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Scientific Foundation SPIROSKI

CITIM

8th International Conference
CANCER IMMUNOTHERAPY & IMMUNOMONITORING

CITIM 2025

March 31st – April 3rd 2025

BUCHAREST, ROMANIA



REDEFINING CANCER THERAPY

WWW.CANCERIMMUNO.ORG

Dear participants,

Welcome to the 8th International **Cancer Immunotherapy and Immunomonitoring Conference**!

This meeting is jointly organized by the International and Local Organizing Committees, and we have worked together to make this conference as informative and enjoyable as possible for you. We are looking forward to many superb presentations and discussions over the next few days that will allow you to consider new hypotheses and findings, present your own data and thoughts, and establish new collaborations and friendships. In addition to the outstanding basic and clinical science that will be presented, we hope that magnificent setting of the Hotel International close to the center of beautiful Bucharest will contribute to making this a very delightful and inspiring conference.

During this meeting, we will discuss cellular and molecular mechanisms of immune regulation in the tumor microenvironment that direct initiating and effector phases of antitumor immunity, as well control the efficacy and usefulness of anticancer immunotherapeutic modalities. We will also focus on recent clinical trial results and discuss the lessons we learned from preclinical evaluation of novel therapeutic approaches and immunomonitoring of patients with cancer receiving different modes of therapy. The special emphasis will be placed on topics presenting new ideas on how to improve the efficacy of cancer treatment, including combined chemo-radiation-immunotherapy, immunomodulators, and new targets for therapy. Invited plenary speakers and speakers selected from submitted abstracts, as well as session chairs will provide a framework for discussion. This will offer participants a unique opportunity to evaluate the field of tumor immunology and immunotherapy from different perspectives and might recommend new ways of solving existing problems and difficulties.

We hope that we have assembled one of the preeminent conferences on CITIM topics and we anticipate that the plenary sessions, short talks, poster presentations and after-the-meeting discussions will stimulate a breakthrough in our understanding of how to improve the efficiency of immunotherapy for cancer. If we can be of any assistance, please do not hesitate to call upon us. Please enjoy the meeting!

Sincerely,
CITIM-2025 Organizers

16TH YEAR ANNIVERSARY

CITIM 2009 – 2025

Kiev – Budapest – Krakow – Ljubljana – Bucharest – Prague – Tbilisi – Vilnius –



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The members of the organizing Committees of Cancer Immunotherapy and Immunomonitoring (CITIM) Conference express their gratitude and acknowledge the following organizations and companies for their generous support:

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The Institute of Cellular Biology and Pathology "Nicolae Simionescu" in Bucharest is a Romanian research institute, founded in 1979 by Maya and Nicolae Simionescu. Its inauguration featured a Romanian-American workshop attended by Nobel Prize laureates Professors George Palade, Christian de Duve, and Gunter Blobel. It is recognized as a Center of Excellence by the Romanian Academy and the Romanian Ministry of Education and Research. In 2000, it was also designated as a Center of Excellence by the European Community.

As a renowned research institution, ICBP "Nicolae Simionescu" stands out as a trusted partner in scientific collaboration, offering comprehensive support for a wide range of biomedical research projects. With its international recognition as a EU Center of Excellence and a long-standing history of innovation, ICBP-NS is well-positioned to contribute to groundbreaking research in cardiovascular pathobiochemistry and related fields. The institute's state-of-the-art facilities, advanced technological resources, and multidisciplinary expertise create a fertile environment for collaborative efforts, fostering innovation and excellence in scientific research. We welcome new partnerships, providing the highest level of scientific rigor, precision, and impactful results.

Organizer

The Romanian Society of Immunology was formed in 1971, having as founding fathers Prof. Ioan Moraru, director of the "Victor Babeș" Institute in Bucharest, Dr. Victor Gheţie, Dr. Andrei Sulica, and from the "Cantacuzino" Institute Prof. Dr. N. Nestorescu. It was first named the National Commission of Immunology and in 2007 it would become the Romanian Society of Immunology (Societatea de Imunologie din Romania - SIR). The first international scientific event organized by SIR was in 1973, in Sinaia, an event organized in collaboration with the International Union of Immunological Societies (IUIS). In 1976 SIR adhered to the European Federation of Immunological Societies and in 2016 SIR was hosted by "Victor Babeș" National Institute of Pathology. Now-a-days SIR has 120 active members from the main research institutes and universities. Almost every year SIR organized annual research meetings bringing together world-class international and national immunology researchers, including early career and established researchers.

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Trima Events started as a small company in 2006, when we thought of a different approach, of communication strategies and novel events, to which results play the most important role. To us, as an agency, what matters the most is that, at the end of the day, the client is happy and satisfied. Either you're planning a small-scale event of 10 people or a conference with hundreds or even thousands of participants, Trima Events is ready to handle all the details of your event - the perfect venue, invitations, catering, registration, set-up, branding, photo & video, printing, multimedia, transportation and accomodation. This way, planning and organizing an event will become a pleasant experience for you. Let our team handle the details while you enjoy the event!

VISITING BUCHAREST, ROMANIA

Dear Colleagues,

It is with great pleasure to welcome you to Bucharest for an exciting scientific meeting – CITIM 2025 and to experience this dynamic city. Bucharest is a fascinating blend of historic charm and modern energy, often referred to as the "Little Paris of the East" for its elegant architecture and cultural richness. The city boasts a unique mix of Belle Époque buildings, grand boulevards, and vibrant neighborhoods, offering a captivating glimpse into Romania's rich history and contemporary spirit.





The legend of Bucharest

This year marks Bucharest's 566th year since it was first officially attested in a document issued by Vlad the Impaler (Vlad Țepeș). Bucharest's most famous legend is about its supposed founder, Bucur, a shepherd. According to folklore, Bucur lived in the area with his flock and was known for his kindness and generosity. There are different versions of the story, but the most common one says that he built a small church and a settlement along the Dâmbovița River. His name, Bucur, means "joy" in Romanian, which is why the city was named București, meaning "city of joy".



Weather in Bucharest

Bucharest has a continental climate, with hot summers, cold winters, and mild springs and autumns. Summers can be hot and dry, with temperatures often exceeding 35°C (95°F), while winters are cold and snowy, sometimes dropping below -10°C (14°F). Spring and autumn are pleasant, with moderate temperatures and occasional rain.



Culture in Bucharest

The vibrant streets of Bucharest's Old Town reveal a fascinating blend of history and modern energy. Stroll past grand Belle Époque buildings, centuries-old churches, and lively cafés that bring the city's past and present together. But Bucharest has more to offer than its charming architecture. It is a hub of creativity, home to renowned theaters, experimental art galleries, and a thriving music scene. Free events fill the calendar, from street performances to cultural fairs. When the sun sets, Bucharest transforms into a city that never sleeps, with a booming nightlife scene where locals and visitors dance until dawn in stylish bars and underground clubs.



CONFERENCE SPEAKERS

Adit Ben-Baruch
Tel Aviv University, Israel

Tak W. Mak
Princess Margaret Cancer Centre, University of Toronto, Canada
Institute of Oncology and Immunology, University of Hong Kong

Michael Shurin
University of Pittsburgh, PA, USA

Viktor Umansky
German Cancer Research Center, Heidelberg, Germany

Alon, Ronen
Weizmann Institute of Science

Banciu, Manuela
Babes-Bolyai University

Baniyash, Michal
Hebrew University

Bilyy, Rostyslav
Danylo Halytsky Lviv National Medical University

Mlynska, Agata
National Cancer Institute

Neagu, Monica
Victor Babes National Institute of Pathology

Nishimura, Michael
Loyola University Chicago

Dace Pjanova
Riga Stradiņš University

Van der Burg, Sjoerd H.
Leiden University Medical Centre

Voronov, Elena
Ben-Gurion University of the Negev

Witz, Isaac
Tel Aviv University

Elkabets, Moshe
Ben-Gurion University of the Negev

Erin, Nuray
Akdeniz University

Fridlender, Zvi G.
Hebrew University

Gaipl, Udo S.
Friedrich-Alexander University of Erlangen-Nuremberg

Herrmann, Martin
Uniklinikum Erlangen

Jablonska, Jadwiga
University Hospital Essen

Jewett, Anahid
UCLA

Keisari, Yona
Tel Aviv University

Lundqvist, Andreas
Karolinska Institute

Pawelec, Graham
University of Tübingen

Porgador, Angel
Ben-Gurion University of the Negev

Rivoltini, Licia
Fondazione IRCCS Istituto Nazionale dei Tumor

Vita Pasukoniene
National Cancer Institute of Lithuania

Flavio Salazar Onfray
Universidad de Chile

Vannucci, Luca
Institute of Microbiology

CITIM 2025

CITIM 2025 AGENDA

March 31st - April 3rd
Bucharest, Romania



DAY 1

March 31st 2025

13:00 **OPENING CEREMONY**

13:30 - 14:30 **KEYNOTE LECTURE** *Michael Shurin*
Chairman: Voronov
Schwann Cells and the Neuro-Immune Axis in the Tumor Microenvironment

14:30 - 15:00 **COFFEE BREAK**

15:00 - 17:15 **SESSION 1: COMPLEX INTERACTIONS AT CANCER**
Chairmen: Yefenof, Keisari

15:00 - 15:30 Interactions Between Schwann Cells and Metastatic Breast Carcinoma (*Nuray Erin*)

15:30 - 16:00 Epigenetic Modulation Drives Inflammatory Responses in Sarcoma (*Lundqvist*)

16:00 - 16:30 Preemptively generated B cell memory accommodates future virus mutants – implications for cancer? (*Kirchenbaum, CTL*) (online, pre-recorded lecture)

16:30 - 17:00 Antigen-Specific Peripheral Immune Cell Dynamics Predicting Clinical Responses to Checkpoint Blockade in Melanoma (*Pawelec*)

17:00 - 17:15 **15 MINUTES SESSION CHANGE**

17:15 - 18:00 **SPONSOR SESSION: TECHNOLOGICAL ADVANCES FOR CANCER IMMUNO-RESEARCH**
Chairman: Butoi

17:15 - 17:40 Transforming Cancer Research: Utilizing High Content Screening and AI-Driven Cell Culture for Tailored Oncology Solutions *(de Bruin, Molecular Devices)*

17:40 - 18:00 Turning the spotlight on immune cell research: exploring FluoroSpot applications and beyond (*Solà, Mabtech*)

18:30 WELCOME RECEPTION
Hotel International Lobby (ground floor)

DAY 2

April 1st 2025

09:00 - 10:00 **KEYNOTE LECTURE** *Tak W. Mak*
(online streaming)
Chairman: Bilyy
Metabolic and Neural Regulation of Immunity and Cancer

10:00 - 11:10 **SESSION 2: ADVANCES IN THERAPY**
Chairman: Baniyash
10:00 - 10:25 Lessons Learned from Using TCR and CAR Gene Modified T Cells for Treating Cancer Patients (*Nishimura*)
10:25 - 10:50 Salvage TEIPP-Vaccination in Checkpoint-Resistant Lung Cancer (*van der Burg*)
10:50 - 11:10 Resipher: Real-Time, Long-Term OCR Monitoring for Deeper Insights (*Lu, Lucid scientific*)

11:10 - 11:30 **COFFEE BREAK**

11:30 - 13:30 **SESSION 3: TUMOR MICROENVIRONMENT**
Chairmen: Ben-Baruch, Gaipl
11:30 - 11:55 Deciphering Immune Tumor Subtypes Through Microenvironment Profiling (*Mlynska*)
11:55 - 12:20 The Tumor Immune Microenvironment as a Target for Nano-Therapies and Patient-Tailored Treatments (*Vannucci*)
12:20 - 12:45 Immunotherapy of cancer: are we already there? (*Porgador*)
12:45 - 13:10 New Concepts and Challenges in Radio-Immunotherapies (*Gaipl*)
13:10 - 13:30 Tissue transglutaminase as a new immunomodulatory target in ovarian cancer tumor microenvironment (*Sima*)

13:30 - 14:30 LUNCH BREAK

14:30 - 16:30 **SESSION 4: NEUTROPHILS AND NETS IN CANCER**
Chairman: Rivoltini, Lundqvist
14:30 - 15:00 The role of NETs and their aggregates in disease (*Herrmann*)
15:00 - 15:30 Neutrophil Extracellular Traps (NETs) in cancer and beyond (*Fridlender*)
15:30 - 16:00 TRIMELVax: A Novel Innate Immune-Engaging Vaccine for Malignant Melanoma Targeting Neutrophil-Driven Antigen Presentation and T-Cell Activation (*Salazar-Onfray*)
16:00 - 16:30 Targeting STAT3 in Neutrophils Alters Their Immunoregulatory Activity in Cancer (*Jablonska*)

16:30 - 18:00 SESSION 5: POSTERS & SNACKS

DAY 3

April 2nd 2025

08:30 - 09:30 **KEYNOTE LECTURE Adit Ben-Baruch**

Chairman: Hurwitz

Partners in crime: TNFα-based networks regulating cancer progression

09:30 - 10:35 **SESSION 6: IMMUNE MODULATING CELLS**

Chairmen: Pašukonienė, Porgador

09:30 - 09:55 The Role of Tumor Microenvironment IL-1β in Colorectal Cancer (*Voronov*)

09:55 - 10:20 Cancer Therapeutic Strategies to Rewire Tumor Microenvironment (*Banciu*)

10:20 - 10:35 Case reports and review of immune checkpoint inhibitor therapy and vitiligo in advanced cutaneous melanoma (*Pjanova*)

10:35 - 11:00 **COFFEE BREAK**

11:00 - 13:00 **SESSION 7: CYTOKINES, CHEMOKINES AND IMMUNE MODULATORS**

Chairmen: Jewett, Nishimura

11:00 - 11:30 Insights in genetic and epigenetic traits in cutaneous melanoma - new therapy targets (*Neagu*)

11:30 - 12:00 Development of an AXL/PD-1 BiCE as a Novel Immunotherapy Agent (*Elkabets*)

12:00 - 12:15 CD19 Chimeric Antigen Receptor (CAR) modified cytokine killer cells compared to classical CD19 CAR-T: advantages and disadvantages (*Karaman*)

12:15 - 12:30 Investigating RUNX2 KO in B16 melanoma cells as a potential strategy to enhance in vivo tumor response to therapeutic approaches (*Dobre*)

12:30 - 13:00 CD8 T cell immune monitoring: where the rubber hits the road (*Lehmann, CTL*) (online, pre-recorded lecture)

13:00 - 14:00 LUNCH BREAK

14:00 CITY TOUR

DAY 4

April 3rd 2025

08:30 - 09:30 **KEYNOTE LECTURE** *Viktor Umansky (streaming online)*
Chairman: Pašukonienė
MDSCs as Major Inducers of Immunosuppression in Melanoma

09:30 - 10:35 **SESSION 8: MYELOID CELLS & CANCER**
Chairmen: Neagu, van der Burg

09:30 - 10:00 ICAM-1 on cDC1 promotes the survival of stem-like memory CD8 T cells generated in tumor draining lymph nodes (*Alon*)

10:00 - 10:15 The Involvement of MDSCs-Bacteria Crosstalk in Colitis as a Predisposing Factor for Colitis-Associated Colorectal Cancer (*Reuven*)

10:15 - 10:35 Introducing Platinum® Pro: Unleashing the Power of Next-Generation Protein Sequencing in Your Lab (*Kudolo, QuantumSI*)

10:35 - 11:00 **COFFEE BREAK**

11:00 - 13:00 **SESSION 9: INFLUENCING SYSTEMIC AND CHRONIC INFLAMMATION**
Chairmen: Jablonska, Pawelec

11:00 - 11:30 Immune Landscape in Chronic Inflammation: Complications and Risks (*Baniyash*)

11:30 - 11:45 Live-Attenuated Influenza Viruses Co-Expressing BPV1 E6 and E7 Peptides Eradicate BPV1/2 Infection *(Hainisch)*

11:45 - 12:15 Tumor Destruction In Situ as a Tool to Trigger a Robust Anti-Tumor Immune Response (*Keisari*)

12:15 - 12:30 Novel Drugs Targeting for Immunogenic Cancer Cell Death (*Bilyy*)

12:30 - 12:45 LL-37: A powerful booster of ex vivo-prepared DC-based vaccines in prostate cancer patients and the TRAMP mouse model, without additional benefit from anti-PD1 therapy (Krausova)

12:45 - 13:00 A new pathway towards immunotherapy supported radiology for targeted Boron Neutron Capture Therapy (Spohr)

13:00 - 14:00 LUNCH BREAK

DAY 4

April 3rd 2025

14:30 - 16:00 **SESSION 10: TRANSLATIONAL RESEARCH HIGHLIGHTS & PERSPECTIVES**

Chairman: Mlynska, Vannucci

14:30 - 15:00 Immunomonitoring of patients with hepatic cancer undergoing liver transplantation after immunotherapy unravels an unexpected synergism between trained immunity and T cell memory (Rivoltini)

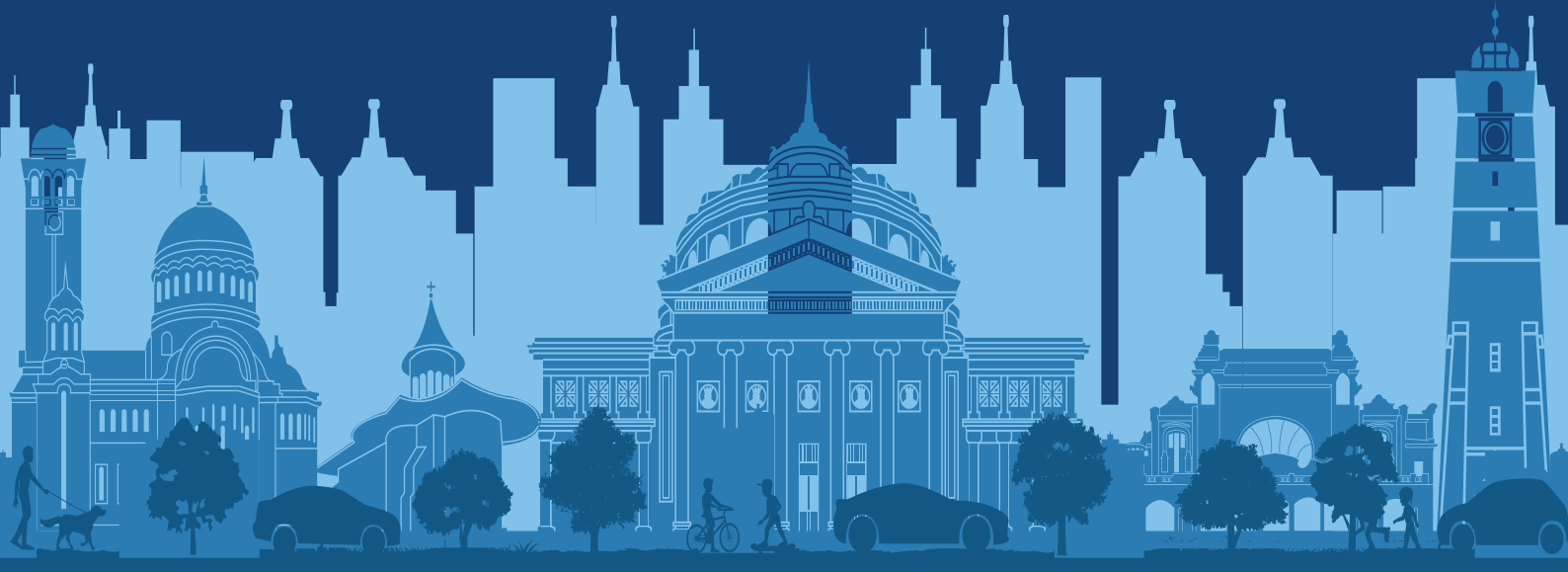
15:00 - 15:30 First-in-Human Clinical Study Demonstrating the Safety and Efficacy of NK101 Supercharged NK Cells (*Jewett*) (online, pre-recorded lecture)

15:30 - 16:00 Cancer Ecosystem – Quo Vadis? (*Witz*)

16:00 CLOSING CEREMONY

18:00 BANQUET, Hanul lui Manuc Restaurant

CITIM 2025 ABSTRACTS





KEYNOTE LECTURES

Scientific Foundation SPIROSKI, Skopje, Republic of North Macedonia
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Section: *Keynote lectures*

Schwann cells and the neuro-immune axis in the tumour microenvironment

Michael R. Shurin

Departments of Pathology and Immunology, University of Pittsburgh Medical Center and University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

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***Correspondence:** Michael R. Shurin. Departments of Pathology and Immunology, University of Pittsburgh Medical Center and University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA. E-mail: shurinmr@upmc.edu
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Abstract

Solid tumor innervation is an important compartment of the tumor microenvironment, which regulates tumor growth, spreading, and metastasis formation directly or indirectly via tumor-infiltrating immune cells, endothelial cells, and fibroblasts. Our data focusing on the mechanistical analysis of the intratumoral neuro-immune axis revealed that Schwann cells, the main glial cells of the peripheral nervous system covering neuron dendrites and axons, can stimulate tumor cell motility and spreading and can attract and up-regulate the immunosuppressive activity of immune regulatory cells – DCs, MDSCs, and Treg cells. The protumorigenic and immunomodulatory activity of Schwann cells in the tumor microenvironment is mediated, at least in part, by Schwann cell-derived exosomes. For instance, our recent data showed that human Schwann cells up-regulated human lung cancer cell proliferation, motility, and invasiveness by exosomal microRNA-21-5p, as was assessed in several in vitro experiments. Furthermore, animal model studies revealed that Schwann cell exosomal miRNA-21-5p could significantly increase metastasis formation. These and other results support the development of a mechanism-based Schwann cell-targeting approach as a novel neuroglia-centric antitumor therapeutic strategy.

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MDSCs as major inducers of immunosuppression in melanoma

Viktor Umansky

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

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***Correspondence:** Viktor Umansky. 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.
E-mail: v.umansky@dkfz.de
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Abstract

Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of myeloid cells enriched and activated in tumor microenvironment under chronic inflammatory conditions. They substantially contribute to immunosuppression in many cancer types including melanoma and represent thereby a valuable therapeutic target. It has been recently demonstrated that such MDSC accumulation in the tumor-bearing host could be mediated not only by a long-term production of soluble inflammatory factors, leading to an impairment of normal differentiation of myeloid cells but also via the acquisition of immunosuppressive properties by mature myeloid cells. This conversion of normal myeloid cells into MDSC could be influenced by extracellular vesicles (EV) secreted by tumor cells that contain a broad range of proteins, mRNA, microRNA and lipids and are considered as mediators of intercellular communication. Furthermore, the EV cargo, including HSP90□, S100A8/A9 and HMGB1 could contribute to this process of MDSC generation. The mechanisms of conversion of mature myeloid cells into MDSC and possible approaches for MDSC targeting in preclinical mouse tumor models and in melanoma patients will be discussed.

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Metabolic and Neural Regulation of Immunity and Cancer

Tak W. Mak

Princess Margaret Cancer Centre, University of Toronto and Centre of Oncology and Immunology, University of Hong Kong

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***Correspondence:** Tak W. Mak. Princess Margaret Cancer Centre, University of Toronto and Centre of Oncology and Immunology, University of Hong Kong. E-mail: Tak.Mak@uhn.ca
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Abstract

It has become clear that not only multiple molecular mechanisms, both intrinsic and extrinsic, but also numerous metabolites converge to alter core cellular metabolism during tumorigenesis. This state of affairs has made it difficult to analyse precisely the roles that key metabolites play in immune cell functions and/or cancer. Fortuitously, the finding that certain mutations in particular metabolic enzymes, such as isocitrate dehydrogenase, can give rise to malignancies such as gliomas, acute myeloblastic leukemia (AML) and angioimmunoblastic T cell lymphoma (AITL) give us avenues to comprehending metabolic and epigenetic pathways that are important in both in tumorigenesis and immune responses. Another metabolite that plays an important linking role between a seemingly unconnected biological process and immunity is acetylcholine. Although choline has been known for decades to be involved in neurotransmission, its deficiency has been shown to cause liver cancer. Our laboratory has reported several findings supporting the intriguing proposition that neurotransmitters like norepinephrine and acetylcholine may be key components that tie the neuronal system to the regulation of immune responses. In this presentation, I will discuss our findings dissecting the roles of important metabolic elements and neurotransmitters in immune cell regulation and diseases such as infections, autoimmune diseases and cancer development.

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Partners in crime: TNFα-based networks promoting cancer progression.

Adit Ben-Baruch

The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel

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***Correspondence:** Adit Ben-Baruch. The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel. E-mail: aditbb@tauex.tau.ac.il
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Abstract

Tumor necrosis factor α (TNFα) has been characterized as a potent pro-inflammatory cytokine, being responsible for the anti-tumor effects of Coley's toxins, reported at the beginning of the 20th century. However, extensive research of TNFα indicated that continuous presence of TNFα in tumors contributes to chronic tumor inflammation, and that the cytokine is a potent tumor-promoting and metastasis-enhancing factor.

Along these lines, we have demonstrated the ability of TNFα to induce a large variety of pro-metastatic functions in breast tumor cells and in stromal cells, acting in cooperativity with other tumor-supporting factors of the tumor microenvironment and with intracellular oncogenes. The signaling pathways mediating these collaborative activities of TNFα with other cancer-promoting elements have been identified in our studies, revealing a rich array of molecular setups acting under different stimulatory conditions.

Our current studies focus on the roles and regulation of TNFα receptors, TNFR1 but mainly TNFR2, in breast cancer progression. Reports that analyzed all breast cancer subtypes as a whole demonstrated metastasis-supporting roles for TNFR2 in breast cancer; in contrast, our research has addressed specifically the most aggressive triple-negative subtype of breast cancer (TNBC) and found that high TNFR2 expression by tumor-infiltrating lymphocytes (TILs) and by TNBC cells in patient tumors was significantly connected to improved patient prognosis.

Following up on these findings, we demonstrated that in chemotherapy-treated TNBC tumors the CD8+ TNFR2+ TIL population was associated with reduced tumor growth in syngeneic mice. Our recent observations suggest that these CD8+ TNFR2+ TILs carry cytotoxic functions, and that chemotherapy leads to selection or preferred proliferation of CD8+ TNFR2+ TILs that have the ability to eradicate cancer cells.

Moreover, our ongoing studies provide evidence to strong tumor-promoting activities of TNFR1 in TNBC cells, while TNFR2 has opposing and protective roles. We identified regulatory functions for WT TNFR2 and for a SNP-TNFR2 variant (depending on the conditions), leading to reduced tumor cell spontaneous and directed migration, lower release of pro-inflammatory chemokines and ICAM-1 expression, and down-regulation of pro-inflammatory signaling.

The observations of our recent studies suggest that in TNBC tumors TNFR2 has metastasis-inhibitory functions and thus it may not be a suitable therapeutic target in TNBC. Rather, we propose that TNBC patients should be treated by TNFR1-specific modalities, while sparing TNFR2; under such conditions, CD8+ TNFR2+ TILs may acquire more efficient anti-tumor activities in the presence of inhibitory immune checkpoints, and the metastatic potential of TNFR2+ tumor cells may be reduced.



INVITED LECTURES

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ICAM-1 on cDC1 promotes the survival ofstem-like memory CD8 T cells generated in tumor draining lymph nodes

Nehora Levi, Stav Kozlovski, Marina Kizner, Moshe Biton, Ronen Alon

Department of Immunology and Regenerative Biology, The Weizmann Institute of Science, Rehovot, Israel.

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***Correspondence:** Ronen Alon Department of Immunology and Regenerative Biology , The Weizmann Institute of Science, Rehovot, Israel.
E-mail: ronen.alon@weizmann.ac.il,
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Abstract

Dendritic cells (DCs) cross-present tumor antigens to cognate T cells in tumor-draining lymph nodes (TdLNs). ICAM-1 is a key adhesion molecule involved in the formation of diverse cell-cell contacts between immune cells as well as blood vessels and stromal cells. We have recently found that ICAM-1 expressed by lymph node DCs can stabilize antigen-dependent contacts with naïve CD8 T cells but is dispensable for CD8 T cell differentiation and proliferation into effector and memory lymphocytes generated in various models of vaccination and virus infections. To follow the role of DC ICAM-1 in CD8 proliferation and differentiation triggered by tumor expressed neoantigens, we orthotopically implanted breast cancer E0771 cells expressing the model OVA neoantigen in immunocompetent mice conditionally deleted of ICAM-1 expression in all CD11c expressing -conventional DCs (cDCs), monocyte derived DCs (mDCs) and pDCs. Strikingly, in contrast to viral challenges, the tumor challenge drove naïve tumor-antigen specific CD8 differentiation almost exclusively into T-stem-like central memory T cells (T_{SL}) with high expression of L-selectin (CD62L), PD-1, and the transcription factor TCF-1 implicated in T cell stemness and proliferative potential. Early CD8 activation and differentiation into these central memory T cells remained normal in these mice. This indicated that tumor antigen transfer by migratory DCs to lymph node DCs and its initial cross presentation to naïve T cells do not require ICAM-1 expression by all types of DCs. Furthermore, deletion of ICAM-1 selectively in XCR1 conventional type 1 dendritic cells (cDC1), specialized in tumor antigen uptake and cross priming of tumor specific T cells in tumor draining lymph nodes, did not impair early tumor specific CD8 activation and differentiation. Nevertheless, the maintenance of resident self-renewing stem-like tumor specific CD8 memory cells in the tumor draining lymph nodes was diminished in the absence of ICAM-1 expression by cDC1. We currently explore the possibility that ICAM-1 deficiency in these DCs impairs the stability of survival niches generated by Ag-specific CD8 T_{SL} and cDC1. Our results highlight a specialized instructive role of cDC1 ICAM-1 in antigen dependent maintenance of a pool of lymph node stem-like tumor-specific lymphocytes. This pool is a main source of tumor specific CD8 T cells which egress the draining lymph nodes and home into primary tumors, where they differentiate into short-lived terminally differentiated effector T cells (CTLs) critical for tumor eradication.
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Correlation of the tumor HLA-I expression (with LOH-HLA) with the immune response and clinical outcomes in NSCLC patients after perioperative neoadjuvant chemo-immunotherapy

Natalia Aptsiauri[1,2*], Francisco Perea[2], Marta Molina-Alejandre[3], Federico Garrido[1,2], Mariano Provencio[3], Francisco Ruiz-Cabello[1,2], Alberto Cruz-Bermúdez[1]

[1]*Department of Biochemistry, Molecular Biology III and Immunology, School of Medicine, University of Granada, Granada, Spain.*
[2] *Biosanitary Research Institute of Granada (ibs.GRANADA), Granada, Spain*
[3] *Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain.*

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***Correspondence:** Natalia Aptsiauri. Department of Biochemistry, Molecular Biology III and Immunology, School of Medicine, University of Granada, Granada, Spain.Biosanitary Research Institute of Granada (ibs.GRANADA),Granada,Spain. E-mail:naptsiauri@ugr.es
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Abstract

Total or selective loss of tumor antigen presentation caused by the loss of HLA class I (HLA-I) haplotype due to the loss of heterozygosity at chromosome 6 (LOH-HLA) may result in reduced repertoire of antigenic peptides presented to cytotoxic T-cells and is known to facilitate cancer immune evasion. LOH involving HLA genes has been linked to increased resistance to immunotherapy, cancer recurrence and poor survival after treatment with checkpoint blockade therapy. Previous studies have shown that neoadjuvant chemo-immunotherapy (CIT) is effective in NSCLC tumors with low tumor mutational burden (TMB). However, there is no data on perioperative chemo-immunotherapy efficacy or response mechanisms in the context of HLA-I defects.

In the present study, using SNP arrays, we demonstrated that LOH-HLA is a common event in cancer samples with a prevalence of a copy neutral type of LOH (CN-LOH) that affects entire chromosome 6 or 15 and involves chromosomal duplications. LOH-6 and was associated with homozygous HLA genotype and partial loss of HLA-I expression. Moreover, we analyzed the baseline HLA-I expression and LOH-HLA at diagnosis in correlation with the characteristics of the tumor microenvironment (TME) and clinical outcomes in a cohort of 24 patients with NSCLC after perioperative chemo-immunotherapy (nivolumab plus chemotherapy). We integrated HLA-I tumor status with molecular data (PD-L1, TMB, TCR repertoire, TILs populations, bulk RNA-seq, spatial transcriptomics) and clinical outcomes (pathological response and survival data) to study the activity of perioperative CIT considering HLA-I defects.

Baseline LOH and/or decreased HLA protein levels occurred in 41.7% of tumors and were categorized as HLA-deficient status. These tumors showed a “desert”-like tumor microenvironment at baseline, with lower PD-L1 levels and immune cell infiltrate. Despite their “cold” microenvironment, perioperative CIT induced similar complete pathological response rates, as well as 3-year progression-free survival (PFS) and overall survival (OS) rates, in both HLA-deficient and HLA-positive tumors. HLA-deficient tumors had B-cell aggregates forming tertiary lymphoid structures (TLS), CD4+T cells in a close location with HLA class II positive cells, and activated CD8+T cells. These data suggest a positive important role of TLS and the coordinated action of helper and cytotoxic T-cells in tumor rejection. All patients with TMB high and HLA-positive tumors were free of disease progression and alive at 3 years.

In conclusion, our findings highlight the impact of antigen presentation status on the immune microenvironment of NSCLC, and its potential synergy with TMB as a promising prognostic biomarker for long-term survival following perioperative CIT. It can help to unveil a potential response mechanism to be therapeutically enhanced in non-responsive tumors.

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Cancer Therapeutic Strategies to Rewire Tumor Microenvironment

Laura Patras[1], Alina Sesarman[1], Giorgiana Negrea[2], Stefan-Mihai Dragan[2], Marta-Szilvia Meszaros[1], Emilia Licarete[1], Valentin Florian Rauca[1], Lavinia Luput[1], Marius Alupei [1] , Alina Porfire[3], Manuela Banciu[1,4]

[1] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology and Center of Systems Biology, Biodiversity and Bioresources, Babes-Bolyai University, Cluj-Napoca, Romania
[2] Doctoral School in Integrative Biology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania
[3] Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Haţieganu, Cluj-Napoca, Romania
[4] UBBMed- School of Health, Babes-Bolyai University, Cluj-Napoca, Romania

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***Correspondence:** Manuela Banciu Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology and Center of Systems Biology, Biodiversity and Bioresources, Babes-Bolyai University, Cluj-Napoca, Romania, Doctoral School in Integrative Biology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania.
E-mail: manuela.banciu@ubbcluj.ro
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Abstract

The main objective of our research is to develop tumor-targeted therapies that modulate the tumor microenvironment (TME) to enhance therapeutic efficacy while minimizing side effects. Specifically, our focus lies in two key strategies: targeting tumor-associated macrophages (TAMs) to supress their protumor functions and utilizing extracellular vesicles (EVs) derived from dendritic cells to enhance intratumor drug delivery and immunotherapy.

We have previously explored the potential of statins, such as simvastatin (SIM), which exhibit antitumor effects at high doses. However, the systemic use of these high doses is limited due to severe side effects. To overcome this challenge, we incorporated SIM into long-circulating liposomes (LCLs) that have a natural tropism for the main key cell players in tumor microenvironment- tumor-associated macrophages (TAMs) [1]. In murine models of melanoma (B16.F10) and colon carcinoma (C26), our results demonstrated that LCL-SIM significantly enhanced antitumor activity compared to free SIM. Notably, LCL-SIM exerted its effects through a mechanism dependent on the tumor type. Thus, in melanoma-bearing mice, LCL-SIM inhibited TAM-driven oxidative stress and metastatic potential, primarily through the suppression of HIF-1α [1]. In contrast, in colon carcinoma-bearing mice, LCL-SIM reduced tumor growth via direct cytotoxic effects on tumor cells and by modulating the microenvironment, including anti-inflammatory and anti-angiogenic actions [2].

In parallel, our research has investigated EVs' ability to selectively deliver cytotoxic drugs that makes them ideal candidates for tumor-targeted drug delivery. In our recent studies, EVs derived from melanoma cells, when encapsulating DOX, demonstrated superior tumor growth inhibition compared to standard DOX liposomal formulations [3]. Furthermore, a combination of DOX-loaded EVs and SIM-loaded, IL-13-functionalized liposomes showed promising antitumor effects by disrupting the communication between TAMs and tumor cells [4].

Building on this, we are exploring EVs derived from activated dendritic cells as potential carriers for enhancing the efficacy of anti-PD-L1 immunotherapy in melanoma. This research aims to harness the natural properties of EVs to modulate the TME, enhance immune responses, and improve therapeutic outcomes in cancer treatment.

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References:

Alupei M.C., Licarete E., Patras L., et al.: Cancer Lett. **2015** 356 (2): 946-952.
Luput L, Licarete E, Drotar DM, et al. J Cancer **2018** 9(2): 440-449".
Patras L, et al., Cancer Biol Ther. **2022** 23(1).
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Immune Landscape in Chronic Inflammation: Complications and Risks

Prof. Michal Baniyash*

The Lautenberg Center for Immunology and Cancer Research
Israel-Canada Medical Research Institute Faculty of Medicine, The Hebrew University, Jerusalem, Israel

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***Correspondence:** Michal Baniyash The Lautenberg Center for Immunology and Cancer Research Israel-Canada Medical Research Institute Faculty of Medicine, The Hebrew University Jerusalem, Israel.
E-mail: michalb@ekmd.huji.ac.il
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Abstract

Controlled inflammatory responses are essential for restoring homeostasis following an insult. However, unresolved stimuli or dysregulated responses can lead to chronic inflammation, resulting in profound alterations in hematopoiesis, particularly within the myeloid lineage (myelopoiesis). These disruptions destabilize immune balance, triggering systemic shifts in immune cell phenotypes and functions that affect both local inflammation sites and peripheral tissues, with significant implications for disease progression. Our studies explore how chronic inflammation reshapes immune functions and disrupts niches of the bone marrow, gut, and peripheral tissues, creating harmful environments that drive complications. In the gut, chronic inflammation during inflammatory bowel disease (IBD), such as ulcerative colitis, compromises the epithelial barrier and facilitates dysbiotic bacterial invasion into the tissue. This process, along with the recruitment of immune cells such as myeloid-derived suppressor cells (MDSCs) to the intestine, disrupts homeostasis and heightens the risk of colitis-associated colorectal cancer (CAC). Intriguingly, the intricate interactions between MDSCs and dysbiotic microbiota and their outcomes in CAC remain poorly understood. Using a murine model of CAC, we demonstrate that CAC-bearing mice exhibit significantly elevated levels of highly immunosuppressive MDSCs alongside microbiota alterations. Within the tumor microenvironment, recruited MDSCs and invading bacteria localize in close proximity, revealing a complex MDSC-microbiota crosstalk. Our findings show that bacteria directly interact with and engulfed by MDSCs, enhancing their suppressive activities and inhibiting their differentiation into mature, beneficial myeloid cells, further impairing immune responses.
Our findings underscore the plasticity of MDSCs and their pivotal role in disease progression, highlighting MDSC-microbiota interactions as critical drivers of immune dysregulation and tumor growth in CAC. By unraveling these mechanisms, we provide new insights into potential therapeutic strategies targeting MDSC-microbiota crosstalk to mitigate inflammation-driven cancer progression.

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Novel Drugs Targeting for Immunogenic Cancer Cell Death

Galyna Bila[1,2], Anton Arkhypov [3], Insa Klemt [3] , Andriy Mokhir[3], Rostyslav Bilyy*[1,2]

[1]*Friedrich-Alexander-University of Erlangen-Nürnberg (FAU), Department of Chemistry and Pharmacy, Organic Chemistry II, 91058 Erlangen, Germany*

[2] *Danylo Halytsky Lviv National Medical University, Department of Histology, Cytology and Embryology, 79010 Lviv, Ukraine*

[3] *Institute of Cellular Biology and Pathology "Nicolae Simionescu", 8 B.P.Hasdeu street, Sector 5, Bucharest, Romania*

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***Correspondence:** Rostyslav Bilyy Friedrich-Alexander-University of Erlangen-Nürnberg (FAU), Department of Chemistry and Pharmacy, Organic Chemistry II, 91058 Erlangen, Germany, Danylo Halytsky Lviv National Medical University, Department of Histology, Cytology and Embryology, 79010 Lviv, Ukraine. E-mail: r.bilyy@gmai.com
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Abstract

The unique characteristics of lysosomes in cancer cells, including their number and stability, make them an attractive target for cancer-specific therapies. However, no lysosome-targeting drugs have been approved for clinical use. Here, we describe a novel therapeutic approach utilizing reversible thiol binder 11, which selectively accumulates in the lysosomes of cancer cells. Thiol binder 11 increases lysosomal reactive oxygen species (ROS), leading to lysosomal destabilization, disruption, and subsequent immunogenic cancer cell death—effects that are not observed in healthy cells.

In a murine sarcoma Nemeth-Kellner (NK)/Ly-RB model, treatment with thiol binder 11 significantly extended the lifespan of mice from 21 to 85 days and resulted in complete cures in 40% of the cohort. Remarkably, the cured mice developed tumor-specific antibodies, and a subsequent challenge with NK/Ly-RB cells led to 100% survival, compared to 0% in the control group of naïve mice.

Ex vivo analyses revealed that neutrophils from the spleens of cured animals contributed to the antitumor response by producing neutrophil extracellular traps (NETs), further supporting the immune-mediated effects.

In conclusion, thiol binder 11 exerts a direct antitumor

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Immunotherapy targeting mutant calreticulins in myeloid blood cancer

Stefan N. Constantinescu*

Ludwig Cancer Research Brussels Laboratories, de Duve Institute, Université catholique de Louvain, Brussels, Belgium
Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK

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***Correspondence:** Stefan N. Constantinescu Ludwig Cancer Research Brussels Laboratories, de Duve Institute, Université catholique de Louvain, Brussels,Belgium Ludwig Institute for Cancer Research,Nuffield Department.
E-mail: stefan.constantinescu@bru.licr.org
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Abstract

Myeloproliferative neoplasms (MPNs) comprise of group of hematopoietic stem cell diseases where acquired mutations lead to excessive formation of myeloid cells, red blood cells, platelets and granulocytes. Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary and Secondary myelofibrosis (MF) are the most prevalent MPNs. The phenotypic driving mutations are the acquired unique somatic *JAK2* V617 mutation, *MPL*/*TpoR* (thrombopoietin receptor mutations) and calreticulin (*CALR*) mutations. The latter are +1 frameshift mutations in exon 9 which lead to a new positively charged sequence in the place of the negatively charged C-terminus of wild type CALR, and lacking the ER retention KDEL sequence. Our group established that these mutants are inducing MPNs (15-20% of ET and 27% of MF) via binding in the ER and dimerizing the TpoR, which is then transported in a partially immature state to the cell surface by the rogue chaperone function of mutant CALR. Exposure at the cell-surface is obligatory for oncogenic signal of TpoR in the absence of Tpo ligand. Since the novel sequence is constituted of 36 novel amino-acids that should function as a tumor antigen we examined the possibility of using immunotherapy against this mutant sequence. In addition to the cell-surface exposed tumor antigen, we report that structure of the N-terminus non-mutated region of CALR slightly differs between wild type and mutant CALR. We used vaccination and showed that naive mice C57-B6 mice respond with antibody and T cell responses to immunization with CALR mutant peptide. In contrast, *Calr* del52 knock-in animals or transplanted animals expressing both wild type Calr and *Calr* del52 knock-in mutant do not respond. We discuss alternate vaccination methods that might be able to break this tolerance. Furthermore, we found that the mutant CALR is also secreted from cells of the clone that do not express TpoR. At levels equivalent to those in patients the circulating mutant CALR acts as a rogue cytokine, forming complexes with endogenous cell-surface mutant CALR and TpoR, but not with TpoR alone, explaining why the mutant CALR only stimulates clonal expansion and not polyclonal thrombocytosis. The circulating mutant CALR will interact with antibodies that recognize the tumor antigen and may exert a sink effect at low antibody doses. Based on our structural studies examining by deuterium exchange mass spectrometry we propose several precise sequences to be targeted by immunotherapy and discuss recent efforts using anti mutant CALR antibodies or bispsecific approaches.

References:

Pecquet C., Papadopoulos N., Balligand T., Chachoua I., Tisserand A., Vertenoeil G., Nedelec A., Vertommen D., Roy A., Marty C., Nivarthi H., Defour J.P., El-Khoury M., Hug E., Xu E., Zagrijtschuk O., Fertig E., Marta D., Gisslinger H., Gisslinger B., Schalling M., Casetti I., Rumi E., Pietra D., Cavalloni C., Arcaini L., Cazzola M., Komatsu N., Kihara Y., Sunami Y., Edahiro Y., Araki M., Lesyk R., Buxhofer-Ausch V., Heibl S., Pasquier F., Havelange V., Plo I., Vainchenker W., Kralovics R. & Constantinescu S.N. Secreted mutant calreticulins as rogue cytokines in myeloproliferative neoplasms. *Blood* **141**(8): 917-929, 2023.

Papadopoulos N., Nédélec A., Derenne A., Şulea T.A., Pecquet C., Chachoua I., Vertenoeil G., Tilmant T., Petrescu A.J., Mazzucchelli G., Iorga B.I., Vertommen D. & **Constantinescu S.N.** Oncogenic CALR mutant C-terminus mediates dual binding to the thrombopoietin receptor triggering complex dimerization and activation. *Nature Communications* 14(1): 1881, 2023

Balligand T., Achouri Y., Pecquet C., Gaudray G., Colau D., Hug E., Rahmani Y., Stroobant V., Plo I., Vainchenker W., Kralovics R., Van den Eynde B., Defour J.P. & **Constantinescu S.N.** Knock-in of murine Calr del52 induces essential thrombocythemia with slow-rising dominance in mice and reveals key role of Calr Exon9 in cardiac development. *Leukemia*, **34**(2):510-521, 2020.

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Development of a AXL/PD-1 BiCE as novel immunotherapy agent

Ksenia M Yegodayev, Moshe Elkabets*

The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel

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***Correspondence:** Moshe Elkabets The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
E-mail: moshee@bgu.ac.il
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Abstract

Myeloproliferative neoplasms (MPNs) comprise of group of hematopoietic stem cell diseases where acquired mutations lead to excessive formation of myeloid cells, red blood cells, platelets and granulocytes. Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary and Secondary myelofibrosis (MF) are the most prevalent MPNs. The phenotypic driving mutations are the acquired unique somatic *JAK2* V617 mutation, *MPL*/*TpoR* (thrombopoietin receptor mutations) and calreticulin (*CALR*) mutations. The latter are +1 frameshift mutations in exon 9 which lead to a new positively charged sequence in the place of the negatively charged C-terminus of wild type CALR, and lacking the ER retention KDEL sequence. Our group established that these mutants are inducing MPNs (15-20% of ET and 27% of MF) via binding in the ER and dimerizing the TpoR, which is then transported in a partially immature state to the cell surface by the rogue chaperone function of mutant CALR. Exposure at the cell-surface is obligatory for oncogenic signal of TpoR in the absence of Tpo ligand. Since the novel sequence is constituted of 36 novel amino-acids that should function as a tumor antigen we examined the possibility of using immunotherapy against this mutant sequence.

In addition to the cell-surface exposed tumor antigen, we report that structure of the N-terminus non-mutated region of CALR slightly differs between wild type and mutant CALR. We used vaccination and showed that naive mice C57-B6 mice respond with antibody and T cell responses to immunization with CALR mutant peptide. In contrast, *Calr* del52 knock-in animals or transplanted animals expressing both wild type Calr and *Calr* del52 knock-in mutant do not respond. We discuss alternate vaccination methods that might be able to break this tolerance. Furthermore, we found that the mutant CALR is also secreted from cells of the clone that do not express TpoR. At levels equivalent to those in patients the circulating mutant CALR acts as a rogue cytokine, forming complexes with endogenous cell-surface mutant CALR and TpoR, but not with TpoR alone, explaining why the mutant CALR only stimulates clonal expansion and not polyclonal thrombocytosis. The circulating mutant CALR will interact with antibodies that recognize the tumor antigen and may exert a sink effect at low antibody doses. Based on our structural studies examining by deuterium exchange mass spectrometry we propose several precise sequences to be targeted by immunotherapy and discuss recent efforts using anti mutant CALR antibodies or bispsecific approaches.

References:

Pecquet C., Papadopoulos N., Balligand T., Chachoua I., Tisserand A., Vertenoeil G., Nedelec A., Vertommen D., Roy A., Marty C., Nivarthi H., Defour J.P., El-Khoury M., Hug E., Xu E., Zagrijtschuk O., Fertig E., Marta D., Gisslinger H., Gisslinger B., Schalling M., Casetti I., Rumi E., Pietra D., Cavalloni C., Arcaini L., Cazzola M., Komatsu N., Kihara Y., Sunami Y., Edahiro Y., Araki M., Lesyk R., Buxhofer-Ausch V., Heibl S., Pasquier F., Havelange V., Plo I., Vainchenker W., Kralovics R. & Constantinescu S.N. Secreted mutant calreticulins as rogue cytokines in myeloproliferative neoplasms. *Blood* **141**(8): 917-929, 2023.

Papadopoulos N., Nédélec A., Derenne A., Şulea T.A., Pecquet C., Chachoua I., Vertenoeil G., Tilmant T., Petrescu A.J., Mazzucchelli G., Iorga B.I., Vertommen D. & **Constantinescu S.N.** Oncogenic CALR mutant C-terminus mediates dual binding to the thrombopoietin receptor triggering complex dimerization and activation. *Nature Communications* 14(1): 1881, 2023

Balligand T., Achouri Y., Pecquet C., Gaudray G., Colau D., Hug E., Rahmani Y., Stroobant V., Plo I., Vainchenker W., Kralovics R., Van den Eynde B., Defour J.P. & **Constantinescu S.N.** Knock-in of murine Calr del52 induces essential thrombocythemia with slow-rising dominance in mice and reveals key role of Calr Exon9 in cardiac development. *Leukemia*, **34**(2):510-521, 2020.

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Interactions between Schwann cells and metastatic breast carcinoma: contact-dependent and independent effects

Nuray Erin*, MD, Ph.D

Akdeniz University, School of Medicine, Dept. Of. Medical Pharmacology, Antalya/Türkiye

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***Correspondence:** Nuray Erin Akdeniz University, School of Medicine, Dept. Of. Medical Pharmacology, Antalya/Türkiye
E-mail: nerin@akdeniz.edu.tr
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Abstract

Schwann cells and may enhance cancer growth and metastasis. Breast carcinoma is a heterogeneous disease and our previous studies demonstrated further heterogeneity in site-dependent metastasis. Specifically cells isolated from liver metastasis of 4T1 mammary carcinoma cells (4TLM) demonstrate distinct phenotypic features leading to diverse tumor microenvironment compared to 4T1 mammary carcinoma cells metastasized to brain (4TBM). In accordance, non-metastatic/hardly metastatic cells obtained from 4T1 mammary carcinoma (67NR cells) interacts with their microenvironment differently. Hence, we examined the effects of secretomes obtained from 67NR, 4TBM and 4TLM cells on primary Schwann cells obtained from mouse sciatic nerve, specifically focusing on inflammatory cytokine release. Secretomes of liver metastatic subset of mammary carcinoma cells (4TLM) induced striking increases in IL-6 secretion, which was markedly higher than IL-6 secretion induced by secretomes of 4TBM and 67NR cells. Similarly, only 4TLM secretomes induced remarkable increases in inflammatory chemokines KC and MIP-2. Monocyte Chemoattractant Protein-1 (MCP-1) (also called CCL2), involved in migration of monocyte, is highly secreted by 4TLM cells but not by 4TBM and 67NR cells. Secretomes of 4TLM cells also induced robust increases in MCP-1 secretion from Schwann cells. Interestingly metastatic cells decreased IL-1b secretion from Schwann cells.

Next we determined how co-culturing of Schwann cells with carcinoma cells alter cytokine/chemokine secretion in order to determine contact-dependent changes. Schwann cells were co-cultured with cancer cells at 1/5, ½.5 and 1/1 ratio and secretomes were analyzed for cytokine secretion. Interestingly, co-culturing markedly suppressed IL-6 secretion from Schwann cells. Secretion of chemokines such as KC and MCP-1, however increased following co-culturing.

Lastly we have performed proteomic analysis of Schwann cell secretomes in the presence and absence of secretomes from cancer cells. Peptides were analyzed using the Orbitrap Fusion mass spectrometry instrument (Thermo Scientific) coupled with the Dionex Ultimate 3000 RSLCnano system. For bioinformatics analysis, the Proteome Discoverer (PD) program was used. As the Venn diagram shows, 446 proteins were identified in secretomes of unstimulated Schwann cells. Interestingly 363 of these proteins were also found in metastatic and non-metastatic breast cancer cells. This was surprising given the differences in cell lineages. Analysis of the 363 common proteins by KEGG pathways revealed several important carcinoma-associated pathways, such as the HIF-1 signaling pathway. The 37 proteins unique to Schwann cells pointed out two reactome pathways: Regulation of Insulin-like Growth Factor transport and post-translational protein phosphorylation. Protein content of secretomes of Schwann cells stimulated with secretomes of cancer cells differed from unstimulated Schwann cells such that 13 proteins were markedly downregulated while over 200 proteins were upregulated. We are in process of analyzing these results.

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Neutrophil Extracellular Traps (NETs) in cancer and beyond

Nuha Al-Sharif, Mohamad Qaisi, Naomi Kaisar, Dan Padawer, Osnath Bohana, Yael Volman, Zvi G. Fridlender*

Hadassah-Hebrew University Medical Center, Jerusalem, Israel

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***Correspondence:** Zvi G. Fridlender Hadassah-Hebrew University Medical Center, Jerusalem, Israel
E-mail: Fridlender@hadassah.org.il
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Abstract

Neutrophils play a major role in tumor biology. The main effects described by us and others of neutrophils in cancer are directly on tumor cells, or indirectly by affecting the stromal immune system. An exciting relatively novel phenomenon described is the neutrophils' ability to release webs of fibrous decondensed chromatin named neutrophil-extracellular traps (NETs). Originally characterized as a novel antimicrobial mechanism, NETs were later described also in the context of cancer, but the exact effects of NETs on tumor and immune cells, and the importance of NETs production (NETosis) in cancer progression is still not clear.

In the current work we characterize the release of NETs from neutrophils during cancer progression, and the effect that NETs confer on tumor and immune cells, comparing the effects of NETs from healthy donors and from lung cancer patients.

We isolated NETs from blood samples of lung cancer patients and healthy donors. Combining multiple approaches such as confocal microscopy, ELISA and flow cytometry and more we tested the impact of human NETs on tumor cell viability and migration ("wound repair"), and tumor-related T-cell proliferation, and activation.

We found that neutrophils from lung cancer patients (LC) produce smaller amounts of NETs Compared to healthy donors (HD) were found to be cytotoxic to A549 lung cancer tumor cells, though NETs from both HD and LC are capable of killing tumor cells and inhibit their motility. NETs were able to show increased activation of T-cells. Activation of CD8 T-cells was at least partly dependent on the presence of DNA, whereas activation of both CD4 and CD8 continued at least to some extent after prolonged heat and use of DNase, suggesting a metabolic mechanism. Furthermore, NETs increased proliferation of CD8 T-cells, somewhat more when originated from HD compared to LC. The mechanism of this effect was also suggested to be through a metabolite.

We next assessed different inhibitors of NETosis in vitro and investigated their efficacy in vivo models of cancer. Interestingly we found that inhibition of ROS production didn't lead directly to reduction of NETosis as it's generally accepted, suggesting an alternative pathway for NETosis. Using a physiologic activator of NETosis that acts through the TLR4 receptor and ROS production we noted in a mouse model of lung cancer that primary tumor growth was inhibited, while the metastatic process was increased.

Our continuous studies on the mechanisms of production of NETs in cancer and beyond as well as the understanding of their effects on both tumor and immune cells shed a light on the role of this exciting, relatively recently described phenomenon in cancer, and could aid in the development of new strategies to direct the immune system against the tumor.

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New concepts and challenges in radio-immunotherapies

Udo S. Gaipl*

Translational Radiobiology, Department of Radiation Oncology, Uniklinikum Erlangen, Erlangen, Germany

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***Correspondence:** Udo S. Gaipl Translational Radiobiology, Department of Radiation Oncology, Uniklinikum Erlangen, Erlangen, Germany
E-mail: udo.gaipl@uk-erlangen.de
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Abstract

During the last decades it has become obvious that radiation has manifold biological consequences ranging from DNA damage to local and systemic immune alterations. Thereby, it has taken a long time to understand how local radiation exposure leads to immune changes that are not just immune suppressive ones. Cells that are exposed to radiation react with a stress response and, when the trigger is strong enough, undergo cell death. In these processes the cells interact with the immune system with consequences on molecular, cellular and organism level. In the talk connection between radiation-induced DNA damage, DNA damage responses and immunological consequence will be elucidated. The dependence of immunological consequences of radiation exposure on dose, dose rate (FLASH-radiotherapy), fractionation, and the tumor micro-environment including lymph nodes will be outlined. Immune signatures being prognostic for radio-immunotherapies will be introduced and challenges to make use of such signatures for understanding radiation-specific immune alterations that persist will be discussed, taking also into consideration the dynamics of immune reactions. Ultimately, it should become clear that radiation and the immune system belong together and that radiation-induced immune changes can and should be used therapeutically for solid tumors in combination with distinct immune therapies such as immune checkpoint inhibition, vaccination and CAR T cell therapy.

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The role of NETs and their aggregates in disease.

Christine Schauer[1], Maximilian Dölling[1], Martin Herrmann*[2]

[1] Internal Medicine, University Clinic, Erlangen, Germany.
[2] Department of General-, Visceral-, Vascular- and Transplant Surgery, University Hospital Magdeburg, Magdeburg, Germany

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***Correspondence:** Martin Herrmann Department of General-, Visceral-, Vascular- and Transplant Surgery, University Hospital Magdeburg, Magdeburg, Germany
E-mail: martin.herrmann@medma.uni-heidelberg.de
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Abstract

Neutrophil extracellular traps (NETs) are structures released by neutrophils in response to infections or tissue damage. They are made of decondensed chromatin, histones, various antimicrobial proteins, and a plethora of enzymes, growth factors, chemokines, and cytokines. Importantly, NETs generated in high neutrophil densities tend to form aggregates that often reach large sizes (1). Whereas solitaire NETs are rapidly degraded, these NET aggregated can persist for many months and even years. In this time NETs accumulate further compounds like complement and fibrin. In certain locations aggregated NETs calcify. Recent research has linked NETs to various diseases, including cancer.
NETs display various tumor modifying features and can (I) create a "sticky" scaffold in the bloodstream that promotes the growth of metastatic cells (II) degrade the extracellular matrix and release factors for tumor cell growth and invasion. (III) stimulate neo-angiogenesis (IV) shield (cancer) cells from immune attack (V) promote an immunosuppressive environment (VI) form immune thrombi as well as fibrin and platelet aggregates.
In formalin-fixed paraffin-embedded tissues the decondensed, extracellular DNA of NETs can reliably be differentiated from condensed nuclear DNA by immunodetection with anti-DNA IgM. Using high-resolution stimulated emission depletion (STED) microscopy, we showed that citrullinated histone H3 (H3cit) closely associated with the de-condensed extracellular DNA of NETs.
In patients with colon cancer we detected NETs preferentially only in the tumour centres or both in the centre and the invasive front. Citrullinated NETs were associated with high histopathological tumour grades and lymph node metastasis.
In vitro, NETs increased mRNAs of mesenchymal markers vimentin and fibronectin and epithelial-mesenchymal transition promoting transcription factors ZEB1 and Slug (SNAI2); the epithelial markers E-cadherin (CDH1) and epithelial cell adhesion molecule (EPCAM) were downregulated. This indicates that NETs have activated an epithetlial-mesenchymal transition-like process in these cells and may contribute to the metastatic progression of CRC.
(1) Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Schauer C, ….. Bilyy R, ….., & Herrmann M.; Nat Med. 2014 May;20(5):511-7. doi: 10.1038/nm.3547. cited 977 times.

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Targeting STAT3 in neutrophils alters their immunoregulatory activity in cancer

Jadwiga Jablonska*

Department of Otorhinolaryngology, University Hospital Essen, Essen, Germany

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***Correspondence:** Jadwiga Jablonska Department of Otorhinolaryngology, University Hospital Essen, Essen, Germany.
E-mail: jadwiga.jablonska@uk-essen.de
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Abstract

Myeloid cells are a major component of immune cells infiltrating tumors, interacting with each other, with tumor cells and other stromal cells, and demonstrating a prominent plasticity. Neutrophils, as a population of such cells, contribute to cancer progression via multiple mechanisms, including stimulation of angiogenesis and the regulation of adaptive immune responses. Importantly, neutrophils were demonstrated to be associated with poor clinical outcome in patients with different kinds of cancer, including head and neck cancer, renal cancer, non-small-cell lung carcinoma or melanoma. Enrichment of neutrophils was also associated with metastases in various tumor entities.

Activity of neutrophils strongly depends on the cytokines or growth factors present in the microenvironment. Since these cells are highly plastic and their functionality can be easily altered, they are ideal target for anti-tumor therapies. Since we observe that elevated STAT3 is associated with pro-tumoral activity of neutrophils, we tested here the approach to functionally reprogram these cells by the inhibition of STAT3 signaling pathway. We could show that such STAT3 inhibition solely in neutrophils led to decreased tumor growth and metastasis in cancer, which was accompanied by a significant expansion of $CD8^+$ cytotoxic T cells in tumors and tumor-draining lymph nodes (TDLNs). Comprehensive multiparameter analyses of tumors and TDLNs revealed a significant enrichment of $CD8^+$ T cell subsets with strongly cytotoxic activity, which effectively killed cancer cells *in vitro*. These findings provide novel insights in therapeutic avenues and suggest neutrophil-targeted STAT3 inhibition as a promising opportunity for cancer immunotherapy.

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First in Human Clinical Study Demonstrating the Safety and Efficacy of NK101 (supercharged NK cells) in the Treatment of Patients with Cancer

Anahid Jewett*

University of California School of Dentistry, Los Angeles, USA

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***Correspondence:** Anahid Jewett University of California School of Dentistry, Los Angeles, USA
E-mail: ajewett@ucla.edu
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Abstract

Natural killer (NK) cells are well-known to lyse tumor cells, but their anti-tumor activity is impaired in tumor microenvironment. In addition, the lack of adequate expansion and decreased persistence in cancer patients and diminished function, with the existing strategies have hampered the field and has shown little efficacy in clinical trials. Moreover, the reduced number and function of NK cells in cancer patients has led to a lower success rate in developing adaptive treatment using autologous NK cells. To overcome these problems, we designed a cancer immunotherapeutic strategy using allogeneic healthy donor-derived supercharged NK cells (NK101). We previously have demonstrated the preclinical efficacy and safety of NK101 in tumor-bearing humanized-BLT mice. In this presentation, we report the results of the first two cancer patients and one patient suffering from deep vein thrombosis (DVT) infused with NK101. The therapy demonstrated an excellent safety profile, with no adverse side effects or any discomfort to either patient post-infusion. In contrast, patients reported an unusual increase in energy levels, disappearance of aches and pains and increase in well-being. Patients were monitored for 180 days post-therapy, and post-infusion blood analysis of patients revealed a positive immune response in the absence of any kind of adverse safety events. Post-infusion clinical presentation revealed early signs of clinical efficacy, with several validated data points confirming NK101's ability to target and significantly decrease the tumor load in cancer patients. In addition, the percentages and function of NK cells rose in patients significantly and remained high for the duration of the studies. The early clinical findings are consistent with preclinical results obtained using NK101 cells that demonstrated the ability to arrest tumor progression and restore immune function.

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Tumor destruction in situ as a tool to trigger a robust anti-tumor immune response

Yona Keisari*

Department of Clinical Microbiology and Immunology, Faculty of Medical and Health sciences, Tel Aviv University, Tel Aviv, Israel

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***Correspondence:** Yona Keisari Department of Clinical Microbiology and Immunology, Faculty of Medical and Health sciences, Tel Aviv University, Tel Aviv, Israel
E-mail: ykeisari@tauex.tau.ac.il
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Abstract

The role of the immune response in tumor development and treatment is a complicated issue. Many elements are involved such as the variety of tumor types, the organism genetics, the complexity of the immune response, metabolism, age, and the microbiome to name a few.
The goal of cancer immunotherapy is to induce tumour regression, eradicate minimal residual disease, establish lasting antitumour memory and avoid non-specific or adverse reactions. Yet, even though about 70 years elapsed since the discovery of tumor antigens, the success of therapeutic cancer vaccines has been very limited and variable. It mainly resulted from the heterogeneity of the tumor and its microenvironment, the presence of immunosuppressive cells and molecules, and tumor escape mechanisms. The failure to develop effective specific tumor vaccines reverted the attention to an observation, which is now 70 years old, “the abscopal effect”. This effect states that irradiation of a tumor site can cause the decrease in size of distant tumor foci [1], an effect which was eventually attributed to the activation of anti tumor immunity.
There is a growing volume of data from both clinical and non-clinical studies that various modalities of *in situ* tumor destruction (ablation) can be a source of tumor antigens that otherwise would be difficult to expose to boost anti-tumor immune responses. The ensued immune reactivity can be further strengthened by both immune and non-immune modulators (2).
Tumor ablation is defined as the direct application of chemical, thermal, radiation or electrical energy to a specific focal tumor to achieve eradication or cytoreduction inducing cellular necrosis. To this effect we developed a novel alpha radiation-based tumor treatment termed “Diffusing alpha emitters Radiation Therapy-DaRT”, which can cause fast tumor cell destruction within several days and trigger specific anti-tumor immunity [3].
The advantages of tumor destruction *in situ*, which facilitate triggering anti-tumor immunity include:
1. Tissue damage which attracts inflammatory cells and causes the release of inflammatory cytokines.
2. The extensive cell death release large quantities of tumor-associated antigens in the context of danger-associated molecular patterns (DAMPs) to the immune system.
3. Elimination of the requirement to identify tumor-specific antigens for each patient.
There are several types of measures which can be taken to promote anti-tumor immunity following *in situ* tumor ablation: i) immune potentiation agents, and ii) agents to counteract suppressive mechanisms.
These findings prompt new ablation modalities and immunological manipulations which will increase the arsenal of the medical staff and promote cancer treatment.
1. Mole, R. H. (1953). Whole body irradiation; radiobiology or medicine? Br J Radiol, 26(305), 234-41.
2. Keisari, Y. (2017). Tumor abolition and antitumor immunostimulation by physico-chemical tumor ablation. *Frontiers Biosc* (Landmark edition), 22, 310-347.
3. Keisari, Y., Popovtzer, A., Kelson, I. (2020). Effective treatment of metastatic cancer by an innovative intratumoral alpha particle-mediated radiotherapy in combination with immunotherapy: A short review. *Journal of Physics*: Conference Series, 1662, 01201.

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Epigenetic modulation drives inflammatory responses in sarcoma

Mireia Cruz De los Santos, Andreas Lundqvist*

Department of Oncology Pathology, Karolinska Institutet, Stockholm, Sweden.

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***Correspondence:** Andreas Lundqvist Department of Oncology Pathology, Karolinska Institutet, Stockholm, Sweden.
E-mail: andreas.lundqvist@ki.se
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Abstract

Compared with adult tumors, childhood malignancies are typically driven by a relatively small number of mutations, frequently in genes encoding epigenetic regulators. High-grade osteosarcoma (OS) is an aggressive bone malignancy characterized by massive genomic rearrangements and one of the most prevalent cancers in young adults. However, the role of epigenetic reprogramming in OS remains unclear. In this study, we sought to investigate the potential of histone modifications as prognostic and therapeutic biomarkers in OS. An *in silico* predictive algorithm of histone modification landscape from RNA sequencing data revealed that genome-wide levels of acetylation of the lysin 27 in histone 3 (H3K27ac) inversely correlated with survival. Notably, H3K27ac levels positively correlated with immune-related signatures indicating an inflamed tumor microenvironment. To investigate if H3K27ac expression influences inflammatory responses, the histone deacetylase 1-3 (HDAC) inhibitor Entinostat was used to induce H3K27ac expression in sarcoma tumors. Compared with untreated sarcoma tumors, Entinostat treatment resulted in higher infiltration of tissue resident T cells in *ex vivo* patient-derived sarcoma spheroids and in an *in vivo* model of OS. Mechanistically, our preliminary results highlight the involvement of the Hippo pathway mediators YAP1 and VGLL3 in Entinostat-mediated tumor infiltration of T cells. Together, these findings show that H3K27ac drives T cells infiltration into sarcoma. Furthermore, genome wide H3K27ac levels may be used to predict survival in OS. In addition, H3K27ac levels are associated with immune-related signatures and could be explored to promote inflammatory responses in sarcoma.

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Deciphering immune tumor subtypes through microenvironment profiling

Agata Mlynska[1,2*], Egle Zymantaite[1,3], Austeja Butkute[1,3], Neringa Dobrovolskiene[1], Jan Aleksander Krasko[1,2], Olha Karaman[1], Karolina Suveizde[1], Beatrice Gudaite[1,3], Margarita Zvirble[1,3], Emilija Paberale[1,3], Nijole Matuseviciene[1], Vita Pasukoniene[1]

[1] *Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania*
[2] *Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania*
[3] *Life Sciences Center, Vilnius University, Vilnius, Lithuania*

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***Correspondence:** Agata Mlynska Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania, Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania. E-mail: agata.mlynska@nvi.lt
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Abstract

Different patterns of immune and stromal interactions within the tumor microenvironment significantly impact tumor behavior and response to treatment. Recent research has identified distinct immune subtypes—immune desert, excluded, and inflamed—each associated with specific mechanisms that impede anti-tumor immune responses. In the era of precision medicine, accurately stratifying patients into these subtypes is essential for optimizing cancer management and personalizing immunotherapy. The research team at National Cancer Institute in Lithuania addresses critical gaps in this field by focusing on several key areas. Our first focus is on developing reliable and accessible tools for determining patient immune subtypes. We are integrating transcriptomics and histology data to create a robust subtyping pipeline, which we are validating with real-world data and streamlining for clinical use. Another aspect of our work involves creating translatable preclinical models for cancer immunotherapy through utilizing 3D cell cultures and biocompatible scaffolds. We are developing druggable syngeneic murine tumor models that accurately reflect the three immune subtypes, enhancing the relevance of preclinical studies. Using both pharmaceutical and non-pharmaceutical approaches, we are tailoring immunomodulatory interventions to address the unique characteristics of each immune subtype. Joint efforts in these research areas could generate translatable insights, contributing to more precise cancer therapies and overcoming resistance mechanisms.

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Insights in genetic and epigenetic traits in cutaneous melanoma - new therapy targets

Monica Neagu [1,2,3*], Dobre Elena-Georgiana [1, 3], Carolina Constantin[1,2], Mihaela Surcel[1], Adriana Munteanu[1], Sabina Zurac [1,2,4]

[1]*Immunology Laboratory, Victor Babes National Institute of Pathology, Bucharest, Romania;*
[2]*Pathology Department, Colentina Clinical Hospital, Bucharest, Romania;*
[3]*Doctoral School, University of Bucharest, Bucharest, Romania;*
[4]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

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***Correspondence:** Monica Neagu Immunology Laboratory, Victor Babes National Institute of Pathology, Bucharest, Romania; Pathology Department, Colentina Clinical Hospital, Bucharest, Romania; Doctoral School, University of Bucharest, Bucharest, Romania.
E-mail: neagu.monica@gmail.com
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Abstract

The purpose of our study was to evaluate the genetic alterations that could affect the EGFR-RAS-RAF pathway in search of new druggable genetic targets.
Materials and methods. Paraffin embedded samples from patients diagnosed with skin melanoma (n=22) were evaluated using droplet digital PCR (ddPCR) in comparison to benign nevi (n=15) and normal skin samples (n=15). Samples were diagnosed using histology and immunohistochemistry (IHC) evaluation. The study was operated in accordance with the principles of the Declaration of Helsinki (according to Annex 4, National Law 104/2004 and application HG451/2004, amended in 2013), and with the approval of the Local Ethics Committee of Colentina Clinical Hospital, Bucharest, Romania (approval no. 25/2017). Patients and control subjects were informed of the study protocol and provided written informed consent prior to enrollment. The mutational status of healthy skin, nevi and melanoma specimens was assessed by ddPCR using *BRAF V600, NRAS G12/G13, NRAS Q61, KRAS Q61* and *EGFR* exon 19 deletions Screening Assays according to manufacturer's instructions.
Results. The samples subjected to ddPCR were analyzed also on RT-PCR and we found a perfect correlation between the data obtained on both technologies (Spearman correlation coefficient = 0.9913).We have found over 86% of the benign nevi harboring BRAF V600 mutations, while only 56% of skin melanomas harbored the same mutation. In melanomas BRAF mutations significantly associated with Breslow index and tumor infiltrating lymphocytes, whereas *NRAS* mutations (*NRAS* Q61 and *NRAS* G12/G13) correlated with Breslow index and the mitotic index. Particularly, malignant transformation to a BRAF wild-type melanocytic tumor occurred in one of our cases involving a benign nevus with BRAF V600 positivity. ddPCR technique is sensitive so that we detected have in a normal skin sample NRAS G12/Q61 mutation, while the benign nevus excised from the skin harbored BRAF mutation. The high mutation profile of benign nevi was associated with a high expression of cell cycle regulatory proteins that could explain the melanocyte's arrested cell cycle within a benign nevus.
Conclusion. The molecular profiling of tumorigenesis is important to follow the dynamics of melanocyte transformation towards melanoma and moreover can orient the patient therapy.

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Lessons Learned from Using TCR and CAR Gene Modified T Cells for Treating Cancer Patients

Michael I. Nishimura

Department of Surgery, Loyola University Chicago, Maywood, IL 60153, USA

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***Correspondence:** Michael I. Nishimura Department of Surgery, Loyola University Chicago, Maywood, IL 60153, USA.
E-mail: michaelinishimura59@gmail.com
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Abstract

The recent FDA approval of various CAR, TCR, and TIL therapies has only increased the excitement over T cell based adoptive transfer therapies. We have manufactured TCR and CAR T cells to treat a total of 29 patients with melanoma (N=7), clear cell renal cell carcinoma (N=15), and non-Hodgkins lymphoma (N=7). While there were objective clinical responses in the three patient populations with the NHL patients treated with CD19 CAR T cells had the best outcomes. What is clear is that the genetically modified T cells can persist for years but they become nonfunctional, probably due to the host environment. Results from our trials support this notion and will be discussed with potential solutions to overcoming the immune suppressive tumor microenvironment.

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Myeloid-derived Suppressor Cells EN-RAGE Antitumor Immunity

Suzanne Ostrand-Rosenberg[1], Tom Hueckstedt[2], Karl Sanders[2]

[1] *Division of Microbiology and Immunology, Department of Pathology, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA*
[2] *George E. Wahlen Department of Veterans Affairs Medical Center, Division of Respiratory, Critical Care, and Occupation Pulmonary Medicine, Department of Internal Medicine, University of Utah, Salt Lake City, UT, USA*

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***Correspondence:** Suzanne Ostrand-Rosenberg Division of Microbiology and Immunology, Department of Pathology, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA.
E-mail: srosenbe@umbc.edu
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Abstract

Immune checkpoint inhibitors (ICI) have radically altered cancer therapy and enable delayed tumor progression and in some cases cures for some cancer patients. However, ICI efficacy is mixed depending on the type of cancer and individual cancer patients. Myeloid-derived suppressor cells (MDSCs) are potent inhibitory cells that are present in most cancer patients where they suppress T cell and NK cell-mediated antitumor immunity. They are likely to contribute to the lack of effect of ICI, and therefore, eliminating MDSCs may enhance the efficacy of ICI. MDSCs are activated by multiple proinflammatory mediators that are typically present in many cancers, and MDSCs are homeostatically regulated so that depletion of MDSCs in circulation or within solid tumors increases their rate of production from the bone marrow.
Multiple clinical trials have been conducted using drugs targeting individual mediators that either eliminate MDSCs in the periphery or neutralize the suppressive activity of MDSCs. Unfortunately, the trials have been largely ineffective in limiting MDSCs and enhancing ICI-induced antitumor immunity. Given the homeostatic regulation of MDSCs and the variety of proinflammatory molecules in individual patients capable of inducing MDSC differentiation, optimal reduction of MDSCs will likely require blocking the relevant proinflammatory mediators and preventing the development of MDSCs in bone marrow.
Signal transduction through The *Receptor for Advanced Glycation Endproducts (RAGE)* activates many of the pro-inflammatory mediators that are established activators of MDSC. Our studies in mice have established RAGE as a receptor on MDSC bone marrow progenitor cells and have demonstrated that blocking RAGE on murine bone marrow stem cells prevents the development of MDSC. Studies by others have demonstrated that RAGE inhibitors also directly suppress the growth of RAGE-expressing tumor cells. Therefore, we hypothesize that RAGE may be a key receptor on myeloid progenitor cells for driving the differentiation, accumulation, and function of MDSC, and that inhibitors of RAGE may be efficacious therapeutics for use in conjunction with ICI.

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Antigen-specific peripheral immune cell dynamics predicting clinical responses to checkpoint blockade in melanoma

Graham Pawelec*

Department of Immunology, University of Tübingen, Tübingen, Germany and Health Sciences North Research Institute, Sudbury, ON, Canada

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***Correspondence:** Graham Pawelec Department of Immunology, University of Tübingen, Tübingen, Germany and Health Sciences North Research Institute, Sudbury, ON, Canada.
E-mail: graham.pawelec@uni-tuebingen.de
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Abstract

Many years ago, we hypothesized that melanoma patients possessing T cells reacting to cancer antigens would do better than those who did not. To test this, we cultured PBMC from conventionally-treated metastatic melanoma patients with short (6 month) vs. long (18 month) survival with mixtures of peptides representing the shared cancer antigens NY-ESO-1, Melan-A, MAGE-A3 and survivin. After 12 days, cultures were restimulated with the same antigens and 24 h later intracellular cytokines measured by flow cytometry. The results were consistent with there being a clinical benefit of possessing T cells reactive to NY-ESO-1 and Melan-A but not MAGE-A3 or survivin, and patients possessing T cells reactive to both NY-ESO-1 and Melan-A did better than those with T cells responding to either alone. A dissection of the nature of the responding cells indicated that the majority of T cells responding to NY-ESO-1 was CD4+ whereas the majority responding to Melan-A was CD8+. Responding cells producing predominantly proinflammatory cytokines (IFN, TNF) were associated with clinical benefit, whereas IL 4 production was associated with poorer survival. Following these results, recent studies have focussed on whether similar findings apply to stage IV melanoma patients treated with anti-PD-1 antibodies, or a combination of anti-PD-1 and anti-CTLA-4 antibodies. Patients´ PBMCs were tested before the start of checkpoint blockade (baseline, BL) and at a mean of 44 days during ICB (follow-up, FU). In the discovery cohort from 3 clinical centers (n=92) and a validation cohort (n=49), it was confirmed that the presence of NY-ESO-1 and Melan-A-reactive T cells at BL before ICB conferred clinical benefit, both in terms of PFS and OS. Intriguingly, it was further shown that a *decrease* of these reactive cells at day 44 FU was associated with better survival than either their retention in the blood or their de novo appearance. This was interpreted to suggest sequestration of the reactive cells in the tumour, for which some limited evidence has accrued. By combining such analyses with other blood biomarkers (of which primarily BL and FU lactate dehydrogenase and myeloid-derived suppressor cell dynamics, along with extraordinarily high titers of IgG specific for human herpesvirus 5 (CMV) were associated with clinical outcome), we may approach more closely to the aim of providing robust individualized immune monitoring methods informative for individual patient outcomes.

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Immunotherapy of cancer: are we already there?

Angel Porgador*

The Shraga Segal Dept. of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

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***Correspondence:** Angel Porgador The Shraga Segal Dept. of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
E-mail: angel@bgu.ac.il
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Abstract

Recent status in cell-based cancer therapy, humoral-based cancer therapy and cancer vaccines will be discussed in short; This will be followed by presentation of data in chimeric antigen receptor (CAR)-therapy employing targeting moieties based on modified ERBB receptors' ligands. The activity and efficacy of modified EGF-like domains derived from NRG1 (ligand to ErbB3 (HER3) receptor) and betacellulin (ligand to ErbB1 (EGFR) receptor) ligands as targeting moiety in CAR therapy will be presented.

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Immunomonitoring of patients with hepatic cancer undergoing liver transplantation after immunotherapy unravels an unexpected synergism between trained immunity and T cell memory.

Licia Rivoltini*, Sherrie Bhoori, Michela Dosi, Francesca Rini, Luca Lalli, Stefano Bergamini, Agata Cova, Elena Daveri, Veronica Huber, Martina Stroscia, Paola Frati and Vincenzo Mazzaferro

Departments Fondazione IRCCS Istituto Nazionale dei Tumori, 20133 Milan, Italy

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***Correspondence:** Licia Rivoltini Departments Fondazione IRCCS Istituto Nazionale dei Tumori, 20133 Milan, Italy.
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Abstract

Immunotherapy (IT) with immune-checkpoint inhibitors is becoming a universal strategy for cancer treatment. However, most solid malignancies require the combination of IT with standard therapies to achieve consistent clinical benefit. Nevertheless, the synergistic mechanisms underlying the efficacy of these different combinatorial regimens, particularly in term of immunological outcomes, are still poorly defined. IT should be combined with other oncological treatments based on a scientifically-sounded rationale to possibly potentiate, and not jeopardize, the IT-mediated priming of anti-tumor immunity. An extreme example of this concept is represented by hepatocellular carcinoma (HCC), in which IT is starting to be administered with liver transplantation (LT). Indeed, such a combination has been considered in recent clinical studies with rather promising results in terms of efficacy. Notwithstanding, the immunosuppressive regimens required for avoiding graft rejection could heavily penalize T cell responses induced by IT. Hence, how could such a combination therapy be clinically effective? This is the topic we have been recently addressing with the ImmunoXXL study (NCT05879328), a prospective clinical trial in which multimodal immunomonitoring has been applied to HCC patients undergoing IT+LT or receiving the two regimens alone. Data obtained so far suggest the occurrence of an unexpected immune synergism based on the ability of LT to sustain IT-primed T cell memory through the engagement of trained immunity, thus shedding light on the rationale of transplant oncology in solid tumors.

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TRIMELVax: A Novel Innate Immune-Engaging Vaccine for Malignant Melanoma Targeting Neutrophil-Driven Antigen Presentation and T-Cell Activation

Flavio Salazar-Onfray[1,2,3]

[1] *Disciplinary Program of Immunology, Faculty of Medicine, University of Chile, Chile;*
[2] *Millennium Institute for Immunology and Immunotherapy, University of Chile, Chile;*
[3] *Division of Infectious Diseases, Karolinska University Hospital, SE-171 77 Solna, Sweden.*

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***Correspondence:** Flavio Salazar-Onfray Disciplinary Program of Immunology, Faculty of Medicine, University of Chile, Chile; Millennium Institute for Immunology and Immunotherapy, University of Chile, Chile; Division of Infectious Diseases, Karolinska University Hospital, SE-171 77 Solna, Sweden.
E-mail: fsalazaronfray@gmail.com
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Abstract

TRIMELVax is an innovative immunotherapeutic vaccine for malignant melanoma, which combines heat shock-conditioned human melanoma cell lysates with a mollusk hemocyanin as an adjuvant. Preclinical studies demonstrated significant effectiveness in reducing tumor growth and improving survival in mice with melanoma and colorectal tumors. However, the precise immunological mechanisms behind its anti-tumor effects have remained unclear. We focused on understanding how TRIMELVax induces inflammation at the injection site, recruits innate immune cells, triggers DC migration to draining lymph nodes, and affects tumor microenvironment and growth. To achieve this, C57BL/6 mice were injected in the footpad with TRIMELVax, PBS, or control vaccines, and then the skin and the popliteal lymph node were harvested for qPCR or FACS analysis. Results showed that TRIMELVax rapidly induced a specific pattern of proinflammatory cytokines and chemokines, leading to a differential acute innate immune response in the injection site. Neutrophils, type 1 macrophages, monocytes, cDC1, Langerhans cells, and monocyte derived-DCs were recruited to the footpad, while type 2 macrophages decreased. This early inflammation facilitated a superior migration of cDC1 and a new subtype of antigen-presenting neutrophils to the draining lymph node compared to controls. Depleting neutrophils before vaccination reduced DC migration, especially cDC1, to the draining lymph node and suppressed TRIMELVax's ability to control tumor growth, demonstrating that vaccine-induced neutrophils play a fundamental role in TRIMELVax's mechanism of action. At the tumor microenvironment level, it was observed that TRIMELVax induces a more intense infiltration of CD8+ T lymphocytes than controls, with a low level of exhaustiveness and greater cytotoxic capacity. A phase I clinical trial in twenty nivolumab-refractory patients showed that TRIMELVax is well tolerated with no grade 3-5 side effects and induces a Delayed-Type hypersensitivity reaction against melanoma antigens after four doses of the vaccine with an impact on disease stability and patient survival. In summary, TRIMELVax triggers a rapid and potent activation of the innate immune system, which seems crucial for a more effective adaptive immune response against aggressive melanoma tumors. These results underscore the promise of TRIMELVax as a potential immunotherapeutic approach for melanoma treatment.

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The exercise of mobilizing the immune system in cancer patients

Per thor Straten*

Center for Cancer Immune Therapy (CCIT), Department of Oncology, University Hospital Herlev, Herlev, Denmark

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***Correspondence:** Per thor Straten Center for Cancer Immune Therapy (CCIT), Department of Oncology, University Hospital Herlev, Herlev, Denmark.
E-mail: Per.Thor.Straten@regionh.dk
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Abstract

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 and also correlate with response to check point inhibition (CPI) therapy. We recently demonstrated that tumors in exercising mice show increased immune cell infiltration, in turn associated with an over 60% reduction in tumor incidence and growth across several murine tumor models. Mechanistically we could show that efficacy was dependent on NK cells, which were engaged through an epinephrine-dependent mobilization. Together these results link exercise with improved immunological control of tumor progression, suggesting that exercise could be a beneficial partner for immunotherapy. We translated these findings to clinical testing and recently finalized a clinical trial where exercise was combined with immunotherapy (HI AIM NCT04263467). HI AIM was a randomized controlled trial (70 patients, 1:1) for patients with metastatic non-small cell lung cancer. Patients in the treatment arm, receive an exercise-intervention consisting of supervised and group-based exercise training, comprising primarily intermediate to high intensity interval training three times per week over 6 weeks. Primary outcome was mobilization of circulating NK cells (TBNK kit assay). Secondary outcome other circulating immune cells, infiltration of immune cells in tumor, inflammatory markers, aerobic capacity measured by VO_2 max test, physical activity levels and quality of life measured by questionnaires, and clinical outcomes. All patients received standard oncological treatments; checkpoint inhibitors, checkpoint inhibitors combined with chemotherapy or oncological surveillance. Blood samples and biopsies (ultrasound guided), harvested before, during and after the 6-week training program, will form basis for immunological measurements of an array of immune cells and markers. Preliminary analyses, based on examination of blood samples from 15 patients in the treatment group, adrenalin and nor-adrenalin demonstrated rapid increase as did T and NK cell numbers and frequencies. High intensity interval training in lung cancer patients, concurrent with standard treatment is feasible and safe. The applied exercise regiment led to acute increase in (nor-)adranalin as well as T and NK cells. Further analyses will include extensive serum and immune cell analyses, as well as correlations to the clinical data of the patients.

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The tumor immune microenvironment as a target for nano-therapies and patient-tailored treatments.

Vannucci, Luca [1*]; Rajsiglova, L [1,2]; Stakheev, D [1,4,5]; Lukac P [1, 2]; Tenti P [1, 2], Sushystskyi L [1,6]; Babic M [3]; Krausova, K [1,4,5]; Kalkusova, K [4, 5]; Taborska P [4, 5]; Sojka L [4, 5]; Makovicky P [7]; Ceci PP [8]; Falvo E [8]; Svoboda J [1]; Hadraba D [9]; Vondrasek D [9]; Mucciolo G [10]; Cappello P [10]; Smrz, D [1, 4, 5]

[1] *Czech Acad Sci, Inst Microbiol v.v.i., Lab Immunotherapy, Videnska 1083, Prague 4, Czech Republic;*
[2] *Charles Univ Prague, Dept Cell Biol, Fac Sci, Prague, Czech Republic;*
[3] *Czech Acad Sci, Inst Macromol Chem, Prague, Czech Republic;*
[4] *Charles Univ Prague, Fac Med 2, Dept Immunol, Prague, Czech Republic;*
[5] *Univ Hosp Motol, Prague, Czech Republic;*
[6] *University of Chemistry and Technology, Dept Saccharides and Cereals, Fac of Food and Biochem Technol, Prague, Czech Republic;*
[7] *Univ Ostrava, Inst Histology and Embryology, Med Fac, Ostrava, Czech Republic;*
[8] *Italian National Res Council IBPM-CNR; Inst of Mol Biol and Path, Rome, Italy;*
[9] *Czech Acad Sci, Inst Physiol v.v.i., Dept Biomathematics, Prague, Czech Republic;*
[10] *University of Turin, Dept Mol Biotech and Health Sciences, Turin, Italy.*

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***Correspondence:** Vannucci, Luca Czech Acad Sci, Inst Microbiol v.v.i., Lab Immunotherapy, Videnska 1083, Prague 4, Czech Republic.
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Abstract

Cancer is a complex illness with local to systemic expression. Cancer cells in relation with the constitutive components of the tissue from which they develop and the immune system elements, that interact with them to ablate the cancer cells and promote tissue repair, form the tumor microenvironment. The interactions between all these elements decide the tumor evolution either toward its elimination (inflammation, immune recognition and direct cytotoxicity) or its establishment and progression (inversion of the immune response, chronic and smoldering inflammation, immune cell exhaustion and establishment of an immune suppressive and tissue remodeling environment). The alteration of the tissue collagen scaffold influenced by the immune environment continuous modifications collaborates to impede adequate access to immune cells and drugs and solicitate epithelial to mesenchymal transition allowing metastatic cell behavior. Under these conditions, the altered immune environment can bias the efficacy of the treatments and favor the tumor heterogeneity. Many therapeutic approaches were developed, from surgery to chemo- and radiotherapy until the recent advances in immunotherapy. However, if the therapeutic approach is systemic, sensible side effects can accompany the treatment because of the involvement also of non-tumoral tissues. Therefore, from the last years of the XX century, a progressive interest and improvement in technical possibilities started to focus on targeting therapies using monoclonal antibodies (e.g. against cellular pathways, growth-factors, immune check-point molecules) and/or organic/inorganic nano-constructs (e.g. ferritin-based, iron-based nanoparticles) studied for directly affect the cancer cells or other microenvironment components (immune cells). Focusing the intervention to the pathological component and selectively foster the anticancer response is the aim of modern oncology. In this presentation, we will highlight some of our observations done on components of the microenvironment favoring the tumor escape and our experience of tumor targeting by nanoparticles in experimental models looking to achieve more tumor and antigen specific treatments for enhancing the anti-cancer immune response, possibly with a patient-tailored approach.

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The role of tumor microenvironment IL-1β in Colorectal Cancer

Noy Machluf-Kaz[1], Sami Stalin[1], Guy Barsky[2], Marina Bersudsky[1], Ron N. Apte[1], Lelyan Moadi[3], Sondra Turjeman[3], Omri Koren[3], Elena Voronov[1*]

[1]The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben Gurion University of the Negev, Beer Sheva, Israel
[2]Surgery B Department, Soroka Medical Center, Ben Gurion of the Negev, Israel
[3]Bar-Ilan University Azrieli Faculty of Medicine, Safed, Israel

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***Correspondence:** Elena Voronov The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben Gurion University of the Negev, Beer Sheva, Israel. E-mail: elena@bgu.ac.il
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Abstract

Colorectal cancer (CRC) is the third leading cause of cancer, with high mortality due to metastasis development, mostly in the liver. Treatment with chemotherapy frequently results in chemoresistance, showing the importance of developing novel therapeutic approaches.
Inflammation has been shown to be a major factor in the development of many cancers, including CRC. Interleukin-1β (IL-1β) and Interleukin-1α (IL-1α), in their secreted form are abundant in the tumor microenvironment, where they can promote tumor growth, or (in some instances) can be involved in anti-tumor activities. In this study, we assessed the role of IL-1 in tumor progression.
After orthotopical injection of MC38 cells, wild-type (WT) mice developed local CRC with progression to liver metastasis. In contrast, in IL-1β knockout (KO) mice, there was retardation of tumor growth with no metastatic lesions in the liver. It is noteworthy that patterns of tumor growth in mice deficient in IL-1α are similar to those of WT mice. We assessed how IL-1β signaling affects various inflammation factors in the tumor microenvironment (TME) and its effects on colon microbiota. We found a significant decrease in inflammation in TME deficient in IL-1β. In addition, IL-1β is intensively involved in chemokine expression and thus in recruitment of inflammatory cells into TME. Reduced inflammation in IL-1β KO mice leads to a shift in colon microbiota, and we observed the prevalence of phyla that were shown to have some anti-carcinogenic properties.
Next, we assessed whether effects of IL-1β on CRC are specifically dependent on the origin of microenvironment. Thus, mice were injected intra-splenic with MC38 cells and experimental liver metastases were assessed. In this model, no differences or even a higher metastasis rate was observed in IL-1β KO mice in comparison to WT mice. These findings suggest that the effects of IL-1β in CRC are dependent on the site of tumor inoculation and specific TME immune response.
In conclusion, we suggest that IL-1β signaling is strongly connected to the local microenvironment and especially to the microbiome. IL-1β supports inflammation by inducing a cascade of other pro-inflammatory molecules, which recruit pro-inflammatory immunosuppressive cells into TME. This inflammatory environment affects the colon microbiota, which becomes pro-tumorigenic. Inhibition of IL-1β can prevent these changes, improve disease outcome and alleviate side effects of chemotherapy.

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Targeting Metabolic Programming in the Tumor Microenvironment

Jonathan M. Weiss[1*], Erika M. Palmieri[1], Marieli Gonzalez-Cotto[1], Ian A. Bettencourt[1], Shilpa Kurian[2], Barry O'Keefe[2], Daniel W. McVicar[1]

[1]*Cancer Innovation Laboratory, Center for Cancer Research, National Cancer Institute, Frederick MD USA*
[2]*Natural Products Branch, Center for Cancer Research, National Cancer Institute, Frederick MD USA*

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***Correspondence:** Jonathan M. Weiss Cancer Innovation Laboratory, Center for Cancer Research, National Cancer Institute, Frederick MD USA. E-mail: weissjo@mail.nih.gov
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Abstract

In cancer, metabolic adaptations such as fatty acid oxidation are frequently associated with immune suppression, oxidative stress and tumor promotion. Using unbiased metabolomics and quantitative PCR, we found that itaconate, the product of the decarboxylation of cis-aconitate, was among the most highly upregulated metabolites in tumor-associated macrophages of the peritoneal space. We show that itaconate is important for fatty acid beta-oxidation promotion of oxidative phosphorylation and generation of mitochondrial reactive oxygen species in macrophages that facilitate tumor growth. Genetic interference with itaconate production confirms a pro-tumoral role for itaconate and highlight itaconate as a therapeutic target for oncology. To evaluate this, we screened a library of over 21,000 compounds using a high throughput primary screen to identify compounds capable of interacting with Irg1, the protein which catalyzes itaconate production. Inhibitory or activating activity was next confirmed by mass spectrometry using both *in vitro* reactions and cell-based assays. Finally, tumor-bearing mice were treated with the most promising candidate compounds. Using this approach, we identified multiple compounds capable of significantly reducing peritoneal tumor burden and are evaluating them for dependency on itaconate inhibition. Our findings reveal metabolic-based pathways which highlight the feasibility of using newly identified compounds for the treatment of peritoneal cancers.

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Investigation of cell therapies: a role for TIL and/or DC vaccines?

Stina L Wickström*

Department of Oncology-Pathology and Department of clinical neuroscience, Karolinska Institutet, Stockolm Sweden
Theme Cancer, Patient Area Head and Neck, Lung and Skin, Karolinska University Hospital, Stockholm, Sweden.

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***Correspondence:** Stina L Wickström Department of Oncology-Pathology and Department of clinical neuroscience, Karolinska Institutet, Stockolm Sweden Theme Cancer, Patient Area Head and Neck, Lung and Skin, Karolinska University Hospital, Stockholm, Sweden. E-mail: stina.wickstrom@ki.se
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Abstract

One category of immunotherapy, cell therapy, describes the process of infusing living cells, either via adoptive cell therapy (ACT) of cytotoxic cells or as a vaccine-based on specialized antigen presenting cells, i.e. dendritic cells (DC). ACT in the form of tumor-infiltrating lymphocytes (TIL) has shown encouraging results in patients with metastatic melanoma. However, clinical effect of TIL therapy has so far not been proven in other solid tumors, including gynaecological cancers. To further improve clinical responses in patients with melanoma we implemented a unique treatment strategy of TIL therapy combined with a DC-based cancer vaccine, clinical trial MAT02 (NCT01946373). The results are encouraging showing one patient with a strong partial response (PR, 8 years without disease progression), two patients with a complete response (CR, 7and 6 years), and one patient with a mixed response (4/4 fully treated patient responding). Cervical cancer has been shown to have an influence of reactive oxygen species (ROS) in tumor progression and that patients in later stages of cancer have ROS levels creating a hostile tumor microenvironment. We have established a strategy to render human cytotoxic lymphocytes resistant towards ROS by Nrf2-activation of intrinsic anti-oxidant responses. We hypothesize that a Nrf2 activation in TIL before infusion could enhance the efficacy of adoptive cell therapy within these patients. Our Aim is to initiate the first TIL trial for cervical cancer patients in Sweden, during 2025.
Unfortunately, clinical trials using DC vaccine as a monotherapy has shown it to be safe but with an overall response rate rarely exceeding 15%, leaving room for improvement. We are currently evaluating different pre-treatment conditions for generation of whole tumor lysate aiming to improve the activation capacity of a therapeutic whole tumor lysate DC vaccine. For this, several protocols for generation of whole tumor lysates are compared, including the “golden-standard”, pre-treatment protocols used in clinical trials, as well as novel pre-treatment protocols. Our preliminary data shows that modification of the pre-treatment of the tumor cells before generation of tumor lysate is enhancing the capacity of the DC vaccine to activate CD8+ T cells. We are currently focusing on the mechanism behind the increased the immunogenicity observed for some tumor lysates.
We aim to be able to use an improved DC vaccine in combination with checkpoint inhibitors (ICI) in the near feature to treat patients with endometrial or cervical cancer, who now receives ICI within the standard of care program in Sweden.

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Cancer Ecosystem - Quo Vadis?

Isaac P. Witz*

The Shmunis School of Biomedicine and Cancer Research The George S. Wise Faculty of Life Sciences Tel Aviv Universit

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***Correspondence:** Isaac P. Witz The Shmunis School of Biomedicine and Cancer Research The George S. Wise Faculty of Life Sciences Tel Aviv Universit.
E-mail: isaacw@tauex.tau.ac.il

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Abstract

Findings related to the Tumor Microenvironment (TME – referred hereafter as the Cancer Ecosystem (CES)) play an essential role in cancer diagnosis and treatment thereby contributing to recent achievements.
There are, however, crucial issues that are ignored or not adequately emphasized by the research community. Among the major issues are: The unique features of each tumor type—such as tissue of origin, anatomic location, metabolism, the dynamic nature of the CES as manifested by a temporal co-evolution of cancer and non-cancerous cells, intra-tumor and inter-tumor heterogeneity that influence therapy sensitivity and resistance. In order to address these issues, we should abandon reductionism and adopt a more holistic approach to uncover fundamental programs common to malignant diseases. Given the profound diversity between cancer types, achieving a holistic view of the entire spectrum of cancer diseases is unattainable at present. Instead, a holistic approach to specific cancer types (or subtypes) may be applied.
AI and technology-based solutions hold the potential to meet the challenges of holism in cancer research.

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A new pathway towards immunotherapy-supported radiology for targeted Boron Neutron Capture Therapy (BNCT)

Klaus M. Spohr[1], Gabriel P. Bleotu[1], Mirabela Cristea[3], Domenico Doria[1], Alexandra Ivan[3,4], Marius Jurca[1], Vincent Lelasseux[1], Cristina Marin[1], Viorel Nastasa[1], Valentin Ordodi[3,4,5], Virgil Paunescu[3,4], Mara Popovici[1], Sivliu Stanciu[6], Stefan Stoenescu[6], Calin Tatu[3,4], Ada Telea[3], Chieh-jen Yang[1], Maria-Iulia Zai[1].

[1]*Extreme Light Infrastructure - Nuclear Physics, "Horia Hulubei" National Institute for R & D in Physics and Nuclear Engineering, Magurele, Romania*
[2]*Romania & School of Computing, Engineering and Physical Sciences, University of the West of Scotland*
[3]*OncoGen, Center for Gene and Cellular Therapies, "Pius Brinzeu" Clinical Emergency County Hospital, Timisoara, Romania*
[4]*UMFVBT - Department of Functional Sciences, "Victor Babes", University of Medicine and Pharmacy Timisoara, Timisoara, Romania*
[5]*UPT - Department of Applied Chemistry, Organic and Natural Compounds Engineering, Polytechnic University of Timisoara, Timisoara, Romania*
[6]*Institutul de Cercetari Nucleare Pitesti (ICN), Mioveni, Romania*

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***Correspondence:** Klaus M. Spohr. Extreme Light Infrastructure - Nuclear Physics, "Horia Hulubei" National Institute for R & D in Physics and Nuclear Engineering, Magurele, Romania
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Abstract

Background: We present our novel, patented paradigm of immunotherapy-supported radiology designed to augment conventional FDA-approved Boron Neutron Capture Therapy (BNCT). This innovative approach employs immunocompetent cells as vectors for boron nanoparticles, thereby facilitating targeted radiological treatment. Our methodology addresses the evasive propensity of 'cold' solid tumors, which often remain undetected due to their integration within fibroblast-rich micro environments devoid of distinct markers.
Methods: By loading boron into immunocompetent cells, these entities function as highly selective delivery agents—akin to nanorobots—capable of transporting therapeutically sufficient payloads (80 μg/tumor) of the stable isotope ^{10}B directly into malignant cells or their immediate vicinity, priming them for BNCT. The subsequent treatment method involves exposing the patient to a relatively benign beam of low-energy, 'epithermal' neutrons (E_n ~ 0.025 eV) generated by a compact particle accelerator. The therapeutic efficacy hinges on the nuclear reaction $^{10}B + n \rightarrow {}^4He + {}^7Li$, which occurs with a high cross-section and is impervious to any tumor-supporting microenvironment. The energy released via Linear Energy Transfer (LET) by the resultant lithium and helium ions is confined to a cellular scale (~9 μm), inducing apoptosis with remarkable precision. **Hallmark:** This spatially restricted energy deposition minimizes collateral damage to adjacent healthy tissue, the hallmark aim of our approach. Our patented boron-loading mechanism employing liposome-mediated endocytosis enables the incorporation of approximately 10^9 boron atoms into a single immunocompetent cell without compromising its biological functionality. In vitro experiments conducted at the neutron-providing nuclear reactor at the ICN in Pitesti corroborate the immense potential of this immunotherapy-supported BNCT paradigm.
Conclusion: We conclude that this pioneering strategy can potentially become a transformative advancement in targeted oncological therapies of recalcitrant solid tumors.

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Tissue transglutaminase as a new immunomodulatory target in ovarian cancer tumor microenvironment

Livia E. Sima[1#§*], Siqi Chen[2#], Horacio Cardenas[1#], Guangyuan Zhao[1], Yinu Wang[1], Cristina Ivan[3], Hao Huang[1], Bin Zhang[2,4], Daniela Matei[1,4,5]

[#]These authors contributed equally
§Currently in the Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania
[1]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, USA;
[2]Department of Medicine; Hematology/Oncology Division, Feinberg School of Medicine, Northwestern University, Chicago, USA;
[3]Department of Experimental Therapeutics, Center for RNA Interference and Non-Coding RNA, The University of Texas MD Anderson Cancer Center, Houston, USA;
[4]Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, USA;
[5]Jesse Brown VA Medical Center, Chicago, USA

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***Correspondence:** Livia E. Sima. Currently in the Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania. Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, USA.
E-mail: livia_e_sima@yahoo.com; lsima@biochim.ro
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Abstract

Tissue transglutaminase (TG2) is a multifunctional protein with roles in multiple diseases including cancer. It was found overexpressed in many solid tumors in the past two decades. In ovarian cancer, it emerged as a potential therapeutic target due to its involvement in processes like metastasis and chemo- and radiotherapy resistance. Generally, TG2 expression in cancer cells has been extensively studied and linked to increased tumor progression. However, its role in the host was less studied. Our research explored the anti-tumor immune response using a TG2KO syngeneic ovarian cancer mouse model, as compared to wild-type animals. We observed decreased tumor burden and increased survival upon i.p. injection of ID8 mouse ovarian cancer cells in TG2KO mice, as compared to wild-type. In the absence of TG2 in the host, an increased infiltration of $CD8^+$ T cells in ascites was evidenced by FACS, while myeloid cells were less present. The TG2KO $CD8^+$ T cells showed increased activation and increased effector function. Moreover, these cells showed an increased cancer cell killing capacity. At the molecular level, this phenotype was supported by attenuated STAT3 phosphorylation. Cancer cells in ascites collected from TG2KO mice showed a gene signature corresponding to IFN-γ response. Overall, these data show decreased tumor progression and increased effector phenotype in $CD8^+$ T cells when TG2 is absent in the host (Sima LE et al. (2021) Journal for ImmunoTherapy of Cancer 9, e002682). When looking to confirm these results in human OC tumor microarrays (TMAs), we found an inverse correlation between human stromal (but not tumor) TG2 expression and $CD8^+$ T cells infiltration by IHC staining followed by StrataQuest image cytometry. Using Opal-based multiplex IHC staining, we identified TG2 in a subset of cancer associated fibroblasts (CAFs). TG2 was previously reported as a non-toxic druggable target involved in “outside-in” signaling downstream of integrins. Further, we used inhibitors developed towards TG2-fibronectin interaction (Sima LE et al. (2019) Mol Cancer Ther 18, 1057-1068) to evaluate how they affect the cancer cells-fibroblasts cross-talk. This treatment prevented the self-assembly of heterospheroids. This is relevant for the situation in the tumor microenvironment, where we found TG2-FN interaction present in both stroma and tumor areas. These results support the potential use of TG2 directed agents in ovarian cancer therapy, as we propose TG2 as a new immunomodulatory target. Our current efforts also include using FLASH radiation to sensitize cancer cells to immunotherapy.

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The Involvement of MDSCs-Bacteria Crosstalk in Colitis as a Predisposing Factor for Colitis-Associated Colorectal Cancer

Or Reuven*, Michal Baniyash

The Concern Foundation Laboratories, The Lautenberg Center for Immunology and Cancer Research, Department of Immunology and Cancer Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

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***Correspondence:** Or Reuven. The Concern Foundation Laboratories, The Lautenberg Center for Immunology and Cancer Research, Department of Immunology and Cancer Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
E-mail: or.reuven2@mail.huji.ac.il
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Abstract

Inflammatory Bowel Disease (IBD), composed of Crohn's Disease (CD) and Ulcerative Colitis (UC), represents a chronic inflammatory condition that is associated, in some cases, with a predisposition of the host to Colitis-Associated Colorectal Cancer (CAC). Both IBD and CAC are characterized by immune system dysregulation and changes in intestinal microbiota composition (dysbiosis). The dysbiotic bacteria invade the intestinal tissue and are found to interact with diverse immune cells of which one key population is represented by Myeloid-Derived Suppressor Cells (MDSCs).

MDSCs are a heterogeneous population of immature myeloid cells that, under normal conditions, give rise to immune cells from the myeloid lineage. However, under pathologies associated with chronic inflammation, these cells become highly polarized with enhanced suppressive activity, suppressing both the innate and adaptive immune systems by different routes.

Our general aim is to better understand the involvement of MDSC-Bacteria crosstalk in colitis as a predisposing factor for CAC development.

Herein, we demonstrate that IBD is characterized by elevated levels of MDSCs with enhanced suppressive activities, which can promote progression to CAC by inducing persistent immunosuppression and a proinflammatory environment in the colon during the IBD stage. In the intestines of IBD individuals, we found that MDSCs can often be seen in close proximity to tissue-invading bacteria, resulting in a polarization of these cells toward a highly suppressive and proinflammatory phenotype.

Suppression mediated by the MDSCs is linked to the secretion of proinflammatory cytokines, nitric oxide, the arrest of T cell proliferation, and downregulated levels of CD247, a key signaling molecule expressed by T cells. Since MDSCs play a major role in the dysregulation of the immune system, different combinatorial therapeutic strategies to recuperate the host's health and regress the disease were tested, aiming at targeting the MDSCs. To this end, we tested different metabolites and identified a potent metabolite inhibiting the bacteria-induced MDSCs polarization. The effects of this metabolite on MDSCs are currently under investigation, using proteomics analysis through the exploration of the downstream mechanism. These studies will help discover novel pathways that mediate the immunosuppression induced by MDSC upon their interaction with bacteria, towards the development of specific anti-MDSC therapeutic modalities.

Our long-run aims are to alleviate intestinal inflammation by manipulating MDSC-induced immunosuppression, combat bacteria-induced MDSC polarization, and study the underlying mechanisms of unique metabolites as MDSC inhibitors.

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Case reports and review of immune checkpoint inhibitor therapy and vitiligo in advanced cutaneous melanoma

Dace Pjanova*, Simona Donina

Riga Stradins University, Science Hub, Riga, Latvia

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***Correspondence:** Dace Pjanova. Riga Stradins University, Science Hub, Riga, Latvia.
E-mail: dace.pjanova@rsu.lv
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Abstract

Vitiligo is a relatively benign autoimmune condition characterized by the destruction of melanocytes, leading to depigmentation of the skin. While primarily considered a cosmetic disorder, emerging evidence suggests that vitiligo may have important prognostic implications in oncology, particularly in melanoma. Several studies have reported an association between vitiligo development and treatment response in advanced melanoma patients undergoing immunotherapy. Although vitiligo expansion does not directly correlate with tumour regression, it has been linked to prolonged progression-free survival (PFS) and shows a trend toward improved overall survival (OS). This suggests that vitiligo may serve as an immune-related biomarker indicative of a favourable clinical course during immunotherapy.

Here, we present two cases of advanced melanoma (BRAF wild-type) treated with pembrolizumab, emphasizing the complex interplay between vitiligo and immune-mediated responses to treatment. **Case 1:** a 77-year-old male diagnosed with stage IIIC metastatic melanoma developed vitiligo during pembrolizumab treatment. Despite the persistence of metastatic disease, he has maintained stable disease over an extended period, suggesting a potential link between vitiligo onset and durable disease control. **Case 2:** a 68-year-old male with metastatic melanoma developed long-term eyelash poliosis, a localized depigmentation phenomenon, during pembrolizumab therapy. Similar to the first case, he has exhibited stable disease, reinforcing the hypothesis that vitiligo and related depigmentation phenomena may be associated with favourable immunotherapy outcomes.

These cases, alongside accumulating literature, support the potential role of vitiligo as a biomarker for prolonged survival in melanoma patients undergoing anti-PD-1 therapy. A deeper understanding of vitiligo expansion, its mechanistic connection to anti-tumour immunity, and its clinical implications could enhance prognostic assessment and aid in optimising the management of immune-related adverse events during immunotherapy.

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LL-37: A powerful booster of ex vivo-prepared DC-based vaccines in prostate cancer patients and the TRAMP mouse model, without additional benefit from anti-PD1 therapy

Kateřina Krausová[1,*,#], Dmitry Stakheev[1,#], Pavla Táborská[1], Milan Reiniš[2], Romana Mikyšková[2], Michal Podrazil[1], Jiřina Bartůňková[1], Daniel Smrž[1]

[1]*Department of Immunology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic*
[2]*Laboratory of Immunological and Tumour Models, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic*

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***Correspondence:** Kateřina Krausová. Department of Immunology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic.
E-mail: katerina.krausova@lfmotol.cuni.cz
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Abstract

Background: Prostate cancer (PCa) is one of the most common cancers in men. As a “cold tumor,” it exhibits low immunogenicity. Therefore, dendritic cells vaccines (DC) have been developed to enhance adaptive immunity. However, the efficacy of *ex vivo*-produced DC remains suboptimal. The antimicrobial peptide LL-37 has shown various immunomodulatory effects. Our previous studies demonstrated that LL-37 enhances the performance of DC produced from the peripheral blood of healthy volunteers.
Aims: In this follow-up study, we aimed to further investigate the therapeutical potential of LL-37/DC alone or in combination with anti-PD1 therapy using PCa patients’ samples and a syngeneic mouse model. Furthermore, we explored the performance of LL-37/DC under GMP conditions.
Methods: Human DC were prepared from leukaphereses of 10 biochemical recurrence (BR) PCa patients. The reactivity and cytotoxicity of these DC to the PCa cell line PC3 were assessed using flow cytometry and fluorescent microscopy. Mice bone marrow-derived DC from B6 TRAMP mice were inoculated subcutaneously into TRAMP tumor-bearing mice (n=9). The tumor growth and survival rates were monitored. Finally, we produced DC under GMP conditions and evaluated their performance *in vitro*.
Results: LL-37/DC-primed lymphocytes from BR PCa patients exhibited a two-fold increase in the expression of both IFN-γ and TNF-α and caused a three times higher contraction of co-cultured PC3 cells compared to DC prepared without LL-37. The increase in the LL-37/DC efficacy was not caused by enhanced DC maturation, as the expression levels of HLA-DR, CD83, CD86, and CD80 remained similar. Adding an anti-PD1 antibody did not improve either DC or LL-37/DC efficacy. Finally, we demonstrated that LL-37/DC prepared under GMP conditions was also significantly more reactive and caused two and a half times higher contraction of the co-cultured PC3 cell line compared to DC prepared without LL-37.
In the TRAMP mice model, LL-37/DC exhibited significantly higher expression of CD80, extended the mice survival by 30 days, and slowed down the tumor growth as compared with the DC prepared without LL-37. The anti-PD1 therapy had no additional effect.
Conclusions: We demonstrated that LL-37 is a promising compound that enhances the antitumor potential of DC both *in vitro* and *in vivo*. Furthermore, this improved potential is preserved even when DC are produced under GMP conditions. On the other hand, using anti-PD1 therapy showed no additional therapeutic impact. Taken together, LL-37/DC offers potentially advantageous treatment for patients with cold tumors, who typically respond poorly to immune checkpoint inhibitor therapy.

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CD19 Chimeric Antigen Receptor (CAR) modified cytokine killer cells compared to classical CD19 CAR-T: advantages and disadvantages

Olha Karaman*[,1,2], Madeline Kim[2], Gina Scurti[2], Jan Aleksander Kraśko[1], Oliwia Bochnacka[2], Suzanne Quinn[2], Vita Pasukoniene[1], Michael I. Nishimura[2]

[1]*Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania*
[2]*Department of Surgery, Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL*

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***Correspondence:** Olha Karaman. Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania Department of Surgery, Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL.
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Abstract

Chimeric antigen receptor (CAR) T-cell therapy has demonstrated remarkable efficacy and unique long-term outcomes in treating relapsed/refractory hematological malignancies in both pediatric and adult patients. However, a subset of patients remains unresponsive, with disease progression continuing despite treatment. Moreover, CAR-T therapy has shown limited success in treating solid tumors.

Accumulating evidence on the role of various lymphocyte subpopulations in antitumor immunity suggests that cytokine-induced killer (CIKs) cells represent a promising alternative for adoptive cell therapy. A key advantage of CIKs is their low toxicity and the presence of unrestricted MHC cytotoxicity against both hematological and solid malignancies.

In our study, we hypothesize that modifying CIKs with CARs would enhance their antitumor efficacy compared to unmodified CIKs. Using a unique retroviral vector containing the CD34 or CD14 marker gene, we successfully generated CD19 CAR-expressing lymphocyte populations, including both classically activated T lymphocytes and CIKs. We then compared their functional properties by analyzing lymphocyte subpopulations based on markers such as CD3, CD4, CD8, CD56, CD62L, and CD45RA, as well as their ability to specifically recognize CD19-positive targets. Additionally, we assessed cytokine production, including IFN-γ (via ELISA and intracellular staining), IL-2, TNF-α, and IL-10 (via intracellular staining).

Our findings indicate that CIKs can be efficiently modified using a retroviral vector while maintaining functional activity. CD19 CAR-CIK populations contained higher numbers of NKT ($CD3^+CD56^+$) and $CD3^+CD8^+$ lymphocytes compared to CD19 CAR-T cells. However, the proportion of naïve-like T cells ($CD62L^+CD45RA^+$) and effector memory RA^+ T cells ($CD62L^-CD45RA^+$) remained similar in both CAR-modified populations. Additionally, CD19 CAR-CIKs exhibited a slightly increased ability to produce IFN-γ and TNF-α in response to $CD19^+$ tumor cells. Despite that, key questions remain regarding the functionality of CD19 CAR-CIKs in an immunosuppressive tumor microenvironment, particularly in the presence of high levels of TGF-β, IL-10, and PD-L1, which may inhibit their activity or lead to exhaustion. Our future research will focus on overcoming these challenges to enhance the therapeutic potential of CAR-CIKs.

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Live-Attenuated Influenza Viruses Co-Expressing BPV1 E6 and E7 Peptides Eradicate BPV Infection, thus Significantly Reducing Tumour Recurrence in Horses after Surgery

Edmund K. Hainisch*[1], Christoph Jindra[1], Markus Wolschek[2], Thomas Muster[2], Sabine Brandt[1]

[1]*Research Group Oncology and Equine Surgery, Equine Clinic, Veterinary University Vienna, Austria*
[2]*BlueSky Immunotherapies GmbH (Ltd.), Vienna, Austria*

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***Correspondence:** Edmund K. Hainisch. Research Group Oncology and Equine Surgery, Equine Clinic, Veterinary University Vienna, Austria
E-mail: edmund.hainisch@vetmeduni.ac.at
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Abstract

Background, aim: It is well accepted today that papillomaviruses can induce mucocutaneous tumours in vertebrates. Given the high metastatic potential of some of these tumours in humans, and their tendency to recur following treatment, there is a need of more effective therapeutics.
Methods: Following the generation of live-attenuated influenza viruses co-expressing bovine papillomavirus type 1 (BPV1)-specific E6 and E7 peptides, we assessed the efficacy of this immunotherapeutic vaccine prototype in a natural tumour model, i.e., horses bearing BPV1 or 2-induced skin tumours termed sarcoids. So far, 21 horses with mild and moderate disease repeatedly received intralesional injections with the vaccine. Ten horses with severe disease (multiple/aggressive sarcoids; 64 in total) were treated by combined therapy, i.e., injections with the vaccine and electrosurgical tumour excision. Where possible, a follow-up was carried out by photo-documentation and BPV1/2 PCR-screening of hair roots/dandruff of the tumour sites.
Results: Nine of 21 horses with mild or moderate disease showed complete tumour regression (CR) after six months, and all samples collected from seven individuals scored BPV1/2-negative. Further nine horses exhibited partial sarcoid regression (PR) that is still ongoing, and harboured BPV1 or 2. Three horses showed stable disease and BPV1/2 infection. In one of these horses, repeated surgery and additional booster injections led to freedom from sarcoids and BPV1/2.
From the 10 severely affected horses, nine showed CR six months after combined therapy. Three recurrent lesions in two horses were eradicated by repeated immunotherapy or combined therapy. This led to 8/9 horses being tumour- and BPV1/2-free meanwhile.
Conclusion: Sarcoid recurrence following standard therapy is a common event because current treatments do not target BPV1/2 infection. Flu-BPV1-E6E7-immunotherapy was effective in 86% of mild and moderate-type sarcoids as well as eradicating BPV infection underlying the disease. Similarly, combined therapy led to CR in 9/10 cases and significantly reduced the risk of post-treatment recurrence due to eradication of BPV infection: only 3/64 treated sarcoids recrudesced, corresponding to a unique recurrence rate of only 4.7%.

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Investigating RUNX2 KO in B16 melanoma cells as a potential strategy to enhance in vivo tumor response to therapeutic approaches

Elena-Georgiana Dobre*[1], Mauro Voi[2], Elisa Orlandi[2], Carola de Martinis[2], Carolina Constantin[1], Donato Zipeto[2], Maria-Teresa Valenti[2], Monica Neagu[1]

[1]*Immunology Laboratory, "Victor Babes" National Institute of Pathology, Bucharest, Romania*
[2]*Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy*

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***Correspondence:** Elena Georgiana Dobre. Immunology Laboratory, "Victor Babes" National Institute of Pathology, Bucharest, Romania.
E-mail: dobregeorgiana_95@yahoo.com
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Abstract

Background: Cutaneous melanoma (CM) is a heterogeneous and highly metastatic disease with unpredictable clinical behaviour, for which the most effective pharmacological strategies are still being sought. In the present study, we describe the workflow for RUNX2 KO generation in B16 melanoma cells, an approach that according to preliminary data from international databases may contribute to defusing CM resistance to targeted and immunotherapies.
Methods: The role of the RUNX2 gene in CM was investigated using the TIMER2.0 (http://timer.cistrome.org/) and TISIDB (http://cis.hku.hk/TISIDB/) databases. Two gRNAs targeting exon 4 of RUNX2 were designed and cloned into the px459v2.0 plasmid, as previously described[1]. The B16 melanoma cells were transfected with 2.5 µg of plasmid DNA using Lipofectamine 3000 in DMEM (without antibiotics). After one day, cells were selected with complete DMEM containing 1 µg/ml puromycin for six days. Cells were further subjected to monoclonal cell isolation by seeding 0.3 cells/well in 96-well plates. Genomic DNA was extracted from the transfected bulk cell population and isolated clones and PCR-amplified with primers spanning the gRNA-target region. RUNX2 KO was confirmed by Western blot.
Results: According to the TIMER2.0 and TISIDB databases, RUNX2 shows positive correlations with the most notorious immune inhibitors within the CM tumour microenvironment and is overexpressed in CM patients unresponsive to immune and targeted therapies. Therefore, we hypothesize that orchestrating RUNX2 KO in melanoma may be a promising strategy to improve tumour response to therapeutic approaches. Five B16 clones were subjected to Sanger sequencing and all showed editing events. In particular, they showed an in-frame deletion within the Runt domain of the RUNX2 gene. In addition, these five clones showed no RUNX2 protein expression by Western blot.
Conclusions: Our study presents the workflow for obtaining genetically engineered melanoma cells that can be further exploited to dissect the biological roles of RUNX2 in syngeneic B16 melanoma mouse models.
References:
[1]Deiana M. et al., 2018. New insights into the Runt Domain of RUNX2 in melanoma cell proliferation and migration. *Cells*. 7(11):220.

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Air Pouch Models for Immunotherapy Evaluation of ROS-Enhancing Anticancer Compounds

Galyna Bila [1,2], Andriy Mokhir, [3] Rostyslav Bilyy [1,2]

[1] *Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
[2] *Danylo Halytsky Lviv National Medical University, Department of Histology, Cytology and Embryology,Ukraine*
[3] *Friedrich-Alexander-University of Erlangen-Nürnberg (FAU), Department of Chemistry and Pharmacy, Organic Chemistry II,Erlangen, Germany*

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***Correspondence:** Galyna Bila. *Institute of Cellular Biology and Pathology "Nicolae Simionescu",8 B.P.Hasdeu street, Sector 5, Bucharest, Romania Danylo Halytsky Lviv National Medical University, Department of Histology, Cytology and Embryology, 79010 Lviv, Ukraine*
E-mail: galyna.bila@gmail.com
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Abstract

The air pouch model has emerged as a valuable in vivo platform for investigating the complex interplay between the immune system and tumor development, making it particularly relevant for evaluating novel anticancer immunotherapies. This study highlights the utility of the air pouch model for preclinical assessment of reactive oxygen species (ROS)-enhancing compounds aimed at inducing immunogenic cancer cell death (ICD) and eliciting antitumor immune responses.

The air pouch, an experimentally induced subcutaneous cavity, rapidly becomes infiltrated with diverse immune cells, including neutrophils, macrophages, eosinophils, and mast cells, creating a localized environment conducive to studying cellular interactions with administered agents and tumor cells. Injection of monosodium urate crystals (MSU) into the air pouch cavity stimulate abundant neutrophil infilatration and formation of aggregated neutrophil extracellular traps (NETs). Introduction of cancer cells, such as the Nemeth-Kellner (NK/Ly-RB) myeloblastoma cell line, into the air pouch allows for the observation of tumor growth within an immunocompetent host. Here we for the first time demonstrate the dynamics of tumor solification inside the formed airpouch, tumor growth dynamics and morphological changes. Animal survival was also evaluated. Furthermore, the early interaction of immune cells, notably neutrophils, with the inoculated tumor cells and the formation of NETs, cytokine production can be readily assessed in this model.

We leveraged the air pouch model to evaluate the efficacy of ROS-modulating anticancer agents with immunotherapeutic potential. For instance, the compound NCure50, a reversible thiol binder that increases lysosomal ROS, demonstrated significant antitumor effects in the NK/Ly-RB air pouch model, extending the lifespan of treated mice and even leading to cures in some cases. Activation of lysosomal ROS resulted in formation of oxidized neoadjuvants. Importantly, surviving mice developed anti-tumor antibodies and exhibited neutrophil-mediated tumor targeting, including NET formation, indicating the induction of systemic and local antitumor immunity [1].

These findings underscore the air pouch model's capacity to provide critical insights into the in vivo efficacy of ROS-enhancing anticancer compounds and their ability to stimulate antitumor immunity. The model allows for the evaluation of local immune responses, such as NET formation, and the subsequent development of systemic immunity, paving the way for the identification and optimization of novel immunotherapeutic strategies against various cancers.

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Enhancing tumor drug delivery: targeting nucleolin with liposomes for improved therapeutic efficacy

Delia Boteanu*, Maria Anghelache, Mihaela Turtoi, Manuela Călin

**Department of Medical and Pharmaceutical Bionanotechnology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*

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***Correspondence:** *Department of Medical and Pharmaceutical Bionanotechnology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania.* E-mail: delia.boteanu@icbp.ro
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Abstract

Nucleolin (NCL) is a multifunctional protein that plays a significant role in cancer development and progression. Nucleolin's expression on both endothelial and cancer cells makes it a strategic target for cancer therapy and drug delivery applications. This study aims to develop dexamethasone-loaded liposomes (LP-Dexa) coated with an affinity peptide for NCL (F3/LP-Dexa) to target cancer and endothelial cells and reduce tumor-associated inflammation. LP-Dexa were prepared using lipid film hydration followed by the extrusion method and functionalized with F3 peptide via thiol-maleimide linkage. Internalization by bEnd.3 cells (endothelial cells isolated from brain tissue derived from a mouse with endothelioma) was assessed using immunofluorescence and flow cytometry at various intervals. NCL protein and gene expression were analyzed post-treatment using immunofluorescence, flow cytometry, and real-time PCR. The anti-inflammatory effect was evaluated by measuring monocyte chemoattractant protein-1 (MCP-1) expression via Western blot. NCL expression increased two-fold in TNF-α-activated bEnd.3 cells compared to controls. F3/LP-Dexa showed efficient internalization and significantly reduced NCL mRNA and MCP-1 protein levels without inducing cytotoxicity at lower dexamethasone concentrations. Targeting NCL with F3/LP-Dexa offers a novel strategy for intracellular drug delivery to both cancer cells and activated endothelial cells, potentially reducing tumor growth and vascular inflammation while advancing therapeutic options for cancer.

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Influence of stemness inhibitors on the immunomodulatory properties of colorectal cancer

Austeja Butkute[1,2*], Marius Baltramonaitis[2], Simona Malmige[3], Adas Darinskas[1], Vita Pasukoniene[1], Agata Mlynska[1,4]

[1]*Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania*
[2]*Life Sciences Center, Vilnius University, Vilnius, Lithuania*
[3]*STEAM Center, Vilnius University, Vilnius, Lithuania*
[4]*Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania*

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***Correspondence:** Austeja Butkute. *Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania; Life Sciences Center, Vilnius University, Vilnius, Lithuania* . E-mail: austejabutkute@gmail.com
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Abstract

Colorectal cancer (CRC) is a heterogeneous disease characterized by a diverse tumor microenvironment comprising immune and non-immune components. Acquired mutations and phenotypic plasticity in the tumor microenvironment create a diversity of cells whose interactions contribute to tumor progression and create resistance to anticancer therapy through various activated mechanisms. At the molecular level, four subtypes of colorectal cancer (CMS1-4) have been validated, differing in their aggressive characteristics and immune infiltration. With the rapid development of oncology science and the development of new cancer treatment strategies, more attention is given to the microenvironment surrounding cancer cells. Since the tumor is densely infiltrated by host immune cells, especially macrophages, it could be a promising target for developing new treatment strategies. This study aims to explore how inhibition of CRC cell stemness properties with specific inhibitors could modulate macrophage polarization, shifting the immune balance from protumoral M2 type to the antitumoral M1 type. We evaluated the effect of four stemness inhibitors - salinomycin, SB-431542, JIB-04, and napabucasin - on CMS4 CRC cell lines (HCT116 and SW620) and human peripheral blood-derived macrophages in an indirect co-culture model. Our results demonstrate that CMS4 CRC cell lines induced distinct macrophage polarization patterns, with HCT116 promoting M2 macrophages and SW620 leaning toward M1 profile. The combination of stemness inhibitors reduced stemness markers (CD133, CD44) in colorectal cancer cells and shifted macrophage polarization toward an M1 phenotype, particularly in co-culture with HCT116. These findings suggest that stemness inhibitors not only target cancer cells but also modulate the immune microenvironment by reprogramming macrophages.
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Adipokines and gut microbiome shape the outcome of cutaneous melanoma: preliminary insights

Carolina Constantin*[1,2], Elena-Georgiana Dobre[1], Adriana Narcisa Munteanu[1], Mihaela Surcel[1], Sabina Andrada Zurac[1,2,3], Monica Neagu[1,2]

[1] *Immunology Laboratory, "Victor Babeș" National Institute of Pathology, Bucharest, Romania;*
[2] *Pathology Department, Colentina Clinical Hospital, Bucharest, Romania*
[3] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

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***Correspondence:** Carolina Constantin. Immunology Laboratory, "Victor Babeș" National Institute of Pathology, Bucharest, Romania. Pathology Department, Colentina Clinical Hospital, Bucharest, Romania
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Abstract

An emerging area of research in cutaneous melanoma is related to the interference of associated co-morbidities such as obesity as an important metabolic alteration, and microbiome characteristics. Such alterations occur in chronic inflammation, immune evasion, and carcinogenesis, impacting antitumor immunity and response to therapy. Increasing data regarding the effect of metabolic disorders in tumor progression associate high levels of adipokines as having an influence on the tumor microenvironment and immune response. A series of cytokines/chemokines support chronic inflammation in cancer in conjunction with metabolic imbalance. Among adipokines, a key hormone, *leptin* may mediate the effects of obesity on melanoma progression, having a direct effect on vital signaling pathways (e.g., JAK2/STAT3 and the MAPK/FOS cascade) and influencing T lymphocyte responses through the transition to the T helper-1 phenotype. Such soluble modulators of cancer dysbiosis can be integrated into complex melanoma study protocols that can encompass investigation of the microbiome at different stages of the disease or for monitoring the effects of therapy. Advanced molecular exploration technologies (e.g., NGS) provide refined data in a complex, multidimensional approach in dermato-oncology.
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Dietary fiber pectin from papaya fruit (Carica papaya) modulates gut microbiota in a mouse model of colon carcinogenesis

Janaina L S Donadio*[1], Prof. João Paulo Fabi[2]

[1]*Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*
[2]*Faculty of Pharmaceutical Sciences, Food Research Center (FORC), University of São Paulo, Brazil*

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***Correspondence:** Janaina L S Donadio. *Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*
E-mail : janaina.donadio@biomed.cas.cz
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Abstract

Introduction: Growing evidence suggests that the gut microbiota, mainly modulated by diet, is involved in CRC development. Pectins, a class of highly fermentable dietary fibers, can modulate the gut microbiota which depends upon the ripening stages of the fruit.
Objective: This work aimed to investigate the differential effects of ripe and unripe papaya pectins on gut microbiota composition in a mouse model of colon carcinogenesis.
Methods: Male Wistar rats were divided into 4 groups fed ripe and unripe papaya pectins and subjected to AOM injections. Animals were euthanized after 10 weeks. Aberrant crypt focus (ACF) and SCFA production were analyzed in the colon. Metagenomic sequencing was done using Nanopore sequencing technology. The AOM model is known to increase the infiltration of neutrophils and M2 macrophages in the tumor microenvironment, as well as decrease the cytotoxicity of CD8+T cells and increase the Tregs in the tumor microenvironment, contributing to tumor growth.
Results: Ripe papaya pectin intake reduced the number of ACF with 1 and 2 crypts. Acetate production was higher in animals fed ripe and unripe papaya pectin. Ripe papaya pectin increased richness in microbiota alpha diversity. The ripe papaya pectin group and the negative control group shared a similar bacterial profile. The intake of ripe papaya pectin increased the abundance of *Lactobacillus* and *Limosilactobacillus* while the intake of ripe papaya pectins increased the abundance of *Rombutsia*. The AOM experimental group had an increase in the abundance of *Mediterraneibacter*. The lactobacillus especially is known to interat
Conclusion: These results suggest that the intake of ripe papaya pectin had a potential preventive effect on colon carcinogenesis development by decreasing the number of ACF, increasing SCFA production, and increasing the abundance of *Lactobacillus*, a well-known probiotic strain that promotes health benefits to the host. This could be beneficial in future preventive measures to reduce CRC development using a natural component of the diet rich in dietary fiber.
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CRC: colorectal cancer, AOM: azoxymethane, SCFA: short chain fatty acids, ACF: aberrant crypt focus.

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Development and Characterization of a Bispecific Antibody Targeting EGFR and AXL

Sarah A. Eisdorfer[1*], Moshe Elkabets[1]

[1]The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva.

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***Correspondence:** Sarah A. Eisdorfer. The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva
E-mail: sarahadi@post.bgu.ac.il
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Abstract

Traditional cancer therapies often overlook the dynamic nature of cancer by utilizing single targets, considering only homogenous tumors, not considering the tumor microenvironment, and failing to overcome common issues such as induced and acquired resistance to therapy. The development of new therapies that address all these concerns remains a challenge in oncology today, and there is a need for single low-dose therapies that can specifically address heterogenous and complex populations. We propose a bispecific antibody targeting EGFR and AXL can address this gap, while considering cytotoxicity and avoiding off-target effects.

EGFR is a receptor commonly overexpressed in many tumors and a known target in cancer therapy, however, many anti-EGFR treatments have detrimental off-target side effects for healthy cells. Additionally, resistance to anti-EGFR drugs is a common occurrence after prolonged treatment. One known mechanism of anti-EGFR resistance involves cross-talk between EGFR and the AXL receptor, a member of the TAM family of tyrosine kinase receptors which is also overexpressed in many tumors as well as in their immunosuppressive tumor microenvironments. AXL expression in oral cancers is known to mediate epithelial-mesenchymal transition through the PI3K pathway and has been shown to be associated with poor prognosis. New data from spatial tumor analysis of human patient derived xenografts shows huge diversity of expression of EGFR and AXL, both between tumors and within different cells of the same tumor where the expression levels of the two receptors appear to complement each other.

This research includes the development and characterization of an IgG-like bispecific antibody targeting both EGFR and AXL receptors which we hypothesize can not only eliminate the immunosuppressive tumor microenvironment and tumors with heterogenous expression of the two targets, but could also prevent the common complication of acquired resistance as well as improve specificity and biodistribution of the therapy to reduce off-target effects.

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Toll Like Receptor 7/8 agonists antitumor effects on NK cells in murine melanoma model

Gheorghita Isvoranu [1*], *Mihaela Surcel* [1], *Adriana Narcisa Munteanu* [1], *Ana-Maria Enciu* [1], *Marioara Chiritoiu-Butnaru* [3], *Gabriela Chiritoiu* [3], *Cristian Munteanu* [2], *Monica Neagu* [2]

[1] *"Victor Babeș" National Institute of Pathology, Animal Husbandry, Bucharest, Romania*
[2] *Institute of Biochemistry of the Romanian Academy, Department of Bioinformatics & Structural Biochemistry, Bucharest, Romania*
[3] *Institute of Biochemistry of the Romanian Academy, Department of Molecular Cell Biology, Bucharest, Romania*

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***Correspondence:** : Gheorghita Isvoranu, "Victor Babeș" National Institute of Pathology, Animal Husbandry, Bucharest, Romania
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Abstract

Background: Toll-like receptors (TLRs) are a subclass of molecules that regulate both innate and adaptive immune cells, and this ability makes them attractive targets for cancer immunotherapy. Synthetic TLR agonists, such as imidazoquinolines, activate TLR7/8 receptors on various immune cells and offer exciting perspectives for cancer therapy.
Aim: In this study we aimed to investigate the effects of TLR7/8 agonists, imiquimod and gardiquimod, on NK cell phenotype and tumor growth in a mouse model of melanoma.
Methods: C57BL/6J mice were injected subcutaneously with B16-F10 melanoma cells on day one. The TLR7/8 agonists used were imiquimod (IMQ), gardiquimod (GDQ) and imiquimod cream (ALDARA), and treatment was administrated after tumor development. IMQ and GDQ were administrated intratumorally on days 8, 10, 15, and 18, while ALDARA was applied topically starting on day 14, for 5 consecutive days. The untreated control group received phosphate-buffered saline solution. Tumor size was measured weekly to determine the impact of treatment. To examine the effects of TLR7/8 agonists on NK cells, mice were euthanized on day 21 and spleens were harvested and assessed immediately using flow cytometry analysis for NK cell surface markers. A control group of healthy mice was considered.
Results: ALDARA treatment induced significant inhibition of tumor growth, while no significant differences were observed in the IMQ and GDQ groups, except for one mouse that received GDQ. In this case, a tumor regression was observed and after two months the tumor started to grow. At necropsy, macroscopically visible melanoma metastases were observed on the lungs. A decrease in the percentage of NK1.1+ and CD49+ cells in the spleen was observed in all groups compared with healthy mice. Evaluation of NK cell phenotype showed an increase of CD69+ and B220+CD11c+NK1.1+ cells in all treated groups. Analysis of NK cell subsets indicated a decrease of the mature cell subset and an increase of the immature cell subset in tumor-bearing mice, with or without treatment, except for the ALDARA treatment group.
Conclusions: Our study showed that treatment with TLR7/8 agonists induced a slowing of tumor growth and activated NK cells. These data can provide new insights into the knowledge of the NK cell phenotype and function in solid tumors treated with TLR7/8 agonists and also new approaches in cancer immunotherapy.

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Inhibition of dendritic cell maturation in 3D and 2D colon carcinoma cell models

Pavol Lukáč[1,2,*], Lenka Rajsiglová[1], Dmitry Stakheev[1,3], Paolo Tenti[1,2], Kateřina Krausová[1,3], Leonid Sushytskyi[1], Daniel Smrž[1,3], Luca Vannucci[1]

[1] *Laboratory of Immunotherapy, Institute of Microbiology of the CAS v.v.i., Prague, Czech Republic*
[2] *Department of cells biology, Faculty of Science, Charles University, Prague, Czech Republic*
[3] *Department of Immunology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic*

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***Correspondence:** Pavol Lukáč. Laboratory of Immunotherapy, Institute of Microbiology of the CAS v.v.i., Prague, Czech Republic. Department of cells biology, Faculty of Science, Charles University, Prague, Czech Republic
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Abstract

Dendritic cells (DCs) play an important role in the immune response against cancer. Numerous studies indicate that tumor microenvironment affects the phenotype and function of DCs. The aim of the work was to determine how three-dimensional (3D) versus two-dimensional (2D) mixed-cells tumor models (mouse CT26 colon cancer cells mixed with mouse 3T3 fibroblasts) can impact on DCs maturation. Three-days old spheroids of 200µm diameter were used, formed by mixing CT26 and 3T3 cells cultivated in 3D Petri Dish™ (microtissues™) molds. DCs were isolated from BALB/c mouse femurs and added to mixed-cell 3D spheroids and 2D mixed-cell monolayer, in coculture. DCs control group and Wnt-inhibited DCs (by XAV939) were also used. After 48 hours of coculture, half of the DCs were collected and analyzed by flow cytometry. Splenocytes were added to the remaining half of the DCs, cocultured for another 48 hours, and T-cell activation analysis was performed by flow cytometry. Here we show that mixed colorectal carcinoma spheroids have significantly higher immunoinhibitory properties than 2D mixed cell culture. Inhibition of Wnt signaling pathway doesn't affect the maturation of DCs but decreases the expression of checkpoint molecules (PD-1, Tim3) on CD4+ T-cells primed by XAV939-treated DCs.

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Molecular mechanisms of communication between innate immune cells and vascular cells in cancer and cardiovascular diseases

Razvan D Macarie*, Monica M Tucureanu, Letitia Ciortan, Mihaela Vadana, Andreea Mihaila, Miruna L Naie, Ana Maria Gan, Ileana Manduteanu, Elena Butoi

Inflammation Department, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

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***Correspondence:** Razvan D Macarie. Inflammation Department, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania . E-mail: razvan.macarie@icbp.ro
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Abstract

The immune-vascular crosstalk is a critical factor in cancer and cardiovascular diseases, driving disease progression through inflammatory, remodeling, and angiogenic pathways. Despite recent progress, the underlying molecular mechanisms that govern the interplay between vascular and immune cells remain elusive. Understanding these interactions provides opportunities for developing novel therapeutic strategies targeting immune and vascular components to improve treatment efficacy and patient outcomes.

In this context, our work aimed to investigate the communication between innate immune cells (monocytes-macrophages) with endothelial cells and smooth muscle cells (SMC). Using different models of cellular cross-talk between monocyte/macrophages and vascular cells, our data reveal that: ***i)*** upon their interaction with monocytes, the molecular signatures of *endothelial cells* suffer transcriptomic changes in processes related to cytoskeleton regulation, focal adhesions, cellular junctions, and cell adhesion, as evaluated by RNAseq analysis, leading to changes in endothelial cells adherence to extracellular matrix, endothelial permeability, monocyte adhesion, and transmigration; ***ii)*** the *SMC-monocyte* interaction induced, in each cell type, an increased mRNA and protein expression of TNFα, IL-1β, IL-6, CX3CR1, MMP-2 and MMP-9, dependent by fractalkine - CX3CR1 axis dependent and by MAPK signaling and activation of NF-kB and AP-1 transcription factors; ***iii)*** when *SMC communicated with macrophages* (MAC), the SMC exhibits increased expression and activity of metalloproteases MMP-9, MMP-1, and augmented secretion of VEGF. Moreover, the conditioned media collected from MAC–SMC co-culture promoted endothelial cell tube formation in Matrigel, signifying an increased angiogenic effect. In addition, MAC exhibited a general increased expression of chemokines (with VCC-1 and GRO-α having the highest fold increase) and of interleukins such as IL-5 (~8-fold) and IL-6 (~4-fold) in MAC. When the cross talk was realized in the presence of resistin (adipose-derived cytokine), a predominant increase of interleukins (the highest fold increase: IL-6, IL-27, IL-23 and IL-5) and an augmented expression of chemokines such as MIP-1β, GRO-α and CCL1 was obtained. Cell communication in the presence of ficolin-2 (a pattern recognition molecule that activates the lectin complement pathway, contributing to innate immunity), induced pro-inflammatory phenotypes in both cell types gained with increased MCP-1, IL-6 and TLR4, in SMC (by activating ERK/MAPK and NF-KB signaling pathways) and IL-1β, IL-6, and MIP-1β in MAC, ensuing enhanced monocyte transmigration.

All these data demonstrate that communication between immune cells and vascular cells affects factors and molecules that could alter processes such as inflammation, tissue remodeling, and angiogenesis, features that could directly dictate the regulation of both, cancer progression and cardiovascular diseases.

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Androgen receptor signaling mediated chronic inflammation drives sex disparity in HNSCC progression and therapy response

Divyasree Marripati*, Moshe Elkabets

The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva.

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***Correspondence:** Divyasree Marripati. The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva
E-mail: divyasree@bgu.ac.il
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Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is a highly prevalent and lethal cancers worldwide with a higher incidence in men than in women. Molecular analysis across various cancers have classified HNSCC into the group of cancers with strong sex-based effect. However, the underlying cellular and molecular mechanisms for these sex-related differences in susceptibility and therapeutic response in HNSCC is rather limited.
Chronic inflammation plays a key role in HNSCC progression and is influenced by many factors that includes sex hormone signaling which modulates both the tumor microenvironment (TME) and systemic immune responses. Recent studies have highlighted the importance of androgen receptor (AR) signaling contributing to immune suppression, increasing the tumor development and inflammatory responses in males. However, the multifaceted relationship between AR and key inflammatory mediators such as IL-1β and COX-2 (PTGS2, prostaglandin-endoperoxide synthase 2) which are crucial regulators of inflammation in HNSCC and may contribute to sex disparities, remains unexplored. Therefore, comprehensive investigations in well characterized clinical and pre-clinical models are essential to identify the sex-specific prognostic and therapeutic targets, addressing a critical unmet clinical need with significant translational impact.
Aims: This study aims to investigate the role of androgen receptor signaling in modulating inflammation within the TME and its contribution to immune suppression and tumor progression in males, with the goal of enhancing sex-specific therapeutic strategies.
Methods: In this study, we generated pre-clinical mouse models using 4-Nitro Quinoline-1-Oxide (4NQO) - a tobacco surrogate and cancer cell lines derived thereof to address the mechanism of sexual dimorphism in HNSCC. To uncover the complexity of the TME in HNSCC, we utilized spatial imaging techniques in preclinical mouse tumors. Immunohistochemistry (IHC) was used to stain for AR, COX-2 in clinical and pre-clinical tumor samples. Additionally, we evaluated the synergistic potential of AR inhibition and anti-PD1 therapy in HNSCC in pre-clinical mouse models.
Results: 4NQO exposure revealed significant sex-based differences, particularly in IL-1b wildtype (+/+), but not knockout (-/-) mice and between castrated vs. sham operated male mice, indicating role for sex hormones in HNSCC pathogenesis. Our findings suggest that COX-2 staining patterns differed by sex, with male patients showing predominant cancer cell expression, whereas in females preferentially expressed in stromal cells of TME. Spatial multiplex imaging has showed females with a robust active immune surveillance and males with more immune suppressive microenvironment. Interestingly, blocking the AR signaling with Enzalutamide in immune-competent male mice altered immune dynamics whereas no effect was observed in immune-compromised mice suggesting an immune regulatory role of AR. Finally, combination of enzalutamide with anti-PD1 led to complete tumor regression in the mice models bearing 4NQO cell line tumors. This study highlights AR signaling as a key regulator of TME in HNSCC. Hence, targeting AR may reduce inflammation driven immune suppression in males thereby enhancing the efficacy of immunotherapy in HNSCC.

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Depletion of Myeloid Cells Reduces Tumorigenesis Driven by a High-Protein Diet in a Colitis-Associated Cancer Model

Veronika Motúzová*, Janaina L S Donadio, Michal Kraus, Štěpán Coufal, Maliha Rizwan, Miloslav Kverka

Laboratory of Cellular and Molecular Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

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***Correspondence:** Veronika Motúzová. Laboratory of Cellular and Molecular Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic.
E-mail: veronika.motuzova@biomed.cas.cz
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Abstract

Diet shapes the tumor microenvironment in colorectal cancer by modulating the reactivity of the immune system. We investigated the immunomodulatory properties of increased dietary protein intake in the early phase of tumorigenesis in a mouse model of colitis-associated cancer.

BALB/c mice were fed a synthetic control diet (CD) or a high-protein diet (HPD) and subsequently treated with azoxymethane and dextran sodium sulfate to induce colitis-associated cancer. The involvement of macrophages in tumorigenesis was examined by depleting them with clodronate-loaded liposomes. Immunophenotyping was performed by flow cytometry, and cytokine production in Peyer's patches (PPs) was measured by ELISA. Colonoscopic screening was performed to assess tumor characteristics.

HPD-fed mice developed more numerous and larger tumors and had a lower proportion of systemic CD8+ T cells. However, clodronate-mediated depletion of macrophages during early tumorigenesis significantly reduced tumor incidence and restored CD8+ T cells. In the spleens of clodronate-treated mice, a proinflammatory macrophage subpopulation was partially depleted, along with a significant reduction in neutrophils, while NK, NKT and DC cells increased. In addition, HPD-fed mice had elevated proinflammatory TNFα, IL-17, IL-1β and S100A8 in PPs, with TNFα and IL-17 even higher in clodronate-untreated mice.

Our findings demonstrated that HPD contributes to intestinal inflammation and exacerbates tumor development driven either by macrophage or neutrophil stimulation.

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Immunomodulatory properties of nickel-doped ferric-based nanoparticles in vitro

Lenka Rajsiglova*[1], Dmitry Stakheev[1,2], Katerina Krausova[1,2], Pavol Lukac[1,3], Paolo Tenti[1,3], Michal Babic[4], Daniel Smrz[1,2], Luca Vannucci[1]

[1]*Laboratory of Immunotherapy, Institute of Microbiology of the CAS, v.v.i; Prague; Czech Republic*
[2]*Department of Immunology, Second Faculty of Medicine, Charles University and Motol University Hospital;*
[3]*Department of cell biology, Faculty of Science, Charles University; Prague; Czech Republic*
[4]*Institute of Macromolecular Chemistry of the CAS, v.v.i.; Prague; Czech Republic*

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***Correspondence:** Lenka Rajsiglova. Laboratory of Immunotherapy, Institute of Microbiology of the CAS, v.v.i; Prague; Czech Republic
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Abstract

Iron-based nanoparticles (NPs) represent small nanocarriers made of iron oxides. These nanoparticles include magnetite NPs (Fe_3O_4), maghemite NPs (γ-Fe_2O_3), or mixed ferrites (metal-doped iron oxide NPs; MFe_2O_4, where M can be Co, Ni, Zn, or Mn). Due to their unique characteristics, including biocompatibility, reported selective toxicity towards cancer cells, magnetic imaging capabilities, and hyperthermic properties, they are regarded as a promising modality for cancer treatments. In this context, it is crucial to assess the impact of these NPs on immune anti-cancer responses, as immunosuppressive effects on immune cells are undesirable in cancer therapies and may compromise the outcome of the treatment.

For this purpose, we initially investigated the direct anti-cancer effects of FN NPs (ferric oxide-based NPs doped with Ni) on the CT26 (murine colorectal adenocarcinoma) cell line. To evaluate the immune response to the treated cancer cells, syngeneic bone marrow-derived dendritic cells (BMDCs) were pulsed with the lysates of FN NP-pretreated CT26 cells. These BMDCs were maturated overnight and analyzed by flow cytometry for expression of maturation markers. A portion of the prepared BMDCs was additionally co-cultured with T cells, and their activation was assessed by examining the production of IFN-γ and TNF-α, along with the expression of PD-1 and TIM-3 using flow cytometry.

Our experiments demonstrated that FN nanoparticles exhibit a direct, dose-dependent toxic effect and an increased apoptotic rate in the CT26 cancer cell line. No immunosuppressive effects were detected concerning the expression of maturation markers of FN NP-CT26-pulsed and maturated BMDCs or their functional capacity to activate syngeneic T cells. The activation of T cells was demonstrated by the production of pro-inflammatory IFN-γ and TNF-α. Moreover, no changes were detected in the expression of PD-1 and TIM-3 checkpoint molecules linked with the exhausted T cell phenotype.

In conclusion, the evaluated FN NPs displayed direct anti-cancer toxicity within our experimental model while preserving the immune response to the cancer cells. These findings imply that the nanoconstruct may be a viable candidate for anti-cancer interventions.

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Innovative Vanadium (V)-Based Drug: Preliminary In Vitro and In Vivo Antitumor Investigations

Mihaela Turtoi*, Maria Anghelache, Geanina Voicu, Florentina Safciuc, Mariana Deleanu, Manuela Călin

Medical and Pharmaceutical Bionanotechnologies Department, Institute of Cellular Biology and Pathology "Nicolae Simionescu" of the Romanian Academy, Bucharest, 050568, Romania.

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***Correspondence:** Mihaela Turtoi. Medical and Pharmaceutical Bionanotechnologies Department, Institute of Cellular Biology and Pathology "Nicolae Simionescu" of the Romanian Academy, Bucharest, Romania.
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Abstract

Several metal-based compounds have been synthesized and tested for their antitumor properties, as current Pt-based cytostatic drugs have not achieved the expected pharmacological outcomes.
In this study, we evaluated the antitumor effects of our newly developed macrocyclic tetranuclear oxovanadium (V) compound, with the chemical formula $[(V^{V}O)(L)(CH_3O)]_4$ (abbreviated Van), where L represents the deprotonated form of the Schiff base 3-methoxysalicylidenvaline (Turtoi M et al., 2021). We investigated the effects of Van on inducing apoptosis and pyroptosis in the human hepatocarcinoma (HepG2) cell line, as well as its potential to reduce the tumor volume *in vivo*.
The synthesis and physicochemical characterization for Van, its stability in cell culture media, its cellular uptake, interaction with albumin, and effects on cell viability, oxidative status, DNA integrity, and cell cycle of HepG2 cells have been previously reported (Turtoi M et al., 2021). The mechanism by which Van exerts its antitumor activity was further investigated by determining the activity of caspase 3 and analyzing the protein expression levels of the anti-apoptotic protein Bcl-2, the pro-pyroptotic proteins NLRP3 and caspase 1, as well as Interleukin (IL)-1β, in comparison to cisplatin. Additionally, the ability of Van to reduce the size of solid tumors was initially evaluated through the subcutaneous injection of HepG2 cells in C57BL/6J mice. The antitumor treatment started 48 hours after tumor induction, involving three injections given either intravenously (i.v.) or intratumorally (i.t.), with each injection administered every three days. The doses were either 1 mg/kg BW Van or 1 mg/kg BW cisplatin. All animals were euthanized one-month post-anti-tumor therapy using an overdose of ketamine and xylazine. The tumors were then excised and histologically analyzed by Trichrome Masson's staining.
Our results demonstrated that Van can produce a pro-apoptotic effect by increasing caspase 3 activity and reducing the synthesis of the anti-apoptotic protein Bcl-2, similar to the effects of cisplatin. Furthermore, Van exhibited a greater ability to activate the pyroptotic signaling pathway compared to cisplatin by enhancing the expression of NLRP-1, caspase 1, and IL-1β protein expression in HepG2 cells. The preliminary *in vivo* results evidenced that Van effectively prevents the formation of subcutaneous solid tumors following i.v administration and reduces the tumor volume after i.t injection compared to control animals.
Our findings highlight the remarkable antitumor efficacy of $[(V^{V}O)(L)(CH_3O)]_4$, underscoring its potential as a powerful therapeutic agent.
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Establishment of cancer immunotherapy in vitro model

Jana Veprkova[1*], Andrej Besse[1,2], Lenka Sedlarikova[1], Lenka Besse[1,2]

[1]*Department of Biology, Faculty of Medicine, Masaryk University Brno, Czechia*
[2]*Department of Oncology and Hematology Kantonsspital St. Gallen St. Gallen, Switzerland*

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***Correspondence** Jana Veprkova. Shurin. Department of Biology,Faculty of Medicine, Masaryk University Brno, Czechia.
E-mail: jana.veprkova@med.muni.cz
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Abstract

T cells play a central role in cancer immunotherapy, yet the lack of standardized *in vitro* models limits the reproducibility and translational potential of immune-cancer cell interaction studies. Existing models often lack reproducibility due to variability in experimental conditions, immune cell sources, and functional assays.

To address this gap, we established a robust and reproducible *in vitro* co-culture model, which serves to evaluate efficacy of the immune cell-based cytotoxicity using peripheral blood mononuclear cells (PBMCs) and multiple cancer cell lines. Healthy donor PBMCs supplemented with IL-2 are co-cultured with (educated) or without (naïve) cancer cell lines for two weeks. The model cell lines are equipped with a luciferase reporter, enabling real-time quantification of immune-mediated killing through assessment of luminescence intensity.

Single-cell RNA sequencing analysis of educated vs. naïve immune cells revealed favorable expansion and differentiation of $CD8^+$ cytotoxic T cells while compromised expansion of $CD4^+$ helper T cells. Moreover, the strong upregulation of Granzyme and Perforin expression in $CD8^+$ T cells is indicative of cytolytic function.

Initially validated using the pancreatic cancer cell line PANC-1, the model was further applied to lung cancer (A549) and multiple myeloma (AMO-1), demonstrating its versatility across malignancies. Despite the MHC non-restricted nature of PBMC cytotoxicity—due to lack of HLA compatibility—PBMCs exhibited a 40-80% increase in selective killing of the cancer cell line to which they were educated, compared to non-educated conditions.

While the use of healthy donor PBMCs poses a potential limitation in clinical settings due to the risk of graft-versus-host disease (GVHD), this model provides a highly adaptable and reproducible *in vitro* platform for studying T-cell mediated anti-cancer responses and optimizing immunotherapeutic approaches.

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Collagen IV-Targeted Nanoparticles for Cardiac Tumor Drug Delivery

Geanina Voicu*, Florentina Safciuc, Cristina Ana Mocanu, Ruxandra Anton, Manuela Calin

Department of Medical and Pharmaceutical Bionanotechnology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

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***Correspondence:** Geanina Voicu. Department of Medical and Pharmaceutical Bionanotechnology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania.
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Abstract

Patients with cardiac tumors have a limited prognosis and the only available treatments are tumor resection surgery, radiation and chemotherapy, with poor expected outcomes. Therefore, it is necessary to identify promising therapeutical strategies for targeting dysregulated molecules in the extracellular matrix characteristic of cardiac tumors. Collagen IV is a promising target for cardiac tumor treatment, due to its high expression in cardiac vasculature, fibrotic cardiac tissue, and the tumor stroma. We aim to synthesize collagen IV-targeted nanoparticles (Col-NPs) as a novel drug delivery system in cardiac tumors. Lipid NPs containing the phospholipids DOPG, POPC and Mal-PEG-DSPE were synthesized using the reverse-phase evaporation technique. The obtained lipid NPs were coupled on their surface with collagen IV-binding peptides via a maleimide-cysteine bond. Col-NPs were characterized for their hydrodynamic diameter and Zeta potential. The biocompatibility and accumulation of Col-NPs in the heart were assessed in a mouse model with increased collagen IV expression in cardiac tissue. The mice were injected intravenously with fluorescently labeled Col-NPs to investigate their localization in organs using an IVIS Spectrum Caliper 200 Imaging System. For the safety evaluation of Col-NPs, biochemical plasma parameters (cholesterol, triglycerides, urea, creatinine, transaminases) were assessed with commercial kits. Organ tissues were analyzed histopathologically using hematoxylin-eosin staining. Col-NPs have a hydrodynamic diameter of ~ 140 nm and a Zeta potential of ~ -35 mV. After intravenous administration, both Col-NPs and untargeted NPs (Scr-NPs) primarily accumulated in the liver and spleen of the mice. When examining the hearts of mice, a greater accumulation of Col-NPs compared to Scr-NPs was observed. There was no evidence of renal or hepatic toxicity, and Col-NPs were found to be biocompatible. Additionally, no pathological lesions were detected in the liver, lung, spleen, and kidney of mice injected with Col-NPs. This targeted approach has the potential to not only improve therapeutic efficacy by facilitating the accumulation of the drug at the tumor site in sufficient concentrations to exert its effects but also to reduce the required dosage, thereby minimizing systemic toxicity.

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Soluble PD-1/PD-L1 in prostate cancer: immune cell associations and clinical implications

Margarita Žvirblė[1,2,*], Žilvinas Survila[1], Neringa Dobrovolskiene[2], Agata Mlynska[2], Vita Pašukonienė[2]

[1] *Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania*
[2] *Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania*

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***Correspondence:** Margarita Žvirblė. *Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania; Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania*
.E-mail: margarita.zvirble@gmail.com
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Abstract

Background:
Soluble PD-1 (sPD-1) and PD-L1 (sPD-L1) are emerging biomarkers in prostate cancer (PCa), yet their cellular origins and interactions within the tumor microenvironment (TME), particularly with NK cells, immunosuppressive Tregs, and MDSCs, remain poorly understood. This study also explores biomarker combinations for better clinically significant PCa stratification.
Methods:
A total of 88 PCa patients (pT2 and pT3) were enrolled. Plasma levels of sPD-1 and sPD-L1 were measured using ELISA. A control group of 41 healthy individuals was also included. Immune cell populations were characterized by flow cytometry. All parameters were evaluated preoperatively and three months post-radical prostatectomy (RP). Clinically significant PCa was defined as cases with International Society of Urological Pathology (ISUP) ≥ 3. Written informed consent was obtained from all participants.
Results:
sPD-1 exhibited a weak positive correlation with NK cells ($r=0.27$, $p=0.03$) in whole cohort, suggesting NK cells as a source of sPD-1. Higher initial sPD-L1 levels were significantly associated with shorter progression-free survival (PFS) ($p<0.05$), additionally in the biochemical recurrence (BCR) group, sPD-L1 showed a positive correlation with Tregs ($r=0.73$, $p<0.05$), highlighting their coordinated role in cancer progression. Postoperative changes in sPD-L1 divided patients into two distinct groups: one showed a decrease ($p<0.001$), indicating tumor-derived origins, while the other exhibited an increase, suggesting an alternative source ($p<0.05$). In patients with a postoperative decrease in sPD-L1, no correlation was found with pretreatment Tregs and MDSC subsets. However, strong associations between total G-MDSCs and M-MDSCs ($r=-0.51$, $p=0.002$) and between G-MDSC% and M-MDSC% ($r=0.49$, $p=0.001$) were observed, indicating a dynamic interplay between these myeloid subsets, which could contribute to immunosuppressive mechanisms regulating sPD-L1 levels. Moreover, a negative correlation between sPD-L1 and M-MDSC% was found in the BCR group ($r=-0.72$, $p<0.05$). The combination of preopreative levels of sPD-L1 with the G-MDSC fraction (best result: AUC=0.93, specificity 100%) demonstrated superior results for clinically significant prostate cancer stratification compared to the M-MDSC fraction. Combinations with Tregs showed poor predictive value.
Conclusions:
Our findings indicate that sPD-1 is primarily derived from NK cells, while sPD-L1 levels are modulated by Tregs and interactions between MDSC subsets. These insights contribute to a deeper understanding of immune evasion in PCa and may facilitate the development of biomarker-driven approaches for disease monitoring and targeted therapies.

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