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Modelling immunotherapy response and toxicity in cancer

final annual conference

2026

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Table Of Contents

Programme

Day 1

Wednesday, 20 May 2026

AGENDA

8.30-9.00	Registration for the Core Group meeting (Core Group members only) **NIB registration desk**
9.00-12.00	IMMUNO-model Core Group Meeting (Core Group members) **NIB conference room**
12.00–13.00	Lunch and NIB Lab tour (Core Group members) **NIB gallery**
Start of the official program (All attendees)	
12.00–13.00	Registration **NIB registration desk**
13.15–13.20	Welcome speech local organizers and NIB director **NIB conference room**
13.20–13.30	Welcome speech Eva Martinez-Balibrea, action chair **NIB conference room**
13.30–15.30	**TGRC session 1**: Gender Equal Opportunities and the Sex Gap in Immunology and Immunotherapy **NIB conference room** Chairs: Hanne Haslene-Hox, SINTEF, Norway & Catarina A. Rodrigues, IPO Porto, Portugal
13.30–14.00	Invited speaker: **Berna Özdemir** (Bern University Hospital, Switzerland) Talk title: Sex and gender differences in immunotherapy efficacy and toxicity- what is the evidence?
14.00–14.30	Invited speaker: **Sonja Loges** (University Medical Centre Mannheim, Germany) Talk title: Sex Differences in NSCLC
14.30–15.00	Invited speaker: **Camila Consiglio** (Lund University, Sweden) Talk title: Decoding sex differences in human immunity using systems immunology

15.00–15.30	**Roundtable discussion** **Uzma Hasan**, The Lyon Immunotherapy for Cancer Laboratory, France Gianmarco Contino, University of Birmingham, United Kingdom
15.30–16.15	Coffee break & **Industry** meetings **NIB gallery**
16.15–18.30	**TGRC Session 2:** Industry Presentations and Networking **NIB conference room** Chairs: Denis Collins, Dublin City University, Ireland and Marta Maleszewska, University of Warsaw, Poland
16.15–16.22	INITIO CELL: **Devrim Pesen Okvur**
16.25–16.32	Labena d.o.o.: **Uršula Prosenc Zmrzljak**
16.35–16.42	Omega d.o.o.: **Samy Dufour**
16.45–16.52	Axion Biosystems: **Aniello Lombardi**
16.55–17.02	RWD Life Science Co., LTD: **Fan Yu**
17.05–17.12	Abbmira Therapeutics AG: **Marc Creus**
17.15–17.22	28DIGITAL: **Sonay Goneli**
17.25–18.25	Open discussion with Industry
18.30–22.00	IMMUNO-model Core Group Meeting Dinner, **Core Group members**

Day 2

Thursday, 21 May 2026

AGENDA

Start of Scientific Program (all attendees)	
8.15–9.15	Registration **NIB registration desk**
	Scientific Session 1: Immuno-models in hematologic tumors WG4 **NIB conference room** Chair: Nuno R. dos Santos, i3S, University of Porto, Portugal
9.15–10.00	Keynote lecture **Elisa Oricchio**, EPFL, Switzerland Talk title: Use tissue explants to decode tumor evolution and response to therapies
	Scientific Session 2: In vivo cancer immunotherapy models–WG2 **NIB conference room** Chair: Doreen Lau, Brunel University of London, UK
10.00–10.45	Keynote lecture **Erik Aarntzen**, Radboud University Medical Centre, the Netherlands Talk title: Imaging CD8 T-cells in vivo: the potential of PET imaging as biomarker technology
10.45–11.25	Coffee break & Poster session WG2+WG4 **Lab tour** **NIB gallery**
	Scientific Session 3: Advances in immuno-models **NIB conference room** Chair: Eva Martinez-Balibrea, Germans Trias i Pujol Research Institute, Spain
11.25–12.10	Keynote lecture **Jerôme Galon**, INSERM, Université Sorbonne, France Talk title: The immune contexture in the era of cancer immunotherapies
12.10–13.25	Lunch & Poster session WG2+WG4 **NIB gallery**
	Scientific Session 4: Immuno-model in hematologic tumors WG4 **NIB conference room**

	Chair: Laura Belver, Josep Carreras Leukaemia Research Institute, Spain
13.25–13.55	Invited speaker: **Emanuele Azzoni**, University of Milano-Bicocca, Italy Talk title: From prenatal origins to novel therapeutic strategies targeting inflammatory pathways: leveraging pre-clinical models of Juvenile Myelomonocytic Leukemia (JMML)
13.55–14.25	Invited speaker: **Jordi Minguillón**, CIEMAT/IIS-FJD and IdiPAZ/CNIO, Madrid Talk title: Preclinical and Clinical Research on CAR T-Cell Therapy for Pediatric Leukemia
14.25–14.35	Short talk: **Adrian Bogdan Tigu**, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania Talk title: Synergizing Immunotherapy and Proteasome Inhibition in Mantle Cell Lymphoma
14.35–15.15	Coffee break & Poster session WG2+WG4 **NIB lab tour** **NIB gallery**
	Scientific Session 5: *In vivo* cancer immunotherapy model WG2 **NIB conference room** Chairs: Eleni Douni, Department of Biotechnology Agricultural University of Athens/Institute for Bioinnovation, B.S.R.C. "Alexander Fleming, Greece and Renata Stripecke, University Hospital Cologne, Germany
15.15–15.45	Invited speaker: **Renata Stripecke,** University Hospital Cologne, Germany Talk title: Humanized mice and engineered CAR-T cells
15.45–16.15	Invited speaker: **Gustavo Alviter-Raymundo,** University of Cambridge, UK Talk title: Humanised mouse models for pre-clinical investigation of cancer immunotherapies
16.15–16.25	Short talk: **Alfredo Pherez Farah,** University of Padova, Italy Talk title: Myeloid-t cell therapy interactions reshape the tumor microenvironment to sustain tumor control
16.25–16.55	**WG2 and WG4 showcase and discussion** **NIB conference room** Chair: Emmet Mc Cormack, Laura Belver
17.00–17.10	**GROUP PHOTO**
18.00–22.00	**NIB welcome reception:** Social dinner

Day 3

Friday, 22 May 2026

AGENDA

Scientific Program (all attendees)	
8.30–9.00	Registration **NIB registration desk**
	Scientific Session 6: *In vitro* and *ex vivo* cancer immunotherapy models WG1 **NIB conference room** Chair: Vinton Cheng, University of Birmingham, UK Hanne Haslene-Hox, SINTEF, Norway
9.00–9.45	Keynote lecture **Frederik De Smet**, KU Leuven, Belgium Talk title: From patient tissue to in vivo models: multiscale analysis of tumour-immune interactions in brain tumours
09.45–10.15	Invited speaker: **Jordane Divoux**, CLCC François Baclesse, France Talk title: The TRIPLEX study: use of patient-derived tumor organoids as an innovative tool for precision medicine in triple-negative breast cancer
10.15–10.45	Invited speaker: **Kristina Kromer,** Institute of Human Biology, Switzerland Talk title: Modelling the development of tumor-associated macrophages (TAMs) in colorectal cancer using a complex human *in vitro* system of the tumor microenvironment
10.45–10.55	Short talk: **Ferran Grau Leal**, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain Talk title: Implementation of Patient-Derived Organotypic Tumor Spheroids (PDOTS) from Primary and Metastatic Colorectal Cancer for Ex Vivo Immunotherapy Testing
10.55–11.35	Coffee break & Poster session WG1+WG3 **NIB gallery**
	Scientific Session 7: Immuno-models in solid tumors WG3 **NIB conference room** Chair: Rosalinda Sorrentino, University of Salerno, Italy and Rebeca Sanz Pamplona, Institute for Health Research Aragon (IISA), Spain

11.35–12.20	Keynote lecture **Marinka Žitnik,** Harvard University, USA Talk title: Empowering cancer research with AI
12.20–12.50	Invited speaker: **Natalia Bednarz-Knoll**, Medical University of Gdańsk, Poland Talk title: Imaging flow cytometry for decoding circulating tumor and normal cell interactions: implications for immunothrepy monitoring
12.50–13.20	Invited speaker: **József Dudás**, Innsbruck Medical University, Austria Talk title: Advances form the COST Action CA21135 "Modelling immunotherapy response and toxicity in cancer" for the translational research in ENT-clinic in Innsbruck, Austria
13.20–13.30	Short talk: **Fatih Özefe,** Izmir Institute of Technology, Turkey Talk title: Perfusion-based Melanoma-Immune Microenvironment (MIMe) Platform for Modeling Adoptive Immune Response
13.30–14.00	Lunch & Poster session WG1+WG3 **NIB lab tour** **NIB gallery**
14.00–14.30	**WG1 and WG3 showcase and discussion** ***NIB conference room*** Chair: Lukasz Skalniak, Marleen Ansems
14.30–15.30	**Interactive session:** Immuno-model showcase, achievements, closure and future **Poster awards** **NIB conference room** Chair: Eva Martinez-Balibrea, Germans Trias i Pujol Research Institute, Spain

Abstracts of keynote lectures

1. Use tissue explants to decode tumor evolution and response to therapies

Elisa Oricchio[1]

[1]*EPFL, Switzerland*

Tumors are highly heterogeneous, with unique molecular alterations and interactions with their microenvironment, making it difficult to choose the most effective therapy for each patient. To improve personalized treatment, patient-derived models such as xenografts and organoids have been developed, but current approaches have important limitations—especially for B-cell lymphomas, where models often fail to replicate the complex structure and diversity of lymphoid tissue. To address this gap, we developed an ex vivo culture system using patient lymphoma tissue fragments that preserve the original tissue architecture and cellular composition. This model allowed rapid testing of multiple therapies and showed selective drug sensitivity. Notably, in most cases where ex vivo results were compared with actual patient treatments, the model successfully predicted clinical outcomes, highlighting its potential as a tool for personalized therapy selection in B-cell lymphomas. We are now exploiting this system to understand the origin of lymphoma and its evolution.

2. Imaging CD8 T-cells in vivo: the potential of PET imaging as biomarker technology

Erik H.J.G. Aarntzen[1,2]

[1]*Department of Nuclear Medicine and Molecular Imaging, University medical center Groningen, Groningen, The Netherlands*

[2]*Department of Medical Imaging, Radboud university medical center, Nijmegen, The Netherlands*

Blood- and tissue-based assays, together with fluorescence microscopy imaging, have been instrumental in understanding cancer-immune cell and immune-immune cell interactions. For example, the presence and intra-tumoral distribution of tumor-infiltrating anti-cancer cytotoxic CD8 T-cells is an established predictor of the efficacy of immune therapy (1-7). Albeit these assays can characterize aspects of CD8 T-cells at cellular and molecular level, they have limited potential to investigate a critical aspect of CD8 T-cell behaviour during immune therapy: inter-organ trafficking. Positron emission tomography (PET) tracers targeting CD8 T-cells, e.g. [89Zr]Zr-crefmirlimab berdoxam, may quantitatively assess CD8 cell distributions at whole body level (8-16), allowing to address inter-organ trafficking under different iimmune-mediated conditions. In this presentation, I will discuss the potential of CD8 PET imaging to develop into an imaging biomarker technology, much complementary to other biomarker technologies, and how PET-derived data can feed into multi-compartmental models describing immune cell interactions.

References:

1. Smyth, M. J., Ngiow, S. F., Ribas, A., and Teng, M. W. (2016) Combination cancer immunotherapies tailored to the tumour microenvironment. Nat Rev Clin Oncol 13, 143-158

2. Tumeh, P. C., Harview, C. L., Yearley, J. H., Shintaku, I. P., Taylor, E. J., Robert, L., Chmielowski, B., Spasic, M., Henry, G., Ciobanu, V., West, A. N., Carmona, M., Kivork, C., Seja, E., Cherry, G., Gutierrez, A. J., Grogan, T. R., Mateus, C., Tomasic, G., Glaspy, J. A., Emerson, R. O., Robins, H., Pierce, R. H., Elashoff, D. A., Robert, C., and Ribas, A. (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515, 568-571

3. The immune contexture in the era of cancer immunotherapies

Jérôme Galon[1]

[1]*Director of Research, French National Institute of the Health and Medical Research (INSERM), Chief of laboratory of Integrative Cancer Immunology, Cordeliers Research Center, Sorbonne Université, Université Paris-Cité, Paris, France*

We demonstrated the critical tumor-microenvironment parameters determining the dissemination to distant metastasis. We found that the combination of immune parameters associating the nature, the density, the functional immune orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host-immune reaction on patients' prognosis. We defined these parameters as the "immune contexture". We characterized the immune landscape within human tumors, and showed the importance of several adaptive immune cells. We further demonstrated the significant role of Immunoscore and immunoediting in affecting metastatic clonal dissemination. We hence proposed a "parallel immune selection model" of tumor evolution incorporating the effects of the immune system in shaping and driving metastatic spread. We proposed a continuum of cancer immunosurveillance from pre-cancer to metastasis, and highlighted the role of the immune contexture to predict response to several types of immunotherapies, including CAR-T cells. Early intervention are limited by our understanding of how carcinogenesis transforms the pre-invasive epithelium and its microenvironment before the carcinoma stage. We described the sequence of molecular and cellular changes leading to cancer formation and the co-evolution of the earliest immune response. We revealed that immune sensing, infiltration and activation of immune cells, immune escape, and microenvironment re-organisation occur early in pre-cancer. Leveraging the immune contexture and the mechanisms of immune modulation for individuals at risk of developing cancer could influence cancer prevention.

4. From patient tissue to in vivo models: multiscale analysis of tumour-immune interactions in brain tumours

Frederik De Smet[1]

[1]*KU Leuven, Belgium*

Tumour-immune interactions critically shape disease progression and therapeutic response in brain tumours, yet are difficult to capture across biological scales. We describe an integrated modelling framework that combines spatially resolved omics of patient tumours with complementary in vitro and in vivo systems. Spatial profiling reveals distinct immune architectures and macrophage states associated with standard and immunotherapeutic treatments. These observations are functionally interrogated using cocultures and zebrafish tumour avatars, enabling real-time visualisation of tumour-immune dynamics in vivo. This multiscale approach provides a versatile platform to study mechanisms of immunotherapy response and resistance in brain tumours.

5. Empowering cancer research with AI

Marinka Žitnik[1]

[1]*Harvard University, USA*

In this talk, I will present artificial intelligence approaches that support cancer research across four biological scales, from molecules to patients. At the molecular scale, ProCyon integrates protein sequence and structure to predict function, model phenotypic effects, and help characterize poorly understood regions of the proteome that may shape tumor–immune interactions. At the cellular scale, PINNACLE combines single-cell transcriptomic data with protein interaction networks to study how drugs and perturbations remodel immune and tumor cell states, and to identify therapeutic targets with cell type specificity. At the therapeutic scale, TxAgent serves as an AI co-pilot that draws on clinically validated knowledge across drugs developed since 1939 to support therapeutic reasoning, combination strategies, and hypothesis generation in oncology. At the patient scale, COMPASS predicts response to immune checkpoint blockade across cancers and treatment settings, while also identifying biological concepts linked to response and resistance, with potential relevance for patient stratification, biomarker discovery, and trial design. I will highlight how these systems are evaluated using patient cohorts and experimental studies in vitro and in vivo. More broadly, this work points to a new role for AI in cancer research: not only as a tool for prediction, but as a partner in generating hypotheses, prioritizing experiments, and helping researchers study anti-tumor immunity across scales.

Abstracts of invited lectures

1. Sex and gender differences in immunotherapy efficacy and toxicity- what is the evidence?

Berna C. Özdemir[1]

[1] *Inselspital, Bern University Hospital, University of Bern, Switzerland*

Reports on sex differences in the efficacy of immune checkpoint inhibitors show conflicting results. Early meta-analyses suggested a greater survival benefit in males compared with female patients. However, subsequent analyses and more recent data have not confirmed a consistent or clinically relevant difference in efficacy across tumor types and treatment settings. Overall, any sex-based effect on treatment benefit appears modest and context-dependent. In contrast, evidence on toxicity is more consistent. Multiple studies indicate that female patients are more likely to develop immune-related adverse events and to discontinue treatment due to toxicity. Differences in the type and severity of adverse events remain less clearly defined and vary across studies.

Interpretation of these findings is limited by methodological issues, including underrepresentation of women in clinical trials, lack of prespecified sex-stratified analyses and reporting of toxicity data by sex and frequent and conflation of sex and gender.

Future clinical trials must ensure sufficient enrollment of female patients, prespecified sex-stratified analyses and systematic reporting of efficacy and toxicity data.

2. Decoding sex differences in human immunity using systems immunology

Camila Consiglio[1]

[1]*Department of Laboratory Medicine, Data-Driven Life Science (DDLS), Lund University, Sweden*

Immune-mediated diseases exhibit marked sex bias. Men display more severe infections and higher rates of cancer, while women exhibit heightened vaccine responses and prevalence of autoimmunity. Sex hormones are emerging as key regulators of the immune system, yet the precise mechanisms underlying these effects remain largely undefined. Systems-level investigation of the immune system during periods of significant hormonal shifts offers an innovative strategy to dissect how sex hormones regulate immunity. We have previously shown that exogenous testosterone shifts human immune function by inducing a pro-inflammatory profile while downregulating cellular and molecular pathways involved in antiviral immunity in individuals undergoing masculinizing hormone therapy. The Consiglio Lab is now investigating mechanisms of testosterone regulation of human immunity by profiling diverse human cohorts characterized by variations in testosterone levels and signaling. Androgen deprivation therapy (ADT) is the standard of care treatment for hormone-sensitive prostate cancer, yet how ADT directly impacts immune cells is not fully understood. We profiled blood immune cells of prostate cancer patients before and following one month of ADT using scRNAseq and spectral cytometry. We observe that short-term androgen suppression reduces the percentage of Tfh cells and the expression of CXCR5 and CCR6 in B cells, which are important players in humoral immune responses. Immune cells are also impacted transcriptionally, with ADT downregulating pathways related to inflammatory responses in B cells and monocytes, IFNγ responses in CD4 T cells, and IFNα responses in monocytes. Further analyses reveal the suppression of the androgen response pathway in a variety of immune subsets, suggesting that ADT immune modulation may be directly mediated by testosterone's action on immune cells. While testosterone generally shows immunosuppressive effects, our results also support a role for inhibition of androgen signaling in broadly suppressing important pathways in innate and adaptive immunity, with potential implications to cancer progression. Decoding the molecular and cellular pathways driving immune sexual dimorphism is a crucial step toward precision medicine and can enable the development of sex-informed immunotherapies against cancer, infections, and other immune-mediated diseases.

3. From prenatal origins to novel therapeutic strategies targeting inflammatory pathways: leveraging pre-clinical models of Juvenile Myelomonocytic Leukemia (JMML)

Giulia Quattrini[1], Cristiana Barone[1], Alessandro Muratore[1], Gloria Zambelli[1], Filipa Timóteo-Ferreira[1], Thea Milanesi[1], Tiphanie Durfort[1], Mahdieh Naghavi Alhosseini[1], Silvia Bombelli[3], Deborah D'Aliberti[1], Valentina Sangiorgio[4], Cristina D'Orlando[1], Raffaella Meneveri[1], Barbara Vergani[1], Rocco Piazza[1,2], Silvia Brunelli[1,2], **Emanuele Azzoni**[1,2]

[1]*School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*

[2]*Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy*

[3]*Human Technopole, Milan, Italy*

[4]*Hematology Division and Bone Marrow Unit, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico San Gerardo dei Tintori, Monza, Italy.*

Juvenile Myelomonocytic Leukemia (JMML) is a rare pediatric myeloproliferative neoplasm driven by RAS-pathway mutations, approximately half of which arise during fetal life. Despite extensive genetic characterization, the cellular context in which JMML-initiating mutations act remains incompletely understood.

To investigate how the timing and cellular context of oncogenic $Kras^{G12D}$ activation shape disease initiation and progression, we developed a genetically engineered mouse model enabling activation of JMML-associated $Kras^{G12D}$ in defined subsets of embryonic hematopoietic stem and progenitor cells (HSPCs).

Our data show that embryonic HSPCs display differential susceptibility to $Kras^{G12D}$. Introduction of the mutation in embryonic hematopoietic stem cells (HSCs) resulted in the most aggressive and fully penetrant JMML-like disease in adult mice. Analysis of prenatal stages immediately following mutation acquisition revealed the emergence of a pre-leukemic state in the fetal liver, characterized by expansion and functional alteration of HSPC compartments and hallmark JMML features such as GM-CSF hypersensitivity. In addition, these fetal HSPCs showed transcriptional and epigenetic reprogramming, including metabolic rewiring and Nfkb1-driven inflammation. These data identify a previously unrecognized pre-leukemic stage in JMML, directly supporting its *in utero* origin.

In adult mice, $Kras^{G12D}$-driven JMML was associated with a pronounced myeloid-biased differentiation program and transcriptional and epigenetic changes indicative of an enhanced inflammatory signature, such as the enrichment of FOS and FOSL2 transcription factors, consistent with a functional role of inflammation in JMML pathogenesis. Based on these findings, we initiated in vivo pharmacological modulation of inflammatory signaling targeting innate immune pathways. Initial analyses indicate measurable modulation of disease-associated inflammatory features, supporting the therapeutic relevance of this approach. To comprehensively

assess treatment-associated changes, transcriptomic and proteomic analyses are currently ongoing.

Our data establish a robust experimental framework to dissect how oncogenic $Kras^{G12D}$ signaling intersects with developmental hematopoiesis and inflammation in JMML, providing a foundation for future functional and translational studies. In particular, this model offers a unique platform to interrogate how oncogenic inflammation shapes the immune microenvironment and to evaluate immunomodulatory and immunotherapy-based strategies targeting innate immune pathways in JMML and related leukemias.

4. Preclinical and Clinical Research on CAR T-Cell Therapy for Pediatric Leukemia

Jordi Minguillón[1,2], Berta González-Martínez[3], Víctor Galán-Gómez[3], Isabel Mirones-Aguilar[4], Sara Naharro[1,5], Alicia Martín-Ayuso[1], Esther Díaz-Maroto[1], Marta Ibáñez-Navarro[1], Lucía Fernández[1], Antonio Pérez-Martínez[1]

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[2]*Advanced Therapies Mixed Unit, CIEMAT/IIS-FJD, Madrid, Spain*

[3]*Pediatric Hemato-Oncology Department, La Paz University Hospital, Madrid, Spain.*

[4]*Advanced Cell Therapy Unit, La Paz University Hospital, Madrid, Spain.*

[5]*Molecular Biology Department, Universidad Autónoma de Madrid, Madrid, Spain*

Chimeric antigen receptor (CAR) T-cells targeting CD19 have demonstrated remarkable outcomes in pediatric patients with relapsed or refractory B-cell acute lymphoblastic leukemia (r/r B-ALL). However, relapse remains common, and novel multi-targeted strategies are being developed to address this limitation, although they are still in early clinical trial phases. For other high-risk, non-B-cell leukemias, such as T-ALL and AML, CAR T-cell therapies targeting alternative markers such as CD5 or CD7 for T-ALL and CD123 or CD33 for AML, are emerging as highly promising; nevertheless, these treatments remain in early clinical stages and are not yet available in our country.

Here we present our experience in both clinical and translational research aimed at treating pediatric patients with hematologic malignancies. From the clinical side, ten patients with r/r B-ALL were treated with tandem anti-CD19/CD22 CAR T-cells on a compassionate use basis in La Paz Hospital, previously reported to be safe and with promising preliminary efficacy (Gonzalez-Martínez B, eBiomedicine 2025). We will update the current status of these patients as well as the additional ones treated in an open Phase I clinical trial (REALL_CART trial, NCT06709469, EudraCT 2023-509723-41-01). From the preclinical side, we are developing new CAR T-cell approaches targeting different markers present in non-B-cell neoplasms. *In vitro* activation, transduction, expansion and cytotoxicity experiments demonstrate that our CAR T-cells are effective killing T-ALL leukemia models such as Jurkat and MOLT4 cells, and AML models such as OCI-AML3. The first preliminary *in vivo* experiment for T-ALL, using NSG immunodeficient mice and Jurkat cells, also shows better tumor control and prolonged survival in mice infused with some of these CAR T-cells.

Our findings highlight promising clinical data with tandem anti-CD19/CD22 CAR T-cells in pediatric patients with r/r B-ALL in the context of compassionate use and a Phase I clinical trial. Also, promising CAR T-cells for the treatment of pediatric non-B-cell malignancies are underway. Further *in vivo* experiments and GMP validation are needed to move on to clinical validation in our center.

5. Humanized mice and engineered CAR-T cells

Renata Stripecke[1]

[1]*University Hospital Cologne, Germany*

Immuno-oncology products developed for clinical purposes and administered in humans (such as antibodies, CAR-T cells, RNA vaccines) should ideally be tested in in vivo models that predict human immune responses (to asses potency or toxicity). Immunodeficient mice transplanted with human hematopoietic cells develop cell lineages of the human immune system (myeloid, B and T cells). These ”humanized mice” have emerged in the last decade as important testing platform and are used by academic labs, biotech and industry. We develop new methods to improve the human immune development in ”humanized mice”.

Chimeric antigen receptor T-cells (CAR-T) contain an engineered receptor to target and kill cancer or infected cells. Our patented proof-of-concept is EBVgp350-CAR-T-cells currently in clinical trials and targeting cancer cells infected with Epstein-Barr Virus (EBV). We are currently developing gene editing strategies using CRISPR to manufacture multi-specific CAR-T-cells to treat additional pathologies. Our goal is to make the edited CAR-T-cells safer, more potent and persistent. We are also generating and testing “off-the-shelf” CAR-T-cells for allogeneic use to allow faster availability.

6. Humanised mouse models for pre-clinical investigation of cancer immunotherapies

Gustavo Alviter-Raymundo[1], Bethany Ruth Bareham[1], Daniel Trajkovski[1] and Kourosh Saeb-Parsy[1]

[1]*Department of Surgery, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, CB2 0QQ, UK*

Cancer immunotherapy has revolutionised modern cancer treatment by harnessing the body's own immune defence to detect and eliminate cancer cells. While effective against haematological malignancies, solid tumours remain challenging due to heterogeneous patient response, limited infiltration into the tumour microenvironment, and therapy-associated toxicity that can offset the initial therapeutic benefit. Current in vitro and ex vivo models, including 3D cell culture systems, organoids, and microfluidic platforms, are gaining complexity to better recapitulate physiological and pathological conditions observed in vivo. Despite this, in vivo models still largely rely on murine immune systems or allogeneic human immune system reconstitution, both of which fail to fully capture immunophenotypic variation and patient-specific heterogeneity in immune responses.

The Kourosh Saeb-Parsy (KSP) group has established various humanised mouse models to investigate the function, safety, and immunogenicity of regenerative cell therapies as well as cancer immunotherapies. Depending on the research aims, these humanised mice can be reconstituted with either partial or complete human immune system, encompassing adaptive and/or innate immune compartments. Humanised mice bearing both a human immune system and patient-derived tumours, as organoids or PDX, represent some of the most advanced models for recapitulating pathophysiological conditions in a personalised context, enabling pre-clinical evaluation of cancer therapies and their associated immune responses in vivo.

7. The TRIPLEX study: use of patient-derived tumor organoids as an innovative tool for precision medicine in triple-negative breast cancer

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Background: Triple negative breast cancers (TNBC) represent 15% of all breast cancers and are associated with higher risk of relapse and shorter median survival. These aggressive tumors require neoadjuvant treatments for which the reference regimen include chemotherapy combined with immune checkpoint blockers (ICB) given the immunogenicity of TNBC. Despite these treatments, a fraction of patients show resistance associated with increased risk of relapse after surgery. Early identification of these patients is thus of critical relevance to adapt patient care and improve survival.

Methods: Our team set up the TRIPLEX protocol to evaluate the potential of using patient derived tumor organoids (PDTO) to predict the response of TNBC patients to treatment. This non-interventional trial allows to access biological material usable to produce PDTO, increasingly described to recapitulate the architecture and molecular features of pa tumors. Exposition of PDTO to treatment during functional assays could thus predict the response of matched patient, a correlation easily assessable given the early access to the clinical response of patients after neoadjuvant treatment.

Results: After 3 years of inclusion (93/163 patients), TRIPLEX brings initial lessons. First, establishment of PDTO lines from tumor biopsies appears more challenging than expected. From the 88 samples received, only 1 led to long term PDTO. Despite this, 44% of samples showed early PDTO which were progressively lost during culture.

Given these results, our team is working to: 1) identify biological and clinical markers impacting PDTO establishment and 2) develop miniaturization protocols to run functional assays on early PDTO. Obtaining tumor specific T cells usable to run cocultures with PDTO is also a pre-requisite to evaluate ICB response. First attempts have been made to isolate, expand and phenotype tumor infiltrating leucocytes with variable efficacy between patients.

8. Modelling the development of tumor-associated macrophages (TAMs) in colorectal cancer using a complex human *in vitro* system of the tumor microenvironment

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Liver cancer is a major global health concern, ranking as the sixth most diagnosed cancer and the third leading cause of cancer-related deaths. Circular RNAs (circRNAs), a class of covalently closed RNAs, have emerged as promising biomarkers and RNA-based therapeutic in oncology. Through analysis of public microarray datasets, we identified hsa_circ_0062682 as differentially expressed in hepatocellular carcinoma (HCC) tissue. Functional characterization confirmed its oncogenic role in HCC cell lines. Transcriptomic analyses revealed widespread gene expression changes upon modulation of hsa_circ_0062682, and proteomic profiling identified its interaction with YBX1, a known oncogene. Interestingly, hsa_circ_0062682 was significantly downregulated in both tumour tissue and plasma in the Slovenian HCC cohort, characterized by non-viral, metabolic, and alcohol-related aetiology. To validate and expand these findings, we performed long- and short-read sequencing of circRNAs from paired tumour and adjacent non-tumor tissues. A meta-analysis of published circRNA datasets revealed limited overlap in differentially expressed circRNAs, highlighting platform-specific detection biases. Finally, we correlated tissue and plasma circRNA profiles to assess the biomarker potential of circRNA for liquid biopsy applications. In conclusion, before circRNAs can be implemented in clinical practice, a deeper understanding of inter-patient variability and rigorous validation and standardization of analytical procedures are essential.

This work was funded by the Slovenian Research and Innovation Agency (ARIS) grants J3-4513, P1-0390, IP-0022 (CFGBC), ESFRI-ELIXIR, the Ph.D. grants, and HPC RIVR consortium and EuroHPC JU by providing computing resources of the HPC system Vega at the Institute of Information Science.

9. Imaging flow cytometry for decoding circulating tumor and normal cell interactions: implications for immunothrepy monitoring

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Circulating tumor cells (CTCs) are cancer cells shed from primary or metastatic lesions into the bloodstream and represent key mediators of tumour dissemination and metastasis. Beyond their individual metastatic potential, CTCs can interact with normal cells, including immune cells, forming heterotypic clusters that may promote survival in circulation, immune evasion, and enhanced metastatic competence. These interactions are particularly relevant in the context of cancer immunotherapy, where systemic immune-tumor crosstalk critically influences therapeutic response and resistance.

Imaging flow cytometry (imFC) offers a powerful approach for characterising such rare circulating events by integrating high-throughput flow cytometry with high-resolution fluorescence imaging. This technology enables multi-marker, multi-parameter phenotyping of single cells and cell clusters while preserving spatial and morphological information. imFC allows the identification of heterogeneous CTC subtypes, including epithelial and epithelial-mesenchymal transition (EMT)-associated phenotypes, and their individual features (such as protrusions or micronuclei) as well as the visualisation and quantitative analysis of CTC interactions with normal and immune cells and the stereometry of CTC clusters, all capabilities that extend beyond conventional flow cytometry or microscopy, and standard phenotyping of CTCs.

Here, we outline imFC-based strategies to visualise and quantify CTC-normal and CTC-immune cell clusters in tumor draining vein and peripheral blood samples. This newly implemented workflow originates from experience in CTC research, tumour dissemination, and imFC, resulting in the library of >5500 single and clustered CTCs from >1000 cancer patients and healthy donors blood samples. This grounded the foundation for CTC Atlas (www.ctcatlas.org), the first open-access platform which presents CTC images, combines it with matched molecular data, and illustrate how this approach can systematically interrogate tumour–host interactions during haematogenous spread and within the broader tumour dissemination ecosystem.

While clinical application in immunotherapy settings is still emerging, imFC has strong potential as a translational tool for immuno-oncology. The ability to directly visualise and phenotype CTC-immune cell interactions may support patient stratification, longitudinal monitoring, and mechanistic studies of immune-tumor dynamics under immunotherapeutic pressure. Ultimately, integrating imFC-based CTC analysis into liquid biopsy workflows may contribute to the development of novel biomarkers for improved diagnostics and monitoring of cancer patients undergoing immunotherapy.

key words: solid tumors, metastatic progression, liquid biopsy, circulating tumor cells, clusters, interactions, imaging flow cytometry, immunotherapy

Funding: This study was supported by National Science Center, Poland (#2020/39/B/NZ5/01258) and Ministry of Science and Higher Education (#NdS-II/SP/0398/2023/01). For representative images of CTCs and their clusters, please, see: CTC Atlas (www.CTCAtlas.org).

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10. Advances form the COST Action CA21135 "Modelling immunotherapy response and toxicity in cancer" for the translational research in ENT-clinic in Innsbruck, Austria

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Introduction. In the Department of Otorhinolaryngology and Head and Neck Surgery, we treat patients with head and neck squamous cell carcinoma (HNSCC) and salivary gland tumours, and are responsible for providing effective therapies. In addition to the rapid development of precision surgery and radiotherapy, immunotherapy offers effective potential for treatments. On the one hand, head and neck tumours might activate anti-tumour immune responses; on the other, they develop primary and secondary resistances.

Methods. To understand how tumour cells are killed by or evade the immune system, we have established and use personalised patient-based models, as organotypic slice cultures (SC), personalised cell lines and organoids. The organotypic cultures contain a fully functional local immune system, which we challenge by immune activation and immune checkpoint inhibitor treatments.

Results. SC revealed the importance of hypoxia/HIF1-α/VEGFA in immune evasion. In samples of nine of 22 patients, immunostimulatory beads did not activate an immune response. In samples of eight of 20 patients, successful activation of the local immune system caused the induction of SerpinB9 activity in tumour cells and counteracted the effective immune response.

The immune activation in salivary gland tumours was limited to a subpopulation of patient samples, where SC were established as a major research tool.

Conclusions. As ex vivo animal-free approaches, our models provide individual investigations of activators and modifiers of the local immune response, offering material for further detailed analysis. Our models accurately reflect the clinical challenges of limited efficiency of immunotherapy and provide a reliable platform for research aimed at improving outcomes. Despite the personalised, patient-based research and inter- and intra-patient heterogeneities, the outcomes reveal recognisable trends and significant results.

Short talks from industry

1. Advancing Human-Relevant Microphysiological Systems for Cancer and Drug Discovery

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Microphysiological systems (MPS) are transforming preclinical research by enabling human-relevant, mechanistically informed models that bridge the persistent gap between conventional in vitro assays, animal studies, and clinical outcomes. In this presentation, I will highlight a portfolio of next-generation MPS platforms developed to model cancer invasion, chemotaxis, immune interactions, tissue-specific metastasis, extravasation, multi-organ pharmacokinetics/pharmacodynamics (PK-PD), and high-throughput drug response. Using our invasion/chemotaxis (IC-chip), distance-dependent interaction (DDI-chip), and extravasation-on-chip systems, we demonstrate how breast cancer cells, macrophages, stromal cells, and patient-derived circulating tumor cells engage in context-dependent paracrine, juxtacrine, and microenvironment-specific signaling. These platforms successfully recapitulate tissue-specific homing behaviors toward lung, liver, bone, and brain microenvironments.

We further introduce immunology-focused MPS, including immune-cell infiltration models and systems integrating mouse lung and brain tissues to study cancer–immune–stromal crosstalk. A multi-organ-on-chip (MOC) approach is presented to capture organ-dependent drug metabolism and toxicity, enabling PK-PD profiling using minimal volumes and human-relevant physiological scaling. Finally, we showcase DRCHIP, a patent-pending open-top microphysiological system that provides simultaneous dose-response and drug-combination screening without external pumps or complex equipment, reducing time and cost up to 15-fold compared to traditional organ-on-chip technologies.

Together, these cell- and disease-agnostic platforms provide versatile, scalable, and animal-free solutions for metastasis research, immuno-oncology, multi-organ studies, and precision medicine applications. They support the emerging regulatory shift toward human-based testing and offer powerful tools for accelerating drug discovery and de-risking clinical translation.

2. Spatial Transcriptomic Profiling to Distinguish Primary Cholangiocarcinoma from Metastatic Pancreatic Adenocarcinoma in the Liver

Uršula Prosenc Zmrzljak[1]

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Distinguishing between primary intrahepatic cholangiocarcinoma (CCA) and metastatic pancreatic ductal adenocarcinoma (PDAC) in the liver remains a significant challenge in clinical pathology. Due to their shared histological features and the lack of highly specific routine immunohistochemical markers, definitive diagnosis is often difficult, yet crucial for determining the appropriate treatment strategy.

In this study, we utilized the high-resolution Xenium *In Situ* spatial transcriptomics platform to identify novel molecular signatures that can differentiate these two entities. We analyzed a diverse set of nine tissue samples, including healthy liver and pancreas, hepatocellular carcinoma (HCC), primary pancreatic cancer, and specifically focused on the comparison between primary cholangiocarcinoma and metastatic pancreatic cancer within the liver.

Our analysis focused on mapping cell-type-specific differential expression patterns and the spatial organization of the tumor microenvironment. By examining the immune system's landscape and its interaction with malignant cells, we aimed to uncover distinct spatial niches characteristic of each tumor type. This spatial transcriptomic atlas provides a deeper understanding of the cellular architecture in the hepatic niche and identifies potential candidate markers to overcome current diagnostic limitations in distinguishing primary from secondary hepatobiliary malignancies.

3. Comprehensive solutions for the formulation and spatial characterization of 3D immune models with Thermo Fisher distributed by Omega D.O.O.

Samy Dufour[1]

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Three-dimensional (3D) cell culture systems, including organoids, spheroids, and tumoroids, are rapidly becoming widely used in immune modeling. By recapitulating tissue architecture, cellular heterogeneity, and diffusion-driven gradients, these models provide a physiologically relevant framework to investigate complex mechanisms such as immune cell infiltration, activation, and therapeutic response. However, standardizing both the generation and analysis of these complex microenvironments remains a key challenge.

To address this, Thermo Fisher Scientific provides a comprehensive suite of solutions supporting each step of the workflow, enabling assay customization while improving reproducibility and scalability. The development of biologically relevant models begins with reliable cell sourcing and engineering capabilities (e.g., primary cells, iPSCs). Establishing the 3D microenvironment is supported by optimized matrices and cultureware that promote tissue-like organization and consistent structure formation (e.g., Gibco™ Geltrex™ Organoid-Qualified Reduced Growth Factor Basement Membrane Matrix, Thermo Scientific™ Nunclon™ Sphera™ plates). Differentiation and immune functionality are driven by specialized media and reagents tailored to 3D systems (e.g., Gibco™ OncoPro™ Tumoroid Culture Medium Kit, PeproTech™ recombinant cytokines).

Once established, extracting meaningful biological insights requires robust and spatially resolved characterization. Advanced imaging plays a central role by enabling both structural and functional analysis of complex 3D models. Brightfield imaging allows rapid monitoring of spheroid size and morphology, while fluorescence-based approaches enable multiplexed analysis of immune and cellular markers. Thermo Fisher Scientific streamlines these workflows by combining high-performance imaging platforms with validated reagents, following a philosophy of user-friendly systems. Automated systems (e.g., Invitrogen™ EVOS™ M7000 Imaging System) enable fast, reproducible imaging for routine monitoring and live cell analysis. High-content confocal screening platforms (e.g., Thermo Scientific™ CellInsight™ CX7 LZR Pro High Content Screening Platform) enable automated, multiparametric imaging of thick 3D samples. Finally, spatial imaging solutions (e.g., EVOS™ S1000 Spatial Imaging System) enable up to 9-color imaging for fast detailed tissue mapping.

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4. Real-Time Label-Free Kinetic Analysis for Cancer Immunotherapy: From CAR T Potency to Cardiotoxicity Monitoring

Aniello Lombardi[1]

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Current preclinical cancer immunotherapy models rely on endpoint assays that provide only snapshots of immune cell activity, missing critical kinetic information such as CAR T exhaustion, effector:target dynamics, and tumor regrowth patterns. Axion BioSystems' Maestro platform enables continuous, real-time, label-free monitoring of immune cell cytotoxicity through impedance-based technology.

The Maestro Z system tracks complete killing kinetics of CAR T, NK cell, and ADCC-mediated responses in physiologically relevant 3D tumor models across 96- and 384-well formats without interfering with native immune biology. The complementary Maestro MEA platform addresses on-target, off-tumor toxicity through real-time electrophysiological monitoring of CAR T-mediated cardiotoxicity in iPSC-derived cardiomyocytes.

This dual-platform approach provides the complete preclinical picture: tumor killing efficacy combined with functional safety assessment, meeting regulatory requirements for next-generation immunotherapy development.

5. Real-Time Improving Reproducibility in Cancer Immunotherapy Models: Practical Workflow Solutions

Fan Yu[1]

[1] *RWD Life Science Co., LTD*

Cancer immunotherapy research relies on robust preclinical models, but variability in experimental workflows can impact data consistency and translational relevance.

This presentation will highlight practical approaches to improving reproducibility across key steps of immuno-oncology workflows, including tumour monitoring, tissue processing, cell counting, and histological analysis.

The session discusses simple and practical insights to support more consistent and reliable preclinical research outcomes.

6. MAVERIC: Enabling reliable and rapid preclinical evaluation of macrophage-targeting immune modulatory drug candidates with vascularized in vitro 3D human lung cancer

Marc Creus[1]

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MAVERIC is an applied research initiative aimed at transforming how macrophage targeting immunomodulatory drug candidates are evaluated in the preclinical phase. The project brings together Living Networks Sp. z o.o., Abbmira Therapeutics AG, and the Mossakowski Medical Research Institute of the Polish Academy of Sciences to develop a vascularized 3D human lung cancer model that more accurately reflects the tumor microenvironment than conventional 2D systems. By integrating tumor associated macrophages (TAMs) into a physiologically relevant, perfused in vitro platform, MAVERIC enables more reliable, rapid, and predictive assessment of emerging immunotherapies designed to modulate macrophage behavior in lung cancer. This approach addresses a critical bottleneck in oncology drug development—poor translational fidelity of standard preclinical assays—by providing a human relevant system capable of capturing complex immune–tumor–vascular interactions. The resulting platform is expected to accelerate candidate selection, reduce development risk, and support the advancement of next generation macrophage directed therapies under the Swiss Polish Cooperation Programme's 2025 Research and Innovation call.

Abstracts of short lectures

1. Myeloid-T cell therapy interactions reshape the tumor microenvironment to sustain tumor control

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T cell–based immunotherapies show great promise, yet their efficacy is limited by toxicity and exhaustion, both involving their crosstalk with the TME. To dissect the bidirectional influence of therapeutic T cells and the TME, we combined an immunocompetent adoptive T cell therapy model (EG.7-OVA tumors treated with ex vivo stimulated OT-I T cells) with uLIPSTIC, a genetically encoded proximity-labeling system[1,2]. By relying on SrtA^{+} OT-I donor mice and *Cd40*$^{G5/G5}$ tumor-bearing hosts, we directly mapped in vivo interactions between therapeutic T cells and TME populations.

At 72 hours post-therapy, flow cytometry revealed major myeloid remodeling, including expansion of inflammatory Ly6C^{high} macrophages. Multiplex cytokine analysis of restimulated TME suspensions showed a strong myeloid signature, hallmarked by CCL3, CCL4, and CCL5. uLIPSTIC labeling demonstrated direct contacts between therapeutic OT-I cells and multiple myeloid subsets, specially Ly6C^{high} macrophages, cDC1, cDC2, and moDCs. We next interrogated whether these interactions were antigen dependent. Using EL4 tumors lacking OVA, labeling was largely abrogated, pointing towards antigen driven interactions. Mixed bone marrow chimeras containing *Cd40*$^{G5/G5}$ *B2m*$^{+/+}$ and *Cd40*$^{G5/G5}$ *B2m*$^{-/-}$ marrows suggested an MHC-I-dependent initial cross-presentation event followed by antigen independent interactions.

Single cell RNA sequencing revealed the selective expansion of inflammatory clusters in treated samples, such as *Nos2* macrophages and interferon stimulated DCs. CITE-seq analysis confirmed specific myeloid clusters engaging in OT-I interactions and revealed potential interacting molecules. Preliminary data on conditional depletion of selected myeloid populations showed impaired complete tumor elimination upon OT-I therapy, highlight the relevance of this communication loop in tumor outcome. Additional efforts are currently being implemented to dissect the role of MHC-I complex cross dressing and describing with higher temporal resolution the TME reshaping dynamics.

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2. Implementation of Patient-Derived Organotypic Tumor Spheroids (PDOTS) from Primary and Metastatic Colorectal Cancer for Ex Vivo Immunotherapy Testing

Ferran Grau-Leal[1,2], Eva Martínez-Balibrea[1,2]

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Introduction: We will present a model using ex vivo culture of patient-derived organotypic tumor spheroids (PDOTs) from liver metastases and primary colorectal cancer (CRC) tumors to evaluate treatments.

Materials and methods: Fresh primary and metastatic CRC specimens were mechanically dissociated, filtered serially (100 µm then 40 µm), and the 40–100 µm spheroid fraction was recovered by reverse washing. Spheroids were embedded in extracellular matrix (Vitrogel and Matrigel) and cultured in microfluidic devices (AIM Biotech) or IBIDI 96-well plates. Single-cell suspensions (<40 µm) were analyzed by flow cytometry (FVS780 viability; CD45, CD3, CD4, CD8). PDOTS were treated with staurosporine, chemotherapy (5-FU 30 µM + oxaliplatin 10 µM), and/or immunotherapy (pembrolizumab 10 µg/mL + ipilimumab 10 µg/mL). Viability readouts were optimized from acridine orange/propidium iodide to DAPI/PI and finally DAPI + caspase-3/7. For immunofluorescence, antibodies targeting CDX2, CD45, phalloidin and DAPI.

Results: A workflow for PDOTS generation from both primary and metastatic CRC was established, with viability imaging showing viable spheroids only in a subset of cultured samples. Flow cytometry revealed patient variability within viable cells (FVS− dead cells: 48.0±39.2% vs FVS+ live cells: 44.25±40.1%) and immune live cells (CD45+ 14.25±14.61%, CD3+CD4+ 3.94±9.57%, CD3+CD8+ 2.46±12.67%; mean±SD). Confocal imaging confirmed tumour architecture (central lumen) and immune infiltration (CD45+ cells adjacent to CDX2+ tumour epithelium). In cultures with sufficient material for dosing, live/dead imaging after 72 h indicated treatment-associated cytotoxicity, with increased PI-positive signal under FUOX (and variably under ipilimumab+pembrolizumab) relative to IgG control. DAPI + caspase-3/7 reduced artefacts from AO/PI autofluorescence and PI staining of luminal debris.

Conclusion: PDOTS were implemented as a rapid CRC ex vivo platform that maintains microenvironmental features for short-term chemo- and immunotherapy testing. Limitations included limited tissue and viability issues with pre-treated metastases; future plans involve expanding cohorts and standardizing matrices and media.

3. Synergizing Immunotherapy and Proteasome Inhibition in Mantle Cell Lymphoma

Adrian Bogdan Tigu[1], Diana Gulei[1], Madalina Nistor[1], Diana Cenariu[1], Ximena-Maria Muresan[1], Richard Feder[1], David Kegyes[1], Ciprian Tomuleasa[1]

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Introduction: Mantle Cell Lymphoma (MCL) is a distinct subtype of Non-Hodgkin Lymphoma, characterized by a proliferation of lymphocytes in the mantle zone of lymphoid follicles. Immune therapies targeting ROR1 in MCL are an emerging area of investigation, given ROR1 's selective expression in malignant B cells, and mostly absent in adult tissues. The use of CAR-T cells targeting ROR1 and Zilovertamab demonstrated meaningful efficacy in R/R MCL. Proteasome inhibitors (PIs) can enhance the efficacy of targeted therapies in MCL, disrupting the protein homeostasis, inhibition of NF-kB signaling and inducing apoptosis. The combination of ROR1 inhibitors with PIs can result in enhanced apoptosis and inhibition of key survival pathways.

Methods: Cytotoxicity assay was assessed using CCK8 for all PIs, CAR-T and Zilovertamab at 24, 48 and 72h. The efficacy of each therapy was assessed first by CCK8 and confirmed by flow cytometry and Western Blat assays. The *in vivo* model of Mantle cell lymphoma was generated using Z138 cell line modified to express Luc2+. The bioluminescence was evaluated by IVIS for all animals under anesthesia.

Results and discussions: The PIs were tested at a low dose, to avoid induced programmed cell death, these doses were priming the tumor cells to maintains their ROR1 on the membranes, as the MFI suggest s. Zilovertamab at a low dose induced ROR1 internalization and at long exposure the cells lowered the MFI, while adding PIs the MFI for ROR1 was restored.

Conclusions: The use of PIs in combination with CAR-T and Zilovertamab showed good results on *in vitro* setup, and the synergizing therapy will be tested on the MCL animal models.

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4. Perfusion-based Melanoma-Immune Microenvironment (MIMe) Platform for Modeling Adoptive Immune Response

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Tumor microenvironment (TME) is a complex and dynamic network of diverse cell types and extracellular matrices. The interactions between tumor and immune cells in TME dictate a fate of malignancy, determining whether it is suppressed or continues to progress. Although its significance, conventional models *(in vitro* and *in vivo)* present critical limitations in accurately recapitulating tumor-immune interactions. Herein, we introduce "MIMe-on-a-Chip", a perfusion based organ-on-chip (OoC) designed to mimic Melanoma-Immune Microenvironment by simultaneously emulating disease pathology and physiological fluid dynamics. MIMe-on-a-Chip features two chambers, representing the melanoma TME and the lymph node (LN) connected by a microbridges for T-cell recruitment. Finite element simulations were employed to optimize shear stress and flow parameters in MIMe-on-a-Chip, and results confirmed that shear stress created in MIMe-on-a-Chip is similar to *in vivo* TME and LN. After modeling, MIMe-on-a-Chip was produced using PDMS replica molding. Next, TME-LN axis was recreated by embedding melanoma spheroids and dendritic cells (DCs) into three-dimensional (3D) collagen scaffold in Chamberl along with T cells seeded into Chamber2 and were perfused through main channel. Results confirmed the generation of uniform 3D melanoma spheroids (175-200 µm; Sphericity Index: 0.98). Also, the synthesis of melanin remained comparable to 2D cultures, 3D spheroids exhibited significantly higher E-cadherin expression. Furthermore, LPS-treatment provided the maturation of DCs via the overexpression of MHCII, CD86, and CD80. Notably, mDCs exhibited increased CCR7 expression and distinct spatial localization patterns (in melanoma-DC co-culture) compared to immature DCs, suggesting active migration potential towards LN. These findings motivated us to investigate T-cell mediated anti-tumoral activity against melanoma in MIMe-on a-Chip, as part of ongoing study.

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Abstracts of posters

1. Engineering 3D Models to Recapitulate the Multiple Myeloma Bone Marrow Microenvironment

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In multiple myeloma (MM) and its precursor conditions monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) malignant plasma cells remodel the bone marrow microenvironment (BME), impairing osteogenic differentiation and promoting disease progression. Conventional *in vitro* systems fail to recapitulate the structural and biophysical complexity of the BME, including matrix stiffness, a key regulator of stromal fate and tumor progression. We engineered advanced 3D hydrogel and bioprinted MM niche models to provide a physiologically relevant platform for studying mesenchymal stromal cell (MSC) dysregulation.

A 3D MM niche model was generated using hydrogel-based biomaterial printed through 3D high-precision microvalve extrusion bioprinting on the RegenHU 3D Discovery platform. Myeloma cells (JJN3) and hTERT-MSCs were embedded in collagen-based bioinks to produce spatially organized constructs with CAD defined geometries. Rheological analyses confirmed printability properties and enabled fine control of matrix stiffness within a physiologically relevant bone marrow range. 3D constructs were cultured for up to 10 days and assessed for viability, morphology, and proliferation. Cytocompatibility was evaluated using LDH and Live/Dead assays. To validate biological relevance, non-hematopoietic BME cells from 16 bone biopsies (MGUS, SMM, MM) were profiled using single-cell RNA sequencing (10x Genomics).

The 3D constructs showed high cytocompatibility and sustained cell viability and proliferation over time. Importantly, tunable matrix stiffness supported physiologically relevant cell organization and mechanotransduction, critical for modeling the MM niche.

Single-cell analysis (42,823 cells) revealed stromal to osteoblast differentiation trajectories and identified two pre-osteoblastic populations: a dysfunctional

immunosuppressive subset and a WISP2⁺ pro-osteogenic subset. The latter progressively decreased from MGUS/SMM to MM and was inversely associated with tumor burden. The 3D platform will be interrogation of these interactions under defined mechanical conditions. We present a preliminary reproducible 3D bioprinted model of the MM bone marrow niche integrating biological and mechanical cues. By combining tunable stiffness, spatial organization, and patient-derived single-cell data, this platform could more accurately replicate the interactions of the tumor microenvironment and serves as a powerful tool for mechanistic studies and preclinical applications in multiple myeloma.

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2. Assignment of gene markers to immune cells based on single-cell RNA-seq data, using a machine learning assessment

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The human hematological and immune systems consist of many different cells that perform highly specialized functions. Because of the importance of their activity, this study investigated the cell types and subtypes that comprise this complex system. Single-cell RNA sequencing (scRNA-seq) was used to analyze and evaluate the markers that best define each cell population and each cell-type. First, we developed an optimized computational workflow for analyzing large scRNA-seq datasets. Then, we used this workflow to identify gene markers of the different cell types present in bone marrow (BM) and peripheral blood (PB). Using this strategy, we analyzed three different single-cell datasets to find specific cell markers. To do so, first we searched with standard CD marker genes, then with genes that encode membrane proteins, and finally with all detected protein-coding genes. This approach allowed us not only to confirm known CDs that best mark some specific cell types (e.g., monocytes, B cells, NK cells, etc), but also to evaluate the ability of new genes to distinguish cell types or subtypes not well identified by CDs. Finally, we applied a machine learning method (Random Forest) to test the accuracy of the different markers found. As a result of all this work, we have found and propose specific and robust gene signatures to identify different types and subtypes of hematological and immune cells.

Keywords: single-cell, gene expression, RNA sequencing, cell type, immune cell, hematopoietic cell, bioinformatics, computational biology, artificial intelligencia, machine learning

3. Survival-Based Immune Stratification Reveals Distinct Immunometabolic Signatures in TCGA-LAML

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Acute Myeloid Leukemia (AML) exhibits profound immune heterogeneity, yet survival-linked immune modeling remains insufficiently characterized. Given the emerging role of immunometabolic pathways in shaping anti-tumor immunity, we aimed to define survival-driven immune signatures in AML using integrative in silico modeling.

RNA-seq and clinical data from TCGA-LAML were analyzed. Overall survival (OS) data were curated and harmonized. Hallmark immune gene sets (MSigDB) were quantified at the single-sample level using gene set–based scoring approaches. A survival-informed immune risk score was generated through dimensionality reduction of immune-related expression patterns. Patients were stratified into high- and low-risk groups based on optimal cutpoints derived from survival modeling. Differential immune pathway activity and effector gene signatures were compared between groups. Multivariate Cox proportional hazards models were used to evaluate independent prognostic contributions.

Preliminary analyses demonstrate significant immune heterogeneity across AML patients. Survival-driven stratification identifies biologically distinct subgroups characterized by differential activation of interferon signaling, inflammatory response, and metabolic reprogramming pathways. Notably, kynurenine-AHR axis–associated signatures and NK effector-related modules display divergent patterns across survival groups, suggesting context-dependent immune suppression mechanisms. Cox modeling indicates that composite immune scores outperform individual pathway metrics in survival prediction.

Our findings support a survival-based immune modeling framework to dissect AML heterogeneity. This approach enables identification of prognostically relevant immunometabolic programs and may guide rational immunotherapeutic or metabolic intervention strategies. Ongoing analyses include pathway-level drug repurposing predictions and validation in independent cohorts.

4. *STAG2*-mutated hematological cancers display characteristic microenvironmental changes with therapeutic implications for immunotherapy

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Immunotherapies have made remarkable improvements in treating solid tumors, yet their potential in combating leukemias remains largely unexplored due to the affected immune system. Some leukemic precursor states, such as Myelodysplastic Syndromes (MDS), are ideal for disease interception to prevent transformation to acute myeloid leukemia (AML). Therefore, MDS represents an exciting example for potential immunotherapies. Nevertheless, the pro-inflammatory changes in the bone marrow microenvironment in MDS, and their implications for immune cell functions remain unclear.

Our research tries to explain how changes at the chromatin level translate to an altered immune cell microenvironment in MDS patients with a focus on STAG2, a nuclear structural protein, encoded by an X-linked gene. STAG2 forms a unique ring-like structure, bridging enhancers and promoters while also playing a crucial role during cell division and is often mutated in MDS. Unfortunately, effective cell culture models that mirror MDS are scarce. However, co-cultures combining mesenchymal stem cells (MSCs) with MDS cells offer a partial understanding of the disease's characteristics.

We analyzed whole transcriptome sequencing data from bone marrow samples of a cohort of 753 MDS patients including 48 samples with *STAG2* mutations. In addition, we generated 4 MDS/AML cell lines without STAG2 by using CRISPR/Cas9. Our data indicate that STAG2-mutated myeloid cancer cells become independent from environmental signals by a deregulated cytokine expression and receptor abundance, promoting differentiation arrest and securing cell proliferation of leukemic cells. Similarly, STAG2-mutated cells were less-sensitive to the differentiation-inducing signals from MSCs. Moreover, STAG2-mutated myeloid cells showed impaired immune cell functions by downregulation of HLA molecules. Importantly, a patient cohort analysis indicated that STAG2-mutated MDS patients die mostly from transformation to AML or chronic inflammatory conditions.

Taken together, we provide insights into specific immune pathways that might be of relevance for developing effective immune-based treatments for MDS.

5. Antibody blockade of the PSGL-1 glycoprotein impairs B-cell lymphoma progression by enhancing T-cell responses

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Non-Hodgkin B-cell lymphomas are a heterogeneous group of hematologic neoplasms resulting from the clonal proliferation of B cells. Despite advances in immunotherapy and CAR-T therapy, most non-Hodgkin lymphomas remain unresponsive to immune checkpoint inhibitors. In this context, there is a need to develop innovative immunomodulatory approaches to improve the management of patients with lymphoma. The P-selectin glycoprotein ligand-1 (PSGL-1) is a transmembrane glycoprotein recently described as a potential immune checkpoint that negatively regulates T-cell function and promotes T-cell exhaustion in murine cancer models. In this study, we investigated the therapeutic potential of PSGL-1 antibody targeting in B-cell lymphoma. Our results demonstrated that PSGL-1 blockade in immunocompetent mouse models with B-cell lymphoma results in decreased tumor progression as well as the recruitment and activation of T cells. Next, we aimed to decipher the mechanism of action of anti-PSGL-1 treatment and explore its effect on the tumor microenvironment in B-cell lymphomas. Preliminary results showed that PSGL-1 is expressed in several immune populations of the lymphoma microenvironment, not only in the mouse model but also in diffuse large B-cell lymphoma (DLBCL) patients. Simultaneously, bioinformatic analysis of scRNA-seq data from four DLBCL patients revealed a strong correlation between expression of the *SELPLG* gene, which encodes PSGL-1, and expression of the *VSIR* gene which encodes the V-domain Ig suppressor of T-cell activation (VISTA) protein. VISTA has recently been identified as a PSGL-1 receptor in acidic microenvironments. Moreover, we found that *SELPLG* expression is predominant in the T-cell lineage, whereas *VSIR* is mainly expressed in macrophages. We hypothesize that the interaction between PSGL-1 and VISTA in these immune populations may contribute to the mechanisms of immunosuppression. Since PSGL-1 was highly expressed in lymphoma-infiltrating T cells, we assessed whether PSGL-1 antibody blockade could hinder lymphoma progression in their absence. In fact, PSGL-1 targeting of lymphoma-bearing T cell-deficient mice did not hinder tumor progression. These findings indicate that T cells are the key mediators of the anti-PSGL-1 therapeutic effect and reinforce the notion that PSGL-1 plays a crucial role in modulating the immune response in B-cell lymphomas, highlighting it as a promising therapeutic target. Further studies will provide new insights for the development of more effective immunotherapeutic strategies against lymphoma.

6. Dual Immunotherapy with Multi-Epitope Nanovaccine and Anti-PD-1 for Robust Anti-Tumor Immunity against Melanoma

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Cancer nanomedicines have been a focus of extensive investigation to overcome existing challenges regarding dose-limiting toxicities, suboptimal patient responses and immune-related adverse effects. While peptide-based vaccines offer safe and tumor-specific response, their efficacy is hindered due to low immunogenicity and fast degradation profile of peptides. Therefore, there is a critical need for multifunctional adjuvant platforms to elicit robust anti-tumor immunity. In this study, anti-tumor and immunomodulatory efficacy of nanovaccine platform, incorporating TLR4/saponin agonists and tumor-specific multi-epitopes (SIINFEKL, Trp-2 or PADRE), was investigated in prophylactic and therapeutic murine melanoma models, both as monotherapy and in combination with anti-PD-1 therapy. The outcomes were assessed by tumor regression, multi-epitope T-cell responses and immune cell infiltration. The nanovaccine significantly inhibited tumor growth in both B16-OVA and B16-F10 prophylactic models. This response was mediated by induction of antigen-specific IFN-$\gamma^{+}CD8^{+}$ cells and intratumoral infiltration of $CD8^{+}$ T cells, macrophages and dendritic cells, reprogramming tumor microenvironment (TME) from a cold to a hot phenotype. In B16-F1O therapeutic model, nanovaccine monotherapy significantly regressed tumor growth, attributing high PD-1 expression on intratumoral $CD8^{+}$ T cells. The combination therapy with nanovaccine and anti PD-1 eliminate established tumors in mice about 21 days after tumor inoculation. The superior response enhanced by dual immunotherapy was characterized with intratumoral $CD8^{+}$ T cells, dendritic cells and M1-like macrophages. These findings demonstrate that nanovaccine serve as potent adjuvant platform in cancer immunotherapy by inducing functional antigen-specific cytotoxic T cell responses with long term memory, reducing tumor growth all in prophylactic and therapeutic melanoma models. Dual immunotherapy with nanovaccine and anti-PD-1 offers a potential strategy for overcoming therapeutic resistance in cancers.

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7. The lung-specific microenvironment: an opportunity for NK cell-based immunotherapy

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Metastatic disease is the main cause of cancer-related deaths. Although immunotherapy is a promising strategy, its success against metastases remains limited. We previously identified a cluster of "High-Immune" metastases that share an inflammatory, immunogenic phenotype, susceptible to immunotherapy. This cluster mainly includes lung metastases, regardless of the primary tumor of origin. On the contrary, most liver metastases exhibited a "Low-Immune" phenotype.

To further investigate this issue, we focused on the gastrointestinal tumors colorectal cancer (CRC) and pancreatic cancer (PDAC), two cancer types that frequently metastasize both in the lungs and the liver.

First, transcriptomic analyses using public datasets revealed that lung metastases from gastrointestinal tumors have a higher infiltration of NK cells and increased expression of NK-related genes, as compared to less immunogenic liver metastases or primary tumors.

In vitro, we studied the cytotoxicity of NK-92 in two different models: organoids derived from lung metastasis CRC patients, and cell cultures derived from spontaneous lung metastasis induced in an orthotopic PDAC mouse model. In both, NK-92 exhibited dose-dependent cytotoxicity. RNA-seq analyses of organoids and matched tissues are underway to identify markers involved in NK cell resistance.

To further evaluate the role of NK cells in tumor progression, we studied the expression of immune markers in established cell lines. HLA-E, a marker involved in NK evasion, was highly expressed in ASPC1, which also presented a higher resistance to NK-92.

Overall, lung metastases present a characteristic immune microenvironment in which NK cells have an essential role, supporting NK-based immunotherapy as a potential strategy against lung metastasis.

8. Radiation quality shapes immune responses: Transcriptional insights for immune-informed radiotherapy

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In radiotherapy (RT), both X-rays and neutrons are relevant in the treatment of malignant diseases. However, they exert distinct effects on the highly radiosensitive hematopoietic system, which may modulate systemic immune responses. Such differences could help explain the limited success of multimodal treatment strategies combining RT with immune checkpoint inhibitors, particularly with regard to achieving systemic “abscopal” effects. Neutrons are relevant as secondary particles in proton and highly energetic photon therapy and as therapeutic agents in boron neutron capture therapy, and they also modulate immune responses in blood lymphocytes. Understanding radiation-induced immune modulation is therefore critical for optimizing dose, fractionation, and treatment timing. To address this, we compared genome-wide transcriptional responses in human whole blood exposed ex vivo to X-rays (0-4 Gy) or simulated fission-spectrum neutrons (0-1 Gy). RNA sequencing was performed 6 h and 24 h post-irradiation.

Both radiation qualities induced significant dose-dependent transcriptional changes, with neutrons eliciting a markedly stronger response. A shared core signature of DNA damage and cell cycle–related genes was activated by both radiation types. Temporal dynamics differed between radiation qualities. After X-ray exposure, the number of differentially expressed genes (DEGs) increased from 6 h to 24 h, whereas neutron-induced DEGs declined over time, indicating distinct response kinetics. Both radiation types induced distinct and shared transcriptional signatures. Immune-related transcriptional changes after X-rays were mainly observed at higher doses, while neutrons triggered immune modulation already at low doses and across the entire dose range. Moreover, neutrons induced a stronger anti-inflammatory transcriptional profile at matched doses and time points.

This study shows radiation quality dependent immune modulation, suggesting potential implications for optimizing RT regimens, and supporting the development of immune-informed RT strategies. Together, this underscores the importance of sparing the blood system during RT to maximize the synergistic antineoplastic effects of concomitant immuno-oncology.

9. Radiotherapy-Induced Activation and Immunomodulatory Reprogramming of Tumour Endothelial Cells

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Tumour vasculature is frequently disorganised, resulting in poor perfusion and hypoxia that limit radiotherapy (RT) efficacy. Although irradiation (IR) affects both cancer cells and the tumour microenvironment, its impact on tumour endothelial cells (TECs) remains incompletely understood. Because IR can induce apoptosis and activation of tumor endothelial cells, as well as vascular normalisation, we investigated IR-driven vascular responses with emphasis on endothelial activation and immune modulation. Human (HUVEC, EA.hy926, Hulec5a) and murine (bEnd.3, 2H11, SVEC4-10) endothelial cells exposed to single doses of 0–10 Gy showed dose-dependent decrease in proliferation and increased cell death. Transcriptomic profiling of HUVECs following 2 or 5 Gy revealed downregulation of cell-cycle pathways and upregulation of immune-response and endothelial activation signalling pathways, confirmed by immunofluorescence staining. RNA sequencing of TECs isolated from irradiated MC38 tumours demonstrated similar immune-associated reprogramming, including increased expression of Vcam1, Vwf, Cd47, and Il6. These findings were validated in CT26 murine colon carcinoma tumours treated with either 15 Gy or 5 × 5 Gy using immunofluorescence for endothelial activation and T cell markers. Spatial transcriptomics of CT26 tumours after fractionated RT identified increased CD8+, CD4+, and endothelial activation marker–positive (CHST4+, GLYCAM+, NTAN1+) regions, confirmed by immunohistochemistry. Comparable analyses were performed on paired human tumour biopsies obtained before and after neoadjuvant 5 × 5 Gy RT. Endothelial responses to irradiation have been further validated in a vascular-on-a-chip model. Overall, IR reduces endothelial proliferation and survival while inducing immune-relevant transcriptomic reprogramming of TECs, consistent with enhanced endothelial activation and potential promotion of anti-tumour immunity.

10. Reconstructing the tumor immune microenvironment on a chip: a primary cell-derived model for studying T cell migration in colorectal cancer

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The immune system plays a pivotal role in cancer progression and response to therapy, with T cells acting as central mediators of anti-tumor immunity. Despite significant progress, the mechanisms governing T cell migration within the tumor microenvironment (TME) remain poorly understood. The inherent complexity and limited accessibility of *in vivo* systems make these processes difficult to study, driving the development of advanced *in vitro* models. In this context, the use of primary cells is particularly critical, as it preserves the physiological heterogeneity and authenticity of native tissues, ensuring that experimental outcomes closely reflect *in vivo* conditions. Here, we present a three-dimensional *in vitro* approach to investigate T lymphocyte migration in colorectal cancer. Using organ-on-a-chip technology, we established a microphysiological system consisting of three interconnected channels: an epithelial channel lined with a 3D tubular monolayer derived from healthy or tumor mouse colon organoids, a central stromal channel containing primary intestinal fibroblasts embedded in a collagen-Matrigel hydrogel, and an immune channel where primary T cells are introduced. This architecture mimics the spatial organization of the colonic wall and enables the study of T cell migration through the stromal compartment toward the epithelial tumor region. We demonstrate that tumor-derived organoids retain key pathological hallmarks within the chip, including enhanced proliferative capacity, reduced differentiation, and the formation of aberrant crypt-like structures. In parallel, we developed an automated imaging and analysis workflow for real-time tracking and quantitative assessment of immune cell migration dynamics. Our findings reveal that tumor-associated factors modulate T cell motility and trajectories, underscoring the critical influence of TME on immune cell behavior. This organ-on-a-chip model provides a versatile platform for dissecting immune-tumor interactions and offers a physiologically relevant tool for advancing the understanding of T cell migration in cancer.

11. Establishing Ovarian Cancer Patient AVATAR Models from Malignant Ascites and Debulking Solid Tumor Tissue to Study Immunotherapy Approaches

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Introduction: Ovarian cancer remains one of the most lethal gynecological malignancies, characterized by intraperitoneal dissemination, a complex tumor microenvironment and limited therapeutic options. Although immune checkpoint inhibitors (ICIs) have demonstrated efficacy in multiple cancer entities, clinical responses in ovarian cancer remain modest. This underscores the urgent need for physiologically relevant preclinical models that faithfully recapitulate the ovarian cancer tumor microenvironment (TME) and its interaction with the immune system. To address this challenge, we are developing patient derived, three dimensional Ovarian Cancer Avatar models using both primary malignant ascites and solid tumor tissue obtained during debulking surgery or ascites drainage at the University Medical Center Hamburg Eppendorf (UKE).

Methods: Our aim is to establish culture conditions to preserve the original cellular composition and heterogeneity of the ascites and tumor tissue, including the expression of key immune checkpoint molecules (ICM), thereby enabling more accurate functional assessment of responses to ICIs alone or in combination. Our workflow includes: (i) phenotyping of the initial cell populations in ascites and debulking solid tumor tissue; (ii) assessment of immune checkpoint expression levels using multiparametric flow cytometry (mFC); (iii) cultivation and treatment of patient samples with ICIs, followed by functional response analyses, including phagocytosis assays.

Results: Patient derived ascites and tumor samples exhibited substantial interpatient heterogeneity in their cellular composition. Notably, differences in immune cell subtype prevalence were observed between ascites and tumor tissue. CD4+ T cells were the predominant immune cell population in ascites, whereas the microenvironment of the solid tumor tissue was primarily dominated by myeloid cells, followed by CD4+ T cells. Furthermore, tumoroids derived from debulking solid tumor tissue displayed a heterocellular architecture containing infiltrating immune and non-immune cells. For ascites samples, co culture conditions were successfully established using supplemented autologous ascites to preserve the initial cellular composition and ICM expression. For solid tumor derived samples, a Matrigel based co culture system is currently under development to preserve tumoroids immune cell interactions for functional testing. Given the observed expression of immune

checkpoints on macrophages - including, MARCO, TIM-3, CD39, and “don’t eat me” signaling pathways such as CD47-SIRPα - we are currently establishing a phagocytosis assay to functionally evaluate macrophage responses to immune checkpoint inhibition, representing a potential immunotherapeutic strategy in ovarian cancer.

Conclusion: The patient derived AVATAR models provide a platform for individualized assessment of responses to immune checkpoint blockade while accounting for the distinct tumor microenvironments of ascites and solid tumor tissue. In the long term, this approach may contribute to the development of more patient oriented and personalized immunotherapeutic strategies to improve treatment efficacy in ovarian cancer.

12. Elucidating the mechanism of action of a first-in-class covalent small-molecule PD-L1 inhibitor

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The PD-1/PD-L1 immune checkpoint is an important target in cancer immunotherapy. Constant effort is being made to design and synthesize new inhibitors of this interaction, with better characteristics compared to the already available clinical monoclonal antibodies. So far, most small-molecule PD-L1 inhibitors developed in this vein have been reported to cause PD-L1 dimerization and internalization. The compound that we have developed also causes dimerization, but through covalent bonding with PD-L1, which has not been achieved before. This change in interaction type leads to noticeable differences in the inhibitory activity of the compound, as well as the fate of the PD-L1 protein in cells. The aim of this work was to investigate the mechanism of action of the covalent inhibitor in more detail. We show that after treatment with both the covalent and non-covalent (reference) compound, the localization of PD-L1 in cells changes, but the localization pattern depends on which compound was used. We also show that the covalent compound is able to cause dimerization of PD-L1 both in *cis* and in *trans*, as well as potentially lead to increased trans-endocytosis of this protein across cells.

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13. Establishing a Preclinical CRC Patient-Derived Organoid Pipeline for Neoantigen Validation

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In colorectal cancer (CRC), particularly in microsatellite-stable (MSS) tumours, low tumour mutational burden (TMB) limits the number of mutation-derived neoantigens and reduces the efficacy of personalised immunotherapy. Although sequencing enables in silico neoantigen prediction, only a small fraction of candidates are truly immunogenic, highlighting the need for robust preclinical models for functional validation. Patient-derived organoids(PDOs) faithfully recapitulate tumour features and represent a promising platform for this purpose.

To address this, we developed a CRC-PDO-based pipeline integrating organoid establishment and characterisation with neoantigen prediction and immune validation. The workflow includes PBMC collection, DNA/RNA extraction from primary tumours and matched organoids, exome and transcriptome profiling, identification of expressed tumour-specific somatic mutations, HLA typing, neoantigen prediction, peptide synthesis, and immunogenicity assessment by ELISpot and tumour-reactive co-culture assays.

Out of 170 CRC tissues collected for PDO establishment, 143 PDOs were successfully generated, and 46 cases were prioritised for this workflow. Among them, 31 cultures showed sustained growth, including 15 with matched peripheral blood mononuclear cells. DNA and RNA were obtained from primary tumour tissue and matched healthy adjacent tissue, as well as from tumour PDOs. In tumour PDOs, DNA sequencing has been completed in 6 cases and RNA sequencing in 15 cases, with the remaining samples under analysis. Initial profiling of the sequenced PDOs revealed marked molecular and pathological heterogeneity, encompassing MSS and MSI tumours as well as conventional, mucinous, medullary and serrated subtypes. TMB ranged from intermediate levels in MSS cases (15-19 mut/Mb) to hypermutated profiles (104-143 mut/Mb). HLA class I typing identified recurrent HLA-A02 and HLA-A24 alleles.

Together, these preliminary results support the feasibility of our CRC-PDO platform as a clinically relevant preclinical framework for integrated molecular profiling, HLA-informed neoantigen prioritisation, and functional validation, with potential to advance personalised vaccine-based immunotherapy strategies in CRC.

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14. A Dynamic OTSC-MIVO® Platform for Immunomodulatory Combination Therapy Modeling in PDAC

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy characterized by late diagnosis, rapid progression, and profound resistance to conventional and immunotherapeutic treatments, largely driven by its immunologically cold tumor microenvironment. The limited efficacy of immunotherapy in PDAC highlights the need for physiologically relevant preclinical models that preserve tumor-stroma-immune interactions. Organotypic tumor slice cultures (OTSCs) preserve native tumor architecture, including stromal and immune components. The dynamic MIVO® millifluidic system mimics physiological flow conditions, enabling the assessment of therapeutic responses under more physiologically relevant conditions. The primary objective of this study was to establish this integrated preclinical model and evaluate a combination treatment strategy in a dynamic setting.

OTSCs were generated from orthotopic pancreatic tumors established in C57BL/6 mice by implantation of 100,000 KPC cells. Tumors were sliced using a Compresstome® VF-510-0Z (Precisionary Instruments) and cultured in the MIVO® chamber (React4Life) under controlled double-flow conditions with continuous medium circulation driven by a peristaltic pump at 37°C and 5% CO_2. Static cultures were maintained in parallel. OTSCs were treated with an epigenetic priming strategy followed by immunotherapy to evaluate combinatorial therapeutic effects under dynamic conditions. Tissue integrity, immune cell presence, apoptosis, and proliferation were assessed by H&E staining, immunohistochemistry, and resazurin-based viability assays.

Dynamic cultivation preserved tissue architecture, cellular heterogeneity, and immune cell presence, while maintaining low baseline apoptotic activity. Treatment induced a reduction in proliferative activity compared to untreated controls, supporting the feasibility of this platform for evaluating combination therapeutic strategies.

Overall, the MIVO®-based dynamic OTSC platform represents a physiologically relevant and innovative model for studying combination immunotherapy in PDAC and may improve the predictive value of preclinical therapeutic testing.

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15. Integrated In Silico and Experimental Development of Dual HDAC6/ROCK Inhibitors as Immunomodulatory Anticancer Agents

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Histone deacetylase 6 (HDAC6) plays a pivotal role in regulating antitumor immunity. HDAC6 influences antigen uptake and presentation by antigen-presenting cells (APCs) and regulates the activity of $CD8^+$ cytotoxic T lymphocytes, regulatory T cells, and natural killer (NK) cells. Additionally, HDAC6 is involved in the control of STAT3 signaling, fibrinogen-like protein 1 (FGL1), and PD-L1 expression, contributing to tumor immune evasion. In parallel, inhibition of Rho-associated coiled-coil containing protein kinases (ROCK1 and ROCK2) has been shown to enhance APC phagocytic activity and promote antitumor immune responses. ROCK inhibition is also associated with modulation and stabilization of PD-L1, highlighting a complex interplay between cytoskeletal dynamics and immune checkpoint regulation. Given their complementary immunomodulatory and antimetastatic effects, developing dual HDAC6/ROCK inhibitors represents a promising strategy to enhance cancer immunotherapy and overcome therapeutic resistance.

The primary objective of this study was to establish a detailed and robust in silico protocol for the rational design of HDAC6/ROCK multitarget inhibitors, followed by comprehensive experimental validation. An integrated drug discovery approach combining ligand-based drug design (LBDD) and structure-based drug design (SBDD) was employed. The computational workflow included molecular docking studies and the development of predictive 2D-QSAR models using machine learning algorithms. Selected compounds were synthesized and evaluated in validated enzymatic and cell-based assays.

Compounds C-9, C-35, and C-40 demonstrated pronounced antitumor, anti-migratory, anti-invasive, and immunomodulatory effects, surpassing the activity of fasudil and tubastatin A. Mechanistic investigations revealed induction of early apoptosis, cell cycle arrest, and downregulation of PD-L1 expression. Furthermore, C-35 upregulated MICA expression, while C-9 reduced PVR levels, enhancing tumor immunogenicity. Collectively, these findings provide a strong foundation for further

optimization of dual HDAC6/ROCK inhibitors as next-generation immunomodulatory anticancer agents.

16. Targeting EGFR and STAT3 in *PEA3*-overexpressing prostate cancer: insights from 3D coculture and CAM models

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Introduction: Prostate cancer (PCa) is the second most frequently diagnosed cancer worlwide and a leading cause of cancer-related death in men. Genomic rearrangements leading to overexpresion of the PEA3 ETS transcription factors *ETV1* (~8%) or *ETV4* (~2%) define molecular subtypes of PCa associated with poor prognosis. Recently, we demonstrated that EGFR and STAT3 activation associates with *ETV1* aggressiveness, and that co-inhibition with Erlotinib and TTI-101 reduces growth in 3D models. In this study, we aim to leverage therapeutic utility of combined EGFR and STAT3 inhibition to *ETV4*-overexpressing prostate carcinomas using advanced 3D cell models mimicking tumor-stromal interactions, and chorioallantoic membrane (CAM) preclinical models.

Methods: Therapeutic potential of combined EGFR+STAT3 inhibition was evaluated in 3D co-cultures combining varying ratios of GFP-stable PC3-derived models (*ETV4*-overexpression and *ETV4*-silencing) and PKH26-labelled PCa-associated fibroblasts (PCAFs). Treatment imapct on microtumors' growth was evaluated using high content screening. For *in vivo* studies, matrigel-embedded PC3 cells were grafted onto the CAM at embryonic day 8 (ED8). At ED13, tumors were treated with Erlotinib+TTI-101/vehicle for 48 hours. Analyses were performed at ED15, including tumor perimeter *in ovo* using ImageJ and angiogenesis levels *ex ovo* through manual vessel counting and AI-based segmentation software (IKOSA), collecting vessel-level parameters. After imaging, CAM-derived tumors were either processed fresh for LC-MS/MS proteomic profiling or FFPE for IHC.

Results: *ETV4*-overexpressing spheroids exhibited marked growth suppression under combined EGFR+STAT3 inhibition. Therapeutic afficacy was maintained in the

presence of stromal cells, even at high PCAFs:PC3 cell ratios. In CAM studies, *ETV4*-overexpression was associated with a treatment-specific reduction in blood vessel number – not observed in *ETV4*-silenced microtumors or tumor-free controls. Preliminary analysis of CAM-derived tumors by LC-MS/MS identified Lysozyme C (cLyz), an innate immune marker, in protein extracts, confirmed by IHC. Ongoing analysis will clarify whether cLyz levels are associated with *ETV4* expression and/or combined treatment.

Conclusion: This work supports therapeutic efficacy of combined EGFR and STAT3 inhibition for *ETV4*-overexpressing PCa, and suggests potential effects on stromal and vascular components of the TME.

17. Impact of the hypoxic and acidic tumor microenvironment on the cargo and immuno-modulatory effects of EVs released by multidrug resistant non-small cell lung cancer cells

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality. Notwithstanding significant advances in clinical management, long-term survival remains limited due to the development of multidrug resistance (MOR). Extracellular vesicles (EVs) are nanosized cell-released particles that mediate intercellular communication within the tumor microenvironment (TME). Hypoxia and acidosis have been consistently linked to increased EVs release. This work aimed to study the impact of the hypoxic and acidic TME on the cargo and immuno-modulatory effects of EVs released by MDR NSCLC cells. For that, two pairs of counterpart drug-sensitive and MDR NSCLC cell lines were used (NCI-H460/NCI-H460/R; A549/A549-CDR2). EVs were isolated (by differential centrifugation) and characterized according to the MISEV2023 guidelines (using Nanoparticle Tracking Analysis, Transmission Electron Microscopy, Western blot-WB), and their protein content assessed by mass spectrometry-based proteomics. Our data showed that 101 proteins were enriched in EVs from MDR NCI-H460/R cells released under hypoxia, while 48 proteins were enriched under acidosis, when compared to normoxia. Equally, 66 and 85 proteins were decreased under hypoxic and acidic conditions, respectively. PANTHER analysis revealed a decline in proteins involved in B and T cell activation. The most relevant differentially expressed proteins were validated by WB. Proteomic analysis of EVs released from drug-sensitive NCI-H460 cells showed an opposite expression trend for immune-related proteins. These findings were further validated in A549/A549-CDR2 cells by WB. Co-culture of activated human T cells with MDR NCI-H460/R cell-derived EVs released under hypoxic conditions resulted in increased immune checkpoint expression.

This was accompanied by an expansion of TEMRA and effector memory compartments, along with a decline in central memory and naive subsets, indicating T cell exhaustion. Overall, this work suggests that MDR cell-derived EVs released under hypoxic conditions may contribute to immunomodulation and have an impact on immunotherapy efficacy. Ongoing work aims to confirm this hypothesis.

18. Modeling Natural Killer Cell–Glioblastoma Crosstalk in Physiologically Relevant *in Vitro* Systems

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Glioblastoma (GB) is the most commonly diagnosed primary brain cancer in adults. Its aggressiveness results in limited treatment options and poor patient prognosis. Natural killer (NK) cell-based immunotherapy is a promising approach for GB treatment. NK cells are cells of the innate immunity and can eliminate a spectrum of target cells, including glioblastoma stem cells (GSCs), which are intrinsically resistant to standard therapeutic approaches and drive GB recurrence. However, the tumor microenvironment of GB is highly immunosuppressive, which may significantly hamper the anti-tumor functions of NK cells. To gain deeper insight into the interactions between GB and NK cells, we established three distinct physiologically relevant *in vitro* model systems. Firstly, spheroids were established from either GSCs or differentiated GB cells in Celvivo ClinoReactors and were subsequently co-cultured with NK-92 cells under static conditions. NK-92 cells more efficiently infiltrated spheroids of differentiated GB cells, but their cytotoxicity was higher against GSC spheroids. In line with prominent NK-92 infiltration, spheroids of differentiated cells secreted higher levels of immunomodulating and NK cell attracting cytokines. Secondly, NK-92 infiltration towards GB was studied in a dynamic MIVO® microfluidic platform mimicking the blood flow and influx of immune cells into the tumors, which confirmed higher NK-92 cell attraction of differentiated GB spheroids compared to GSC spheroids. Thirdly, the ability of NK-92 cells to detect and eliminate GB cells was studied in advanced GB assebloids, i.e., co-cultures of brain organoids and GB spheroids, mimicking the invasive infiltration of GB cells into healthy brain parenchyma. Overall, our *in vitro* model systems provide a foundation for future investigations into the complex crosstalk between GB and NK cells.

19. Progresses in the sustainable design of PD-L1 inhibitors

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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 step. Subsequent transformations will lead to novel inhibitors.

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20. Cysteine cathepsins in models of tumour microenvironment

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Cysteine peptidases are enzymes known to play a role in the development and spread of cancer. However, the development of cancer depends not only on cancer cells but also on cells in the tumour microenvironment (TME). Therefore, for successful regulation of peptidases as antitumour targets understanding their role in TME is necessary. To achieve this, models based solely on cancer cells are insufficient; stroma cells, immune cells, and endothelial cells must also be included. However, 2D cell models are not the best to replicate the physiological pathological state of cancer in the human body as closely as possible and to understand the role of peptidases in different cell population. Instead, we can use 3D models of spheroids and organoids, which are much more accurate representations of the biological state. Our study's objective was to create 3D cell models of breast cancer, which included both breast cancer cells and cells from the TME, that would serve as the foundation to investigate the expression of cathepsin in breast cancer TME.

To address our objective, we prepared co-culture models and spheroids of cancer cells, stromal cells and macrophages which are used as immune cells from TME. Two methods were used to create the spheroids: liquid overlay and hanging drop. The protein levels and activity of the observed cathepsins in different cell populations were assessed by western blot and enzyme activity assay.

According to our findings, stromal cells impact the protein levels and activity of cysteine cathepsins in TME. During generation of spheroid models, we concluded the formation of spheroids differs among cell lines and the scaffold and stromal cells can enhance spheroid formation. Furthermore, the spheroid creation technique must be tailored to each breast cancer cell line independently. These spheroid models may be applied to evaluation of cathepsin directed anti-tumour therapy and future investigations into the mechanisms behind the progression of cancer.

21. OASIS: Oncological AtlaS of Immunotherapy response signatures

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The wide use of omics sciences, such as transcriptomics, is generating an unprecedented amount of biological data to be analyzed and interpreted. However, access to these datasets can be complex and time consuming, particularly for non-computational researchers.

The objectives of this Immuno-model Data Gathering Task Force were: i) to implement a systematic search strategy to collect publicly available immuno- oncology clinical and pre-clinical transcriptomic datasets and ii) to develop a graphical interface for the user-friendly interrogation of such omics data.

Candidate datasets were selected from public repositories (Gene Expression Omnibus and ArrayExpress), through a set of pre-defined queries, which results were evaluated by members of this taskforce (at least two expert researchers per query). Datasets fulfilling the following criteria were selected for downstream use: bulk or single-cell transcriptomic datasets from pre-clinical or clinical samples receiving immunotherapy, alone or in combination with other treatments.

To achieve the second goal, an application was developed in R using Shiny packages, which facilitates the creation of user-friendly web interfaces. SQL (Structured Query Language) was used to manage the database enabling users to easily access, query, and work with the data. Among its functionalities, the Shiny app allows users to compare gene expression between responding and non-responding patients using visualizations such as box plots or heatmaps or download gene expression tables. Additionally, it enables users to explore the data and select subsets of patients.

In conclusion, this project is contributing to create an "user-friendly" public database on immunotherapy treated patients which can be used to share and compare data or results, enable the download or upload of data, facilitating the optimization and use of available data in the immuno-oncology field.

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22. Immune deconvolution of ovarian tumors reveals distinct immune phenotypes

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Ovarian cancer (OC) is an entity in which the success of immune checkpoint inhibitors (ICIs) remains limited. However, the innate immune system, in particular tumor-associated macrophages (TAMs) and dendritic cells may represent a novel and encouraging therapeutic target. The aim of this study is to cluster patients based on their myeloid microenvironment and to identify biomarkers of response, and subsequently validate these findings in suitable preclinical models.

To address this objective, a total of 258 samples from 226 patients with serous ovarian cancer at different disease stages were profiled by RNA sequencing (RNA-seq), including 26 primary tumors, 14 ascites samples, and 218 metastatic lesions. Patients included both treatment-naïve and previously treated cases. Among the cohort, a subset of samples corresponded to paired specimens from the same patients. RNA-seq data were processed using a standardized pipeline including quality control and alignment, followed by normalization for downstream analyses. Principal Component Analysis (PCA) was performed to assess sample clustering according to clinical variables. Immune cell infiltration was estimated from transcriptomic data using the *ConsensusTME* deconvolution tool to identify immune hot profiles. Survival analyses were conducted to evaluate the association between macrophage abundance and patient prognosis. The identified macrophage populations will undergo detailed analysis to determine the most prominent immune checkpoint molecules, guiding the development of combination blocking strategies. These approaches will be validated in an ex vivo patient-derived model developed as part of the collaborative ioAVATAR initiative.

23. Integrative extracellular vesicle liquid biopsy profiling reveals immune modulation and response pathways during immunotherapy

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Immune checkpoint inhibitors (ICIs) modulate anti-tumor immunity by restoring T-cell activity, yet patient responses and immune-related adverse events (irAEs) remain highly heterogeneous. Understanding systemic immune modulation and identifying minimally invasive models to monitor response and toxicity are critical unmet needs. Extracellular vesicles (EVs) and peripheral immune cells provide complementary liquid biopsy-based platforms to study immune dynamics, with EVs uniquely capturing therapy-induced changes through their protein cargo and surface signatures.

This ongoing prospective study includes immunotherapy-naïve patients with solid tumors, with peripheral blood collected at baseline and 4 ± 2 weeks after initiation of anti-PD-1/PD-L1 therapy. EVs are isolated by size-exclusion chromatography and characterized according to MISEV2023 guidelines. Proteomic profiling by LC-MS and EV immunophenotyping by flow cytometry are performed alongside multiparametric flow cytometry of peripheral immune cells to define cellular subsets, activation states, and regulatory phenotypes.

EV characterization confirmed efficient isolation, minimal contamination, and consistent detection of canonical markers. Following ICI initiation, we observed an increase in EV concentration and size, consistent with enhanced vesicle release during systemic immune activation. Immunophenotyping revealed expansion of EV subsets enriched in immune- and checkpoint-related markers, including components of the PD-1/PD-L1 axis, with pronounced inter- and intra-patient variability indicative of differential immune modulation. Proteomic analyses showed enrichment of

pathways associated with T-cell activation, antigen presentation, and inflammatory responses, suggesting a potential connection to early mechanisms of immune-related response. Importantly, EV signature shifts paralleled dynamic changes in circulating immune cell populations, including activation of effector T-cell subsets and modulation of myeloid compartments, supporting coordinated systemic immune reprogramming.

Together, these findings support EVs as a minimally invasive model for monitoring immune modulation during ICI therapy and for exploring mechanisms underlying response heterogeneity and irAEs. Ongoing integrative analyses aim to validate EV-based signatures as predictive tools for therapeutic outcomes in cancer immunotherapy.

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24. Reprogramming colorectal cancer *N*-glycosylation to enhance innate- like anti-tumor immunity

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Colorectal cancer (CRC) is a leading cause of cancer-related mortality, with rising incidence in young adults and limited benefit from current immunotherapies. In mismatch repair-deficient (MMRd) CRC, immune escape often involves HLA class I downregulation, which impairs $CD8^+$ T-cell recognition. In contrast, innate-like lymphocytes (e.g., NK cells) can act independently of HLA, offering an alternative route for tumor control. Beyond classical stress-ligand recognition, these cells also integrate glycan-binding properties that can modulate their function. Tumor glycosylation is emerging as a major regulator of immune recognition, but its impact on innate-like responses in CRC remains unclear.

We hypothesize that CRC glycan signatures tune innate-like lymphocyte recruitment and function, contributing to immune escape. To address this, we integrated *in silico* analysis of CRC patient datasets with glycoengineered murine CRC models and coculture assays with innate-like lymphocytes.

In silico, HLA-high tumors showed higher glycosylation and higher inferred scores of innate-like immune populations. Notably, within HLA-low tumors, expression of selected glycogenes correlated positively with these innate-like populations, whereas in HLA-high tumors a selected glycogene correlated negatively, suggesting that HLA context conditions how tumor glycosylation associates with innate-like immunity.

Functionally, cocultures of murine MC38 CRC cells (mock vs selected glycogene knockout) with innate-like lymphocytes revealed that glycogene-deficient MC38 cells had reduced viability and increased active caspase-3, indicating greater susceptibility to innate-like cytotoxicity. Correspondingly, cocultured lymphocytes upregulated activation markers such as CD25 and a specific NK-receptor, and a selected glycan-binding protein (GBP). This is consistent with an enhanced immune engagement and activation.

In vivo, glycoengineered CRC tumors were smaller, with increased infiltration of innate-like lymphocytes and an activated intratumoral phenotype.

Together, these preliminary data support a direct, context-dependent role for tumor glycan remodeling in shaping innate-like immunity in CRC - particularly when HLA-mediated $CD8^+$ T-cell recognition is compromised - and motivate glycan-targeted strategies to potentiate innate-like anti-tumor responses.

25. Immunomodulatory and Anti-Tumor Effects of *Alchemilla smirnovii Juz* in Experimental Breast Cancer Models

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Natural plant-derived compounds represent a promising source of biologically active molecules with potential immunomodulatory and anticancer properties. *Alchemilla smirnovii* Juz. (AS), a medicinal herb traditionally used in herbal medicine, has not been extensively investigated for its immunological and antitumor activities. The present study aimed to evaluate the anticancer effects of AS, with particular focus on its influence on immune-related and inflammatory pathways, using both in vitro and in vivo breast cancer models.

In vitro experiments were performed on hormone receptor–positive MCF-7 and triple-negative MDA-MB-231 breast cancer cell lines. Real-time cell analysis (xCELLigence system) demonstrated that AS moderately inhibited cell proliferation and migration, suggesting potential anti-metastatic activity. The extract exhibited low cytotoxicity and limited induction of apoptosis, indicating a predominantly cytostatic effect. In MCF-7 cells, AS treatment showed inhibitory activity toward HER2-related signaling pathways.

In vivo studies were conducted using a dimethylbenz[a]anthracene (DMBA)-induced breast cancer model. Administration of AS, particularly in combination with inhibitors of L-arginine metabolic pathways, resulted in enhanced therapeutic effects. Molecular analysis revealed increased tumor necrosis factor-α (TNF-α) levels in tumor tissue and circulation, alongside elevated interleukin-2 (IL-2) levels, suggesting modulation of immune-associated signaling. Treatment also reduced the expression of cyclooxygenase-2 (COX-2), matrix metalloproteinase-2 (MMP-2), and vascular endothelial growth factor A (VEGF-A) in tumor tissue, blood, and lungs, indicating suppression of pro-inflammatory, pro-metastatic, and pro-angiogenic factors within the tumor microenvironment.

Collectively, these findings suggest that AS exerts multi-target antitumor effects partly through modulation of inflammatory mediators and immune-related pathways, including L-arginine–associated mechanisms. These results support further investigation of AS as a potential modulator of the tumor microenvironment and as a complementary approach in cancer immunotherapy-oriented strategies.

26. 3D High-Grade Serous Ovarian Cancer Spheroid Models Reveal Clinically Relevant Chemoresistance Mechanisms and a Validated Platform for Tumour Microenvironment Modelling

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High-grade serous ovarian cancer (HGSOC) remains the most lethal gynaecological malignancy, with over 70% of patients relapsing after platinum-based chemotherapy. A key barrier to developing effective immunotherapy strategies is the absence of biologically faithful preclinical models that recapitulate tumour architecture, oxygen-nutrient gradients, and the EMT programmes known to drive immune cell exclusion.

We established and characterised 3D in vitro HGSOC models using ultra-low attachment (ULA) spheroid culture and extrusion-based 3D bioprinting in laminin-enriched bioink, across four genomically distinct cell lines (OVCAR-4, OVSAHO, COV362, Kuramochi). Models were benchmarked against 2D cultures using morphological analysis, RT-qPCR (22 genes across EMT/ECM/Wnt/DDR/angiogenesis pathways), flow cytometry, immunocytochemistry, and total RNA sequencing with Celligner transcriptomic alignment to TCGA HGSOC patient subtypes.

3D models spontaneously developed rim-core architecture and activated EMT programmes (VIM, SNAI1/2, ZEB1/2, TWIST1/2, MMP2/9, WNT11) absent in 2D cultures. Celligner analysis showed that 3D transcriptomes cluster significantly closer to the mesenchymal HGSOC patient subtype that most strongly associated with immune exclusion and poor prognosis. Carboplatin IC_{50} values in 3D (63–284 µM) matched clinical plasma concentrations, with resistance driven by a cytostatic G0/G1 arrest mechanism. Post-treatment upregulation of WNT11, ZEB1/2, and TWIST2 was geometry-dependent and correlated with shorter progression-free survival in TCGA HGSOC patients (HR=1.36–1.93, n=726–771).

These biologically validated 3D models reproduce the spatial architecture and transcriptomic landscape of immune-excluded HGSOC tumours, making them a relevant foundation for future co-culture studies incorporating stromal and immune cell populations.

27. From Method Development to Clinical Monitoring: Circulating Tumor Cell Detection Pipeline in Early Breast Cancer Modelling

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Circulating tumor cells (CTCs) and CTC clusters are considered key mediators of distant metastasis. While homotypic clusters consist exclusively of tumor cells, heterotypic clusters include tumor cells together with immune cells, platelets, or stromal components, potentially enhancing survival, immune evasion, and metastatic capacity. In early breast cancer (eBC), CTCs and clusters have historically been regarded as rare events, evaluated using marker-based isolation approaches.

To improve detection sensitivity and enable longitudinal monitoring, we established a standardized CTC detection pipeline within a prospective study of high-risk eBC patients undergoing neoadjuvant systemic therapy (NST). Peripheral blood was collected via vascular access port (VAP) at predefined treatment time points (before NST, mid-NST and after NST). CTC enrichment was performed using a size- and deformability-based, antibody-independent microfluidic platform, followed by morphological evaluation.

Using this approach, CTCs were identified in all evaluated patients, and CTC clusters were detected more frequently than previously reported in eBC. Both CTC numbers and cluster incidence declined during NST. Notably, CTC clusters persisted in a subset of patients despite achieving pathological complete response (pCR).

These preliminary findings suggest that refined detection methodologies may reveal a higher prevalence of CTC clusters in eBC and indicate a potential role for CTC clusters in treatment response and minimal residual disease. Ongoing follow-up will clarify their prognostic significance and association with long-term outcomes.

28. 3D Microfluidic Model Uncovers Dynamic Crosstalk Between Natural Killer Cells and Glioblastoma

Tina Kolenc Milavec[1,2], Anamarija Habič[1,2], Pia Žižek[1], Špela Kladnik[1], Urban Švajger[3,4], Andrej Porčnik[5], Metka Novak[1], Barbara Breznik[1]

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Glioblastoma (GB) is the most common primary brain tumor in adults and is associated with a very poor prognosis. Among emerging therapeutic strategies, natural killer (NK) cell–based immunotherapy has shown considerable promise. NK cells can naturally recognize and eliminate cancer cells, but in the highly immunosuppressive GB microenvironment, their function becomes severely impaired. A better understanding of the interactions between GB and NK cells is therefore essential for the development of effective therapies and for overcoming treatment resistance.

To investigate these interactions, we established a 3D glioblastoma spheroid model, which more closely mimics the *in vivo* tumor, using either differentiated glioblastoma cells (GBM) or glioblastoma stem cells (GSCs). The spheroids were co-cultured with NK-92 cells, revealing ratio-dependent NK cell cytotoxicity against GB cells. Notably, NK cells exhibited significantly greater cytotoxicity toward GSCs than toward GBMs, despite infiltrating GBM spheroids more efficiently. These findings were consistent with cytokine expression analyses showing that GBMs secrete higher levels of immune cell chemoattractants compared to GSCs.

To better reflect *in vivo* conditions with dynamic influx of immune cells into the tumor, we next transferred our GB model into MIVO® microfluidic platform (React4Life). In this system, spheroids were embedded in Matrigel® and cultured in a chamber positioned above a microcirculation of NK cells. Similar to observations in the static model and *in vivo* tumors, NK cell infiltration into GB spheroids remained limited overall, with lower infiltration observed in GSC spheroids compared to GBM spheroids. In addition, NK cell viability was reduced in the presence of GSC spheroids, suggesting that interactions of NK cells with GSCs may induce NK cell death.

We successfully established 3D and microfluidic tumor models for studying GB–NK cell interactions *ex vivo*. Our results elucidate that GB-NK crosstalk regulates NK cell activity, and that it is GB phenotype specific. Interrupting this crosstalk could improve the response of GB cells to immunotherapy and should be further explored.

29. Understanding glioblastoma immune microenvironment for personalized therapy

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Glioblastoma remains a highly lethal disease despite the current standard-of-care, which includes maximal surgical resection followed by radiotherapy and temozolomide treatment. A major obstacle in developing new therapies and studying treatment responses is the absence of *in vitro* models that faithfully capture the complexity of patient tumors and allow visualization of the tumor microenvironment.

To overcome these limitations, we generated patient-derived GB organoids that closely resemble their parental tumors, preserving tumor heterogeneity and resistance to clinical therapies. In parallel, we applied spatial transcriptomics to characterize molecular changes within the tumor immune microenvironment.

Modern cancer research increasingly highlights the need to understand the dynamic and multifaceted nature of the tumor microenvironment as a whole. Achieving this requires advanced technologies and innovative *in vitro* models that enable real-time visualization of cellular composition and interactions in response to therapeutic interventions.

This work was supported by the Slovenian Research Agency (grants P1-0245, J3-4504, J3-2526, NC-0023, N3-0394, NC-25002, Young researcher grants 10040137, 10040147), the EU Program of Cross-Border Cooperation for Slovenia-Italy Interreg TRANS-GLIOMA and by the HE project Twinning (CutCancer; 101079113).

30. Modeling prostate cancer-induced bone metastasis and evaluation of therapies in TgRANKL mice

Christos-Chrysovalantis Syrigos[1,2], Eleni-Dimitra Lioki[1,2], Melina Dragolia[2], Vasileios Ntafis[2], Martina Rauner[3] and **Eleni Douni**[1,2*]

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Bone metastases are highly prevalent in patients with advanced prostate cancer, with bones being the most frequent site of prostate-driven metastasis. Receptor activator of nuclear factor-κB ligand (RANKL) plays a pivotal role in prostate cancer metastasis to bone by promoting osteoclastogenesis. Anti-resorptive agents, such as denosumab, which directly inhibits RANKL activity, and zoledronic acid, which induces osteoclast apoptosis, have been employed as a therapeutic strategy to reduce skeletal-related events (SREs) associated with bone metastasis. In this study, we examined the metastatic potential of prostate cancer cells in the bones of osteoporotic TgRANKL male mice, which overexpress the human RANKL gene. Additionally, we investigated the effects of anti-resorptive drug treatment in the progression of bone metastases. To assess metastatic bone lesions, RM1-BM mouse prostate cancer cells, recognized for their bone-metastatic potential, were injected systemically into the caudal tail artery of syngeneic TgRANKL and wild-type (WT) male mice. Due to stable transduction of RM1-BM cells with the firefly luciferase gene, metastatic progression was monitored through in vivo bioluminescence imaging. Histological analysis and microCT analysis were performed to evaluate metastatic bone lesions. Our results demonstrated increased incidence and burden of bone metastasis and more severe bone loss in TgRANKL mice compared to their WT littermates. Subsequently, we investigated the potential of denosumab and zoledronic acid as prophylactic treatments to prevent bone metastasis. Both imaging and histological analysis indicated that these anti-resorptive agents effectively prevented bone metastasis and inhibited osteolysis. In conclusion, we have established an in vivo mouse model of prostate cancer-induced bone metastasis for the preclinical application and evaluation of novel therapeutics including immunotherapies.

This research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 5th Call for HFRI PhD Fellowships (Fellowship Number: 19290).

List of keynote lectures

Lecture	Presenter name	Presenter surname	Talk title
1	Elisa	Oricchio	Use tissue explants to decode tumor evolution and response to therapies
2	Erik	H.J.G. Aarntzen	Imaging CD8 T-cells in vivo: the potential of PET imaging as biomarker technology
3	Jérôme	Galon	The immune contexture in the era of cancer immunotherapies
4	Frederik	De Smet	From patient tissue to in vivo models: multiscale analysis of tumour-immune interactions in brain tumours
5	Marinka	Žitnik	Empowering cancer research with AI

List of invited lectures

Lecture	Presenter name	Presenter surname	Talk title
1	Berna	C. Özdemir	Sex and gender differences in immunotherapy efficacy and toxicity- what is the evidence?
2	Camila	Consiglio	Decoding sex differences in human immunity using systems immunology
3	Emanuele	Azzoni	From prenatal origins to novel therapeutic strategies targeting inflammatory pathways: leveraging pre-clinical models of Juvenile Myelomonocytic Leukemia (JMML)
4	Jordi	Minguillón	Preclinical and Clinical Research on CAR T-Cell Therapy for Pediatric Leukemia
5	Renata	Stripecke	Humanized mice and engineered CAR-T cells
6	Gustavo	Alviter-Raymundo	Humanised mouse models for pre-clinical investigation of cancer immunotherapies

7	Jordane	Divoux	The TRIPLEX study: use of patient-derived tumor organoids as an innovative tool for precision medicine in triple-negative breast cancer
8	Kristina	Kromer	Modelling the development of tumor-associated macrophages (TAMs) in colorectal cancer using a complex human in vitro system of the tumor microenvironment
9	Natalia	Bednarz-Knoll	Imaging flow cytometry for decoding circulating tumor and normal cell interactions: implications for immunothrepy monitoring
10	József	Dudás	Advances form the COST Action CA21135 “Modelling immunotherapy response and toxicity in cancer” for the translational research in ENT-clinic in Innsbruck, Austria

List of short talks from industry

Lecture	Presenter name	Presenter surname	Talk title
1	Devrim	Pesen Okvur	INTIO CELL: Advancing Human-Relevant Microphysiological Systems for Cancer and Drug Discovery
2	Uršula	Prosenc Zmrzljak	Labena d.o.o.: Spatial Transcriptomic Profiling to Distinguish Primary Cholangiocarcinoma from Metastatic Pancreatic Adenocarcinoma in the Liver
3	Samy	Dufour	Omega d.o.o.: Comprehensive solutions for the formulation and spatial characterization of 3D immune models with Thermo Fisher distributed by Omega D.O.O.
4	Aniello	Lombardi	Axion Biosystems: Real-Time Label-Free Kinetic Analysis for Cancer Immunotherapy: From

			CAR T Potency to Cardiotoxicity Monitoring
5	Fan	Yu	RWD Life Science Co., LTD
6	Marc	Creus	Abbmira Therapeutics AG
7	Sonay	Goneli	28DIGITAL

List of short lectures

Lecture	Presenter name	Presenter surname	Talk title
1	Alfredo	Pherez-Farah	Myeloid-t cell therapy interactions reshape the tumor microenvironment to sustain tumor control
2	Ferran	Grau-Leal	Implementation of Patient-Derived Organotypic Tumor Spheroids (PDOTS) from Primary and Metastatic Colorectal Cancer for Ex Vivo Immunotherapy Testing
3	Adrian	Bogdan Tigu	Synergizing Immunotherapy and Proteasome Inhibition in Mantle Cell Lymphoma
4	Fatih	Ozefe	Perfusion-based Melanoma-Immune Microenvironment (MIMe) Platnform for Modeling Adoptive Immune Response

List of posters

Poster number	Presenter Name	Presenter Surname	Poster title
1	Cristina	Manferdini	Engineering 3D Models to Recapitulate the Multiple Myeloma Bone Marrow Microenvironment
2	Javier	De Las Rivas	Assignment of gene markers to immune cells based on single-cell RNA-seq data, using a machine learning assessment
3	Can	Türk	Survival-Based Immune Stratification Reveals Distinct Immunometabolic Signatures in TCGA-LAML
4	René	Winkler	STAG2-mutated hematological cancers display characteristic microenvironmental changes with therapeutic implications for immunotherapy
5	Nuno R.	dos Santos	Antibody blockade of the PSGL-1 glycoprotein impairs B-cell lymphoma progression by enhancing T-cell responses
6	Nilgun	Yakubogullari	Dual Immunotherapy with Multi-Epitope Nanovaccine and Anti-PD-1 for Robust Anti-Tumor Immunity against Melanoma
7	Paula	Martín Rubio	The lung-specific microenvironment: an opportunity for NK cell-based immunotherapy
8	Ahmed	Hassan	Radiation quality shapes immune responses: Transcriptional insights for immune-informed radiotherapy
9	Boštjan	Markelc	Radiotherapy-Induced Activation and Immunomodulatory Reprogramming of Tumour Endothelial Cells
10	David	Bartolomé-Català	Reconstructing the tumor immune microenvironment on a chip: a primary cell-derived model for studying T cell migration in colorectal cancer
11	Sarah	Pottmeier	Establishing Ovarian Cancer Patient AVATAR Models from Malignant Ascites and Debulking Solid Tumor Tissue to Study Immunotherapy Approaches
12	Agnieszka	Maślanka	Elucidating the mechanism of action of a first-in-class covalent small-molecule PD-L1 inhibitor
13	Diogo Rafael	Da Cruz Ribeiro	Establishing a Preclinical CRC Patient-Derived Organoid Pipeline for Neoantigen Validation
14	Ivana	Hlavenova	A Dynamic OTSC-MIVO® Platform for Immunomodulatory

			Combination Therapy Modeling in PDAC
15	Milan	Beljkas	Integrated In Silico and Experimental Development of Dual HDAC6/ROCK Inhibitors as Immunomodulatory Anticancer Agents
16	Elsa	Gomes Paiva	Targeting EGFR and STAT3 in PEA3-overexpressing prostate cancer: insights from 3D coculture and CAM models
17	Helena	Branco	Impact of the hypoxic and acidic tumor microenvironment on the cargo and immuno-modulatory effects of EVs released by multidrug resistant non-small cell lung cancer cells
18	Anamarija	Habič	Modeling Natural Killer Cell–Glioblastoma Crosstalk in Physiologically Relevant in Vitro Systems
19	Philippe	Bertrand	Progresses in the sustainable design of PD-L1 inhibitors
20	Marjeta	Lipužič	Cysteine cathepsins in models of tumour microenvironment
21	Eduardo	Aranda Cañada	OASIS: Oncological AtlaS of Immunotherapy response signatures
22	Ester	Raigón Gómez	Immune deconvolution of ovarian tumors reveals distinct immune phenotypes
23	Catarina A.	Rodrigues	Integrative extracellular vesicle liquid biopsy profiling reveals immune modulation and response pathways during immunotherapy
24	João	Pereira	Reprogramming colorectal cancer N-glycosylation to enhance innate-like anti-tumor immunity
25	Hayarpi	Javrushyan	Immunomodulatory and Anti-Tumor Effects of *Alchemilla smirnovii Juz* in Experimental Breast Cancer Models
26	Vesna	Kokondoska Grgič	3D High-Grade Serous Ovarian Cancer Spheroid Models Reveal Clinically Relevant Chemoresistance Mechanisms and a Validated Platform for Tumour Microenvironment Modelling
27	Tanja	Jesenko	From Method Development to Clinical Monitoring: Circulating Tumor Cell Detection Pipeline in Early Breast Cancer Modelling
28	Tina	Kolenc Milavec	3D Microfluidic Model Uncovers Dynamic Crosstalk Between Natural Killer Cells and Glioblastoma

29	Metka	Novak	Understanding glioblastoma immune microenvironment for personalized therapy
30	Eleni	Douni	Modeling prostate cancer-induced bone metastasis and evaluation of therapies in TgRANKL mice

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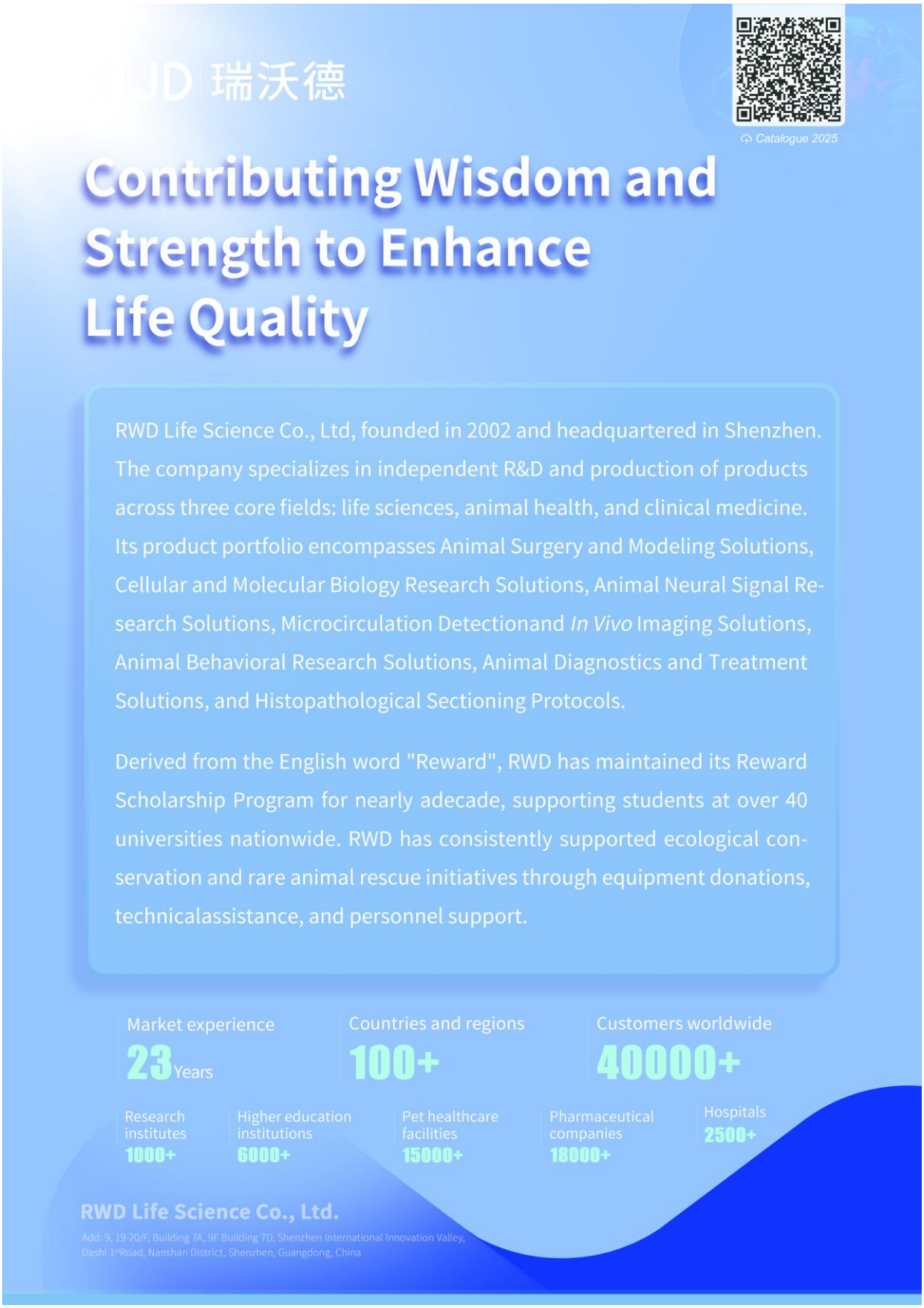
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Labena

Established in 1993, Labena Group is a family-owned business headquartered in Slovenia with offices in Croatia, Serbia, Bosnia & Herzegovina, North Macedonia, Montenegro and sicer 2025 also in Italy. The company started as sales and service support of highly specialised laboratory, manufacturing and process equipment utilized in the pharmaceutical and food industry, as well as industry of materials, research institutes and academia. To date Labena has developed into a fast growing and agile organisation with 4 major business divisions: (1) Division for Distribution, Servicing and Application support for Laboratory Equipment; (2) Organisation dedicated to the development, design, manufacturing and assembly of laboratory furniture; (3) Contract Research and Development Organisation for (Bio)Pharma and Biotech industries; (4) Corporate Ventures Accelerator named *Labena Ventures*.

Labena progressed to a path of major growth and diversification in 2016, with the acquisition of a Contract Research Organisation (CRO) laboratory, certified for Good Manufacturing Practice (GMP), accredited by the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA). When acquired, the CRO employed 12 people altogether, and comprised of 'Analytical Laboratory' focused on design and development of protocols for drug manufacturing, and on analytical testing of active pharmaceutical ingredients and generic medicines, and their conformity with the European and US Pharmacopoeia. Today the CRO employs over 60 colleague, of which more than 20 hold a PhD.

Labena d.o.o. in Slovenia, together with its CRO division, is also a holder of ISO 9001:2015 certificate and a holder of GMP certificate, as well as licence-holder for manufacturing and testing of medicines.

In 2017 the company invested into expansion of the CRO by building a **'Molecular Biology Laboratory'**, and later on in 2019 also a **'Protein Biochemistry Laboratory'**, focused on downstream and also upstream process characterisation and development for manufacturing of biopharmaceuticals (such as monoclonal antibodies). Today, **the Molecular Biology Laboratory** focuses on the development of processes for the production of biologics (such as monoclonal antibodies) and gene and cell therapies. It also carries out contract research and development projects for the (bio)pharmaceutical industry and conducts analytical testing of medicines. CRO operations are growing with addition of Bioinformatics and newly established Microbiology laboratories which are to be fully GMP certified. The acquisition of the CRO and investment in its growth propelled Labena's expansion from selling and servicing equipment into research and development projects with medium and large (bio)pharmaceutical companies globally.

Today Labena group has grown to over 140 employees and is further expanding in areas of research and development services with the opening of the environmental DNA laboratories and with the opening of its clinical diagnostic laboratory, BIA Genetics in Zagreb, Croatia.

With the launch of Labena's Grant Challenge project in 2017, Labena started extensively investing in fostering collaboration with institutions, faculties, companies, and start-ups in areas of life-sciences by awarding research grants, providing its knowhow, resources, facilities and equipment to research and development ideas with most prominent commercial potential. But we are not stopping here: In February 2023 we at Labena took innovation-focused approaches a step further and aunched our **Labena Ventures** division. Labena Ventures is a unique corporate accelerator programme focused on globally scaling disruptive innovation in areas of biotech, healthtech, pharmatech and medtech, currently accelerating 34 promising startups from around the globe.

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EXECUTIVE SUMMARY: Abbmira Therapeutics AG

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The Abbmira founding team includes three seasoned biotech entrepreneurs. This core team is supported by a growing network of translational and clinical experts, and a strong foundation in immunology, oncology and medicinal chemistry.

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