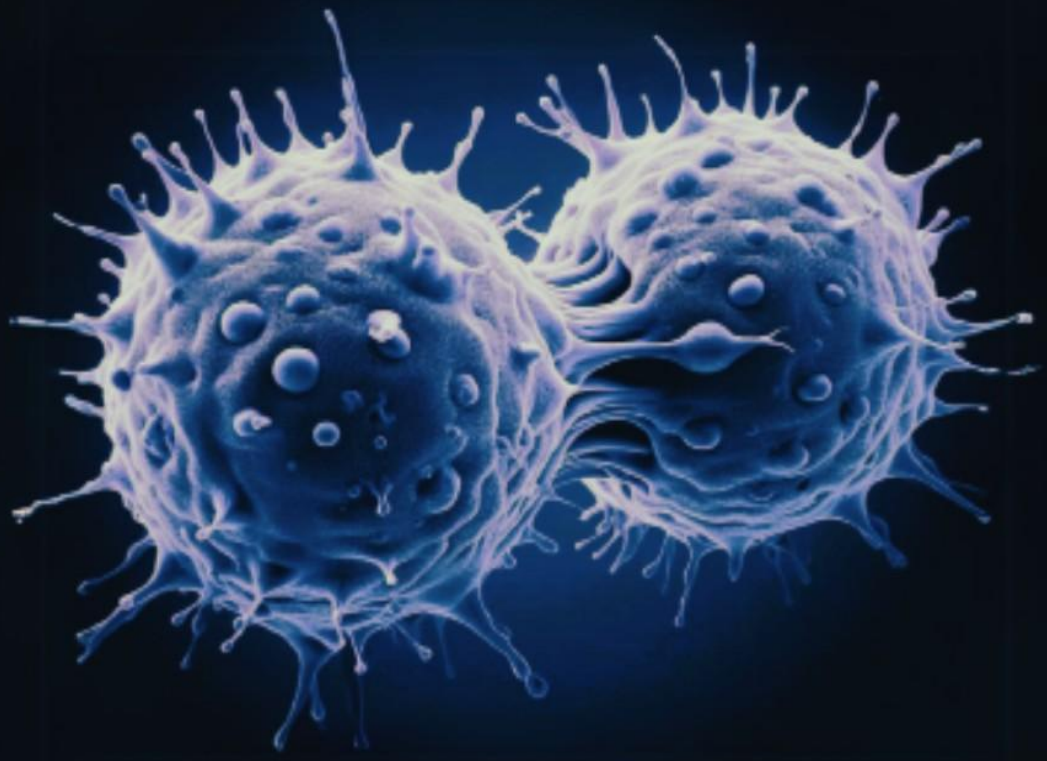
IMMUNO-model

Third Annual Conference

Modeling Immunotherapy for Cancer:

Bridging Research to Clinical Application



13-14 May 2025

University of Warsaw, Poland

PROCEEDINGS BOOK

University of Warsaw Biological and Chemical Research Centre

Faculty of Biology University of Warsaw

University of Warsaw

cost European Cooperation in Science & Technology

Funded by the European Union

COST Action IMMUNO-model CA21135 (https://www.immuno-model.eu/) aims to foster research and innovation in preclinical immuno-oncology models with the ultimate goal of advancing the treatment of cancer patients by improving their outcomes and quality of life.

IMMUNO-model brings together European researchers from diverse sectors (academia, clinical, industry) with the common goal of establishing a Network that endorses immuno-oncology research by specifically promoting the sharing, standardization, and application of immunotherapy preclinical models.

This Proceedings Book is based upon work from COST Action IMMUNO-model CA21135, supported by COST – European Cooperation in Science and Technology (https://www.cost.eu/).

COST is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research.

WELCOME LETTER

Dear Participants,

It is with great pleasure that we welcome you to the **Third IMMUNO-model Annual Conference**, held under the theme *"Modeling Immunotherapy for Cancer: Bridging Research to Clinical Application"*, taking place on May 13–14, 2025, at the University of Warsaw, Poland.

This event brings together researchers, clinicians, and industry professionals from around the world to foster the exchange of knowledge and to advance preclinical modeling in cancer immunotherapy. Organized within the framework of the COST Action CA21135, the conference serves as a dynamic platform to discuss the latest innovations, challenges, and opportunities in the field of Immuno-Oncology.

The scientific program will feature sessions dedicated to ***in vitro***, ***in vivo***, ***ex vivo***, and ***in silico*** models, reflecting the full breadth of approaches used to understand and develop immunotherapies. In addition to keynote lectures by renowned speakers and selected abstract presentations, the conference will host meetings of the different Working Groups of the IMMUNO-model network, providing further opportunities for collaboration and strategic discussions.

We thank you for joining us and wish you a productive and inspiring conference.

The Organizing Committee

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COMMITTEES

Organizing Committee

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Parycja Ściślewska – University of Warsaw, Poland

Katarzyna Głuchowska – Molecure, Poland

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PROGRAMME

Tuesday, May 13th 2025

Arrival of Attendees & Registration		**8:30 – 9:00**
Opening Session	**Eva Martinez-Balibrea, Marta Maleszewska**	**9:00 – 9:15**
Session 1 – *In vitro* models (part I) Chairs: Eva Martinez-Balibrea, Metka Novak		**9:15 – 11:00**
Keynote speaker	**David Fernandez-Antoran** *Immuno-Epithelioids: In vitro preclinical modelling of immunotherapy treatments*	9:15 – 10:00
Invited Talk 1	**José Alexandre Ferreira** *Glycoengineered Cells and GlycoAvatars: Unraveling the Glycocalyx and Cancer-Immune Cell Interactome for New Therapeutic Strategies*	10:00 – 10:30
Invited Talk 2	**Lucia Gabriele** *3D Technology for Investigating Cancer-Immune System Crosstalk and Drug Testing*	10:30 – 11:00
Coffee Break and Poster session		**11:00 – 11:30**
Session 1 – *In vitro* models (part II) Chairs: Vladimir Jurisic, Catarina A. Rodrigues		**11:30 – 13:00**
Invited Talk 3	**Natalia Marek-Trzonkowska** *On the way to cellular therapy of non-small cell lung cancer - putting the puzzles together*	11:30– 12:00
Invited Talk 4	**Thomas Sommermann (Dynamic42 - Organ-on-chip)** *Dynamic vascularized organ-on-chip models for studying immunotherapies and tumor immune interactions*	12:00 – 12:30
Short Talk 1	**Emanuela Senjor** *Evaluation of cathepsin V inhibition on glioblastoma stem cell proliferation and NK cell cytotoxicity from 2D to 3D perfused models on chip*	12:30 – 12:45
Short Talk 2	**Silvia López Borrego** *Effect of EVs containing different alleles of MICA on NKG2D Modulation and Lytic Granule Mobilization in Natural Killer Cells*	12:45 – 13:00
Lunch Break		**13:00 – 13:45**

Poster Session		**13:45 – 14:45**
Session 2 – *In vivo* models (part I) Chairs: Rosalinda Sorrentino, Marta Maleszewska		**14:45 – 16:30**
Keynote speaker	**Bozena Kaminska** *Dissecting the immune microenvironment of experimental brain tumors with single-cell and spatial transcriptomics.*	14:45 – 15:30
Invited Talk 1	**Catarina Brito** *3D culture strategies to model cancer microenvironment dynamics and therapeutic responses*	15:30 – 16:00
Invited Talk 2	**Michal Mikuła** *Patient-derived tumor models for functional precision oncology and cancer research*	16:00 – 16:30
Coffee Break and Poster session		**16:30 – 17:00**
Session 2 – *In vivo* models (part II) Chairs: Vinton Chang, Doreen Lau		**17:00 – 18:15**
IMMUNO-model updates	**Leticia Oliveira Ferrer** *Patient Taskforce* **Denis Collins** *Challenges in Modelling Immunotherapies: A TGRC Update*	17:00– 17:30
Short Talk 1	**Jakub Godlewski** *Predicting Immunotherapy Responses in Glioblastoma: An Integrated Evaluation of EVOV Vaccine-like Particles in Preclinical Models*	17:30 – 17:45
Short Talk 2	**Tianren Shi** *Determinants of immune infiltration in triple-negative breast cancer identified by multi-omic integration of large clinical cohorts*	17:45 – 18:00
Short Talk 3	**Marco Barreca** *Leveraging public pan-cancer transcriptomic data for immune-oncology: systematic collection and graphical tool for analysis*	18:00 – 18:15
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Social event – Dinner at the restaurant "Warszawski Sen"		**20:00 – 23:00**

Wednesday, May 14th 2025

Session 3 – *In silico* modelling Chairs: Perter Rashkov, Marco Bareca		**9:00 – 11:00**
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Invited Talk 2	**Björn Rotter (GenXPro GmbH)** *RNA from FFPE-tissue and liquid biopsies for clinical decision support- challenges and solutions*	9:30 – 10:00
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Short Talk 1	**Lucia Juhásiková** *Exploring Immunotherapy in Pancreatic Ductal Adenocarcinoma: Insights from Organotypic Slice Cultures*	10:30 – 10:45
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Coffee Break and Poster session		**11:00 – 11:30**
WG2 & WG4 meetings		**11:30 – 12:30**
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Invited Talk 1	**Gunes Esendagli** *Migration and tumor-infiltration capacities of adoptive T cells: of mice and microchannels*	12:30– 13:00
Invited Talk 2	**Nataliia Beztsinna** *Patient-Derived Organoids and Fresh Ex Vivo Tissue Cultures as Translational Models for Immuno-Oncology Drug Testing*	13:00 – 13:30
Closing Remarks		**13:30 – 14:00**
Farewell Lunch		**14:00 – 15:00**

CONFERENCE VENUE

The **venue of the Conference** is the:
Centrum Nauk Biologiczno-Chemicznych, Uniwersytetu Warszawskiego
ul. Żwirki i Wigury 101, 02-089 Warszawa
Aula C

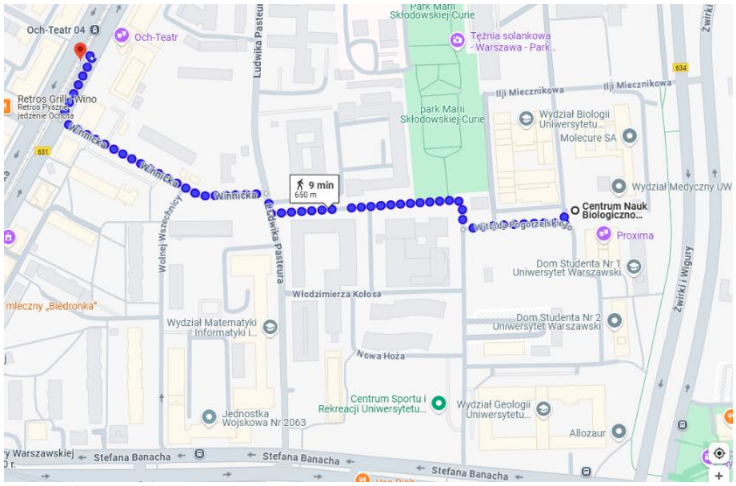
Google Maps link: https://g.co/kgs/ZJGd3Zx

- **Coming by public transport**

To find your convenient connection from anywhere in Warsaw use: http://warszawa.jakdojade.pl

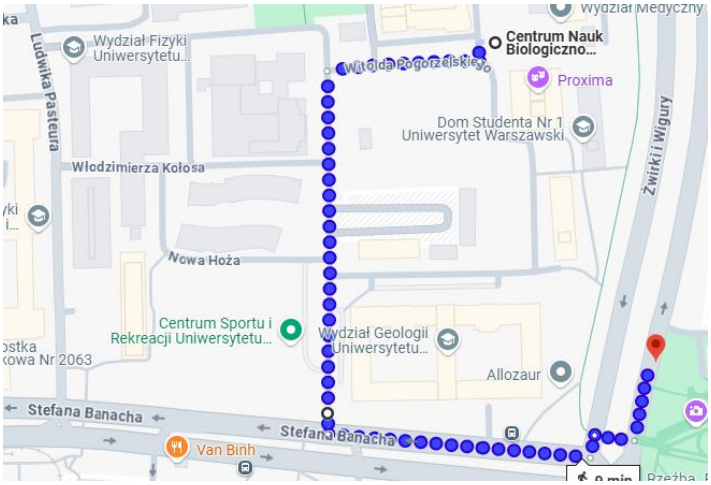
- ***from the Central Station:***

The most convenient way to get to the Conference Venue from the Central Station is by taking tram no 9. You leave at the “Och-teatr” stop, which is ca. 500 m from the conference venue (along Winnicka Street), then turn right and immediately left into a small pedestrian street.



- ***from the Warsaw Chopin Airport:***

Coming directly from the airport, you should take the bus no. 175 or 188 and leave at the "Banacha Szpital" stop. Go along Banacha str. and after ca. 200 m turn into no name street closed for cars with a barrier (first left).



- **Coming by taxi:**

It is, of course, possible to take a taxi waiting outside the arrival hall at the airport (ELETAXI: 0048 22 8111111, MPT: 0048 19191). The trip to the CNBCh or city center should cost around 40-50 Polish złotych (≈10 Euro) and take about 15-30 min, depending on the traffic. You can pay with a credit card in most taxis.

Social event:

On May 13th, for those previously registered, the dinner will take place at the restaurant "**Warszawski Sen**"
Google Maps link: https://g.co/kgs/heLAtGi

The conference venue provides internet access for all attendees.

Network: **CNBC-PUB**

Password: **CNBC_ZwirkiWigury_101**

Network: **AULA_C**

Password: **aula_c_2016**

Session 1 – *In vitro* models (part I)

Immuno-Epithelioids: *In Vitro* Preclinical Modelling of Immune-Epithelial Crosstalk During Treatments

Authors and Affiliations

Alberto Pradilla Dieste[1], Philippa Samella[1], Kasandra Malasi[1], Michelle Miniter[1], Inês Ferreira[1], Jose Antonio Valverde-Lopez[1], Carmen Sancho Serra[2], Roser Vento-Tormo[2], Kourosh Saeb.Parsy[3], Krishnaa T. Mahbubani[3], David Fernandez-Antoran[1,4]

1. The Gurdon Institute, University of Cambridge, Cambridge, UK
2. The Wellcome Sanger Institute, Hixton, UK
3. Cambridge Biorepository for Translational Medicine (CBTM) – NHS, Cambridge, UK
4. ARAID Foundation, IIS-Aragon, Zaragoza, Spain

Abstract

To study the mechanisms that underlie disease processes in humans, the community largely relies on *ex vivo* approaches. However, modelling tissue behaviour *in vitro* represents a major challenge. Most approaches fail to recapitulate *in vivo* features, and results are challenging to extrapolate to the clinic. Developments in complex 3D models, such as organotypic cultures and organoids, partially mimic tissue architecture and microenvironment, but they lack the self-maintaining capacity of adult tissues, limiting their potential as a platform to study biological processes over the long-term. To realise the translational potential of *ex vivo* methods, there is a pressing need to develop models that recapitulate the complexity of human tissue.

We have recently developed an innovative *in vitro* system (Epithelioids) that supports the long-term maintenance of 3D epithelial cultures derived from both mouse and human tissues and recapitulate the structural organisation, cellular heterogeneity, and dynamic behaviour of *in vivo* tissue, providing a robust and versatile platform to study human biology.

We have further refined this technology to accurately recapitulate the tissue microenvironment, enabling us to model not only tumour-normal interactions but also the original immune infiltration. This advancement establishes Epithelioids as a powerful *in vitro* platform for studying chemo, radio, and immunotherapies, providing a valuable tool for benchmarking diagnostic and therapeutic strategies. By integrating functionalised and engineered scaffolds, immune-Epithelioid closely mimic the *in vivo* tissue microenvironment, significantly enhancing our capacity to investigate immune responses and the efficacy of immunotherapies *in vitro*.

This work was supported by grants from the CRUK-RadNet 28870, NC3Rs NC/X000885/1, Newton Trust RG8930 and Wellcome 106149.

Glycoengineered Cells and GlycoAvatars: Unraveling the Glycocalyx and Cancer-Immune Cell Interactome for New Therapeutic Strategies

Authors and Affiliations

José Alexandre Ferreira[1,2,3]

1. Experimental Pathology and Therapeutics Group, Research Center of IPO-Porto (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC Raquel Seruca), Porto, Portugal
2. School of Medicine and Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal
3. GlycoMatters Biotech, Espinho, Portugal

Abstract

The cell surface glycocalyx undergoes dynamic changes in cancer, giving rise to aberrant, immature glycosylation patterns that promote immune evasion and tumor progression. Our research integrates glycoengineering technologies with glycomics and glycoproteomics to decode and therapeutically target the cancer glycocode. Using CRISPR/Cas9-edited human and murine cancer cell lines, we model immature glycosylation signatures commonly found in solid tumors. These engineered systems enable systematic investigation into how aberrant glycosylation shapes key cancer hallmarks, including invasion, metastasis, and immune suppression, with a central focus on its impact on antigen-presenting cells and T lymphocyte engagement. To further dissect these interactions, we have developed GlycoAvatars, a platform of magnetic beads coated with plasma membrane glycoproteins from glycoengineered cancer cells. These bead-based mimetics replicate tumor-specific glycosylation and serve as a high-throughput tool for mapping the cancer–immune cell interactome. Using this system, we have identified membrane-bound and intracellular proteins involved in glycan-mediated immune signaling, revealing novel therapeutic nodes, several of which have demonstrated efficacy in preclinical models. We are now translating these insights into first-in-class cancer vaccines designed to induce durable anti-tumor immunity by targeting tumor-associated glycoepitopes. Collectively, these advances establish a translational framework linking glycobiology to immune-based therapies, paving the way for novel strategies in precision oncology and next-generation cancer immunotherapy.

This work was supported by Portuguese Foundation for Science and Technology (FCT) Principal Researcher contract 2022.08311.CEECIND and project RESOLVE (DOI: 10.54499/PTDC/MED-OUT/2512/2021). FCT is co-financed by the European Social Fund under the Human Potential Operation Programme from the National Strategic Reference Framework.

3D Technology for Investigating Cancer-Immune System Crosstalk and Drug Testing

Authors and Affiliations

Stefania Parlato[1], Giulia Silvani[2], Arianna Mencattini[3], Adele De Ninno[4], Caterina Lapenta[1], Alessandra Fragale[1], Laura Lattanzi[1], Giuseppina D'Agostino[5], Giulia Romagnoli[1], Simona Donati[1], Stefania Rossi[1], Irene Canini[1], Maria Rosaria Venturino[1], Farnaz Dabbagh Moghaddam[4], Davide Caprini[4], Susanna Falcucci[6], Maria Vincenza Chiantore[6], Paola Di Bonito[6], Annamaria Gerardino[4], Eugenio Martinelli[3], Carlo Massimo Casciola[2], Luca Businaro[4], Lucia Gabriele[1]

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Abstract

The tumor microenvironment (TME) is a dynamic ecosystem where immune and cancer cells interact, influencing tumor growth and immune evasion. Effective immunotherapies must modulate the immune cells within the TME to boost the body's antitumor response. However, the TME heterogeneity and adaptability, along with its immunosuppressive nature, pose significant challenges in developing universally effective treatments. To address these challenges, experimental models that mimic the heterogeneity and dynamism of human tumors are crucial. 3D culture systems, including spheroids, organoids, and microfluidic models, provide valuable platforms for studying the TME complexity, enabling the tracking of cancer-immune interactions, tumor progression, and immune responses over time. Tumor-on-chip models, in particular, represent an innovative approach to recreate the real-world clinical setting, providing a robust tool for testing new therapies and advancing our understanding of tumor immunology. We created innovative Immune_System-Tumor-on-a-chip devices that closely replicate the human TME. One such platform includes two cancer chambers and a central immune chamber, enabling a competition assay to study the preferential migration of immune populations, such as dendritic cells (DCs), toward drug-treated cancer cells over untreated ones. To reproduce the TME complexity, 3D tumor spheroids were loaded into the chip to follow their interactions with immune cells or their morphological alterations in response to anti-cancer drug treatments. The second model features a microvascular structure with a lumen lined by endothelial cells, creating a physiologically relevant tumor model. It enables the study of DC migration through the endothelial barrier to interact with melanoma cells, simulating immune response to tumor growth.

In conclusion, these on-chip platforms allowed to reveal that the immunosuppressive nature of the TME presents a significant challenge for effective DCs migration and initiating robust anti-tumor immunity. The combination of romidepsin, an epigenetic agent, with type I interferon (IFN), which is known to enhance DC functionality and counteract immune suppression, offers a promising therapeutic strategy. By improving DC recruitment to the TME and promoting better tumor-DC interactions, this

combination has the potential to overcome the immunosuppressive barriers within the TME and significantly enhance the anti-tumor immune response. This approach could represent a critical step forward in cancer immunotherapy, fostering more effective immune-mediated tumor elimination.

Session 1 – *In vitro* models (part II)

On the Way to Cellular Therapy of Non-Small Cell Lung Cancer – Putting the Puzzles Together

Authors and Affiliations

Ines Papak[1&], Martyna Muszczek[1&], Artur Piróg[1], Jakub Faktor[1], Anna Biernacka[1], Zuzanna Urban-Wójciuk[1], Magdalena Szczepanowska[2], Magdalena Sitkiewicz[2], Witold Rzyman[2], Sachin Kote[1], Natalia Marek-Trzonkowska[1,3]

1. International Centre for Cancer Vaccine Science, University of Gdansk, Kładki 24, 80-822, Gdańsk, Poland
2. Department and Clinic of Thoracic Surgery, Medical University of Gdańsk, ul. Mariana Smoluchowskiego 17, 80-214 Gdańsk, Poland
3. Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdańsk, Dębinki 2, 80-210, Gdańsk, Poland

& - equal contribution

Abstract

Cancer is characterized by genome instability and high diversity even in patients with the particular type of cancer. Therefore, there is need for novel and individualized anti-cancer strategies. Our team have been working on immune therapy of non-small cell lung cancer (NSCLC). NSCLC accounts for 85% of all lung cancer cases and is the leading cause of death among both men and women with malignancies. NSCLC is also characterized by high tumour mutational burden (TMB), as this type of cancer develops mostly in smokers and past smokers due to mutagenic impact of tobacco smoke. Therefore, we assumed NSCLC to be a perfect target for immunotherapy.

To address these issues we aimed to explore how various immune cell populations recognize and respond to diverse cancer cells. Simultaneously, we aimed to test how *in vitro* and *in vivo* models affect repertoire of peptides presented by MHC class I molecules of NSCLC.

We have established 2D and 3D cell cultures of NSCLC obtained from clinical samples. Simultaneously, the same cancer samples have been implanted into NSG mice to establish patient derived xenograft models (PDX). In parallel PBMC (peripheral blood mononuclear cells) and TILs (tumour infiltrating lymphocytes) have been phenotyped, expanded and tested in functional assays. Each time freshly retrieved cancer and healthy lung samples have been also processed and analysed with mass spectrometry for identification and quantification of peptides presented by MHC class I molecules (immunopeptidome). The same procedure has been repeated after cancer expansion *in vitro* and *in vivo* and the repertoire of presented peptides has been compared.

We have observed that cancer cells derived from the same patient but expanded in different model differed significantly in terms of repertoire of MHC class I peptides. Nevertheless, we have identified several cancer unique peptides which were shared by the primary tumours and the models.

These peptides seems to be promising candidates for development of antigen specific T cells which will serve as a tool against lung cancer.

In addition we observed heterogeneity including stage of differentiation of the cells present within NSCLC tumours. These differences were associated with various immune response against these subsets, including complete lack of recognition of low-differentiated cancer cells by T cells.

In summary, our observations underline the importance of understanding the diversity of populations comprising the cancer microenvironment, as well as the impact of a cancer model chosen for studying immune responses against cancer. *In vitro* and *in vivo* testing of anti-cancer therapies is a standard pathway towards the clinical trial. Nevertheless, without understanding how these models affect the cancer biology and immunogenicity we have low chances to defeat cancer. Our observations suggest that one type of immune cell can not be an efficient weapon against all subsets of cancer cells. However, purposeful selection of target neoantigens is probably the future of immunotherapy.

This work was supported by the following projects:
1. "International Centre for Cancer Vaccine Science" carried out within the International Research Agendas Program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund
2. "Science for Welfare, Innovations and Forceful Therapies (SWIFT)", project no. FENG.02.01-IP.05-0031/23; funded by European Funds for Smart Economy 2021-2027 (FENG) Priority FENG.02 Innovation-friendly environment, Measure FENG.02.01 International Research Agendas
3. "Enhancing Cancer Vaccine Science for New Therapy Pathways (CANVAS)" funded by the European Commission, within Horizon Europe Twinning program; HORIZON-WIDERA-2021-ACCESS-03, grant agreement no. 101079510

Dynamic Vascularized Organ-On-Chip Models for Studying Immunotherapies and Tumor Immune Interactions

Authors and Affiliations

Thomas Sommermann[1], Anne-Kathrin Bothe[1], Joanka Nowacka[1], Knut Rennert[1], Martin Raasch[1]

1. Dynamic42 GmbH, Jena, Germany

Abstract

Dynamic vascularized organ-on-chip models provide an innovative platform for studying immunotherapies and tumor-immune interactions. These microfluidic systems integrate perfused vascular networks with tumor microenvironments (TME), enabling detailed exploration of immune cell dynamics, including tumor infiltration, immune cell polarization, and the efficacy of immune therapeutics. Such models have the potential to complement, and even surpass, traditional animal experiments.

In this talk, we will discuss the general setup of cancer-on-chip systems, highlighting a Pancreatic Ductal Adenocarcinoma (PDAC)-on-chip platform developed to investigate dynamic drug administration, immune infiltration, and interactions within the TME. Key challenges and opportunities in replicating a realistic TME and employing diverse readout strategies will be addressed. Additionally, we will showcase the capabilities of organ-on-chip models in studying immunotherapies, exemplified by the T cell modulatory CD28-antibody TGN1412. Mouse models for the study of cancer immunology provide excellent systems in which to test biological mechanisms of the immune response.

SELECTED ORAL PRESENTATION

Evaluation of Cathepsin V Inhibition on Glioblastoma Stem Cell Proliferation and NK Cell Cytotoxicity From 2D to 3D Perfused Models On Chip

Authors and Affiliations

Emanuela Senjor[1], Ana Mitrović[1,2], Marko Jukić[2], Matic Proj[2], Stanislav Gobec[2], Barbara Breznik[3], Janko Kos[1,2], Milica Perišić Nanut[1]

1. Department of Biotechnology, Jozef Stefan Institute, Ljubljana, Slovenia
2. University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia
3. National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana Slovenia

Abstract

Glioblastoma is one of the most aggressive brain tumors, characterized by cellular heterogeneity, invasive and therapy-resistant tumor stem cells, and an immunosuppressive microenvironment. Since current therapy, including surgery, radio- and chemotherapy, is not effective, new therapeutic approaches, including immunotherapy, are being rapidly explored. Natural killer (NK) cells are crucial for immune control of cancer, as they trigger apoptosis of target cells via the perforin-granzyme pathway without prior activation. This pathway is regulated by cathepsins (C,H, L), whose function is inhibited by cystatin F, an endogenous cathepsin inhibitor. Elevated cystatin F expression in glioblastoma is linked to poorer prognosis. Its activation from inactive dimer to active monomer occurs via cathepsin V, which is often overexpressed in cancer. Cathepsin V has elastolytic activity, is associated with cellular proliferation, reduced expression of adhesion molecules, infiltration of tumor-associated macrophages, contributing to poorer prognosis in glioblastoma patients. Targeted inhibition of cathepsin V could reduce tumor cell proliferation and enhance NK cell cytotoxicity. We developed a selective, reversible and non-covalent cathepsin V inhibitor (compound 7) through virtual chemical library screening and tested it in standard 2D and advanced 3D cellular models.

We demonstrated that compound 7 effectively inhibits the proliferation of glioblastoma stem cells (NCH-421k) using flow cytometry, under standard culture conditions. Additionally, compound 7 inhibited the growth of spheroids derived from NCH-421k cells under static conditions as well as when the spheroids were cultured in perfused microfluidic chips. We examined the effect of compound 7 on the activation of cystatin F in NK cells isolated from healthy donors. NK cells that were exposed to compound 7 (20 μM, 18 hours) had less active monomeric cystatin F. Consequently, they exhibited improved cytotoxic activity against individual NCH-421k cells in a cytotoxicity assay. We also tested the effect of compound 7 on the cytotoxic activity of NK cells in 3D conditions, where we similarly confirmed that NK cells treated with compound 7 had improved cytotoxic activity against tumor spheroids compared to control NK cells both in static and perfused condition.

Targeting cathepsin V represents a promising strategy for addressing proliferation of tumor cells and enhancing the cytotoxic potential of NK cells by reducing the immunosuppressive effect of cystatin F.

This work was supported by grants Z3-50102, J3-2516 and P4-0127 awarded by Slovenian Research and Innovation Agency.

SELECTED ORAL PRESENTATION

Effect of EVs Containing Different Alleles of MICA on NKG2D Modulation and Lytic Granule Mobilization in Natural Killer Cells

Authors and Affiliations

Silvia López-Borrego[1], Karen Toledo-Stuardo[2], Luis A Pedroza[3], Frederique van-den-Haak[3], Yu Li[3], MC Molina[2], Jordan S Orange[3], Mar Valés-Gómez[1]

1. Department of Immunology and Oncology, National Centre for Biotechnology (CNB), Spanish National Research Council (CSIC), Madrid, Spain
2. Faculty of Medicine, Immunology Program, Institute of Biomedical Sciences (ICBM), Universidad de Chile, Santiago, Chile
3. Department of Pediatrics, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY, United States

Abstract

The activating immune receptor NKG2D is expressed at the surface of human αβ CD8+, γδ T cells and NK cells, and plays and important role in the recognition of tumours and pathogen-infected cells. NKG2D-ligands (NKG2D-L), MICA/B and ULBPs, are usually expressed in transformed or infected cells. Release of NKG2D-L by metalloprotease cleavage or in extracellular vesicles (EVs) represents an immune evasion mechanism related to cancer progression. Comparison of MICA*008 and MICA*019 alleles previously demonstrated that membrane attachment via a GPI or transmembrane domain resulted in preferential recruitment to EVs or metalloprotease susceptibility, respectively. Here, we have studied the biology of several MICA alleles (MICA*002, MICA*009, MICA*011) and demonstrated that transmembrane variants can also be incorporated in EVs. When comparing the effects of cell-surface or EV-bound MICA on NK cells after NKG2D interaction, we observed differential NKG2D receptor downmodulation based on the MICA allele and its presentation form. Moreover, both cell-surface and EV-associated MICA were transferred and internalized by NK cells, but with different kinetics. Analysis of NK cell immune synapse by confocal microscopy shows lytic granule convergence in NK cells confronted with MICA-transfectant cells, which disappears in NKG2D-KO NK cells. Lytic granules did not converge when NK cells were incubated with MICA-EVs in suspension, although they did when MICA-EVs were attached to the bottom of the chamberslide. Interestingly, lytic granule convergence was induced in NK cells when confronted with MICA-coated beads (diameter of 4.5 µm) in suspension, indicating that MICA crosslinking may be critical for NKG2D signaling. These results suggest that the way MICA is presented has remarkable consequences in immune regulation. As many therapeutic strategies consider interfering with the NKG2D system, it is important to understand the immune effects of different presentations of the same ligand on cancer elimination.

This work was supported by grants PID2021-123795OB-I00 and FPU20/07300.

Session 2 – *In vivo* models (part I)

Dissecting the Immune Microenvironment of Experimental Brain Tumors with Single-Cell and Spatial Transcriptomics

Authors and Affiliations

Bozena Kaminska[1]

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Abstract

The tumor microenvironment (TME) plays important role in tumor endurance and response to therapies. TME of malignant brain tumors (gliomas) is characterized by a large diversity of different cell types, including endothelial cells, neurons, astrocytes, and a variety of immune cells such as microglia, tumor-associated macrophages, and tumor-infiltrating lymphocytes and various non-cellular components. Advancements of single-cell techniques provide powerful means to systemically profile the multiple-omic status of the TME at a single-cell resolution, revealing the phenotypes and functionalities of disease-specific cell populations. There is large heterogeneity in the immune landscape in human tumors with different genetic alterations. IDH1 mutations are among the initial events in tumorigenesis and mutant IDH1 enzymes via the production of the oncometabolite 2-hydroxyglatarate (2-HG) exert pronounced effects on immune composition of TME. We aimed to dissect immune cell heterogeneity in experimental gliomas having various genetic alterations with the mutated NRAS or overexpression of PDGFB and different IDH1 status (wild type of mutated). We immunosorted CD45+ cells from sham operated or tumor bearing mice, and used Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) to identify cells and cellular states. Computational analysis revealed the presence of 34 cell clusters among immune CD45+ cells in tumors. Contribution of specific cells and states was distinct in tumors with distinct genetic alterations. Comparison of many marker genes demonstrated that IDH1 mutant gliomas have less cytotoxic T lymphocytes than wild type gliomas, and we noticed the inhibition of cell cycle processes in T cells suggesting deeper immunosuppression in IDH1 mutant gliomas. The observed differences were validated by flow cytometry and immunocytochemistry. We conclude that TME of IDH1 mutant gliomas is highly immunosuppressive and devoid of T effector cells that would limit responses of those tumor to immunotherapy. The experimental tumor models reveal underlying mechanisms of immune dysregulation and define new targets for immunomodulators to test new therapies.

Studies were supported by the funding from the Polish National Science Center 2020/39/B/NZ4/02683 (BK).

3D Culture Strategies to Model Cancer Microenvironment Dynamics and Therapeutic Responses

Authors and Affiliations

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Abstract

Targeted, combinatorial, and immunomodulatory therapies, such as antibody-drug conjugates (ADCs) and immunomodulatory antibodies (Abs), are powerful weapons against tumor cells and immune cells within the tumor microenvironment (TME). Therefore, it is critical that the evaluation of such therapies is conducted in pre-and co-clinical models recapitulating the complex cellular and molecular crosstalk of the TME while ensuring robustness and scalability. The difficulties in recapitulating these heterogeneous microenvironments with human cell models, with mature cell functionality and without the confounding effects of heterologous extracellular matrices, are a bottleneck in deciphering the crosstalk mechanisms and predicting their impact on therapeutic efficacy.

To overcome these limitations, we have been developing 3D cell models of disease, applying advanced cell culture approaches (3D culture, co-culture, cell immobilization) and systems (perfusion, bioreactors) to human pluripotent stem cells, other patient-derived cells, and human cell lines. By integrating cell biology, biochemical, imaging, and omics approaches, we can depict the dynamic modulation of a specific cell microenvironment over time or in response to a therapeutic challenge.

To build in critical hallmarks of the TME, we devised a solid tumor heterotypic 3D cell culture platform (3D-3), exploring microencapsulation within an inert biomaterial (alginate) and stirred-tank culture systems. TME cellular components, e.g., fibroblasts and immune cells were added to tumor cell line-derived tumor spheroids. This strategy built up specific disease microenvironments, with the secretion and accumulation of cytokines and retention of extracellular matrix components that fostered immunomodulatory interactions of cancer-associated fibroblasts and tumor-associated macrophages. As a proof-of-concept, we employed the developed 3D-3 TME cell models to address the potency and specificity of ADCs and immunomodulatory mAbs. We explored distinct models with incremental cellular composition and a panel of molecular and cellular readouts to pinpoint the mechanism of action of each therapeutic modality.

Patient-Derived Tumor Models for Functional Precision Oncology and Cancer Research

Authors and Affiliations

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Abstract

Functional Precision Oncology (FPO) is a transformative approach in cancer research, addressing critical gaps left by traditional genomics-driven methods. While genomic profiling identifies mutations, it often fails to predict drug responses due to tumor heterogeneity, epigenetic background, and dynamic resistance mechanisms. The FPO approach directly tests patient-derived (PD) tumor models, i.e., organoids, tumoroids, PD xenografts (PDXs) against therapies, capturing functional vulnerabilities overlooked by standard genetic testing. At MSCI, we have been developing and advancing (humanization, genomic and transcriptomic characterization) these models for more than a decade, establishing a first-in-Poland living biobank of PDXes and oncological preclinical platform to provide access for academic and industry partners, and in future, tailored treatment strategies to patients. Our FPO approach with ground-state stem cell tumoroid cultures combined with high-content screening microscopy (HCS) enables scalable ex vivo drug sensitivity testing with a short turnaround time to guide therapeutic interventions. However, widespread adoption of FPO requires addressing scalability, cost, and standardization through prospective clinical trials to fully realize their potential in transforming cancer care.

Session 2 – *In vivo* models (part II)

SELECTED ORAL PRESENTATION

Predicting Immunotherapy Responses in Glioblastoma: An Integrated Evaluation of EVOV Vaccine-like Particles in Preclinical Models

Authors and Affiliations

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Abstract

Glioblastoma remains one of the most challenging cancers to treat due to its aggressive behavior and immunosuppressive microenvironment, thus underscoring an urgent need for effective personalized immunotherapy. This study evaluated the prognosis and immune activation induced by vaccine-like particles—oHSV (oncolytic Herpes virus 1)-infected glioma stem cell (GSC)-derived extracellular vesicles (EVOV) - compared to control EVs. The goal was to validate integrated preclinical *ex vivo* and *in vivo* immuno-oncology models capable of accurately predicting personalized cancer immunotherapy responses, ultimately classifying the therapeutic potential of preclinical approaches. A tiered strategy using an array of preclinical assays was used to associate the immune cells' phenotype with the antitumor effect. An *ex vivo* monoculture approach, priming PBMCs with vaccine-like particles, was used to evaluate their impact on immune cell subpopulation activity. The subsequent ex vivo 3D organoid co-culturing assay of particle-primed PBMCs and GSCs assessed the interaction between immune and cancer cells. The *in vivo* phase used humanized NSG mice with PBMCs and vaccine-like particles administered systemically, allowing observation of immune cell dynamics in response to the intracranially implanted GSC tumors. EVOV particle treatment elicited robust immune activation across all models. In *ex vivo* monocultures, flow cytometry 17-marker platform revealed significant activation of NK, dendritic, and CD4+ as well as CD8+ T cells. In 3D organoids, increased activation of NKT and CD8+ T cells, along with enhanced monocyte-to-dendritic cell differentiation, correlated with elevated GSC death. RarecyteTM 15 marker co-expression imaging demonstrated substantial infiltration of granzyme B- and TBX21-expressing CD8+ T-cell alongside a reduced population of PD1+ inhibitory cells. Single-cell RNA sequencing-based assignment of immune scores further delineated immune-responsive GSC subpopulations. In the *in vivo* model, EVOV treatment reduced exhausted PD1+CD8+ T cells and increased activated helper T cells in peripheral blood analyzed by flow cytometry, while 3D Genomics Vision HD confirmed significant immune infiltration at the tumor site with a notable decrease in PD1+ inhibitory cells. These integrated *ex vivo* and *in vivo* approaches demonstrated that EVOV vaccine-like particle treatment significantly enhanced immune activation and antitumor cytotoxicity, effectively predicting personalized immunotherapy responses in preclinical glioblastoma models. These findings thus support the prognostic utility in

predicting personalized responses to immunotherapeutic interventions for glioblastoma of *ex vivo* organoids preclinical models in lieu of *in vivo* models.

This work was supported by NSC Poland grants: 2021/41/N/NZ6/02682, 2022/45/B/NZ5/04226, 2020/39/B/NZ5/02893, 2021/41/B/NZ6/02436.

SELECTED ORAL PRESENTATION

Determinants of Immune Infiltration in Triple-Negative Breast Cancer Identified by Multi-Omic Integration of Large Clinical Cohorts

Authors and Affiliations

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Abstract

Tumour immune infiltration (TII) has been demonstrated to be associated with better prognosis and better response to chemotherapy in triple-negative breast cancer (TNBC). The relatively high levels of TII have generated a strong rationale for implementation of immunotherapy in this breast cancer subtype. However, the mechanisms driving TII remain poorly understood.

Here we aimed to identify factors associated with TII and potentially distinguishing immune “cold” from “hot” tumours by extracting from multi-omic data a set of high-level features defining the molecular portrait of the tumour and its microenvironment (TME), particularly the immune compartment.

Genomic and transcriptomic data from TNBC TCGA (The Cancer Genome Atlas Program, n=209) and FUSCC (Fudan University Shanghai Cancer Center, n=462) datasets were used. From transcriptomic data, single sample scores were computed using singscore for the Cancer Hallmark and the ConsensusTME genesets. Expression of selected genes was evaluated and an expression-based Homologous Recombination Deficiency score (eHRD) was computed. From copy number and mutational data, we computed: tumour mutational burden (TMB), MATH heterogeneity score, ploidy, HRD, mutational status of selected genes and 10 cancer driver pathways. Presence of copy number (CN), single nucleotide substitution (SBS), insertion/deletion (ID) mutational signatures was quantified using SigProfilerAssignment. Association of immune-related features with all the other features was evaluated by Spearman correlation for continuous features or Wilcoxon test for categorical features. Results from the two datasets were integrated, overall p-values computed and adjusted for multiple testing. Associations with adjusted p-value <0.001 were considered significant.

Features quantifying immune cell types were highly correlated one another ($\rho>0.87$), indicating their concomitant presence in the tumour, although with quantitative variability. Activation of glycolysis, NOTCH, WNT and Hedgehog signalling associated with low TII ($-0.71>\rho>-0.32$). Contrary to other cancer types, high TMB did not induce TII. HRD scores did not associate with TII, while a weak negative correlation was found with eHRD ($-0.145>\rho>-0.39$). Signature SBS13 (APOBEC) and CN21 (of unknown aetiology) showed weak positive and negative association with TII, respectively. Genomic alterations in cancer genes and cancer pathways associated with TII in the TCGA but in most cases were not confirmed in FUSCC.

Our analysis unveiled significant associations between TII and either tumour or TME features, suggesting possible strategies to promote TII. However, the contribution of each feature is relatively small, suggesting a complex picture warranting further exploration.

SELECTED ORAL PRESENTATION

Leveraging Public Pan-Cancer Transcriptomic Data for Immuno-oncology: Systematic Collection and a Graphical Tool for Analysis

Authors and Affiliations

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Abstract

Development of omics technologies has generated a growing amount of publicly available molecular profiles. Such goldmine could be interrogated to generate or validate hypotheses on new biomarkers or therapeutic targets. However, access and analysis of such datasets, in particular for non-computational experts, could be challenging. Hence, the goals of this work were to: i) implement a Systematic Search Strategy to collect publicly available transcriptomics datasets from immuno-oncology related experiments, ii) facilitate access to such resource by developing a graphical interface for the analysis.

The project was divided into a pilot and main phase. The first focused on setting the basis of the Systematic Search Strategy, i) collecting information to define the search boundaries, ii) developing a code to interrogate the data repositories and collect the query results and iii) determining the criteria for manual curation and dataset selection by expert researchers. In the main phase, queries were run on the two selected repositories (Gene Expression Omnibus and ArrayExpress) and the query results are being evaluated by the members of the established taskforce to identify datasets matching the selection criteria. To allow the graphical-based data mining and multiple unsupervised and supervised analyses, a Shiny app is under development.

The pilot phase highlighted that a manageable number of candidate datasets per cancer type could be found, without needing to narrow down the search with specific keywords. Evaluation by multiple researchers of the same query results emphasized the need for clear selection criteria to reduce discrepancies. Such criteria could be summarized in: bulk or single-cell transcriptomic datasets from pre-clinical or clinical samples receiving immunotherapy, alone or in combination with other treatments.

In the main phase, queries were run for over 20 distinct cancer types. Candidate datasets collected with each query are being evaluated by at least two expert researchers. Statistics to thoroughly describe the collected datasets will be computed and presented at the meeting. The collected datasets will be made available for analysis through an online open-access and user-friendly graphical tool. Examples of the tool capabilities will be presented.

This project is contributing to create knowledge-sharing channels that could foster the connection between basic, translational, and clinical investigators, supporting the development, optimization and use of preclinical models for immunotherapy research.

This work was supported by Immuno-model COST Action VMG, in the extended call from September 23rd to October 21st, 2024.

Session 3 – *In silico* modeling

In Silico Simulations of Cancer-Immune Interactions to Aid Clinical Trial Design and Execution

Authors and Affiliations

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Abstract

Simulations are key tools for training and planning purposes in many high-stakes areas, such as aviation, civil engineering, and the military. Although the stakes of many clinical trials are similarly high, researchers largely rely on classic statistical methods to design their trials and conduct their power analyses. I will make the case that mechanistic simulations are powerful and underutilized tools in the area of trial design. I will illustrate this approach by presenting a mechanistic model of tumor development and immune response and illustrate its application to the design of a biomarker discovery study, a power analysis for a cancer immunotherapy trial, and as an element of a teaching activity for undergraduate students. Since there is a rich foundation of established mathematical models for various cancer types and treatments, I hope that such applications can leverage this foundation for the benefit of both patients and researchers.

This work was supported by a Young Investigator Grant (10620) from the Dutch Cancer Society and by NWO grant VI.Vidi.192.084.

RNA from FFPE-Tissue and Liquid Biopsies for Clinical Decision Support – Challenges and Solutions

Authors and Affiliations

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Abstract

While DNA is pivotal for molecular diagnostics and precision medicine in cancer quantitative RNA data are still playing a minor role in the field, despite decencies of research and millions of transcriptome datasets. Recent developments and insights into the tumor microenvironment and the complex interplay between the immune system and tumors however demonstrate that quantitative RNA data is becoming increasingly important for therapeutic decision support. However, robust and reliable gene expression analyses from FFPE are required for standardized and reliably diagnostics. We developed MACE-Seq, a sensitive 3' mRNA Sequencing method for the analyses of FFPE-derived RNA and demonstrate its versatility for tumor RNA-analyses in combination with a robust scoring method, making clinically relevant RNA-information more accessible.

Potential Utility of Induced Translocation of Engineered Bacteria as a Therapeutic Agent for Mounting a Personalized Neoantigen-Based Tumor Immune Response

Authors and Affiliations

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Abstract

The success of immune checkpoint inhibitors (ICIs) in cancer immunotherapy has emphasized the critical role of neoantigens in eliciting tumor-specific immune responses. However, many breast tumors, especially luminal and triple-negative subtypes, exhibit low tumor mutational burden (TMB) and limited neoantigen expression, reducing their responsiveness to current immunotherapeutic strategies.

The NEOANTITUM project explores a novel therapeutic approach using genetically modified bacteria as vehicles for the systemic presentation of tumor-specific neoantigens. By leveraging the natural phenomenon of intestinal bacterial translocation, induced via dextran sulfate sodium (DSS), we aim to deliver engineered bacteria carrying DNA encoding tumor neoantigens into the systemic circulation. This potentially promotes interaction with antigen-presenting cells and triggers a robust and specific anti-tumor immune response.

The study employs syngeneic orthotopic murine models of breast cancer (EO771 and 4T1 lines) characterized by known mutations and neoantigens. Bacterial vectors were designed to express selected neoantigens in either the periplasm or surface, followed by oral inoculation in mice pretreated with DSS. Preliminary analyses using Red Fluorescent Protein (RFP) confirm successful translocation and expression, and ongoing histological and immunological evaluations aim to determine therapeutic efficacy.

This strategy represents an innovative and safer alternative to intravenous bacterial vaccination, enabling localized, controlled immune activation through the digestive route. Our findings provide a proof of concept for neoantigen-based bacterial immunotherapy in low-TMB tumors and offer promising avenues for personalized cancer treatment.

This work was supported by grant INV/23/20/CON by CAPREOLUS INVESTIGACIONES, A.I.E. and its participants, under the call "Financiación estructurada de proyectos de I+D por Agrupaciones de Interés Económico (A.I.E.)", in collaboration with INVENTIUM.

SELECTED ORAL PRESENTATION

Exploring Immunotherapy in Pancreatic Ductal Adenocarcinoma: Insights from Organotypic Slice Cultures

Authors and Affiliations

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive pancreatic cancer with a five-year survival rate of just 3% in advanced-stage patients. The tumour's dense stroma, a part of the tumour microenvironment (TME), plays a key role in tumour progression and resistance to treatment. Therefore, epigenetic drugs could be promising agents for sensitizing TME towards other therapies, such as immunotherapy. Organotypic slice cultures (OTSCs) retain cellular diversity, offering a more accurate model for evaluating immunotherapy. In this study, we developed OTSCs from KPC cell line-derived tumour xenografts and primary PDAC to assess the effectiveness of the epigenetic drug decitabine, anti-hPD1-Pem-hIgG1 and their combination.

C57BL/6 mice were orthotopically implanted into the pancreas with 100,000 KPC cells to derive tumour xenografts. OTSCs were generated from these tissues and a freshly resected human tumour from a 79-year-old patient. Tumour specimens were sectioned using a Compresstome® VF-510-0Z. Cultures were incubated at 37°C for 5 days and maintained in RPMI 1640 medium. OTSCs derived from mice were treated with monotherapy of decitabine, anti-PD-1 neutralizing antibody, and their combination. Patient OTSCs were treated with monotherapies of decitabine and anti-hPD1-Pem-hIgG1. After treatment, OTSCs were fixed in formalin and processed for hematoxylin and eosin (H&E) staining together with IHC using antibodies against cleaved caspase 3 (CC3), Ki67, granzyme B, CD4, and CD8.

Throughout the cultivation process, all OTSCs maintained their viability and structure. H&E staining was performed to verify tissue preservation, while CC3 staining demonstrated that apoptosis was minimal. Additionally, the analysis of immune markers showed the presence of immune cells within the tissue slices. These markers highlighted the infiltration of immune cells, offering valuable insight into the immune response within the tissue and its potential role in therapy response.

OTSC is an excellent model for the preservation of tumour cytoarchitecture and modelling tumour-immune interactions. Their potential for testing immunotherapy makes them a promising tool in preclinical research and potentially in precision therapy.

This work was supported by grants APPV-21-0197, APPV-20-0143, TRANSCAN2023-1858-117, APD0045, APP0602, COST Action grants CA21135, E-COST-GRANT-CA21135-59b2e900, CA21116, and projects No. 09I01-03-V04-00073.

SELECTED ORAL PRESENTATION

Immunotherapy: Revolutionizing Cancer Treatment through Personalized Approaches

Authors and Affiliations

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Abstract

Immunotherapy (IT) has revolutionized cancer treatment, particularly in melanoma, thanks to immune checkpoint inhibitors. However, clinical responses remain limited to a small number of patients, highlighting the need to develop more personalized IT strategies. Our project, conducted within the TIFEX platform (Tumor Immune Function *Ex vivo*) at the Cancer Immunotherapy Laboratory of Lyon (LICL), aims to better understand the effects of IT on the tumor microenvironment (TME) and to develop approaches to study intratumoral immune responses.

We employ an innovative organotypic culture of live tumor slices (250 µm) *ex vivo* to analyze the immune component of the TME at the single-cell level. This cutting-edge approach combines spatial in situ analyses using multiplex immunofluorescence (mIF) with *ex vivo* functional analyses using spectral flow cytometry and multiplex assays. We investigate immune responses induced by immunotherapies (checkpoint inhibitors, TLR agonists) in fresh tumor samples, primarily focusing on oral cavity squamous cell carcinomas (OSCC) and skin cancers, including melanoma.

Our preliminary data demonstrate successful isolation of an average of over 147,000 total cells per tumor slice from OSCC and 186,000 viable cells per slice from skin tumors. Spectral flow cytometry enabled the identification and characterization of immune subpopulations, such as conventional dendritic cells (cDC1, cDC2), plasmacytoid dendritic cells (pDC), and natural killer (NK) cells. We are currently developing specific spectral panels to better characterize T/NK populations as well as macrophage-neutrophil populations in cell suspensions obtained from PCTS (precision-cut tumor slices). mIF analysis confirmed the ability to accurately detect and localize these immune populations within tumor tissue, including rare subsets like cDC1 and NK cells.

Our results revealed a general decrease in immune and tumor cell populations after 24 hours of culture, likely due to cell migration out of the tissue or tissue distress. Despite this limitation, the technique remains relevant for studying cellular dynamics within the TME. By characterizing in situ and *ex vivo* responses of diverse immune populations, we aim to distinguish between immunologically reactive and non-reactive tumors and to uncover mechanisms of treatment resistance. Ultimately, this platform will enable the validation and comparison of new ITs and may potentially predict patient responses. TIFEX will benefit biotech companies and researchers involved in the development of immuno-oncology molecules, as well as clinicians and cancer patients.

Session 4 – *Ex vivo* modeling

Migration and Tumor-Infiltration Capacities of Adoptive T Cells: Of Mice and Microchannels

Authors and Affiliations

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Abstract

Even though inflamed tumors, which are characterized with high T-cell infiltration and noticeable interferon-γ (IFN-γ) signaling, tend to better respond to the immune checkpoint inhibitors (ICI), the functional status of T cells and suppressive assets of the tumor microenvironment determine the success of the therapy. Type 1 helper T (Th1) cells play a critical role in anti-tumor immune responses by enhancing antigen presentation and cytotoxic responses. Nevertheless, both cytotoxic T lymphocytes (CTL) and Th1 cells are prone to immune regulation which occurs due to continuous stimulation and chronic exposure to inflammatory factors. Under these circumstances, the T cells acquire a hyporesponsive or exhausted state. Exhaustion in Th1 cells, which is functionally characterized by progressive loss of IFN-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-2 cytokine production and proliferation, are also identified with a unique epigenetic landscape. The presence of multiple inhibitory receptors such as PD-1, CTLA-4, TIM-3, and LAG3 has been acknowledged as an asset of exhausted T cells. On the other hand, an underestimated facet of T cell exhaustion is cellular paralysis; exhausted T cells lose their directional motility which decreases immune infiltration of the tumors. In addition, the efficacy of adoptive T cells immunotherapies such as CAR-T and TIL infusions are restricted due to deficits in tumor homing and infiltration. Therefore, monitoring the T cell recruitment towards malignant tissues carries pivotal importance for improving immune intervention therapies. This talk will focus on T cell motility assays employing patient-derived xenograft (PDX) mouse models and microfluidic systems for testing the potential of ICI and adoptive T cell immunotherapy approaches.

This work was supported by Hacettepe University Scientific Research Projects Coordination Unit and by The Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Patient-Derived Organoids and Fresh *Ex Vivo* Tissue Cultures as Translational Models for Immuno-Oncology Drug Testing

Authors and Affiliations

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Abstract

Advancing immuno-oncology drug development requires preclinical models that replicate the complexity of the human tumor microenvironment (TME) and predict clinical outcomes. Patient-derived organoids (PDOs and PDXOs) retain genetic and morphological features of the original tumors and, when combined with immune cells and fibroblasts, can model a reconstituted TME. We also utilize a 3D *Ex Vivo* Patient Tissue (EVPT) Platform, which preserves native TME from fresh *ex vivo* tumor samples. Both models—native and reconstituted—have distinct strengths, and we present several case studies across various cancer types including ovarian, breast, lung, prostate, melanoma, and head and neck cancers showcasing both capabilities and benchmarking the technologies against each other.

Tumor tissues were processed within 24 hours, embedded in protein-rich hydrogel, and treated with drug panels in 384-well plates for 5–7 days. Phenotypic responses were quantified using High Content Imaging (HCI). Drug sensitivity profiles were generated for each patient sample based on responses to immunomodulatory agents (anti-PD1, anti-CTLA4, anti-PD-L1, STING agonists) and immunostimulatory controls (SEA and CD3/CD28 beads). Among 51 NSCLC samples tested, 30 (59%) showed tumor volume reduction following SEA treatment. Pembrolizumab was tested on 34 samples, with 10 (29%) responding—aligning with its known clinical response rate (~25%). Responder samples also exhibited elevated IFN-γ, IL-2, and GZMB levels in supernatants, along with high immune cell infiltration as confirmed by FACS, IF, or IHC.

To assess immune sensitivity in PDO and PDXO models, healthy donor PBMCs—either naïve or activated with SEA or CD3/CD28 beads—were stained and added to 3D cultures. For EGFR-targeting immunotherapy evaluation, models were co-cultured with CAR-T cells or PBMCs plus T cell engagers. HCI captured immune infiltration and organoid killing, complemented by cytokine profiling of co-culture supernatants via MSD multiplex assays. Data from 100 models revealed variable sensitivity to immune-mediated killing, reflecting the heterogeneity of human cancers. EGFR-targeted T cell engagers reduced organoid volumes dose-dependently and elevated inflammatory cytokines like IFN-γ. However, HCI-based killing assays provided more specific functional readouts. Similarly, EGFR CAR-T cells enhanced T cell infiltration and killing, with consistent responses across both CAR-T and T cell engager treatments.

Selecting the most suitable preclinical model for (immuno-)oncology drug testing depends on factors like biological relevance, TME representation (native or reconstituted), reproducibility, scalability, and cost. Here we presented a comprehensive pipeline—from complex *ex vivo* fresh tissue cultures (EVPT) for clinically relevant testing, to fully characterized PDO models co-cultured with allogeneic or autologous PBMCs for high-throughput mechanistic screening.

01. Targeting PD-L1 with First-In-Class Covalent Small Molecules

Authors and Affiliations

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Abstract

The PD-1/PD-L1 immune checkpoint is one of the most common targets in cancer immunotherapy. However, the only PD-L1 inhibitors approved for clinical use so far are in the form of monoclonal antibodies, which come with certain disadvantages. One alternative approach focuses on designing small-molecule compounds with inhibitory activity against PD-L1. Those compounds could rival monoclonal antibodies in various aspects, such as the cost and ease of production, as well as tissue penetration. Moreover, other than simply blocking the PD-1/PD-L1 interaction, small-molecule compounds can modulate the amounts of PD-L1 present on the cell surface by affecting its internalization and degradation, maturation in the ER or transport to the Golgi.

This work presents a novel compound with the ability to covalently bind and dimerize the PD-L1 protein, leading to blockade of PD-1/PD-L1 immune checkpoint, as well as PD- L1 re-location. We show, through a cell-based immune checkpoint blockade (ICB) assay,that our compound leads to disruption in the PD-1/PD-L1 interaction. The PD-L1 blockade is also confirmed by flow cytometry analysis. The covalent nature of the PD-L1 dimers is shown by western blotting.

Imaging of cells with fluorescently labelled PD-L1 indicates that treatment with the compound leads to changes in the distribution of the protein in cells.

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02. Investigation of EBV-Associated Gene Expression Changes in T Cells and Their Role in Immune Dysregulation and Oncogenesis

Authors and Affiliations

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Abstract

Epstein-Barr virus (EBV) is a well-established oncovirus implicated in several malignancies, including Burkitt lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma. Recent evidence also suggests a role for EBV in immune-mediated disorders such as celiac disease (CD). Notably, 25% of 20 patients developed Transglutaminase Antibodies (TGA) following EBV infection, suggesting a potential increase in CD risk. Additionally, active EBV infection has been detected in 70.5% of biopsy specimens from patients with refractory celiac disease (RCD) compared to 16.6% in typical CD, indicating EBV's role in disease progression and immune dysregulation. Given EBV's ability to manipulate host immune responses and promote lymphoproliferative disorders, understanding its impact on T cell gene expression could provide novel insights into EBV-driven immune pathologies and oncogenesis. This study aims to investigate EBV-associated gene expression changes in T cells and their potential role in both immune dysregulation and oncogenic transformation by analyzing publicly available datasets.

An *in silico* approach was used to analyze differentially expressed genes (DEGs) in T cells from two datasets in the Gene Expression Omnibus (GEO): GSE214174 (acute EBV infection) and GSE168527 (celiac disease). Bioinformatics analyses included Robust Multi-array Average (RMA) normalization, identification of DEGs, pathway enrichment analysis, gene set enrichment analysis, and network analysis. We identified five key genes exhibiting significant expression changes in both EBV infection and celiac disease. Among them, OAS1 and PICALM were highlighted due to their essential roles in immune regulation and cancer-related pathways. OAS1, a key antiviral response gene, has been linked to EBV-driven lymphomagenesis and immune modulation. PICALM, which is involved in cellular trafficking and apoptosis, has been associated with hematological malignancies and immune dysfunction. These findings suggest that EBV-induced alterations in T cell gene expression may contribute to both immune dysregulation in CD and EBV-related oncogenesis.

This study highlights the dual role of EBV in promoting immune dysregulation and oncogenesis by altering T cell gene expression. The involvement of OAS1 and PICALM in both EBV-associated immune disorders and malignancies warrants further investigation as potential biomarkers or therapeutic targets in EBV-driven pathologies.

03. Investigation of EBV-Associated Gene Expression Changes in T Cells and Their Role in Immune Dysregulation and Oncogenesis

Authors and Affiliations

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Abstract

Antitumor treatments that activate the immune system to recognize and destroy cancer cells have been the most significant advancements in recent oncology therapies. However, for these treatments to be more effective, the tumors must present sufficient tumor-specific neoantigens. One way to overcome this is to lower the activation threshold of the immune system. In addition, it has been observed that bacterial translocation, characteristic of inflammatory diseases, allows colonic mucosal bacteria to reach the lymph nodes and bloodstream, facilitating the presentation of certain antigens to immune cells.

The goal of this project is to use modified bacteria containing DNA encoding tumor neoantigens as vehicles to induce bacterial translocation, thereby presenting these neoantigens to the immune system in a systemic context that enables an effective and specific response against the tumor. A preclinical study will be conducted in an orthotopic murine model (BALB/c) of triple-negative breast cancer using the 4T1 syngeneic cell line, in which neoantigens have been previously identified. This will allow the evaluation of the safety and antitumor efficacy of inducing bacterial translocation to express these neoantigens. This translocation will be induced by local colitis using dextran sulfate sodium (DSS).

For the initial approach, *Escherichia coli* with constitutive Red Fluorescent Protein (RFP) expression was used to study bacterial translocation and the immune response after DSS-induced colitis. Mice were either treated with 5% DSS in water *ad libitum* for 5 days or untreated (Group 3, control). Bacteria were inoculated via gastric gavage on days 0 (Group 2 and Group 3) and +3 (Group 1). Finally, on day +24, animals were anesthetized, exsanguinated by cardiac puncture, and euthanized by cervical dislocation. The organs were collected for histopathological analysis, and fecal samples were collected to assess the degree of bacterial colony implantation in the colon through PCR amplification. Immune study results showed a weak antibody response against RFP in Group 2. Therefore, in the next assay, *Listeria monocytogenes* (attenuated strain) or *Enterococcus gallinarum* will be used to enhance the immune response.

In conclusion, this project establishes a basis for personalized treatment of breast cancer based on the induction of translocation of genetically modified bacteria expressing neoantigens identified in the primary tumor.

This work was supported by grant NEOANTITUM, Catholic University of Murcia.

04. Targeting GARS1 and Metabolic Signaling: A Novel Immunomodulatory Strategy for Cancer Therapy

Authors and Affiliations

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Abstract

Immunotherapy has transformed cancer treatment, yet many patients fail to achieve durable responses due to resistance mechanisms within the tumor microenvironment (TME). Fraisinib, a small-molecule inhibitor of glycyl-tRNA synthetase (GARS1), induces metabolic and immune regulatory changes. Our integrative approach, combining proteomic, transcriptomic, and miRNA analyses, reveals that Fraisinib significantly alters pathways involved in antigen presentation, inflammatory signaling, and metabolic stress adaptation. Differentially expressed proteins (DEPs) at 24, 48 and 72 hours post-treatment include key regulators of immune activation, highlighting its potential role in reshaping the TME. Additionally, miRNA profiling suggests Fraisinib-driven modulation of immunoregulatory networks, with key miRNAs associated with T-cell activation and cytokine secretion.

Preclinical in vivo studies demonstrate that Fraisinib significantly reduces tumor growth in murine models, achieving up to 89% tumor volume reduction at optimized dosing regimens. Importantly, Fraisinib exhibits a favorable toxicity profile, with no significant alterations in body weight, hematological parameters, or vital organ function. These results suggest that Fraisinib acts as a potent and well-tolerated agent, effectively modulating the TME and enhancing immune responsiveness.

Notably, our findings indicate that metabolic rewiring through nucleotide derivatives plays a pivotal role in immune modulation, influencing T-cell priming and macrophage function. These results position Fraisinib as a promising candidate for combination strategies with checkpoint inhibitors and metabolic immunotherapies.

This work was supported by grants from the Italian Ministry of health (5X1000 and Ricerca Corrente).

Fraisinib is a patented compound developed by GIAM Pharma International Sarl (Monthey, CH, patent number WO2018061045A1 and patent pending PCT/IB2023/054699).

05. Investigation of the Local Immune Microenvironment in Organotypic Slice Cultures of Head And Neck Squamous Cell Carcinoma

Authors and Affiliations

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Abstract

Novel ongoing clinical trials in locally advanced head and neck squamous cell carcinoma (HNSCC) concern neoadjuvant immune checkpoint inhibitor (ICI); anti-PD1 IgG4 antibody (Pembrolizumab) treatments, followed by surgery, and by adjuvant ICI cycles combined with, if required, standard of care. In this treatment relation the investigation of therapy naïve surgical biopsy samples gained an increased significance.

This study has been approved by the local ethic commission, biopsy sampling and tissue cultures were done with the patients´ consent. Biopsies were taken during diagnostic pan-endoscopy. Fresh tissue samples were sectioned with a Precisionary Compresstome® (Ashland, MA, USA) and precision cut 300 µm tissue slices (SC) were cultured in 1 ml defined Keratinocyte medium for 5 days. On days 1 and 2 SC were treated with 30 µl Miltenyi Biotech T cell Stimulation and Expansion Kit mixture containing anti-Biotin MACSiBead Particles and biotinylated antibodies against human CD2, CD3, and CD28. Alternatively, slices were treated with 1 µg/ml anti-PD1 human antibody, Pembrolizumab biosimilar. On day 3 of culture the medium was changed and the tissue slices were further cultured up to day 5. The SC were fixed in formalin, paraffin embedded, sectioned and used for immunohistochemistry (IHC) of Granzyme B (GrB) and of cleaved caspase 3 (CC3). The IHC intensities were quantified using image cytometry. CC3 signals were attributed to tumor cells by morphometric tools in HistoQuest and StrataQuest programs of TissueGnostics, Vienna, Austria.

The T cell activation kit achieved increase of GrB-positive cells and GrB immunohistochemical intensities in the majority of the patients´ SC. The activation of GrB was accompanied by increase of CC3-positive tumor cells in several SC, whereas, in tissues containing sustained, stabilized Slug (SNAI2) protein levels, the GrB activation was not followed by CC3-induction in tumor cells. Anti-PD1 treatment was started now, and we have results only on SC of few patients, including ones where the percent of GrB-positive cells increased 2.9-times over the control, and the percent of CC3-positive tumor cells increased 6.22-times. In SC of several patients the anti-PD1 did not achieve activation of GrB and neither CC3-increase in tumor cells.

Organotypic slice culture allows a short-term model for experimental immunotherapy testing in the original tumor tissue. This system contains the original tissue architecture and the local immune system. The results are patients-specific and the model might allow the investigation of predictive molecular and clinical parameters for immune stimulation and induced tumor cell death. Increase of GrB signal and GrB-positive cells seemed to be a good marker for immune stimulation, which was accompanied by CC3-positive reaction in tumor cells.

This work was partly supported by ViraTherapeutics, Neu-Rum, Austria. The supporter did not have influence on the study.

06. Exploring the Role of Immuno-Epigenetics in Prostate Cancer

Authors and Affiliations

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Abstract

Although prostate cancer (PCa) is one of the most incident malignancies, it currently lacks treatment options for advanced forms of disease. In PCa, epigenetic dysregulation has been shown to play a crucial role in tumor development, progression and patient prognosis. Beyond regulating tumor development, recent research has highlighted the pivotal role of epigenetics in shaping immune cell function and tumor cell recognition. Herein, we aimed to increase prostate cancer immunogenicity by targeting one of the most relevant epigenetic enzymes in PCa tumorigenesis – EZH2. This enzyme is responsible for the trimethylation of histone 3 at lysine 27 (H3K27me3), which leads to transcriptional repression.

EZH2 and H3K27me3 expression was assessed in PCa patient tissues by immunohistochemistry, displaying increased expression with disease progression. Inhibition of EZH2 in PCa cells was able to increase the expression of NK cell activating ligands, particularly NKG2D ligands, essential for anti-tumor immune responses. Remarkably, CUT&RUN revealed H3K27me3 binding at the promoter region of those ligands, underscoring their epigenetic regulation. Furthermore, EZH2 inhibition led to an increase in NK cell-mediated killing and of IFN-g secretion upon co-culture of NK cells and PCa cells. In addition, this effect decreased upon NKG2D blocking, underlining this improved anti-tumor immune response to be NKG2D-dependent.

Our results suggest that harnessing epigenetic mechanisms is a promising approach to improve the immune recognition of prostate tumor cells, paving the way for novel strategies in the treatment of PCa.

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07. Investigation of Monocyte Amoeboid Movement Using Alginate-PLL Hydrogel in a Lab-On-a-Chip

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Abstract

Monocytes are highly mobile cells with amoeboid migration properties, enabling them to navigate through extracellular matrix (ECM) pores and play critical roles in immune responses and inflammation, relevant to both health and disease states.
Most existing models utilize hydrogels such as collagen and Matrigel, which are animal-derived, expensive, and susceptible to degradation by enzymes secreted by monocytes. Alginate, a non-biodegradable material, can be modified with positively charged poly-L-lysine (PLL), offering distinct advantages for studying cell motility. This project aims to explore the effects of alginate-PLL complexes on cell migration in three-dimensional (3D) environments using lab-on-chip (LOC) systems, providing a biologically compatible model.
The ICS-Chip device, fabricated from PDMS, features three continuous reservoirs separated by single capillary burst valves and a user-friendly open-top design. This innovative platform facilitates the straightforward placement of hydrogel in the central channel while enabling observation of cell movement dynamics in response to chemoattractants in the side channels. The migration of cells can be tracked, offering valuable insights into cellular behavior under environmental stimuli.
In this study, green fluorescently labeled U937 human monocytes were encapsulated in alginate-PLL hydrogel, and their migration towards serum-free and serum-containing channels was observed. Cells were imaged using confocal microscopy, and data were analyzed with Fiji and R-Studio. Results showed that monocytes exhibited significant migration towards serum-containing media compared to serum-free media.
Future studies will expand the application of the ICS-Chip to investigate amoeboid cell migration in various cell types. This platform holds potential for cancer research, immunotherapy, and modeling immune responses, presenting an innovative and sustainable alternative in LOC technology.

08. Deciphering the Crosstalk Between Immune and Tumoral Cells in Colorectal Lung Metastasis Using Spatial Transcriptomics

Authors and Affiliations

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Abstract

Previous studies in the group using transcriptomic data suggested that lung metastases were more immunogenic than hepatic metastases in patients with colorectal cancer (CRC). This could have important implications since these patients could be treated with immunotherapy.

The aim is to characterize lung metastases by analyzing spatial transcriptomics, to decipher the molecular crosstalk and to identify differential gene expression between immune and tumor regions.

FFPE tissue samples from eight patients CRC lung metastases were analyzed using Visium CytAssist Spatial Transcriptomics Kit (10x Genomics). Spatial transcriptomics allows the analysis of gene expression while preserving tissue structure, providing a more detailed view of the tumor molecular biology. Libraries were sequenced using Illumina Hiseq 400 and data were analyzed with Space Ranger, Loupe Browser and Seurat. Differential gene expression analysis was performed between tissue areas, and the molecular crosstalk between clusters was evaluated using TALKIEN.

Bioinformatic analysis allowed for thorough quality control of the samples, correlating gene expression with histology and grouping the samples into expression clusters. Afterwards, TALKIEN was used to identify molecular interactions between clusters.

In conclusion, spatial transcriptomics is a valuable tool that provides a deeper understanding of complex tissues such as lung metastases, allowing identification of differentially expressed genes and molecular crosstalk between tumor regions.

This work was supported by grants APVV-23-0198, PI22/01930, and CNS2022-136176.

09. Gene Set Enrichment Analysis Reveals Immune Pathway Alterations in Taxane-Resistant Breast Cancer Models

Authors and Affiliations

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Abstract

Breast cancer is the second most diagnosed tumor according to the Global Cancer Observatory in 2022. It can be classified based on its molecular characteristics into luminal A and B subtypes, which are characterized by the presence of estrogen and progesterone receptors; the HER2 subtype, which is positive for this proto-oncogene; and the triple-negative breast cancer (TNBC) subtype, a highly heterogeneous subgroup characterized by the absence of estrogen receptors, progesterone receptors, and HER2. The treatment of patients depends on the tumor type and presence of specific molecular markers at diagnosis. One of the main challenges in TNBC treatment is the limited availability of targeted therapies. Although immune checkpoint inhibitors, such as pembrolizumab and PARP inhibitors, have expanded therapeutic options for specific TNBC subgroups, taxanes remain a cornerstone of treatment. However, the emergence of taxane resistance remains a major hurdle, leading to poor prognoses and decreased survival rates.

To better understand the mechanisms underlying taxane resistance, cell lines from two breast cancer models, luminal (MCF7) and triple-negative (MDA-MB-231), have been progressively adapted to increasing concentrations of docetaxel for three years, which is known for its ability to inhibit microtubule depolymerization. Both parental and resistant cell lines were established, and total RNA was extracted using the Direct-zol RNA MiniPrep kit and sequenced using the Illumina NextSeq 2000 platform. Using bioinformatics analyses with the BEAVR tool, we performed Gene Set Enrichment Analysis (GSEA) to identify immune-related enriched pathways, with the aim of uncovering potential alterations in the immune system associated with resistance acquisition.

In the triple-negative model (MDA-MB-231), we found alterations in TCR signaling and transcriptional regulation of granulopoiesis. In contrast, in the luminal model (MCF7), interferon alpha and beta signaling pathways were altered.

This work was supported by grants (MEFASCINAS, Universidad Católica San Antoni.

10. Engineering of Cancer Microenvironments with Embedded Interconnected Microcapillary Networks via *In Vitro* Magnetic Assembly of Endothelial-Cell 'Seeds'

Authors and Affiliations

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Abstract

Despite significant developments in endothelial-cell (EC) manipulation techniques, a proper in vitro model of a functional microvasculature with controlled local interconnectivity under well-defined global architecture is still lacking. Here, we report the generation of such controlled multi-scale vascular networks via manipulation of tens of sprouting EC 'seeds'. We exploit magnetic patterning to assemble EC-coated superparamagnetic microbeads into ordered arrays and establish effective growth rules governing the development of interconnectivity and directionality of the networks depending on the applied seed-seed spacing. The EC-seed-based approach offers a range of advantages over conventional EC-manipulation techniques including: (i) expedited sprouting, (ii) spatial control over interconnections, (iii) reduction in cell consumption by
>100x, and (iv) native high-throughput format. We co-develop multiparametric morphometric analysis tool, introduced previously and demonstrate high-content assessment of drug-induced vascular remodeling, and cancer cell invasion in 3D tumor microenvironments (Fig. 1). Overall, we propose a uniquely precise and standardized vascular-microtissue engineering tool with applications, e.g., in angiogenesis research, high-throughput drug testing including personalized therapies, and with possible extension to immune microenvironments and organ-on-chip approaches.

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11. Mechanism of Biomimetic Virus-Like Nanoparticles in Triggering Immune Response Elucidated by Proteomics

Authors and Affiliations

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Abstract

Mesoporous silica nanoparticles (MSNs) are promising for cancer therapy due to their mesoporous structure, high surface area, biocompatibility, and functionalization potential. In cancer immunotherapy, MSNs serve as immune adjuvants and nanocarriers for delivering therapeutic antigens. Inspired by pathogens, virus-like mesoporous silica (VLP) particles with biomimetic topologies were designed to enhance cellular uptake. However, the interaction mechanism between the nanoparticles and immune cells has been poorly understood. Proteomics is a powerful tool for the large-scale study of proteins for understanding biological processes and disease therapy mechanisms. It has been widely used in nanomedicine to identify protein corona formed on NPs and protein biomarkers for targeted drug delivery.

VLPs were synthesized via a microemulsion growth method, functionalized with PEG and amine silane, and characterize particles and conduct in vitro study. Proteomics analyses, including pathway enrichment, protein interaction networks, and heatmaps, were carried out to understand the mechanism of VLPs in activating immune response.

In this study, we examined the impact of spike length on immune responses. Compared to spherical particles, the VLPs with longer spikes (30 nm) showed higher cellular uptake efficiency. Proteomic analysis revealed that the expression levels of immune-related proteins including TRAF6 and PIAS4 proteins enhanced. Pathway analysis identified the involvement of RIG-I-like receptor, Chemokine, MAPK, NF-κB, Toll-like receptor, B cell receptor signaling pathway and Th1 and Th2 cell differentiation pathways in immune response. Additionally, VLPs upregulated CD86, CD80, and HLA-DR on antigen-presenting cells, highlighting their potential as immunoadjuvants for enhancing antigen delivery and immunotherapy.

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12. PD-L1 Blockade Increases Elimination of Cancer Cells by Lymphocytes in a New *In Vitro* Model

Authors and Affiliations

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Abstract

Targeting such immune checkpoint molecules (ICMs) as PD-1, PD-L1, or CTLA4 with therapeutic antibodies is now a strategy of choice for the treatment of several types of cancers. The design of new molecules as drug candidates targeting ICM requires reliable in vitro models for proper and accurate testing of their bioactivity before progressing toward in vivo studies.

In this report, a new cell-based co-culture model is presented, in which RKO cancer cells are contacted with primary lymphocytes to provide a platform for the evaluation of the anticancer potential of T-cells in the function of PD-1/PD-L1 blockade. The new methodology allows for verifying not only the reactivation of immune cells by the tested molecules, but also the biological effects of this reactivation, i.e. (1) T-cell proliferation, (2) expression of T-cell surface markers, and (3) T-cell-mediated killing of cancer cells. Additionally, the viability of T cells is monitored providing premises for potential direct toxicity of the treatment. All these parameters are measured within a single flow cytometry experiment, providing reproducible, highly informative results that provide a complete view into the PD-L1-targeting potential of tested molecules.

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13. Approaching the Extracellular Matrix to Modulate the Tumor Immune Landscape: NIDOGEN-1 as a Novel Immunomodulatory Factor

Authors and Affiliations

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Abstract

Recent studies have highlighted the critical role of extracellular matrix (ECM) components in immune regulation during tumour progression. Our studies using melanoma and mammary tumour models revealed that tumour blockade induced by theab sence of the metalloproteinase *Adamts1* led to an increased vascular deposition of its substrate, the basement membrane glycoprotein NIDOGEN-1 (NID1). Notably, NID1 overexpression in an immunocompetent melanoma model similarly suppressed tumour progression, mirroring the phenotype observed in *Adamts1*-deficient tumours. These tumours exhibited significant alterations in immune infiltration, with an enriched population of antitumorigenic macrophages and an overall pro-inflammatory microenvironment. Further characterization of tumour-infiltrating macrophages identified a previously unrecognized phenotype with features reminiscent of an M1-like antitumorigenic state. *In vitro* studies confirmed that full-length NID1, but not its fragments, promoted M1-like macrophage polarization, primarily mediated by αvβ3 integrin signalling.

Importantly, RNA-seq analysis from our tumour models, integrated with datasets from two large human melanoma cohorts, revealed a novel gene signature associated with favourable prognosis and increased M1-like macrophage abundance. These findings establish NID1 as a novel tumour suppressor with immunomodulatory properties and reinforce the ECM as a critical player in tumour immunity. Our ongoing studies aim to explore NID1 as a potential immunomodulatory therapeutic strategy in oncology.

This work was supported by grants PID2019-104416RB-I00 (Ministerio de Ciencia, co-financed by FEDER) and PE-0225-2018 (Junta de Andalucía) to JCRM.

14. Visualization of Glioblastoma Immune Microenvironment for Personalized Therapy

Authors and Affiliations

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Abstract

Glioblastoma remains a lethal disease despite current standard treatment with maximal surgical resection, radiation, and temozolomide therapy. One significant challenge hindering drug development and the study of GB therapeutic response is the lack of an appropriate in vitro model that accurately represents the complexity of patients' tumors and allows visualization of the tumor microenvironment. To address these limitations, we established patient-derived GB organoids that mimic parental tumors, reflecting tumor heterogeneity and resistance to clinical therapies. Additionally, we employed spatial transcriptomics to evaluate changes in the tumor microenvironment at the molecular level. Cancer research emphasizes the importance of understanding the intricate and dynamic nature of the entire tumor microenvironment. This requires utilizing novel techniques and in vitro models that enable real-time visualization of tumor cellular composition and cell interactions in response to therapeutic interventions.

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15. DNA Methylation Drives Uveal Melanoma Progression by Regulating Immune Evasion and an Immunosuppressive Microenvironment

Authors and Affiliations

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Abstract

Uveal melanoma (UM) is an aggressive malignancy originating from the pigmented layers of the eye. Metastases develop in up to 50% of patients, leading to poor survival outcomes due to the limited efficacy of existing treatments. UM employs multiple immune evasion mechanisms, rendering it resistant to conventional immunotherapy. Understanding the epigenetic regulation of immune-related genes may offer new insights into immune escape mechanisms and identify potential therapeutic targets.

We performed an integrative analysis on 25 UM tumor samples, combining genome-wide DNA methylation profiling (Illumina Infinium Methylation EPIC array, 850K) with gene expression analysis (Agilent array) to assess methylation-driven transcriptional changes. Key findings were validated in an extended cohort of 58 UM tissues using RT-PCR and pyrosequencing.

We identified 944 differentially expressed genes in high-risk UM tumors, including 45 upregulated and 20 downregulated DNA methylation-regulated genes associated with immune functions. Upregulated genes spanned key functional categories, including immune-related genes, growth factors/receptors, extracellular matrix components, intracellular signaling molecules, and metabolic/transport proteins, underscoring their roles in tumor progression and immune regulation. Conversely, downregulated genes indicated disruptions in immune signaling, cell communication, extracellular matrix remodeling, and transcriptional regulation, with VIM downregulation suggesting altered mesenchymal identity. Notably, six of the top ten genes (EDNRB, IL12RB2, CALHM2, TLR1, AHNAK2, RNF43) play key roles in immune evasion and metastasis, with IL12RB2 promoting anti-tumor immunity via T and NK cell activation and TLR1 linking innate immune responses to poor-prognosis predictor monosomy 3. AHNAK2 may further contribute to immune signaling and tumor immune escape.

Our results confirm that DNA methylation plays a crucial role in UM progression, influencing immune-related pathways, tumor microenvironment immunosuppression, adhesion, migration, and calcium homeostasis. The identification of differentially methylated immune-associated genes provides new insights into UM immune evasion. Given UM's resistance to conventional treatments, targeting epigenetic modifications could enhance the efficacy of immunotherapy in UM.

This work was supported by grants VEGA 2/0027/24, VEGA2/0165/25, APVV-17-0369 and APVV-23-0294.

16. Identifying Molecular Regulators of Pro-Tumorigenic Reprograming in Glioma-associated Microglia

Authors and Affiliations

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Abstract

Microglia, the immune cells of the brain, faces a singular challenge regarding brain tumors development. In the oncogenic context, this brain guardians can be reprogramed and assume a tumor-supportive phenotype which sustains tumor growth. Glioblastoma (GB), the deadliest among brain tumors, is known for creating an immunosuppressive niche associated to the reprograming of microglia and infiltrating monocytes/macrophages. Besides, the oncogenic reprograming of glioma-associated myeloid cells (GAMs) allows tumor expansion and resistance, and the presence of activated microglia is associated with a more aggressive phenotype of GB. Despite the advances in dissecting molecular phenotypes of these cells in recent years, mechanisms underlying the pro-tumoral reprogramming of microglia are poorly understood. We aimed to explore molecular mechanisms involved in microglia reprogramming by exploring multi-omics datasets combining RNA-seq, ATAC-seq, and ChIP-seq for several histone modifications of glioma-associated myeloid cells (CD11b+) sorted from the brains of Gl261 glioma-bearing mice at different time-points during tumor progression. We explored transcriptomic and epigenetic profiles and identified transcription factors (TFs) as potential master regulators along with target genes. Computational prediction analysis showed upregulation of IRF7 (from interferon regulatory factors family) and other candidates in microglia at day 7th after implantation. IRF7 levels increased in BV2 microglial cells at the first 3 h of co-culture with glioma cells, as confirmed by Western blot analysis. Moreover, we found activation of STAT and IRF-dependent pathways as demonstrated by an increase in luciferase reporter constructs driven by STATs and IRFs. Immunostaining analysis demonstrated an increase of IRF7 signal in the nucleus of BV2 treated with glioma condition media (GCM), suggesting its activation and translocation to the nuclear compartment. Another candidate identified by in-silico analysis was Ets2, and although its expression did not change significantly at the protein level in BV2 cells upon co-culture with GL261 cells, the Ets2-interacting protein ID3 (inhibitor of differentiation 3) was increased, suggesting the activation of a specific regulatory mechanism involving those molecules. The presented data illuminate the involvement of IRF7 and ID3 in microglia reprogramming by glioma.

This work was supported by grants HIT GLIO.

17. Advances in the Sustainable Design of PD-L1 Inhibitors

Authors and Affiliations

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Abstract

The replacement of compounds from fossil origin in various chemical applications and the access to greener transformation paths are two challenges in chemistry for this century. Furanic derivatives like furfural are produced from cellulose and furfural itself, or its reduced derivative furfuryl alcohol, are two key intermediates to access many oxygenated compounds with lot of applications in the industry. Recently our group has demonstrated that furfural can be transformed to aromatic compounds in various ways.
In the context of immune check point inhibitors biphenyl-based compounds with high inhibitory activity against PD-L1 have been described by BMS.[4] All compounds described since this discovery have mainly in common this biphenyl key group that is responsible for the principal mode of binding to the dimeric PD-L1 target.
We are currently developing from our previous results an alternative access to biaryl compounds from furanic derivatives that could potentially be used to design sustainable PD-L1 inhibitors, already known or novel ones. The two key reactions consist in a Diels-Alder reaction between the furanic derivatives and various activated alkenes or alkynes, followed by an aromatization step. Subsequent transformations will lead to novel inhibitors.

This work was supported by Ligue Nationale Contre le Cancer, Centre National de la Recherche Scientifique.

18. Timing of Melanoma Brain Metastasis Dictates the Immunomodulatory Effect and Efficacy of Systemic Anti-CD40 Agonist Therapy

Authors and Affiliations

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Abstract

Melanoma brain metastasis (MBM) affects nearly 50% of patients with advanced melanoma. Despite progress in systemic therapies, particularly immune checkpoint inhibitors, intracranial responses remain highly variable. The CD40/CD40L axis and its pleiotropic effects on the anti-tumoral immune response has the potential to improve MBM treatment. We investigated whether timing of MBM seeding, in a two-site mouse model of MBM, impacts the efficacy of anti-CD40 agonism (αCD40).

$2x10^5$ B16F1-cOVA murine melanoma cells were subcutaneously (s.c.) injected in the right flank in C57BL/6 mice (5F, 5M). After 3 days, $1x10^5$ B16F1-cOVA cells were intracerebrally (i.c.) injected into the right striatum under stereotaxic guidance ('early MBM' cohort). A separate cohort of C57BL/6 mice (5F, 5M) had s.c. injection of B16F1-cOVA cells, followed by an i.c. injection of B16F1-cOVA cells after 5 days ('late MBM' cohort). Mice were randomly allocated to receive either an αCD40 or an isotype IgG antibody (control) intraperitoneally on days 4 (D4) and 7 (D7) post-i.c. injection (D0). S.c. tumor volume and survival data were serially collected. Separately, tumor-bearing mice treated with either αCD40 or IgG (n = 4 per early/late MBM groups) were euthanised day 9 (D9) post-i.c. injection and tissues (blood, brain tumor [BT], and flank tumor [FT]) were collected for immune profiling by spectral flow cytometry.

The early MBM cohort showed a significant s.c. tumor response to αCD40 therapy with a mean D9:D4 s.c. tumor volume ratio of 2.81 (SD±2.05) vs 16.51 (±13.10) in the IgG group (unpaired t-test; p=0.0497). The late MBM cohort showed no significant difference in s.c. tumor response between αCD40 or IgG (D9:D4 ratio 1.52±1.00 vs 1.20±0.83; p=0.5999). Survival increased in early MBM following αCD40 vs IgG (median 20 days vs 13 days; logrank test, p=0.0078), which was not replicated in late MBM (median 21 days vs 19 days; p=0.7717). Systemic αCD40 led to reduced circulating B cells, and increased monocytes, compared to IgG treatment. In the BT, there were higher infiltration of CD8 T cells (normalised to microglia), including OVA+ CD8 T cells, and CD4 T cells in early MBM treated with αCD40 vs IgG. Systemic αCD40 induced more infiltrating SIRP1α+ cDC2 cells than XCR1+ cDC1 cells and vice versa in the IgG group, in both the BT and FLT.

Systemic αCD40 therapy improved survival of MBM-bearing mice, with a shorter interval between s.c. and i.c. implantation; an effect abrogated by later MBM seeding. Systemic αCD40 therapy induced specific changes in the BT infiltrative immune profile, which may explain the distinct response between the early and late BM cohorts. These findings have implications on the experimental modelling of MBM and provide insight into variability of MBM response to immunomodulating therapies.

This work was supported by a grant from the Focused Ultrasound Foundation.

19. Advancing Cancer Therapy: Macrophage-Drug Conjugates for Solid Tumor Treatment

Authors and Affiliations

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Abstract

The treatment of solid tumors, such as glioblastoma, faces significant challenges due to poor drug delivery and the immunosuppressive tumor microenvironment. Our recent findings using macrophages as cellular vehicles for targeted drug delivery offer promising solutions to these obstacles. We have identified a novel process involving the transfer of hemoglobin (Hb) from macrophages to cancer cells via extracellular vesicles. This process enables the use of macrophages and Hb-drug conjugates for cancer therapy. This mechanism bypasses traditional Hb degradation pathways and represents a previously unreported method of intercellular transfer. In addition, we have developed a macrophage drug conjugate (MDC) platform. It utilizes ferritin-drug complexes that, when loaded into macrophages, are transferred directly to cancer cells through direct contact and immune synapse-like structures. This approach has shown efficacy in several preclinical solid tumor models, with macrophages acting as efficient carriers for various anticancer drugs. In addition, we have shown that MDCs not only deliver cytotoxic payloads directly to tumor cells but also reprogram the immunosuppressive glioblastoma tumor microenvironment to enhance immune activation and induce durable T cell-mediated anti-tumor responses. Taken together, these findings underscore the versatility of macrophages in drug delivery, their ability to modulate the tumor microenvironment, and their potential for clinical translation, offering a transformative strategy for the treatment of solid tumors.

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20. Spatial Mapping the Immune Landscape of Prostate Cancer Throughout Disease Progression

Authors and Affiliations

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Abstract

Prostate cancer (PCa) is the second most common and the fifth deathliest cancer in men worldwide. Its heterogeneous nature and the scarcity of therapeutic approaches for more aggressive disease subtypes impact clinical outcomes. PCa is typically considered an "immune cold" tumor with low immune infiltration, enriched in immunosuppressive and dysfunctional immune cells. Nevertheless, a comprehensive analysis of the immune landscape throughout disease progression and its association with PCa prognosis is still lacking. In this project, we implemented a multiplex-based spatial characterization of the PCa immune contexture across disease progression. Therefore, localized PCa (ISUP grade 1-5), paired non-tumor adjacent, castration-resistant PCa (CRPC), and neuroendocrine PCa (NEPC) formalin-fixed paraffin-embedded (FFPE) samples were selected. Whole-slide multiplex immunohistochemistry was performed using an Akoya Opal Kit with a panel of five antibodies: anti-CD20, anti-CD3, anti-CD8, anti-CD4, and anti-FoxP3. Slides were scanned using PhenoImager® HT, and images were analyzed with Phenochart, inForm®, and QuPath. PCa exhibited higher immune cell infiltration than non-tumoral tissues, with CRPC as the most infiltrated disease subtype. CD4+ T cells were the most prominent immune population, followed by B cells, CD8+ T cells, and Tregs, in all disease subtypes. Remarkably, as the disease progressed, there was an enrichment in T cell subsets, contrary to B cells. Particularly in localized PCa, immune cells predominantly infiltrated intratumoral epithelial areas, with ISUP grade 5 displaying the highest level of Tregs. Notably, heightened CD3+ and CD4+ T cell levels were significantly associated with worse overall survival in PCa. Despite being regarded as a "cold tumor", we found PCa clearly infiltrated by B and T cells. Surprisingly, higher CD3+ and CD4+ T cell levels associated with worse PCa outcomes.
These results deepen our understanding of PCa immune landscape, paving the way for improved patient stratification and personalized treatment.

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21. Analysis of Gene Regulation in Glioma-associated Myeloid Cells During Murine Glioma Progression

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Abstract

Tumor microenvironment (TME) plays a critical role in tumor progression. Myeloid cell infiltrating brain tumors are reprogrammed by the tumor and acquires new properties and inhibit antitumor immunity. We hypothesized that reprograming involves epigenetics changes that drive distinct expression patterns and understanding its regulation holds a key to restoring proper host defenses. We generated and performed analysis of multi-omics data (RNA-seq, ATAC-seq, and histone modifications: H3K4me3, H3K27ac, H3K27me3) from CD11b+ cells sorted from GL261 glioma-bearing mouse brains at several time-points (0, 7, 14, 21 days) during tumor progression. We identified distinct patterns of changes in gene expression and chromatin openness, which can broadly be categorized into down-regulation or up-regulation during tumor progression, with the down-regulated genes related to homeostatic functions of microglia, and up-regulated genes involved in immune functions of both microglia and infiltrating monocytes / macrophages. For many genes, changes in gene expression were associated with concurrent or earlier changes of chromatin openness and histone modification in their promoter regions. Starting from the collected experimental data and combined with published pre-computed data on cis-regulatory regions and transcription factor binding sites (TFBSs) motifs, we performed an integrative analysis of gene regulation. This analysis identified several transcription factors (TFs) as key putative regulators of specific temporal patterns of transcription and/or changes in chromatin openness in tumor-associated myeloid cells, as well as candidate target genes of these TFs. Analysis of the timing of the predicted activity of these regulators suggested that some of them are specific for microglia or for monocytes/macrophages, while other operate in both these cell lineages. This conclusion was corroborated and extended by comparison with our published single-cell RNA-seq data from the same experimental model.

22. Mathematical Model for Non-Monotone Dose Response to the PD-L1 blockade *In Vitro*

Authors and Affiliations

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Abstract

We present a model for T-cell re-activation under the action of therapeutic compounds targeting the PD-1/PD-L1 interaction. The effector T cells in the assay express luciferase upon TCR-mediated activation, which is diminished by the presence of PD-1/PD-L1 interaction provided by co-cultured artificial antigen-presenting cells. Upon PD-L1 blockade with tested compounds added for 6 hours at different dose concentrations, the activation of effector T cells is restored, reflected by increased luminescence signals. The resulting dose response curve is non-monotone due to the existence of two separate and antagonizing effects, i.e. specific activation of T cells and unspecific toxicity, observed separately, but also overlapping at a certain range of the compound concentration.

A mathematical model is used to estimate the concentration for maximum level of activation and the EC50 concentration. The model presents a mechanism for the temporal change in the activity of the Firefly Luciferase over the course of the experiment as measured by the strength of the luminescent signal based on the following assumptions: 1. under the action of the compound, cells upregulate the luminescent signal in a dose-dependent manner; 2. the toxicity effect is modelled as a removal of cells and of the luminescent signal.

Parameters of the model are estimated from experimental data and are used to estimate the drug concentration corresponding to the maximum level of T-cell activation and the EC50 concentration.

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23. *STAG2* Deficiency in Leukemia Disrupts Microenvironmental Interactions with Potential Implications for Therapy

Authors and Affiliations

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Abstract

STAG2 encodes a crucial component of the cohesin complex and is frequently mutated in Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML). Loss of STAG2 leads to altered enhancer-promoter contacts, resulting in deregulated gene expression. Importantly, STAG2-mutated leukemia patients often face poor clinical outcomes. We wanted to elucidate how STAG2 loss impacts the interaction of leukemic cells with the bone marrow microenvironment and affects the response to microenvironmental stimuli. We analyzed whole transcriptome sequencing data from bone marrow samples of a cohort of 753 MDS patients including 48 samples with STAG2 mutations to identify mutation-associated gene signatures. Moreover, we performed single cell RNA sequencing from bone marrow of MDS patients with or without STAG2 mutations to uncover expression changes at the cell type level. To mimic STAG2 loss-of-function mutations in vitro, we generated multiple MDS/AML cell lines (MDS-L, OCI-AML5, and THP-1) with STAG2 deletion using CRISPR-Cas9. Cell lines were used for further transcriptomic and functional characterization, including co-culture with mesenchymal stromal cells. Our results show that STAG2-deficient leukemic cells exhibit transcriptomic alterations associated with cell-to-cell communication, immune receptor activity, and membrane proteins. We consistently found downregulation of genes encoding class II HLA complexes in STAG2-depleted leukemic cells. Moreover, multiple inflammatory pathways were deregulated in MDS patients with STAG2 mutation which we were able to trace back to conformational changes in the three-dimensional chromatin structure. A cell-to-cell communication analysis suggests impaired interferon-mediated T cell engagement by monocytes underlining improper reaction to external signals in STAG2-mutated cells. Finally, our co-culture experiments indicate that mesenchymal stromal cells can release the differentiation block of leukemic cells, such as OCI-AML5, but this phenotype was lost in STAG2-deficient leukemic cells. Taken together, STAG2 loss reshapes the transcriptome of leukemic cells with implications for interaction with cells of the bone marrow niche which might provide an advantage driving clonal expansion and highlights the potential for targeting STAG2-related pathways as a therapeutic approach to improve outcomes for MDS patients with STAG2 mutations.

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24. Biomimetic Nanovaccines for Cancer Immunotherapy: Enhancing Immune Activation with Virus-like Nanoparticles

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Abstract

Cancer immunotherapy has revolutionized cancer treatments. Challenges remain in long term immune response and specificity. Biomimetic cancer nanovaccines offer a strategy to improve the immune response. We have developed virus-like mesoporous silica nanoparticles (VLPSi), which can be coated with cancer cell membranes (CCM) to create biomimetic cancer nanovaccines (CCM-VLPSi). These nanoparticles are covered with spikes, which mimic viral morphology, facilitating efficient antigen uptake and immune activation. CCM´s tumor-associated antigens activate immune cells to recognize and target the cancer cells efficiently. Additionally, the biomimetic surface enhances nanoparticle stability, prolongs circulation time, ultimately boosting vaccine efficacy.

Our in vitro studies demonstrated that CCM-VLPSi significantly upregulated key lymphocyte activation markers (CD25, CD69) on human peripheral blood NK and T cells compared to cytokine stimulation after 24-hour stimulation. Additionally, VLPSi with optimized spike length (30 nm) can upregulate co-stimulatory molecule expression (CD86, CD80, HLA-DR) on dendritic cells, enhancing antigen presentation.

Our findings suggest that VLPSi-based cancer nanovaccines could serve as dual-function platforms, acting both as antigen carriers and immune adjuvants. For future aspects, utilizing patient-derived CCM, these biomimetic cancer nanovaccines have potential for personalized immunotherapy, offering a novel approach to overcoming tumor heterogeneity and improving clinical outcomes in cancer treatment

This work was supported by a grant from Finnish Cancer foundation (decision number 230130).

25. Stimulate mRNA Readthrough Driving Cancer to Synthesize its Own Vaccine

Authors and Affiliations

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Abstract

A critical cellular surveillance mechanism that recognizes and eliminates aberrant RNAs containing premature termination codons (PTC) is termed as the nonsense-mediated mRNA decay (NMD). The NMD pathway, including UPF1, UPF2, UPF3a, and UPF3b, is over-produced in human cancers and can stimulate immune escape thus the NMD pathway is a recognized drug target for cancer therapy, but there are no current clinical tools to validate this for use in human populations. Inhibitors that can modulate NMD activity offer critical tools for understanding the mechanism and physiological functions of the NMD pathway, and they also have the potential for treating certain genetic diseases and cancer. Our approach to the treatment of diseases caused by PTC mutations is the inhibition of NMD, which is regulated by UPF1. We focus on the core functions of UPF1 to find novel ATP mimetics that can be used as a chemical tool to define the function and drugability of the machinery. Such novel molecules targeting UPF1 functions can increase premature stop codon readthrough to enable the production of mutated proteins, which eventually enhances the probability of mutant peptides (neoantigens) loaded over the MHC class I and presented to T-cell receptors resulting in triggered immune response. In this sense, NMD (UPF1) inhibitors stimulate the tumour cell to make its own vaccine. Most cancer vaccines in clinical trials exploit mutated proteins; these vaccines include peptide products, viral assembly of genomic encoded mutated peptides, or mRNA synthesis that encodes mutated peptides in a patient specific manner. However, our approach to stimulate PTC readthrough with a small molecule, that drives the tumour to synthesize its own novel mutated peptides, is itself a novel approach for developing cancer vaccines. The advantage of NMD inhibition over standard vaccine approaches is that this chemical tool that inhibits UPF1 can be used in a wide range of patients without the need to use the current vaccine pipeline-the current vaccine pipeline is; sequencing patients genomic DNA; creating the vaccine based on mutated proteins that dock into MHC Class I; and injecting the vaccine into the patient. The investigation of the interaction network of UPF (UPF1, UPF2, UPF3a, and UPF3b) genes by experimental assays bring novel insights into the assembly of this multi-protein complex and other inhibitors of NMD that could impact on cancer vaccinology.

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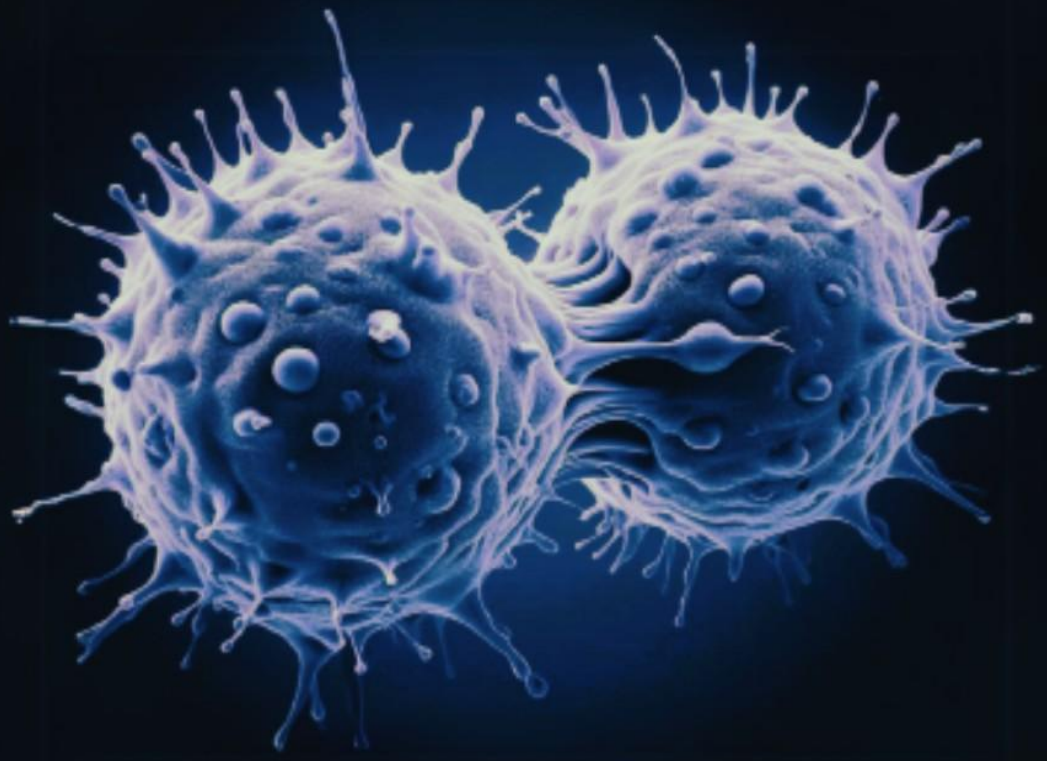

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