PIVAC-23

28th–30th September 2023

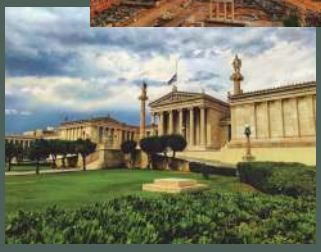
St. Savvas Cancer Hospital, 171 Alexandras Avenue 11522
Athens - Greece

22nd International Conference on Progress in Vaccination Against Cancer

Athens

Organized by
"St. Savvas" General Cancer - Oncology Hospital of Athens








Scientific Program

PIVAC-23
28th–30th September 2023
St. Savvas Cancer Hospital, 171 Alexandras Avenue 11522, Athens - Greece

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ΤΟΜΕΑΣ ΚΟΙΝΩΝΙΚΗΣ ΠΟΛΙΤΙΚΗΣ

ΠΜΣ: ΔΙΟΙΚΗΣΗ ΚΑΙ ΔΙΑΧΕΙΡΙΣΗ ΥΠΗΡΕΣΙΩΝ ΥΓΕΙΑΣ ΚΑΙ ΚΟΙΝΩΝΙΚΗΣ ΦΡΟΝΤΙΔΑΣ

WELCOME LETTER

We welcome you to the PIVAC meeting "The 22nd International Conference on Progress in Vaccination Against Cancer" (PIVAC-23), 28th - 30th September 2023, Athens, Greece at the Saint Savvas Cancer Hospital, 171 Alexandras avenue, 11522, Athens.

PIVAC 23 aims to bring together scientists working on translational and clinical cancer research for three days of presentations on the most recent advances in these fields including (i) immunotherapies (cellular, immune checkpoint blockade, vaccines); (ii) innate immunity and tumor control; (iii) host- tumor interactions in the tumor microenvironment; (iv) in vitro and in vivo models for cancer immunotherapies (v) combinational cancer therapy approaches; and (vi) prognostic and predictive biomarkers in tumors and immune cells.

Improved knowledge of genomic and molecular alterations in tumors has significantly contributed to improved clinical outcomes. However, many hurdles remain to be overcome. Alterations in the tumor genome are associated not only with immune stimulation but also with an immune suppressive microenvironment and tumor immune escape mechanisms. Currently, various promising novel approaches are being employed to improve the efficacy of immunotherapies alone or in combination with targeted therapies, often by seeking to reverse the immunosuppressive environment. Additionally, prognostic signatures are being sought in order to provide a basis for tailored immunotherapies.

During the meeting there will be plenty of time for fruitful discussions between speakers and participants to encourage the emergence of new collaborations.

On behalf of the Organizing Committee, we would like to thank you for your participation in this Meeting.

The Meeting's Chairs

Olga Balaoura, *President of the Board of Directors of Saint Savvas Cancer Hospital*

Dr. Constantin N. Baxevanis, *Scientific Director Cancer Immunology and Immunotherapy Center, Cancer Research Center, Saint Savvas Cancer Hospital*

George Georgiou, *Deputy Director of Finance, Saint Savvas Cancer Hospital*

Prof. Graham Pawelec, *Department of Immunology, University of Tübingen, Tübingen, Germany*

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THURSDAY, SEPTEMBER 28

09:00-09:15 *Welcome Address*

Session 1: Cancer Biomarkers for Prognosis and Prediction (Part I)

Chairs: **J. Bartunkova (CZE) - C.N. Baxevanis (GRE)**

09:15-09:45 "Peripheral blood biomarkers predicting clinical response to checkpoint blockade in melanoma"
G. Pawelec *(Department of Immunology, University of Tübingen, Germany)*

09:45-10:15 "SSX family members play different roles in the early development of ovarian cancer: new targets for therapy"
Barbara-Ann Guinn *(Centre for Biomedicine, Hull York Medical School, Kingston, UK)*

10:15-10:45 "Predictive value of TCR-Vβ profile for Durvalumab (anti-PDL1)-based immunotherapy in patients with NSCLC"
M. Goulielmaki *(Cancer Immunology and Immunotherapy Center, Cancer Research Center, St Savvas Cancer Hospital, Athens, Greece)*

10:45-11:15 *Coffee break*

Session 2: Cancer Biomarkers for Prognosis and Prediction (Part II)

Chairs: **Barbara-Ann Guinn (UK) - M. Goulielmaki (GRE)**

11:15-11:45 "Immune correlates of response to immunotherapy in glioblastoma"
E.M. Inderberg *(University of Oslo, Norway)*

11:45-12:15 "Emerging onco-fetal immunomodulatory targets and immunomodulators"
Joerg Wischhusen *(University Hospital Würzburg, Germany)*

12:15-12:45 "Predictive biomarkers for breast cancer: the AE37 vaccine paradigm"
C.N. Baxevanis *(Cancer Immunology and Immunotherapy Center, Cancer Research Center, St Savvas Cancer Hospital, Athens, Greece)*

12.45-13.15 "The immune contexture in ovarian cancer: the impact on disease outcome and response to immunotherapy"
J. Bartunkova *(Department of Immunology, Charles University and Motol University Hospital, Prague)*

13:15-14:15 *Lunch break*

Session 3: Cancer Immunotherapies (Part I)

Chairs: **R. Offringa (GER) - P. Verginis (GRE)**

14:15-14:45 "Long-term survival benefit by adding helper peptides to a melanoma vaccine: next steps for personalized vaccines"
C.L. Slingluff *(Department of Surgery, University of Virginia, USA)*

14:45-15:15 "Exploiting the cystine/glutamate antiport xCT to improve the immune response against breast cancer"
F. Cavallo *(Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy)*

15:15-15:45 "Targeting cancer immortality in the clinic: A happy marriage between the cancer vaccine UV-1 and checkpoint inhibitors"
E.B. Ellingsen *(Ultimovacs ASA, Norway)*

15:45-16:00 *Short break*

Session 4 : Understanding TCR specificity and cross-reactivity for predicting epitope immunogenicity

Chairs: **B. Seliger (GER) - G. Adema (NED)**

16:00-16:30 "Structure Function Relationships Between the T Cell Receptor and Target Recognition"
M. Nishimura *(Loyola University Chicago, USA)*

16:30-17:00 "How reliable are predictions of CD8 T cell epitope recognition? Studies of the anti-HCMV response suggest, they are far from that"
P.V. Lehmann *(Research and Development, Cellular Technology Ltd. (CTL), Ohio, USA)*

17: 00-18:10 **SHORT PRESENTATIONS**

Chairs: **E.M. Inderberg (NOR) - S. Lucas (BEL)**

17: 00-17:10 **O1-1 Exploiting the crosstalk between TLR2 and the cysteine/glutamate antiporter xCT for the development of a new combined therapy for breast cancer**
C. Cossu[1], A. Di Lorenzo[1], E. Bolli[1], V. Franceschi[2], G. Donofrio[2], F. Cavallo[1] and L. Conti[1]
[1] Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy
[2] Department of Medical Veterinary Sciences, University of Parma, Parma, Italy

17: 10-17:20 **O1-2 Chimeric DNA vaccination against the chondroitin sulfate proteoglycan 4: an effective strategy to induce targeted immunity for melanoma and osteosarcoma treatment**
Federica Riccardo[1]*, Lidia Tarone[1]*, Davide Giacobino[2], Mariateresa Camerino[2], Selina Iussich[2], Giuseppina Barutello[1], Maddalena Arigoni[1], Laura Conti[1], Elena Quaglino[1], Soldano Ferrone[3], Emanuela Morello[2], Paolo Buracco[2], Federica Cavallo[1]
[1] Department of Molecular Biotechnology and Health Sciences, University of Turin; Turin, Italy;
[2] Department of Veterinary Sciences, University of Turin; Turin, Italy;
[3] Department of Surgery, Massachusetts General Hospital, Harvard Medical School; Boston, Massachusetts, USA.

17: 20-17:30 **O1-3 Synovial sarcoma, X breakpoint family members cause epithelial- mesenchymal transition changes in ovarian cancer**
Grayson, K.C.,[1] Khan, G.N.,[2] Hardman, M.J.[1] & Guinn, B.A.[1]
[1] Centre for Biomedicine and Hull Molecular Imaging Centres, Hull York Medical School, University of Hull, United Kingdom.
[2] Current address: Lancaster Medical School, University of Lancaster, England, United Kingdom.

17: 30-17:40 **O1-4 miRNAs are serum biomarkers that predict poor risk outcomes in adults with acute myeloid leukaemia at diagnosis**
E. Brown[1], D.M. Fletcher[1], M. Mortoglou[2], L. Davis[3], G.J. Mufti[4], S. Caserta[2], K.I. Mills[3], K.H. Orchard[5], P. Uysal-Onganer[2], B.-A. Guinn[1]*
[1] Centre for Biomedicine, Hull York Medical School, University of Hull, Cottingham Road, Kingston-upon-Hull;
[2] Cancer Mechanisms and Biomarkers Research Group, School of Life Sciences, University of Westminster, London;
[3] Patrick G. Johnson Centre for Cancer Research, Queen's University Belfast, Lisburn Road, Belfast;
[4] Department of Haematological Medicine, King's College London and King's College Hospital NHS Foundation Trust, Denmark Hill, London;
[5] Department of Haematology, University Hospital Southampton NHS Foundation Trust, Tremona Road, Southampton, UK.

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17: 40-17:50 **O1-5 T-cell receptor and immune-response gene studies in tumor microenvironment and peripheral blood in head and neck cancer patients**
Panagiota Batsaki[1], Maria Goulielmaki[1], Andriana Razou[2], Athanassios Sakellaridis[2], Niki Arnogiannaki[3], Angelos D. Gritzapis[1], Constantin N. Baxevanis[1], Sotirios P. Fortis[1]
[1] Cancer Immunology and Immunotherapy Center, Cancer Research Center, Saint Savvas Cancer Hospital, Athens, Greece.
[2] Ear-Nose-Throat Head and Neck Surgery Department Saint Savvas Cancer Hospital, Athens, Greece.
[3] Department of Surgical Pathology, Saint Savvas Cancer Hospital, Athens, Greece.

17: 50-18:00 **O1-6 Multiplexed immunofluorescence and spatial quantification of GARP expression and TGF-ß1 signaling in human tumors**
Van Meerbeeck P.[1], Maatougui D.[1], Vaherto N.[1], Benhaddi F.[1], Rouaud L.[2], Noel A.[2], van Baren N.[1], Lucas S.[1]
[1] Université Catholique de Louvain, Institut de Duve, GECE unit, Brussels, Belgium
[2] Université de Liège, GIGA, Tumours and development biology unit, Liège, Belgium

18: 00-18:10 **O1-7 Immunotherapy by Cytokine-Induced Killer cells and Bacterial Lectin is a promising antitumor treatment of breast and pancreatic cancers: an in vitro study**
Olha Karaman[1,2], Jan Aleksander Kraśko[2], Nadiia Cheremshenko[1], Olexandra Lykhova[1], Irma Krasaitienė[2], Vita Pašukonienė[2], Vasyl Chekhun[1]
[1] R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology NAS Ukraine, Kyiv, Ukraine
[2] National Cancer Institute, Vilnius, Lithuania

FRIDAY, SEPTEMBER 29

Session 5: The Tumor Microenvironment and Regulation of Antitumor Immunity

Chairs: **G. Pawelec (GER) - P. Thor Straten (DEN)**

09:00-09:30 "Microenvironment, lymphocytes and tumor cells in breast cancer"
B. Seliger *(Martin Luther University Halle-Wittenberg, Germany)*

09:30-10:00 "Conceptualization of the antigen presenting cell landscape in solid tumors"
M. Tsoumakidou *(Biomedical Sciences Research Center 'Alexander Fleming', Vari, Greece)*

10:00-10:45 *Coffee break*

10:45-11:15 "Adjuvants and immune checkpoints to prime and boost Anti-tumor Immunity in situ"
G. Adema *(Radboud University Medical Center, The Netherlands)*

11:15-11:45 "Inflammatory responses in the TME in common solid tumors"
I. Pateras *(2nd Department of Pathology, "Attikon" University Hospital, Medical School)*

11:45-12:15 "The exercise of TAMing on the immune system"
P. Thor Straten *(University of Copenhagen, Denmark)*

12:15-13:30 *Lunch break*

Session 6: Cancer Immunotherapies (Part I)

Chairs: **R. Offringa (GER) - P. Verginis (GRE)**

13:30-14:00 "Biomarkers of PD1 immunotherapy in lung cancer patients"
P. Verginis *(Laboratory of Immune Regulation and Tolerance, University of Crete Medical School, Heraklion, Greece)*

14:00-14:30 "Targeting complement in cancer: where do we stand"
D.C. Mastellos *(Division of Biodiagnostic Science and Technologies, National Center for Scientific Research "Demokritos", Athens, Greece)*

14:30-15:00 "CAR T-cell therapy"
G. Vassilopoulos *(Department of Hematology, Larissa University Hospital, University of Thessalia, Larissa, Greece.)*

15:00-15:15 ***Short break***

Session 7: Immunotherapies and Immune Resistance

Chairs: **F. Cavallo (ITA) - C.L. Slingluff (USA)**

15:15-15:45 "Development of novel cancer immunotherapy drugs in the context of the DKFZ-Bayer Alliance: oral small molecules targeting intracellular immune checkpoints"
R. Offringa *(University Hospital Heidelberg & German Cancer Research Center, Germany)*

15:45-16:15 "GARP: TGF-b1 blockade for the immunotherapy of solid tumors or blood cancers"
S. Lucas *(de Duve Institute, Université Catholique de Louvain, Brussels, Belgium)*

16:15-16:45 "Cytotoxic T cells, DC vaccine's and the immunoproteasome; improving ACT therapy"
S. Wickström *(Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden)*

16:45-17:00 ***Short break***

Session 8: Characterizing the antigen-specific memory B cell repertoire

Chairs: **Greg Kirchenbaum (USA) - Stephen Todryk (UK)**

17:00-17:30 "Monitoring memory B cells provides insights into humoral immunity that measurements of circulating antibodies do not reveal"
Greg Kirchenbaum *(Research and Development Department, Cellular Technologies Limited, Cleveland, USA)*

17:30-18:00 "Defining the magnitude, Ig class/subclass utilization, affinity, and cross-reactivity of the memory B cell repertoire"
Stephen Todryk *(Department of Applied Sciences, Northumbria University Newcastle, UK)*

18:00-19:00 **SHORT PRESENTATIONS**

Chairs: **M. Goulielmaki (GRE) - I. Pateras (GRE)**

18:00-18:10 **O2-1 Live attenuated influenza viruses co-expressing bovine papillomavirus 1 (BPV1) antigens for the treatment of equine skin tumours and underlying papillomavirus infection**
Edmund K. Hainisch[1], Christoph Jindra[1,2], Sabine Brandt[1]
[1] Research Group Oncology (RGO), University of Veterinary Medicine, Vienna, Austria, [2] Division of Molecular Oncology and Haematology, Karl Landsteiner University of Health Sciences, Krems an der Donau, Austria

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18:10-18:20 **O2-2 Investigating the role of the cystine/glutamate antiporter xCT in the extracellular vesicles-mediated modulation of the pre-metastatic niche**
Antonella Iacoviello[1], Roberto Ruiu[1], Marta Tapparo[2], Claudia Landi[3], Maddalena Arigoni[1], Federica Cavallo[1]
[1] Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino, Italy [2] Department of Medical Sciences, Molecular Biotechnology Center, University of Torino, Turin, Italy [3] Functional Proteomics Lab, Department of Life Sciences, University of Siena, 53100 Siena, Italy

18:20-18:30 **O2-3 Teneurin-4 exerts a protumorigenic role in triple-negative breast cancer**
Giuseppina Barutello, Giulia Peppino, Federica Riccardo, Elisabetta Bolli, Maddalena Arigoni, Federica Atzori, Betul Deniz, Federica Cavallo, and Elena Quaglino
Department of Molecular Biotechnology and Health Sciences, University of Torino, Molecular Biotechnology Center "Guido Tarone, Torino, Italy

18:30-18:40 **O2-4 Adult B-cell acute lymphoblastic leukaemia (B-ALL): enriched pathways identify new targets for leukaemia stem cell immunotherapy**
Eithar Mohamed[1], Sara Goodman[1], Leah Cooksey[1], Ken I. Mills[2], Kim H. Orchard[3], Barbara-ann Guinn[1]*
[1] Centre for Biomedicine, Hull York Medical School, University of Hull, HU6 7RX; [2] Patrick G. Johnson Centre for Cancer Research, Queen's University Belfast, Lisburn Road, Belfast BT9 7AE; [3] Department of Haematology, Southampton University Hospital, Tremona Road, Southampton, SO16 6YD, U.K.

18:40-18:50 **O2-5 The role of SSX in the early development of ovarian cancer**
Alice Fearn, Kelly Grayson, Holly N. Wilkinson, Barbara-ann Guinn
Centre for Biomedicine, Hull York Medical School, University of Hull, Hull, HU6 7RX.

18:50-19:00 **O2-6 The TCR Vβ repertoire composition as a predictive biomarker of immunotherapy efficiency in non-small cell lung cancer patients**
Panagiota Batsaki[1], Sotirios P. Fortis[1], Angelos D. Gritzapis[1], Anastasia Xagara[2], Athanasios Kotsakis[2,3], Constantin N. Baxevanis[1] and Maria Goulielmaki[1]
[1] Cancer Immunology and Immunotherapy Center, Cancer Research Center, Saint Savvas Cancer Hospital, Athens, Greece [2] Laboratory of Oncology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Thessaly, Greece [3] Department of Medical Oncology, University General Hospital of Larissa, Larissa, Thessaly, Greece

19:00 ***Concluding Remarks***

SATURDAY, SEPTEMBER 30

EXCURSION: Athens – Mycenae – Nafplion (start: 09:00am)

Invited Speakers

Gosse Adema *(Radboud University Medical Center, The Netherlands)*

Jirina Bartunkova *(Department of Immunology, Charles University and Motol University Hospital, Prague)*

Constantin N. Baxevanis *(Cancer Immunology and Immunotherapy Center, Cancer Research Center, St. Savvas Cancer Hospital, Athens, Greece)*

Federica Cavallo *(Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy)*

Espen Basmo Ellingsen *(Ultimovacs ASA, Norway)*

Maria Goulielmaki *(Cancer Immunology and Immunotherapy Center, Cancer Research Center, St. Savvas Cancer Hospital, Athens, Greece)*

Barbara-Ann Guinn *(Centre for Biomedicine, Hull York Medical School, Kingston, UK)*

Else Marit Inderberg *(University of Oslo, Norway)*

Greg A. Kirchenbaum *(Center for Vaccines and Immunology, University of Georgia)*

Paul V. Lehmann *(Research and Development, Cellular Technology Ltd. (CTL), Ohio, USA)*

Sophie Lucas *(de Duve Institute, Université Catholique de Louvain, Brussels, Belgium)*

Dimitrios C. Mastellos *(Division of Biodiagnostic Science and Technologies, National Center for Scientific Research "Demokritos", Athens, Greece)*

Michael Nishimura *(Loyola University Chicago, USA)*

Rienk Offringa *(University Hospital Heidelberg & German Cancer Research Center, Germany)*

Ioannis S. Pateras *(2nd Department of Pathology, "Attikon" University Hospital, Medical School, National and Kapodistrian University of Athens, Greece)*

Graham Pawelec *(Department of Immunology, University of Tübingen, Germany)*

Barbara Seliger *(Institute of Medical Immunology, Martin Luther University Halle-Wittenberg, Germany)*

Craig L. Slingluff *(Department of Surgery, University of Virginia, USA)*

Per thor Straten *(University of Copenhagen, Denmark)*

Stephen Todryk *(Faculty of Health and Life Sciences, Northumbria University at Newcastle)*

Maria Tsoumakidou *(Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece)*

Georgios Vassilopoulos *(Department of Hematology, Larissa University Hospital, University of Thessalia, Larissa, Greece)*

Panagiotis Verginis *(Laboratory of Immune Regulation and Tolerance, University of Crete Medical School, Heraklion, Greece)*

Stina Wickström *(Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden)*

Joerg Wischhusen *(University Hospital Würzburg, Germany)*

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ABSTRACTS

Exploiting the crosstalk between TLR2 and the cysteine/glutamate antiporter xCT for the development of a new combined therapy for breast cancer

C. Cossu[1], A. Di Lorenzo[1], E. Bolli[1], V. Franceschi[2], G. Donofrio[2], F. Cavallo[1] and L. Conti[1]

[1] Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy
[2] Department of Medical Veterinary Sciences, University of Parma, Parma, Italy

Background: Breast cancer is still the leading cause of cancer death in women, due to relapses and metastases. Therefore, the development of innovative combined therapies able to target key cancer-inducing or cell-sustaining pathways is needed. We have previously demonstrated that mammary cancer stem cells (CSCs) overexpress the cystine-glutamate antiporter xCT and Toll-Like Receptor (TLR)2, which are key players in cancer cell self-renewal and resistance to chemotherapy. Therefore, xCT and TLR2 targeting could be a promising strategy for breast cancer therapy, and a deeper characterization of their crosstalk may lead to the setup of effective combination therapies for breast cancer.

Methods: The effects exerted by TLR2 activation on xCT expression and function were analyzed *in vitro* on mouse and human breast cancer cell lines in which TLR2 was activated with endogenous or bacteria-derived ligands or silenced. Moreover, xCT expression was analyzed in transgenic BALB/c-NeuT spontaneous mammary tumor model ($TLR2^{WT}$-neuT and $TLR2^{KO}$-neuT mice) and on cell lines derived from their tumors. The efficacy of a combined inhibition of TLR2 and xCT, in association or not with chemotherapy, was tested *in vitro* on these cell lines and *in vivo*.

Results: TLR2 induced the upregulation of xCT in breast cancer cells, and its absence decreased xCT expression both *in vitro* and *in vivo*. Since xCT is a key regulator of intracellular redox balance, TLR2 downregulation induced an increase of intracellular reactive oxygen species in breast cancer cells. TLR2 inhibitors synergized with xCT inhibitors in hindering breast cancer cell viability and inducing their apoptosis, and these results were increased by the association with doxorubicin. Moreover, TLR2 inhibition and chemotherapy combined with xCT vaccination results in an increased anti-tumor effect in vivo.

Conclusions: We demonstrated that TLR2 promotes breast CSC self-renewal, cancer progression and that it upregulates xCT expression in breast cancer cells, protecting them from oxidative stress. The use of TLR2 inhibitors in association with xCT immunotargeting and chemotherapy may represent a more effective combination therapy for breast cancer.

Chimeric DNA vaccination against the chondroitin sulfate proteoglycan 4: an effective strategy to induce targeted immunity for melanoma and osteosarcoma treatment

Federica Riccardo[1]*, Lidia Tarone[1]*, Davide Giacobino[2], Mariateresa Camerino[2], Selina Iussich[2], Giuseppina Barutello[1], Maddalena Arigoni[1], Laura Conti[1], Elena Quaglino[1], Soldano Ferrone[3], Emanuela Morello[2], Paolo Buracco[2], Federica Cavallo[1]

[1] Department of Molecular Biotechnology and Health Sciences, University of Turin; Turin, Italy;
[2] Department of Veterinary Sciences, University of Turin; Turin, Italy;
[3] Department of Surgery, Massachusetts General Hospital, Harvard Medical School; Boston, Massachusetts, USA.

* These Authors equally contributed to the work

Background: Melanoma (MM) and pediatric osteosarcoma (OSA) are aggressive tumors for which standard therapies are poorly effective, mostly due to mechanisms of resistance and severe side effects. The chondroitin sulphate proteoglycan (CSPG)4 has emerged as an ideal immunotherapeutic target. It exhibits limited expression in healthy tissues, while it is highly expressed in MM and OSA, with a key oncogenic role, potentially contributing to therapy-resistance. Consequently, anti-CSPG4 vaccination arises as an appealing strategy, alone or in combinatorial approaches, to stimulate patient's own immune system, inducing a specific and long-lasting anti-tumor immune response.

Methods: Since the CSPG4 is a non-mutated self-antigen, hence poorly immunogenic, we generated a hybrid plasmid derived partly from the human (Hu) and partly from the dog (Do)-CSPG4 to overcome host's unresponsiveness. We tested the safety, immunogenicity, and anti-tumor potential of HuDo-CSPG4 electrovaccination in mice challenged with transplantable human or murine tumors, in dogs with surgically-resected, naturally-occurring CSPG4^{+} MM and OSA and in a human surrogate *in vitro* assay.

Results: HuDo-CSPG4 electrovaccination was immunogenic and endowed with an anti-tumor potential in MM and OSA preclinical mouse models. In canine MM and OSA patients the electrovaccination was safe, immunogenic and with a beneficial effect, significantly increasing the overall survival of vaccinated dogs as compared to controls, treated with conventional therapies alone. Both cellular and antibody responses were induced and correlated with canine patients' survival. HuDo-CSPG4 vaccine-induced antibodies prompted CSPG4 internalization and down-regulation, resulting in the impairment of CSPG4-dependent proliferation and migration of canine MM and OSA cells. HuDo-CSPG4 was also able to induce a cytotoxic response in a human setting *in vitro*.

Conclusions: These results provide the rationale for proposing HuDo-CSPG4 vaccination as an effective strategy to break immune tolerance and treat CSPG4+ MM and OSA patients. On these findings, our focus is directed toward the development of anti-CSPG4 vaccination with an emphasis on rational combinatorial approaches (including chemo-, targeted- and immune-therapies) to overcome resistance and to target tumor cells from multiple angles, aiming for a durable clinical response.

Synovial sarcoma, X breakpoint family members cause epithelial- mesenchymal transition changes in ovarian cancer

Grayson, K.C.,[1] Khan, G.N.,[2] Hardman, M.J.[1] & Guinn, B.A.[1]

[1] Centre for Biomedicine and Hull Molecular Imaging Centres, Hull York Medical School, University of Hull, United Kingdom.
[2] Current address: Lancaster Medical School, University of Lancaster, England, United Kingdom.

Background: Ovarian cancer (OC) is considered the deadliest gynaecological malignancy and contrary to other malignancies, the mortality rate has only improved only marginally over the last 50 years. A delay in diagnosis due to the asymptotic nature of OC contributes to almost half of patients being diagnosed in the late stages when the five-year survival rate is below 40%. Due to the limitations of current diagnostic techniques and the importance of detecting OC in the early stages, the search for new biomarkers and a better understanding of OC disease progression is paramount. The epithelial-mesenchymal transition (EMT) is a biochemical process by which a polarized epithelial cell can assume a mesenchymal cell behaviours and provides a source of cells involved in the cancer phenotype. HOM-MEL-40/SSX2 is a cancer-testis antigen, present on the X-chromosome which has been shown to play a role in a broad range of cancers, but to date has not been examined in OC.

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Methods: Immunohistochemistry of OC and healthy adjacent tissue arrays were performed to determine SSX2A, SSX2B, SSX3 and SSX4 expression in OC samples. Plasmid overexpression of SSX family members were used to investigate the individual function of four SSX variants in the epithelial OC cell line, OVCAR3, *in vitro* and through RNA-sequencing.

Results: Expression of SSX family members were observed at higher levels in OC patient samples than normal adjacent tissue, and when compared to CA125, WT1 and HE4. Following the generation of OVCAR3-SSX overexpression variants, we showed that all, except OVCAR3-SSX2B, had increased proliferation. SSX2A and SSX3 overexpression were both associated with increased SLUG expression, an EMT related transcription factor ($p<0.05$ and <0.01, respectively). Increased SSX3 expression was associated with an increase in transcription of the mesenchymal marker Vimentin ($p<0.05$) and the epithelial marker E-cadherin ($p<0.0001$).

Conclusions: *In vivo* and *in vitro* validation showed that the SSX genes convey complimentary and individual effects on OVCAR3 cells and contribute to its cell proliferation and EMT. The SSX family may be novel contributors to disease progression in OC which requires further investigation.

miRNAs are serum biomarkers that predict poor risk outcomes in adults with acute myeloid leukaemia at diagnosis

E. Brown[1], D.M. Fletcher[1], M. Mortoglou[2], L. Davis[3], G.J. Mufti[4], S. Caserta[2], K.I. Mills[3], K.H. Orchard[5], P. Uysal-Onganer[2], B.-A. Guinn[1]*

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Background: Acute myeloid leukaemia (AML) is a rare, heterogenous and often difficult to treat disease. A variety of cytogenetic changes and, increasingly, single gene mutations can predict outcomes for AML patients at disease presentation. We previously identified genes that were differentially expressed between risk subgroups in adults and children with AML (1). Several of these genes encoded microRNAs (miRNAs) that regulate gene transcription and translation by targeting specific mRNAs for silencing or degradation. Recently their importance has emerged as regulators of gene expression in health and disease with roles as novel cancer biomarkers and potential therapeutic targets.

Methods: We have examined the miRNAs that are differentially expressed between risk subgroups using The Cancer Genome Atlas (TCGA) and analysed the pathways they act on using DAVID. We have further confirmed the expression of these miRNAs in AML samples using qPCR.

Results: We identified cellular miRNA-378G, miRNA-486-1, miRNA-1915 and miRNA-8086 as differentially expressed (≥two-fold) when comparing intermediate vs good, or poor vs good, risk subgroups in The Cancer Genome Atlas dataset (2). Using UCSC Xena Browser, these miRNAs were found significantly upregulated in poor risk subgroups compared to intermediate and good risk ($p=0.016$, 2.011×10^{-12}, 0.019 and 0.02, respectively).

The potential of these miRNAs to act as biomarkers of risk subgroups were analysed using Receiver Operating Characteristics. miRNA-486-1, miRNA-1915 and miRNA-8086 returned Area Under the Curve (AUC) scores of 0.68, 0.72 and 0.81, respectively, in intermediate vs good risk subgroups. Combined, these miR-

NAs were an effective prognostic biomarker (AUC = 0.94). miRNA-378G and miRNA-1915 returned AUC scores of 0.78 and 0.75, respectively, and 0.89 combined, in good vs poor risk subgroups.

In addition, miRNA-378G, miRNA-486-1 & miRNA-1915 were linked to known pathways with targets implicated in various malignancies including JAK-STAT, FOXO, Wnt, MAPK, BCL-2, PTEN and BRAF.

Conclusions: We have identified a number of miRs that impact on pathways known to play key roles in AML and act as biomarkers for disease prognosis at diagnosis. Future work will determine the capacity of these miRNAs to act as therapeutic targets.

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T-cell receptor and immune-response gene studies in tumor microenvironment and peripheral blood in head and neck cancer patients

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Background: Worldwide, head and neck cancer (H&N) accounts for approximately 900,000 new cases and over 400,000 deaths annually. An emerging tumor microenvironment (TME) is a complex and continuously evolving entity. T-cell receptor (TCR) repertoire and levels of expression of immune-related genes (IRGs), were widely introduced as important tools to reveal the status of the immune response intratumorally, as well as in the circulation. Furthermore, genetic alterations could be used as molecular targets for treatment, as well as biomarkers with prognostic utility. The aim of this study was to investigate the tumor mutational load, along with alterations in IRGs expression levels and in the TCR repertoire, in the peripheral blood and TME of H&N cancer patients.

Methods: DNA and RNA were isolated from peripheral blood and from tumor tissue (Formalin-fixed paraffin-embedded sample-FFPE) of 26 H&N cancer patients, followed by Next-Generation Sequencing (NGS). The Ion Oncomine™ TCR Beta-SR Assay was used in order to identify and measure the clonal expansion of T-cells. The IRG profiling was studied using a panel of 398 immune-related genes (Oncomine™ Immune Response Research Assay). Alterations in the TCR repertoire and in IRG expression levels were evaluated in the peripheral blood and the TME. The genetic alterations in the TME were detected using the RingCap® Human Pan-Cancer Drive Gene Mutations Kit.

Results: We found 80 IRGs that were down- and 72 IRGs that were up- regulated in the TME compared to matched peripheral blood samples. In a similar fashion, we identified alterations in the frequencies of TCR clonotypes between the circulating and infiltrating T-cells. We also detected hotspot variants in 38 common tumor driver genes, among which point mutations and small fragment insertion and deletion mutations in the TME.

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Conclusions: Differences in TCR repertoire and in IRGs expression between TME and circulation could potentially act as dynamic biomarkers in H&N cancer. The mutations detected could be variants with clinical significance or potential drug targets. These findings need to be further confirmed in larger patients' cohorts.

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Multiplexed immunofluorescence and spatial quantification of GARP expression and TGF-ß1 signaling in human tumors

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Background: TGF-ß1 plays a deleterious role in cancer, notably by inhibiting anti-tumor immunity. All cells can produce TGF-ß1 in a latent but inactive form. The latter is formed of the mature TGF-ß1 non-covalently associated to the latency associated peptide (LAP). Specific cell types can also produce latent TGF-ß1 in a complex with GARP, a transmembrane protein. In a process called TGF-ß1 activation, the mature TGF-ß1 is released from the LAP, which can then act on its receptor.

However, which GARP-expressing cells among regulatory T cells (Tregs) and/or blood endothelial cells (BECs) can activate TGF-ß1 in human tumors is unknown. It is also unknown whether T cells could be the targets of TGF-ß1 in human tumors, leading to immunosuppression.

Methods: We analyzed, by multiplexed immunofluorescence, 164 tumors of several types (cutaneous melanoma metastases, colon, lung, breast and urothelial carcinomas) and 10 tonsils. When it was possible, we had access to normal tissues matched with the tumors. We quantified and located cells and vessels. We computed the distances between the cells targeted by the TGF-ß1 to their nearest GARP-expressing cell.

Results: The frequency of GARP-expressing Tregs among all cells was the same in tonsils (1x10-3) than in tumors ($2x10^{-4}$ – $2x10^{-3}$). About 50% of colon cancers presented a high frequency of GARP-expressing Tregs (>$4x10^{-4}$), greater than in the other tumor types (12-32%). We observed less frequent GARP-expressing Tregs in normal tissues (0-$7x10^{-5}$) as compared to tumors. Density of GARP-expressing blood vessels was homogenous across all samples (25-64 GARP+ blood vessel/mm^2). The frequency of cells targeted by TGF-ß1 (pSMAD2+ cells) was lower in tonsils (1%) than in tumors (1-4%). No correlation was observed between the frequency of pSMAD2+ cells with that of GARP-expressing cells.

We observed that pSMAD2+ T cells were closer to their nearest Tregs than they would be by chance in all type of cancer.

Conclusions: In each type of cancer, GARP-expressing Tregs were as frequent in tumors as in tonsils.

T cells could be a preferential target of TGF-ß1 produced by GARP-expressing Tregs in tumors.

Immunotherapy by Cytokine-Induced Killer cells and Bacterial Lectin is a promising anti-tumor treatment of breast and pancreatic cancers: an in vitro study

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Background: Breast and pancreatic cancers remain a serious problem for clinical oncologists due to the presence of aggressive forms resistant to therapy. Limited treatment options for these forms of cancer drew researchers to immunotherapy and adoptive immunotherapy in particular. One of the more interesting options are cytokine-induced killer T-cells (CIKs): a heterogeneous mixture of cells with the phenotype of T, NK- and NKT- cells. The clinical benefit of using CIKs as monotherapy and in combination with other methods was confirmed for patients with lung, stomach and ovarian cancer. However, the problem of avoiding the negative impact of tumor microenvironment on CIKs remains relevant. We hypothesize that lectins, products of microbial origin, contribute to maintaining the antitumor activity of CIKs. Moreover, lectins could be used to specifically target malignant cells due to the distinct glycolconjugate signature on their surface.

The aim of this work was to investigate *in vitro* the antitumor effects of CIKs and bacterial lectin against breast and pancreatic tumor cells.

Methods: The lectin was obtained from liquid culture of lectin-producing B. subtilis strain IMV B-7724 in IEPOR (Ukraine). CIKs were obtained from peripheral blood of healthy donors in NCI (Vilnius). K562 cells were used as a standard NK-sensitive line for cytotoxicity testing. *In vitro* models included pancreatic cancer cells (Panc-1) and breast cancer cells with different molecular subtypes and sensitivity to doxorubicin: MCF-7 (p53wt, ER+, HER-2/neu-), MCF-7/Dox, and MDA-MB-231 (p53mut, ER-, HER-2/neu -). Cytotoxic activity was measured with CCK8 and cell phenotype was established with FACS.

Results: Lectin increases the cytotoxic activity (CTA) of CIKs against K562 by 23.0% (effector-target ratio 5:1) and 13.2% (20:1) and contributes to the preservation of CTA during 6 days of co-cultivation. MDA-MB-231 and Panc-1 cells were the most sensitive to CIKs. Addition of lectin slightly increased the cytotoxic activity of CIKs, especially against pancreatic cells.

Conclusions: Results show promising effect of CIKs and lectin in aggressive tumors such as triple-negative breast and pancreatic cancer. The ability of lectin to contribute to the preservation of the CTA of CIKs in an aggressive tumor microenvironment suggests combination therapy.

Live attenuated influenza viruses co-expressing bovine papillomavirus 1 (BPV1) antigens for the treatment of equine skin tumours and underlying papillomavirus infection

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Background: Equine sarcoids (ES) are semi-malignant skin tumours caused by closely related bovine papillomaviruses (BPV) 1 and 2. ES do not metastasise, however a high rate of recurrence following surgery poses challenges for treatment. Persistent BPV1/2 infection of the whole integument is thought to be a main contributor to this. BPV1/2 (episomal DNA) can be detected by PCR in intact skin close to lesions and in the whole integument of ES affected horses. We have generated human influenza (Flu) A and B viruses deficient for the interferon-antagonist NS1 (dNS1) and co-expressing BPV1 E6 and E7 peptides as innovative immunotherapeutic vaccines. dNS1-FluA/B-E6E7 was shown to be safe and immunogenic in horses.

Methods and Results: A clinical study in 29 -BPV1/2 PCR-positive ES affected horses, intralesionally injected with the vaccines showed an overall response rate of 62%. Complete regression (CR) was observed in 14 and partial response in four horses. In 10 horses with CR, long term follow up was possible. No recurrencies were detected 26 to 44 months after treatment. Most remarkably, 10/10 horses tested PCR negative for BPV1/2 from scrapings collected from the sites of former sarcoids.

To further improve the therapeutic response in severely affected horses dNS1-FluA/B-E6E7 treatment is combined with surgery. dNS1-FluA/B is injected intralesionally into one or two ES. On days 12 -14 all ES are surgically removed. Intra-muscular booster injections are given on day 28 and a further 2 months later. Data for three patients are available so far. Patient #1 with >20 ES is free from recurrences 8 months after treatment and 4 index lesion sites tested repeatedly and consistently BPV1/2 PCR-negative so far. Patients #2 and #3 are free from recurrencies and 50% of index lesion sites tested BPV1/2 PCR-negative two weeks after surgery.

Conclusions: Our data indicate that dNS1-FluA/B-E6E7 has the potential to effectively treat ES and importantly underlying BPV infection. Translation of obtained insights into immunotherapy of HPV16-induced cervical lesions is in progress.

Investigating the role of the cystine/glutamate antiporter xCT in the extracellular vesicles-mediated modulation of the pre-metastatic niche

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Background: Breast cancer is the most common malignancy among women worldwide, and metastasis is the primary cause of treatment failure and mortality. The cystine/glutamate antiporter xCT is upregulated in breast cancer and plays a role in the metastatic process. Indeed, we have highlighted that its lack in cancer cells reduces lung metastasis formation and alters immune infiltration in the lungs even before metastasis occurs. Interestingly, it was reported that xCT is involved in the release of extracellular vesicles (EV) by tumor cells through a glutamate-dependent pathway, and EV are recognized as important mediators of pre-metastatic niche formation.

Methods: To study the role of xCT in the EV release we used 4T1 cells and xCT^{KO} 4T1 cells, previously generated using CRISPR/Cas9 technology. EV were isolated from cell culture media of xCT-proficient and xCT-deficient cells through ultracentrifugation and analysed through Nanoparticle Tracking Analysis. EV were lysed to evaluate the presence of exosomal markers and xCT using Western Blot. 2D electrophoresis was performed to analyse differences in EV protein cargo.

Results: The absence of xCT in 4T1 cells reduces cell migration *in vitro* and lung metastasis *in vivo*. This is accompanied by an altered immune cell recruitment in the pre-metastatic niche. In line with this, we observed that the absence of xCT in 4T1 cells reduces the release of small EV and alters the composition of their cargo.

Additionally, we observed that xCT is present in EV derived from xCT WT 4T1 cells. The next step of this work will be the evaluation of the existence of a causal link between xCT-mediated EV release and the metastatic process, with a particular focus on the formation of the pre-metastatic niche.

Conclusion: The absence of xCT diminishes the metastatic potential of breast cancer cells, thereby influencing the formation of a pro-metastatic niche. Moreover, xCT is involved in the release of EV and in the regulation of their cargo. These results provide the basis to better elucidate the mechanism through which xCT modulates the formation of the pre-metastatic niche.

Teneurin-4 exerts a protumorigenic role in triple-negative breast cancer

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Background: Teneurin-4 (TENM4) is a transmembrane protein whose mutations and rearrangements have been identified in several tumors. However, the functional roles that TENM4 plays in cancer are still unknown, and contrasting evidence suggests its involvement as a tumor-suppressive or pro-tumoral protein. We identified TENM4 among the overexpressed transmembrane proteins in triple-negative breast cancer (TNBC) stem cell-enriched tumorspheres. Meta-analysis of breast cancer datasets shows that TENM4 mRNA is up-regulated in invasive carcinoma specimens compared to healthy breast tissues and that TENM4 high expression correlates with shorter relapse-free and overall survival in TNBC patients.

Methods and Results: We previously demonstrated that TENM4 plays a key role in cancer stem cells' self-renewal and migratory ability in murine and human TNBC cells and now we are investigating the role of TENM4 exploiting a preclinical cancer model of TNBC. A significantly lower number of lung metastasis was observed in mice bearing tumors induced by TENM4-deficient 4T1 injection as compared to 4T1 wild-type (WT) cells, pointing to a protumoral role of TENM4 in TNBC. This finding fits with data revealing the attenuated migration and invasive capacity of TENM4-deficient cells compared to the WT counterpart.

Altogether, these data suggest the possible use of TENM4 as a target for effective anti-cancer therapies. Indeed, we demonstrated that TENM4 targeting through DNA vaccination is safe and effective in inducing specific cellular and humoral immune responses. Experiments evaluating the impact of the anti-TENM4 vaccination against the growth of murine TNBC are still ongoing.

Finally, we explored the possibility of TENM4 conveyance through extracellular vesicles (EVs) from TNBC tumors. A significantly higher amount of TENM4 protein was found in the plasma of TNBC tumor-bearing mice as compared to the healthy ones. Similar results have been obtained by analyzing plasma-derived EVs from a cohort of TNBC patients.

Conclusions: Taken together these findings suggest the protumorigenic role of TENM4 in TNBC and the potential to exploit TENM4 as a new biomarker for breast cancer.

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Adult B-cell acute lymphoblastic leukaemia (B-ALL): enriched pathways identify new targets for leukaemia stem cell immunotherapy

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Background: Adult B-cell acute lymphoblastic leukaemia (aB-ALL) is a haematological malignancy that is characterised by abnormal differentiation and proliferation of lymphoid progenitors in the bone marrow and extra-medullary sites. AB-ALL is rare and has a poor prognosis with a 5-year survival rate of 20%. First remission is achieved for most patients with the current treatment but relapse is common with a high associated mortality. However, new treatments such as immunotherapy are required to extend remission and prevent relapse to improve patient survival rates.

Methods: We identified aB-ALL antigens through the analysis of previously published microarrays (1,2). We identified antigens recognised by antibodies in patient but not healthy donor sera in protoarrays (3), and by SEREX (unpublished data) as well as the Cancer Testis Antigen database (http://www.cta.lncc.br/). We determined which antigens were associated with patient survival (BloodSpot.eu) and the upstream molecular pathways of each antigen using Enrichr.

Results: We found that the Wnt, Hippo and TGF pathways were highly represented ($p<0.02$) by the antigens identified. The top five antigens were examined to determine their expression in aB-ALL subtypes and their association with patient survival. SOX4 was upregulated in all types of B-ALL ($p<0.001$) but the expression was not associated with survival ($p=0.104$), while TGFB1 and ROCK1 were associated with survival ($p=0.0008$, and 0.001, respectively). ROCK1 was highly expressed in all types of B-ALL ($p<0.001$ except t(8;14) and t(1;19)). SOX4, ROCK1, YAP1, SMAD3 and TEAD4 were verified by qPCR but only TEAD4 showed significant transcript upregulation when comparing B-ALL and healthy donors ($p=0.01$).

Conclusions: We have identified five antigens that are differentially expressed in aB-ALL compared to healthy donor samples, and whose above and below median levels are associated with patient survival. Future studies will examine how these antigens and/or their pathways can be targeted by immunotherapeutic strategies.

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The role of SSX in the early development of ovarian cancer.

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Background: Ovarian Cancer (OC) affects around 1 in 78 women in their lifetime and is often diagnosed late due to its vague symptoms. The 5-year survival rates are as high as 95% when diagnosed at stage I but drop to as low as 15% at stage IV, showing the dire need for further treatment options. The cancer-testis antigen, SSX2 has been shown to be expressed in stage I and II OC, at higher frequencies than either CA125 or HE4.

Recent evidence has indicated that the most common and aggressive form of epithelial OC (HGSOC) derive from the STIC cells of the fallopian tubes. Here we aim to determine whether SSX can act as an early event in OC by transfecting immortalised fallopian tube cells with SSX cDNAs.

Methods: Immortalised fallopian tube cells, named FT194, were transfected with either SSX2A, SSX2B, SSX3, SSX4, or the vector alone and grown into stable cell lines. Stable transfections were confirmed using qPCR and immunocytochemistry. The biological effects of the transfections were investigated by examining cell doubling time (trypan blue exclusion assays), foci formation (using crystal violet staining), cell adhesion (onto collagen-coated plates) and cell migration (scratch assays).

Results: In the first experiment the cell line variants, vector control and parental cells all showed the same doubling time. However, in subsequent experiments when the cells had been passaged for a longer period FT194-SSX4 and FT194-SSX2A began to show a shorter doubling time and tolerated a higher cell density. Cells transfected with SSX3 and SSX4 were significantly more adhesive than the vector control ($P<0.05$) with SSX4 transfected cells being the most adhesive compared to all other variants, and control cell lines. All FT194-SSX cell variants appeared to close the scratch area faster than the parental or vector control cell line, but not as fast as those cells transfected with SSX4 ($p<0.05$).

Conclusions: Overexpression of SSX, specifically SSX4, exacerbates the cancer phenotype in immortalised fallopian tube cells, suggesting that SSX proteins could play a role in the early development of OC. Future studies will investigate how different levels of SSX protein affect the cells' cancer phenotype over time.

The TCR Vβ repertoire composition as a predictive biomarker of immunotherapy efficiency in non-small cell lung cancer patients

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Background: Immunotherapy is widely utilized for the treatment of solid tumors including non-small cell lung cancer (NSCLC). Considering that immune checkpoint inhibitors (ICIs) act through reinvigorating the endogenous antitumor T-cell immunity, facilitating cancer cell recognition and elimination by specific T-lymphocytes, it can be assumed that the TCR Vβ repertoire will change upon treatment with ICIs. The aim of this study is to investigate immunotherapy-induced changes in the peripheral TCR Vβ repertoire of NSCLC patients.

Methods: Thirty-six PD-L1(+), stage III NSCLC patients, eligible to receive durvalumab post radio-chemotherapy, have been recruited in this study (study group). Another twenty-one patients were assigned to receive either chemoradiotherapy or immunotherapy alone, or combined immuno-/chemioradiotherapy (control group). DNA was isolated from the peripheral blood of patients in the study group at baseline (before immunotherapy), and where available, at 1st evaluation (three months after immunotherapy initiation) and at the end of the treatment course or at disease progression. DNA was also extracted from the available tumor tissue samples of patients belonging in both groups. Next-generation-sequencing was performed and the Ion Oncomine™ TCR Beta-SR Assay was used for the identification and quantification of T-cell clonal expansion.

Results: Our findings demonstrate alterations in the peripheral TCR Vβ repertoire post immunotherapy. Based on clonotype counts three months after treatment initiation, two groups of patients were identified, those with i) decreased or ii) increased number of TCR clonotypes. Significant changes were observed regarding the frequencies of distinct TCR clonotypes at the different time points of the study. New TCR clonotypes with increased frequencies emerged in fourteen patients, while eight patients had clonotypes that disappeared at 1st evaluation. Significant differences were also identified between the patients' periphery and tumor.

Conclusions: Significant alterations in the TCR Vβ composition were identified post durvalumab treatment. The emergence of new TCR clonotypes and the extinction of others were also important findings. Such alterations in the TCR Vβ repertoire may hold potential as dynamic biomarkers of response to treatment.

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