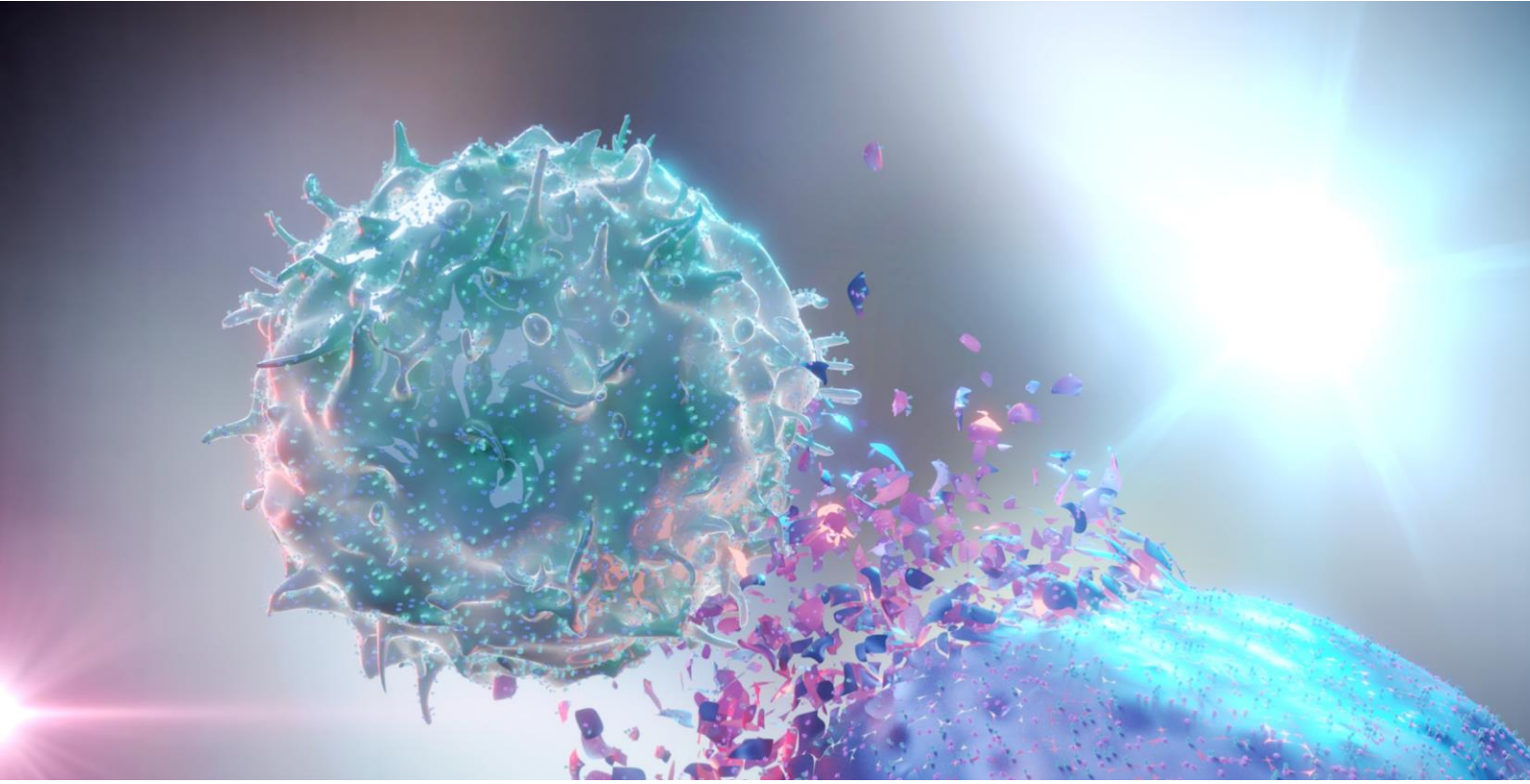
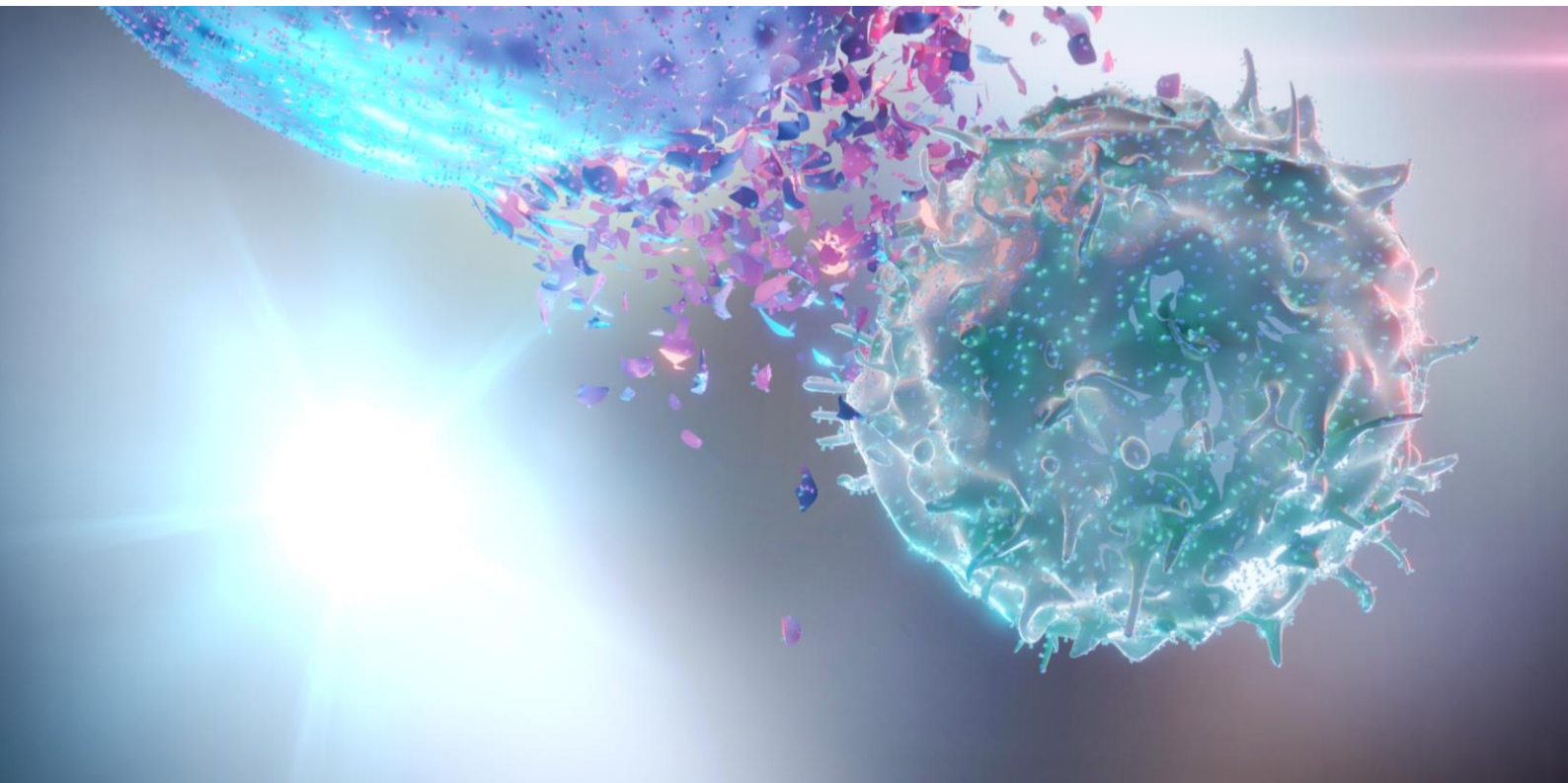


IMMUNO-model Second Annual Conference

From Pre-clinical Models to the Search for Biomarkers in Immuno-Oncology

Book of Abstracts

Bratislava, May 13-15, 2024



IMMUNO-model Second Annual Conference: From Pre-clinical Models to the Search for Biomarkers in Immuno-Oncology
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Bratislava, May 13-15, 2024

Compiled by
Viera Horváthová Kajabová
The authors are responsible for the content of the contributions.

Published by

ISBN 978-80-972111-7-2
EAN 9788097211172

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

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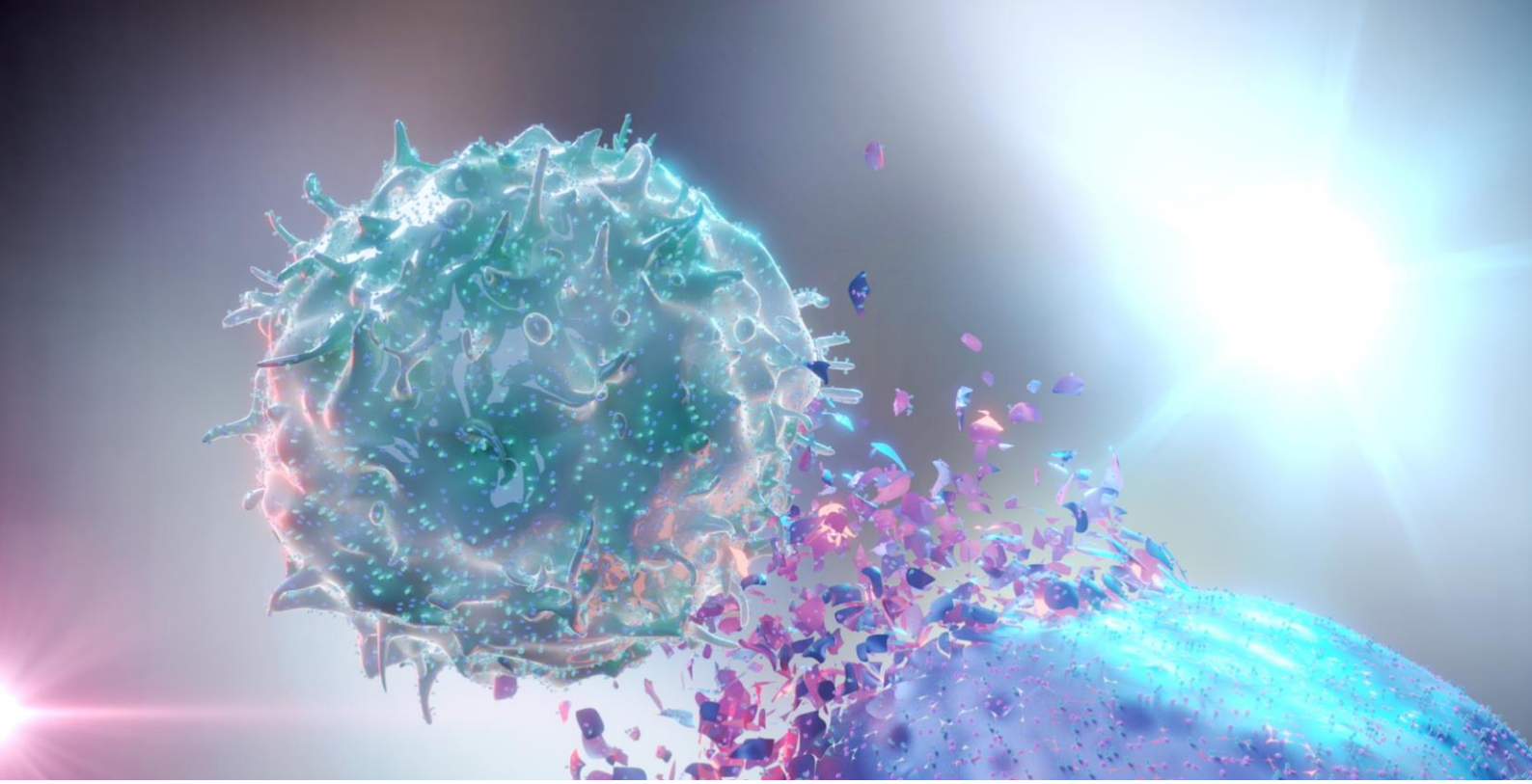

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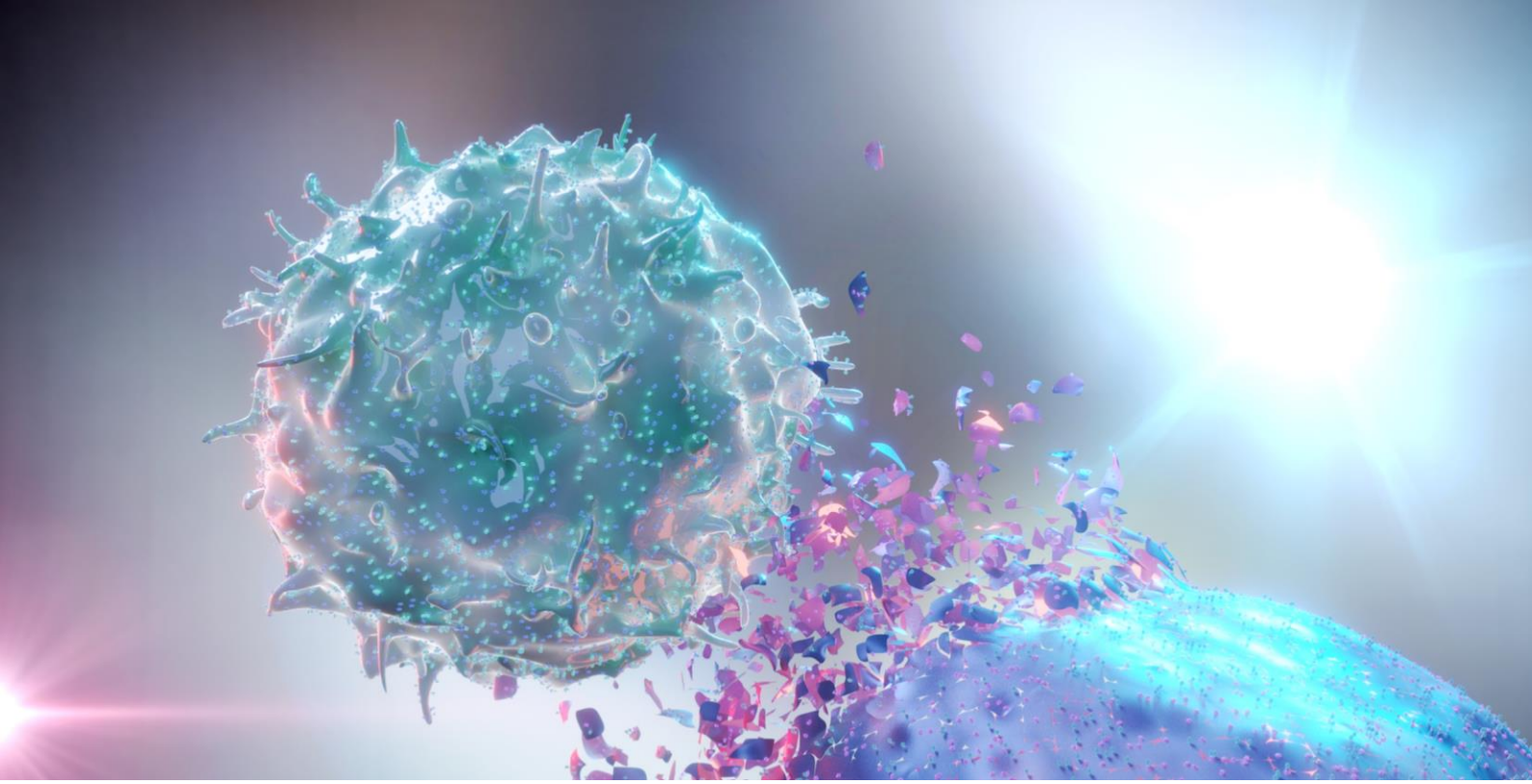
IMMUNO-model
Modelling immunotherapy response and toxicity in cancer



Program

Monday, May 13th , 2024

14:00 - 18:30 **Core group meeting:** only for core group members
(Venue: Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava)

14th and 15th May, 2024

"IMMUNO-model Second Annual Conference: From Pre-clinical Models to the Search for Biomarkers in Immuno-Oncology"
(Venue: HubHub, Twin City C, Mlynské nivy 7816/14, Bratislava)

Tuesday, May 14th, 2024

08:00 - 8:30 Registration

08:30 - 8:45 Welcome
Silvia Pastoreková, General Director of the Biomedical Research Center of the Slovak Academy of Sciences
Eva Martínez Balibrea, IMMUNO-model COST Action Chair

08:45 - 9:30 Opening Keynote Lecture
Leticia Oliveira-Ferrer: IO-Resp: A collaborative project for prediction of treatment response to immuno-oncological agents in ovarian cancer

Scientific session WG1

Chairs: Hanne Haslene-Hox, Lucia Kučerová

09:30 - 10:45 **Bruno Sarmento:** Multicellular 3D immunospheroid models for preclinical development of nanomedicines
Marcin Krzykawski: Novel 3D Cell Culture Models for Immuno-Oncology studies
Uzma Hasan: Functional and spatial analyses of patient-derived tumor fragments to dissect response and resistance mechanisms ex vivo to immune checkpoint inhibitors in cancers

10:45 - 11:15 Coffee break with Poster session WG1 + WG2

Selected abstracts

11:15 - 12:00 **Ana Mitrovic**: Inhibition of cathepsins B and X as an approach to impair cancer stem cells
Marta Maleszewska: Epigenetic inhibitors as potential agents modulating immune response in glioma
Metka Novak: Glioblastoma organoid model as a personalized tool for standard and immuno-therapy research

12:00 - 12:05 **Emanuela Senjor:** Introducing the IMMUNO-model Protocol Database Task Force

12:05 - 13:00 Discussion about the WG1 activities led by Devrim Pesen Okvur & Cristiana Tanase

13:00 - 14:00 Lunch break

Scientific session WG2
Chairs: Emmet McCormack, Lukasz Skalniak

14:00 - 15:15 **Mangala Srinivas**: In vivo imaging for quantitative tracking of immune cells
Calum Leitch: Utilising preclinical animal models and single-cell analysis for cancer immunotherapy studies
Juan Carlos Rodriquez-Manzaneque: Unveiling new extracellular matrix immunomodulatory actions within the tumor microenvironment: the axis ADAMTS1/NIDOGEN1

Selected abstracts
15:15 - 16:00 **Serkan Sen**: Do Statins Influence Prognosis in Patients With Advanced Non-Small Cell Lung Cancer Treated With Nivolumab?
Chiara Calarusso: Identification of mechanism/s of resistance to immunotherapy using a mouse model of low tumor mutational burden
Eleni Douni: Modeling breast cancer and bone metastasis in TgRANKL mice

16:00 - 16:30 Coffee break with Poster session WG1 + WG2

16:30 - 17:30 Discussion about the WG2 activities led by Johannes Haybaeck & Emmet McCormack

18:30 Dinner (WERK restaurant)

Wednesday, May 15th, 2024

08:30 - 9:15 Keynote lecture
Emmanuel Donnadieu: Predicting safety and efficacy of engineered T cells using an ex vivo human model

Scientific session WG3
Chairs: Marleen Ansems, Denis Collins

09:15 - 10:30 **Anguraj Sadanandam**: Stratification of a Panel of Mouse Models of Pancreatic Cancer using Immune Landscape and Extra-cellular Matrix to Predict Response to Synthetic IL-12 Therapy (online lecture)
Denis Collins: Investigating the immune profile of early stage HER2+ breast cancer patients receiving chemotherapy and HER2-targeted therapies

Osman Ugur Sezerman: Multiomics AI models to predict immunotherapy response and biomarkers

10:30 - 11:00 Coffee break with Poster session WG3 + WG4

Selected abstracts

11:00 - 11:45 **Lucia Kučerová**: DNase treatment affects tumor angiogenesis and augments cytotoxic effect of cisplatin in chemoresistant germ cell tumors
Ana Maria Enciu: Various patient-derived Glioblastoma 3D tumor models and their downstream application opportunities
Talip Zengin: In-Silico Cancer Immunology Cohort Discovery Using TCGAnalyzeR

11:45 - 12:45 Discussion about the WG3 activities led by Marleen Ansems & Maurizio Callari

12:45 - 13:45 Lunch break

Scientific session WG4

Chairs: Laura Belver, René Winkler

13:45 - 15:00 **Christian P. Pallasch**: Therapy Resistance in TP53-deficient B-cell Malignancies - the role of EVs and PD-L1 suppressing Macrophages (online lecture)
Magdalena Winiarska: The CAR costimulatory domain influences the process of CAR-CD19 resistance development in lymphoma and B-ALL
Víctor M. Díaz Cortés: Pre-clinical validation of humanized CAR-Ts cells in the industry: experiences from the bench to the bedside

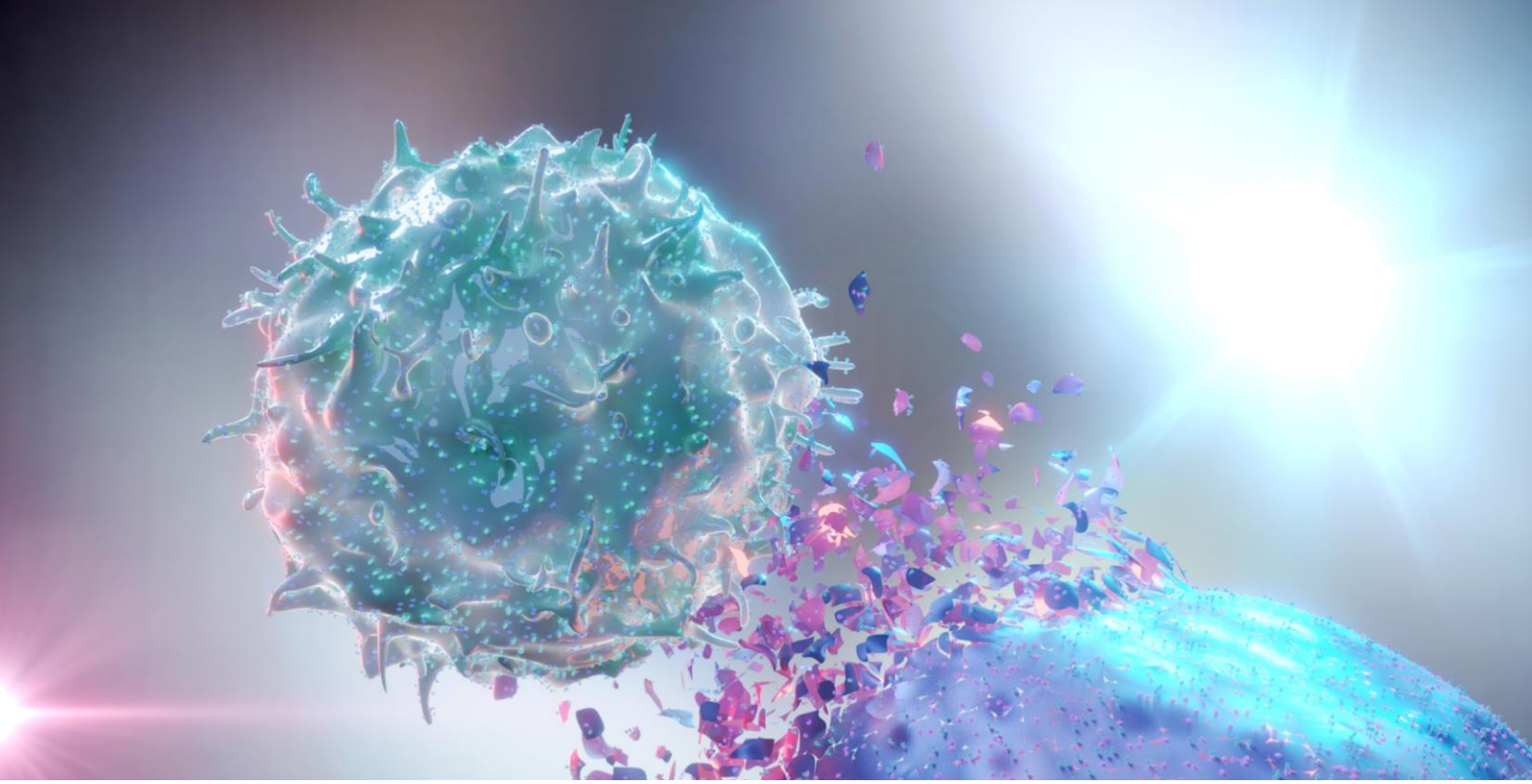
Selected abstracts

15:00 - 15:45 **Jessica Dal Col**: Exploring the role of microRNAs in intercellular communication during Immunogenic Cell Death
Anamaria Bancos: Retinoid Differenciating agents induce Maturation of MDS cells in vitro
Klaudyna Fidyt: The identification of potential targets for CAR-based therapy using transcriptomic and proteomic approaches

15:45 - 16:15 Coffee break with Poster session WG3 + WG4

16:15 - 17:15 Discussion about the WG4 activities led by Laura Belver & Rui Bergantim

17:15 - 17:30 Closing remarks
Eva Martínez Balibrea



Invited speakers

Leticia Oliveira-Ferrer

University Medical Center Hamburg-Eppendorf, Department of Gynecology, Hamburg, Germany

2021	Apl. Professor
Since 2016	Head of the Research Laboratory of the Clinic for Gynecology, University Hospital Hamburg-Eppendorf
2012 – 2016	Research Group Leader at the Clinic for Gynecology, University Hospital Hamburg-Eppendorf
2013	Habilitation in Experimental Oncology with venia legend in Biochemistry at the medical faculty of the University of Hamburg
2009 - 2012	Research Group Leader: Angiogenesis II, Department of Oncology, Haematology and BMT, University Hospital Hamburg-Eppendorf
2006 - 2009	Postdoctoral fellow at the Department of Oncology, Haematology and BMT, University Hospital Hamburg-Eppendorf
2002 - 2006	Postdoctoral fellow at the Institute of Anatomy I, University Hospital Hamburg-Eppendorf
2001	PhD (Dr. rer. nat.), Organic Chemistry Department, University of Hamburg, Germany
1990 – 1995	Studies of Chemistry at the University of Oviedo, Spain

The title of the lecture: **IO-Resp: A collaborative project for prediction of treatment response to immuno-oncological agents in ovarian cancer**

Emmanuel Donnadieu

Institut Cochin, INSERM, Université de Paris, Paris, France

Dr. Emmanuel Donnadieu Ph.D, is a group leader at INSERM Institut Cochin in Paris. Working in the field of Immunology for more than 15 years, he has accumulated a thorough experience in cell signalling and cellular imaging related to T cell physiology in lymphoid organs and tumours. His work uses fluorescence imaging techniques and ex vivo human 3D models to monitor T cell activities in human tumors. His current projects aim at targeting the tumor microenvironment to improve CAR T cell therapy. He belongs to several national and international networks including a European H2020 consortium on CAR T cells.

The title of the lecture: **Predicting safety and efficacy of engineered T cells using an ex vivo human model**

Bruno Sarmento

i3S - Instituto de Investigação e Inovação em Saúde Universidade do Porto, Porto, Portugal

Bruno Sarmento is Principal Investigator, Group Leader and member of the Board of Directors at i3S – University of Porto. Bruno Sarmento is also invited Associated Professor at IUCS-CESPU. His research is focused on developing functionalized nanomedicines, namely nanoformulations for mucosal and target delivery. He has also specialized in mucosal tissue engineering models to validate functionalized nanomedicines and to perform in vitro/in vivo correlation. He published 480 papers in international journals (total citations in Scopus 21000; H-index 73). He has extensive experience in mentoring PhD and Post-doc researchers, and attracted direct competitive funding worth more than 25 M€, at national and international levels. Bruno Sarmento has a strong involvement in EU projects, being WP coordinator in HORIZON-RIA 101057491-GENEGUT, 814558-2 RESTORE and ERA-Chair 951723-MOBILIsE, and coordinator of Litwin IBD Pioneers Program 937924. Bruno Sarmento was the first Chair of the Nanomedicine and Nanoscale Delivery Focus Group of the Controlled Release Society (CRS) and is now Director-at-Large of CRS and member of CRS College of Fellows.

The title of the lecture: **Multicellular 3D immunospheroid models for preclinical development of nanomedicines**

Marcin Krzykawski

Real Research Sp. z o.o., Krakow, Poland

Real Research founder and CEO, PhD in medical sciences, with 14 years of experience in 3D cell culture: extensive knowledge about developing 3D cell culture models and using them in drug discovery. Marcin received several business trainings like “Ignite” at Judge Business School, Cambridge University, “IMPULSE” business training at the Cambridge Maxwell Center, and Kauffman Foundation business training, among others. He is an alumnus of FNP (Polish Foundation for Science), co-author of three patents, and principal investigator of many R&D projects.

The title of the lecture: **Novel 3D Cell Culture Models for Immuno-Oncology studies**

Uzma Hasan

Inserm at the Centre International de Recherche en Infectiologie, Lyon, France. The Lyon Immunotherapy for Cancer Laboratory – LICL, Centre Leon Berard, Lyon, France

For the last ten years, I have been working in the field of innate immunity and cancer. My career path started at Kings College then at St Bart's and Royal London Medical School where I was awarded her PhD in Immunology. My post-doctoral training was based at Schering Plough under the direction of Giorgio Trinchieri working in the field of Toll Like Receptors. I worked on HPV, EBV and HBV immune escape from innate

receptor sensing at IARC, WHO, France. During my stay, I also won an EMBO fellowship to work in the lab of Ruslan Medzhitov, Yale. These experiences led to forming a theme within Inserm in which I continue to understand how chronic diseases can alter immune responses.

I currently have two posts; one is as a PI at the Centre International de Recherche en Infectiologie (CIRI) focused on studying oncoviral deregulation of innate immunity. Here, I work on several projects that linked immunological characterization in HBV infected patients, using CRISPR screens to examine the molecular pathways that altered by its viral proteins. I have recently won grants to study the role of HBV on NK and B cells in patients and will supervise a study in collaboration with the Civil Hospitals in Lyon and Limoges. My other post is at the cancer hospital in Lyon (Centre Leon Berard) at the Lyon Immunotherapy for Cancer Laboratory (LICL). The creation of the LICL in Lyon is to expand our knowledge by characterizing immune responses in cancer patients who receive immunotherapy. The arrival of the LICL with its cutting-edge technologies and scientific valorization from bench to bedside is the main driver that has led me to join and help coordinate projects. My missions as coordinator are to facilitate the running, valorization, funding as well as to solidify interactions between clinicians, researchers and industry for smooth running of the LICL. I work closely with the LICL director Christophe Caux, help manage the in-silico studies he heads, support the translational cytometry research platform (Innovation in Immunomonitoring and Immunotherapies) run by Christine Caux and the Multiplex Immuno-Fluorescence In Situ (MIFIS) platform lead by Bertrand Dubois. I will also help to establish the Tumor Immune Functions EX VIVO platform with Nathalie Bendriss-Vermare.

The title of the lecture: **Functional and spatial analyses of patient-derived tumor fragments to dissect response and resistance mechanisms ex vivo to immune checkpoint inhibitors in cancers**

Mangala Srinivas

Wagenigen University and Research, Wageningen, Netherlands

Mangala is Professor and Head of the Department of Cell Biology and Immunology at Wageningen University & Research (WUR; the Netherlands), since 2021. She is also C.S.O. and co-founder of Cenya Imaging B.V. Mangala completed her PhD at Carnegie Mellon University (USA), after her accelerated B.Sc. (Hons.) from the National University of Singapore. Before joining WUR, Mangala worked for GE Healthcare in Strategy, Search and Evaluation, where she helped identify and evaluate emerging technologies in imaging. She worked to unify different company arms, forming a coherent strategy in imaging, winning an internal "Impact" award. Prior to that, Mangala was at Radboud University Medical Center for about 12 years, initially as a postdoc. She also spent a year full-time for Cenya, with some freelancing as an independent scientific consultant. Mangala's work focuses on agents for in vivo imaging of selected cells. Mangala's early work established 19F Magnetic Resonance Imaging (MRI) for quantitative in vivo cell tracking; she published the first paper on this topic. More recently, her group works on customisable nanoparticles for imaging and advanced personalised medicine applications, incl. cell therapies and immunomodulation. These nanoparticles can be produced at GMP-grade for clinical

use. Her group employs a range of imaging modalities, including fluorescence, MRI, PET, SPECT, ultrasound, and photoacoustic imaging in different disease models, especially cancer and cardiovascular disease. This work has received support from prestigious grants such as an NWO VENI, ERC Starting Grant, ERA-NET CVD grant, ERC Proof of Concept grants, and H2020 PHOENIX, a large EU grant. Her team won the Dutch Venture Challenge in 2015, leading to the creation of Cenya Imaging B.V.

Overall, Mangala's team has advanced a new type of imaging agent from development and fundamental characterisation to clinical readiness and commercialisation. Research on tracking cell migration is vital for understanding biological processes and manipulating them. Trafficking of immune cells is crucial for their function. The field has taken on added importance with the advent of cell-based therapies. However, we often lack a mechanistic understanding of these complex processes. Thus, noninvasive imaging is necessary to study cell populations.

At WUR, her work involves immunomodulation through multifunctional nanoparticles, and noninvasive imaging. WUR's uniquely wide range of animal and plant provides a perfect playground for imaging studies. As Chair, she is directly responsible for the Department, overseeing career development, finances, education, and management. Her Department teaches several large courses, reaching about 2000 students/year, and currently consists of about 30 people. Mangala served as Chair for two full terms at the Young Academy of Europe (YAE), and then as Outgoing Chair (2020/21). She also served as Category Chair for the European Molecular Imaging Meetings, twice. Mangala was shortlisted for the WMIS WIMIN Outstanding Leadership award in 2019. Through the YAE, she has promoted early academics, focusing on Open Science, rewards and recognition, and diversity and inclusion. She was involved in the EU project, CALIPER, focusing on gender equality in academia, and has published a few articles in the broadly-read magazine, Nature, to raise awareness. She also serves on the Scientific Advisory Board of Open Research Europe. Most importantly, Mangala is a busy mother of 3 young children, who constantly inspire (frustrate, and amuse) her.

The title of the lecture: **In vivo imaging for quantitative tracking of immune cells**

Calum Leitch

University of Bergen/ Kinn Therapuetics, Bergen, Norway

Calum Leitch from Glasgow, Scotland. Leitch studied Molecular and Cellular Biology as an undergraduate and received a master’s degree at the University of Glasgow. In 2012, Leitch entered a PhD program at the University of Bergen in Norway. The PhD work was performed in the group of Prof. Bjørn Tore Gjertsen and focused on the identification and development of small molecule therapies for the treatment of acute myeloid leukemia. From 2017 Leitch has been employed by Kinn Therapuetics a CRO specializing in preclinical models for cancer therapy. Since 2023 Leitch has been employed as researcher in the group of Prof. Emmet McCormack at the University of Bergen. His project is focused on developing novel models for testing immunotherapy in ovarian and hematological malignancies.

The title of the lecture: **Utilising preclinical animal models and single-cell analysis for cancer immunotherapy studies**

Juan Carlos Rodriquez-Manzaneque

GENYO - Centre for Genomics and Oncological Research, Granada, Spain

Dr Rodriguez-Manzaneque obtained his PhD in Biochemistry and Molecular Biology at the Universidad Complutense de Madrid (Spain). During his postdoctoral training at Harvard Medical School and UCLA (USA), he was introduced studying the extracellular matrix and its relevance in angiogenesis and tumor progression. Later, he started leading his own group, first in Vall d'Hebron Research Institute (Barcelona, 2002-2008), and finally in GENYO (Granada, 2008-actually) as the Principal Investigator of the Proteases and Extracellular Matrix group. During this time, he has approached a deep characterization of the proteolytic activity of ADAMTS proteases. On top of the identification of various substrates of this family of proteases and its activity on complex in vitro assays, he later initiated studies using animal tumor models. Significantly, Dr Rodríguez-Manzaneque's team is contributing to new perspectives of ECM remodeling, affecting tumor plasticity and neo-vascularization, and more recently, with direct modulatory implications in the tumor immune compartment. Among the human cancers studied by this group there are melanoma, sarcoma, glioblastoma, and breast and lung cancer. Importantly, their studies are paradigm of the diverse activity of extracellular proteases, contributing as tumor inducers in some cases, and tumor suppressors in others. Furthermore, such contributions already highlighted the importance of the microenvironment to support such paradoxical activities. Later work performed in genetically modified murine models allowed the identification of specific roles depending of stromal and tumoral origin, including relevant findings that involve the immune response. Unveiling the contribution of ADAMTS1 and some of its substrates to educate macrophages is one of his current focus, in coordination with pan-cancer bioinformatic analysis to determine the relationship between matrisome-related signatures and the efficacy of immunotherapies.

The title of the lecture: **Unveiling new extracellular matrix immunomodulatory actions within the tumor microenvironment: the axis ADAMTS1/NIDOGEN1**

Denis Collins

Dublin City University, School of Biotechnology, Dublin, Ireland

Denis Collins is a translational research scientist. Denis has a degree in Biochemistry from University College Cork. He graduated from Dublin City University with his PhD on drug resistance in lung cancer in 2008. Since then, Denis has been awarded an Irish Research Council Enterprise Partnership Scheme Postdoctoral Fellowship and a Roche Postdoctoral Fellowship to support his research. He is a past Chair of the Junior Council of the Irish Association for Cancer Research (IACR) and is presently a member of the IACR Senior Council.

Denis is currently The Caroline Foundation Senior Research Fellow and Co-PI on a Science Foundation Ireland Strategic Partnership Programme Award, ACORN, that is studying the potential of small molecule tyrosine kinase inhibitors as treatments in multiple cancer types including breast, lung, gastric, oesophageal and skin cancer.

His research team is also funded by the Cancer Clinical Research Trust and the Irish Research Council. These studies investigate new antibody drug conjugate

combinations and the immune response mediated by NK cells and monoclonal antibody therapies in breast cancer. Immune-based biomarker studies using patient blood and tumour are underway in lung, breast, gastric/oesophageal and skin cancers.

The title of the lecture: **Investigating the immune profile of early stage HER2+ breast cancer patients receiving chemotherapy and HER2-targeted therapies**

Osman Ugur Sezerman

Department of Biostatistics and Bioinformatics, Institute of Health Sciences, Acıbadem University, Istanbul, Türkiy

B.Sc. Electrical Engineering, M.Sc. Biomedical Engineering Dept. Bosphorous University and PhD at Boston University Biomedical Engineering Dept. Established the first Bioinformatics Graduate and undergraduate programs in Turkey at Sabanci University then moved to Acibadem University School of Medicine to establish the first Clinical Bioinformatics Program . He is specially focused in developing algorithms for Multi Omics data analysis, Identification of individualized disease aetiology and therapy targets via network based analysis of omics dataanalysis, , personalized medicine and wellness bioinformatics; more than 170 publications. More than 1500 citations. Involved in several EU projects ;European Joint Project on Rare diseases project (EJPRD) responsible for developing pathway based analysis tools for Rare disease data. IT Future of Cancer ITFOC ERA-Net project on personalized Cancer, DigiTwins Flagship application, CHARME Cost Action on Harmonization of Standards, BEAT-Primary Ciliary D COST Action to name a few.

He is also the founder of three SMEs Epigenetiks Inc where he is providing services to several hospitals on omics data analysis for diagnostics and personalized therapy .The other two companies Eternans Ltd. U.K, which is designing peptide based drugs for Cancer, COPD and CKD and Nurture Welness LLC USA which is providing omics data and AI based personalized wellness services.

The title of the lecture: **Multiomics AI models to predict immunotherapy response and biomarkers**

Christian P. Pallasch

University of Cologne, Center for Molecular Medicine Cologne, Köln, Germany

Education and Professional Career
2019: Professor (W2) “Tumor microenvironment and therapy resistance” Univ. of Cologne
2018: Attending Physician, Dep. I for Internal Medicine
2017: Venia legendi for Internal Medicine (Habilitation), University of Cologne
2015: Board Certificate Internal Medicin, Hematology and Oncology
since 2012: Research Group Leader at the Medical Clinic I of the University Hospital Cologne
2009 - 2012: Postdoctoral Fellow, MIT, Department of Biology, Koch Institute, Advisor: Prof. M.T. Hemann, PhD
2004 - 2009: Postdoctoral Research Fellow University of Cologne, Germany, Advisors: Prof. Dr. C.M. Wendtner and Prof. Dr. M. Hallek
1999 - 2003: MD thesis, Institute for Human Genetics, University of Saarland Homburg/Saar (Grade: Summa cum laude, highest honors) Advisor Prof.Dr. E. Meese, Scholar at the Graduiertenkolleg “Cellular regulation and growth”
1996 - 2003: Medical school, (Final grade: Sehr gut, highest honors), University of Saarland Homburg/Saar, University of Oslo, Unversity of Cologne)
Awards and Distinctions
2019: Medical Faculty Award
2015: NRW-Junior Research Group Grant
2010: Research fellowship of the Deutsche Forschungsgemeinschaft
2004: “Eduard-Martin-Preis” by the University of Saarland
2000: DFG research scholarship by Graduiertenkolleg Homburg/Saar
1997: Talented Student Scholarship (Begabtenförderung) “Evangelisches Studienwerk Villigst”

The title of the lecture: **Therapy Resistance in TP53-deficient B-cell Malignancies - the role of EVs and PD-L1 suppressing Macrophages** (online lecture)

Magdalena Winiarska

Mossakowski Medical Research Institute, Warsaw, Poland

Magdalena Winiarska was awarded a PhD by the Medical University of Warsaw (MUW) in 2008 and DSc in 2018. She was a visiting scientist to the lab of Prof. Efremov, International Centre for Genetic Engineering and Biotechnology, Italy, Cancer Laboratory led by Prof. Olive, Institut Paoli Calmettes, Aix-Marseille Université, France. She has secured funding in several grants funded by Polish (National Science Centre, National Centre for Research and Development) and European (ERC Starting Grant) institutions. During her career, she was a mentor of more than 20 graduate students and supervisor of five PhD students.

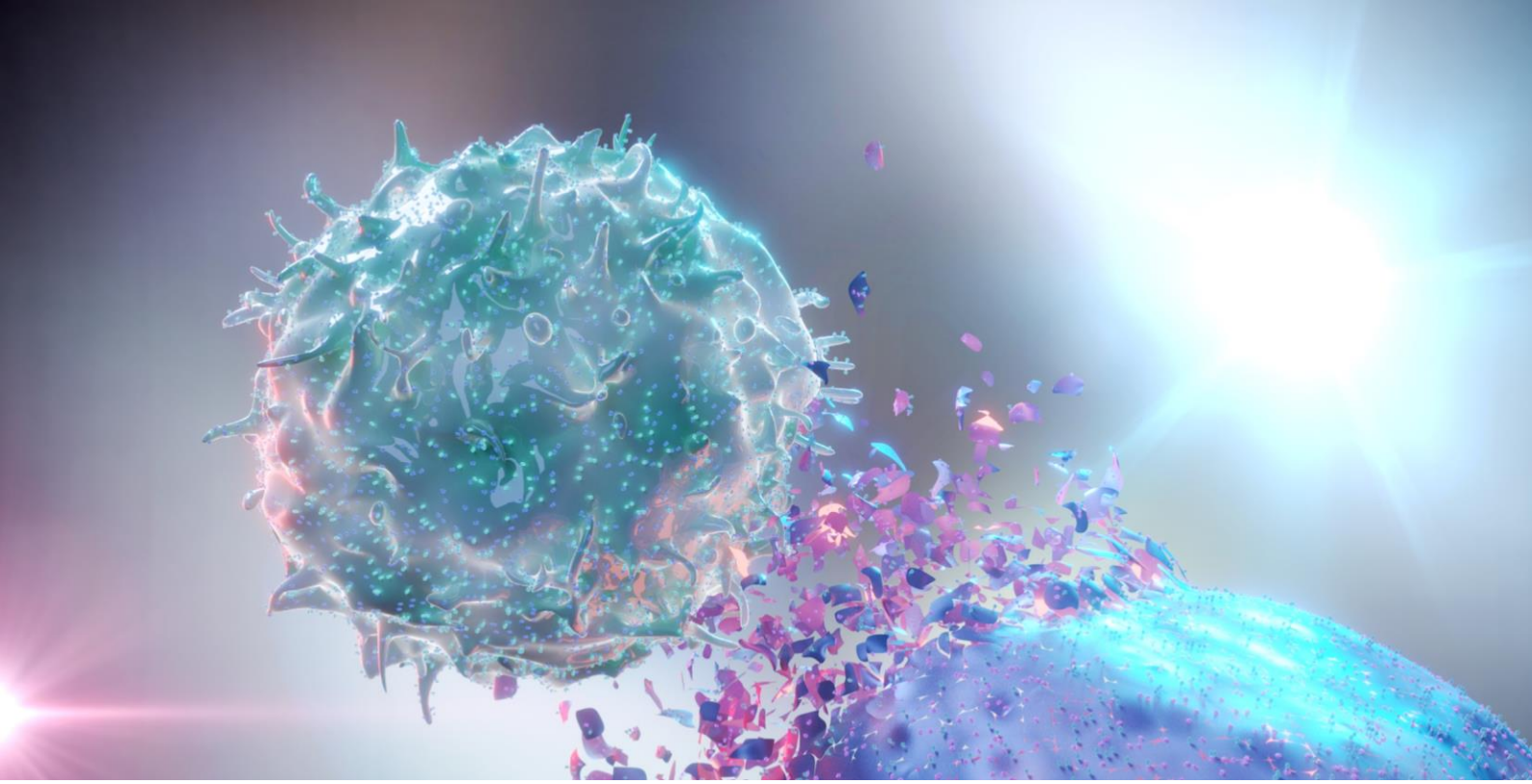
The title of the lecture: **The CAR costimulatory domain influences the process of CAR-CD19 resistance development in lymphoma and B-ALL**

Víctor M. Díaz Cortés

Research Director of OneChain Immunotherapeutics, S. L., Barcelona, Spain

During my PhD, I had the privilege of working at renowned institutions such as the Hospital Vall d'Hebron in Barcelona and the New York University School of Medicine (NYU). My research focused on studying the role of proteases in stimulating the growth, invasion, and angiogenesis of pancreatic cancer cells. Following my PhD, I was awarded a "Marie Curie" Postdoctoral contract by the European Union, which brought me to the San Raffaele Biomedical Science Park in Milan where I applied to characterize several homeobox protein complexes. After this first postdoctoral, I returned to Spain to pursue a second postdoctoral position at IMIM-Hospital del Mar focused on studying the process of epithelial-to-mesenchymal transition (EMT) and its role in cancer progression. After completing my postdoctoral training, I joined Universitat Pompeu Fabra as a Lecturer in 2009 and later transitioned to the position of Assistant Professor in 2015, as an independent researcher. My work primarily revolved around elucidating the mechanisms and pathological implications of Snail1 ubiquitination, degradation, and stabilization. In 2020, I made a bold career move and transitioned to the private industry as the Research Director and Torres Quevedo Researcher at OneChain Immunotherapeutics, a groundbreaking Spin-Off company from the prestigious “Josep Carreras Institute against Leukemia”, dedicated to developing innovative immunotherapies using CAR-T cells to combat haematological neoplasms. My primary accomplishments in this role include obtaining Orphan Drug Designations from both the EMA (EU/3/21/2535) and FDA (DRU-2023-9582) for a CAR-T therapy targeting acute lymphoblastic leukemia/lymphoblastic lymphoma. I have also participated in generating the clinical dossier (IMPD) which received a positive evaluation for a clinical trial (CT) by the “Agencia Española de Medicamentos y Productos Sanitarios” (AEMPS, EudraCT 2021-002333-42). This CT is an autologous therapy “first-in-human”. I am currently directing R&D processes at OneChain where I am proud to participate in several national competitive projects from the Spanish Ministry of Science and Innovation and to coordinate an EU international consortium aiming to develop an allogeneic platform for cell therapies (HORIZON-EIC-TRANSITION project, 101113067-CARxALL).

The title of the lecture: **Pre-clinical validation of humanized CAR-Ts cells in the industry: experiences from the bench to the bedside**



Speaker Abstracts

IO-Resp: A collaborative project for prediction of treatment response to immuno-oncological agents in ovarian cancer

Leticia Oliveira-Ferrer[1], Louisa Hell[1,2], Marius Witt[3], Yi Ding[1], Kathrin Eylmann[1], Maila Rossberg[1], Walter Fiedler[3], Jürgen Kupper[2], Barbara Schmalfeldt[1], Franziska Brauneck[3*], Tabea Sturmheit[2*] Jasmin Wellbrock[3*]

[1] *Department of Gynecology, University Hospital Hamburg-Eppendorf, Hamburg, Germany;*
[2] *2cureX GmbH, Hamburg, Germany;*
[3] *II. Medical Clinic and Polyclinic (Oncology), University Hospital Hamburg-Eppendorf, Hamburg, Germany*

Ovarian cancer (OC) is the most lethal gynecological malignancy, with an expected 5-year mortality rate of >60%. Due to the lack of symptoms at early stages, most patient are in an advanced stage at the time of diagnosis, and display a substantial heterogeneity between the primary tumor site, extensive intraperitoneal metastases and frequently accompanying ascites tumor cells.

In OC, although preclinical studies have shown promising results, the efficacy of immunotherapy has been rather moderate showing encouraging clinical results only in a small subgroup of patients. Omics analyses have further identified an immunologic OC subtype associated with a more favorable prognosis. These data suggest that certain OC subpopulations are suitable for immunotherapeutic approaches. Thus, the aim of this joint project was to gain a comprehensive insight into the characteristic immune profiles of OC patients and provide a basis for the subsequent functional evaluation of potential therapeutic candidates.

Solid tumor tissue samples, ascites and peripheral blood (PB) were collected from patients with OC during debulking surgery in our institution. Tumor cell aggregates were extracted from solid tumor and ascites samples and characterized with regard to their stem cell properties and immune cell markers using multiparametric flow cytometry (MFC). In addition, the phenotype and expression of co-regulatory markers of the immune cell compartment in PB, ascites and tumor tissue, if available from the same patient, were assessed by MFC. Finally, using different approaches we performed in vitro functional tests to explore the role of diverse immune checkpoints in the anti-tumorogenic effect of macrophages and CD8+ T cells.

In the tumor cell compartment, we found that ligands for co-inhibitory T-cell-receptors PD-L1 and PVR, as well as phagocytosis-associated molecules CD47 and Calreticulin were higher expressed by tumor cells isolated from ascites, rather than from solid tumors. When considering the tumor stem cell characteristics, tumor aggregates from solid tumors reached higher plasticity scores than those from malignant ascites, meaning they showed higher expression of plasticity markers (CD10, CD24, CD90, CD133). Interestingly, tumor cell subpopulations displaying ≥1 plasticity marker, independently of the compartment, showed significantly higher levels of the investigated immunological targets (PD-L1, PD-L2, PVR, PVRL2, CD47). Regarding the immune cell population, PB and ascites of patients with OC contain an increased frequency of immune suppressive tumor associated macrophages (TAMs) expressing M2 markers such as CD163, CD204 and CD206, and a decreased frequency of M1 macrophages.

Interestingly, OC-derived M2 TAMs showed an increased expression of the co-inhibitory receptors TIGIT, TIM-3 and LAG-3. In this context, blockade of TIGIT with a monoclonal antibody led to repolarization of TIGIT+ M2 TAMs into M1 macrophages, and in consequence to augmented phagocytic capacity when combined with CD47 blockade in vitro. Further, we identified an OC-characteristic CD8+ T cell population defined by high PD-1 levels and increased co-expression of TIGIT and CD39. TIGIT blockade in vitro showed no significant effects on T cells, whereas blocking the enzymatic activity of CD39 led to enhanced proliferation and activation of CD8+ T cells and increased IL-2, TNF-α, Granzyme A and B secretion.

We identified two promising immunotherapeutic approaches for OC, namely the blockade of TIGIT to further enhance anti-CD47-mediated phagocytosis and the blockade of CD39 enzymatic activity leading to the re-activation of CD8+ T cells. Additionally, our data indicate a more pronounced expression of immune markers in tumor cells derived from ascites than in those from the solid tumor, pointing to a patient subpopulation that could especially benefit from personalized immunotherapy. In this context, patient-specific co-cultures of ascites-derived tumor and immune cells could provide an opportunity for the identification of patients who are likely to respond to immune-oncological agents.

Multicellular 3D immunospheroid models for preclinical development of nanomedicines

Bruno Sarmento

i3S - Instituto de Investigação e Inovação em Saúde Universidade do Porto, Portugal

In the ever-evolving landscape of biomedical research, 3D Cell Culture (3DCC) systems have emerged as pivotal tools for mimicking *in vivo* environments and fostering more physiologically relevant cellular responses. It is widely known that 3DCC models may play a crucial role in preclinical studies thus reducing costs of drug testing and avoiding the excessive need for animal models. In our study, we presented a potency of 3D co-cultures of cancer cells and PBMCs (Peripheral Blood Mononuclear Cells) and we indicated their use in further stages of drug efficacy testing and immuno-oncology studies.

We used protein-based hydrogel LifeGel® for creating 3D cell co-culture of cancer and immune cells. Thus, we demonstrated the new *in vitro* model on which the potency of immunotherapeutic antibodies: immune checkpoint inhibitors, was verified. The viability of 3D structures was measured with colorimetric CellTiter-Blue assay (Promega). The morphological parameters of tumor spheroids after co-culture with immune cells were assessed using AnaSP software. Furthermore, we confirmed that not only immortalized cancer cells but also 3D structures of patient-derived tumoroids or patient-derived xenograft models may be used in co-culture with immune cells for drug screening and immuno-testing purposes. We also confirmed the phenomena that the 3D structures grow on top of the hydrogel making them accessible to large molecules like: antibodies, Antibody-Drug-Conjugates (ADCs), cytokines, CAR-T therapy, oncolytic viruses, etc.

In the realm of cancer research, 3D cell cultures showcase a predictive power in assessing the mechanistic effects and anticancer effects of drugs that rivals traditional *in vivo* research models. By providing a biomimetic microenvironment, the hydrogels facilitate the formation of complex cellular structures, enabling reliable representation of tissue architecture and cellular interactions. LifeGels® biophysical parameters can be modified: hardness, density and elasticity, making a solution adapted to every type of 3D cell structures. Moreover, the unique property of our approach is to use 3DCC in immune-oncology research as high-throughput screening (HTS), high-content analysis and fully-automated processing.

Novel 3D Cell Culture Models for Immuno-Oncology studies

Justyna Kocik-Krol, David Earnshaw, Renata Krzykawska, Marcin Krzykawski

Real Research, Krakow, Poland

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Functional and spatial analyses of patient-derived tumor fragments to dissect response and resistance mechanisms *ex vivo* to immune checkpoint inhibitors in cancers.

Mathilde Persch, Justine Berthet, Thibault Andrieu, Christine Caux, Axel Nkodia, Christine Bardin, Sophie Leon, Severine Tabone-Eglinger, Christine Ménétrier-Caux, Bertrand Dubois, Christophe Caux, Nathalie Bendriss-Vermare*, Uzma Hasan*.

The Lyon Immunotherapy for Cancer Laboratory (LICL). Le Centre de Recherche en Cancérologie de Lyon (CRCL, UMR Inserm 1052 – CNRS 5286) Centre Léon Bérard. Bureau: Cheney B 3rd floor - Centre Léon Bérard, 69373. Cedex Lyon 08

**co-last.*

Immunotherapy (IT) has revolutionized cancer treatment. Several types of IT, including immune checkpoint inhibitors, give a durable clinical response, but their efficacies vary, and only subsets of cancer patients can benefit from them. Immune infiltrates in the tumor microenvironment have been shown to play a key role in tumor development and affect the clinical outcomes of cancer patients as well as their response to IT.

We currently possess expertise to analyse tumour cell death and proliferation in response to therapeutic agents on fresh human tumour slices (250µm) for up to 72h. This *ex vivo* assay allows to maintain the preserved TME architecture as found in the patient. At the LICL, we have recently embarked on an initiative to evaluate immune profiles in both tonsil tissue and various human cancer types. This involves utilizing ex vivo tissue or tumor cultures. In these cultured slices, we meticulously characterize immune cell populations using advanced techniques such as multispectral flow analysis and imaging. Here we will discuss our findings; showing optimisation of technics and the production of our proof-of-concept data in immune monitoring.

The goal is to examine the early immunological response in response to IT such as: 1) the baseline properties of the tumor, 2) the dynamics of the treatment response, 3) the functional importance of specific cell types or cytokines in the treatment response, and 4) the impact of innovative IT as single treatment or in combination with other therapies. This will facilitate the achievement of our long-term objectives is in accordance with the CLB/CRCL's "bench to bedside" policy.

This work is supported by La Ligue Contre le Cancer.

Inhibition of cathepsins B and X as an approach to impair cancer stem cells

Ana Mitrović[1,2], Damijan Knez[2], Izidor Sosič[2], Stanislav Gobec[2], Janko Kos[1,2]

[1] *Department of Biotechnology, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia*
[2] *Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia*

Tumour recurrence and resistance to conventional therapies continue to be the major challenge affecting the success of cancer treatment. This is mainly due to the presence of a small population of cancer stem cells (CSCs). Efficient targeting of CSCs could result in prolonged patients' disease-free survival or full recovery, and is therefore a promising strategy for developing new therapeutics for cancer treatment. Lysosomal cysteine cathepsins are important enzymes involved in multiple stages of tumour progression and have been identified as targets for cancer therapy. An important contribution is attributed to cathepsins B and X, which are unique due to their carboxypeptidase activity and compensational role they play between each other.

We have shown that the protein levels and activity of cathepsins B and X are increased in CSCs isolated from different breast cancer cell lines based on their ability to form tumorspheres compared to single adherent differentiated tumour cells. The increased activity of both cathepsins can be selectively regulated at multiple levels, including by specific small molecule inhibitors. Here, we demonstrated the effect of selective, reversible small molecule inhibitors of cathepsins B and X on CSCs isolated from breast cancer cell lines based on their ability to form tumorspheres. Cathepsin B and X inhibitors affected the phenotype of CSCs by decreasing the expression of stemness markers and markers of mesenchymal cell phenotype. We also showed that inhibition of cathepsin B and X affects signalling pathways in CSCs that are important for tumour progression, among them several signalling kinases Additionally, the effect of cathepsin B and X inhibition on CSCs was also demonstrated in functional assays that mimic tumour progression processes.

In summary, the results of this study show that inhibition of cathepsin B and X has effects on CSCs and represents a promising strategy to reduce a pool of CSCs. The use of cathepsin B and X inhibitors is thus a promising approach to improve existing antitumor therapy.

This work was supported by grants J3-3071 to AM and P4-0127 to JK from Slovenian Research and Innovation Agency (ARIS).

Epigenetic inhibitors as potential agents modulating immune response in glioma

Marta Maleszewska[1,2], Aleksandra Steranka[2], Magdalena Smiech[2], Michał Dąbrowski[2], Bozena Kaminska[2]

[1] *Department of Animal Phyliology, Faculty of Biology, Warsaw University, Warsaw, Poland*
[2] *Nencki Institute of Experimental Biology, Laboratory of Molecular Neurobiology, Warsaw, Poland*

Immunotherapy is emerging as a promising approach to completely eradicate cancer. However, it exerts low responses in glioblastoma patients. GBM is a "cold" tumor, which lacks infiltration of functional T and NK cells and actively suppress their activities. This highly immunosuppressive microenvironment is generated by tumor cells and tumor-associated myeloid cells, such as microglia and recruited regulatory monocytic cells. Microglia and peripheral macrophages accumulate in malignant glioblastomas (GBMs), but instead of launching anti-tumor responses, these cells are polarized to immunosuppressive phenotype which lasts a long time and contributes to a "cold" immune microenvironment of GBMs. Transcriptome analysis of microglial primary cultures exposed to glioma-conditioned medium (GCM) or lipopolysaccharide (LPS) revealed activation of distinct signaling and metabolic pathways resulting in different patterns of gene expression. As these two phenotypes are relatively stable we sought to determine if changes in gene expression could be mediated by epigenetic mechanisms and if epigenetic enzyme inhibitors could modulate immune responses of microglia.

We demonstrate that microglia pre-exposed to glioma attain an "epigenetic memory" and display reduced responses after stimulation with LPS. Inhibitory histone marks – H3K9me3 and H3K27me3 manifested 24h after GCM exposure at gene regulatory regions and associate with consolidation of acquired phenotype. To investigate the role of histone modifications in microglia polarization, cells were exposed to GCM or LPS alongside selected inhibitors. Inhibitors targeting enzymes responsible for H3K9me3 demonstrated greater efficacy in impeding the effects of GCM compared to those targeting H3K27me3. Hence, H3K9me3 appears pivotal in stabilizing the immunosuppressive phenotype. These findings underscore the significance of epigenetic modifications in microglia polarization, emphasizing that inhibition of histone-modifying enzymes prevents the acquisition of a distinct phenotype. As epigenetic alterations are reversible, this presents promising avenues for the utilization of epigenetic inhibitors in glioma immunotherapy.

This work was supported by grants NN 301786240 and HOMING PLUS/2011-3/7.

Glioblastoma organoid model as a personalized tool for standard and immuno-therapy research

Metka Novak[1], Bernarda Majc[1], Anamarija Habič[1,2], Marta Malavolta[3], Miloš Vittori [4], Andrej Porčnik[5], Roman Bošnjak [5], Jernej Mlakar[6], Alenka Matjašič[6], Andrej Zupan[6], Marija Skoblar Vidmar[7, 8], Tamara Lah Turnšek [1], Aleksander Sadikov [3], Barbara Breznik[1]

[1] *Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, 1000 Ljubljana, Slovenia*
[2] *Jožef Stefan International Postgraduate School, Nanosciences and Nanotechnologies, 1000 Ljubljana, Slovenia*
[3] *University of Ljubljana, Faculty of Computer and Information Science, 1000 Ljubljana, Slovenia*
[4] *University of Ljubljana, Biotechnical Faculty, Department of Biology, 1000 Ljubljana, Slovenia.*
[5] *Department of Neurosurgery, University Medical Centre Ljubljana, 1000 Ljubljana, Slovenia*
[6] *Institute of Pathology, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia*
[7] *Institute of Oncology, University Medical Centre Ljubljana, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia*
[8] *Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia*

Glioblastoma remains a lethal disease despite current standard treatment with maximal surgical resection, radiation, and temozolomide therapy. One aspect that hinders drug development and study of GB therapeutic response is the lack of an appropriate model that represents the complexity of patients' tumours.

To address these limitations, we investigated the effects of irradiation and TMZ using novel GB organoids that reflect the tumour heterogeneity and resistance to clinical therapies. Furthermore, several novel therapies are currently under investigation, of which immunotherapy with natural killer (NK) cells holds great potential.

Therefore, we have established a 3D glioblastoma model that can be used for high-throughput evaluation of NK cell therapy effect and to study the patient-specific interactions between GB cells and NK cells.

This work was supported by the Slovenian Research Agency (grants P1-0245, P2-0209, J3-4504, J3-2526, NC-0023, Young researcher grants 10040137, 10040147) and by the EU Program of Cross-Border Cooperation for Slovenia-Italy Interreg TRANS-GLIOMA. The research was funded by HE project Twinning (CutCancer; 101079113) and funding from the EU's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie - Innovative Training Network 2020, N° 956394.

Introducing the IMMUNO-model Protocol Database Task Force

Emanuela Senjor[1], Vinton Cheng[2], Christina Sakellariou[3], Leticia Oliveira Ferrer[4], Emmet Mc Cormack[5], Laura Belver[6], Marleen Ansems[7], Maria Dudau[8], Devrim Pesen Okvur[9], Barbara Breznik[10], Eva Martinez-Balibrea[11]

[1] *Jozef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia*
[2] *Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom*
[3] *Department of Immunotechnology, Lund University, Lund, Sweden*
[4] *University Medical Center Hamburg-Eppendorf*
[5] *Department of Clinical Science, University of Bergen, Norway*
[6] *Josep Carreras Leukemia Research Institute, Barcelona, Spain*
[7] *Radboud Institute for Molecular Life Science, Netherlands*
[8] *Biochemistry-Proteomics Department, Victor Babes National Institute of Pathology, Bucharest, Romania*
[9] *Izmir Institute of Technology, Turkey*
[10] *National Institute of Biology, Ljubljana, Slovenia*
[11] *Catalan Institute of Oncology, Barcelona, Spain*

IMMUNO-model Cost Action was established to connect researchers in the field of preclinical immuno-oncology models. The primary aim of IMMUNO-model Cost Action is to promote collaboration and sharing of knowledge to contribute to the successful application of immunotherapy preclinical models. One of the objectives is to collect immuno-model protocols from Cost Action members and publish them in an open-access database to facilitate preclinical immuno-oncology research. The Protocol Database Task Force was established to lead these efforts. Protocols in the database will adhere to a standardized template to include all necessary information for the successful implementation of the protocol. Protocol collection from Cost Action members will be documented, to ensure traceability. Important tasks for the task force will also include the engagement of Cost Action members to contribute with protocols and dissemination of the database, in coordination with IMMUNO-model Working Group 5.

The IMMUNO-model Core Group has conducted a survey to assess the types of protocols available among the Cost Action members. The survey responses (71) focused on in vitro protocols (67.6%), derived from human material (67.6%), solid tumors (85.9%), with colorectal (15.5%), breast (14%), lung (11.3%) and pancreatic (7%) being the most common tumor types. Hematologic tumors represented 11.3% of responses. The most common biological source for tumor models is primary cancer cells (38%), followed by cancer cell lines (36.6%). Meanwhile, in 12.7% of provided responses, primary immune cells are incorporated into the model. 25.3 % of responses characterized the protocols as immuno-model protocols, and 26.8% characterized them as sample processing, the remaining 47.9 % remain uncategorized. Overall, these results provide valuable insight into the possible protocols to be submitted into the database, laying a foundation for the database creation. In summary, the creation of a publicly available database of protocols will benefit the immuno-oncology scientific community by fostering collaboration, knowledge exchange and supporting new scientific discoveries.

This work was supported by IMMUNO-model CA21135.

In vivo imaging for quantitative tracking of immune cells

Mangala Srinivas

Wagenigen University and Research, Cell Biology and Immunology, Wageningen, Netherlands

My group works with polymer-entrapped perfluorocarbon (PFC) nanoparticles for in vivo imaging using 19F Magnetic Resonance Imaging (MRI), ultrasound, fluorescence and nuclear imaging. These particles have been spun-out to a company for clinical imaging, and production at GMP grade. PFCs are simultaneously lipophobic and hydrophobic. The production process, consisting of a triphasic continuous, microfluidic system, results in particles of about 200 nm diameter with a fractal, multicore structure. We have applied these particles to tracking various cell types in vitro and in vivo in a range of disease models, in a longitudinal and quantitative manner, and are approved for a clinical trial in the NL. Some cell types that we have labelled and imaged are primary human dendritic cells (moDCs, pDCs, mDCs), T cells (incl. CAR T cells), macrophages and stem cells, using a range of imaging modalities, such as quantitative in vivo 19F MRI, fluorescence and PET.

Utilising preclinical animal models and single-cell analysis for cancer immunotherapy studies

Calum Leitch[1,2], Cara Wogsland[1], Satwinder Kaur Singh[3], Katrin Kleinmanns[2], Erik Manting[3], Bjørn Tore Gjertsen[2,4], Mihaela Popa[1]

[1] *KinN Therapeutics AS, Jonas Lies vei 91B 5021, Bergen, Norway*
[2] *Centre for Cancer Biomarkers CCBIO, Department of Clinical Science, University of Bergen, 5021 Bergen, Norway*
[3] *Mendus AB, Galileiweg 8, 2333 BD Leiden, The Netherlands*
[4] *Department of Medicine, Haematology section, Haukeland University Hospital, 5021 Bergen, Norway*

The arrival of immunotherapy has presented a new set of challenges for preclinical animal models in oncology research. Models require a functional immune system and optimised study design to accurately assess the immune-cancer interactions. Kinn Therapeutics AS is a contract research organisation (CRO) specialising in preclinical services for cancer therapy development. In recent years, our activity has reflected the ongoing clinical transition towards immune modulating drugs and biologicals. DCP-001 (Vididencel) is a dendritic cell vaccine designed to manage residual disease in haematological malignancies. We have assessed the vaccine's preclinical efficacy in humanised mouse models of acute myeloid leukaemia (AML). NSGS mice were selected for their superior reconstitution of the human immune system and ability to support the growth of the DCOne leukaemia reporter cell line, DCOne. Efficacy studies were performed in CD34+ humanised mice to assess the anti-leukemic capacity of DCP-001. Disease progression was monitored by tumour volume and bioluminescence imaging. Chimerism was evaluated by longitudinal sampling and flow cytometry analysis of mCD45, hCD45, hCD19 and hCD3. Mass cytometry characterisation of tumour tissue confirmed T cell infiltration in animals exposed to the vaccine. In addition to model development, we have utilised single-cell analysis to better support immunotherapy studies. Mass cytometry using a panel of 21 antibodies has enabled high-dimensional immunophenotyping of our AML-PDX models to identify cell surface markers for CAR-T therapy. In a syngeneic model of melanoma using B16F10 mice, a 36-marker mass cytometry antibody panel was used to characterise immune cells within the tumour microenvironment following administration of a novel peptide therapy. This approach has been translated to the clinical setting where we support an ongoing clinical trial in AML by performing mass cytometry analysis on patient blood and bone marrow samples, detecting immune cell modulation as early as four hours post-drug administration.

This work was supported by the Kinn Therapeutics and its sponsors and in partnership with the University of Bergen.

Unveiling new extracellular matrix immunomodulatory actions within the tumor microenvironment: the axis ADAMTS1/NIDOGEN1

JC Rodríguez-Manzaneque, R Caracuel-Peramos, FJ Rodríguez-Baena, S Redondo-García, JA Villatoro-García, and P Carmona-Sáez

GENYO. Center for Genomics and Oncological Research, Granada, Spain

Recent research highlighted the contribution of extracellular matrix (ECM) in general, and ADAMTS remodeling proteases in particular, in inflammation and immune responses. Our group developed various syngeneic tumor models in wild type (WT) and *Adamts1* knockout (Ats1-KO) mice. Significantly, these studies exhibited the relevance of the protease *Adamts1* as a pro-tumorigenic agent altering vascular and immune-related pathways. Among its known substrates, we observed a significant increased deposition of the basement membrane *Nidogen 1* (Nid1) in vessels of Ats1-KO tumors.

More specific studies in the melanoma syngeneic model revealed that Nid1 overexpression also blocked tumor progression, disclosing a phenotype that overlaps, at least partially, with the absence of *Adamts1*. Tumor immune infiltrates showed important alterations, emphasizing an enhanced presence of antitumorigenic M1-like macrophages and a global inflammatory landscape. To uncover the mechanisms of Nid1 to educate macrophages, we corroborated in vitro that only intact Nid1 promoted an M1-like macrophage polarization, mainly mediated by the αvβ3 integrin present in these immune cells.

Our findings are shedding new light on how extracellular matrix in general, and the basement membrane constituent Nidogen 1 in particular, is able to modulate the myeloid-related microenvironment, mainly regulating immune infiltrates and macrophage populations relevant for tumor progression. Ongoing analyses are assessing RNAseq data from our tumor models, aimed to discover an ECM-associated gene signature, and explore its relevance in human melanoma and pan-cancer datasets for prognostic and therapeutic applications. Importantly, these studies are intimately linked with relevant initiatives to advance in the understanding of myeloid cell-specific information in the context of cancer and further diseases in which the contribution of the vascular basement membrane deserves to be deeply investigated.

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.

Do Statins Influence Prognosis in Patients With Advanced Non-Small Cell Lung Cancer Treated With Nivolumab?

Yasar Culha[1] , Serkan Sen[2], Meltem Baykara[1], Tulay Koken[3]

[1]Department of Medical Oncology, Medicine Faculty, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey
[2]Department of Medical Laboratory Techniques, Ataturk Vocational School of Health Services, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey
[3]Department of Medical Biochemistry, Medicine Faculty, Afyonkarahisar Health Sciences University, Afyonkarahisar

Immune checkpoint blockade has taken its place in first- or second-line treatment regimens, with or without chemotherapy, in advanced NSCLC (non-small cell lung cancer) without a target mutation and with high PD-L1 expression. Studies have shown that statins significantly suppress the progression of NSCLC and effectively convert the immune-cold tumor environment into an inflamed phenotype. Based on this, we aimed to evaluate the prognostic effect of statin use in NSCLC patients who received Nivolumab treatment in our center.

Forty patients with locally advanced or metastatic NSCLC who received Nivolumab treatment between June 2018 and July 2023 were included in the analyses. Patients were categorized into statin-user and nonstatin-user groups. The time from the start of Nivolumab to progression was defined as progression-free survival (PFS), the time from diagnosis to death was defined as overall survival (OS), and the time from the start of Nivolumab to death was defined as overall survival after Nivolumab (OS1).

The median follow-up period was 17.6 months (IQR: 13.5-25.1). The mean age was higher in the statin users group, at 68.8 ± 4.3 years ($p = 0.006$). The mean LDL cholesterol of the statin users group was 94.3 ± 27.0 mg/dL, significantly lower ($p = 0.046$). Disease progression developed in 4 (33.3%) patients in the statin-users group during the ongoing follow-up period, while this number was 21 (75%) in the non-statin-users group ($p = 0.033$). The two groups had no significant difference in median PFS. The OS1 was 10.2 months in the group using statins and 7.9 months in the group of nonstatin users ($p = 0.51$). With statin use, the risk of progression was significantly reduced in both univariate ($p=0.017$) and multivariate ($p=0.017$) logistic regression analysis.

In patients treated with Nivolumab in advanced NSCLC, statistically, significantly less disease progression was observed in the statin users group. Statin use has been identified as an independent factor in reducing the risk of progression. In the study by Mao et al., better response rates and better overall survival times were found using statins in a similar patient group. Similarly, in the study of Omori et al., better overall survival was found. Another study found a higher overall response rate in patients using statins and a significant risk reduction in PFS in multivariate analysis (HR: 0.52, 95% CI: 0.29-0.93, $p = 0.03$) was detected, but no significant difference was found in OS. Our study found no significant difference in the median PFS and OS.

Identification of mechanism/s of resistance to immunotherapy using a mouse model of low Tumor mutational burden (TMB)

Chiara Colarusso, Anna Falanga, Simone Di Caprio, Emmanuel D'Andria, Michela Terlizzi, Rosalinda Sorrentino

Department of Pharmacy (DIFARMA), University of Salerno, Fisciano (Salerno), Italy

High tumor mutational burden (TMB) has been recently associated to a favorable clinical response to immune-checkpoint inhibitors (ICIs) (Chan TA et al., 2019). This is mainly due to the expression of tumor antigens that once ICs are inhibited, CD8+ T cells can exert their cytotoxic activities, blocking tumor progression. Instead, patients characterized by low TMB are less responsive to ICIs.

Thus, in order to identify molecular or cellular mechanism/s to pharmacologically target, we are currently investigating the tumor microenvironment (TME) after the administration of ICIs combined ICI with chemotherapy. To pursue this goal, we took advantage of a K-Ras/p53 mutated (KP) mouse model of lung adenocarcinoma, recapitulating key features of low TMB (Salehi-Rad R et al., 2021), compared to high TMB mouse model.

We found that the tumor mass of KP mice treated with ICI+chemotherapy was populated by tissue-like memory B cells, a subset of B cells known to express patterns of homing and inhibitory receptors (Negera E et al., 2017). These cells play a similar role as exhausted CD8+ T cells, the latter correlated to tissue-like memory B cells.

Modeling breast cancer and bone metastasis in TgRANKL mice

Evi Gkikopoulou[1,2], Christos Chrysovalantis Syrigos[1,2], Melina Dragolia[1], Vasileios Ntafis[1], Martina Rauner[3], Eleni Douni[1, 2]

[1] *Institute for Bioinnovation, B.S.R.C. "Alexander Fleming", Athens, Greece*
[2] *Department of Biotechnology, Agricultural University of Athens, Athens, Greece*
[3] *Department of Medicine III, Faculty of Medicine, Dresden, Germany*

Receptor activator of nuclear factor-κB ligand (RANKL) is known for its crucial role in bone remodeling and immune regulation. Recently, RANKL has been associated with the development of breast tumors, promotion of cancer cell migration and bone metastases, while the involvement of the immune system in RANKL-mediated carcinogenesis remains unclear.

In order to investigate the function of RANKL in mammary cancer and skeletal metastasis, we generated transgenic mice overexpressing human RANKL (TgRANKL). By injecting E0771 mammary cancer cells orthotopically in abdominal mammary glands of the mice, we established a breast cancer model. Our results revealed that TgRANKL mice exhibit an earlier onset of tumor formation and increased tumor burden compared to wild-type (WT) mice, that is attenuated by prophylactic administration of Denosumab, an anti-human RANKL monoclonal antibody. Additionally, we established a skeletal metastasis model by injecting E0771 cells systemically in mice, demonstrating that transgenic mice exhibited excessive bone metastases as well as severe osteolysis and cortical bone loss in their hind legs compared to WT mice. Prophylactic administration of Denosumab reduced bone metastasis and skeletal-related events in TgRANKL mice by suppressing osteoclast activity.

In conclusion, we have established in vivo mouse models of breast cancer and bone metastasis for the preclinical application and evaluation of novel therapeutics including immunotherapies.

This work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 05346).

Predicting safety and efficacy of engineered T cells using an ex vivo human model

Emmanuel Donnadieu

Institut Cochin, INSERM, U1016, CNRS, UMR8104, Université de Paris. Email: emmanuel.donnadieu@inserm.fr

Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has shown remarkable clinical efficacy against advanced B cell malignancies. This clinical success has generated urgent interest in the development of new CARs and the extension of CAR T cell therapy to solid tumors that are, up to now, refractory to this strategy. Prior to initiating clinical trials, model systems in which CAR T cells can be characterized and tested for their potency and safety should be in place.

To date, few models perfectly recapitulate the human immune system and tumor microenvironment, and some models have revealed CAR T limitations that were contradicted or missed entirely in other models. Thus, careful model selection is a crucial step in evaluating CAR T cell treatment and a major issue in the field of cancer immunotherapy. We have established a unique pre-clinical imaging platform based on slices from fresh human tumors that allows investigating, in a preserved tumor microenvironment, the efficacy of CAR-T cells. The possibility to use such a model to assess toxicity (e.g., on-target off-tumor) of engineered T cells will also be discussed.

Stratification of a Panel of Mouse Models of Pancreatic Cancer using Immune Landscape and Extra-cellular Matrix to Predict Response to Synthetic IL-12 Therapy

Ragulan C[1], Herpels M[1,2] , Hari PS[1], Lampis A[1] Gorrini C[1,3], Matthew H[4], Wennerberg E[1], Ishihara J[2], Sadanandam A[1]

[1] *Division of Molecular Pathology, The Institute of Cancer Research, London, United Kingdom*
[2] *Department of Bioengineering, Imperial College London, United Kingdom*
[3] *University of Leeds, United Kingdom*
[4] *Leeds Teaching Hospitals NHS Trust, United Kingdom*

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer type with poor survival rates. It is characterised by an immunosuppressive microenvironment and desmoplastic stroma, which contains abundant extracellular matrix (ECM). Here, we classified PDAC patient and mouse models into immunologically distinct subgroups with a unique ECM content. We consequently exploited this ECM enrichment for targeted pro-inflammatory cytokine, interleukin-12 (IL-12), delivery engineered to a collagen binding domain (CBD) protein. The CBD protein has a high affinity for collagen and can deliver IL-12 intratumourally to reduce toxicity and enhance efficacy. Using patient samples, we developed a novel gene signature *(PDACi)* that is capable of classifying PDACs into distinct subgroups. We characterised their collagen content and performed a cross species analysis to identify ECM-rich, immune desert syngeneic mouse models (n=15). We tested CBD-IL12 in combination with anti-PD1 in multiple of these orthotopic models (n=5) and analysed tumours using flow cytometry, ELISA, spatial profiling (PhenoCycler, Akoya), DNA-barcoding and transcriptome analysis.

The *PDACi* signature identified 4 subgroups of which two are immune desert/myeloid-enriched. These two subgroups also contain significantly more ECM. The CBD-IL-12+anti-PD1 combination therapy significantly prolonged the survival of mice bearing such immune desert ECM-enriched tumours and it significantly reduced liver metastatic burden. Using flow cytometry and Spatial profiling, an increase in effector $CD4^+$ T cells was observed. These T cells were found to be mainly T-helper (Th)-1 cells whilst the Th17 cells were significantly reduced following CBD-IL-12+anti-PD-1 therapy. The DNA-barcoding analysis showed that the cancer cells seeded in the liver as micro-metastases in treated mice, whereas macro-metastases only found in control mice. On the other hand, the immune-enriched mouse models did not respond to the therapy.

We established a new classification in patients and mouse models that are available to model different therapies, including immunotherapy, in PDAC. CBD-IL-12 in combination with anti-PD-1 selectively remodelled the landscape of a subset of PDAC models, highlighting the need for patient selection prior to immunotherapy. Further studies are required to understand the mechanism of action behind the observed results.

Investigating the immune profile of early stage HER2+ breast cancer patients receiving chemotherapy and HER2-targeted therapies.

Denis M Collins[1], Nicola Gaynor[2], Stephen F Madden[3], Sinead Toomey[4], Ji Qiu[5], Jaine Blayney[6], Alfonso Blanco[7], Jean Fletcher[8], Damien Kaukonen[3], Javier Sánchez Ramírez[2], Elaine Kay[9], Darran O'Connor[10], Ausra Teiserskiene[11], Alex J Eustace[1], Naomi Walsh[1], Richard Kennedy[12], Joshua LaBaer[5], William Gallagher[13], Bryan Hennessy[14], John Crown[15]

[1] *Dublin City University, School of Biotechnology, Dublin, Ireland.*
[2] *Dublin City University, Cancer Biotherapeutics Research Group - School of Biotechnology, Dublin, Ireland.*
[3] *Royal College of Surgeons in Ireland, Data Science Centre- School of Population Heath Sciences- RCSI University of Medicine and Health Sciences, Dublin, Ireland.*
[4] *Royal College of Surgeons in Ireland, Medical Oncology Group- Department of Molecular Medicine, Dublin, Ireland.*
[5] *Arizona State University, BioDesign Institute, Tempe- AZ, United States.*
[6] *Queen's University Belfast, Patrick G Johnston Centre for Cancer Research, Belfast, United Kingdom.*
[7] *University College Dublin, Flow Cytometry Core Technology- UCD Conway Institute, Dublin, Ireland.*
[8] *Trinity College Dublin, School of Biochemistry and Immunology- Trinity Biomedical Sciences Institute, Dublin, Ireland.*
[9] *Beaumont Hospital, Department of Pathology- RCSI University of Medicine and Health Sciences, Dublin, Ireland.*
[10] *Royal College of Surgeons in Ireland, School of Pharmacy & Biomolecular Science, Dublin, Ireland.*
[11] *Cancer Trials Ireland, RCSI House- 121 St. Stephen's Green, Dublin, Ireland.*
[12] *Queen's University Belfast, School of Medicine- Dentistry and Biomedical Sciences-Patrick G Johnston Centre for Cancer Research, Belfast, United Kingdom.*
[13] *University College Dublin, UCD School of Biomolecular and Biomedical Science, Dublin, Ireland.*
[14] *Beaumont Hospital, Dept of Medical Oncology, Dublin, Ireland.*
[15] *Saint Vincent's University Hospital, Dept. of Medical Oncology, Dublin, Ireland.*

The HER2+ breast cancer (BC) subtype accounts for 15-20% of all BCs. There are eight HER2-targeted therapies approved for treating HER2+ BC including monoclonal antibodies (mAbs), antibody drug conjugates (ADCs) and tyrosine kinase inhibitors (TKIs). Trastuzumab (T) was the first mAb therapy approved for the treatment (tx) of HER2+ breast cancer. Trastuzumab targets the extracellular domain of HER2, directly inhibiting HER2 signalling activity but also engaging innate immune cells like natural killer (NK) cells through its Fc receptor, eliciting antibody-dependent cell-mediated cytotoxicity (ADCC). Our work investigates the engagement of trastuzumab with the immune system, focussing on the impact of trastuzumab-based therapy regimens on the immune response and the potential impact of immunotherapies like the anti-PD-1 immune checkpoint inhibitor pembrolizumab on response to T. Here we provide an overview of our comprehensive immune-based translational investigation of blood and tissue samples from the Irish Phase II ICORG 10-05 HER2+ BC neo-adjuvant (Neo-Adj) clinical trial. Plasma, serum, peripheral blood mononuclear cells (PBMCs) and formalin fixed paraffin embedded (FFPE) tumour biopsies were obtained

from the ICORG 10-05 (NCT01485926) HER2+ BC clinical trial (n=88) comparing T, Lapatinib (L) and TL in combination with Neo-Adj chemotherapy (docetaxel/carboplatin). Activated NK cell gene expression profiles were generated from tumour RNA-Seq data (CIBERSORT). PBMCs were assessed for ex vivo T-ADCC (Guava flow cytometer) in co-culture assays. Circulating PBMCs were immunophenotyped - CD3, CD4, CD8, CD56, CD19, CD14, CD56 (CytoFLEX platform). Fc receptors were genotyped (Agena MassArray technology). Preliminary serum tumour auto-antibody (AAb) profiling was carried out (HD-NAPPA platform) and plasma was profiled for chemokines (Multiplex ELISA). Tumour infiltrating lymphocyte (TIL) data and clinico-pathological data were available. Data were assessed pre- and post-tx and by pathological complete response (pCR). Tumour NK gene cell signatures and peripheral immune cells (T cell/NK cell/monocyte, B cells) displayed significant alterations post Neo-Adj therapy. These changes were primarily confined to the No pCR cohort. Varying Fc receptor engagement/activation capacity as assessed by FcR genotype status did not associate with pCR. Neo-Adj tx attenuated ex vivo T-ADCC of PBMCs. Potential biomarkers of response were identified including a subset of No pCR patients with pembrolizumab-sensitive PBMCs. Lower pre- and post-tx levels of the chemokine CCL17 were associated with the pCR cohort. AAbs targeting p53 were detected in patients with p53 tumour mutations. AAbs associated exclusively with pCR, partial response and non-responders were detected. Translational studies from the ICORG 10-05 trial have identified distinct immune profiles associated with pCR, as well as potential biomarkers of response to HER2-targeted therapy/chemotherapy for exploration in larger datasets. Investigator-led clinical trials with in-built translational studies are an essential component of cancer research conducted in academic institutions.

We wish to acknowledge the patients who took part in ICORG 10-05 and Cancer Trials Ireland, formerly the All-Ireland Co-operative Oncology Research Group (ICORG), sponsors of the ICORG/CTRIAL-IE 10-05 (NCT01485926) clinical trial. This work was funded by the Caroline Foundation and the Cancer Clinical Research Trust CHY12210, and the Irish Cancer Society Collaborative Cancer Research Centre BREAST-PREDICT (CCRC13GAL).

In-Silico Modelling for Immunotherapy Response Prediction in Multiple Cancer Types Using Multi-Omics Data

Mustafa Samet Pir[1] , Osman Uğur Sezerman[1, 2]

[1] *Department of Biostatistics and Bioinformatics, Institute of Health Sciences, Acıbadem University, Istanbul, Türkiye*
[2] *Department of Biostatistics and Medical Informatics, School of Medicine, Acıbadem University, Istanbul, Türkiye*

In recent years, immunotherapy emerged as a promising method for cancer therapy. However, only a small proportion of the patients who are eligible for immunotherapy can benefit from the treatment. In addition, current biomarkers associated with immunotherapy response are often not reliable and fail to predict the response. Therefore, it's crucial to develop computational models to predict immunotherapy response and to uncover new markers and associated pathways. For this, we have developed machine learning models for advanced clear cell renal cell carcinoma (ccRCC), urothelial cancer, melanoma, and breast cancer. In each case, we used publicly available WES and RNA-seq data to train models with Random Forest, Gradient Boosting, SVC and XGBoost algorithms. The models showed varying performance, with the best performer was Random Forest in each data with the AUCs between 0.82 and 0.93. We also performed differential expression and pathway enrichment analysis to find novel markers and pathways which may potentially play a role in the immunotherapy resistance.

DNase treatment affects tumor angiogenesis and augments cytotoxic effect of cisplatin in chemoresistant germ cell tumors

Kucerova L[1,2], Udvorkova N[2], Majtanova K[2], Vlkova B[3], Pastorek M[3], Celec P[3], Kalavska K[1,4], Mego M[1,4].

[1] - Translation Research Unit, Faculty of Medicine Comenius University and National Cancer Institute, Bratislava, Slovakia, [2] - Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center, Bratislava, Slovakia, [3] - Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [4] - 2nd Department of Oncology, Faculty of Medicine, Comenius University and National Cancer Institute, Bratislava, Slovakia.

Platinum-based therapy has been used in the treatment of testicular germ cell tumors (TGCT) with high efficacy for over 40years. Disease relapse due to the drug resistance remains a clinical problem in recurrent refractory GCT (rrGCT). Recent studies suggested the potential of deoxyribonucleases (DNases) to work as anticancer agents by potentiation of cytotoxic effect of chemotherapy. We hypothesized, that combining DNase with cisplatin (CPT) might augment its cytotoxic effect in TGCT, like the effect observed for other tumor types. We examined the effect of recombinant human DNase I (Pulmozyme®) treatment alone and in combination on the xenografts derived from the chemoresistant variant of the human embryonal carcinoma NT2 CisR cells.

There was no significant effect on the proliferation of TGCT cells *in vitro*. Combination treatment with rhDNase also did not affect the response to CPT, tumor cell migratory or invasive properties. Next, the rhDNase and CPT treatment efficacy was tested in the presence of human neutrophils as these host cells pose a potential source of the extracellular DNA. Direct coculture resulted in augmentation of CPT cytotoxic effect by 200U/mL of rhDNase in the presence of neutrophils even in the cisplatin resistant EC cell line variant NLR-NT2 CisR, choricarcinoma model cells NLR-JEG3 CisR and yolk sac tumor cells NLR-NOY1 CisR.

To investigate this finding *in vivo*, we used immunocompromised NSG mice, which retain the innate neutrophil function, as the host for human xenografts. Animals were randomized to 4 groups: control, CPT alone, Pulmozyme® alone or the combination of the two. Median tumor weight in the rhDNase treated animals was not significantly different from the untreated controls (206 mg vs. 308 mg, respectively). However, the combination treatment resulted in lower median tumor weight of 49 mg vs. control ($p \geq 0.01$). The effect of combination treatment on significant prolongation of animal survival in the CPT + Pulmozyme® treated group was achieved. We have identified highly significant effect of the treatment on the tumor angiogenesis based on the immunohistochemical analysis and enumeration of CD31 marker. We will discuss the potential mechanism and in vitro modelling of the complex interactions within the tumor microenvironment upon the chemotherapeutic treatment in combination with exogenous DNase supplementation. The prominent effect observed in this preclinical study *in vivo* warranties the use of the rhDNase for the combination therapy in rrGCT.

This work was supported by grants APVV-20-0158, APVV-21-0197 and VEGA 2/01124/21.

Various patient-derived Glioblastoma 3D tumor models and their downstream application opportunities

Ana - Maria Enciu[1,2], Elena Codrici[1,2], Ionela-Daniela Popescu[1], Maria Dudău[1], Emanuel Tudor Fertig[1,2], Maria Savoaia[2], P.Mugur Rădoi[2,3], Cristiana Tanase[1,4]

[1] Victor Babeș National Institute of Pathology, Bucharest, Romania;
[2] Carol Davila University of Medicine and Pharmacy, Bucharest, Romania;
[3] National Institute of Neurology and Neurovascular Disease, Bucharest, Romania;
[4] Medical Research Institute "Nicolae Cajal", Titu Maiorescu University, Bucharest, Romania.

Background: Glioblastoma is an aggressive form of brain tumour, with no resolution, despite recent advancement in understanding the molecular mechanisms. In order to find novel therapy targets, more suitable models are to be implemented and standardized. **Aim**: to develop and implement a protocol for patient-derived glioblastoma spheroids/organoids, best fit for plasmid-based transfection. **Material and methods**. Glioblastoma biopsies were harvested in high-glucose DMEM medium, kept at 4-8°C for up to 4 hours then processed by mechanical trituration and enzymatic digestion in Accutase (2 min at 37°C). The suspension was filtered through a 40µ mesh and the resulting filtrate incubated in ultralow adhesion plates, Matrigel Organoid or dextran-based hydrogels. Spheroids were nucleofected with GFP plasmid to assess efficiency and penetrability of transfection. **Results**. Of the tested methods, ultralow adhesion incubation yielded tumour spheroids with the highest efficiency, of macroscopic size, compatible with downstream imagistic detection and transfection. However, they failed to recapitulate the infiltrative nature of the glioblastoma, which was achieved in various hydrogels, such as Matrigel Organoid and precast gradient hydrogel plates (Merck). Incubating spheroids in hydrogels restricted their size and slowed growth. **Conclusion**. For morphology assessement, biomarker detection and fast and highthrouput analysis, ultralow attachment plates are the most convenient method.

This work was supported by the Core Program within the National Research, Development and Innovation Plan, 2022–2027, with the support of MCID, project no. 10N/01.01.2023, PN 23.16.02.03 and Program 1—The Improvement of the National System of Research and Development, Subprogram 1.2—Institutional Excellence—Projects of Excellence Funding in RDI, Contract No. 31PFE/30.12.2021 and CA21135.

In-Silico Cancer Immunology Cohort Discovery Using TCGAnalyzeR

Tuğba Önal-Süzek[1], Talip Zengin[2], Başak Abak Masud[1]

[1] Department of Bioinformatics, Faculty of Science and Engineering, Mugla Sitki Kocman University, Mugla 48000, Türkiye;
[2] Department of Molecular Biology and Genetics, Mugla Sitki Kocman University, Mugla 48000, Türkiye.

The human capacity for visual parallel processing expedites the comprehension of intricate clinical data patterns. This ability is particularly crucial for clinicians dealing with relatively large datasets, wherein visualization tools play a pivotal role in converting raw data into clinical decisions by managing inherent complexity and monitoring patterns interactively in real-time. The extensive size and data diversity of The Cancer Genome Atlas (TCGA) database present challenges for clinicians and biologists in effectively harnessing this valuable resource.
In our study, we conducted a comprehensive re-analysis of five molecular data types-mutation, transcriptome profile, copy number variation, miRNA, and methylation data from TCGA—pertaining to approximately 11,000 cancer patients across all 33 cancer types. In additon, we imported and curated the existing TCGA patient cohorts from the literature into a free web application named TCGAnalyzeR. This tool offers an integrative visualization of pre-analyzed TCGA data, featuring several innovative modules:

1. Identification of simple nucleotide variations with driver prediction.
2. Detection of recurrent copy number alterations.
3. Analysis of differential expression in tumor versus normal tissues, including pathway and survival analysis.
4. Integration of TCGA clinical data, incorporating metastasis and survival analysis.
5. Inclusion of external subcohorts from literature, curatedTCGAData, and BiocOncoTK R packages.
6. Determination of internal patient clusters through iClusterPlus R package or signature-based expression analysis of the five molecular data types.

TCGAnalyzeR successfully combines multi-omics, pan-cancer TCGA data with approximately 120 subcohorts from the literature. The platform includes clipboard panels, empowering users to create custom subcohorts, compare against existing external subcohorts (such as MSI, Immune, PAM50, Triple Negative, IDH1, miRNA, metastasis, etc.), and visualize cohort-centric or gene-centric results interactively
During the presentation, we will demonstrate the efficiency of TCGAnalyzeR in analyzing the large number of existing immune subcohorts among 11,000 patients representing 33 cancer types.

This work was supported by grants TÜSEB 4583.

The CAR costimulatory domain influences the process of CAR-CD19 resistance development in lymphoma and B-ALL

Magdalena Winiarska

Mossakowski Medical Research Institute, Warsaw, Poland

Therapy with CD19-CAR-T-cells has greatly improved the treatment of patients with relapsed/refractory B-cell-derived malignancies, however around 35-55% of responding patients eventually relapse. Since factors contributing to resistance are still actively investigated, we generated in vitro models of B-ALL/lymphoma cells resistant to CD19-CAR-T-cells by exposing tumor cells repeatedly to either 4-1BB or CD28-CD19-CAR-T-cells.

We observed costimulatory domain-dependent differences in CD19 antigen loss upon contact with CAR-T-cells. Prolonged exposure to CD19-4-1BB-CAR-T-cells inevitably and independently of the cell line origin (B-ALL/lymphoma) led to resistance development. The mechanism underlying this phenomenon varied from selective FMC63 epitope loss to total CD19 protein downregulation. Simultaneously, CD19-CD28-CAR-T-cells did not induce antigen-escape-mediated resistance. Although they reduced the FMC63 epitope in tumor cells, the remaining levels were sufficient to retain tumor cells' sensitivity to CD19-CAR-T cells. By showing different mechanisms of resistance our results underline the need to monitor CD19-CAR-T-cell-treated patients for FMC63 levels with antibodies recognizing this specific epitope. Moreover, our models serve not only to understand CD19 regulation, but also help to identify the changes accompanying/driving the resistance, such as the downregulation of CD21, ICAM-1, and CD58.

A comprehensive surfaceome analysis of our models offers a unique opportunity to investigate the factors contributing to resistance, a methodology previously unexplored and inaccessible within clinical studies. In this context, our results, consistent with recent real-world clinical comparisons, in which CD28-based axi-cel demonstrated significantly improved responses and longer progression-free survival than 4-1BB-based tisa-cel in lymphoma patients, help to understand better the mechanisms underlying this phenomenon.

Pre-clinical validation of humanized CAR-Ts cells in the industry: experiences from the bench to the bedside

Víctor Manuel Díaz Cortés

OneChain Immunotherapeutics, S. L., Parc Científic de Barcelona, Barcelona, Spain

Advanced cell therapies have gained important prominence in recent years. The use of chimeric antigen receptor (CAR)-T cell treatments has been highly beneficial in the treatment of B-cell acute lymphoblastic leukaemia/lymphoma (B-ALL). Unfortunately, advances to combat relapsed/refractory T-cell acute lymphoblastic leukaemia (T-ALL) have been very limited. T-ALL has a dismal outcome, and no previous effective targeted immunotherapies has been reported and has remained challenging because the shared expression of target antigens between CAR-Ts and T-ALL blasts leading to CAR-T cell fratricide.

OneChain Immunotherapeutics is a clinical stage CAR-T therapy company that emerges as a spin-off derived from the Josep Carreras Leukemia Institute. OneChain has developed OC-1, a humanized CAR-T cell autologous therapy targeting CD1a that it is expressed exclusively in cortical T-ALL (coT-ALL), a major subset of T-ALL, and retained at relapse. This product has received a designation as orphan medicinal product by the EMA and FDA indicated in patients with refractory or relapsed T-cell acute lymphoblastic leukaemia/lymphoblastic lymphoma (TALL/LL) and is now part of a phase I clinical trial (https://clinicaltrials.gov/study/NCT05679895) conducted by the Hospital Clinic de Barcelona and the Hospital Sant Joan de Déu. OneChain current work will be summarized regarding other autologous CAR-T products against T-ALL, B-ALL and Glioblastoma Multiforme and the generation of an allogeneic platform of gamma delta T-cells.

This work is part of the projects CPP2021-008508, CPP2022-009759 and PTQ2020-011056 financed by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR. Project funded by the European Union's EIC Transition programme under Grant Agreement nº 101113067.

Exploring the role of microRNAs in intercellular communication during Immunogenic Cell Death

María Julia Lamberti[1], Annunziata Nigro[2], Loredana Leggio[3], Maria Ravo[4], Ilaria Salvato[2], Francesca Mensitieri[2], Barbara Montico[5], Nunzio Iraci[3], Viviana Izzo[2], Vincenzo Casolaro[2], Cristiana Stellato[2], Jessica Dal Col[2]

[1]Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, INBIAS-CONICET, Río Cuarto 5800, Córdoba, Argentina;
[2]Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy
[3]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
[4]Genomix 4 Life Srl, Baronissi, Salerno, Italy
[5]Immunopathology and Cancer Biomarkers, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Aviano, Italy

Immunogenic Cell Death (ICD) is a therapy-induced phenomenon where dying tumor cells release signals that trigger an immune response especially through the interaction with dendritic cells (DCs). Previously we demonstrated that the combination of retinoic acid (RA) and interferon-α (IFNα), a well-characterized ICD inducer, significantly upregulated two microRNAs: miR-212-3p and miR-4284 involved in immune processes in lymphoma cells. Herein, we aimed to unravel specifically the contribution of ICD-induced miRNAs in the interaction between dying tumor cells and DCs.

We obtained immunogenic tumor cell lysates (iTCLs) from RA/IFNα-treated lymphoma cell lines, as reservoirs of tumor antigens and adjuvants for loading monocyte-derived DCs. Cytotoxic T cells generated by autologous iTCLs-pulsed DCs more efficiently recognized and specifically lysed lymphoma cells *in vitro* than cells exposed to DCs pulsed with TCLs from untreated tumor cells (CTRL-TCL-pulsed DCs). Through next-generation sequencing, we confirmed the presence of both miR-212-3p and miR-4284 in iTCLs and in iTCL-pulsed DCs. Validation assays by real-time PCR showed that only miR-212-3p resulted upregulated in iTCLs-pulsed DCs compared to CTRL-TCL-pulsed DCs. Functional network analysis unveiled interactions between miR-212-3p and predicted mRNA targets, notably within cell surface receptor signaling pathways, like the IL-1 pathway. This finding was further supported by cytokine secretion patterns evaluated through multiplex assays. To investigate whether the two microRNAs identified in iTCLs also contribute to the cross-talk between tumor cells and DCs during *in situ* ICD, we explored their presence in tumor extracellular vesicles (EVs) as mediators of intercellular communication. We compared EVs isolated through ultracentrifugation of the culture medium from untreated *vs* RA/IFNα-treated lymphoma cells. This revealed an increase in concentration and size of EVs released by ICD-treated tumor cells detected by nanoparticle analyzer. Interestingly, real-time PCR showed significant increase of miR-212-3p levels in EVs derived from RA/IFNα-treated tumor cells. Overall, our findings underscore the potential role of microRNAs and their transport in EVs in orchestrating immune mechanisms during experimental ICD and potentially in clinical settings. Understanding these mechanisms could have significant implications for regulating key events in the immune response against cancer.

Retinoid Differenciating agents induce Maturation of MDS cells in vitro

Anamaria Bancos[1,2], Diana Cenariu[1], Adrian Bogdan Tigu[1], Rares Drula[1], David Kegyes[1], Mareike Peters[1], Gabriel Ghiaur[1,4] , Ciprian Tomuleasa[1,2,3,4]

[1]Medfuture Research Center for Advanced Medicine, Iuliu Hatieganu University of Medicine and Pharmacy Cluj Napoca, Cluj Napoca, Romania
[2]Department of Hematology, Iuliu Hatieganu University of Medicine and Pharmacy Cluj Napoca, Cluj Napoca, Romania
[3]Department of Hematology, Ion Chiricuta Clinical Cancer Center Cluj Napoca, Cluj Napoca, Romania
[4]Johns Hopkins University, 1650 Orleans St. Room 243, Baltimore, MD 21231, USA

Myelodysplastic syndromes (MDS) are considered heterogeneous hematopoietic stem cell (HSC) disorders characterized by bone marrow (BM) dysplasia and peripheral cytopenias. CyP26 activity in the BME protects normal and malignant HSCs from ATRA-induced differentiation and contributes to drug resistance. Malignant cells can hijack control of stromal CYP26 activity to create permissive microenvironments.

In vitro cultivation of MDSL cells and OP9 cells, treated with different ATRA concentrations (10e-8M, 10e-8M + R115866) and AM80 10-9M to induce differentiation. Talarozol (R115866), an inhibitor that blocks CyP26 was added to the ATRA treatment in the experimental design, at 1nM cc-tion. The experiment was set up, in EPO/G-CSF media, MDS-L cells were cultured with or without stroma OP9, in 3 successive days. Flow cytometry evaluation. The processed experimental samples are labelled with 5 antibodies: CD45PerCP-Cy5.5: CD36 FITC, CD38 APC H7, CD71 APC, GlyA PE and the mean flourescence intensity is calculated. Colony forming units are performed to evaluate and validate the maturation of MDS cells following retinoic treatment, by counting the number of colonies formed in each plate. 500 cells from each combination MDSL no stroma and MDSL co-cultivated with stromal OP9 on 6 well plates on methylcellulose for 14 days, have been counted.

CD45 staining isolates the leucocyte population out of the analyzed cells; from here, specific antibodies that prove maturation of MDSL cells are used: CD36 FITC, CD38 APC H7, CD71 APC, GlyA PE. There is a shift in the positive population of mature cells dependent of the ATRA treatment concentration. Best results are observed at 10e-8M ATRA. The number of colonies formed is counted in triplicate for all wells and compared in Graph Prism 8.0. The number of colonies formed decreases, once the differentiation of MDSL cells is induced due to the retinoic acid treatment, in the presence/absence of stroma.

Since myelodysplastic cells mature slower in the presence of stroma than those grown without stroma by the addition of Talarozol to the experimental design we could prove *ex vivo* that R115866 blocks CyP26, thus therapy resistance can be eliminated *in vitro*. The ATRA inactivation is stronger on MDSL in the presence of stroma OP9, which proves that Cyp26 is involved in limiting the production of the active ATRA, *in vitro*.

The research was financed by Project PN-III-P4-ID-PCE-2020-1118 and International collaborative grant of the European Economic Space between Romania, Iceland and Norway 2014–2021, "Continuous Flow Interchange of Communication and Knowledge in Biomedical University Research" (grant number 21-COP-0034).

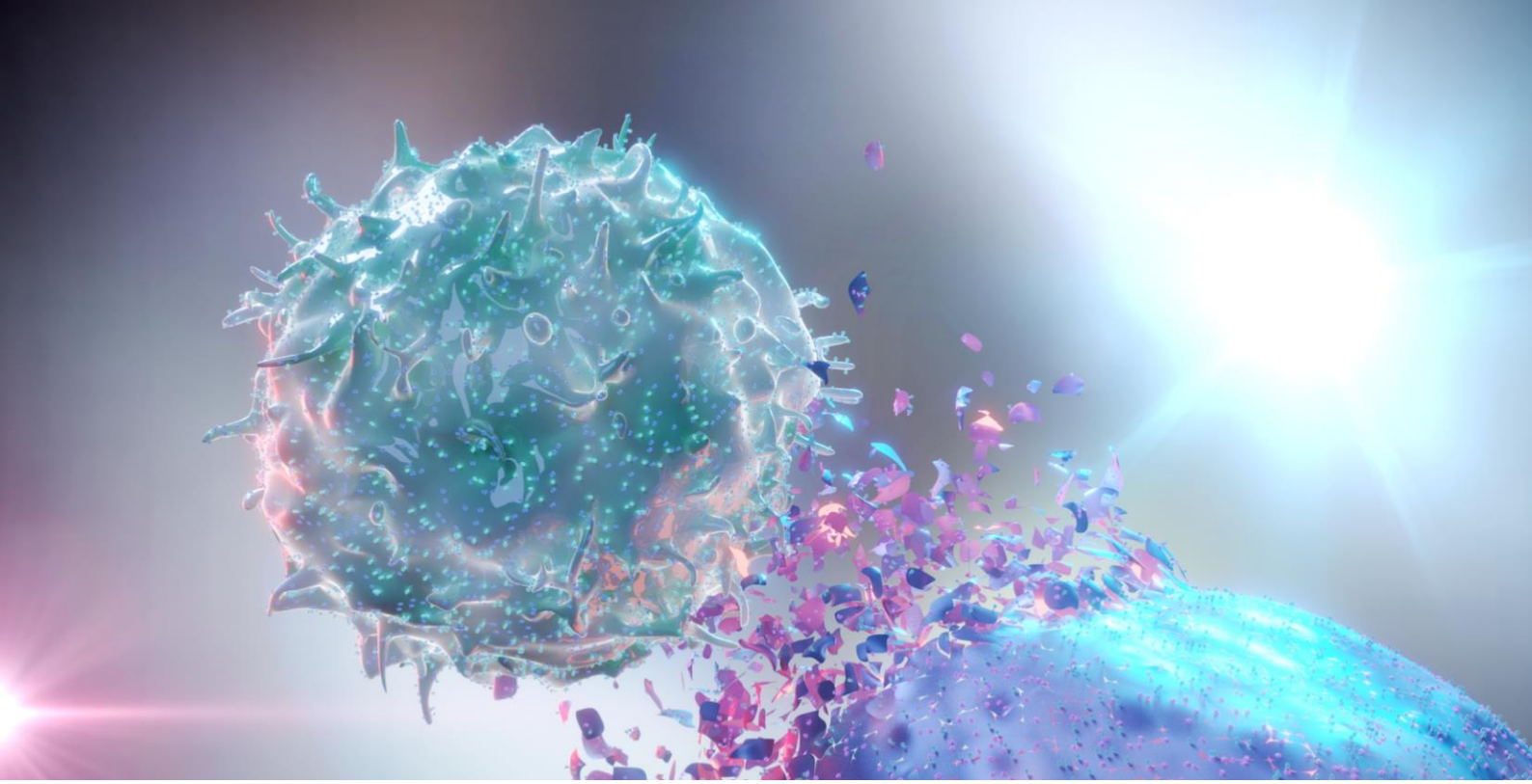
The identification of potential targets for CAR-based therapy using transcriptomic and proteomic approaches

Klaudyna Fidyt[1], Katsiaryna Marhelava[1], Marta Krawczyk[1,2,3], Monika Pepek[1], Agata Malinowska[4] , Bianka Swiderska[4], Agata Pastorczak[5], Agnieszka Kraft[6], Malgorzata Firczuk[1,2], Magdalena Winiarska[1,2]

[1]Department of Immunology, Medical University of Warsaw, Warsaw, Poland, [2]Laboratory of Immunology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland, [3]Doctoral School of Translational Medicine, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland, [4]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, [5]Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Lodz, Poland, [6]Laboratory of Functional and Structural Genomics, Centre of New Technologies, University of Warsaw, Warsaw, Poland

Although the introduction of CD19-targeting chimeric antigen receptor (CAR)-T-cell-based immunotherapy became a turning point in the treatment of various B-cell-derived malignancies, a significant proportion of patients continue to experience disease progression after receiving this clinically approved cell therapy. The reasons for that are still under investigation, however, among the most common are CAR-T-cell dysfunctions, suppressive tumor microenvironment, and tumor intrinsic features resulting in, for example, target antigen escape. One of the strategies to overcome CD19-negative relapses is to find alternative targets on malignant cells and design novel CARs. Herein, we implemented two distinct methods for target identification on B-cell acute lymhoblastic leukemia (B-ALL) and lymphoma cells, such as bioinformatics-transcriptomic and proteomic approaches. Given the malignant cell origin, in the transcriptomic approach, we focused on B-cell specific genes. Accordingly, we re-analysed available microarray datasets and identified the genes of interest via differential gene expression analysis. Further, based on the surfaceome databases, we selected 24 genes coding for membrane proteins and designed the panels for flow cytometry to evaluate their expression. The flow cytometry analysis was performed on various parental B-ALL/lymphoma cell lines, as well as cell lines with established resistance to known therapeutic strategies, including CD19 CAR-T. Aware of the pitfalls of the transcriptomic approach, and the fact that cell lines do not represent heterogeneity observed in patients, we optimized the technique for characterization of patient-derived cells' surfaceome by biotin labeling of membrane proteins followed with tandem mass spectrometry analysis. Specifically, as target cells we used B-ALL patient-derived xenografts previously generated in immunodeficient mice. Based on both approaches, we identified proteins already investigated in the CAR-T-cell field, including CD19, CD22, CD72, CD79A, and CD79B, as well as those not yet explored as potential targets for CAR-T therapy. For selected proteins we created CAR-T-cells and validated their antitumor activity in preclinical tests.

This work was supported by the National Centre for Research and Development within POLNOR program NOR/POLNOR/ALTERCAR/0056/2019 (PI: Magdalena Winiarska).



Poster Abstracts

POSTERS

WG1 (*In vitro* models) + WG2 (*In vivo* models) - 14th May 2024

P1 Muhammet Karaman, Department of Molecular Biology and Genetics, Faculty of Science, Kilis 7 Aralik University, Kilis, **Turkey**
A Novel Immunological Target for Early Diagnosis and Therapy of Cancer; Neonatal Variant of Voltage-gated Sodium Ion (Na+) Channel (nNaV1.5)

P2 Ginés Luengo-Gil, Department of Pathology and Laboratory Medicine, Santa Lucía University Hospital, Institute for Biomedical Research from Murcia (IMIB), Cartagena, **Spain**
Ex vivo systems as surrogates for the evaluation of the response to immunotherapy in patients with colorectal cancer

P3 Anna Loginova, Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
Investigating Impact of Galectins on Cancer: Comprehensive Approach

P4 Nikoleta Mojzesova, Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, **Slovakia**
Gene expression changes induced by green tea polyphenol epigallocatechin-3-gallate in human colorectal cancer cell lines

P5 Devrim Pesen Okvur, Department of Molecular Biology and Genetics, Faculty of Science, Izmir Institute of Technology, Izmir, **Turkey**
Lab-on-a-chip device (LOC) for determining drug dose response

P6 Sweta Rani, Department of Science, School of Science and Computing, South East Technological University, Waterford, **Ireland**
In vitro 3D culture models in drug resistant breast cancer research

P7 Emanuela Senjor, University of Ljubljana, Faculty of Pharmacy, Ljubljana, **Slovenia**
Open Source Image Analysis Pipeline For Measuring Cell Viability, Proliferation and Immune Cell Infiltration Into 3D Cancer Models

P8 Emanuela Senjor, Jozef Stefan Institute, Department of Biotechnology, Ljubljana, **Slovenia**
Introducing the IMMUNO-model Protocol Database Task Force

P9 Lukasz Skalniak, Jagiellonian University, Faculty of Chemistry, Department of Organic Chemistry, Krakow, **Poland**
New co-culture models for evaluating the potency of PD-1/PD-L1-targeting immune checkpoint inhibitors

P10 Silvia Tyčiaková, Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, **Slovakia**
Overexpression of TNFα affects tumorigenicity and differentiation in colorectal cancer cell lines

P11 Maria Urbanova, Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, **Slovakia**
The Cancer-Associated Fibroblasts Modulate the Drug Response of Pancreatic Ductal Adenocarcinoma

P12 Wujun Xu, Department of Technical Physics, University of Eastern Finland, **Finland**
Cell membrane coated biomimetic nanoparticles for targeted cancer therapy

WG3 (Solid tumors) + WG4 (Hematologic tumors) - 15th May 2024

P13 Marco Barreca, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, **Italy**
Tumour ecosystem and molecular dynamics in clinical triple-negative breast cancer depend on the chemotherapy regimen

P14 Philippe Bertrand, IC2MP UMR CNRS 7285, Poitiers University, **France**
Assessment of New HDAC Inhibitors for Immunotherapy of Malignant Pleural Mesothelioma

P15 Philippe Bertrand, IC2MP UMR CNRS 7285, Poitiers University, **France**
Towards sustainable synthesis of PD-L1 inhibitors: Accessing birayl systems from renewable biobased furanic derivatives

P16 Lucie Janstová, Department of Modern Immunotherapy, Institute of Hematology and Blood Transfusion, Prague, **Czechia**
Modeling complex leukemia microenvironment in vitro for advanced pre-clinical cytotoxicity testing of novel cell-based immunotherapy

P17 Urška Kamenšek, Institute of Oncology Ljubljana, Ljubljana, **Slovenia**
Mutational burden and tumor immune status as predictive factors for in situ vaccination by TNFα and IL-12 gene electrotransfer

P18 María Julia Lamberti, Departamento de Biología Molecular, INBIAS, Universidad Nacional de Río Cuarto, Río Cuarto, Cordoba, **Argentina**
Unraveling the interplay between BRAFV600E mutation, immune landscape, and immunogenic cell death in melanoma: Implications for therapeutic strategies

P19 Katarína Lopušná, Institute of Virology, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, **Slovakia**
PD-L1 is highly expressed in Mantle cell lymphoma cell lines

P20 Melis Denizci Öncü, Life Sciences, Marmara Research Center, The Scientific and Technological Research Council of Turkey, Kocaeli, **Turkey**
CDR Grafting: Implications for Anti-Angiogenic Therapy in Solid Tumors

P21 Paula Martin-Rubio, IIS Aragón, Zaragosa, **Spain**
NK cell-based immunotherapy against lung metastases from gastrointestinal tumors: a therapeutic opportunity

P22 Nour Islem Yanis Saidani, Aix Marseille University, Marseille, **France**
Adhesion GPCRs as modulators of immune functions and potential targets for solid tumors

P23 Nuno Rodrigues dos Santos, i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, **Portugal**
PSGL-1 as a novel target for B-cell lymphoma immunotherapy

P24 Lenka Trnkova, Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, **Slovakia**
Decitabine in the treatment of therapy-resistant breast cancer

P25 Natália Udvorková, Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, **Slovakia**
Novel treatment of chemoresistant germ cell tumors using immunotherapy – based antibody – drug conjugates

P1 A Novel Immunological Target for Early Diagnosis and Therapy of Cancer; Neonatal Variant of Voltage-gated Sodium Ion (Na^+) Channel (nNaV1.5)

Muhammet Karaman,

Department of Molecular Biology and Genetics, Faculty of Science, Kilis 7 Aralik University, Kilis, TURKEY

Early cancer diagnosis improves survival and treatment outcomes significantly. In the early stages of their development, prior to metastatic dissemination, tumors exhibit increased vulnerability to therapeutic therapies. Cancer diagnosis involves various methods and techniques, including: Imaging test, Biopsy, Blood tests and Molecular/Genetic test. Predilection towards a specific diagnostic modality such as the type and location of the cancer, as well as the individual patient's circumstances. Among the methods, Imaging is often considered the cornerstone of cancer diagnosis. Notably, fluorescence imaging methods have become increasingly popular in addition to classical imaging methods. Fluorescence imaging is used not only in diagnosis but also in tumour surgery. The process named as tumour-targeted fluorescence-guided surgery.

Tumour-targeted fluorescence dye binds to an appropriate binding site to ensure adequate localization of the fluorophore within the target tissue. Facilitated by the selective binding of fluorescent dyes to tumour-associated molecular targets, this approach facilitates precise demarcation of neoplastic foci amidst healthy tissue, concurrently enabling histopathological subtype delineation through the utilization of tissue-specific markers. The neonatal variant of voltage-gated sodium ion (Na^+) channel (nNaV1.5) is a tissue-specific marker for many cancer cells such as breast, colon and ovary, melanoma, astrocytoma, neuroblastoma, and oral squamous cell carcinoma. Although various small molecules and antibodies have been developed to block the channel for cancer treatment, therapeutic targeting of this channel remains constrained by the absence of a comprehensive three-dimensional structural blueprint, precluding the rational design of pharmacological agents or antibodies tailored to nNaV1.5. In this study, in order to shed light on the structure of the neonatal variant, we examined the structures of other In this study, in order to shed light on the structure of the neonatal variant, we examined the structures of other voltage-gated sodium ion channels are its close neighbors and revealed their differences and similarities.

P2 Ex vivo systems as surrogates for the evaluation of the response to immunotherapy in patients with colorectal cancer

Pablo Conesa-Zamora[1], Ana Hurtado[2], Ginés Luengo-Gil[1], Alberto Sánchez-Espinosa[1], Daniel Torres-Moreno[1], Fátima Postigo-Corrales[2], Ana B. Arroyo[1], Silvia Montoro-García[2], Begoña Alburquerque-González[2], Joaquín Roca-González[3], Dolores Ojados-González[3], Tuula Salo[4], Jorge Moscat[5], María D. López-Abellán[1], Azadeh Fahim[6], Björn Rotter[6], José García-Solano[1].

[1] Department of Pathology and Laboratory Medicine, Santa Lucía University Hospital, Institute for Biomedical Research from Murcia (IMIB), Cartagena, Spain. [2] Life Science Campus, San Antonio Catholic University from Murcia Region (UCAM), Murcia, Spain. [3]Biomedical Engineering Grade, Polytechnical University from Cartagena (UPCT), Cartagena, Spain. [4]Department of Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland. [5]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, USA. [6]GenXPro, Biomarkers & Translational Medicine, Frankfurt am Main, Germany

In colorectal cancer (CRC) the impact of immune checkpoint inhibitors (ICIs) hav not been as great as in other cancers because, with the exception of those with microsatellite instability (MSI), it is not frequent that CRC presents a high number of neoantigens that make ICI as effective. In any case, it is being seen that there is not a total correlation between MSI and a good response to ICI, with stable cases for microsatellites (MSS) that could benefit from this therapy. In addition, the determination of the tumor mutational burden (TMB) involves the complex analysis not always relating to response to lCIs. The aim of the project is to develop organoid-based *ex vivo* culture systems to assess response to immunotherapy using either microfluidic chips or 3D bioprinting using a REGEMAT 3D platform. The organoids will come from the C57BL/6 mouse and will be of three types presenting an increasing number of mutations per Mb; MSS, MSI and mutated in POLE. In parallel, organoids from patients with CRC showing MSI, who are candidates for treatment with ICIs, will be established. As *in vivo* models to compare the usefulness of the *ex vivo* systems, both a syngeneic murine model in which the organoids will be implanted orthotopically as well as the response of the patient with CRC MSI to treatment with ICI will be used. Given the experience of the research team in the development and characterization of fascin inhibitors (with two patents and one non-commercial clinical trial) and the role that this protein has recently shown in the evasion of the immune response, we will test, in these systems, the effect of fascin inhibitors in the reversal of the anergic tumor phenotype. For these aims, the research team has established more than 50 CRC patient-derived organoids encompassing different histological (serrated, mucinous, medullary) and molecular subtypes (MSI, MSS, BRAF or KRAS mutated, BRAF/KRAS WT). An RNAseq-based algorithm for predicting the best treatment response to each organoid has been developed and is being validated in vitro.

This work was supported by a grant by the Spanish Ministry of Health (Instituto de Salud Carlos III) ref: PI23/00601 and the European Commission (REVERT project) ref: GA848098

P3 Investigating Impact of Galectins on Cancer: Comprehensive Approach

Anna Loginova, Vladimír Křen, Pavla Bojarová

Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ-14220 Prague 4, Czech Republic

Galectins, a family of carbohydrate-binding lectins, regulate cellular processes such as proliferation, apoptosis, adhesion, and migration by interacting with glycan structures on cell membranes and/or extracellular matrix components, thereby modulating cellular events [1]. In cancer, the overexpression of galectins (mainly Gal-3) has been increasingly linked to tumor progression, invasion, and resistance to therapies, including radiotherapy. In addition, the galectin profile within tumors is frequently altered across various cancer types.

The present contribution provides an overview of an effective workflow of *in vitro* techniques used in cancer research to investigate the role of galectins. This workflow was effectively applied in our laboratory [2] to evaluate a potential of galectins as a target for anti-cancer therapy. It combines techniques for monitoring of apoptosis of T cells incited by galectins, for assessment of migration and proliferation of cancer cells as well as for studying the effectivity of galectin binding to the cancer cell surface in the presence of various glyco-inhibitors.

We believe that a comprehensive exploration of galectin role in various cancer types and their interaction with the immune microenvironment of the tumor using diverse *in vitro* methods could lead to the discovery of novel strategies for targeted immunotherapy.

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Support from the grant project LUC23148 is gratefully acknowledged.

P4 Gene expression changes induced by green tea polyphenol epigallocatechin-3-gallate in human colorectal cancer cell lines

Nikoleta Mojzesova[1,2], Martina Poturnajova[1], Silvia Tyciakova[1], Miroslava Matuskova[1]

[1]*Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Department of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava*

In recent years, cancer research has focused on the anticancer effect of phytochemicals. Natural substances, including epigallocatechin-3-gallate (EGCG), interact with DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) and alter the expression of oncogenes and tumour suppressor genes through epigenetic mechanisms. Aldehyde dehydrogenases 1 (ALDH1) have been described as molecular markers. Investigation of their expression regulation would allow us to unhide new signalling pathways and molecules that may influence cell migration and metastasis formation.

We revealed that overexpression of the *ALDH1A1* gene is associated with increased tumorigenicity and the ALDH1A3 isoform is linked with increased chemoresistance and metastatic potential in colorectal cancer (CRC). We also focused on the effect of EGCG on the panel of CRC-derived cell lines HT-29, HCT 116, FUR (5-fluorouracil resistant cell line derived from HT-29) and pts80 (patient-derived cell line). We observed that EGCG modulates the expression of *ALDH1A1* and *ALDH1A3* genes. The mechanism of alteration of ALDH1 expression by EGCG is still unknown. One of the possible mechanisms is the epigenetic regulation.

We cultivated cells in various concentration gradients of EGCG to determine their non-toxic concentrations. Subsequently, cells were cultured in IC_{10} and IC_{20} of EGCG, and RT-qPCR revealed significant gene expression changes in all analysed cell lines.

The effect of plant-derived alternative anticancer drugs on gene expression may help in personal treatment design.

This work was supported by the Slovak Research and Development Agency under the contract APVV-21-0296, by (CRC) the Slovak Academy of Sciences through DoktoGrant APP0477, and the Slovak Cancer Research Foundation.

P5 Lab-on-a-chip device (LOC) for determining drug dose response

Ramazan Temizkan, Devrim Pesen Okvur

Department of Molecular Biology and Genetics, Faculty of Science, Izmir Institute of Technology, 35430 Izmir, Turkey

It is important to determine the dose that is effective and at the same time has minimum side effects. It is also important to determine toxic doses as well as doses that do not result in an effect. In classical 2D cell culture, serial dilutions are often used to test different doses of drugs. However, this approach is prone to pipetting errors. A more critical disadvantage is the lack of a physiologically relevant microenvironment. One solution is to use lab-on-a-chip (LOC) devices that can generate gradients of drugs. Such current devices are complex in structure, require flow and are difficult to fabricate. Here we provide a novel, easy to fabricate LOC that can determine cell viability to different drug doses in a single LOC, in the absence of flow, in physiologically relevant conditions, designated as the DRCHIP. We investigated the diffusion profile of small molecule drugs in DRCHIP using Alexa488 as an avatar with VCell simulations and fluorescence microscopy imaging, both of which were in agreement. We then determined the cell viability of MDA-MB-231 breast cancers cells to different doses of paclitaxel in the DRCHIP using Calcein-AM and Alamar Blue. As expected, regions in the DRCHIP with higher dose of paclitaxel exhibited lower cell viability. Our results show that the DRCHIP can be used to determine drug dose response in a physiologically relevant microenvironment.

DRCHIP is a patent pending product of Initio Cell Biyoteknoloji A.S. of which Devrim Pesen Okvur is co-founder and CTO.

P6 In vitro 3D culture models in drug resistant breast cancer research

Shauna Coleman[1], Thanh Hoa Vo [1,2], Jai Prakash Mehta[1,3], Sweta Rani[1,2]

[1] Department of Science, School of Science and Computing, South East Technological University, Cork Road, Waterford, X91 K0EK, Ireland. [2] Pharmaceutical and Molecular Biotechnology Research Center, South East Technological University, Cork Road, X91 K0EK, Waterford, Ireland. [3] Department of Applied Science, South East Technological University, Kilkenny Road, R93 V960, Carlow, Ireland.

Background: High incidences of breast cancer and mortality is a major public health problem. Although HER-targeted drugs have improved patient outcomes, innate/primary and acquired resistance presents substantial clinical challenges. Acquired resistance is a result of genomic changes in the cancer cell and monitoring them is not possible using routine methods. For the HER-targeted drug to kill the cancer cells in a solid tumour, it must travel through the tumour via blood vessels. Distribution of these drugs are greatly hindered by the hypoxic and acidic conditions of the tumour microenvironment. Three-dimensional (3D) cell culture models will better represent the tumour characteristics including, cell-to-cell contact, cell-to-extracellular matrix interaction (ECM), growth and hypoxic environment that results in changes in gene expression and drug responsiveness. **Aim**: The aim of this study is to grow the HER-acquired drug resistance cells in 3D and characterise their morphology and phenotype. **Experimental design and methods**: In our body, the cells are in a 3D environment. To mimic the conditions in our body, we grew the cells in a 3D culture. Several methods were explored for 3D culture including, hanging drop method, growing cells ultra-low attachment surface, coating 96-well plates with poly-2- hydroxyethyl methacrylate (polyHEMA) and matrigel. Hanging drop method and 96 well plate with ultra-low attachment surface was found to be the best and easy way to grow the cells in 3D culture. Morphological and phenotypic studies were carried out on 3D cultured cells and compared to 2D cultured cells. Drug-induced toxicity was also assessed in 3D and compared to 2D cultured cells. **Results**: Primary emphasis of our research is HER2 overexpressing breast cancer. HCC1954 and SKBR3 cells were made resistant to HER-targeted drug lapatinib and neratinib. Aged cells were grown as control. Spheroid formation capacity of drug resistant and aged HCC1954 and SKBR3 cells were studied. SKBR3 cells were found to form bigger spheroid compared to HCC1954 cells when seeded at same cell concentration. IC50 concentration of neratinib was determined in drug resistant cells grown in 2D and 3D culture. Cells cultured in 3D provides biologically relevant data as it reflects the poor drug penetration as in the tumour mass. **Conclusion**: Cells grown in 3D culture are better mimic of in vivo physiology. Finding the best method that suits your study might be hard but 3D culture is the best method to achieve biologically relevant data.

This work is supported by funds from South East Technological University, President's Scholarship (WD-2022-14-WSCH).

P7 Open Source Image Analysis Pipeline For Measuring Cell Viability, Proliferation and Immune Cell Infiltration Into 3D Cancer Models

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

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

Recent advances in cancer research have highlighted the importance of three-dimensional (3D) cancer models, which better mimic the tumor microenvironment compared to traditional 2D cell cultures. Immunotherapy has emerged as a promising approach for cancer treatment, however, evaluating critical endpoints of immunotherapy in 3D models remains challenging due to the complexity of analyzing 3D datasets. We have developed an image analysis pipeline for quantifying cell viability, proliferation, and immune cell infiltration into 3D cancer models in multichannel confocal microscopy images with multiple z-stacks for the analysis of the effects of NK cell cytotoxicity modulation by inhibition of cathepsin V (CTSL2). CTSL2, a lysosomal cysteine peptidase, is implicated in cancer, particularly affecting tumor progression and prognosis and in immune surveillance. CTSL2 plays a key role in the activation of cystatin F, an endogenous proteinase inhibitor, regulating granule-mediated cytotoxicity of NK cells.

The spheroids were prepared using glioblastoma stem cells (NCH-421k) with hanging drop method. The GBM cells were labelled with CFSE. NK cells (unstained or stained with Cell tracker) were added to the spheroids and treated with CTSL2 inhibitor. The cells were imaged on the day of the addition of NK cells to the spheroids and after three days with confocal microscopy. Images were analysed using ImageJ with StarDist and 3D Suite plugins and AnaSP software. AnaSP software was used for the segmentation of z-projection of brightfield images of spheroids. Then the area of segmented objects was used to assess spheroid proliferation. For a measurement of cell viability individual CFSE positive cells were segmented based on the fluorescence signal using StarDist. The labeled segmented image was then imported into the 3D manager of the 3D suite where the fluorescence intensities for each object were measured. A short script in R was used to automate the calculation of mean fluorescence intensity for each image. For NK cell infiltration, NK cells were segmented based on the Cell tracker fluorescence as described above. The spheroid channel was used to approximate an ellipsoid corresponding to the spheroid dimensions.

Using the 3D suite plugin we have then calculated the distribution of segmented NK cell objects inside the ellipsoid, which was divided into 10 “layers” of equal volume, ranging from ellipsoid periphery to the centre. Using the image analysis pipeline above, we were able to quantify the effects of cathepsin V inhibition on spheroid proliferation, assess the cytotoxicity of NK cells against GBM spheroids and their infiltration inside the spheroids.

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

P8 Introducing the IMMUNO-model Protocol Database Task Force

Emanuela Senjor[1], Vinton Cheng[2], Christina Sakellariou[3], Leticia Oliveira Ferrer[4], Emmet Mc Cormack[5], Laura Belver[6], Marleen Ansems[7], Maria Dudau[8], Devrim Pesen Okvur[9], Barbara Breznik[10], Eva Martinez-Balibrea[11]

[1] *Jozef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia*
[2] *Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom*
[3] *Department of Immunotechnology, Lund University, Lund, Sweden*
[4] *University Medical Center Hamburg-Eppendorf*
[5] *Department of Clinical Science, University of Bergen, Norway*
[6] *Josep Carreras Leukemia Research Institute, Barcelona, Spain*
[7] *Radboud Institute for Molecular Life Science, Netherlands*
[8] *Biochemistry-Proteomics Department, Victor Babes National Institute of Pathology, Bucharest, Romania*
[9] *Izmir Institute of Technology, Turkey*
[10] *National Institute of Biology, Ljubljana, Slovenia*
[11] *Catalan Institute of Oncology, Barcelona, Spain*

IMMUNO-model Cost Action was established to connect researchers in the field of preclinical immuno-oncology models. The primary aim of IMMUNO-model Cost Action is to promote collaboration and sharing of knowledge to contribute to the successful application of immunotherapy preclinical models. One of the objectives is to collect immuno-model protocols from Cost Action members and publish them in an open-access database to facilitate preclinical immuno-oncology research. The Protocol Database Task Force was established to lead these efforts. Protocols in the database will adhere to a standardized template to include all necessary information for the successful implementation of the protocol. Protocol collection from Cost Action members will be documented, to ensure traceability. Important tasks for the task force will also include the engagement of Cost Action members to contribute with protocols and dissemination of the database, in coordination with IMMUNO-model Working Group 5.

The IMMUNO-model Core Group has conducted a survey to assess the types of protocols available among the Cost Action members. The survey responses (71) focused on in vitro protocols (67.6%), derived from human material (67.6%), solid tumors (85.9%), with colorectal (15.5%), breast (14%), lung (11.3%) and pancreatic (7%) being the most common tumor types. Hematologic tumors represented 11.3% of responses. The most common biological source for tumor models is primary cancer cells (38%), followed by cancer cell lines (36.6%). Meanwhile, in 12.7% of provided responses, primary immune cells are incorporated into the model. 25.3 % of responses characterized the protocols as immuno-model protocols, and 26.8% characterized them as sample processing, the remaining 47.9 % remain uncategorized. Overall, these results provide valuable insight into the possible protocols to be submitted into the database, laying a foundation for the database creation. In summary, the creation of a publicly available database of protocols will benefit the immuno-oncology scientific community by fostering collaboration, knowledge exchange and supporting new scientific discoveries.

This work was supported by IMMUNO-model CA21135.

P9 New co-culture models for evaluating the potency of PD-1/PD-L1-targeting immune checkpoint inhibitors

Lukasz Skalniak[1], Thanh Hoa Vo[2], Agnieszka Maslanka[1,3], Justyna Kocik-Krol[1]

[1] *Jagiellonian University, Faculty of Chemistry, Department of Organic Chemistry, Gronostajowa 2, 30-387 Krakow, Poland*
[2] *Department of Science, South East Technological University, Waterford, Ireland*
[3] *Jagiellonian University, Doctoral School of Exact and Natural Sciences, prof. S. Lojasiewicza 11, 30-348 Krakow, Poland*

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. Within the past years, extensive drug-development efforts have been made to provide non-Ab immune checkpoint inhibitors (ICI), which could substitute for antibodies and expand possible mechanisms of targeting ICMs. This effort largely relies on the development of reliable *in vitro* models for proper and accurate testing of molecule bioactivity and comparing this bioactivity with previously known ICIs.

In this study, we present the development and use of cell-based models designed to provide information on the activation status of T cells in the presence of the tested ICMs, as well as the status of the targeted cancer cells. The models rely on the co-culture of artificial antigen-presenting cells (aAPCs) or cancer cells with primary blood lymphocytes and monitoring of cell fate-related parameters, such as expression of activation markers, proliferation, or survival, with flow cytometry. Optimization steps allowed us to establish a proper co-culture method (direct contact vs. inserts), the number of lymphocytes in co-culture, the duration of co-culture, and the proper parameters to be measured. The models are sensitive to the presence of PD-1/PD-L1-targeting ICMs, as exemplified by the use of the therapeutic antibody, durvalumab.

Established models will serve us to evaluate the potency of the newly synthesized small molecules targeting human PD-1/PD-L1 interaction. They are also ready for the analysis of the dynamics of the activation of lymphocyte populations in response to activation in the presence of therapeutic agents.

This work was supported by grant 2021/42/E/NZ7/00422 from the National Science Centre, Poland.

P10 Overexpression of TNFα affects tumorigenicity and differentiation in colorectal cancer cell lines

Silvia Tyciakova, Peter Makovicky, Valeria Valova, Lucia Rojikova, Miroslava Matuskova

Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia

Introduction

The progression of cancer strongly depends on the tumor microenvironment and antitumor immune surveillance. Tumor necrosis factor α (TNFα), a key inflammatory cytokine, can drive both tumor elimination and promotion, depending on its dose and cancer type. Overexpression of TNFα in engineered tumor cells has a significant impact on their tumorigenicity. It can block the engraftment of tumor cells, creating a 'tumor-resistant' or 'tumor-suppressive' microenvironment.

Material and Methods

Colorectal cancer cell lines HCT 116 and HT-29 engineered to stable overexpress the human *TNFα* gene were used to induce subcutaneous tumors in two immunodeficient mouse strains: athymic Balb/c-nu/nu and SCID/bg mice. Tumorigenicity and tumor growth rate were monitored, followed by histopathological and immunohistochemical analyses of xenografts. Expressions of genes encoding *TNFα*, TRAIL, extracellular matrix proteins, cytokeratins, and aldehyde dehydrogenase were analyzed by reverse transcriptase quantitative PCR.

Results and Discussion

In athymic mice, *TNFα* overexpressing cells were not able to form xenografts and the cells completely lost their tumorigenicity. Athymic mice have defective development of thymus epithelium and they lack immune reaction mediated by T cells (CD4 and CD8 response), but they preserve robust NK cell response. In SCID/bg mice, with no mature T and B cells and lacking NK cells, the *TNFα* overexpressing cells formed rudimentary flat ulcerous xenografts with rapidly reduced size. The tumorigenicity was 50% and 62% for HT-29hTNFα and HCT116hTNFα cells, respectively. Histopathological analysis revealed necrotic lesions, a more differentiated phenotype of tumor cells forming pseudoglandular structures, and more abundant stromal cells. Ki67 marker revealed areas with non-dividing cells and decreased cytokeratin 7 positivity. PCR revealed increased aldehyde dehydrogenase expression.

Conclusion

Careful modulation of tumor microenvironment to the tumor-suppressive one using potent cytokine *TNFα* and controlled stimulation of antitumor immunity can be helpful in the future cancer treatment approach.

This work was supported by grants VEGA 2/0185/21 and APVV-21-0296.

P11 The Cancer-Associated Fibroblasts Modulate the Drug Response of Pancreatic Ductal Adenocarcinoma

Maria Urbanova[1], Verona Buocikova[1], Marina Cihova[1], Monika Burikova[1], Lucia Juhasikova[1], Jana Jakubechova[2], Bozena Smolkova[1]

[1] *Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Dubravska Cesta 9, 845 05 Bratislava, Slovakia*
[2] *Stem Cell Preparation Department, St. Elisabeth Cancer Institute, Bratislava, Slovakia*

Ductal Adenocarcinoma (PDAC) is characterized by the intricate interplay of factors within the tumor microenvironment (TME), where cancer-associated fibroblasts (CAFs) play a significant role. Recent studies emphasize the potential of epigenetic modulation to reshape the PDAC TME, thereby enhancing the efficacy of chemotherapy. Our objective was to develop a preclinical *in vitro* model that reflects the PDAC TME more accurately and could serve for the evaluation of combination targeting strategies. This includes exploring the use of decitabine (DAC) to remodel PDAC and improve sensitivity to conventional therapies.

The culture of stable cancer-associated fibroblasts (CAFs) originated from a grade 1 PDAC sample of a 67-year-old woman and was maintained in low-glucose DMEM supplemented with 6% platelet extract. The CAFs were characterized by immunofluorescence staining of selected markers and directly co-cultured with stable PDAC cell line MIA PaCa-2, representing the more aggressive basal-like subtype. The *in vitro* co-culture model was then treated with the combination of the epigenetic drug DAC with standard chemotherapy gemcitabine.

Our CAFs line exhibited myofibroblastic properties (myCAFs), as evidenced by high expression of α-SMA and PDGF-α proteins. MIA PaCa-2 cells co-cultured with CAFs responded better to standard chemotherapy gemcitabine alone, as well as to combination treatment, compared to MIA PaCa-2 tumor cells alone. Interestingly, the CAFs alone were also highly resistant to both treatments, indicating that cell interactions in co-culture modulate its drug response.

Our findings indicate, that CAFs present in PDAC tumors modulate the treatment response, and the myCAF phenotype could positively influence drug response.

The project was supported by APP0337, APP0497, APVV-20-0143, and APVV-21-0197 grants.

P12 Cell membrane coated biomimetic nanoparticles for targeted cancer therapy

Wujun Xu[1], Lizhi Liu[2], Vesa-Pekka Lehto[1]

[1]*Department of Technical Physics, University of Eastern Finland, 70210, Finland*

[2]*Children's Hospital, Boston MA 02115, Harvard University, USA*

Introduction: Cell membrane (CM)–coated biomimetic nanoparticles (NPs) have attracted a lot of attention in nanomedicines due to their unique biological features such as immune evasion, prolonged blood circulation, and homologous tumor targeting [1]. The present study aims to thoroughly investigate the effect of CM coating integrity on tumor targeting [2].

Methods: The CMs of CT26 cells were first extracted using the Dounce homogenizer [1]. A fluorescence quenching assay was proposed to quantitatively probe the integrity of the CM coating. At the end, a novel approach using external phospholipid was designed to improve CM coating efficacy (Fig. 1A). The NPs were characterized with physicochemical methods and evaluated with *in vitro* assays & preclinical animal models *in vivo*.

Results: Our results discovered that most of the biomimetic NPs (>90%) were only partially coated with CM [1], which contradicts the common assumption that the NPs would be completely coated. To address the problem of partial CM coating, the use of external phospholipid increased CM fluidity, promoting the final fusion of CM on the NPs (Figure 1). Consequently, the tumor targeting of the biomimetic NPs was significantly improved by enhancing CM coating efficacy [2].

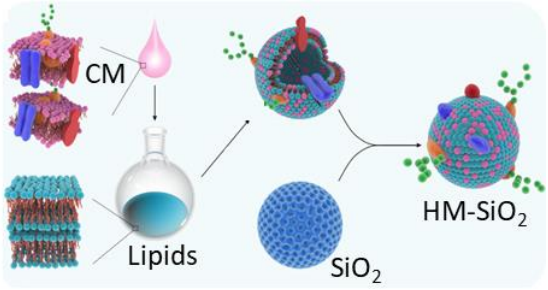

Figure 1. Illustration scheme showing the process of developing biomimetic NPs.

Conclusions:

This research offers innovative perspectives on cell membrane coating technology, paving the way for the strategic development of biomimetic NPs tailored for targeted cancer therapy.

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Acknowledgements

We acknowledge the support from the Research Council of Finland (projects 314412 and 356056).

P13 Tumour ecosystem and molecular dynamics in clinical triple-negative breast cancer depend on the chemotherapy regimen

Marco Barreca[1,2], Marco Mariani[3], Matteo Dugo[3], Barbara Galbardi [3], NeoTRIP consortium, ETNA consortium, Pinuccia Valagussa[1], Daniela Besozzi[6,7], Giuseppe Viale[1,4], Luca Gianni[1], Giampaolo Bianchini[5], Maurizio Callari[1]

[1] *Michelangelo Foundation, Milan, Italy*
[2] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*
[3] *IRCCS San Raffaele Hospital, Milan, Italy*
[4] *IEO, European Institute of Oncology, IRCCS, Milan, Italy*
[5] *UniSR San Raffaele University, Milan, Italy*
[6] *Department of Informatics, Systems and Communication, University of Milano-Bicocca, Milan, Italy*
[7] *Bicocca Bioinformatics, Biostatistics and Bioimaging (B4) Research Centre, Milan, Italy*

Background - Triple-negative breast cancer (TNBC) treatment traditionally relies on chemotherapy, increasingly paired with immune checkpoint inhibitors (ICIs) as the standard of care. Distinct chemotherapeutic agents may differently impact both the tumour and its microenvironment (TME), especially regarding immunomodulation. Despite extensive studies in cancer models, data on clinical tumours are scarce. We aimed to compare the early modulation of cancer pathways, immune-related features, and selected genes in TNBC patients undergoing various neoadjuvant chemotherapy regimens.

Methods – Four TNBC patient cohorts with RNA-seq data from paired core biopsies before and after the first neoadjuvant chemotherapy cycle were analysed. Patients received doxorubicin/cyclophosphamide (AC, n=19), nab-paclitaxel/carboplatin (PNabT, n=97), nab-paclitaxel (NabT, n=17), or paclitaxel (T, n=15). We quantified 82 cancer hallmark, immune, and TME genesets in each sample using the singscore R package, assessing their differential modulation through Student's t-test and ANOVA. Additionally, selected single genes were similarly evaluated.

Results – Comparing on-treatment to pre-treatment expression profiles revealed a general upregulation of immune cell- and immune function-related signatures, coupled with a downregulation of proliferation-related signatures. However, significant quantitative treatment-dependent differences emerged. T, NK, and dendritic cells signatures showed the largest upregulation in tumours receiving AC or NabT, while a decrease was observed in over 20% of tumours treated with PNabT or T, particularly in those with high pre-treatment expression. B, plasma, and mast cells signatures exhibited the highest upregulation in patients on NabT. PNabT and NabT groups demonstrated the most significant downregulation of proliferation signatures. PD-L1 was upregulated in 90% of AC-treated tumours, contrasting with a decrease observed in 40-60% of tumours in the other cohorts.

Conclusions – Our study underscores the early immunomodulatory and chemoattractant effects of neoadjuvant chemotherapy in TNBC. Anthracyclines and nab-paclitaxel alone are associated with a quantitatively stronger immunomodulatory response, revealing potential clinical implications for selecting optimal chemotherapy partners for ICIs in TNBC treatment.

P14 Assessment of New HDAC Inhibitors for Immunotherapy of Malignant Pleural Mesothelioma

BENSAID Douae[1, 2], DEHAME Virginie[1], DESHAYES Sophie[1], ERRAMI Mohammed[2], BERTRAND Philippe[3], BLANQUART Christophe[1]

1. CRCNA, INSERM UMR 892 - CNRS UMR 6299, Nantes University, France
2. Laboratory of Physiology and Physiopathology, Faculty of Sciences of Tetouan, Morocco.
3. IC2MP UMR CNRS 7285, Poitiers University, France

Researchers have long been looking to developing an effective treatment for malignant pleural mesothelioma (MPM), a highly aggressive cancer of the pleura with to date no uniform curative treatment identified. MPM is associated in most cases with asbestos exposure, and its incidence is still increasing despite the use of asbestos has been highly reduced. Epigenetic drugs could be very efficient in terms of cytotoxicity for several types of cancers such as mesothelioma, like Vorinostat (SAHA) in clinical trials. However, they often require high doses to be efficient, inducing toxic and none specific effects which urged the development of new molecules more efficient, less toxic, and acting in low doses.

In this study, we characterized newly synthesized histone deacetylase inhibitors (HDACIs) in comparison to valproic acid (VPA) and SAHA. Toxicity was evaluated on immune cells implicated in anti-tumor immune responses. The immunogenicity of Meso96 cell line treated with HDACIs in combination with the hypomethylating agent was studied by measuring cancer testis antigens expression (CTAs) and recognition of treated Meso 96 cells by a specific NY-ESO-1 CD8 + T-cell clone through measuring IFN-γ production. Finally, the effect of these combinations was tested on the mRNA expression of PD-L1, an inhibitory molecule of the immune response. Whereas all HDACIs were toxic for immune cells at high concentrations, VPA and the hydroxamate ODH presented a particular toxicity towards T-reg and NK cells. All HDACIs increased decitabine-induced CTA expression in Meso96 cells and allowed recognition by CD8+ NY-ESO-1 T-cell clone. Only two compounds, NODB and NODH, poorly induced PD-L1 expression. Finally, on Meso 96 spheroids, the HDACIs VPA, SAHA, ODB and ODH increased decitabine-induced XAGE-1 expression and all HDACIs increased PD-L1 expression.

This work was supported by Ligue Nationale Contre le Cancer, Structure Fédérative de Recherche François Bonamy, Centre National pour la RechercheScientifique et Technique (Morocco), Physiology and Physiopathology Laboratory (Morocco).

P15 Towards sustainable synthesis of PD-L1 inhibitors: Accessing birayl systems from renewable biobased furanic derivatives

RICHIEU Antoine, BERTRAND Philippe

IC2MP UMR CNRS 7285, Poitiers University, France

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.[1][2][3]

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 steps. Subsequent transformations can lead to novel inhibitors.

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

P16 Modeling complex leukemia microenvironment in vitro for advanced pre-clinical cytotoxicity testing of novel cell-based immunotherapy

Lucie Janstová[1,2], Veronika Švubová[1,2], Marek Jedlička, Eva Mašínová[1], Jana Szabová[1], Kateřina Kuglerová[1], Kateřina Kuželová[3], Barbora Brodská[3], Jan Frič[1,4]

[1] *Department of Modern Immunotherapy, Institute of Hematology and Blood Transfusion, Prague, Czechia*
[2] *Department of Cell biology, Faculty of Science, Charles University, Prague, Czechia*
[3] *Department of Proteomics, Institute of Hematology and Blood Transfusion, Prague, Czechia*
[4] *International Clinical Research Center of St. Anne's University Hospital in Brno (FNUSA-ICRC), Pekařská 53, 656 91, Brno, Czechia*

In the context of treating hemato-oncological diseases, we often rely on simplistic cancer cell line cultures for pre-clinical testing. However, this model falls short in replicating the complex human *in vivo* leukemic microenvironment within the bone marrow niche (BMN). To address this, we've developed advanced *in vitro* leukemia 3D model by combining mesenchymal stem cells (MSCs), human umbilical vein endothelial cells (HUVECs), and leukemic cells, provide a more realistic platform for evaluating the cytotoxicity and migration of innate immune cells (NK cells, δγ T cells, iNKT cells etc.), or compare pre-treatment conditions.

For simulating the leukemic BMN in monolayer, we co-cultured human telomerase reverse transcriptase (hTERT) MSCs with hematopoietic stem/progenitor cells (HSPCs) isolated from umbilical cord blood or mobilized blood. BMN spheroids were formed with Aggrewell 400 (STEMCELL Technologies), involving hTERT MSCs, HUVECs, and leukemic cell lines. NK cells, isolated from peripheral blood mononuclear cells, were expanded for 14 days in X-VIVO 20 with Gentamicin (Lonza) and 5% human serum (Capricorn), using irradiated K562 cells and cytokines IL-2 and IL-15 (both R&D).

In the BMN monolayer, NK cells demonstrated specific targeting of K562 cells, albeit with significantly reduced efficiency compared to conventional models. The spheroids effectively facilitate pre-clinical cytotoxicity testing of *in vitro* expanded NK cells while being suitable for migration assays. We showed that NK cells need a recovery period after cryopreservation to fully restore their cytotoxic and migration capabilities. Moreover, we shoved altered migration and killing ability of NK cells cultured in the presence of TGF-β.

In conclusion, model described here holds promise for the development and testing of novel compounds in future clinical trials. They underscore the importance of more intricate leukemia models to better emulate the *in vivo* leukemia microenvironment, which is expected to enhance NK cell effectiveness and refine therapeutic approaches for AML.

This work was supported by Ministry of Health, Czech Republic – conceptual development of research organization (Institute of Hematology and Blood Transfusion – ÚHKT, 00023736).

P17 Mutational burden and tumor immune status as predictive factors for in situ vaccination by TNFα and IL-12 gene electrotransfer

Urška Kamenšek[1,2], Katja Uršič Valentinuzzi[1,2], Boštjan Markelc[1,3], Maja Čemažar[1,4], Vita Šetrajčič Dragoš[5], Gregor Serša[1,3]

[1] *Institute of Oncology Ljubljana, Zaloška cesta 2, SI-1000 Ljubljana, Slovenia*
[2] *University of Ljubljana, Biotechnical Faculty, Jamnikarjeva ulica 101, SI-1000 Ljubljana, Slovenia*
[3] *Faculty of Health Sciences, University of Ljubljana, Zdravstvena pot 5, SI-1000 Ljubljana, Slovenia*

In situ vaccination is an innovative immunotherapy strategy that uses localized ablative techniques to elicit an immune response against tumor antigens released from therapy-killed tumor cells. In our previous work, we proposed the use of intratumoral gene electrotransfer to simultaneously deliver a cytotoxic cytokine tumor necrosis factor-α (TNFα), to facilitate in situ vaccination, along with an immunostimulatory cytokine interleukin 12 (IL-12), to boost the primed immune response. In the current study, we aimed to correlate tumor immune profiles with the local and systemic efficacy of this approach in three syngeneic mouse tumor models: B16F10 melanoma, TS/A mammary adenocarcinoma and CT26 colon carcinoma (Approval No. U344011/2015/43). To assess local and abscopal, i.e., systemic, antitumor efficacy, dual-flank tumor models were established by subcutaneous injection of tumor cells in the right flank of syngeneic mice (primary tumor), followed by subcutaneous injection of a reduced amount of cells (80%) into the left flank 3 days later. Gene electrotransfer was performed in 40 mm^3 primary tumors. Therapeutic efficacy was evaluated by monitoring tumor growth in both treated and untreated tumors. Tumor immune profiles were characterized in untreated 40 mm^3 tumors by determining tumor mutational burden, tumor infiltrating CD4+ and CD8+ lymphocytes, and expression of PD-L1 and MHC-I on tumor cells. The correlation results showed that none of the investigated tumor characteristics directly predicted local treatment success. However, high tumor mutational burden, increased immune infiltration and MHC-I expression were associated with greater systemic (abscopal) efficacy. Thus, we have confirmed that tumor antigen abundance and presentation, as well as the absence of immunosuppressive mechanisms, are important for effective in situ vaccination. These results provide important clues for the future development of in situ vaccination-based treatments and for the selection of tumor types that are most likely to benefit from such treatments.

This work was supported by the Slovenian Research Agency under the scope of the Program P3-0003, grant number J3-8202 and J3-2528.

P18 Unraveling the interplay between BRAFV600E mutation, immune landscape, and immunogenic cell death in melanoma: Implications for therapeutic strategies

Fátima María Mentucci[1], Elisa Ayelén Romero Nuñez[1], Agustina Ercole[1], Valentina Silvetti[1], Jessica Dal Col[2], María Julia Lamberti[1]

[1]Departamento de Biología Molecular, INBIAS, Universidad Nacional de Río Cuarto, Río Cuarto, Cordoba, Argentina; mlamberti@exa.unrc.edu.ar
[2]Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, Baronissi, Salerno, Italy

The intricate interplay between the immune system and melanoma progression poses a challenge in understanding and effectively treating this aggressive malignancy. Melanoma, characterized by a high genomic mutational burden and frequent BRAFV600E mutation, exhibits complex immune evasion mechanisms. Our study delves into the genomic and immune landscape alterations associated with the BRAFV600E mutation in melanoma. Through in silico analyses, we reveal a decreased tumor mutational burden (TMB) and alterations in immune subtypes indicative of immune evasion in BRAFV600E-mutated tumors. Furthermore, we investigate the responsiveness of BRAFV600E-mutated cells to immunogenic cell death (ICD) induction, particularly photodynamic therapy (PDT). Our findings suggest increased susceptibility of BRAFV600E-mutated tumors to PDT-induced cell death, potentially linked to alterations in the interferon-1 (IFN-1) pathway. Specifically, we observe upregulation of IFN-1 pathway activation in response to PDT, highlighting its potential as a therapeutic strategy. Understanding the mechanisms underlying these associations provides insights into optimizing immunotherapeutic approaches for melanoma treatment. Further research into the broader implications of the IFN-1 pathway across different cell death inducers is warranted to enhance our understanding of melanoma biology and therapeutic responses.

This work was supported by Florencio Fiorini para Investigación en Ciencias Biomédicas grant, PICT-2021-GRFTI-00393, PICTO CBA 00034/2022, PIBAA-CONICET, and Jóvenes en Ciencia – MinCyT-Cba.

P19 PD-L1 is highly expressed in Mantle cell lymphoma cell lines

Lopušná Katarína[1], Nižňanská Zuzana[1], Benko Mário[1], Štibrániová Iveta[1], Zezulová Viktória[1], Džubara Jozef[1], Bitala Andrej[1], Vranovský Andrej[2], Benčíková Petra[2], Režuchová Ingeborg[1], Nemčovičová Ivana[1]

[1] Institute of Virology, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia [2] Department of Oncohematology, National Cancer Institute, Bratislava, Slovakia

Immune checkpoint molecules are expressed by various immune cells, they regulate immune responses and immune homeostasis. We recognize two classes of checkpoint receptors, inhibitors and activators. In healthy state antigen presenting cells (APCs) express inhibitory checkpoint molecules, such as Programmed death-ligand 1 (PD-L1). Binding of PD-L1 with its receptor PD-1 on T cells inhibit T cell responses through downregulation of TCR signaling. On the other side, tumor cells have developed sophisticated strategies to evade recognition by the immune system, and one key mechanism involves the manipulation of immune checkpoint molecules. Tumor cells express immune checkpoint molecules to suppress immune responses, e.g. through PD-L1. Their function is to bind with inhibitory receptors expressed by T and B cells and in this way suppress proliferation and effector functions of T and B cells including their antitumor activities in a same way as APCs.

In this work, we evaluated *in vitro* models for Mantle cell lymphoma (MCL), a rare hematologic malignancy, which is difficult to treat and has a median survival around 3- 5 years. Analysis of publicly available RNA-seq data showed that PD-L1 is highly expressed in most of the MCL cell lines, including Maver-1, JVM-13 and Granta-519. In addition, real-time qPCR analysis of PD-L1 expression in various lymphoma cell lines derived from MCL, Chronic lymphocytic leukemia, Chronic myeloid leukemia, Burkitt´s lymphoma and others showed that MCL cell line JVM-2 exhibit the highest expression of PD-L1 from all cell lines tested. To evaluate whether high expression of PD-L1 in MCL cell lines would correlate with clinical data, we analyzed PD-L1 expression in 71-year old MCL patient. We found that expression of PD-L1 in blood sample was increased almost 5-times when compared to healthy donors. These results suggest that MCL cell lines Maver-1, Granta-519, JVM-2 and JVM-13 might serve as pre-clinical model for *in vitro* studies of potential anticancer drugs targeting PD-L1 in Mantle cell lymphoma.

The work was funded by the project No. 1136/01/02, which has received funding from the European Union's Horizon 2020 Research and Innovation Programme on the basis of the Grant Agreement under the Marie Skłodowska-Curie funding scheme No. 945478 - SASPRO 2; by the Scientific Grant Agency of the Slovak Republic (VEGA 2/0063/21); and by the Slovak Research and Development Agency (APVV-19-0376).

P20 CDR Grafting: Implications for Anti-Angiogenic Therapy in Solid Tumors

Melis Denizci Öncü[1], Bertan Koray Balcıoğlu[1], Beytullah Özgür[1] , Hasan Ümit Öztürk[1], Müge Serhatlı[1], Şeyma Işık[2], Berrin Erdağ[3], Gizem Dinler Doğanay[4], Aylin Özdemir Bahadır[1]

[1] *Life Sciences, Marmara Research Center, The Scientific and Technological Research Council of Turkey, Kocaeli, Turkey*
[2] *Medical Biotechnology Department, Acıbadem University, İstanbul, Turkey*
[3] *Health Sciences Department, İstanbul Aydın University, İstanbul, Turkey*
[4] *Molecular Biology and Genetics Department, İstanbul Technical University, İstanbul, Turkey*

Monoclonal antibody therapeutics targeting vascular endothelial growth factor receptor 2 (VEGFR-2) have long been sought after as a potential strategy to inhibit angiogenesis, a critical process in solid tumor growth. However, the development of such antibodies has been challenging, particularly due to issues with solubility and activity. In this study, we address these challenges by engineering a soluble single-chain variable fragment (scFv) against VEGFR-2 using a complementarity determining regions (CDR) grafting technique.

We first engineer an scFv against VEGFR-2, known for its inclusion body formation, and then graft its CDR sequences onto a framework of a more soluble scFv molecule. State-of-the-art prediction tools and molecular dynamics simulations were employed to assess the physicochemical properties of the engineered scFv. Experimental analyses, including expression studies, affinity determination via surface plasmon resonance (SPR), and biological activity assays, were conducted to evaluate the solubility and activity of the engineered molecule. Our computational and experimental results demonstrate that the CDR grafting technique significantly enhances the solubility of the scFv compared to the original molecule. SDS-PAGE and Western blot analyses confirm the increased solubility of the expressed protein, while SPR assays reveal comparable binding affinities to VEGFR-2. Moreover, biological activity assays conducted on human umbilical vein endothelial cells (HUVEC) demonstrate the anti-angiogenic properties of the engineered scFv.

This study highlights the potential of CDR grafting as a strategy to rescue inclusion body-forming scFvs, offering a pathway to develop soluble and active anti-angiogenic therapeutics targeting VEGFR-2. Such advancements hold promise for the development of effective treatments for solid tumors, providing new avenues for anti-angiogenic therapy in cancer patients.

This work was supported by grants TUBITAK 1009 TARAL project (117H001) and TUBITAK TARAL 1003 project (216S387).

P21 NK cell-based immunotherapy against lung metastases from gastrointestinal tumors: a therapeutic opportunity

Paula Martin-Rubio[1], Sandra García-Mulero[3], Eduardo Candeal[1], Andrea Moreno[1], Isabel Villaoslada[1], Pilar Espiau-Romera[1], Alba Royo-García[1], Beatriz Parejo-Alonso[1], Claudia Guerrero[2], Sandra Hidalgo[1], Alberto Villanueva[3], Cristina Santos[4,5], Raúl Embún[6], Gonzalo Hijos[6], Julián Pardo[1,2,7], Nacho Aguiló[2]*, Patricia Sancho[1]* & Rebeca Sanz-Pamplona[1,8]*

[1] IIS Aragón, [2] UNIZAR, [3] IDIBELL, [4] ICO, [5] HCULB, [6] CIBERONC, [7] CIBERINFEC, [8] CIBERESP *Co-corresponding authors*

In recent decades, the live expectancy of cancer patients has improved thanks to advances in research. However, treatment options for metastatic disease, responsible for about 90% of cancer deaths, continue being limited. Immunotherapy has emerged as an interesting therapeutic strategy but, unfortunately, with limited success in the metastatic setting. In our group, we have identified a cluster of "High-Immune" metastases that share an inflammatory, immunogenic phenotype, susceptible to be treated with immunotherapy. This cluster mainly includes lung metastases samples, regardless the primary tumor of origin.

To continue exploring this issue, we focus our studies on the gastrointestinal tumors colorectal cancer (CRC) and pancreatic cancer (PDAC). These cancer types have common features in terms of metastatic colonization and in their mutation patterns, both in primary tumors and in lung metastases. Applying immunomics to transcriptomic data, we found that lung metastases show a higher infiltration of NK cells and higher expression of HLA genes, as compared to the less immunogenic liver metastases. These results suggest that NK cells may control tumor growth in the lungs and that NK cell-based therapy could be an interesting therapeutic approach for lung metastases.

We tested two NK cell-based immunotherapies against lung metastases: 1) administration of NK92 cells and 2) vaccination with BCG, a tuberculosis vaccine that activate cytotoxic immune cells such as NK cells. A proof-of-concept study demonstrated the NK92 cells treatment feasibility in a patient derived orthoxenograft model of a lung metastasis of CRC. All treated mice showed a significant reduction in tumor weight and tumor size in comparison with animals treated with standard chemotherapy. Moreover, our preliminary results in a metastatic orthotopic PDAC model showed that prophylactic BCG vaccination improves survival and decrease metastatic burden, being the inhibition of lung metastases more pronounced than other less immunogenic metastatic sites.

To continue this project, new in vitro and in vivo models of lung metastasis are being stablished. In addition, we are validating our results by spatial transcriptomics technique in human samples. Finally, we want to expand our studies to other cancer types. In conclusion, although further studies are needed, NK cell-based immunotherapy could be an interesting therapeutic option for cancer patients metastasizing in lungs.

P22 Adhesion GPCRs as modulators of immune functions and potential targets for solid tumors

Nour Islem Yanis SAIDANI[1], Kenza Aouar[2]

[1] *Aix Marseille University, Marseille, France, nour-islem-yanis.saidani@inserm.fr*
[2] *Institute of Cancer and Genomic Sciences, Birmingham University, Birmingham, United Kingdom*

Immune checkpoint blockade (ICB) has been a major medical and scientific advance. ICB found success in the management of metastatic solid tumors such as melanoma and non-small cell lung cancer. Although the identified immune checkpoints, PD-1, CTLA4, TIGIT…etc are potently targeted, a large number of patients remain ineligible for the currently marketed ICBs. We identified the adhesion subfamily within the G-protein coupled receptors superfamily as potential targets for the potentiation of the immune system and targeting of malignant cells. RNA-seq of poor prognosis tumors such as pancreatic adenocarcinoma and ovarian cancer show an overexpression of aGPCRs when paired with healthy tissue. Single cell RNA-seq reveal a dual expression of aGPCRs in malignant cells along with infiltrating immune cells. Most notably, the adhesion GPCR ADGRG1 is highly expressed in malignant cells and CXCL13 CD8 cells which have elevated levels of exhaustion markers (PD1, TIM3 and TIGIT). Furthermore, the aGPCR ADGRG6 has been found to be expressed in tumor associated macrophages M2 MMP9 along with malignant cells. Moreover, ADGRG6 overexpression in ovarian cancer is correlated with a significantly decreased overall survival in TP53 mutated patients. Altogether, these results suggest a potential therapeutic strategy to target both malignant cells along with protumoral immune cells of the tumor microenvironment.

This work was supported by grants of the French ministry of higher education and research.

P23 PSGL-1 as a novel target for B-cell lymphoma immunotherapy

Nuno Rodrigues dos Santos[1]

[1] *i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

The P-selectin glycoprotein ligand-1 (PSGL-1) has garnered significant attention as a potential therapeutic target in the treatment of cancer, given its critical roles in mediating leukocyte-endothelial cell interactions and contributing to the immune evasion mechanisms of cancer cells. Our research has revealed dual therapeutic strategies targeting PSGL-1 in lymphoma: antibody-mediated direct lymphoma cell killing and blockade of its immune checkpoint function in T cells. The first approach involves the application of monoclonal antibodies that specifically bind to PSGL-1 on the surface of lymphoma cells, thereby directly inducing cytotoxic effects to eliminate the cancer cells. This method leverages the high expression of PSGL-1 on certain lymphoma subtypes. Treatment of a lymphoma mouse model with a PSGL-1 mAb led to significant tumor growth inhibition.The second strategy focuses on the inhibition of PSGL-1's role as an immune checkpoint molecule. PSGL-1 interaction with its ligands has been implicated in the suppression of T-cell activation and proliferation, a mechanism that lymphoma cells exploit to evade immune surveillance. By blocking this interaction, antibody treatments can restore T-cell functionality and promote an effective anti-tumor immune response, as demonstrated by treatment of human T cells co-cultured with lymphoma cells with a PSGL-1 blocking mAb. In addition, our preclinical studies in a syngeneic mouse model have shown promising results, with PSGL-1 targeted therapies demonstrating significant antitumor activity. These findings underscore the potential of PSGL-1 as a multifaceted therapeutic target in lymphoma, offering new avenues for the development of more effective and targeted cancer immunotherapies.

This work was supported by grants from Gilead Sciences Portugal (Programa Gilead GÉNESE ref. no. PGG/038/2017), and Associação Portuguesa Contra a Leucemia (APCL).

P24 Decitabine in the treatment of therapy-resistant breast cancer

Lenka Trnkova, Verona Buocikova, Monika Burikova, Andrea Cumova, Svetlana Miklikova, Bozena Smolkova, Marina Cihova

Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia

Breast cancer is the most common cancer type among women and represents the second leading cause of female cancer-related deaths. Despite significant advancements in breast cancer management in recent years, the development of therapeutic resistance still challenges the efficacy of current therapies. Epigenetic deregulation has been described as a mediator of therapy resistance, and epigenetic therapy has recently emerged as a potential strategy to enhance response to anti-cancer treatment. Similarly, treatment with the DNA demethylating agent decitabine, either alone or in combination with standard therapy, has shown favorable results in preclinical models. Based on these findings, ongoing clinical studies are focusing on its application to improve response to standard therapies, including chemotherapy and immunotherapy. However, these clinical studies provide controversial results, calling for extended research in this field to provide more tailored therapeutic strategies.

This work presents the development and characterization of chemotherapy-resistant triple-negative breast cancer (TNBC) cell lines, possessing acquired resistance to doxorubicin and paclitaxel. Derived chemotherapy-resistant cancer cells harbor changes in cell proliferation, tumorigenicity, and gene expression profiles compared to their sensitive counterpart. Sensitivity to decitabine was altered in paclitaxel-resistant TNBC cells. *In vitro* and *in vivo* combination treatment revealed a synergistic effect of decitabine combined with doxorubicin in sensitive and paclitaxel-resistant TNBC cells. Sensitive and paclitaxel-resistant tumors of i*n vivo* mice xenografts were used to assess transcriptomic and DNA methylation changes following decitabine monotherapy and combined therapy.

In conclusion, the presented results indicate phenotypic and transcriptomic alterations in derived chemotherapy-resistant TNBC cells and suggest combination therapy with the epigenetic agent decitabine as a possible strategy to sensitize TNBC cells to standard therapy.

This work was supported by grants EraCoSysMed project RESCUER, VEGA 2/0138/20 and VEGA 2/0067/22. We acknowledge Genomics Core Facility of CEITEC Masaryk University and Bioinformatics Core Facility of CEITEC Masaryk University of A4L_ACTIONS, supported by European Union's Horizon 2020 under grant agreement No. 964997.

P25 Novel treatment of chemoresistant germ cell tumors using immunotherapy – based antibody – drug conjugates

Natália Udvorková[1,2], Lucia Kučerová[1,3], Michal Mego[1,3,]

[1] *Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava*
[2] *Faculty of Medicine, Comenius University, Bratislava*
[3] *Translational Research Unit, National Cancer Institute and the 2nd Oncology Clinic of Medical Faculty, Comenius University, Bratislava*

Tumor stem cells are part of the tumor microenvironment and contain specific proteins - markers that are often overexpressed and the signaling pathways they participate in are often deregulated. These markers have thus become specific biomarkers from the point of view of cancer diagnosis and at the same time represent prospective therapeutic targets. Germ cell tumors are the most common malignancy in young men, with an ever-increasing incidence. The treatment of these solid tumors is very successful mainly thanks to chemotherapy based on cisplatin. However, there is a group of patients who show resistance to this type of treatment, relapse and have a poor prognosis. It is these patients who require new treatment procedures.

Several studies demonstrate the efficacy of the Sacituzumab Govitecan ADC (antibody – drug conjugate) immunotherapeutic approach targeting the tumor cell surface marker TROP2 in various tumor types. We detected the surface marker TROP2 on several types of germ cell tumor cell lines. Pilot studies demonstrate the cytotoxic effect of Sacituzumab Govitecan in vitro and antitumor effect in vivo. Our results will be discussed.

The work was supported by the Research and Development Support Agency - grant APVV-20-0158, APVV-21-0197, grant VEGA 2/0124/21 and with the support of the Cancer Research Foundation and the League against Cancer.