PIVAC-17

17th International Conference on Progress in Vaccination Against Cancer

27th–30th September 2017
«Alexandrion» Conference Hall
Loutraki, Corinth, Greece

www.pivac17.eu

Organized by

- The Cancer Immunology and Immunotherapy Center, St. Savas Cancer Hospital
- The Hellenic Pasteur Institute
- The Hellenic Society of Immuno-Oncology

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Day 1 - Wednesday 27 September 2017

13.00 - 20.00 REGISTRATION Entrance Lobby of the Alexandrion Conference Hall

13.00 - 14.30 WELCOME LUNCH

13.00 - 20.45 POSTER EXHIBITION

15.00 - 15.15 WELCOME BY THE MAJOR OF LOUTRAKI
George Gionis

WELCOME BY THE PIVAC -17 CHAIR
Costas Baxevanis *Cancer Immunology and Immunotherapy Center, St. Savas Hospital, Greece*

SESSION 1
Session Chair: **C. Baxevanis**

15.15 - 15.45 **Rafael Solana** *Maimonides Biomedicine Institute of Cordoba (IMIBIC), Reina Sofia Hospital, University of Cordoba, Spain* "New insights on NK cell-based immunotherapy of cancer"

15.45 - 16.15 **Rolf Kiessling** *Karolinska Institutet, CCK, Stockholm, Sweden* "Combining Adoptive Cell Therapy with DC vaccination for therapy of malignant melanoma"

16.15 - 16.45 **Per thor Straten** *Center for Cancer Immune Therapy, Department of Hematology, University Hospital Herlev, Denmark* "Therapy of solid tumors using T cells and running shoes"

16.45 - 17.15 COFFEE BREAK

SESSION 2
Session Chair: **B. Seliger, F. Garrido**

17.15 - 17.45 **Barbara Seliger** *Institute of Medical Immunology, Martin - Luther - University Halle - Wittenberg, Halle, Germany* "Tumor-induced immune escape mechanisms due to deregulation of immune relevant components"

17.45 - 18.15 **Federico Garrido** *Departamento de Bioquimica, Biologia Molecular III e Inmunologia, Facultad de Medicina, Universidad de Granada, Spain* "Cancer Immune Escape: HLA Class I Loss And Tumour Tissue Architecture"

18.15 - 20.00 Proffered Papers

Toos Daemen *Department of Medical Microbiology, Tumor Virology and Cancer Immunotherapy, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands* "Semliki Forest Virus-based anticancer vaccines"

Anne M.H. Larsen *Center for Cancer Immune Therapy (CCIT), Copenhagen University Hospital, Herlev, Denmark* "Tumor extracellular Matrix as a potent regulator of intratumoral immunity"

Christine U. Delle, **Daniel H. Madsen** *Center for Cancer Immune Therapy (CCIT), Dept. of Haematology, Herlev Hospital, Herlev Ringvej, Denmark* "Is the activity of tumor-infiltrating T cells modulated by colon cancer cells?"

Maren Eckey *JPT Peptide Technologies GmbH, Berlin, Germany* "TERS-routine application of a new tool for standardizing functional T-cell assays"

Elena Quaglino *Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy* "TENM4 emerges as a potential target for Triple Negative Breast Cancer Stem Cells immune-targeting"

Barbara-ann Guinn *School of Life Sciences - Biomedical Science, University of Hull, Hull, UK* "New targets for the immunotherapy of adult B-acute lymphocytic leukaemia"

Barbara-ann Guinn *School of Life Sciences - Biomedical Science, University of Hull, Hull, UK* "Can a cancer-testis antigen act as biomarker for early stage ovarian cancer?"

20.00 - 22.00 WELCOME RECEPTION

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Day 2 - Thursday 28 September 2017

SESSION 3
Session Chair: **W.H. Fridman**

09.00 - 09.30 **Efstratios Stratikos**, *National Center for Scientific Research Demokritos, Agia Paraskevi, Athens, Greece* "Modulating intracellular antigen processing for enhancing tumour antigenicity"

09.30 -10.00 **Wolf Herve Fridman**, *INSERM Paris, France; Université Paris Descartes/Paris V, Sorbonne Paris Cité, Paris, France* "Cancer microenvironments: prognostic and theranostic impacts"

10.00 -10.30 **Doriana Fruci**, *Immuno-Oncology Laboratory, Ospedale Pediatrico Bambino, Rome, Italy* "Clinical relevance of tumor-infiltrating immune cells in neuroblastoma"

10.30 -11.00 **Ioannis Pateras**, *Department of Histology and Embryology, University of Athens, Greece* "Biomarkers in Inflammation- relevance to cancer"

11.00 -11.30 COFFEE BREAK

SESSION 4
Session Chair: **C. Figdor**

11.30 -12.00 **Gustav Gaudernack**, *Department of Immunology, Institute for Cancer Research, University Hospital-Radiumhospitalet, Oslo, Norway* "Clinical trials with a second generation hTERT vaccine, UV1. Single agent therapy and combination with checkpoint inhibitors"

12.00 -12.30 **Sjoerd van der Burg**, *Leiden University Medical Center, The Netherlands* "Cancer Immunotherapy, not without vaccination"

12.30 -13.00 **Carl Figdor**, *Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands* "21st Century Cancer Vaccines"

13.00 -13.30 COFFEE BREAK

SESSION 5
Session Chair: **P. Coulie**

13.30 -14.00 **Federica Cavallo**, *Molecular Biotechnology Center, University Turin, Italy* "The cystine/glutamate antiporterxCT: A new cancer stem cell target for anticancer vaccines"

14.00 -14.30 **Pierre Coulie**, *de Duve Institute, Université Catholique de Louvain, Brussels, Belgium* "Immunogenicity of primary breast carcinomas"

14.30 -15.00 **Kostas Kosmatopoulos**, *Vaxon Biotech, Paris, France* "Optimized cryptic peptides: a universal neoantigen therapeutic cancer approach"

15.00 -16.30 LUNCH

Chairs: **F. Cavallo, C. Baxevanis**

16.30 -18.15 Proffered Papers

Simon Klaessens, *Ludwig Institute for Cancer Research, Brussels; Belgium. de Duve Institute, Université catholique de Louvain, Brussels, Belgium* "Tryptophan 2,3-dioxygenase, an enzyme involved in tumour immune escape, is positively regulated by its substrate tryptophan"

Alvaro Lladser, *Laboratory of Gene Immunotherapy, Fundacion Ciencia & Vida, Santiago, Chile* "Vaccination-induced skin-resident and circulating memory CD8+ T cells collaborate to mediate broad protection against cutaneous melanoma"

Marit R. Myhre, *Department of Cellular Therapy, Section for Cancer Immunology, Oslo University Hospital-The Norwegian Radium Hospital, Oslo, Norway* "Exploiting CD4+ T cells for adoptive cell therapy in cancer"

Thomas W. Smith, *Department of Surgery, Loyola University Medical Center; Department of Surgery, Medical University of South Carolina, USA* "Genetically enhanced T cells with IL2Rα lead to increased survival and proliferation"

Marius Strioga, *National Cancer Institute, Vilnius, Lithuania* "Chemoimmunotherapy using autologous dendritic cell vaccines for the treatment of recurrent glioblastoma multiforme"

Raquel Tarazona, *Immunology Unit, University of Extremadura, Cáceres, Spain* "Increased expression of activating receptors and recovery of NK cell function in Acute Myeloid Leukaemia patients after in vitro culture with IL-15"

Claudia Wurzenberger, *Immunocore Ltd., Milton Park, Abingdon, Oxfordshire, UK* "ImmTAC™: From TCR discovery to bi-specific immunotherapeutic agents for the treatment of cancer"

18.15 -18.45 COFFEE BREAK

"Satellite Session by the industry"

18.45 -19.15 **Paul Lehmann**, *Case Western Reserve University, USA* "Tumor antigen recognition by healthy individuals and cancer patients"

19.15 -19.45 **Robert Stad**, *Perkin Elmer*, "Immunophenotyping the Tumor Microenvironment in FFPE Sections"

20.00 -22.00 DINNER

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Day 3 - Friday 29 September 2017

SESSION 6
Session Chair: **V. Umansky**

09.00 -09.30 **Victor Umansky**, *Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany* "Accumulation of myeloid-derived suppressor cells in melanoma microenvironment and their targeting"

09.30 -10.00 **Vincenzo Bronte**, *Department of Medicine, University Hospital, University of Verona, Italy* "Myeloid cells assist tumor progression by molecular mechanisms either dependent or independent from adaptive immunity"

10.00 -10.30 **Suzanne Ostrand-Rosenberg**, *Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, USA* "The good, the bad, and the in-between: immune suppression, obesity, and tumor progression"

10.30 -11.00 COFFEE BREAK

SESSION 7
Session Chair: **S. Ostrand-Rosenberg**

11.00 -11.30 **Dimitrios Mastellos**, *Division of Biodiagnostic Sciences and Technologies, INRASTES, National Center for Scientific Research 'Demokritos', Athens, Greece* "Revising the role of complement in antitumor immunity: challenges and opportunities"

11.30 -12.00 **Benoit van den Eynde**, *Ludwig Institute for Cancer Research, Oxford, UK* "Mechanisms of resistance to cancer immunotherapy"

12.00 -12.15 POSTER AWARDS

12.15 -14.00 LUNCH

SESSION 8
Session Chair: **G. Pawelec**

14.00 -14.30 **Gunter Hämmerling** *Division of Molecular Immunology, German Cancer Research Center Heidelberg, Germany* "Reprogramming of the tumor microenvironment for efficient cancer immunotherapy"

14.30 -15.00 **Graham Pawelec** *Second Department of Internal Medicine, University of Tübingen, Germany* "Immunotherapy of Cancer: triumphs and challenges, and the impact of immunosenescence"

15.00 -15.30 **Cornelis Melief** *Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands* "Combination immunotherapy against cancers expressing viral or neo-antigens"

15.30 -16.00 COFFEE BREAK

SESSION 9
Session Chair: **C. Gouttefangeas**

16.00 -16.30 **Gosse Adema** *Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands* "Immuno-combination therapies for cancer"

16.30 -17.00 **Antonia Dimitrakopoulou-Strauss** *Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Heidelberg, Germany* "Therapy monitoring of immune checkpoint inhibitors in patients with metastatic melanoma with PET-CT"

17.00 -17.30 **Cecile Gouttefangeas** *Group leader at the Institute for Cell Biology, Department of Immunology, Tübingen, Germany* "Immunomonitoring as a tool to identify biomarkers predicting the outcome of immunotherapy-treated patients"

17.30 -19.00 Cancer Immunology and Immunotherapy editorial board meeting

19.00 -20.30 DINNER

Saturday 30, September 2017

Free day - Excursion to Epidaurus old theater, Ancient Mycenae, Nafplion

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Abstracts

Invited Speakers

New insights on NK cell-based immunotherapy of cancer

Rafael Solana[1,2] and Raquel Tarazona[1]
[1]Immunology Unit, University of Extremadura, Cáceres, Spain
[2]IMIBIC - Reina Sofia University Hospital - University of Cordoba, Spain

Natural Killer (NK) cells are innate immune effector cells involved in the first line of defence against virus infected and malignant cells. NK cells distinguish healthy cells from tumour cells by the interaction of activating and inhibitory receptors with their different ligands in the targets. NK cytotoxicity is the result of a fine balance between activating and inhibitory signals triggered by these NK receptors. The expression of activating ligands together with a reduction in the expression of MHC class I molecules on cancer cells activates NK cell killing and release of proinflammatory cytokines such as IFN-γ.

The advances in research in NK cell biology have provided new insights in the clinical applications of these cells in cancer immunotherapy. Cancer is considered a disease of old age and the contribution of age-associated changes of the immune system, immunosenescence, to the increased risk of cancer in old individuals has been discussed for many years. NK cell immunosenescence is characterized by a redistribution of NK cell subsets and a diminished expression of several activating receptors. Altered expression of activating receptors has also been described in young and elderly cancer patients, including patients with acute myeloid leukaemia, probably due to chronic exposure to ligands on tumour cells.

The aim of NK cell-based cancer immunotherapy is to reverse the NK cell dysfunction observed in many cancer patients and to increase and sustain NK cell effector functions. Strategies harnessing the power of NK cells against target tumour cells include activation of endogenous NK cells or transfer of exogenous cells by hematopoietic stem cell transplantation or adoptive cell therapy. In addition, checkpoint blockade of inhibitory receptors and the use of agonist antibodies to stimulate activating receptors are also considered as emerging areas of research. In this context, the effect of immunosenescence should be considered to improve the efficiency of cancer immunotherapy.

Improving Adoptive Cell Therapy (ACT) through combinations with other therapies

Rolf Kiessling[1], Tanja Lövgren[1], StinaWickström[1], Maria Wolodarski[1], Isabel Poschke[2], Maarten A. Ligtenberg[1], Lars Adamson[1], Andreas Lundqvist[1] and Yago Pico de Coaña[1]
[1] Department of Oncology/Pathology, Karolinska institutet and Oncology clinic, Karolinska University hospital, Stockholm, Sweden
[2] Department of Molecular Oncology of Gastrointestinal Tumors, German Cancer Research Center (DKFZ), Heidelberg, Germany

Immunotherapy of malignant diseases is rapidly expanding the therapeutic options for cancer patients. Adoptive cell therapy (ACT) is a potent method of harnessing autologous immune cells, allowing for *ex vivo* manipulation of T cells or NK cells prior to their re-infusion into the patient. ACT includes therapy based on expansion of tumor-infiltrating lymphocytes (TIL) cultured from a surgical resection of the tumor or from peripheral blood mononuclear cells (PBMCs) engineered to become tumor specific. Although no ACT method is yet FDA approved, clinical trials have shown promising results, with TIL therapy of malignant melanoma yielding an overall response (OR) rate around 30-50%. Methods to further improve ACT will be discussed, including combinations with dendritic cell vaccination expressing shared tumor antigens or neoantigens, silencing of PD-1 on the injected T cells or suppressing MDSCs in the treated patient.

Therapy of solid cancers using T cells and running shoes

Per thor Straten, PhD
Center for Cancer Immune Therapy (CCIT), Department of Hematology, University Hospital Herlev & Department of Immunology and Microbiology, University of Copenhagen, Denmark

Cells of the immune system, e.g., CD8 T cells and Natural killer (NK) cells are capable of recognizing and killing cancer cells. Naturally elicited anti-cancer immune responses lead to infiltration of immune cells into tumors and the presence of these cells impact on disease progression, i.e., overall survival of the patient. Moreover, infiltration of T cells in tumors is associated with response to treatment with immune check point inhibitory monoclonal antibodies (mAb), and immune cells in tumors can be directly utilized in therapy by administration of *in vitro* expanded tumor infiltrating lymphocytes (TIL). Thus, ways to increase immune cell infiltration in tumors hold great promise in therapy. We recently demonstrated that voluntary wheel running showed over 60% reduction in tumor incidence and growth across several murine tumor models. Immune cell infiltration including T and NK cells was significantly increased in tumors from exercising mice, and depletion of NK cells blunted the exercise-dependent tumor control. Moreover, NK cells were engaged through an epinephrine-dependent mobilization, and blockade of this pathway blunted the exercise-dependent tumor inhibition. Together these results link exercise with improved immunological control of tumor progression, suggesting that exercise could be a beneficial partner for immunotherapy. ACT using TILs has shown impressive responses in melanoma, however, many patients do not benefit from treatment. To this end, homing of adoptively transferred T cells to tumors may be inadequate. Using lentiviral transduction of T cells for ACT, we have shown that CXCR2 transduced T cells more efficiently home to human melanoma tumors *in vitro* as well as *in vivo* using the NOG mouse model.Thus, clinical application of this approach may increase the clinical efficacy of TIL based ACT. In some cases, however, TILs as a cellular source may not be neither best choice nor possible. We have studied the prospects of using γδ T cells harvested from blood as a source of T cells for ACT. These cells can be easily expanded, and are highly efficient cancer cell killers, and thus represent a potential easily accessible cellular source for ACT.

Novel mechanisms shaping the MHC class I mediated immune escape mechanisms of tumors and its relevance for anti-tumoral immune responses

Barbara Seliger, Juergen Bukur, Claudia Lennicke, Karthikeyan Subbarayan, Claudia Wickenhauser, Alexander Eckert,Daniel Bethmann, Sandra Leisz, Andre Steven, Marifili Lazaridou, Simon Jasinski-Bergner, Katharina Biehl, Diana Handke, Anja Mueller, Bernhard Fox, Chiara Massa, Ofer Mandelboim

Abnormalities of classical and non-classical MHC class I antigens have been reported in *in vitro* models of oncogenic transformation, murine as well as human tumors of distinct origin resulting in escape of tumor cells from T cell-mediated immune surveillance. The underlying molecular mechanisms of these alterations are highly complex and include transcriptional, epigenetic and posttranscriptional control of MHC class I antigens and components of the antigen processing machinery (APM). Next to the identification of transcription factors involved in the regulation of these molecules, epigenetic control appears an important event. While the classical MHC class I pathway is mainly regulated by alterations in the histone acetylation, methylation is an important event leading to suppression of HLA-G. Concerning the posttranscriptional regulation,defects in the IFN signal transduction and enhanced activity of oncogenic signaling cascades influence components of the MHC class I APM in murine and human tumors.In addition, altered expression of immune modulatory microRNAs and RNA-binding proteins affects the expression of MHC class I APM and/or HLA-G. Furthermore, proteins involved in oxidative folding of MHC class I molecules within the ER are able to upregulate MHC class I surface molecules suggesting that the redox status of tumor cells affects tumor immunogenicity. Even more complex is the role of the extracellular matrix protein biglycan (BGN), which is downregulated in HER-2/neu-overexpressing tumor cells. Restoration of its expression renders BGN^{high} $HER\text{-}2/neu^{+}$ cells less tumorigenic in immune competent mice, which was associated with enhanced immune cell responses, a higher frequency of immune effector cells in tumors and peripheral blood and an increased MHC class I surface expression. This effect was due to an altered expression of members of the TGF-β pathway.

The escape of tumor cells from immune surveillance appears to be due to changes in the cellular composition of the tumor microenvironment. Indeed, the frequency, localization and topology of suppressor and effector immune cells is of importance for proper tumor host interaction and in combination with MHC class I APM component expression has a further prognostic value for patients with cancer.

In conclusion, a better understanding of the immune evasion mechanisms is still required for improving T cell-based, individualized immunotherapies, and appears to be of importance in the development of resistances to these therapies, but opens also new venues for therapeutic intervention.

Cancer Immune Escape: HLA Class I loss and tumor tissue architecture

Federico Garrido
Dept. Analisis Clinicos & Immunologia, Hospital Universitario Virgen de las Nieves.
Instituto de Investigacion Biosanitaria, Ibs. Dept. Bioquimica, Biología Molecular III e Inmunologia, Facultad de Medicina, Universidad de Granada, 18014 Granada, Spain.

Most tumor cells derive from MHC-I-positive normal counterparts and remain positive at early stages of tumor development. T lymphocytes can infiltrate tumor tissue, recognize and destroy MHC class I (MHC-I) positive cancer cells ("permissive" phase I). This phase can end in the total destruction of the tumor with no clinical evidence. Alternatively, MHC-I-negative tumor cell variants resistant to T-cell killing can emerge. During this process of T cell immune selection, tumors first acquire a heterogeneous MHC-I expression pattern and finally become uniformly MHC-I negative. At this stage (phase II) tumour cells actively create an immuno supressive microenviroment that prevents intra-tumour T/NK cell infiltration and produces a "non-permissive" encapsulated structure with tumor nodes surrounded by fibrous tissue containing different elements including leukocytes, macrophages, fibroblasts, Tregs, MDSCs etc.The transition from phase I to phase II probably lasts for a short period of time.This phase II is associated with a peculiar tumour tissue architecture well defined by pathologists long time ago but not previously associated with the absence of HLA-I molecules.This tissue structure resembles that observed in different TH2 granulomas in which the pathogen cannot be destroyed but is isolated from the body by a biological barrier. Molecular mechanisms responsible for total or partial MHC-I down regulation play a crucial role in determining and predicting the antigen-presenting capacity of cancer cells. MHC-I downregulation caused by reversible ("soft") lesions can be upregulated by TH1-type cytokines released into the tumor microenvironment in response to different types of immunotherapy. In contrast, when the molecular mechanism of the tumor MHC-I loss is irreversible ("hard") due to a genetic defect in the gene/s coding for MHC-I heavy chains (chromosome 6), beta-2-microglobulin (β2M) (chromosome15) or IFN genes, malignant cells are unable to upregulate MHC-I, remain undetectable by cytotoxic T-cells and continue to grow and metastasize. Based on the tumor MHC-I molecular analysis, it might be possible to define MHC-I phenotypes present in cancer patients in order to distinguish between responders versus non-responders. This highlights the need for designing strategies to enhance tumor MHC-I expression that would allow CTL-mediated tumor rejection (Refs. 1 & 2).

1.-*The absence of HLA class I expression in non-small cell lung cancer correlates with the tumor tissue structure and the pattern of T cell infiltration.*-Francisco Perea, Monica Bernal, Abel Sanchez-Palencia, Javier Carretero, Cristina Torres, Clara Bayarri,Mercedes Gomez-Morales, Federico Garrido and Francisco Ruiz-Cabello.
Int. J. Cancer: 140, 888–899 (2017).
2.-*The Escape of Cancer from T Cell-Mediated ImmuneSurveillance: HLA Class I Loss and Tumor Tissue Architecture.*- Federico Garrido, Francisco Perea, Mónica Bernal, Abel Sánchez-Palencia,Natalia Aptsiauri and Francisco Ruiz-Cabello.Vaccines 5,7 2017.

Modulating intracellular antigen processing for enhancing tumor antigenicity

Efstratios Stratikos
Protein Chemistry Laboratory, INRASTES, National Centre for Scientific Research Demokritos, Athens, 15341, Greece.
Email: stratos@rrp.demokritos.gr

The immunogenicity of tumors is largely dependent on the presentation of tumor-specific antigenic peptides by MHC class I molecules on the cell surface. As a result, current immunotherapy or cancer vaccination approaches can be limited by the low efficacy of presentation of cancer neoantigens, resulting from inefficient intracellular antigen processing. Understanding the mechanisms behind the generation of antigenic peptides is critical for designing pharmacological approaches aimed at enhancing clinical responses to cancer vaccines and immune-checkpoint inhibitors. Antigenic peptides are generated from cellular antigens or neoantigens by complex intracellular proteolytic pathways. A key component of these pathways is the optimization of antigenic peptide length and sequence inside the Endoplasmic Reticulum by resident aminopeptidases, such as ERAP1, ERAP2 and IRAP. The activity of these enzymes has been linked with cancer immune evasion strategies and their silencing can enhance cellular anti-tumor responses making them a tractable target for cancer immunotherapy. First generation inhibitors for these enzymes have been developed and are now in preclinical evaluation for efficacy in enhancing tumor antigenicity.

Cancer Microenvironments: Prognostic and Theranostic Impacts

W.H. Fridman[1-3]
[1]INSERM UMRS1138, Cordeliers Research Centre, Cancer and immune escape laboratory, Paris, France.
[2]Université Paris Descartes, Paris, France.
[3]Université Pierre et Marie Curie, Paris, France.
Wolf H. Fridman Cordeliers Research Centre, INSERM UMRS 1138 and University Paris Descartes, 15 rue de l'Ecole de Médecine, 75006, Paris, France, (herve.fridman@crc.jussieu.fr)

Tumors grow within a complex microenvironmen tcomposed of immune cells, fibroblasts, endothelial cells and other non-malignant cells. The study of the composition of tumor microenvironments has led to classifications with prognostic and theranostic values, as well as to treatments modulating its composition and its functional orientation. Concurrently, molecular classifications of tumors have proposed taxonomies that define groups of patients with different prognosis and which predict responses to treatments.

The density, location and functional orientation of tumor-infiltrating lymphocytes form the immune contexture which composition is positively correlated in most cases with patient's survival. Colorectal cancer represents a paradigm for tumor immunology, as it is the human cancer in which it was exemplified that an adaptive immune response can control tumor growth and metastasis. A high infiltration of Th1/cytotoxic T cells is associated with longer disease or progression free and/or overall survival both in primary and metastatic sites. There are, however, exceptions to this rule such as Renal Cell Carcinoma where high CD8+ T cell infiltration correlates with shorter survival. High infiltration by myeloid cells and fibroblats is generally associated with poor prognosis in cancer.

We developed and validated a method, called MCP-Counter, which allows to simultaneously quantify the proportions of 10 different cellular populations in human tissues and applied it to human cancers. It allowed to establish a microenvironment-based classification of cancers that we correlated with known molecular classifications in Colorectal and clear cell RenalCell Cancers. We confirmed our data by quantifying tumor infiltrating cells by immunohistochemistry.

In Colorectal Cancer, the molecular and immune classifications confirmed that not only Microsattelite Instable (MSI) tumors, but also a subgroup of Microsattelite Stable (MSS) tumors, are characterized by a favorable immune contexture with high Th1/cytotoxic infiltration. Other subtypes exhibited poor immune infiltation or, in the worst prognostic case, high T cell infiltration in the context of a major inflammatory, angiogenic and desmoplastic reaction. Patients with MSI tumor srespond to immunotherapy with anti-PD1 antibodies. Immuno-therapeutic strategies based on the immuno-molecular classifications for the other subtypes of Colorectal Cancer will be discussed.

In clear cell Renal Cell Cancer, we identified a poor prognosis subgroup with high infiltration of CD8+ T cells which express check-point inhibitors in the presence of PDL-1 and/or PDL-2 expressing tumor cells and a high inflammatory context. In addition, using multiparametric immunophenotyping of tumor-infiltrating T cells, we characterized the lymphocyte populations that concur to poor prognosis.

These analyses form the basis of an unification of molecular and immune classifications of human cancers, challenge our current views of the relationship between the composition of the tumor microenvironment and patient's prognosis, and suggest immunotherapeutic approaches that could benefit subgroups of patients in different cancers.

Clinical relevance of tumor-infiltrating immune cells in neuroblastoma

Doriana Fruci
Bambino Gesù Children Hospital, Rome, Italy

The presence of tumor-infiltrating immune cells is crucial for the development of immunotherapies and the prediction of clinical response for many humans.Recently, we have shown that tumor-infiltrating T cells have a prognostic value greater than, and independent of, the criteria currently used to stage neuroblastoma (NB), the most common pediatric extra-cranial solid tumor accounting for 15% of childhood cancer-related death. We defined an immunoscore, based on the different T-cell subsets, that associates with favorable clinical outcome in NB patients. Moreover, we demonstrated that expression of HLA class I in NB cells is inversely correlated with that of PD-L1, and combined together they represent a novel promising prognostic biomarker for predicting clinical outcome in NB patients.

To further dissect the immune heterogeneity of NB microenviroment, we evaluated the density of infiltrating dendritic cells (iDC), which are crucial for drug-induced anticancer immune response,in the same cohort of NB samples, previously characterized for type, density, and composition of tumor T lymphocytes and expression of tumor PD-L1 and HLA class I. As for T cells, high density of iDCs was correlated with the presence of infiltrating T cells, expression of tumor HLA class I and favorable clinical outcome, suggesting that iDCs may be critical for robust tumor control by improving prediction of patient survival.

Melaiu O, et al. Clin Cancer Res. 2017 Mar 7. doi: 10.1158/1078-0432.CCR-16-2601.
Brandetti E, et al. Oncoimmunology. 2017 Apr 20;6(6):e1316439.
Petroni M, et al. Cell Death Differ. 2016 Feb;23(2):197-206.
Mina M., et al. Oncoimmunology. 2015 Apr 2;4(9):e1019981.
Cifaldi L, et al.Cancer Res. 2015 Mar 1;75(5):824-34.
Cifaldi L, et al. Cancer Res. 2011 Mar 1;71(5):1597-606.
Forloni M, et al. Cancer Res. 2010 Feb 1;70(3):916-24.

Biomarkers in inflammation - relevance to cancer

I.S. Pateras
Department of Histology and Embryology, School of Medicine, National Kapodistrian University of Athens, Athens, Greece

The relationship between inflammation and cancer was introduced by Rudolph Virchow who first coined the term "lymphoreticular infiltrate" of tumors. Over the past 20 years our conception about the role of inflammation during

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carcinogenesis has supported Virchow's view. Hence in 2011 in the revised "Hallmarks of Cancer" Hanahan and Weinberg included the term "tumor promoting inflammation" as an enabling hallmark stressing out the significance of inflammation in cancer development. The currently established link between inflammation and cancer opens a new era in clinical practice. The need to establish biomarkers that reflect in a precise manner inflammation in various clinical settings is increasing. A desirable biomarker should have the following features: non-invasive, convenient, rapid, inexpensive, reproducible, high sensitivity and accurate. In the current presentation we are going to demonstrate ongoing biomarkers reflecting the inflammatory status. Furthermore, we are also moving a step forward suggesting novel markers focusing on cancer based on our understanding on the role of the inflammatory process in human physiology and pathology.

Exploiting CD4+ T cells for adoptive cell therapy in cancer

Marit R. Myhre[1], Pierre Dillard[1], Nadia Mensali[1], Sylvie Pollmann[1], Gunnar Kvalheim[1], Gustav Gaudernack[2], Sébastien Wälchli[1], Else M. Inderberg[1]

[1]Department of Cellular Therapy, [2]Section for Cancer Immunology, Oslo University Hospital - The Norwegian Radium Hospital, Oslo, Norway

T-cell based immunotherapy represents an attractive strategy for the treatment of cancer.
Whereas cellular anti-tumour immune responses have typically been attributed to CD8+ T cells, CD4+ T cells play a critical role in tumour elimination and the priming and maintenance of CD8+ T-cell responses. Combining HLA class I- and class II-restricted TCRs for T-cell redirection may provide a more potent therapeutic effect in adoptive T cell therapy.
Furthermore, HLA class II-restricted TCRs may be of therapeutic value both in haematopoietic malignancies and in melanoma where tumour cells frequently express HLA class II.
We have isolated CD4+ T cells reactive against tumour antigens from patients who experienced clinical benefit from treatment with cancer vaccines targeting universal tumour antigens and frequent neoantigens. Strong T-cell responses against the vaccines or unrelated cancer antigens suggesting epitope spreading correlated with enhanced survival and tumour regression in late stage cancer patients.

These HLA class II restricted T-cell clones recognised target cells loaded with long peptides or protein and for some CD4+ T cell clones we could also show direct tumour recognition.
TCRs were expressed in expanded donor T cells by mRNA electroporation or retroviral transduction and found functional in both CD8+ and CD4+ T cells producing TNF-α, IFN-γ with the capacity of target cell killing.
We also show preliminary *in vivo* data for one of our broadly applicable TCRs recognizing a universal antigen, hTERT, presented on one of the most frequent HLA alleles, HLA-DP4.

Cancer Immunotherapy, not without vaccination

Sjoerd H. van der Burg

Human papilloma virus (HPV)-associated oropharyngeal squamous cell cancer (OPSCC) has a much better prognosis than HPV-negative OPSCC and this is linked to dense tumor immune infiltration. Since the viral antigens may trigger potent immunity, we performed an in-depth analysis of tumor-infiltrating immune cells in HPV16-positive and –negative OPSCC patients. In 64% of the HPV16+ tumors type 1 HPV16-specific T cells were present, albeit with a wide range of frequencies and dominated by CD4+ T cells. Their presence was not only strongly related to a better overall survival, a smaller tumor size and less lymph node metastases but also to a type I oriented tumor microenvironment, including high numbers of activated CD161+ T cells, CD103+ tissue-resident T cells, dendritic cells (DC) and DC-like macrophages, as determined by mass cytometry (CyTOF), flow cytometry and immunohistochemistry. Thus, the viral antigens trigger a tumor-specific T-cell response that shapes a favorable immune contexture related to much better survival. Despite these observations, the HPV-specific T cell response, especially that by CD8+ T cells, is not strong enough in immune responders to completely control tumor outgrowth. Furthermore, HPV-specific T-cell reactivity is absent in ~35% of the HPV+ patients. This may explain why still only a percentage of the HPV+ patients respond to checkpoint therapy. Hence, reinforcement of HPV16-specific T cell reactivity by vaccination is expected to boost this process. To test the hypothesis a single arm phase II trial of ISA101, a synthetic long-peptide HPV16 vaccine and nivolumab was conducted in patients with incurable HPV-16+ cancer. Preliminary data suggest that the efficacy of vaccine-induced T cells can be augmented by anti-PD-1 therapy.

Towards the next generation of cancer vaccines

Carl G. Figdor, Gerty Schreibelt, Kalijn Bol, Harm Westdorp, Angela Vasaturo, Altuna Halilovic, Mark Gorris, Yusuf Dolen, Michael Valente, Loek Eggermont, Roel Hammink, Winald Gerritsen, Martijn Verdoes and Jolanda de Vries

Department of Tumor Immunology, Radboud University Medical Center, The Netherlands

During the past decade we have extensively explored dendritic cell (DC)-based cancer vaccines. DC isolated from a patient are loaded with tumor antigen and immune modulators to activate DC to optimize antigen presentation and T cell stimulation. We now know that this form of immunotherapy is safe and more recently we have also started to use natural DC circulating in the blood instead of monocyte-derived DC. In particular myeloid DC and plasmacytoid DC are a powerful combination, now being tested in a phase III trial.

Because with current DC-based vaccinations a new product must be produced for each patient, we have initiated studies to look for alternatives, where we either can target DC *in vivo* or even replace DC by the generation of 'synthetic DC'. During my talk I will elaborate on these novel cancer vaccine developments.

The cystine/glutamate antiporterxCT: A new cancer stem cell target for anticancer vaccines

Federica Cavallo[1]

[1]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino, Italy.

Previous works in mouse models have shown that vaccines are uniquely effective in preventing the progression of early stages of cancer[1]. When the reaction elicited is against a protein required for the neoplastic progression (i.e. against an "oncoantigen"), chances that a tumor evades the vaccine-induced immune reaction are markedly reduced. Preventive vaccines targeting oncoantigens are able to provide a life-long protection against incipient tumors of different kind[2]. By contrast, in the management of minimal residual disease or when they have to contrast cancer recurrences and metastases, the protective potential of vaccines is limited and has to be further enhanced[3].

A new and rational way to enhance the vaccine efficacy is to target oncoantigens expressed by cancer stem cells (CSC), the cancer cell sub-population which initiates and drives carcinogenesis and is a reservoir for the relapse, metastatic evolution and progression of the disease after treatment, representing a major barrier towards effective cancer eradication.

We have recently identified xCT, the light chain of the cystine/glutamate antiporter system x_c^-, as being overexpressed in CSC from $Her2^+$ and triple negative breast cancer (TNBC)[4]. The reported stabilizing interaction

of xCT with a variant isoform of CD44, one of the cell surface markers associated with CSC, further confirms our data on an increased expression of xCT by CSC. The antiporter system xc- enables the intracellular uptake of cystine, which is required for the synthesis of glutathione, a major cellular metabolite that protects against oxidative and chemical injury and exhibits a variety of other cytoprotective effects. xCT may thus represent an ideal target for breast cancer immune-prevention. Indeed, we have recently demonstrated[4] that vaccination against xCT prevents pulmonary metastases formation and delays early stage subcutaneous tumors in mice challenged with syngeneic CSC from TUBO (Her2+) and 4T1 (triple negative) breast cancer cells. Anti-xCT vaccination thus represents a novel approach for the secondary and tertiary prevention of metastatic breast cancer by targeting CSC.

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Presence of tumor-specific cytolytic T cells in human primary breast carcinoma: consequences for immunotherapy

D. Schröder, J. Carrasco, O. Bricard, G. Hames, N. Remy, M. Kevin, J.-L. Canon, C. Galant, M. Berlière, and P. G. Coulie
de Duve Institute, University of Louvain, 1200 Brussels, Belgium.
Department of Medical Oncology, Grand Hôpital de Charleroi, 6000 Charleroi, Belgium.
Breast Clinic, King Albert II Institute, Cliniques Universitaires Saint-Luc, 1200 Brussels, Belgium.

Background: Immunotherapy through stimulatory antibodies targeting the CTLA-4 or PD-1 pathways has a demonstrated clinical efficacy in a fraction of patients with various cancers. It is likely that the main immune effectors of these therapies are CD8+cytolytic T lymphocytes (CTL) recognizing tumor-specific antigens. Highly antigenic tumors such as metastatic melanomas are often immunogenic, i.e. they stimulate spontaneous anti-tumor CTL responses. This immunogenicity, of which the presence of tumor-infiltrating T cells (TILs) is probably a surrogate marker, might be a predictive marker for clinical benefit to immunostimulatory antibodies. The immunogenicity of primary breast carcinomas for $CD8^+$ T cells has not been studied, but the amounts of TILs have been positively correlated with patients' survival. Here we wished to obtain evidence for the presence of tumor-specific $CD8^+$ T cells in TILs from primary breast carcinomas.

Methods: From 6 tumors (2 $ER^+/HER2^-$, 2 $ER^+/HER2^+$, 2 $ER^-/HER2^-$) we isolated TILs and derived a random set of ±100 $CD8^+$T cell clones that we screened for recognition of candidate tumor-specific antigenic peptides selected through tumor exome sequencing and gene expression profiling. These peptides were encoded by mutated genes or by cancer-germline genes.

Results: For 5 tumors, we screened 61-142 T cell clonotypes for recognition of 23-61 candidate mutated peptides, without any positive result. For the last tumor, 6 out of 57 T cell clonotypes recognized 4 out of 109 candidate mutated peptides but not the corresponding wild-type peptides. This tumor contained more mutations than the other four, and displayed microsatellite instability.

Conclusions: Some human primary breast carcinomas are immunogenic, as one tumor contained at least 10% of tumor-specific cells among the CD8+ TILs. Our observation corroborates the association between high mutation burden and CTL response to mutated tumor antigens. The presence of tumor-specific $CD8^+$ suggests that the corresponding patient could benefit from the currently used immunostimulatory antibodies.

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Optimized cryptic peptides: a universal neoantigen therapeutic cancer approach

Kostas Kosmatopoulos
Vaxon Biotech, 3 rue de l'Arrivée 75015, Paris, France.

Optimized cryptic peptide based cancer vaccines share the two main characteristics of neo-antigens : they escape self-tolerance and are strongly immunogeni because they exhibit a high affinity of HLA-I molecules. Moreover, unlike neo-antigens they can be used for a broad vaccination and are not patient or tumor specific. Vx-001 is the first vaccine using optimized cryptic peptides. It targets the universal tumor antigen TERT and is restricted to HLA-A*0201 expressing patients. Vx-001 has been tested in a randomized phase II study in HLA-A*0201 positive patients with TERT expressing metatstatic Non-Small Cell Lung Cancer (NSCLC) who experienced disease control after front line chemotherapy. We observed a very strong correlation between immune and clinical response. More interesting Vx-001 wasclinically very active in patients with non immunogenic tumors who were resistant to immune check-point inhibitors but not in patients with immunogenic tumors who responded to immune check-point inhibitors. Our results also suggested that Vx-001 can turn tumors resistant to immune check-point inhibitors sensitive to these inhibitors.

Melanoma antigen-specific CD8+ cells in healthy donors and melanoma patients

Paul V. Lehmann
Cellular Technology Limited • 20521 Chagrin Boulevard • Shaker Heights, OH USA

Naïve tumor antigen-specific CD8+ cells typically (with few exceptions) occur in very low frequency in blood, and do not secrete IFN-γ or Granzyme B (GzB). Effector CD8+ cells, capable of cytolysis, secrete GzB in addition to IFN-γ, and due to clonal expansion occur in increased frequencies in blood. Resting CD8+ memory cells secrete IFN-γ, but not GzB, also occurring in increased frequencies in blood. Such resting memory cells can re-express GzB within several days upon antigen re-encounter, converting into effector CD8+ cells. Looking for these well established features of CD8+ cells, we performed IFN-γ and GzB ELISPOT assays to measure the frequency of melanoma antigen (MA)-specific CD8+ cells secreting these analytes after antigen stimulation. MA Tyrosinase (Tyr), gp100, Melan/MART-1, MAGE-A3, and NY-ESO were tested on PBMC of healthy donors (HD) and melanoma patients (MP). Of the above MA, Tyr triggered

IFN-γ secreting CD8+ cells in 7 of 21 (33%) of healthy donors within at 24h *ex vivo*. At this time point these CD8+ cells did not produce GzB yet, however they engaged in GzB production by 72h after antigen stimulation. Therefore, Tyr-specific CD8+ cells in healthy controls are clonally expanded resting memory cells (IFN-γ+/GzB-) that can be reactivated to become effector cells (IFN-γ+/GzB+) within 72h. Such resting Tyr-specific CD8+ memory cells could not be detected in eight MP tested so far. Compared to Tyr, a lower percentage of HD displayed responses to gp100 (2/20=10%), Melan/MART-1 (2/20=10%), MAGE-A3 (4/20=20%, and NY-ESO (1/20=5%). None of the 8 MP tested so far responded to these MA. While unresponsive to MA, MP showed unimpaired CD8+ and CD4+ responses to third party antigens suggesting a selective inactivation of MA-reactive CD8+ cells in MP.

Immunophenotyping the Tumour Microenvironment in FFPE Sections

Robert Stad, PhD
Imaging Sales Development Manager EMEA, PerkinElmer

There has been a rapid growth in the field of tumor immunobiology in recent years as a result of recent successes in cancer immunotherapies, and it is becoming clear that immune cells play many sometimes conflicting roles in the tumor microenvironment. However, obtaining phenotypic information about the various immune cells that play these roles in and around the tumor has been a challenge. Existing methods can either deliver phenotypic information on homogenous samples (e.g., flow cytometry or PCR) or morphologic information on single immunomarkers (standard IHC). We present here a methodology for delivering quantitative per-cell marker expression and phenotyping, analogous to that obtained from flow cytometry, but from cells imaged in situ in FFPE tissue sections.

This methodology combines: the sequential multi-marker labeling of up to 8 antigens using antibodies all of the same species in a single section; automated multispectral imaging (MSI) to remove the typically problematic FFPE tissue autofluorescence and correct cross-talk between fluorescent channels; and an automated analysis that can quantitate the per-cell marker expression, determine the cellular phenotype, count these cells separately in the tumor compartment and in the stroma and provide high-resolution images of their distributions.

Accumulation of myeloid-derived suppressor cells in melanoma microenvironment and their targeting

Viktor Umansky, Mareike Grees, Christoffer Gebhardt and Jochen Utikal
Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg and Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany

Melanoma is known for its fast progression and poor response to current therapies.Insufficient anti-tumor reactivity could be induced by the chronic inflammation that results in immunosuppression and cancer progression. Importantly, most of factors involved in chronic inflammatory reactions could be secreted in course of acute inflammation, inducing the stimulation of T cell-mediated immune reactions. However, a long-term maintenance of the same mediators under chronic inflammatory conditions typical for tumor development induces immunosuppression mediated, in particular,by myeloid-derived suppressor cells (MDSC).Using a ret transgenic mouse melanoma model that closely resembles human melanoma, we found in melanoma lesions increased concentrations of various inflammatory factors associated with the accumulation of MDSC that inhibit T cell anti-tumor reactivity. Upon administration of paclitaxel at low, non-cytotoxic doses in tumor-bearing mice, we demonstrated a reduction of numerous inflammatory mediators in melanoma lesions in association with decreased MDSC frequencies and immunosuppressive functions. These led to the restoration of anti-tumor T cell reactivity and to prolonged mouse survival. We have recently established the production of constructs encoding MHC class I molecules that couples the peptide presentation and activation of dendritic cells (DC). Combination of such DC vaccination with low dose paclitaxel resulted in a significant increase in mouse survival together with a stimulation of anti-tumor reactivity of T cells and decreased MDSC activities.

Low dose paclitaxel was also applied in small group of advanced melanoma patients that were resistant to immunotherapy with checkpoint inhibitors. We found that 4 of 9 treated patients displayed stable disease associated with the restoration of T cell anti-tumor reactivity and down-regulation of MDSC immunosuppressive features. Our data suggest that chronic inflammatory mediators and various populations of myeloid cells are of importance for melanoma pathogenesis and should be inhibited to increase an efficiency of melanoma immunotherapy.

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Immune regulatory programs and chemotherapy susceptibility in monocytic myeloid-derived suppressor cells

Vincenzo Bronte
Immunology Section, Department of Medicine, Verona University Hospital.

Myeloid-derived suppressor cells (MDSCs) are characterized by their myeloid origin, heterogeneous cell composition and ability to regulate negatively adaptive and innate immune responses to cancer. In addition, their presence and frequency in the blood of cancer patients is emerging as a potential prognostic marker to monitor clinical outcome and response to therapy. Among different subsets, monocytic (M)-MDSC are extremely susceptible to low dose chemotherapy, to the point that administration of different agents can increase the efficacy of adoptive cell therapy with tumor antigen-specific CD8+ T cells by eliminating *in vivo* the immune suppressive M-MDSCs. Although endowed with different mechanisms of cell damage, the chemotherapeutic agents effective on M-MDSCs share the ability to down-regulate the expression of the same anti-apoptotic molecule. Surprisingly, not only M-MDSCs are poisoned and need to express this protein for their survival but the very same molecule regulates a complex transcriptional program. Human monocytes infected with lentiviral vectors expressing this apoptotic regulator modulated different genes including *IDO1*, PD-L1, PD-L2, *IL-10*, and IL-4Rα. Moreover, this enforced expression generated strongly suppressive MDSCs, able to inhibit both *in vitro* and *in vivo* T cell activation and proliferation, as well as control the severity of established graft versus host disease in xenogenic mouse models. The connection between apoptosis regulation and immune modulation was further exploited in transgenic mice that constitutively express an active form of the gene in the myeloid cell lineage. This novel, unexpected link between apoptosis and immune regulation in monocytes can have potential applications for the development of immune regulatory and cell-based therapeutic strategies, as well as representing a biomarker for cancer patients.

Myeloid-derived suppressor cells (MDSC): Their ying and yang effects in tumor-bearing obese mice

Suzanne Ostrand-Rosenberg
University of Maryland Baltimore County, Baltimore, MD USA

High fat diet (HFD) and obesity are risk factors for both cancer mortality and the risk of developing cancer. There are numerous factors associated with high fat diet and obesity that are thought to facilitate cancer progression. The chronic low grade inflammation that accompanies obesity is one of the major factors. Myeloid-derived suppressor cells (MDSC) are known facilitators of cancer progression that act by suppressing the activation and function of tumor-reactive T cells and through their production of VEGF and matrix metalloproteases that drive neoangiogenesis and metastasis. Because MDSC quantity and function are driven by chronic inflammation, we have hypothesized that HFD and obesity drive the accumulation and suppressive potency of MDSC which subsequently inhibit antitumor immunity and enhance tumor progression. We have tested this hypothesis using tumor-bearing mice on a HFD or low fat diet (LFD). Since metabolic dysfunction is also associated with HFD and obesity, we have assessed both tumor progression as well as the two hallmarks of obesity associated metabolic dysfunction, insulin tolerance and blood glucose levels. Our results demonstrate that HFD enhances the accumulation of MDSC, which in turn, promote metastatic disease and reduce survival by inhibiting T cell-mediated antitumor immunity. MDSC also promote weight gain by increasing adiposity. However, MDSC also have beneficial qualities in HFD mice because they reduce some of the metabolic dysfunction caused by HFD and obesity. Leptin, an adipokine that regulates appetite satiety and is over-expressed in obesity, plays a major role in MDSC effects by regulating the levels of MDSC. Collectively, these studies demonstrate that through the action of leptin, HFD and obesity drive the accumulation of MDSC which have both detrimental and protective effects in tumor-bearing obese individuals.

Revisiting the role of complement in antitumour immunity: challenges and opportunities

Dimitrios C. Mastellos
Division of Biodiagnostic Sciences and Technologies, I/NRASTES, National Center for Scientific Research "Demokritos", Aghia Paraskevi Attikis, Athens 15310, Greece

For many decades complement was merely perceived as an innate immune effector that augments the cytolytic action of anti-tumour antibodies in the context of cancer immunotherapy (e.g. rituximab). Intriguingly, however, accumulating evidence from studies published in the past decade has pointed to a fascinating paradigm shift: the realization that complement activation within the tumour microenvironment can serve a tumour-promoting role by enhancing T cell immunosuppression and fueling chronic inflammation that promotes tumour immune escape, outgrowth and metastasis. Diverse complement effectors and downstream signaling pathways have been implicated in processes ranging from tumour cell anchorage and proliferation to tumour-associated neoangiogenesis, hypoxia, tumor invasiveness, and metastasis. With new insights being gained from a variety of tumour models, it is becoming clear that the contribution of complement to anticancer immune responses is far more complex than originally thought and appears to be largely contextual, depending on distinct factors such as the tumor's genetic landscape, the inherent capacity of tumour cells to produce autologous complement proteins, the nature of the tumour microenvironment, and the magnitude of complement activation.

Current and emerging aspects of complement's contribution to cancer elimination and progression will be discussed, including novel therapeutic targets and strategies with clinical potential. Particular emphasis will be placed on clinical challenges faced in translating research findings to efficacious tumour cytotoxic modalities and on the emerging promise of combinatorial immunotherapies.

Mechanisms of resistance to cancer immunotherapy

Benoit J. Van den Eynde, Marc Hennequart, Jingjing Zhu, Celine Powis de Tenbossche, Stefania Cané
Ludwig Institute for Cancer Research,
de Duve Institute, Universitécatholique de Louvain,
Walloon Excellence in Life Sciences and Biotechnology (WELBIO)
Brussel, Belgium.

Tryptophan-catabolism mediated by indoleamine 2,3-dioxygenase 1 (IDO1) is a prominent mechanism of tumoral immune resistance. IDO1 expression can be induced by interferon-gamma (IFNγ) in many cells, but can also be constitutive in a number of human tumors. Constitutive IDO1 expression can prevent T-cell infiltration and result in "cold" tumors. We have unraveled the mechanism responsible for constitutive IDO1 expression in human tumors. We found that constitutive IDO1 expression depends on COX-2 and prostaglandin E2 (PGE_2), which, upon autocrine signaling through the EP4 receptor, activates IDO1 via the PKC and PI3K pathways. COX-2 expression itself depends on the MAPK pathway, which therefore indirectly controls IDO1 expression. Most of these tumors carry PI3K or MAPK oncogenic mutations, which may favor constitutive IDO1 expression. Celecoxib treatment promoted immune rejection of IDO1-expressing human tumor xenografts in immunodeficient mice reconstituted with human allogeneic lymphocytes. This effect was associated with a reduced expression of IDO1 in those ovarian SKOV3 tumors, and an increased infiltration of $CD3^+$ and $CD8^+$ cells. These results highlight the role of COX-2 in constitutive IDO1 expression by human tumors and substantiate the use of COX-2 inhibitors to improve the efficacy of cancer immunotherapy, by reducing constitutive IDO1 expression, which contributes to the lack of T-cell infiltration in "cold" tumors, which fail to respond to immunotherapy.

In the autochthonous TiRP melanoma model, which is resistant to all forms of immunotherapy, we found that TiRP tumors recruit and activate tumor-specific $CD8^+$ T cells after adoptive T cell therapy (ACT), but then induce apoptosis of these T cells. This does not occur with isogenic transplanted tumors, which are rejected after ACT. Apoptosis of tumor-infiltrating lymphocytes (TIL) can be prevented by interrupting the Fas/Fas-ligand axis, and is triggered by polymorphonuclear-myeloid-derived suppressor cells, which express high levels of Fas-ligand and are enriched in TiRP tumors. Blocking Fas-ligand increases the anti-tumor efficacy of ACT in TiRP tumors, and increases the efficacy of checkpoint blockade in transplanted tumors. Therefore, TIL apoptosis is a relevant mechanism of immunotherapy resistance, which could be blocked by interfering with the Fas/Fas-ligand pathway.

Reprogramming of the tumor microenvironment by danger signals and innate cells for efficient cancer immunotherapy

Günter J. Hämmerling
Tumor Immunology Program, German Cancer Research Center, Heidelberg, hammerling@dkfz.de

Despite the impressive advances made with checkpoint inhibitors, the clinical success of cancer immunotherapy is still limited. Insufficient T cell infiltration into tumors has been identified as a major hurdle and therefore correlates with poor clinical prognosis. Most human and animal tumors are characterized by the formation of an aberrant tumor vasculature which formed a barrier against T cell infiltration. Thus, in various genetic and transplantation mouse tumor models vaccination or transfer of tumor-specific T lymphocytes failed to eliminate tumors, because of the aberrant tumor endothelium. However, induction of an inflammatory microenvironment in the tumor, e.g. bydanger signals such as local low dose (2Gy) irradiation or immunostimulatory TLR ligands resulted in modulation of the tumor vasculature leading to increased T cell infiltration and tumor eradication. Detailed cell analysis of the tumor microenvironment showed that polarization of immunosuppressive tumor macrophages towards immunostimulatory M1-like iNOS+ macrophages was crucial in this process. These M1 macrophages not only attracted effector T cells by secretion of chemoattractants CXCL9 and CXCL10, but also activated the tumor endothelium via production of nitric oxide.

Moreover, co-transfer of T cells together with other chemoattractant producing innate cells such as activated basophils and eosinophils resulted in strong T cell infiltration. Thus, eosinophils emerge as accessory cells that help to attract T cells into tumors rather than killing the tumor directly, as was commonly believed.

In conclusion, for clinical cancer immunotherapy combination approaches need to be considered in which T cell activation is combined with modulators of the suppressive tumor microenvironment and enhancers of T cell infiltration.

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Immunotherapy of Cancer: triumphs and challenges, and the impact of immunosenescence in humans

Graham Pawelec
Second Department of Internal Medicine, Tübingen University, Tübingen, Germany
Health Sciences North Research Institute, Sudbury, ON, Canada
John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, UK

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 (potentially leading to holes in the repertoire for neoantigens) and ii) "exhaustion" of potentially tumor-specific memory T-cells. Encouragingly, but only anecdotally, accumulated clinical experience (thus far, mostly limited to melanoma treated with anti-CTLA-4 antibodies) suggests that advanced aged does not result in decreased responses or increased side effects. However, the fraction of patients experiencing clinical benefit is low with single-agent anti-CTLA-4 treatment, which in the meantime has been more-or-less superseded by anti-PD-1/PDL-1 treatments, where age effects are not yet clear. As newer agents are licensed, 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* using intracytoplasmic flow cytometry to detect 6 cytokines simultaneously. We found that these peripheral immune signatures were equally prognostic in older patients (>80 years of age). We therefore conclude that immunosenescence should not be a barrier to anti-tumor immunity in elderly people treated with immunomodulatory antibodies, at least for responses targeting shared tumor antigens. It remains to be established whether responses to tumor neoantigens are compromised by immunosenescence, which seems *a priori* more likely, given that responses to shared antigens are memory responses whereas neoantigen-specific immunity would require naïve T cells. Given the current emphasis on neoantigen responsiveness, immunosenescence remains an unexplored concern.

Strong T cell responses after vaccination with HPV16 long peptides for late stage cervical cancer are associated with prolonged survival

Cornelis JM Melief
ISA Pharmaceuticals, Leiden, The Netherlands, Leiden University Medical Center, The Netherlands

Therapeutic vaccination with HPV type 16 synthetic long peptides (HPV16-SLP) results in T cell–mediated regression of HPV16-induced premalignant lesions but fails to install effective immunity in patients with advanced HPV16-positive cervical cancer. We showed that HPV16-SLP vaccination in mice and in patients with advanced cervical cancer patients fosters robust HPV16-specific T cell responses, when combined with chemotherapy (Welters et al. Sci. Transl. Med., 2016). We have now completed a chemo-immunotherapy study in 70 patients with late stage HPV16+ cervical cancer (clinical trials.gov NCT02128126). Three HPV16-SLP vaccine doses were given 2 weeks after the second, third and fourth cycle of standard chemotherapy (carboplatin, AUC 6; paclitaxel 175 mg/ m^2). Cohorts of 12 patients each were vaccinated with each of 4 dose levels (20, 40, 100 and 300 µg/ per peptide) of 13 overlapping HPV16 synthetic long peptides (HPV16-SLP) together covering the length of the 2 E6 and E7 proteins. Two additional cohorts of 6 patients each were vaccinated with the most promising doses of 40 and 100 µg/ peptide. Robust vaccine-induced HPV16-specific T cell responses as assessed by interferon-γ Elispot were observed and were sustained until at least 30 days after the 6th cycle of chemotherapy. In addition the chemotherapy augmented recall responses to microbial antigens. Such robust T cell responses were not noted in previous trials when similar patients were vaccinated without timing of vaccination during chemotherapy. A marked and significant positive correlation was observed between the strength of the vaccine-induced immune response and overall survival. No such correlation was observed between the strength of the T cell response against common recall antigens and survival. In addition a remarkably high proportion of patients survived beyond 2 years after the start of therapy. The results suggest that survival duration is directly related to the strength of the vaccine-induced HPV16-specific T cell response and is not due to generally better immuno-competence.

Therapy monitoring of immune checkpoint inhibitors in patients with metastatic melanomas with PET-CT

Antonia Dimitrakopoulou-Strauss
Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Germany

Immune checkpoint inhibitors (ICI) have revolutionized therapy of metastatic melanomas. The first ICI was ipilimumab, a CLTA-4 inhibitor with response rates of approximately 11% and control disease of 22%. The programmed cell death 1 (PD-1) inhibitors, like pembrolizumab and nivolumab led to longer progression-free survival (PFS) and overall survival (OS) rates. In a phase 3 randomized study with 834 patients the 6-month PFS for pembolizumab was 47% vs. 26.5% for ipilimumab. The 12-month survival rates were 74% for pembrolizumab vs. 58 % for ipilimumab. Overall, PD-1 inhibitors demonstrate prolonged survival and less side effects (1).
Molecular imaging techniques, like PET-based imaging (PET-CT and/or PETMRI) with FDG are in use for staging and therapy monitoring of metastatic melanomas. The idea is to early identify non-responders in order to tailor immunotherapy. First data with ipilimumab demonstrate the usefullness of interim FDG PET-CT, after two cycles, for prediction of therapy outcome. The correct prediction rate of the interim FDG PET-CT was 90% (2). A known problem in immunotherapy monitoring is pseudoprogression due to inflammation. We observed a pseudoprogression under ipilimumab therapy in 9% of the patients (2). Furthermore, we demonstrated that the absolute number of new lesions is the most important parameter for the definition of progress. In a recent study we presented new PET based immune related criteria, the so called PERCIMT (**P**ET **E**valuation **R**esponse **C**riteria for **IM**muno**T**herapy) criteria for the evaluation of the FDG PET follow-up studies. According to these criteria progress is defined by the presence of at least 4 new lesions of any size, or of at least 3 new lesions of at least 1 cm in functional diameter or of at least 2 lesions of at least 1.5 cm (3). Overall, FDG PET is a useful tool for immunotherapy individualization in metastatic melanomas.

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A novel, strong adjuvant for peptide vaccination

Cécile Gouttefangeas[1], Anoop Chandran[1], Henning Zelba[1], Daniel Kowalewski[1], Johanna Bödder[1], Markus Löffler[1], Stefan Stevanović[1], Karl-Heinz Wiesmüller[7] and Hans-Georg Rammensee[1]
[1]University of Tübingen and DKFZ partner site Tübingen, Dept. of Immunology and [2]EMC microcollections GmbH, Tübingen, Germany

Efficient peptide vaccination against cancer requires appropriate vaccine antigens and strong adjuvants. We have previously shown that the bacterial lipopeptide Pam3Cys-Ser-Ser coupled to a vaccine peptide acts as a strong adjuvant for priming virus-specific T cells in mice. Such amphiphilic molecules are not water-soluble and laborious to produce in GMP quality for human application. We have now designed a synthetic lipopeptide named XS15 which is a Pam3Cys derivative easily accessible and water-soluble.
In vitro, we observed that XS15 is able to mature dendritic cells and to activate several immune cell subsets. For *in vivo* application, synthetic peptides representing virus-derived HLA-class I and class II epitopes were mixed with XS15, emulsified in Montanide ISA51, and injected subcutaneously to a healthy volunteer. After a single dose, a granuloma developed at the application site. A PET/MRI analysis performed 6 weeks post-injection showed that this granuloma was metabolically highly active.
Elispot assessment of PBMCs obtained 4 and 6 weeks after injection revealed strong, ex *vivo* detectable, vaccine-specific CD4+ and CD8+ T cell responses; in contrast, PBMCs collected before vaccination showed no reactivity against the HLA-class II peptide and very weak recognition of the HLA-class I epitopes. In the granuloma which

was excised after 6 weeks, we found the injected peptides, immune cells and inflammatory cytokines. Importantly, anti-vaccine effector T cells were also detected at increased frequencies within the granuloma-infiltrating cells, as determined by multimer staining, Elispot and intracellular cytokine staining. Finally, vaccine-specific T cell responses were still detectable Günter J. Hämmerling in the volunteer´s PBMCs 14 months after immunization.

In summary, we describe here the first in-man application of a novel adjuvant for peptide vaccination. A large batch of GMP-grade XS15 is being produced and a first clinical trial in the anti-cancer vaccination setting is scheduled for 2018.

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Abstracts

Participants

Semliki Forest virus-based DNA replicon vaccines targeting cervical cancer

Stephanie van de Wall[1,2], Karl Ljungberg[2], Peng Peng Ip[1], Annemarie Boerma[1], Maria L. Knudsen[2], Hans W. Nijman[3], Peter Liljeström[2] and Toos Daemen[1]

[1]Department of Medical Microbiology, Tumor Virology and Cancer Immunotherapy, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. [2]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden. [3]Department of Obstetrics & Gynecology, University of Groningen, University Medical Center Groningen, The Netherlands

Immunotherapeutic strategies targeting HPV are critical for eliciting effective immunity in patients with (pre)malignant cervical lesions. Alphavirus-based strategies are attractive platforms due to the high-level of transgene expression as well as activating both humoral and cellular immunity. Our group pioneered a recombinant viral vector system based on Semliki Forest virus, an alphavirus, encoding for HPV E6 and E7 (rSFVeE6,7) which is now being evaluated in a phase I trial.
The most striking benefit of this SFV replicon-based vaccine platform compared to DNA-based vaccines is its high potency. We now combined the benefits associated with SFV-based replicon vaccines with the benefits of the lower costs and ease of production of DNA vaccines, by developing DNA-launched RNA replicon (DREP) vaccines. We tested the efficacy of these novel DREP cancer vaccines in mice, targeting cervical cancer. Using intradermal delivery followed by electroporation, we demonstrated that prime-boost with DREP encoding for E6,7 (DREP-eE6,7) or a “shuffled” version of E7 (DREP-eE7sh) induced potent therapeutic anti-tumor efficacy with doses as low as 0.05 ug. This is a 1000-fold lower dose as compared to most studies using conventional pDNA as application in cancer immunotherapy.
This DNA replicon strategy could pave the way for clinical translation of DNA vaccines applicable to a wide range of target antigens.

Antitumor efficacy and PET imaging of rationally designed cancer treatments targeting T cell activation, tumor homing and immune suppression

Oana Draghiciu[1], S.V.Hartimath[2], Annemarie Boerma[1], Baukje-Nynke Hoogeboom[1], Erik F.J. de Vries[2], Hans W. Nijman[3], Toos Daemen[1]

[1]Department of Medical Microbiology, Tumor Virology and Cancer Immunotherapy, University of Groningen, University Medical Center Groningen, Groningen, the Netherland; [2]Department Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; [3]Department of Gynecology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

The clinical efficacy of therapeutic cancer vaccines as monotherapies is limited. Effective cancer immunotherapy-likely requires 1) induction of specific immune responses, 2) intratumoral homing of tumor-specific immune cells and 3) suppression of intratumoral immunosuppression. Here, we report on a combination of treatments targeting all of these aspects. Immune responses were elicited using a therapeutic cancer vaccine based on a Semliki Forest virus vector encoding the oncoproteins E6 and E7 of human papillomavirus (SFVeE6,7). Intratumoral homing was induced using low-dose tumor irradiation; sunitinib was applied to eliminate myeloid-derived suppressor cells (MDSC). This triple-treatment combination dramatically changed the intratumoral immune compartment resulting in a 10,000-fold increased effector-to-suppressor ratio. As a result, the triple treatment strongly enhanced the immunotherapeutic antitumor effect, blocking tumor development altogether and leading to 100% tumor-free survival.

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

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

Anti-cancer T cell cytotoxicity is modulated by the surrounding extracellular matrix

Dorota Ewa Kuczek[1], Anne Mette Hvid Larsen[1], Daniel Hargbøl Madsen[1,2]
[1] Center for Cancer Immune Therapy (CCIT), Copenhagen University Hospital, Herlev, Denmark
[2] Presenter of study, Head of Tumor Stroma and Matrix Immunology group at CCIT, Copenhagen University Hospital, Herlev, Denmark

A high tumoral extracellular matrix (ECM) density correlates strongly with poor prognosis in many types of cancer including breast cancer and colon cancer. Previous investigations of the underlying mechanism behind this effect have mainly focused on the potential regulation of cancer cell proliferation and invasiveness. In this study we have investigated if a high ECM density can modulate the immune environment in tumors and thereby limit the ability of tumor infiltrating lymphocytes (TILs) to kill cancer cells.

Using a 3D cell culture system, we have investigated if macrophages or T cells respond to the density of the surrounding ECM. Transcriptomic analysis of macrophages revealed that this cell type indeed responds to the ECM density. Interestingly, this involved changes in several immune modulating molecules including COX-1 and COX-2. The functional implications of these changes are currently under investigation.

T cell culture in different ECM densities did not significantly alter cellular proliferation but whole-trancriptome analysis, however, revealed dramatic changes on the gene-expression level. The changes particularly involved clear downregulation of cytotoxic activity markers and upregulation of regulatory T cell markers. The chemokine profile of the 3D cultured T cells was also dramatically altered by the density of the ECM.

To examine if the observed transcriptional changes had any functional implications, we embedded and cultured TILs isolated from a dissected melanoma in ECM of various densities. Subsequently, the TILs were extracted and assayed for their ability to lyze an autologous melanoma cell line. Consistent with the observed transcriptional changes,TILs that had been cultured in a high density ECM demonstrated a much reduced cytotoxic activity.

The study suggests the existence of a new and conceptually different mechanism of T cell activity regulation, which could have great importance in cancer, and potentially the underlying mechanism could be targeted in order to improve immunotherapy efficiency.

Collagen Density Regulates the Immune Modulatory Activity of Macrophages

Larsen AMH[1], Madsen DH[1,2]
[1] Center for Cancer Immune Therapy (CCIT), Herlev Hospital,DK-2730 Herlev, Denmark
[2] Finsen Laboratory/BRIC, DK-2200 Copenhagen N, Denmark

Immunotherapy is a new promising way of treating cancer, using different components of the immune system, to induce T cells to fight cancer. Unfortunately, an immunosuppressive tumor microenvironment often limits the efficiency of the treatment, through incompletely understood mechanisms. Tumor progression is often accompanied by the deposition of a high-density collagen-rich extracellular matrix. The amount and structure of this new tumor specific collagen has been associated with a poor prognosis of cancer, but the reason for this is still largely unknown. Previous studies have shown that the extracellular matrix can affect various cell types. However, it has not been investigated how the extracellular matrix affects one of the main cellular components of the tumor microenvironment, macrophages. Tumor-associated macrophages often acquire an anti-inflammatory phenotype, which is known to support tumor growth by suppressing the activity of tumor-infiltrating T cells.

We are investigating if tumor collagen promotes T cell suppression through its effects on tumor infiltrating macrophages.

To examine this, macrophages were cultured in 3D collagen gels of low and high densities, mimicking a tumor of good and poor prognosis, respectively. Furthermore, co-culture experiments with isolated murine T cells were conducted in order to investigate the relationship between increased collagen density, anti-inflammatory macrophages, and suppression of T cells.

Promising results have been obtained, revealing how collagen affects the gene expression profile and consequentlyfunctional capacities of macrophages. Macrophages grown in high density collagen gels are capable of inhibiting Tcell proliferation to a greater extent than macrophages grown in low density collagen gels.The obtained knowledge can reveal novel strategies for modulating the immune environment, thus increasing the efficiency of cancer immunotherapy.

Is the activity of tumor-infiltrating T cells modulated by colon cancer cells?

Ch. Delle[1], D.H. Madsen[1]
[1]Center for Cancer Immune Therapy (CCIT), Dept. of Haematology, Herlev Hospital, Herlev Ringvej 75, opgang 81, etage 05, DK-2730 Herlev

In colon cancer a high number of infiltrating T cells is highly associated with a good prognosis. An immunosuppressive tumor microenvironment, however, prevents the T cells from efficiently killing the cancer cells. It is incompletely understood how this immunosuppressive milieu is generated. Earlier publications have indicated mutual influences of the cytokine profiles of cancer cells and macrophages *in vitro* but it was not investigated if these interactions lead to increased T cell suppression. By performing indirect and direct co-culture we investigated the ability of a panel of colon cancer cell lines to affect T cell proliferation. Using BrdU-based flow cytometry analysis we showed that four out of six colon cancer cell lines were able to suppress T cell proliferation in a cell-cell-contact dependent manner. Further co-culture experiments investigated how colon cancer cells (ccc) modulate the phenotype of monocytes. Flow cytometry analysis showed no changes in the expression levels of the immune modulating molecules PD-L1 and PD-L2. Noticeably Cd11b and in particular the mannose receptor were upregulated on monocytes through soluble factors indicating that these monocytes mature and might change into more immunosuppressive M2-polarized macrophages. Performing size fractionation of conditioned media of ccc we could determine the soluble factor(s) responsible for these changes in the fraction between 30-100 kDa. Ongoing experiments involving pre-co-cultivation of monocytes with ccc and then afterwards co-culturing these monocytes with T cells will reveal if the interaction between ccc and monocytes lead to enhanced immunosuppressive activity of the monocytes. In later stages of the project, the immunosuppressive mechanisms may be identified by targeted analysis of factors known to induce an immunosuppressive macrophage phenotype.

The Danish Cancer Society Kræftens Bekæmpelse supported Christine Delle for this project with a scholarship for students.

TERS -routine application of a new tool for standardizing functional T-cell assays

Maren Eckev, Tatjana Teck, Pavlo Holenya, Ulf Reimer, Florian Kern, Holger Wenschuh
JPT Peptide Technologies GmbH, Berlin, Germany

Immunological assays for monitoring antigen-specific T-cell responses like Elispot, ICS, or MHC-multimer staining, are complex and prone to significant variation at all assay stages (e.g. sample acquisition, storage, shipment, processing, and analysis). However, these assays are generally poorly standardized. In particular, the lack of external assay standards reduces the comparability of results between runs and across institutions. A recently established technology provides a new type of reference T-cell sample based on T-cell-receptor-engineering. These T-cell engineered reference samples (TERS) can be adjusted with respect to the frequency of specific TCR-bearing T-cells in the sample, even permitting the use of high and low controls from the same source in parallel. They deliver stable signals over time and can be used in connection with stimulation based assays (Elispot, ICS) or MHC-multimer staining. The recent commercial availability of TERS kits with different TCR-specificities allows users to produce their own tailor-made batches of TERS and is likely to become a game changer in regards to the use external standards for the mentioned assay platforms. Here, we show our initial results produced with commercially available TERS kits, highlighting their usefulness for example in assay calibration and assay monitoring over time. The use of TERS facilitates the detection of even small variations in assay performance and may ultimately allow users to accept or reject assay runs based on an objective control. Our data showcases both the ease of use and the robustness of this approach on different platforms and highlights the advantages associated with proper assay standards.

TENM4 emerges as a potential target for Triple Negative Breast Cancer Stem Cells immune-targeting.

Roberto Ruiu[1†], Maddalena Arigoni[1†], Federica Riccardo[1], Laura Conti[1], Stefania Lanzardo[1], Raffaele Adolfo Calogero[1], Federica Cavallo[1] and Elena Quaglino[1].
[1]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy.
[†]These authors equally contributed to this work.

Triple-negative breast cancer (TNBC) is insensitive to some of the most effective therapies for other breast cancers, including endocrine and HER2-directed therapies, and specific treatment are currently lacking. As patients with TNBC usually experience a quicker relapse and metastatic progression compared to other subtypes, we hypothesized that cancer stem cells (CSC) could play a central role in TNBC. This prompted us to search for TNBC CSC-associated molecules to be used as targets for anti-cancer vaccination.

To enrich the CSC population, we established tumorsphere cultures from murine (4T1) and human (HCC1806) mammary cancer cell lines. RNA-Seq was used to identify differences in gene expression between tumorspheres and their monolayer counterparts. By focusing on upregulated genes, in particular those coding for surface molecules suitable for antibody-inducing vaccination approaches, we selected Teneurin-4 (TENM4) as a candidate target.

Meta-analysis of publicly available datasets revealed that TENM4 mRNA is upregulated in both invasive lobular and invasive ductal carcinoma specimens compared to normal breast, and that high expression of TENM4 in TNBC patients shows a trend of correlation with a shorter relapse-free survival. TENM4 upregulation in tumorspheres was confirmed by both RT-PCR and WB, and TENM4 silencing through RNAi significantly impaired the tumorsphere-forming potential of 4T1 cells. Interstingly, focal adhesion kinase (FAK) expression and phosphorylation are increased in 4T1 tumorspheres compared to parental cells andTENM4 silencing also led to a decrease in the phosphorylation of FAK.

Since FAK has been linked by others to cancer stem-like features and tumor-initiating potential, our results seem thus to indicate that the stem-like status of TNBC cells is accompanied by an increase of TENM4, and FAK may represent the link between TENM4 and cancer-stem cell phenotype. Further experiments will help us to better define the role TENM4 as a potential therapeutic target.

New targets for the immunotherapy of adult B-acute lymphocytic leukaemia

Laurie Freire-Boullosa[a,b], Stephanie Bonney[c], Laurence Orchard[c], Evelien Smits[b], Ken Mills[d], Kim Orchard[c], Barbara-ann Guinn[a*]
[a]School of Life Sciences - Biomedical Science, University of Hull, Hull, [b]Laboratory of Experimental Haematology, Vaccine and Infectious Disease Institute, University of Antwerp, Belgium, [c]Department of Haematology, Southampton University Hospital, Tremona Road, Southampton, SO16 6YD, England, [d]Centre for Cancer Research and Cell Biology, Queen's University Belfast, BT7 7AE, Northern Ireland.

Introduction
Acute lymphoblastic leukaemia (ALL) is a form of leukaemia characterized by excess lymphoblasts in the bone marrow. The most effective treatment to date is allogeneic stem cell transplant which can improve overall survival rates and may in part be due to a 'graft-versus-leukaemia' effect. However few of the cancer antigens have been identified in adult B-ALL which could act as targets for immunotherapy.

Methods
We performed antibody specific profiling on sera samples from nine adult B-ALL patients and nine age and sex-matched healthy donor controls. Signals from 9,000 peptides were analysed on the ProScanArray using ProtoArray® Prospector v5.2 software. The mean value and standard deviation of each signal was calculated to produce a z-score and the five most promising antigens identified. We used an existing microarray dataset (GSE38403)[1] to examine whether the expression of the antigens we had identified as promising, by the proto-array and in silico methods, were frequently expressed in 215 B-ALL patient samples and correlated with survival. RT-PCR and ICC were used to confirm the expression of these genes in our patient samples.

Result
By RT-PCR we have examined a total of 20 different antigens in nine presentation B-ALL patient samples and three healthy volunteers. By RT-PCR we found that the cancer-antigens WT1(n=3/9) and Survivin(n=6/9) were transcribed most frequently in patient samples but not healthy donor samples (n=0/5). By microarray analyses Survivin (p=0.013, ANOVA) and Endoglin (p=0.015, ANOVA) were frequently over-expressed in B-ALL patient samples versus normal PreB cells. We have also found that having either or both HAGE and SSX2IP gene expression were poor prognostic markers for the probability of overall survival, although neither reached statistical significance.

Conclusion
Further analysis of patient samples and healthy donor lymphoblasts will determine whether Endoglin and Survivin remain the most promising targets for the immunotherapy of adult B-ALL.

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Can a cancer-testis antigen act as biomarker for early stage ovarian cancer?

Ghazala Khan[a], Suzanne Brooks[b], Ken Mills[c], Barbara-ann Guinn[d*]
[a]Department of Life Sciences, University of Bedfordshire, Luton LU1 3JJ, [b]Biomedical Imaging Unit, Southampton University Hospital, Tremona Road, Southampton, SO16 6YD, [c]Queens University Belfast, [d]School of Life Sciences - Biomedical Science, University of Hull, Hull, HU6 7RX, U.K.
**B.Guinn@hull.ac.uk*

Introduction: Ovarian cancer (OVC) affects approximately 7000 women in the U.K. every year. It can occur at any age but is most common after menopause. Diagnosis at an early stage of disease greatly improves the chances of effective treatment however, diagnosis tends to be in the later stages of disease when patients present with pelvic or abdominal pain, urinary frequency or urgency, increased abdominal size or bloating. A diagnosis of OVC is usually confirmed by a pelvic examination, transvaginal ultrasonography and detection of carbohydrate antigen 125 (CA125) in the tumour tissue. However, CA125 has proven to be non-specific with variable expression between patients, which appears to work better as part of a panel to improve specificity, sensitivity (100%) and differentiation of OVC from endometriosis.

Methods: Using immunohistochemistry we examined the expression of a panel of tumour antigens including variants of the cancer-testis antigen, PASD1, and ovarian cancer protein (OCP), as well as the standard biomarkers for OVC, CA125, HE4 and WT1, in paraffin-embedded OVC microarrays containing 208 samples. Scoring was performed in a single blinded fashion.

Results: We found OCP to be expressed at an intensity and frequency that exceeded that of CA125, HE4, WT1 or PASD1. To confirm this expression we used two additional commercially-available antibodies that recognised OCP and demonstrated that this expression was reproducible and restricted to OVC with little or no expression in adjacent, healthy ovarian or endometrial tissues, or indeed disease or inflamed endometrial tissue.

Conclusion: We have identified a cancer-testis antigen that is more frequently and more intensely expressed in presentation Stage I and II OVC than CA125, HE4 and WT1. We now wish to determine whether OCP can be detected in stage III and IV OVC at disease presentation.

Tryptophan 2,3-dioxygenase, an enzyme involved in tumour immune escape, is positively regulated by its substrate tryptophan.

Klaessens S[1], De Plaen E[1], Stroobant V[1] and Van den Eynde B[1].
[1]Ludwig Institute for Cancer Research, Brussels ; Belgium. de Duve Institute, Université catholique de Louvain, Brussels, Belgium

Tryptophan catabolism catalysed by enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) represents an important mechanism of peripheral immune tolerance by inhibiting T cell proliferation. The IDO1 gene is expressed in the placenta and is mainly involved in immunologic functions, while TDO2 is expressed in the liver and regulates the systemic concentration of tryptophan. Previously, our group has revealed that many human tumours express constitutively the IDO1 and TDO2 genes and that their activity contributes to tumour immune escape.

Here, we show that the amount of TDO protein in human tumour cell lines which express TDO2 and mouse hepatocytes, is regulated by a feedback mechanism. When tryptophan content decreases in their environment, the amount of TDO decreases significantly, thereby halting tryptophan degradation. We found that it is possible to prevent this decline by treating tumour cells with a high concentration of tryptophan or by inhibiting the enzymatic activity of TDO.

This feedback loop is still active when tumour cells are treated with cycloheximide, a ribosome inhibitor. Consequently, tryptophan concentration influences the activity of TDO by regulating its half-life. Furthermore, we found that some cullin RING ubiquitin ligases and the proteasome are responsible for the degradation of TDO in the absence of tryptophan.

Finally, we show that tryptophan stabilizes the tetrameric conformation of TDO that allows degradation of many substrates at the same time. When the tryptophan concentration falls below a threshold (60-80µM), the structure of TDO evolves into a monomeric conformation. We believe that the tetrameric conformation of TDO could hide a degron recognized by cullin RING ubiquitin ligases.

Together, our results support a feedback mechanism that controls the half-life of TDO, thereby preventing excessive tryptophan degradation in low-tryptophan conditions. Such a mechanism is perfectly in line with the main function of hepatic TDO, which is to control systemic tryptophan levels.

Vaccination-induced skin-resident and circulating memory CD8+ T cells collaborate to mediate broadprotection against cutaneous melanoma

Felipe Gálvez-Cancino[1],Ernesto Lopez[1], Evelyn Menares[1], Ximena Díaz[1], Sofía Hidalgo[1], Pablo Caceres[1], Marcela Alcantara[2], Juliana Idoyaga[2], Alvaro Lladser[1]
[1] Laboratory of Gene Immunotherapy, Fundacion Ciencia & Vida, Santiago,Chile.
[2] Department of Microbiology and Immunology, Stanford University, CA, USA.

Long-lasting memory CD8⁺ T cells have the potential to control primary and disseminated tumors. Resident-memory CD8+ T (Trm) cells stably reside in non-lymphoid tissues and mediate potent protective immunity. However, their specific contribution to antitumor immunity remains poorly understood. Moreover, vaccination strategies that efficiently generate Trm cell responses are expected to improve protection against tumors. Here, we demonstrated that intradermal vaccination with DNA-encoded or DC-targeted protein antigens efficiently induced antigen-specific Trm cells, which accumulated in vaccinated and distant non-vaccinated skin, and were resistant to *in vivo* antibody-dependent depletion. Intradermal, but not intraperitoneal, vaccination generated memory precursors expressing skin-homing molecules and Trm cell responses in skin that suppressed the growth of B16F10 melanoma tumors, independently of circulating memory CD8+ T cells. Interestingly, Trm-mediated rejection of B16F10 melanoma engineered to express a model neoantigen led to the generation of secondary cytotoxic CD8+ T cell responses against a melanoma-derived self-antigen, providing protection against re-challenge with B16F10 cells lacking neoantigen expression. This work highlights the therapeutic potential of vaccination-induced Trm cells against skin malignancies and their cooperative role with circulating CD8+ T cells to further broaden and strengthen antitumor immunity, potentially controlling antigen-loss escape mutants.

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Exploiting CD4+ T cells for adoptive cell therapy in cancer

Marit R. Myhre[1], Pierre Dillard[1], Nadia Mensali[1], Sylvie Pollmann[1],Gunnar Kvalheim[1], Gustav Gaudernack[2], Sébastien Wälchli[1], Else M Inderberg[1]
[1]Department of Cellular Therapy, [2]Section for Cancer Immunology, Oslo University Hospital-The Norwegian Radium Hospital, Oslo, Norway

T-cell based immunotherapy represents an attractive strategy for the treatment of cancer.
Whereas cellular anti-tumour immune responses have typically been attributed to CD8 T cells, CD4+ T cells play a critical role in tumour elimination and the priming and maintenance of CD8 T-cell responses. Combining

HLA class I- and class II-restricted TCRs for T-cell redirection may provide a more potent therapeutic effect in adoptive T cell therapy.
Furthermore, HLA class II-restricted TCRs may be of therapeutic value both in haematopoietic malignancies and in melanoma where tumour cells frequently express HLA class II.
We have isolated CD4+ T cells reactive against tumour antigens from patients who experienced clinical benefit from treatment with cancer vaccines targeting universal tumour antigens and frequent neoantigens. Strong T-cell responses against the vaccines or unrelated cancer antigens suggesting epitope spreading correlated with enhanced survival and tumour regression in late stage cancer patients.
These HLA class II restricted T-cell clones recognised target cells loaded with long peptides or protein and for some CD4+ T cell clones we could also show direct tumour recognition.
TCRs were expressed in expanded donor T cells by mRNA electroporation or retroviral transduction and found functional in both CD8+ and CD4+ T cells producing TNF-α, IFN-γ with the capacity of target cell killing.
We also show preliminary *in vivo* data for one of our broadly applicable TCRs recognizing a universal antigen, hTERT, presented on one of the most frequent HLA alleles, HLA-DP4.

Genetically enhanced T cells with IL2Rα lead to increased survival and proliferation

Thomas W. Smith Jr., David C. Murray, Mark P. Rubinstein, Michael I. Nishimura
Department of Surgery, Loyola University Medical Center; Department of Surgery, Medical University of South Carolina

Adoptive T cell therapy has shown promising outcomes with multiple malignancies, including melanoma. The goal of adoptive T cell transfer is to obtain patient-derived tumor reactive T cells, expand the population *ex vivo*, and transfuse back to the patient. Current difficulties with adoptive T cell therapy center on tumor reactive T cells' competition with endogenous T cells for growth factors and cytokines, leading to decreased function and persistence. To increase efficacy of tumor reactive T cells, patients are given preparative lymphodepletion and high dose IL-2. Lymphodepletion and high dose IL-2 are extremely toxic and morbid, and cause multiple organ dysfunction in a significant portion of patients. Instead of transferring pre-existing tumor reactive T cells, our lab generates tumor reactive T cells by genetically modifying peripheral blood T cells with a tumor reactive T cell receptor (TCR). Our lab has previously characterized two HLA-A2 restricted gp100:209-217 reactive T cell clones, R6C12 and T4H2, with the higher affinity T4H2 TCR being cloned from a $CD3^+$, $CD4^-$, $CD8^-$ T cell while lower affinity R6C12 TCR is cloned from a $CD3^+$, $CD4^-$, $CD8^+$ T cell. The goal of this current study is to enhance the genetically modified T cells' persistence, potentially negating or reducing the need for lymphodepletion and IL-2 therapy, by transducing the IL2Rα and the T4H2 or R6C12 TCRs. Preliminary data has shown co-transduced T4H2 or R6C12 TCR with IL2Rα T cells have similar proliferation and sensitivity when supported with high concentration IL-2, however in low concentration to no IL-2 support, the IL2Rα T cell constructs outcompete the wild type T4H2 or R6C12 TCR. Further *in vitro* and *in vivo* work will help identify the role of cytokine receptors, including IL2Rα, to augment persistence in patients undergoing adoptive T cell therapy.

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Chemoimmunotherapy using autologous dendritic cell vaccines for the treatment of recurrent glioblastoma multiforme

Marius Strioga[1], Alvydas Česas[2,3], Neringa Dobrovolskienė[1], Jan. A Krasko[1,3], Vita Pašukonienė[1]
[1] National Cancer Institute, Vilnius, LITHUANIA
[2] Department of Oncology, Klaipeda University Hospital, Klaipeda, LITHUANIA
[3] JSC "Froceth", Vilnius, LITHUANIA

From May 2013 to May 2016,fourty patients with the first recurrence of glioblastoma multiforme (GBM) were treated at Klaipeda University Hospital and National Cancer Institute (Lithuania). Patients had GBM recurrence >6 months after completion of treatment for primary GBM, including complete tumor resection, followed by chemoradiation therapy and 6 cycles of adjuvant temozolomide monotherapy.

Standard treatment for recurrent GBM included complete tumor resection followed by 6 cycles of temozolomide. Patients in the investigative group (n=20) received standard treatment combined with autologous monocyte-derived, tumor-lysate loaded, mature DCs on the basis of hospital exemption at National Cancer Institute.They received 6 doses of DC vaccine (5×10^6 DCs per dose) injected intradermally and subcutaneously (50:50) in the upper arm. Starting from the 2nd cycle of temozolomide, the initial four vaccine doses were injected on day 14 of each 28-day chemotherapy cycle. The remaining 2 doses were injected monthly after the completion of chemotherapy. Two immune adjuvants were used with DC vaccination, including topical imiquimod 5% crème with each vaccine dose on days 0 and 1 and low-dose (3×10^6 UI) subcutaneous interferon alpha-2a used with the last 2 DC vaccine doses on days 0 and 3.Control group patients (n=20) received standard treatment alone at Klaipeda University Hospital. They were matched to the investigative group patients by age, clinical parameters and performance status.

All patients, experiencing further disease recurrences received the same next-line treatment, including biweekly irinotecan (for 2nd GBM recurrence) and oral lomustine every 6 weeks (for 3rd GBM recurrence). Contrast-enhanced brain MRI was performed every 3 months orat any time point if neurological symptoms manifested indicative of GBM recurrence. Progression-free survival (PFS) was defined as survival from the start of postoperative temozolomide until the 2nd GBM recurrence. Overall survival (OS) was defined as survival from the start of postoperative temozolomide until patient's death or the last evaluation date for living patients.

Results: Median follow-up was 15 months (range 10–42 months). There was no difference in mPFS between the investigative and control groups (7.75 months versus 7 months, respectively). However mOS in the investigative and control groups was 19.5 months versus 13.5 months, respectively (p=0.0001). In the investigative group,12-month, 18-month, 24-month,and 36-month survival rate was 100%, 80%, 30% and 10%, respectively, whereas in the control group 12-month survival rate was 80%, and no patient survived 18 months. At time of analysis (Jun 2017), 13 patients (65%) were alive in the investigative group and 2 patients (10%) in the control group. mOS from the time of diagnosis of primary GBM was 37.5 months in the investigative group and 31 months in the control group (p=0.0001).

Conclusions: our preliminary data indicate that immunotherapy with DCs is an effective treatment option in combination with chemotherapy for patients with resectable recurrent GBM.

Increased expression of activating receptors and recovery of NK cell function in Acute Myeloid Leukaemia patients after *in vitro* culture with IL-15

Beatriz Sanchez-Correa[1], Juan M. Bergua[2], Alejandra Pera[3], Carmen Campos[3], Maria Jose Arcos[2], Helena Bañas[2], Esther Duran[4], Rafael Solana[1,2] and Raquel Tarazona[1]

[1] *Immunology Unit, University of Extremadura, Cáceres, Spain.*
[2] *Department of Haematology, Hospital San Pedro de Alcantara, Cáceres, Spain,*
[3] *IMIBIC - Reina Sofia University Hospital - University of Cordoba, Spain.*
[4] *Histology and Pathology Unit,Faculty of Veterinary, University of Extremadura, Cáceres, Spain*

Natural killer (NK) cells constitute an important area of research for hematologic malignancies, because this subpopulation is able to kill target cells spontaneously without previous sensitization, representing a novel tool for the treatment of cancer. Acute myeloid leukaemia (AML) patients show abnormal NK cytolytic function due in part to decreased expression of activating receptors or defective expression of ligands for NK cell activating receptors on target cells. The increased expression of inhibitory receptors on NK cells also contribute to blast escape from NK cell cytotoxicity. New immunotherapies are focused in identifying factors that could increase the expression of NK cell activating receptors, to counteract inhibitory receptors expression, and therefore, to improve the NK cell cytotoxic capacities against tumour cells.

In this work, we analyse the effect of *in vitro* culture with IL-15 on the expression of NK cell-activating receptors NKp30, NKp46, DNAM-1 and NKG2D. Our results showed that IL-15 increased the surface expression of NKp30 on NK cells from healthy donors and AML patients with the consequent improvement of NK cell cytotoxicity. It has been previously shown that NK cell interaction with immature dendritic cells induces their maturation. Here, we observed that IL-15 can enhance this effect probably mediated by NKp30 upregulation on NK cells.

These results support the relevance of IL-15 to induce functional active NK cells in AML patients with enhanced capacity to destroy leukemic cells and induce DCs maturation. However, the finding of high levels of IL-15 in AML patients and the possibility that IL-15 acts as a growth factor in leukaemia blasts has to be considered for *in vivo* use of this cytokine. A better understanding of IL-15/IL15R axis will allow the identification of novel therapeutic strategies directed to increase IL-15 immunomodulatory properties while avoiding its harmful effects.

ImmTAC™: From TCR discovery to bi-specific immunotherapeutic agents for the treatment of cancer

Claudia Wurzenberger, Rute Sousa, Nathaniel Liddy, Giovanna Bossi, Jane Harper, Joseph Dukes, Samantha Paston, Frayne Bianchi, Tara Mahon, Malkit Sami, Emma Baston, Brian Cameron, Andrew Johnson, Namir Hassan, Annelise Vuidepot and Bent Jakobsen.
Immunocore Ltd., 101 Park Drive, Milton Park, Abingdon, Oxfordshire, OX14 4RY, UK. Website: www.immunocore.com

Immunotherapeutic agents that are able to induce infiltration and activation of T cells into tumours have the potential to eradicate the tumour. However, self-tolerance toward tumour-associated antigens via thymic selection and a suppressive microenvironment of the tumour limit the effectiveness of natural T cells. To overcome poor immunogenicity of the tumour, we have developed ImmTAC molecules (Immune-mobilising monoclonal TCRs Against Cancer); a unique platform of bi-specific molecules composed of an affinity enhanced monoclonal T cell receptor (mTCR) combined with an anti-CD3 scFv, that re-directs polyclonal T cells to destroy cancer cells with high potency and specificity.

In order to generate a specific ImmTAC, we isolate T cell clones that recognise a validated cancer specific peptide-HLA complex. After sequencing the alpha and beta chains, an additional disulphide bond is introduced and both chains are expressed in bacterial cells and refolded as soluble TCRs. Specific binding to the target peptide-HLA is verified *in vitro* by Surface Plasmon Resonance (SPR). Subsequently, we dramatically enhance the affinity of the TCR up to a million-fold by introducing mutations in the complementarity determining regions (CDRs) using phage display.

Our lead clinical candidate, IMCgp100, targets the HLA-A2/gp100$_{280\text{-}288}$ epitope presented by melanoma cells. IMCgp100 is currently in a Phase I/II clinical trial for advanced malignant melanoma and is undergoing pivotal studies for uveal melanoma.

Wharton's Jelly Mesenchymal Stem Cells: A Novel Approach for Cancer Treatment

Devetzi Marina[1], Goulielmaki Maria[1], Adamaki Maria[1], Christodoulou Ioannis[1]* and Vassilis Zoumpourlis[1]*

[1] *Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB), National Hellenic Research Foundation (NHRF), 48 Vasileos Konstantinou Ave., 11635 Athens, Greece*
** Corresponding authors: e-mails: vzub@eie.gr, ichristo@eie.gr*

Abstract

Stem cells form a highly heterogenous population with unique properties. During the last decades, the discovery that mesenchymal stem cells (MSC) are able to migrate towards tumors and interact with cancer cells *in vivo*, a property also known as homing, has highlighted them as putative candidates for use in cancer cytotherapy. Therapeutic strategies based either on cell-to-cell contact or on paracrine effects of MSC, have utilized genetically modified MSC (GM-MSC) as delivery vehicles for anti-cancer molecules and genes, or naive MSC (nMSC) of various origins for cancer treatment. GM-MSC have shown good efficacy in preclinical models but their use has been associated with safety risks. The use of nMSC is also controversial, as they have been reported either to promote or suppress tumor growth. Based on the results of a highly detailed review and meta-analysis of the available bibliographic data conducted by our group, we have concluded that the outcome of cancer cytotherapy approaches is largely dependent on various parameters during combination of MSC population and cancer cell type targets. Subsequently, we have been able to highlight a set of optimal conditions, in which the tumor suppressive action of MSC predominates. According to these observations, we evaluated the paracrine effects of various MSC populations on the proliferation and survival of four selected cancer cell lines in indirect co-culture and 3D culture systems. In turn, we examined the transcriptome of the two cell lines, in which the anti-tumorigenic effect was most prominent, in an effort to exploit the expression pathways and regulatory networks contributing to the observed anti-cancer activity. Total mRNA analysis of cancer cells revealed a significant target dependence of the anti-tumorigenic effects displayed by MSC, which are mediated by different pathways (metabolic, cell cycle and apoptosis regulation, innate immune response) and are initiated by the same MSC secretome-derived stimuli.

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