, engineered collagen-binding immunotherapies demonstrate translational promise as tumor targeting immunotherapeutics.

Regulating mitochondrial metabolism and effector function of CD8 cells through MCJ

Mercedes Rincon
University of Vermont Medical Center,
Burlington, US

Metabolism is currently considered as a major factor that regulates the function of immune cells and influences the course of an immune response. Control of T cell metabolism is emerging as an alternative strategy to modulate the immune response. Metabolism of CD8 cells is reprogrammed during activation. While glycolysis is important for cell expansion, memory CD8 cells primarily use mitochondrial respiration for the generation of ATP. Thus, in those circumstances where the goal is to therapeutically increase CD8 cell immune response, such as during cancer immunotherapy, promoting mitochondria activity without compromising the glycolytic pathway for expansion could be an ideal approach. We have recently identified MCJ (Methylation-Controlled J protein) as an endogenous negative regulator of Complex I of the electron transport chain (ETC) and mitochondrial respiration in CD8 cells in mice. CD8 cells lacking MCJ have elevated mitochondrial respiration and production of mitochondrial ATP, but normal glycolysis. Increased mitochondrial respiration enhances effector functions of CD8 cells, including both cytokine secretion and cytotoxic activity. Importantly, we have shown that MCJ-deficient CD8 cells have superior protective capacity in vivo. Thus, MCJ could be a therapeutic target to increase CD8 cell responses for cancer-targeted immunotherapy.

The gut microbiome in mucosal and systemic inflammatory disease

Randy Longman
Weill Cornell Medicine,
New York, US

The gut microbiome has emerged as a critical regulator of immune responses in health and disease. Alterations in the gut microbiome correlate with inflammatory and metabolic disease as well as the response to cancer therapy. These findings support the possibility that the gut microbiome can regulate the "immune set point" in mucosal and systemic inflammatory diseases, but our understanding of the mechanisms of this regulation in human disease is limited. To evaluate this in patients with inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), we characterized patients with or without the IBD-associated extra-intestinal manifestation of joint inflammation or spondyloarthritis (SpA). By analyzing the IgA-coated microbiome repertoire in these patients, we identified a selective enrichment in the adherent-invasive E. coli (AIEC) pathotype in patients with Crohn's disease-associated SpA (CD-SpA) compared to CD alone. This lecture will discuss our emerging analysis of patient-derived isolates in gnotobiotic and genetic mouse models to define both the microbial and host factors that link mucosal and systemic immunity. This analysis will provide mechanistic insight into microbial regulation, and potential targets, of anticancer immune responses.

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Session #5

Targeting immune system: immunotherapy

Chairperson: ***Joao Monteiro***

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Insights in response to cancer therapy via reverse translation

Jennifer Wargo
The University of Texas MD Anderson Cancer Center,
Houston, US

Treatment with immunotherapy has transformed cancer care, however durable responses are not universal. It is becoming increasingly apparent that many patients will require combination strategies, however optimal combination strategies are not well delineated despite numerous regimens being tested. It is also clear that multiple factors impact therapeutic response, thus single biomarkers (such as tumor mutational burden and PD-L1 expression) are not sufficient in this age of cancer precision medicine.

Tremendous insights are gained in response to cancer therapy via reverse translation, where findings from patients are taken back to the bench to gain mechanistic insights and therapeutic optimization, and then are translated back to patients. This has been performed in retrospective cohorts and prospectively in clinical trials, elucidating the complex underpinnings of therapeutic response and resistance. Such studies have demonstrated the impact of tumor mutational load and factors within the tumor microenvironment on therapeutic response, as well as factors influencing systemic immunity. In addition to this, the microbiome (particularly the gut microbiome) has been demonstrated to have an impact on therapeutic response. Together, these call for an integrated approach to biomarkers for response to cancer immunotherapy with opportunities to guide optimal precision cancer medicine.

We have utilized such an approach in cohorts of patients with locoregional metastatic melanoma treated on neoadjuvant "window" trials. Through these efforts, we have identified known and novel biomarkers or response, as well as putative targets of therapeutic resistance. These will be discussed herein, as will an integrated approach to biomarkers and therapeutic strategies. We will also comment on strategies to modulate the gut microbiome to enhance therapeutic responses.

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Using mouse models to determine mechanisms of anti-cancer immune responses

Marcus Bosenberg
Yale Cancer Center,
New Haven, US

The success of immune therapies in cancer has underscored the need for accurate pre-clinical models for the evaluation of novel therapies and to determine mechanisms of reponse. We have generated a series of genetically diverse syngeneic melanoma cells lines that form tumors following injection into immune competent C57Bl/6J mice. These models represent an ideal set of tools for the study of cancer immunology and response to immune therapies. By grafting into host mice that lack specific components of the immune system, or by use of blocking/depleting antibodies, one can functionally interrogate host requirements for effective anti-cancer immune responses. Tumor cell autonomous genetic requirements can be evaluated by utilizing genetically distinct models, as has been done to illustrate immunosuppressive roles of tumor Wnt signaling or loss of Pten. Additional genes that influence anti-cancer immune responses can be evaluated using biallelic CRISPR-mediated deletion. We have utilized these approaches to define functional elements of checkpoint inhibitor-induced anti-cancer immune responses.

SHORT TALK

Reactive astrocytes act as a local immunomodulatory hub in brain metastasis

Neibla Priego[1], Lucía Zhu[1], Cátia Monteiro[1], Manon Mulders[1], David Wasilewski[1], Wendy Bindeman[1], Laura Doglio[1], Santiago Ramón y Cajal[2,3], Javier Muñoz[4], Coral Fustero-Torres[5], Elena Pineiro[5], Aurelio Hernández-Laín[6], Riccardo Soffietti[7], Joaquim Bosch-Barrera[8,9,10] and Manuel Valiente[1]

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Brain metastasis remains an unmet clinical need since available therapies have a limited impact on the progression of the disease. Our search for altered signaling pathways in pro-metastatic components of the metastasis-associated microenvironment identified reactive astrocytes (RA) as potential target.

A subpopulation of RA located in the vicinity of metastatic lesions activates STAT3 in various experimental models and in the majority of human brain metastases derived from different primary tumors. Genetic and pharmacologic approaches probed that STAT3+ RA are key for the viability of metastasis, especially at advanced stages of brain colonization. Moreover, an orally bioavailable STAT3 inhibitor improved the outcome of the disease locally in experimental models and in a small cohort of lung adenocarcinoma patients with established brain metastasis.

We dissected the pro-metastatic functions of STAT3+ RA that include modulatory roles on the innate and acquired immune systems. Specifically, STAT3+ RA promote the expansion of pro-tumor CD74+ microglia/macrophages while simultaneously decrease the activity of CD8+ lymphocytes that infiltrate metastatic lesions. Both actions combined, allow cancer cells to adapt the otherwise inhospitable brain microenvironment.

Thus, STAT3+ RA not only have a strong potential to become a novel target to challenge brain metastases, but also are a key component of the regulatory mechanisms governing local immunosuppression.

Targeting hepatic stellate cells to ameliorate liver inflammation

SHORT TALK

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Background and aims. Cellular damage and stress cause tissue specific NF-kB activation and inflammation, hallmarks during the progression of cancer. Conversely, stimulation of the immune system has emerged as a promising strategy against cancer. Hence, the cross-talk between the different players involved in inflammation during tumorogenesis has become a very active field of research. In humans, elevated serum ferritin levels are a marker of hepatic inflammation and chronic liver diseases. Previously, we have described that H-Ferritin (FTH1) stimulates pro-inflammatory signalling cascades and the expression of IL1β in hepatic stellate cells (HSCs) in and iron-independent, PKCδ/NF-kB-dependent manner. Now, we identify the FTH1-signalling receptor in HSC and its impact on inflammasome activation.

Methods. We used one-year old ex-breeder rats (equivalent to a forty-year old humans). FTH1 receptor candidates were identified in primary rat HSCs using unbiased and independent pull-down and ligand-receptor glycocapture/mass spectrometry experiments. Candidates were validated by gain- and loss-of-function experiments combined with disruption of the plasma membrane-endocytic trafficking pathways. The FTH1-induced inflammasome activation was assessed via western-blotting, QRT-PCR and ELISA.

Results. This study identifies ICAM1 as the HSC-specific FTH1-receptor mediating secretion of the pro-inflammatory cytokine IL1β. Accordingly, ICAM-1 depletion prevented FTH1-induced IL1β secretion, while ICAM1-GFP overexpression enhanced IL1β transcript levels. In an ICAM1, dynamin-2 and clatrhin-coated pitendocytosis-dependent manner and using early endosomes as the physical signaling platform, FTH1 stimulated NLRP3-inflammasome in HSC. Finally, we show that FTH1 directly induces IL1β expression in mouse liver tissue.

Conclusion. This study identifies ICAM1 as the FTH1 receptor in HSCs to stimulate NLRP3-inflammasome activation and IL1β secretion and postulate FTH1-ICAM1 interaction.

Developing a preclinical platform for the study of immunotherapies in melanoma

Glenn Merlino
Laboratory of Cancer Biology and Genetics, Center for Cancer Research, NCI, Bethesda, US

Eva Perez-Guijarro[1], Zoe Weaver Ohler[2], Rajaa El Meskini[2], Howard Yang[1], Suman Vodnala[3], Cari Graff-Cherry[4], Sung Chin[4], Corinne Rauck[1], Anyen Fon[1], Terry Van Dyke[2], Shyam Sharan[2], Maxwell Lee[1], and Chi-Ping Day[1], **Glenn Merlino[1]**

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[2]Center for Advanced Preclinical Research and
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Melanoma has the highest mortality of all the skin cancers due to its resistance to therapy. Immune checkpoint blockade (ICB) has led to unprecedented durable responses, but only in a subset of patients and commonly associated with severe toxicities. This warrants the identification of predictive biomarkers to better stratify patients for treatment. Recent studies have implicated mutation/neoantigen load with clinical benefit, but these are insufficient to predict individual patient outcome. The lack of reliable preclinical models for mechanistic study in this field impedes the identification of response determinants. We hypothesized that the heterogeneous nature of melanoma accounts for the variable ICB responses and have aimed to incorporate the pathological and immunological diversity of human melanomas in our preclinical studies. We developed four syngeneic mouse melanoma models in a C57BL/6 background using human-relevant genetic modifications and carcinogenic agents. Pathology and gene expression profiling confirmed a broad spectrum of histological features and differentiation states in the models. Comparison of the mutational profiles with TCGA human data revealed that two models cluster together with BRAF mutant and two with BRAF/NRAS/NF1 (triple) wildtype melanomas, indicating they represent distinct patient subtypes. To define their ability to activate the immune response in vivo (immunogenicity), we vaccinated mice with lethally γ-irradiated cells from each model, prior to implanting the corresponding living melanoma cells from the same model. Tumor onset was delayed in two of the models compared with non-vaccinated control mice. Notably, anti-CTLA-4 ICB demonstrated that immunogenic models were overtly sensitive, whereas non-immunogenic models were resistant, suggesting that immunogenicity dictates response to ICB. We further evaluated influential factors by immunostaining and differential gene expression profiling. T cell infiltration and upregulation of inflammatory pathways did not necessarily predict ICB efficacy. Moreover, neoantigen load and antigen presentation functionality did not significantly correlate with ICB response. Overall, our study highlights the complexity of patient melanoma responses to ICB and underscores the importance of using multiple relevant in vivo immunocompetent preclinical models. Notably, our genetically and phenotypically distinct mouse models recapitulate the broad spectrum of human responses to ICB observed in the clinic today, and offer a valuable new platform for mechanistic studies of melanoma.

Dendritic cell targeted vaccines

Nina Bhardwaj
The Tisch Cancer Institute
Icahn School of Medicine at Mt Sinai
New York US

Despite multiple trials, effective therapeutic cancer vaccines are not yet part of our treatment armamentarium. Only a few vaccines and immune modulators have been FDA approved for to treat cancer and the development of effective cancer vaccines remains a significant challenge. This session will discuss the road blocks that face the field and newer emerging strategies including combination therapies that may change the landscape.

Yasmine Belkaid
The National Institutes of Health (NIH),
US

Madrid 9—11 July 2018

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Speakers' Biographies

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Nina Bhardwaj
Director of Cancer Immunotherapy
Professor of Medicine
Ward-Coleman Chair in Cancer Research
The Tisch Cancer Institute
Icahn School of Medicine at Mt Sinai
New York USA

Dr. Bhardwaj was trained in Internal Medicine at the Brigham and Womens Hospital and in Rheumatology at the Hospital for Special Surgery. Her current clinical involvement is related primarily to the immunotherapy of cancer and also includes HIV infection. Dr. Bhardwaj has undertaken several innovative and groundbreaking studies that have established the role of the immune system to combat cancer. In that capacity she interacts closely with physicians with expertise in Infectious Diseases and Hematology and Oncology to translate basic science discoveries from bench to bedside in these populations.

Marcus Bosenberg
Marcus W. Bosenberg, MD, PhD
Professor of Dermatology and Pathology
Yale Dermatopathology Laboratory for Medicine and Pediatrics (LMP)
Yale University
New Haven, US

Marcus Bosenberg M.D., Ph.D., is Professor of Dermatology, Pathology, and Immunobiology at Yale University. He is Co-Leader of the Genomics, Genetics and Epigenetics Program and Director of the Center for Precision Cancer Modeling at Yale Cancer Center, and Director of the Yale SPORE in Skin Cancer. Dr. Bosenberg studies the genetic and cellular changes that result in melanoma, the leading cause of skin cancer deaths. His laboratory has developed several widely utilized mouse models in order to study how melanoma forms and progresses, to test new melanoma therapies, and determine how the immune system can be stimulated to fight melanoma.

Peter Carmeliet
Laboratory of Angiogenesis and Vascular Metabolism,
VIB-KU Leuven Center for Cancer Biology, Department of Oncology KU Leuven,
Leuven, Belgium

Dr. Peter Carmeliet is head of the Laboratory of Angiogenesis and Vascular Metabolism and former director of the VIB-Vesalius Research Center, now called VIB-KU Leuven Center for Cancer Biology. He graduated as Doctor in Medicine in 1984 at the University of Leuven in Belgium, completed his PhD in 1989, and performed a postdoctoral training at the Whitehead Institute, MIT in Cambridge USA. After his return to Leuven in 1992, Dr. Carmeliet started his own research group with a focus on how blood vessels grow (angiogenesis) in health and disease.

Dr. Carmeliet has published over 650 articles (>73.800 citations, h-index: 127). His achievements have been recognized by several honors and awards.

The Carmeliet lab is currently studying how endothelial cells change their metabolism during vascular branching and exploring the therapeutic potential of targeting endothelial metabolism for anti-angiogenic strategies. The role of several key metabolic targets in endothelial cell biology and angiogenesis in vivo is under investigation.

More info: http://www.vibcancer.be/peter-carmeliet
http://www.vib.be/en/research/scientists/pages/peter-carmeliet-lab.aspx

Nabil Djouder
Growth Factors, Nutrients and Cancer Group, CNIO,
Madrid, Spain

Nabil Djouder, born in France, obtained his PhD in Molecular Pharmacology from the University of Strasbourg (France) and the University of Freiburg (Germany), where he worked in the laboratory of K. Aktories. He studied the molecular mechanisms underlying the activation of mast cells by cross-linking high affinity antigen receptors (Fcε-RI) and the involvement of small GTPases from the Rho family in this activation.

In 2001 he moved to Basel (Switzerland) as a postdoctoral research fellow and joined the laboratory of W. Krek at the Novartis Friedrich Miescher Institute. He has since worked in the field of growth control, cancer, and associated metabolic disorders. Most of his research focuses on the mTOR pathway and the integration of growth factors, nutrients, and energy homeostasis.

In 2003 he moved with W. Krek to the Institute of Cell Biology at the Eidgenössische Technische Hochschule (ETH) in Zurich. He became a member of the Competence Centre for Systems Physiology and Metabolic Diseases (CCSPMD). In September 2009 Nabil Djouder joined the CNIO as a Junior Group Leader, establishing his group in the field of Growth factors, Metabolism and Cancer. Nabil Djouder was recently promoted to CNIO Senior faculty.

Thomas F. Gajewski

Thomas F. Gajewski, M.D., Ph.D.
The University of Chicago,
Chicago, US

The focus of Dr. Gajewski's work is on fundamental aspects of anti-tumor immunity and bringing these concepts forward from the laboratory into clinical trial testing in patients. While working on melanoma vaccine strategies, his laboratory uncovered a role for downstream resistance pathways allowing tumor evasion from the immune response. Gene expression profiling and IHC approaches have identified the T cell-inflamed and non-T cell-inflamed tumor microenvironment phenotypes. T cell-inflamed tumors contain tumor antigen-specific T cells but also negative regulatory pathways that have been moved forward as drug targets, including blockade of PD-1/PD-L1 interactions and IDO. Strategies to promote T cell priming and infiltration into non-T cell-inflamed tumors have led to STING pathway agonist development, currently in phase I clinical testing. Genomic characterization of non-T cell-inflamed tumors has revealed oncogene pathways that mediate T cell exclusion, the first of which is the Wnt/β-catenin pathway. Recent work has also identified germline polymorphisms and evidence for commensal microbiota that also regulate anti-tumor immunity, suggesting additional novel ways to facilitate improved immunotherapy outcomes.

Dr. Gajewski has published more than 220 manuscripts and 20 book chapters in these areas, and has presented data at more than 200 scientific conferences. He is past president of the Society for Immunotherapy of Cancer, is founding editor of the Journal for Immunotherapy of Cancer, is current chair of the Cancer Immunopathology and Immunotherapy grant review study section at NIH, has served on the program committees for ASCO and AACR, and is a grant reviewer for the Melanoma Research Alliance. In 2016 he became the first recipient of the American Cancer Society-Jules L. Plangere Jr. Family Foundation Professorship in Cancer Immunotherapy, and was designated a Distinguished Professor at the University of Chicago. In 2017, he has been named the AbbVie Foundation Professor for Cancer Immunotherapy, and received the William B. Coley Award for contributions to the field of cancer immunology. He has had continuous NIH funding for 20 years, and is scientific co-founder of Jounce Therapeutics.

Carola García de Vinuesa
Co-Director, Centre for Personalised Immunology, NHMRC Centre of Research Excellence
John Curtin School of Medical Research,
The Australian National University,
Canberra, Australia

Professor Carola Vinuesa was born in Spain and obtained a medical degree at the University Autonoma of Madrid. She undertook specialist clinical training in the UK and in 2000 was awarded a PhD by the University of Birmingham. A year later she was the recipient of a Wellcome Trust International Travelling prize Fellowship to do postdoctoral work at The John Curtin School for Medical Research in The Australian National University. Since 2006 she has been a group leader. She has been the recipient of several prestigious awards including the Science Minister's Prize for Life Scientist of the year (2008), the Gottschalk Medal of the Australian Academy of Sciences (2009). In 2015, she was elected as a Fellow of the Australian Academy of Science. She is currently Professor of Immunology at the Australian National University and Director of the Centre for Personalised Immunology (CPI), an NHMRC Centre for Research Excellence.

W. Nicholas Haining

W. Nicholas Haining, B.M., B.Ch.
Associate Professor of Pediatrics, Harvard Medical School
Associate Member, Broad Institute
Departments of Pediatric Oncology &
Cancer Immunology and Virology
Dana-Farber Cancer Institute
Boston, MA, US

W. Nicholas Haining, B.M., B.Ch. is a physician-scientist and pediatric oncologist at Dana-Farber Cancer institute. Dr. Haining received his undergraduate and medical degree from Oxford University, UK, and completed his medical training in Pediatrics at Children's Hospital, Boston, and subsequently in Pediatric Hematology/Oncology at Dana-Farber Cancer Institute. He is an Associate Professor of Pediatrics at Harvard Medical School and an Associate Member of the Broad Institute of Harvard and MIT. The Haining lab focuses on understanding the regulation of T cell exhaustion, and defining the mechanisms by which tumor cells evade the immune system. His work has defined some of the key transcriptional and epigenetic regulators of T cell exhaustion using functional genomics and computational biology. He has used in vivo genetic screens to identify immune vulnerabilities of cancer cells in mouse models, which provide a rich source of new immunotherapeutic targets. The long-term goal of these studies is to identify how to manipulate the immune system to improve cancer immunotherapy.

Guido Kroemer
University of Paris Descartes, INSERM U1138, Centre de Recherche des Cordeliers,
Hopital Européen George Pompidou, Gustave Roussy Cancer Campus, Villejuif/Paris, France
The Cordeliers Research Centre (CRC),
Paris, France

Guido Kroemer has made important contributions to medical research through his groundbreaking work in the fields of cell biology and cancer research. He is best known for the discovery that the permeabilization of mitochondrial membranes constitutes a decisive step in programmed cell death. Kroemer has explored the fine mechanisms of mitochondrial cell death control, the molecular pathways that explain the inhibition of cell death in cancer cells, upstream of or at the level of mitochondria, and the mechanisms that make cancer cell death immunogenic. Kroemer has launched and then proven the hypothesis that the immune response against stressed and dying tumor cells dictates the therapeutic success of anticancer chemotherapy, both in mouse models and in cancer patients.

Dan R. Littman

Dan R. Littman, M.D., Ph.D.
The Kimmel Center for Biology and Medicine of the Skirball Institute,
New York University School of Medicine, New York, NY;
Howard Hughes Medical Institute, New York, NY, US

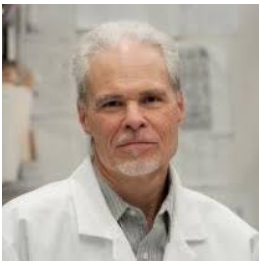
Dr. Littman received his PhD and MD from Washington University in 1980 and was a postdoctoral fellow in Richard Axel's laboratory at Columbia University. He was Professor of Microbiology and Immunology at the University of California, San Francisco, before joining NYU, where he is the Kimmel Professor of Molecular Immunology at the Skirball Institute and an Investigator of the Howard Hughes Medical Institute. Dr. Littman is a member of the U.S. National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts and Sciences, past president of the American Association of Immunologists, and recipient of several scientific awards, including the Ross Prize in Molecular Medicine and the Vilcek Prize in Biomedical Sciences. His laboratory applies molecular and genetics tools to study how T lymphocytes develop and participate in inflammation and how commensal microbiota influence immune homeostasis and pathogenesis.

Randy Longman
Weill Cornell Medicine,
New York, NY, US

Dr. Randy Longman is an Assistant Professor of Medicine at Weill Cornell Medicine and a member of the Jill Roberts Center and Institute for Research in Inflammatory Bowel Disease (IBD). His research focuses on defining the cellular and molecular mechanisms underlying host-microbiota interactions that drive the pathogenesis of mucosal and systemic inflammation in IBD.

Alberto Mantovani
Humanitas Clinical and Research Center,
Rozzano, Milan, Italy

Alberto Mantovani, MD, is Professor of Pathology at the Humanitas University in Milan, and Scientific Director of the Istituto Clinico Humanitas. His attention has been focused on molecular mechanisms of innate immunity and inflammation. He has contributed to the advancement of knowledge in the field of Immunology formulating new paradigms and identifying new molecules and functions. For his research activity he has received several national and international awards, including in 2016 the Triennial OECI Award from the Organization of the European Cancer Institutes and the Robert Koch Award for his contribution to tumor immunology and immunotherapy. The broad impact of his contributions is testified by citations. as of March 2018 he has over 103,000 citations and an H-index of 154 (source: Scopus).



Glenn Merlino

Senior Investigator, Laboratory of Cancer Biology and Genetics Scientific Director for Basic Research, Center for Cancer Research, National Cancer Institute, NIH,
Bethesda, US

Dr. Merlino has contributed through his career to our understanding of receptor tyrosine kinase signaling, oncogenic transformation, transcriptional regulation, cell cycle regulation, multiple drug resistance and genomic instability. Currently, Dr. Merlino is seeking to elucidate the complex molecular/genetic programs governing melanoma genesis and progression through the development and analysis of genetically engineered mouse models. His models are being used to identify the molecular and microenvironmental mechanisms underlying UV induction of melanoma, as well as its metastatic spread. A translational goal is to develop improved preclinical melanoma models to study inherent and acquired resistance to targeted and immune-based therapeutics.

Joao Monteiro

Nature Medicine
Chief Editor
New York, US

Joao received his medical training at the Federal University, in Rio de Janeiro, Brazil, where he also earned a PhD degree, studying mechanisms of Treg function and loss of tolerance to self-antigens in autoimmune diseases. Afterwards he was awarded a postdoctoral fellowship from the Pew charitable trusts and joined the lab of Ron Germain, at the National Institutes of Health, USA, to pursue studies on T cell antigen recognition and in vivo dynamics of the immune responses. He has been serving the scientific community as a professional editor since 2013, when he joined Cell as the primary editor for immunology and translational medicine. Joao joined Nature Medicine as Chief Editor in December 2017. You can follow him on Twitter @immuno_stuff

Graham Pawelec

Graham Pawelec, MA, PhD (Cantab), FGSA,

Second Department of Internal Medicine,
University of Tübingen Center for Medical Research (ZMF),
Tübingen, Germany

Cancer Solutions Program,
Health Sciences North Research Institute,
Sudbury, Canada

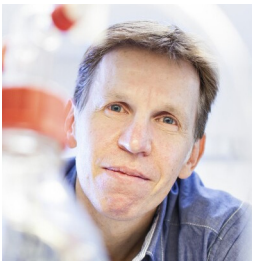
Graham Pawelec received an MA in Natural Sciences in 1978 and a PhD in Transplantation Immunology in 1982 from the University of Cambridge, UK, and the Dr. habil and Venia Legendi from the University of Tübingen, Germany, where he became Professor of Experimental Immunology in 1997. From 1999 to 2017 he led the Tübingen Ageing and Tumour Immunology (TATI) initiative within the Second Department of Internal Medicine, University of Tübingen Hospitals System focussing on immunogerontology in the context of vaccination and cancer immunotherapy. He remains affiliated with the department at the Center for Medical Research, University of Tübingen and is currently also affiliated with the Health Sciences North Research Institute of Canada, Sudbury, ON. His research interests remain centered on vaccination, cancer immunology and immunotherapy, and immunogerontology.

Mercedes Rincon
University of Vermont Medical Center,
Burlington, US

Dr. Mercedes Rincon, Ph.D. is a full Professor in the Department of Medicine, Division of Immunobiology at the University of Vermont. Dr. Rincon received her Ph.D. from the University Autonoma of Madrid, trained as a postdoctoral fellow at Yale University School of Medicine, and joined the University of Vermont in 1996 as an Assistant Professor. She was subsequently promoted to Associate Professor with tenure and full Professor in Medicine in 2009. She has made essential contributions to the understanding roles for the JNK and p38 MAPK pathways in immune responses, including the identification of GSK3β as a novel substrate of p38 MAPK and its involvement in DNA damage responses in T and B cells as well as in the brain. She has worked for over 25 years and has led the development of our understanding of IL-6 as a modulator of CD4 and CD8 T-cell responses and its impact in rheumatoid arthritis, asthma, influenza infection and breast cancer pathogenesis. Her group has recently identified IL-6 as a regulator of mitochondrial Ca^{2+} in CD4 T-cells. For the last ten years she has also focused on a relatively novel molecule MCJ (DnaJC15) as an endogenous brake on Complex I activity and mitochondrial respiration. Pioneering studies from her lab uncovered important roles for MCJ in breast cancer chemoresistance, liver metabolism, and CD8 cell function. With the goal of moving her findings to the clinic, in the recent years Mercedes has broaden her interest and become an entrepreneur herself and promoted biomedical entrepreneurship at the University of Vermont and other institutions. She has established the SPARK program and the I-Trep program at the University of Vermont.

Andrea Schietinger
Memorial Sloan Kettering Cancer Center,
New York, NY, US

Dr. Andrea Schietinger is a basic scientist and tumor immunologist. She received her graduate training at the University of Chicago in the laboratory of Dr. Hans Schreiber where she investigated the genetic creation and biochemical structures of tumor-specific glycopeptidic neoantigens. As a postdoctoral fellow at the University of Washington and the Fred Hutchinson Cancer Research Center (Seattle) in Dr. Phil Greenberg's lab, she studied mechanisms underlying peripheral self-tolerance. Since 2015, Dr. Schietinger is an Assistant Member in the Immunology Program at Memorial Sloan Kettering Cancer Center, and Assistant Professor at Weill Cornell Medical College, New York. Her laboratory studies the molecular and epigenetic programs underlying T cell differentiation and dysfunction in the context of tumors and self-tolerance.



Ton Schumacher

The Netherlands Cancer Institute (NIK),
Amsterdam, Netherlands

Ton Schumacher is Principal Investigator at The Netherlands Cancer Institute, and Professor of Immunotechnology at Leiden University. Research of his lab focuses on the development of novel technologies with which T cell responses can be measured or manipulated, and the subsequent use of these technologies to understand how T cells can recognize and destroy human cancer. Schumacher is recipient of, amongst others, the Queen Wilhelmina Award (2014), San Salvatore Award (2014), Meyenburg Cancer Research Award (2015), and William B. Coley Award (2016). Schumacher is also co-founder of 3 biotechs that focus on the development of novel cancer immunotherapeutics.

Marisol Soengas
Head of the Melanoma Group,
Spanish National Cancer Research Centre (CNIO),
Madrid, Spain

María S. Soengas is the Head of the Melanoma Group and the Dean for Academic Affairs at the *Spanish National Cancer Research Centre* (CNIO) in Madrid. The long-term goal of her team is to translate basic research in melanoma into the clinic by identifying novel tumour markers and drug targets. Her group is particularly interested in mechanisms of cellular stress (involving apoptosis, senescence and lysosomal-mediated degradation) that being selectively deregulated in melanoma, define lineage-specific vulnerabilities for therapeutic intervention (publications in *Science, Cell, Cancer Cell, Nature Cell Biology, Nature Communications*, among others). Her group has also generated first-in-class lymphoreporter mice for non-invasive imaging of pre-metastatic niches in melanoma (*Nature*). Soengas is co-founder of Bioncotech Therapeutics, a *spin off* of the CNIO with a nanoparticle-based agent currently in Phase I clinical trials. Soengas has received numerous fellowships and awards including the *Dana Ashby Young Investigator Award* of the Melanoma Research Foundation, and *the Outstanding Research Investigator Award*. She is also the recipient of the *Elsa Medrano Memorial Award* by the Society for Melanoma Research, which recognizes most influential women scientists in the melanoma field.

Melody Swartz
Institute for Molecular Engineering, University of Chicago,
Chicago, US

Melody A. Swartz is a Professor in the Institute of Molecular Engineering at the University of Chicago, where she holds the William B. Ogden Chair as well as a joint appointment in the Ben May Department for Cancer Research. She holds a BS from the Johns Hopkins University and a PhD from Massachusetts Institute of Technology, both in Chemical Engineering. She undertook postdoctoral studies at Brigham & Women's Hospital in Boston before starting in 1999 as an Assistant Professor at Northwestern University, jointly in the Departments of Biomedical Engineering and Chemical Engineering. In 2003, she was recruited to the Ecole Polytechnique Fédérale de Lausanne (EPFL), where she was promoted to Full Professor in the Institute of Bioengineering and the Swiss Institute for Experimental Cancer Research. Trained as a bioengineer, she uses quantitative approaches in immunobiology and physiology, including biotransport and biomechanics, to develop a deeper understanding of how the lymphatic system regulates immunity in homeostasis and disease, particularly in cancer and chronic inflammation. Her lab applies this knowledge to develop novel immunotherapeutic approaches in cancer, including lymph node-targeting vaccine approaches, as well as in vitro model systems that recapitulate relevant features of the 3D, perfused tumor microenvironment. She is a MacArthur Fellow and member of the American Academy of Arts and Sciences.

Erwin F. Wagner

Erwin F. Wagner, Univ. Prof., Dr. Dipl. Ing.
Director of the Cancer Cell Biology Program
Spanish National Cancer Research Centre (CNIO),
Madrid, Spain

Erwin Wagner obtained his PhD in 1978 for his studies on bacterial genetics in Berlin. He did his postdoctoral training with Beatrice Mintz in Philadelphia (1979-83), became a Group Leader at the EMBL in Heidelberg (1983-88) and from 1988 he was Senior Scientist and Deputy Director at the IMP in Vienna, Austria. Since 2008 he is Vice Director (2008-11) and Director of the Cancer Cell Biology Program at the CNIO in Madrid.

His work focuses on understanding gene functions in mammalian development and disease/cancer/cancer cachexia, employing genetic mouse models for human diseases as well as human patient samples. He has a long-standing focus on defining the complex functions of the AP-1(Fos/Jun) transcription factor complex in inflammation, metabolism and cancer.

Jennifer Wargo
The University of Texas MD Anderson Cancer Center,
Houston, US

Dr. Wargo's career commitment has been to advance the understanding and treatment of disease through science. After completing her medical training and residency, Dr. Wargo started her translational research at Harvard University / Massachusetts General Hospital studying the interface between oncogenic mutations and anti-tumor immunity. At MD Anderson Cancer Center, she is leading research studies in melanoma and other cancer types to understand responses to therapy and to develop novel strategies to combat resistance in genomics and immunotherapy to identify predictive biomarkers of response along with mechanisms of therapeutic resistance that can be targeted (via modulation of the host microbiome).

Laurence Zitvogel
Gustave Roussy Cancer Center,
Villejuif, France

Pr L. Zitvogel, MD (clinical oncology), PhD (tumor immunology), PU-PH Faculty Paris Sud, University Paris XI (Clinical Biology), graduated in Medical Oncology from the School of Medicine of the University of Paris in 1992. She started her scientific career when she was at the University of Pittsburgh in the USA in Michael Lotze's laboratory. She became Research Director at Institut National de la Santé et Recherche Médicale U1015, and Scientific Director of the Immuno-Oncology programmm at Gustave Roussy, the largest cancer Center in Europe. She has been actively contributing to the field of cancer immunology and immunotherapy, and she brought together basic and translational research, including the design of cancer therapies through combined animal studies and Phase I/II patient trials. Her expertise is mainly dendritic cell and innate effector biology and relevance during tumour development as well as exosome-based vaccine designs. She pioneered the concept of immunogenic cell death and showed that chemotherapy, radiotherapy and inhibitors of tyrosine kinase mediate their tumoricidal activity, at least partly through the immune system. Her team discovered the critical role and impact of gut microbiota in cancer immunosurveillance and therapies. She was the recipient of many awards including the National Academy of Medicine, the Translation Research INSERM Prize, the ASCO-SITC, the Brupbacher Awards 2017 and the ESMO Immuno-Oncology Award 2017.

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Poster Session

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Hedgehog regulated immunity in basal cell carcinoma

Sandra Grund, Antal B. Nagy, Richard Weiss, Iris Gratz, **Fritz Aberger**

Department of Biosciences, University of Salzburg, Salzburg, Austria

Genetic activation of Hedgehog (HH)/GLI signaling induces basal cell carcinoma (BCC), a very frequent non-melanoma skin cancer with about 4 million new cases diagnosed per year in the US alone 1,2. Medical therapy of advanced and metastatic BCC involves targeting of the key HH effector Smoothened (SMO) by small molecule SMO-inhibitors. Despite striking therapeutic efficacy, the lack of durable responses and frequent development of drug resistance urgently call for better treatments. Notably, BCC display one of the highest mutation rates of all cancer entities, suggesting that BCC lesions express neoantigens and therefore are immunogenic. Understanding the precise role of HH/GLI signaling in regulating the anti-tumoral immune response will therefore be key to the development of efficient treatments involving a combination of HH/GLI inhibitors and immunotherapy regimens.

Given the high mutational burden of BCC we hypothesize that i) BCC are highly immunogenic and ii) oncogenic HH/GLI signaling drives cancer growth by suppressing the anti-tumoral immune response and also by recruiting inflammatory cells with tumor promoting function. To identify the modulation of the immune system in BCC by the HH/GLI pathway, we generated a BCC mouse model that allows the characterization of the immunological landscape of BCC and the selective depletion of immune cells infiltrating the tumor.

Our results suggest differences in the infiltration of BCC lesions with cells of the adaptive and the innate immune system, paralleled by the differential expression of cytokines and chemokines involved in cell recruitment. The results support rational combination treatments using HH inhibitors together with immune modifying drugs to improve the current limitations of targeted therapy of non-melanoma skin cancer.

Tumor-stroma-immune system crosstalks as viewed in the AA protein-based model for cancer genesis

2

Adouda Adjiri

Department of Physics, Faculty of Sciences, Setif-1 University, Algeria

Tumor formation is a complex process and survival of cancer cells in the primary site as well as in secondary sites during metastases formation, mirrors failures of the immune system in clearing these malignant cells. Tumor stroma that consists basically of nonmalignant cells, including cancer-associated fibroblasts, innate and adaptive immune cells, has been shown to play a crucial role in cancer development. The questions asked here are; how do cancer cells escape immune surveillance? And how stromal cells contribute in creating a supportive microenvironment allowing survival, growth and development of cancer cells; first as single cells then as established tumors?

Based on an in-depth analysis of cancer hallmarks; cancer initiation has been postulated to result from a pathological breakup of a normal protein called here AA protein.

This protein-based model sheds light on all aspects of cancer initiation and development: (i) The breakup of a non-mutated protein could explain the switch a cell's fate from normalcy-to-malignancy; (ii) Following this initial event, DNA mutations begin to accumulate, driving hence cancer progression and metastases formation; (iii) The AA protein breakup is predicted to give rise to two entities A1 and A2, present in cancer cells only, with A2 entity capable of infiltrating cells present in the stoma; (iv) This infiltration could explain how cancer cells hijack stromal cells, using them as a shield, preventing as a consequence immune cells action; (v) A2 entity could also travel and infiltrate normal stromal cells at secondary sites, forming niches for future metastatic cells to land and multiply into new tumors; (vi) Finally, the presence of A2 entity, being self as opposed to nonself, could therefore confuse the immune system that becomes unable to trigger efficient immune action against cancer cells.

This model could open new venues for valuable breakthroughs in cancer research and therapy.

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3

Characterization of molecular mechanisms involved in resistance of melanoma cells to ERK inhibitors

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Melanoma treatment with the BRAF V600E inhibitors (BRAFi) vemurafenib (VMF) or dabrafenib provides therapeutic benefits but the common emergence of drug resistance remains a challenge. Combined treatment with BRAFi and MEK inhibitors such as trametinib (TMT), has improved progression-free survival, but still resistance limits the efficacy of this therapy. Re-activation of the MAPK (RAF/MEK/ERK) pathway is a common resistance response accounting for both monotherapy and combined treatments in melanoma. Targeting ERK itself is a suitable strategy that is currently being investigated and tested in clinical trials in melanoma. In order to anticipate possible resistance responses to ERK inhibitors (ERKi), we have generated BRAF V600E/N-Ras wt (A375 and SK-Mel 28) and BRAF wt/N-Ras Q61R (SK-Mel 103) melanoma cells resistant to SCH772984 (SCH), an Erk1/2 inhibitor. These ERKi-resistant (ER) cells also displayed resistance to VMF, TMT and to combined VMF+SCH and TMT+SCH. Both A375-ER and SK-Mel 28-ER cells exhibited a strong reduction of pErk1/2 and pRSK, whereas SK-Mel 103-ER showed pErk1/2 re-activation. On the other hand, A375-ER and SK-Mel 28-ER cells displayed higher BRAF expression as well as Ras and PI3K-AKT activation than their parental counterparts. However, treatment with SCH and an inhibitor of the PI3K-AKT pathway only modestly reduced resistance. In addition, A375-ER cells showed increased Cdk4 and Cdk6 expression compared to parental cells, but treatment with palbociclib, a Cdk4/Cdk6 inhibitor, or siRNA-mediated Cdk4/Cdk6 silencing did not alter melanoma resistance to SCH. We are currently testing whether other MAP kinases might be involved in the resistance responses of melanoma cells to ERKi.

New and improved methods for characterization of tumour-specific markers in exosomes

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The activation of the immune system mediated by engagement of NKG2D with its ligands is a crucial step in the regulation of both innate and specific immune responses, in particular, in immune recognition of cancer. NKG2D-ligand expression is upregulated when the cells suffer different types of stress, notably tumoral transformation. However, the NKG2D-mediated response can be modulated by the release of these molecules to the extracellular milieu, leading to immune evasion. To generate tools that facilitate investigation of the role of the presence of the NKG2D-ligand MICA in tumour derived-exosomes, we have analysed different methods for the detection and characterization of tumourderived exosomes including newly developed lateral flow devices and bead capturebased flow cytometry tests. Comparison of the different techniques: Western blot, ELISA, flow cytometry and lateral flow, demonstrates that the use of different combination of tetraspanins and tumour markers antibodies can result in very different outcomes when used in the different techniques. In fact, the optimal combinations and concentrations of antibodies for use in each technique had to be optimised separately. These results are the consequence of translating methods originally established for detection of soluble molecules into the detection of vesicles. The implications of these data for the detection of tumour markers in exosomes of biological samples will be discussed.

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Melanoma-driven immune evasion by rewiring dendritic cell differentiation and immunogenicity

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Malignant melanomas are characterized by their inherent potential to metastasize and evade the immune system. Immunotherapy against melanoma has been very successful, but a significant fraction of patients is resistant to these therapies. Understanding how immune tolerant environments are generated and more importantly, how to revert them are pending questions in the melanoma field. Dendritic cells (DCs) are a key immune population to build a proper adaptive response to cancer. However, tumors may impair their function by effecting myelopoiesis or rewiring DCs to a tolerogenic phenotype. Our group has recently identified the heparin-binding factor MIDKINE (MDK) as a pro-lymphangiogenic and pro-metastatic factor. Further analysis of MDK-secretome suggested additional functions in modifying the immune system. In particular, tumors over-expressing MDK have significantly less DCs, as well as, in the draining lymph nodes. Specifically, we found that MDK reduces cDC1 in vivo and in vitro. Conversely, Mdk-deficient mice had higher number of cDC1. Mechanistically, this new function of MDK was linked to transcription factors involved in the development of cDC1. Moreover, we found MDK to affect not only levels, but also functional aspects of DCs, by promoting a tolerogenic phenotype. Together, these data support MIDKINE as a new regulator of immune tolerance, and as such a potential target for therapeutic intervention in melanoma.

Dendritic cell-targeted nanovaccines synergize with immune checkpoint modulators for melanoma therapy

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Immune checkpoint therapy significantly improved the clinical outcome of melanoma treatment. However, results are far from the initially expected. In fact, Programmed cell death protein-1 (PD-1) antibody monotherapy induced effective in 30-40% of advanced melanoma patients. The monoclonal anti-OX40, an immune checkpoint stimulator, member of the tumor necrosis factor receptor family, showed modest monotherapy outcomes in clinical trials. We hypothesized that the immune checkpoint therapy with anti-PD-1/anti-OX-40 - to inhibit tumor immunosuppression and to boost T-cell activity, respectively - could be improved by a cancer nanovaccine, which would increase tumor-associated antigen recognition, processing, and presentation to those T cells. The results enabled the characterization of immune-mediated antitumor responses induced by a multifunctional nano-vaccine combined with anti-PD-1/anti-OX40 in a melanoma mouse model.

Mannose-grafted polymeric nanoparticles (man-NP) were produced as dendritic cell (DC)-targeted nanovaccines to deliver melanoma MART-1 peptide antigens, and the the toll-like receptor ligands CpG and MPLA. Man-NP were spherical-shaped, with an average diameter of 170 nm, narrow polydispersity index, surface charge close to neutrality, and EE > 75%. Man-NP triggered secretion of inflammatory cytokines and induced cytotoxic T-cell activity against melanoma cells in vivo. The combination of man-NP with anti-PD-1/anti-OX40 induced maximal tumor inhibition, leading to 100% of survival 42 days after of tumor inoculation, against 20% obtained for anti-PD-1/anti-OX-40 treatment. In the combination group, 50% of the animals were still alive two months after tumor inoculation, with high percentage of infiltrating lymphocytes within the tumor.

The synergistic combination of nanovaccines with anti-PD-1/antiOX40 provide essential insights to devise alternative combination regimens to improve the efficacy of immune checkpoint.

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Melanoma-secreted exosomes induce immunotolerance and metastasis by an NGFR-dependent signaling

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Metastasis is responsible of 90% of all cancer deaths. Lymph nodes adjacent to the primary tumor are often the first site of metastases. It has been demonstrated that tumor cells induce early changes in the architecture and physiology of tumor draining lymph nodes, even before colonizing the lymph node in a process known as pre-metastatic niche formation. Melanoma-secreted exosomes have been shown to home to lymph nodes supporting metastatic progression although the mechanism is unknown. In this work, we demonstrate that lymphatic endothelial cells (LECs) and subcapsular and medullary macrophages are the main cell types incorporating exosomes in the lymph node. Exosome exposure induces upregulation of lymphangiogenesis-related genes in LECs and lymphangiogenesis *in vivo*. Proteomic profiling identified the neurotrophin receptor NGFR as highly expressed in metastatic melanoma exosomes versus non-metastatic and melanocyte-derived exosomes. NGFR Blocking in tumor and secreted exosomes led to a decrease of melanoma metastasis, lymphangiogenesis and increased DC and T cell recruitment to the lymph node. Furthermore, pharmacological targeting of NGF receptor efficiently decreases LN and lung metastasis in immunocompetent mice. In summary, our results points to NGFR as a relevant mediator of premetastatic niche formation and a promising target for the development of therapeutic approaches in metastatic melanoma.

Shared immune-related genetic susceptibility among atopic conditions, bladder and pancreatic cancers

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The history of asthma and allergies has been associated with a reduced risk of cancers such as pancreas, bladder, among others1,2. A common genetic background has been reported for asthma, allergies, and autoimmune diseases3. Through a bioinformatics approach, we identified a shared genetic background between these conditions and pancreatic cancer4. Using information from large case-control studies we explored the potential genetic link underlying the negative correlation between atopy and pancreatic and bladder cancers. A total of 102,919 genetic variants located in atopy relevant loci previously associated with adult asthma or rhinitis in GWAS at a p.value <=0.05x10-5 were analyzed in 1317 pancreatic cancer cases, 1432 bladder cancer cases and 1795 controls. Adjusted multinomial logistic regression models showed that 143 genetic variants were significantly associated (p.value <0.05) with both cancers in the same direction, being 129 SNPs and 14 indels. Eleven variants were located in intergenic regions, while most variants (N=112) were distributed in introns along 20 genes. Among those, tumor suppressor genes CSMD1 and WWOX were observed containing 43 and 27 significant genetic variants, respectively, pointing to potential mechanisms behind the commonly observed associations between atopy and cancer. A better understanding of cancer-immune associated protective genetic mechanisms has the potential to open new venues into the prevention and treatment of these complex phenotypes.

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New bacterial genotoxin produced in human intestine

Maksym Kitsera

Abstract removed until new update

Serum levels of methylarginines in patients with breast cancer

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Intorduction: L-Arginine Nitric Oxide pathway is the biologic process through which Nitric Oxide (NO) is synthesized from L-Arginine by the enzyme Nitric Oxide Synthase (NOS). Disorders in this pathway had been associated with many diseases, lately including certain cancers. The aim of this study was to investigate the affects of the metabolites and the inhibitors of this pathway on the etiopathogenesis of breast cancer.

Method: 27 early stage breast cancer patients and 27 healthy controls were selected fort his study. Blood samples were taken preoperatively and postoperatively from cancer patients. Asymmetric and symmetric dimethylarginine (ADMA and SDMA, respectively), NG monomethyl-L-arginine (L-NMMA), L-Arginine and L-Citrulline levels were measured with Liquid chromatographytandem mass spectrometry. SDMA/ADMA and Arginie/ADMA ratios are calculated. Preoperative values were compared against healthy controls and postoperative values.

Results: Both ADMA and SDMA values were higher in preoperative cancer patients compared to healthy controls ($p<0.001$ and $p=0.002$ respectively), however SDMA/ADMA ratios were similar in both groups ($p=0.679$). Other parameters were similar in both groups. ADMA values are significantly lower in postoperative period compared to preoperative period ($p<0.01$), however SDMA values are similar ($p=0.829$). SDMA/ADMA ratio is higher in postoperative period ($p=0.047$).

Conclusion: ADMA and SDMA are potentially useful diagnostic biomarkers for breast cancer diagnosis. ADMA and SDMA/ADMA ratio can be useful biomarkers for breast cancer follow-up.

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PD-1/PD-L1 expression and tumor-infiltrating lymphocytes are prognostically favorable in high-grade serous ovarian carcinoma.

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Objective. The prognostic value of PD-1 and PD-L1 in high-grade serous ovarian carcinoma has been studied with ambiguous results. In this study, we map the presence of tumor-associated macrophages (TAMs), tumor infiltrating lymphocytes (TILs) and the expression of PD-L1 and PD-1 and their impact on prognosis in a cohort of 141 women diagnosed with high-grade serous carcinoma of the ovary, fallopian tube and peritoneum. Methods. Immunohistochemical stainings for PD-L1, CD68, CD3 and PD-1 were performed on consecutive TMA sections and their expression was assessed in relation to survival. The effects of the genes encoding PD-L1 (CD274) and CD3 (CD3G) on prognosis were studied in an independent data set, as validation. Results. PD-L1 was expressed mainly by TAMs. PD-L1 expression in TAMs/TILs (but not in tumor cells), PD-1 expression in TILs and CD3 TIL expression were favorable prognostic factors in relation to survival. Further, we identified a group of patients with co-expression of PD-L1 in TAMs and PD-1 in TILs with significantly better prognosis, even after adjusting for age at diagnosis, stage and residual tumor after primary surgery (P=0.008 for PFS and P=0.044 for OS). In the external data set high expression of PD274 (encoding PDL1) was associated with improved 5- and 10-year OS among high-grade serous cancers (P= 0.027 and P= 0.004, respectively). Conclusions. We propose that coexpression of PD-L1 in TAMs and PD-1 in TILs signals an active PD-1/PD-L1/PD-L2 pathway and may provide useful information when selecting patients for PD-1/PDL1 blockade therapy.

ICOS costimulation with targeted agonist aptamer at the tumor site enhances CTLA-4 blockade therapy

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Inducible T-cell costimulator (ICOS) is an immunecheckpoint receptor induced on the surface of preactivated CD4 and CD8 T lymphocytes, but also on Tregs, opening the debate whether ICOS costimulation could be protumorigenic or antitumorigenic. In the last few years ICOS signaling has gained scope as a possible potential marker to predict response to CTLA4 blockade therapy as well as to enhance its therapeutic effect. It has been reported that CTLA4 antibody can deplete Tregs in the tumor leaving the action of ICOS costimulation only on effector T cells. There is no direct accessibility to ICOS agonist monoclonal antibodies leading us to develop alternative aptamer-based ICOS agonist molecules. Aptamers are single-stranded oligonucleotide ligands that can be engineered to act as multivalent "chemical antibodies." Herein we describe that intratumoral injection of an ICOS agonist (IApt8) potentiates anti-CTLA-4 therapy in various tumor models. *In vitro*, experiments with IApt8 resulted in significant activation of CD4 and CD8 T cells measured by proliferation and INF-γ production. *In vivo*, the combination of CTLA-4 blockade with IApt8 induced a 90% of tumor rejection in the murine Hepatocarcinoma model (Hepa 129). In a more challenging tumor melanoma model (B16/F10) the combination leads to significant reduction in the tumor growth. Using this approach we have detected a potent induction of an adaptive immune response against tumor antigens. Moreover, with the aim to treat unreachable disseminated tumor lesions we generated a bi-specific aptamer consisting on the agonistic IApt8 and MRP1 aptamer to target ICOS costimulation to the tumor site. In this scenario CTLA-4 antibody and ICOS-MRP1 bi-specific aptamer can be injected systemically reaching the tumor. B16-MRP1-expressing melanoma bearing mice treated with the combo displayed a significantly reduction in tumor growth, with remarkable infiltration of activated CD8 lymphocytes in the tumor.

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Development of tertiary lymphoid structures in cancer

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Lymphoid aggregates can develop upon infiltration of lymphocytes into tumours. Some of such aggregates form an architecture that resembles that of secondary lymphoid organ (SLO) follicles. These are referred to as tertiary lymphoid structures (TLS). TLS have been shown to support adaptive immunity and, hence, could be beneficial in cancers by driving local anti-tumour immunity. This hypothesis is supported by the observation that cancer patients with high density of tumour-associated TLS have a better prognosis, particularly when TLS contain germinal centres. However, it is not yet known what factors are involved in development of cancer-associated TLS.

In order to generate a mouse model of tumour-associated TLS, Lewis lung carcinoma (LLC) cells, which are inherently not able to promote development of TLS *in vivo*, were genetically modified to overproduce the chemokine CXCL13 or lymphotoxins alpha and beta (LTα and LTβ, respectively); these molecules have established roles in SLO formation. Subcutaneous LLC-CXCL13 or LLC-LTαβ tumours did not develop TLS and grew similarly as control LLC tumours. In contrast, intravenously injected LLC-CXCL13 or LLC-LTαβ cells resulted in rare, poorly defined aggregates of lymphocytes in the metastatic lung that, however, did not contain germinal centres. In future experiments, we will manipulate lung adenocarcinoma cells (KrasG12D; P53 -/-), which inherently support B cell aggregate formation, to overexpress CXCL13 or LTαβ. We expect that these cell lines are more permissive to TLS development compared to LLC.

We also showed that in a model of alum and ovalbumin-induced TLS development in the lungs, TLS formation follows a stepwise process from aggregation of B cells to a more mature phenotype of follicular dendritic cell-positive B cell aggregates. A CXCL13 reporter mouse strain will next be exploited using both the cell lines and the alum model to better elucidate the important cell types in the stepwise process of cancer-associated.

Potential markers of response and resistance to programmed cell death-1 blockade in patients with advanced urothelial cancer

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Background: Immune checkpoint inhibitors have demonstrated clinical benefit in advanced urothelial cancer (UC) patients. However, there are no biomarkers to identify patients that will benefit from this therapy. We studied a small series of UC patients treated with first-line PD-1 checkpoint inhibition in order to analyze their characteristics and patterns of response or resistance to therapy.

Patients and Methods: Eleven UC samples were obtained from patients before undergoing therapy with a programmed-cell death type 1 (PD-1) inhibitor. Patients were classified according to their benefit from therapy in responders (N=5) and non-responders (N=6). Genomic and immunohistochemistry analyses were performed.

Results: Both luminal and basal UC subtypes showed benefit from anti-PD-1 therapy. Tumors from non-responders showed increased mutations in chromatin remodelling genes and the amplification of 3q26-28 region. Transcriptome analyses showed that tumors from responders displayed a significant enrichment of genes associated with interferon γ and α response, TNFα via NFκB, genes upregulated by MYC or E2F, genes involved in G2/M checkpoint and epithelial-mesenchymal transition compared to non-responders. Specific immune cell subsets were present in the tumor microenvironment of tumors from responders and non-responders. Immunohistochemistry showed that none of the immune cell markers analyzed individually was sufficient to discriminate between responders and non-responders. However, the increase in FOXP-3, PD-L1, PD-1, CD8, β2microglobulin and CD68 and the decrease in CD4 and CD163 cells identified UC patients that responded to anti-PD-1 therapy.

Conclusions: Our findings confirm that the evaluation of pre-treatment UC tumor samples provides valuable information that could influence treatment decisions.

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An inducible suicidal allele of Pd-l1 for investigating the immune checkpoint

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The recent success of immunotherapy strategies based on antibodies that block immune checkpoint responses has revitalized the interest in this field. Among them, antibodies that block the interaction between programmed death receptor 1 (PD-1) and its ligand PD-L1 have shown particularly good efficacy in clinical trials and are already approved for the treatment of several tumors including advanced melanoma, lung cancers or Hodgkin's lymphoma. In contrast, some tumors such as pancreatic cancers have shown a poor response to this strategy. While the effects in patients that respond to the therapy have been impressive, there are still important and unanswered questions in this field. Why is the response limited to a subset of the patients? What are the mechanisms that drive PD-L1 expression in tumors and normal tissue? What are the consequences of killing PD-L1 expressing cells for tumors and healthy tissues? To address these and other questions, we have developed an inducible suicidal mouse strain of PD-L1 expression. We are currently using this mouse strain, and cell lines established from it, to investigate the mechanisms that drive PD-L1 expression through genomewide CRISPR-Cas9 screens. In addition, we want to explore the effects of eliminating PD-L1 expressing cells in cancer and normal tissues.

The role of S100A8/A9 proteins in mediating tumour-stroma crosstalk in pancreatic cancer

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Introduction: The secretion of soluble factors enables communication between tumour cells and the surrounding microenvironment and plays an important role in carcinogenesis. S100A8 and S100A9 are calcium-binding proteins expressed in stroma-associated monocytes. Both proteins are known to increase cancer cell proliferation and migration in pancreatic cancer and to contribute to the formation of a pre-metastatic niche at distant sites in lung cancer. The aim of this study was to examine how S100A8/A9 proteins mediate tumour-stroma crosstalk in pancreatic cancer.

Methods: Cytokine profiling of pancreatic cancer cell-derived conditioned media was performed using Bio-Plex Pro 27 Plex Human Cytokine assays. Protein expression and activation of downstream signaling effectors and NF-κB were assessed by western blotting analysis and reporter assays respectively.

Results: Stimulation of cultured pancreatic cancer cells with S100A8 and S100A9 increased the secretion of the pro-inflammatory cytokines IL-8, TNF-α, and FGF. Conversely, pancreatic cancer cell-derived conditioned media and the individual cytokines, TNF-α and TGF-b induced the expression of S100A8 and S100A9 proteins in the HL-60 monocytic cell line and primary human monocytes. S100A8 and S100A9 activated MAPK and NF-κB signaling through activation of the receptor of advanced glycosylation end-product (RAGE).

Conclusion: S100A8 and S100A9 proteins promote specific cytokine secretion from pancreatic cancer cells. Interestingly, a number of these cytokines, in turn, induce the secretion of S100A8 and S100A9 from monocytic cells. These events may create a favorable environment for tumour development and metastases.

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Immunomodulatory and therapeutic properties of dsRNA nanoplexes identified by live imaging of pre-metastatic niches in MetAlert mice

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Melanoma is a paradigm of cancers with a high potential for colonization of lymph nodes, a process usually preceded by neo-lymphangiogenesis. However, removal of sentinel lymph nodes does not necessarily increase patient survival. Therefore, a key pending question in the field is the specific contribution of tumor-induced lymphangiogenesis to immune-evasion and the generation of visceral metastases. Moreover, anticancer agents able to reprogram the immune system against the tumor, blunt melanoma-associated lymphangiogenesis and metastasis in a sustained manner and without secondary effects to the normal vasculature have yet to be identified. We have generated melanoma "Met Alert" mouse models that allow for whole-body imaging of lymphovascular pre-metastatic niches *in vivo*.

Pharmacological studies in these mice identified synthetic long dsRNA nanoplexes as potent inhibitors of melanoma metastasis. Mechanistically, the therapeutic effect of these dsRNA particles was found associated to the re-programing of the immune system towards an anti-tumoral phenotype and the repression of lymphovascular pre-metastatic niches. Anti-tumoral effects included the transcriptional blockade of MIDKINE and the lymphangiogenic factor VEGFR3, features we demonstrated driven by type I interferon responses, and the induction of immunogenic cell death. Ultimately, these compounds inhibited melanoma progression and metastatic relapse after surgery. Importantly, dsRNA nanoplexes showed synergy with immunotherapy treatments (PD-L1). These results are particularly relevant since, derivatives of these dsRNA nanoplexes are now being tested in phase I clinical trials.

Molecular recalibration of PD-1+ antigen-specific T cells as immunotherapy for Hepatocellular carcinoma

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Introduction: Checkpoint inhibitors and adoptive cell therapy provide promising options for treating solid cancers such as hepatitis B-related hepatocellular carcinoma but have limitations. We tested the potential to combine advantages of each approach, genetically re-programming T cells specific for viral/tumour antigens to overcome exhaustion by down-modulating the co-inhibitory receptor PD-1.

Methods: We developed a novel lentiviral transduction protocol to achieve preferential targeting of endogenous low-frequency or TCR-redirected antigenspecific CD8 T cells for shRNA knockdown of PD-1 and tested functional consequences for anti-tumour immunity in 2D and 3D cultures.

Results: Antigen-specific CD8 T cells transduced with LV-shPD-1 consistently had a marked reduction in PD-1 compared to those transduced with a control lentiviral vector. PD-1 could also be down-modulated on liver-resident or T cell receptor (TCR)-redirected T cells. PD-1 knockdown of human T cells rescued anti-tumour effector function and promoted killing of hepatoma cells in a 3D microdevice recapitulating the pro-inflammatory PD-L1hi liver microenvironment. However, upon repetitive stimulation, PD-1 knockdown drove T cell senescence and induction of other co-inhibitory pathways.

Conclusion: We provide proof-of-principle that T cells with endogenous or genetically engineered specificity for HCC viral antigens can be targeted for functional genetic editing. We show that PD-1 knockdown enhances immediate tumour killing but is limited by compensatory engagement of alternative coinhibitory and senescence programmes upon repetitive stimulation.

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E. coli synthetic adhesins targeting human tumor cells toward bacterial immunotherapies

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Anaerobic bacteria, such as Escherichia coli, can colonize and grow preferentially in solid tumors (1). This tumor colonization, along with further engineering of the bacteria, may enhance the immune response to favor the elimination of tumors. In order to improve the tumor colonization and tumor specificity of E. coli, we developed synthetic adhesins (SAs) that can be expressed constitutively on their bacterial surface, allowing E. coli to adhere specifically to antigens expressed on the surface of tumor cells (2). SAs are based on single domain antibodies (VHH or nanobodies) fused to the outer membrane-anchoring domain of Intimin. In previous studies we showed that engineered E. coli bacteria expressing SAs targeting GFP as a model antigen adhered specifically to HeLa cells expressing GFP on the plasma membrane and colonize more efficiently solid tumors generated by HeLa-GFP tumor cells implanted subcutaneously in mice (2).

In this work, we report the generation of engineered E. coli strains expressing SAs targeting actual tumor-associated cell surface antigens expressed in human tumors, such as the human epidermal growth factor receptors EGFR (ErbB1) and HER2 (ErbB2). These membrane proteins are upregulated in many human cancers, including melanomas, breast, colon and bladder carcinomas. We demonstrate surface display of these SAs from single-copy chromosomal expression and specific adhesion of the engineered E. coli strains to human tumor cell lines of different origins. These results set the basis for the preclinical evaluation *in vivo* of engineered bacteria expressing SAs against different human tumors.

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Intratumoral immunotherapy with XCL1 and sFlt3L encoded in recombinant Semliki Forest Virus-derived vectors to foster dendritic cell-mediated T-cell crosspriming

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Multiple lines of evidence indicate a crucial role for antigen cross-presentation by conventional BATF3-dependent type 1 classical dendritic cells (cDC1s) in CD8- mediated antitumor immunity. Flt3L and XCL1 constitute, respectively, a key growth/differentiation factor and a potent and specific chemoattractant for cDC1s. To exploit their antitumor functions in local immunotherapy, Semliki Forest Virus (SFV)-based vectors encoding XCL1 and soluble Flt3L (sFlt3L) were prepared. These vectors readily conferred transgene expression to tumor cells in culture and when engrafted as subcutaneous mouse tumor models. In syngeneic mice, intratumoral injection of SFV-XCL1-sFlt3L (SFV-XF) delayed progression of MC38- and B16-derived tumors. Therapeutic activity was observed but did not exert additive or synergistic effects in combination with anti-PD-1 or anti-CD137 immunostimulatory monoclonal antibodies. Therapeutic effects were abolished by CD8β T-cell depletion and, notably, were markedly enhanced by CD4 T-cell depletion but not by Treg pre-depletion with anti-CD25 mAb. Antitumor effects were abolished in BATF3- and IFNAR-deficient mice. In B16-OVA tumors, SFV-XF increased the number of infiltrating CD8 T cells recognizing OVA. A clear increase of both resident and migratory BATF3-dependent DCs was found in tumor-draining lymph nodes following intratumoral treatment courses but not in the tumor microenvironment. In conclusion, viral gene transfer of sFlt3L and XCL1 is feasible, safe and biologically active in mice, exerting antitumor effects that are potentiated by CD4 T-cell depletion.

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Role of PD-L1/PD-1 immune checkpoint in NK cell-mediated hybrid resistance to parental transplantable hematopoietic lymphoid A20 tumor cells

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NK cells are bone marrow-derived type I innate lymphoid cells responsible for the early anti-viral response and for the rejection of syngeneic transformed cells without the need of prior sensitization due to a mechanism of recognition based on the array of germline-encoded inhibitory and activating receptors expressed on its surface. NK cells display cytotoxic activity against syngeneic tumor cells lacking MHC class I histocompatibility antigens (missing-self theory) and can also detect an altered pattern of expression of surface molecules in syngeneic tumor cells and activate their cytotoxic activity.

We hypothesized that PD-L1 expression on tumor cells or tumor microenvironment may modulate NK cell function in a similar manner to the effect exerted on CD8 T cells mediating anti-tumor responses. In contrast to other tumor models, A20 (H-2d) lymphoid tumor cells escape NK cell-mediated rejection when injected intravenously into F1 (C57BL/6xBalb/c) recipients and we postulated that PD-L1 expression on tumor cells may confer tumor protection against NK cell-mediated tumor rejection.

We first validated a couple of guided RNA targeting exon 3 of mouse PD-L1 (encoding 2 bp of signal peptide and the IgV domain) in an in vitro assay. A guide RNA exhibiting the highest cutting efficiency of the target gene was selected. CRISPR/Cas9 technology using a plasmid encoding the selected guide RNA and a integrase defective second generation lentiviral packaging system was applied to deliver both Cas9 and the guide RNA targeting exon 3 (pLentiV2, F. Zhang) to A20 tumor cells to knock-out mouse PD-L1 gene. A biallelic indel PD-L1 mutation (cell line A20, subclone 4A4/1C6) was identified consisting of 5 bp insertion and 20 bp deletion leading to a frameshift in exon 3 and premature stop codon. Preliminary data from ongoing experiments support the notion that the loss of PD-L1 expression in A20 lymphoma tumor cells makes them susceptible to NK cellmediated rejection. These results suggest that hematopoietic tumor cells use PDL1 to modulate NK-cell mediated hybrid resistance.

SOX9-STAT3 as a novel pathway for immunoresistance and cancer stem cell activity

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Glioblastoma Multiforme (GBM) is the most frequent and lethal primary brain tumor, which exhibits an extremely poor prognosis characterized by an average survival of 15 months. GBM contains a subpopulation of glioma stem cells (GSC) that are crucial drivers of tumor initiation, recurrence and resistance to therapies.

The dysregulation of essential genes for embryo development and stem cell maintenance seems to be a critical feature in GBM development and GSC activity. Therefore, these genes could become potential therapeutic targets in the treatment of GBM. In this sense, we have recently identified that SOX9 (sexdetermining region Y (SRY)-box 9 protein) is a relevant player in glioma stem cell activity and temozolomide resistance(1).

To characterize SOX9 underlying molecular mechanisms, we have performed transcriptomic analysis in patient-derived GSC with SOX9 knockdown. Notably, IFN and JAK2/STAT3 were within the pathways significantly downregulated in SOX9 knockdown cells. Functional studies of SOX9 and STAT3 genetic and pharmacological silencing showed that severely impairs self-renewal and tumor initiation in patient-derived GSCs. Moreover, we found that there is a genetic regulatory loop between them. We also tested their clinical relevance finding that their expression is elevated in biopsies from a subset of patients with overall poor prognosis. In summary, these results demonstrate that SOX9-STAT3 is a critical axis for cancer stem cell maintenance and immunoresistance, postulating that its inhibition is a promising strategy to combat therapy resistance in glioblastoma, idea that might extend to other types of aggressive cancers.

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Antioxidant metabolism regulates CD8+ T memory stem cell formation and antitumor immunity

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Adoptive T cell transfer (ACT) immunotherapy benefits from early-differentiated stem cell memory T (TSCM) cells capable to persist in the long term and to generate potent anti-tumor effectors. Due to their paucity *ex vivo*, TSCM cells can be derived from naïve precursors but the molecular signals at the basis of their generation are ill-defined. We found that less differentiated human circulating CD8+ T cells display substantial antioxidant capacity *ex vivo* compared to more differentiated central and effector memory T cells. Limiting reactive oxygen species (ROS) metabolism with antioxidants during naïve T cell activation hindered terminal differentiation while allowing expansion and generation of TSCM cells. N-acetylcysteine (NAC), the most effective molecule in this regard, induced transcriptional and metabolic programs characteristic of self-renewing memory T cells. Upon ACT, NAC-generated TSCM cells established long-term memory *in vivo* and exerted more potent anti-tumor immunity in a xenogeneic model when redirected with CD19-specific CAR, highlighting the translational relevance of NAC as a simple and inexpensive method to improve ACT.

cGAS expression contributes to tumor immunogenicity by enhancing innate signaling through the transfer of cGAMP to dendritic cells

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Spontaneous antitumor immune responses depend on the local production of type I interferons. In this context, the cyclic GMP-AMP-synthase (cGAS)/ stimulator of interferon genes (STING) pathway is of interest in which tumor-derived DNA is sensed in the cytosol of tumor-infiltrating dendritic cells (DCs). Its activation leads via the second messenger cyclic GMP-AMP (cGAMP) to type I interferon secretion. In other settings, the second messenger cGAMP has been shown to be transferred between cells to induce type I interferons in bystander cells. Together with the fact that activation of STING in the tumor microenvironment promotes anti-tumor immunity, we hypothesized that cGAS/cGAMP expression in tumors contributes to their immunogenicity.

Using *in vitro* co-culture experiments of various tumor cells and bone marrowderived dendritic cells (BMDCs), we identified a clear correlation of cGAS expression in tumor cells and the capacity to induce type I interferons in cocultured BMDCs. Moreover, our data suggest that transfer of cGAMP, but not DNA, from tumor cells to DCs via gap junctions composed of connexin 43 mediates the type I interferon production in DCs.

In experimental murine tumors, edited by CRISPR-Cas9 to abolish cGASexpression, we observed lower numbers of tumor-infiltrating DCs that expressed lower levels of the activation marker CD86, CD40 and MHCII. Furthermore, lysates of cGAS-deficient tumors contained less type I interferons. T lymphocytes and the effector cytokines IFNγ and TNFα were significantly reduced in cGASdeficient tumors. In line with a constricted adaptive immune response, we observed that cGAS-deficient tumors grew faster than their parental counterpart. These data suggest that cGAS contributes to tumor immunogenicity by enhancing innate signaling and priming of adaptive immune responses. Validation of this mechanism could have major implications on cancer prognosis and immunotherapy.

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Regulation of the expression of IL-1R8, a regulatory member of the Interleukin-1 receptor family

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IL-1R8 is an Interleukin-1 receptor (ILR) family member which activates an antiinflammatory program by inhibiting ILR and Toll like receptor (TLR) signaling. In NK cells IL-1R8 acts as checkpoint molecule in cancer and viral infections. The purpose of this study was to dissect the regulation of IL-1R8 expression in leukocytes. ChIPseq analysis suggested that Colony Stimulating Factors (CSF)-inducible Transcription Factors and epigenetic modifications affect IL-1R8 in NK cells and macrophages along maturation and activation. IL-1R8 expression was downregulated during macrophage differentiation. Pro-inflammatory molecules involved in macrophage and NK cell activation down-regulated IL-1R8 expression in human and mice. Prostaglandin E2 and Interleukin-10 (IL-10), which are involved in cancerassociated immunosuppression, counteracted this effect in NK cells and upregulated IL-1R8 in human monocytes and macrophages. RNA-seq analysis, q-PCR and Western Blot also revealed the existence of IL-1R8 truncated forms in human immune cells constituted by the exons coding for the intracellular part of IL-1R8, after M1 polarization. Thus pro-inflammatory stimuli are implicated in conventional isoform IL-1R8 down-regulation, overexpression of truncated forms of the protein, whose biological role is presently unknown. In addition, the up-regulation of IL-1R8 in NK cells by PGE2/IL-10 axis suggests that IL-1R8 could be part of the immunosuppressive activity of these molecules. IL-1R8 acts as a checkpoint molecule tuning antitumor and antiviral NK cell activity. Understanding how IL-1R8 expression in NK cells is affected by the tumor microenvironment is essential in the development of this checkpoint as a potential immunotherapy target.

The immune system controls dormant metastasis in the lungs

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Metastasis is the leading cause of breast cancer-related mortality. This multistep process requires that disseminated tumor cells evade immune surveillance and adapt to an ectopic environment in the metastatic organ. Although metastatic disease can be present already at diagnosis, in some patients metastasis appears months or years after resection of the primary tumor. This is thought to be due to the ability of some tumor cells to remain dormant after metastatic seeding. It is not known how dormancy is controlled although there is evidence for tumor cellintrinsic and -extrinsic mechanisms including immune defense.

To investigate whether and how immune defense controls metastasis to the lungs, we used two breast cancer cell lines derived from the same spontaneous tumor in immunocompetent BALB/c mice. We found that orthotopic 4T1 tumors gave rise to progressive metastatic disease, whereas orthotopic 4T07 tumors did not. However, both tumors gave rise to circulating and disseminated tumor cells. Thus, disseminated 4T07 cells displayed a dormant behavior. We next investigated whether 4T07 cells were dormant in immunodeficient NOD.Cg-Prkdcscid Il2rgtm1 (NSG) mice that lack T, B and NK cells and found that 4T07 tumors induced progressive lung metastasis, suggesting that the immune system essentially controls dormancy. Loss of 4T07 dormancy was also observed in BALB/c mice bearing a contralateral 4T1 tumor, suggesting that 4T1 tumors promote the escape from dormancy of 4T07 cells. We propose that 4T1 tumors compromise immune surveillance by a mechanism that is yet to be unraveled.

Our future plans include to further elucidate which immune cells control dormancy of 4T07 cells and how they do this. Furthermore, we want to understand why the presence of 4T1 tumors impinges on 4T07 dormancy.

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Metabolic inflammation-associated IL-17A causes non-alcoholic steatohepatitis and hepatocellular carcinoma

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Obesity increases hepatocellular carcinoma (HCC) risks via unknown mediators. We report that hepatic unconventional prefoldin RPB5 interactor (URI) couples nutrient surpluses to inflammation and non-alcoholic steatohepatitis (NASH), a common cause of HCC. URI-induced DNA damage in hepatocytes triggers inflammation via T helper 17 (Th17) lymphocytes and interleukin 17A (IL-17A). This induces white adipose tissue neutrophil infiltration mediating insulin resistance (IR) and fatty acid release, stored in liver as triglycerides, causing NASH. NASH and subsequently HCC are prevented by pharmacological suppression of Th17 cell differentiation, IL-17A blocking antibodies, and genetic ablation of the IL-17A receptor in myeloid cells. Human hepatitis, fatty liver, and viral hepatitis-associated HCC exhibit increased IL-17A correlating positively with steatosis. IL-17A blockers may prevent IR, NASH, and HCC in high-risk patients.

Characterization of molecular mechanisms involved in resistance of melanoma cells to ERK inhibitors

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Melanoma treatment with the BRAF V600E inhibitors (BRAFi) vemurafenib (VMF) or dabrafenib provides therapeutic benefits but the common emergence of drug resistance remains a challenge. Combined treatment with BRAFi and MEK inhibitors such as trametinib (TMT), has improved progression-free survival, but still resistance limits the efficacy of this therapy. Re-activation of the MAPK (RAF/MEK/ERK) pathway is a common resistance response accounting for both monotherapy and combined treatments in melanoma. Targeting ERK itself is a suitable strategy that is currently being investigated and tested in clinical trials in melanoma. In order to anticipate possible resistance responses to ERK inhibitors (ERKi), we have generated BRAF V600E/N-Ras wt (A375 and SK-Mel 28) and BRAF wt/N-Ras Q61R (SK-Mel 103) melanoma cells resistant to SCH772984 (SCH), an Erk1/2 inhibitor. These ERKi-resistant (ER) cells also displayed resistance to VMF, TMT and to combined VMF+SCH and TMT+SCH. Both A375-ER and SK-Mel 28-ER cells exhibited a strong reduction of pErk1/2 and pRSK, whereas SK-Mel 103-ER showed pErk1/2 re-activation. On the other hand, A375-ER and SK-Mel 28-ER cells displayed higher BRAF expression as well as Ras and PI3K-AKT activation than their parental counterparts. However, treatment with SCH and an inhibitor of the PI3K-AKT pathway only modestly reduced resistance. In addition, A375-ER cells showed increased Cdk4 and Cdk6 expression compared to parental cells, but treatment with palbociclib, a Cdk4/Cdk6 inhibitor, or siRNA-mediated Cdk4/Cdk6 silencing did not alter melanoma resistance to SCH. We are currently testing whether other MAP kinases might be involved in the resistance responses of melanoma cells to ERKi.

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Staufen 1, a new class of pro-oncogenic factor in melanoma linked to dsRNA signalling

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Cutaneous melanoma is an increasing prevalent cancer, characterized for the extensive changes in the transcriptome. Yet, the specific contribution of RNA binding proteins (RBPs) to melanoma progression is largely unknown. Targeted therapies together with immunotherapy have arisen as promising therapeutic approaches for metastatic patients. However, there is still an important fraction of patients that do not respond. Understanding the mechanisms underlying this resistance may pave the way for new therapeutic strategies. Here we present computational analyses of large clinical datasets together with histological and functional studies, leading to the identification of the dsRNA binding protein STAU1 as a potential novel driver and immune modulator in melanoma. In particular, we have found a high upregulation of STAU1 mRNA and protein levels in human melanoma biopsies (to our knowledge the largest changes reported for an RBP in this tumor type). Mechanistically, analyses in genetically modified mouse models support a contribution of the MAPK pathway to STAU1 expression. Moreover, STAU1 depletion compromises melanoma cell proliferation and invasion in cell culture. Potential targets of STAU1 include the coding transcript for the immune checkpoint blocker PD-L1. Together these data support unanticipated roles of STAU1 in the control of aggressive features of malignant melanoma.

Therapeutic targeting of CD44 in T cell acute lymphoblastic leukemia

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NOTCH1 is a prevalent signaling pathway in T-cell acute lymphoblastic leukemia (TALL), but crucial NOTCH1 downstream signals and target genes contributing to T-ALL pathogenesis cannot be retrospectively analyzed in patients and thus remain ill-defined. This information is clinically relevant, as initiating lesions that lead to cell transformation and leukemia-initiating cell (LIC) activity are promising therapeutic targets against the major hurdle of T-ALL relapse. Here, we describe the generation *in vivo* of a human T-cell leukemia that recapitulates T-ALL in patients, which arises de novo in immunodeficient mice reconstituted with human hematopoietic progenitors ectopically expressing active NOTCH1. This novel T-ALL model allowed us to identify CD44 as a direct NOTCH1 transcriptional target, and to recognize CD44 overexpression as an early hallmark of pre-leukemic cells, which engraft the bone marrow (BM) and finally develop a clonal transplantable T-ALL that infiltrates lymphoid organs and brain. Notably, CD44 is shown to support crucial BM niche interactions necessary for LIC activity of human T-ALL xenografts and disease progression, highlighting the importance of the NOTCH1-CD44 axis in T-ALL pathogenesis. The observed therapeutic benefit of anti-CD44 antibody administration in xenotransplanted mice holds great promise for therapeutic purposes against T-ALL relapse.

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The druggable immune system: drug repositioning in immune transcriptome

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PURPOSE

Immune cells can control the fate of tumor, either promoting its growth or diminishing it (1). The goal of this work is finding drugs that can promote a better immune-environment to improve responses to antitumoral treatments. For instance, avoiding the effect of immunosuppressive T-helper, MDSC or T-reg cells by reverting its signature towards conventional T-reg, reverting the immunosuppressive M2 macrophages signature towards a more favorable macrophage state such M1.

METHOD

We have employed an in-house version of Connectivity Map (2) to predict single drug treatments and revert expression signatures integrating data from L1000, CCLE, GDSC2.0 and CTRP projects, and comprising more than 5,000 compounds and ~4 million drug-drug interactions. We have applied this approach to study 156 selected immunologic gene expression signatures associated to T-reg, T-helper, MDSC and macrophages obtained from MSigDB (3) and scientific literature. The analysis has been performed both for human and mouse signatures.

RESULTS

Using our methodology we have obtained at least one significant signature-drug prediction for 44% of the immune signatures. In total, we obtained 5,472 immune gene expression signature-drug interactions corresponding to 1,081 drugs (FDR < 0.05). To validate our approach, we have manually reviewed some of our predictions checking scientific literature. For instance as previously reported, we predict that PI3K inhibitors (i.e.wortmannin), suppress Treg activity.

CONCLUSIONS

We have built a catalogue of prioritized drug predictions for some highly relevant immune cells involved in tumor's fate that can either promote or revert a give immune signature. Our approach can be extended to predict drug treatments in other curated immunological studies. The final goal is the creation of a database containing predictions of immune signature-drugs interaction that potentially may revert immune cellular states.

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The immune activating receptor NKG2D and its ligands in cancer recognition

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NKG2D is a key receptor for the activation of immune effector cells, mainly Natural Killer cells and CD8+ T lymphocytes, in infection, cancer and autoimmune diseases. However, the ligands recognised by this receptor can be released from stressed cells blocking NKG2D and leading to a decreased cytotoxic immune response. Indeed, the detection of elevated levels of ligands for NKG2D in sera of cancer patients is, in most cases, indicative of bad prognosis. Some NKG2D-ligands are released as soluble molecules while other are recruited to extracellular vesicles and work from our group has defined the diverse biochemical and cellular properties displayed by different NKG2D-ligands that underlie these different modes of release. Recently, we have also reported that directed therapy using vemurafenib, an inhibitor specifically targeting the mutant BRAFV600E, present in many cases of metastatic melanoma, actually impairs immune recognition due to the downmodulation of the ligands for NKG2D from the plasma membrane. Thus, targeted therapies can affect tumour recognition by the immune system. Further studies showed that vemurafenib-treated cells also contain less NKG2D-ligands in exosomes. Although it is now clear that the NKG2D system can be used as a tool for diagnosis and a target for therapy, alone or in combination with checkpoint inhibitors, some questions remain open due to the complexity associated with the existence of a large number of ligands, each one of them displaying distinct biological properties. We propose that to fully understand the roles of NKG2D ligands as stimulators of immune responses versus immune evasion, it will be necessary to consider multiple aspects of this system, including polymorphism, expression at the plasma membrane versus released proteins in plasma, and discrimination between soluble, metalloprotease cut protein and those ligands incorporated into exosomes.

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The CCL8/CCR8 axis maintains an immunosuppressive tumor microenvironment

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Aside from cancer cells, the tumor microenvironment (TME) contains a vast array immune cells, which are known to contribute significantly to tumor growth and dissemination and are therefore considered interesting targets for cancer therapy. Strikingly, detailed studies regarding the role of chemokines and chemokine receptor expression on these tumor-resident immune cells is largely lacking. Our research group has identified that the Chemokine (C-C motif) receptor 8 (CCR8) plays an important role in tumor progression, since several tumor models grew more slowly in syngeneic CCR8-/- mice. This is in accordance with previous studies in cancer patients where CCR8 expression on immune cells was shown to contribute to immune evasion, promote tumor growth and was associated with a worse prognosis in breast cancer patients. In mice, the CCR8-deficient phenotype coincided with the induction of an inflammatory response, a more M1-like phenotype of tumor associated macrophages (TAMs) and the stimulation of antitumoral T-cell immunity. Moreover, the CCR8-specific ligand, CCL8, was shown to be specifically and highly expressed by pro-tumoral M2-like TAMs, whereas CCR8 was shown to be expressed specifically, and highly on regulatory T cells (Tregs) inside the TME and not in the periphery. Furthermore, CCR8 expression on Tregs appeared to correlate with a more activated, pro-tumoral CD25HighOX40High Treg phenotype.

Overall, these results suggest that the CCL8/CCR8 chemokine axis plays a crucial role in maintaining an immunosuppressive tumor microenvironment, acting both on macrophages and T cells. Hence this pathway represents a potential target for tumor immunotherapy.

Bacteria-trained lymphocytes as next generation of cancer immunotherapies

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Esteban Veiga

Spanish National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

Besides classical forms of tumor treatments such as radiation and chemical therapy, an emerging field, revolutionizing the field of oncology, regards the use of immunotherapies. Most patients, however remain resistant to these therapies and secondary effects induce them to leave the treatment. It is therefore necessary to find novel therapies more effective and minimizing side effects.

We have discovered that conventional CD4+ T cells (paradigm of the adaptive immunity) can capture and kill bacteria (Cruz-Adalia et al., 2014). During this process, CD4+ T cells exposed to bacterial PAMPs are "trained" acquiring novel abilities. Trained (tr), CD4+ T cells became potent antigen presenting cells able to (1) cross-present antigens from captured bacteria, activating naïve CD8+ T cells and (2) generating central memory (Cruz-Adalia et al., 2017); activities involved in the removal of tumors. Note that actually there exist huge efforts to generate central memory CD8+ T cells from tumor infiltrating lymphocytes (Sukumar et al., 2017). These effects, together with (3) the massive and localized secretion of inflammatory cytokines by trCD4+ T cells (Cruz-Adalia et al., 2014), which could block the immunosuppressive environment generated by solid tumors, prompted us to hypothesized that trCD4+ T cells could be useful in antitumor therapies. This hypothesis was tested in a proof-of-concept model of aggressive mouse melanoma. Mice vaccinated with trCD4+ T cells were very effectively protected against tumor development (Cruz-Adalia et al., 2017).

We propose to discuss these groundbreaking data with the audience in order to improve our discoveries and further study the antitumor potential of trCD4+ T cells.

Cruz-Adalia, et al. (2014). *Cell Host Microbe* 15, 611–622.
Cruz-Adalia, A., et al. (2017). *Nature Communications* 8, 1591.
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A novel multifunctional polypeptide-based nanosystem as an anti-cancer immunotherapeutic approach for melanoma

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INTRODUCTION

Melanoma is the most dangerous type of skin cancer and novel treatments are needed [1]. Therefore, alternative therapeutics should be devised, isolated or in combination with targeted immunotherapies, to efficiently stimulate specific antitumor responses. Branched polypeptides exhibit advanced engineered complexity and unique structural properties inaccessible to linear polymers that make them ideal constructs to be employed as drug delivery systems with enhanced biological performance [2]. Branched nanoscale systems have the ability to activate immune cells, as dendritic cells (DC) and natural killer (NK) cells, constituting potential platforms to modulate the release profile of loaded molecules, including tumorassociated antigens (TAA), adjuvants and drugs [3]. This work aims to evaluate the *in vivo* anti-tumor efficacy of peptide-1 -conjugated polypeptide (pept-1-BP), with special emphasis on its impact on the modulation of the immune cell function.

RESULTS AND DISCUSSION

BP were synthesized and conjugated with the peptide-1 (pept-1-BP) presenting a terminal cysteine via reductive-sensitive disulfide linker. To address in vitro and *in vivo* studies, Cy5.5 was conjugated to the platform. To evaluate the effect of the conjugate on melanoma tumor growth, B16.F10 cells were implanted subcutaneously into 8-week-old C57BL/6 mice. At day 7, animals were injected with two doses (1-week apart) of 100 µL of PBS, Toll-like receptor ligands CpG (20 µg/dose) and Poly I:C (40 µg/dose) in solution, BP backbone (575 µg/dose) and pept-1-BP (575 µg/dose) mixed with adjuvants. Every 2 days, the weight of the animals and the tumor growth were followed. At day 21, mice were sacrificed and tumor and lymph nodes were collected. Cell suspensions from tumor cells and lymph nodes of each animal were prepared and analyzed for infiltrating lymphocytes (CD45.1, CD3e, CD8α, CD4, CD107, PD-1, CTLA-4) by fluorescence-activated cell sorting (FACS). The BP presented a size of 81.86 ± 1.63 nm and a zeta potential of -45.10 ± 1.72 mV, while pept-1-BP showed a mean average diameter of 104.1 ± 2.21 nm and a zeta potential of -24.8 ± 0.64 mV, with a pept-1 loading efficiency of 8.7 % (w/w). *In vivo* results showed a significant reduction of tumor size in pept-1-BP-treated mice compared with the other groups. In addition, the FACS analysis of infiltrating lymphocytes within tumor site evidenced an increased expression for CD4, CD8α and NK cells.Overall, our results support the promising use of this novel conjugate for the delivery of TAA, as an effective anti-tumor immune therapeutic strategy able to control and overcome tumor growth.

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Hongtao Yu
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Institute for Research in Biomedicine, Barcelona, Spain

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DKFZ German Cancer Research Center, Heidelberg, Germany

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John Rubinstein
The Hospital for Sick Children Research Institute, Toronto, Canada

Monday 26 Feb
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Hebrew University of Jerusalem, Israel

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Friday 20 Apr
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