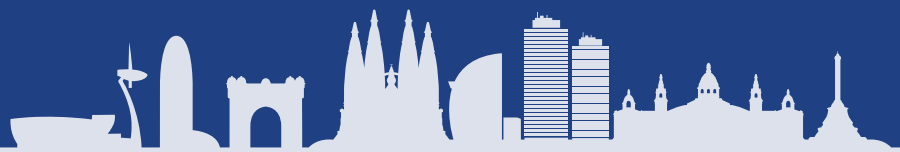
Conference Program and Abstract Book



1st IMMUNO-model COST Action Conference

Exploring the Frontiers of Cancer Immunotherapy: From Models to Breakthroughs

Josep Carreras Leukaemia Research Institute (IJC) – Auditorium

1-2 June 2023. Badalona, Barcelona. Spain

Program

June 1st 2023

IMMUNO-MODEL COST ACTION MANAGEMENT SESSION

8.30 – 9.00 Registration for IMMUNO-Model COST Action members and Invited Speakers

9.00 – 10.30 **IMMUNO-Model COST Action Management Committee meeting**
Josep Carreras Leukemia Research Institute (IJC) Auditorium

10.30 – 10.50 Coffee Break at the IJC rooftop

10.50 – 11.50 **WG1 meeting – In vitro and ex vivo cancer immunotherapy models**
IJC Auditorium
Hosted by WG1 leaders Dr. Devrim Pesen Okvur and Dr. Cristiana Tanase

11.55 – 12.55 **WG3 meeting – Solid tumors**
IJC Auditorium
Hosted by WG3 leaders Dr. Marleen Ansems and Dr. Maurizio Callari

11.55 – 12.55 **WG4 meeting – Hematologic tumors**
Germans Trias i Pujol Research Institute (IGTP) Multipurpose Room
Hosted by WG4 leaders Dr. Laura Belver and Dr. Rui Bergantim

13.00 – 14.00 **WG2 meeting – In vivo cancer immunotherapy models**
IJC Auditorium
Hosted by WG2 leaders Dr. Johannes Haybaeck and Dr. Emmet McCormack

13.00 – 14.00 **WG5 meeting – Communication, events and partnering with industry**
IJC Board of Trustees Room
Hosted by WG5 leaders Dr. Árpád Szöőr and Dr. Barbara Breznik

During the WG meeting time (10.50 – 14.30) the IJC rooftop will be open for IMMUNO-Model COST Action members and Invited Speakers as a networking area. Refreshments and snacks will be available.

June 1st 2023

IMMUNO-MODEL CONFERENCE – SCIENTIFIC SESSION 1

14.00 – 14.30 Registration for all IMMUNO-model Conference participants

14.30 – 14.40 **Welcoming to the participants**

Dr. Manel Esteller

IJC Director

Dr. Jordi Barretina

IGTP Director

Chair: Dr. Marleen Ansems

14.40 – 15.30 **Opening Keynote Lecture**

Dr. David Barbie

Dana Farber Cancer Institute, USA

Improving the precision of cancer immunotherapy using human tumor spheroid culture

15.30 – 16.30 **Selected abstracts**

Dr. Barbara Breznik
National Institute of Biology, Slovenia
Personalized glioblastoma models for immunotherapy research

Dr. Lukasz Skalniak
Jagiellonian University, Poland
Towards the modeling of PD-1/PD-L1 interaction in cellular models

Catarina A. Rodrigues
Portuguese Oncology Institute of Porto (IPO Porto), Portugal
Synergistic antitumoral effect of combined laser thermotherapy and immunotherapy: an exploratory in vivo study

Dr. Rebeca Sanz-Pamplona
Institute for Health Research Aragón (IISA), spain
Adoptive NK cell therapy as a therapeutic opportunity for colorectal cancer lung metastasis

Madeleine Benguigui
Technion – Israel Institute of Technology, Israel
A multi-model preclinical approach identifies interferon-stimulated neutrophils as a biomarker for immunotherapy response in human

16.30 – 16.45 Coffee Break at the IJC lobby

June 1st 2023

IMMUNO-MODEL CONFERENCE – SCIENTIFIC SESSION 1

Chair: Dr. Árpád Szöőr

16.45 – 17.15 **Dr. Silvia Scaglione**
Institute of Electronics, Computer and Telecommunications, italy
An organ-on-chip based approach for 3D cancer tissue culture towards a better understanding of human cancer progression and more predictive drug testing

17.15 – 17.45 **Dr. Emmet McCormack**
University of Bergen, Norway
Modeling and imaging immuno-oncology systems

17.45 – 18.45 Poster session and networking time at the IJC lobby

June 2nd 2023

IMMUNO-MODEL CONFERENCE – SCIENTIFIC SESSION 2

Chair: Dr. Hanne Haslene-Hox

9.00 – 9.30 **Dr. Elena Martínez Fraiz**
Institute for Bioengineering of Catalonia, Spain
Bioprinting as a tool to mimic tissues in vitro

9.30 – 9.40 **Erwan Corcuff**
Janvier Labs, France
3Rs and immunodeficiency models: the best option?

9.40 – 10.40 **Selected abstracts**

Dr. Syed Mian
The Francis Crick Institute, United Kingdom
Myelodysplastic Syndrome bone marrow cells are highly dependent on their niches but also can play an instructive role in modelling their microenvironment

Dr. Nuno Rodrigues dos Santos
Institute for Research and Innovation in Health I3S – University of Porto, Portugal
Antibody blockade of the P-selectin glycoprotein ligand-1 immune checkpoint enhances human T cell activation against lymphoma cells

Dr. Urska Kamensek
Institute of Oncology Ljubljana, Slovenia
Assessment of T cell responses in preclinical tumor models with undefined target antigens

Dr. Anguraj Sadanandam
Institute of Cancer Research, United Kingdom
Bioengineered interleukin-12 enhances immunity in a metastatic PDAC model

Dr. Ondrej Vanek
Charles University – Faculty of Science, Czech Republic
Engineered cytokine/antibody fusion proteins improve delivery of IL-2 to pro-inflammatory cells and promote antitumor activity

10.40 – 11.00 Coffee Break at the IJC lobby

June 2nd 2023

IMMUNO-MODEL CONFERENCE – SCIENTIFIC SESSION 2

Chair: Dr. Laura Belver

11.00 – 11.10 **Dr. Anna Bigas**
Hospital del Mar Medical Research Center – Josep Carreras Leukaemia Research Institute – Biomedical Research Network in Cancer (Ciberonc), Spain
Immune4all: an initiative to develop and validate immunotherapy biomarkers for solid tumors with high mortality

11.10 – 11.40 **Dr. Manel Juan**
Clinic Hospital of Barcelona, Spain
Why academic autologous advanced therapies can be more efficient and less toxic: the ARI CAR-T model

11.40 – 12.30 **Closing Keynote Lecture**
Dr. Silvia Formenti
Weill Cornell Medicine, USA
Challenges in translating the immunogenic potential of radiation therapy

12.30 – 12.40 **Closing remarks**
Dr. Eva Martínez Balibrea
IMMUNO-model COST Action Chair

A final networking time and farewell will take place at the IJC rooftop for all participants (12.40 – 14.00). Refreshments and snacks will be available.

Dr. David Barbie

Dr. David Barbie is Associate Professor at the Dana-Farber Cancer Institute (Harvard Medical School) and Lowe Center for Thoracic Oncology, and Associate Director of the Robert and Renée Belfer Center for Applied Cancer Science. Dr. Barbie graduated from Harvard Medical School. During his medical school years, Dr. Barbie worked as an HHMI Medical Fellow in Dr. Ed Harlow's laboratory at Massachusetts General Hospital. He furthered his training by completing his residency in internal medicine at Massachusetts General Hospital. Dr. Barbie was a medical oncology fellow in the Dana-Farber/Partners CancerCare program, followed by a postdoctoral fellow in Dr. William Hahn's lab at the Broad Institute. In 2010, he obtained a tenure-track independent investigator position at the Dana-Farber Cancer Institute and a clinical role within the Lowe Center for Thoracic Oncology.

Dr. Barbie is recognized as a key opinion leader in the field of antitumor immunity, contributing to a better understanding of the mechanisms of pathologic innate immune signaling and its relation to oncogenesis. Moreover, he has played a critical role in the development and utilization of advanced 3D ex vivo tumor culture technologies to study immune responses in cancer.

Dr. Silvia Formenti

Dr. Silvia Formenti is Chair of the Department of Radiation Oncology at Weill Cornell, Associate Director of the Meyer Cancer Center, and Radiation Oncologist in Chief at New York-Presbyterian Hospital. Dr. Formenti obtained her MD degree from the Faculty of Medicine and Surgery at the University of Milan, where she also did her training in medical oncology, radiology, and radiation oncology. She was awarded a competitive grant from the Italian National Research Council (CNR), which allowed her to join the laboratory of Dr. Malcolm Mitchell at the University of Southern California (USC). Later on, she also obtained an Audrey Meyer Mars Career Development Award from the American Cancer Society to continue her research, and completed her residency and attained board certification in radiation oncology at USC. Dr. Formenti was appointed as Chair of the Department of Radiation Oncology New York University (NYU) Medical Center in 1999, where she had a prolific research activity, publishing numerous scholarly papers in high-impact journals. In 2015, she was named Associate Director of Radiation Oncology at the Meyer Cancer Center and Chair of the newly established Department of Radiation Oncology at Weill Cornell Medical College.

Among her extensive contributions to the field of oncology, Dr. Formenti is considered a pioneer of immunogenic radiotherapy, making groundbreaking contributions that demonstrated that radiation therapy can stimulate the immune system's response against cancer, leading to enhanced tumor control and improved patient outcomes.

Dr. Silvia Scaglione

Dr. Silvia Scaglione is Head of the Laboratory of Tissue Engineering at the Institute of Electronic, Computer and Telecommunications of the National Research Council of Italy (CNR-IEIIT), Professor of Bioengineering at the University of Genoa, and Cofounder and Chief Scientist of React4life. She obtained her PhD in Bioengineering at the University of Genoa in 2005 and was a visiting scientist at the University Hospital of at the laboratory of Dr. Iván Martín. She was postdoctoral associate at Advanced Biotechnology Center in Genoa, before getting an appointment as Head of the Laboratory of Tissue Engineering at the CNR-IEIIT in 2012. In 2016, Dr. Scaglione cofounded React4life, an innovative biotech startup that provides next generation in vitro 3D fluid-dynamic assays as experimental tools for preclinical studies and drug screens.

Dr. Scaglione's research implements biotechnological, engineering and material science approaches with the aim of developing new innovative technologies that can be applied in biomedical research. She is owner of 7 patents and coordinator of a Future Emerging Technology (FET-OPEN) European H2020 project, aiming to develop a 3D device that recapitulates physiological tissue complexity of breast cancer metastasis to the bone. Dr. Scagliones has also been recently nominated as Ambassador of the European Innovation Council (EIC) for her capacity to bring basic research results to the market, generating impact for society.

Dr. Elena Martínez Fraiz

Dr. Elena Martínez Fraiz is Head of the Biomimetic Systems for Cell Engineering Laboratory at the Institute for Bioengineering of Catalonia (IBEC) and Associate Professor at the Faculty of Physics of the University of Barcelona. Dr. Martínez Fraiz obtained her PhD in Physical Sciences from the University of Barcelona in 2001. After graduation, she joined the Swiss Federal Institute of Technology Lausanne (EPFL) as a postdoctoral researcher. In 2003, Dr. Martínez Fraiz became a Ramón y Cajal Investigator at the Barcelona Science Park (PCB), where she was also Head of the Nanotechnology Platform. In 2008, Dr. Martínez Fraiz joined the Nanobioengineering group, led by Dr. Samitier at IBEC, as a senior researcher at IBEC. During this period, she also completed a postdoctoral stay at Imperial College London. In 2013, she was appointed as the Group Leader of the Biomimetic Systems for Cell Engineering Laboratory at IBEC, formally establishing her as an independent researcher.

Dr. Martínez Fraiz work focuses on the development of biomimetic cellular engineering systems for the creation of new in vitro tissue models. To carry out her research, her group employs cutting-edge biofabrication technologies and novel biological tools, such as organoids. Dr. Martínez Fraiz is currently the principal investigator of an ECR-Consolidator project and coordinates a FET-OPEN consortium.

Dr. Emmet McCormack

Dr. Emmet McCormack is Head of Pharmaceutics and leader of the Translational Molecular Imaging in Cancer Group at the Center for Cancer Biomarkers (CCBIO) at the University of Bergen, Adjunct Professor at the University of Tromsø, and Chairman at KinN Therapeutics. Dr. McCormack graduated with honors in Applied Chemistry and Applied Mathematics at the Dublin Institute of Technology and Trinity College Dublin. He did his PhD in Pharmacognosy at Trinity College Dublin, working on the synthesis of anti-cancer drugs targeting acute myeloid leukemia. Upon graduation, he relocated to Norway and joined the laboratory of Dr. Bjørn Tore Gjertsen at the University of Bergen, where he was involved in the development of preclinical models and molecular imaging strategies for drug development in hematological malignancies. Subsequently, Dr. McCormack was awarded a Trond Mohn Foundation starter grant in 2009, which allowed him to establish his own laboratory at the University of Bergen.

Dr. McCormack research is currently dedicated to the advancement of novel preclinical models and imaging strategies for investigating cancer biology and evaluating the efficacy of new drugs. Using approaches in the interface of clinical and basic research, his group works on the generation of novel immunotherapies and in the development of state-of-the-art preclinical models that have a direct impact on cancer research and therapeutics.

Dr. Manel Juan

Dr. Manel Juan is the Head of the Immunology Department at the Clinic Hospital of Barcelona, Chief of the Immunotherapy Platform at the Sant Joan de Déu (SJD) Children's Hospital, Associate Professor at University of Barcelona (UB) Medical School, and leads the Immunogenetics of the Autoinflammatory Response Laboratory at the August Pi i Sunyer Biomedical Research Institute (IDIBAPS). Dr. Juan obtained his MD degree from the UB Medical School and completed his training in Immunology at the Clinic Hospital of Barcelona. During his professional career, he has acquired an extensive international experience at numerous American and European institutions, including the National Jewish Center (Denver, CO, USA), the Benaroya Research Institute (Seattle, WA, USA), the University of Pennsylvania (Philadelphia, PA, USA), and the Herlev Hospital (Herlev, Denmark). In 2016, he was named Director of the Immunotherapy Section at the Clinic Hospital of Barcelona, which was followed by an appointment as Chief of the Immunotherapy Platform at the SJD Children's Hospital in 2017 and as Head of the Immunology Department at the Clinic Hospital of Barcelona in 2020.

Dr. Juan led the team that designed the first academic CAR-T entirely developed in Europe to be approved by a regulatory agency, using their own monoclonal CD19 antibody to modify T-lymphocytes (ARI-0001). Following up on this achievement, Dr. Juan's research continues focused on the development of new clinical immunotherapies based on dendritic cells and CAR-T.

Dr. Barbara Breznik

National Institute of Biology, Slovenia

Personalized glioblastoma models for immunotherapy research

Introduction. Glioblastoma (GBM) remains one of the most lethal malignancies. Recent advances in immunotherapy that prolong survival in a wide range of cancer types have not shown much benefit in GBM patients. Several novel therapies are currently under investigation, of which immunotherapy with natural killer (NK) cells is a promising option. The aim of our study was to establish relevant tumor models that mimic patient's tumor microenvironment to explore GBM resistance to therapy and new treatment approaches, such as NK cell-based therapy.

Methodology. We set up and characterized different 3D GBM models with immune compartment. One approach is co-cultures of tumor cell spheroids with PBMCs/NK cells using established and patient-derived GBM (stem) cells. Tumor cell spheroids were generated using the ClinoStar system. The second approach is patient-derived organoids established from fresh tumor biopsies and cultured for several weeks. Microscopy, immunofluorescence, flow cytometry, qPCR and spatial transcriptomics in combination with markers of GBM cells, immune/stromal cells, proliferation, hypoxia and NK receptor ligands were used to characterize the models.

Results. We optimized a method to produce uniformly sized spheroids from patient-derived GBM cells that can be cultured for several weeks. Several ligands for inhibitory and activating receptors on the surface of NK cells are expressed in GBM spheroids. Decreased cytotoxic activity of NK cells against GBM cells in spheroids was observed when compared with their cytotoxicity against GBM cells grown in flask culture. This was associated with altered ratios between NK cell activating and NK cell inhibitory ligands. Patient-derived organoids preserved the tumor microenvironment of the original tumor and retained the immune cell compartment for 3-4 weeks of culture, including the presence of macrophages, microglia and T cells. We detected the expression of immune checkpoints and secretion of several cytokines and growth factors. The model is now used to determine the effect of standard therapy in combination with immunotherapeutic agents.

Discussion. We have established several 3D GB models that can be used to evaluate the effects of standard and NK cell-based therapies in the context of the tumor microenvironment. Our results highlight the importance of 3D in vitro tumor models for more precise immunotherapy research.
principal investigator of an ECR-Consolidator project and coordinates a FET-OPEN consortium.

Dr. Lukasz Skalniak

Jagiellonian University, Poland

Towards the modeling of PD-1/PD-L1 interaction in cellular models

Introduction. The blockade of the PD-1/PD-L1 immune checkpoint has been proven as a successful strategy for the treatment of multiple cancer types. Currently, the approach relies exclusively on the use of therapeutic monoclonal antibodies, yet extensive studies are conducted to introduce also alternative, small molecule-based medicaments. Progress in the field requires simple, reliable, and reproducible cell-based models which would allow for estimating and comparing the potency of tested molecules before moving to in vivo studies. However, the models used in experimental setups, especially those published in chemical journals, often are far from being convincing, thus generating unreliable data. One of the goals of our work is to introduce new cell-based modes for in vitro testing of experimental immune checkpoint blockade approaches and increase the awareness of the importance of choosing proper models for proper verification of the activity of the synthesized molecules.

Methodology. In our research, we have established new cell lines intended to be used in co-culture models of PD-L1-expressing cancer cells and PD-1-expressing T cell surrogates. The models allow testing the effect of the tested compounds towards both, the PD-L1 of the human and the mouse origin, which facilitates the choice between syngeneic and PD-L1-humanized mouse models. Also, the cell-based models allow for the quantification and comparison of the activity and the toxicity of the tested molecules. The models are composed of the effector cells (Jurkat T cells expressing PD-1 and Luciferase under the control of NFAT-RE promoter), co-cultured with human (RKO) or mouse (B16-F10) cells expressing either the human or mouse PD-L1 and the artificial TCR-Activator molecule. The readout is done based on the luminescence following the addition of the Luciferase substrate.

Results. Using our models, we have tested the activity of several small molecules, macrocyclic peptides, and therapeutic antibodies (atezolizumab, durvalumab, and avelumab) targeting the PD-L1 protein. We found out that while the molecules and peptides effectively blocked the human PD-L1 protein, mouse PD-L1 protein was not blocked at the same concentrations. Surprisingly, we also found out that some therapeutic antibodies (atezolizumab and avelumab) block both variants of the PD-L1 protein, while the other (durvalumab) presents selectivity toward the human version of the protein. Also, using the new model, we have defined the activities of our homemade inhibitors of the PD-L1 protein.

Discussion. In the literature, syngeneic mouse models are often preferred due to lower costs and better model stability. At the same time, often the fact that syngeneic mouse models rely on mouse molecular targets is neglected. Our work indicates that the choice of syngeneic models needs always be reasoned by providing exact characteristics of the activity of the tested molecule toward the mouse molecular target. If the molecule turns out to be inactive toward the mouse target, more complex humanized mouse models should be considered. Convincing the scientists working in the field of medicinal chemistry to choose adequate, reliable, clear, and reproducible models will improve the quality of the published data and allow for the comparison of the potencies of the reported molecules between the articles.

Catarina A. Rodrigues

Portuguese Oncology Institute of Porto (IPO Porto), Portugal

Synergistic antitumoral effect of combined laser thermotherapy and immunotherapy: an exploratory in vivo study

Introduction. The advent of immune checkpoint inhibitors (ICIs) has marked a revolutionary milestone in the field of Oncology. However, their widespread use is impeded by the fact that some patients may develop resistance. Immune-stimulating Interstitial Laser Thermotherapy (imILT) is a promising approach that induces tumor necrosis by exposing cells in the tumor's periphery to heat. Additionally, ImILT facilitates immunological cell death, generating a favorable inflammatory tumor microenvironment. Clinical studies of imILT have shown promising results. Given the promising outcomes of both imILT and ICIs when used independently, combining treatments could address the shortcomings of each and lead to more successful outcomes. This study aims to determine the optimal concentration of anti-PD1 to be combined with imILT and to investigate the benefits of this combination, specifically studying the immunomodulation in the tumor microenvironment in a poorly immunogenic melanoma mice model.

Methodology. Mice were subcutaneously inoculated bilaterally with 5x104 B16F10 cells and were randomly assigned into 5 groups: control, anti-PD1 (5mg/kg and 10mg/kg), imILT and a combination of imILT and 10mg/kg anti-PD1 treatment. The anti-PD1 treatment was administered intraperitoneally in 3 doses, 3 days apart. The imILT treatment was performed for 30 minutes at a steady-state temperature of 46ºC at the tumor margin. Animals' weight and tumors were measured twice a week, until the volume reached 2000mm3, at which point the animals were humanely euthanized. Tumor specimens were collected and processed according to in-house protocols. Flow cytometry (FC) analysis was performed using an 8-color panel of fluorochrome-conjugated monoclonal antibodies to examine various lymphoid and myeloid populations. Cell suspensions were acquired using a FACS Canto II cytometer, and data analysis was carried out using InfinicytTM. Graphical representations and statistical analyses were obtained using GraphPad Prism.

Results. The administration of anti-PD1 10mg/kg, both alone and combined with imILT, improved the median survival compared to the 5mg/kg and the control groups. Our FC results showed trends in changes in the tumor immune cell populations, but not always reaching statistical significance. The anti-PD1 groups presented significant differences in the CD4+ and CD8+ T lymphocytes, leading to a decrease in the CD4+/CD8+ ratio compared to the control. There was a significant increase in the CD8+ effector/memory cells in the anti-PD1 10mg/kg group compared to the control. These results led to the selection of the best anti-PD1 concentration for combination with imILT. The combination treatment with anti-PD1 10mg/kg and imILT showed a similar trend of differences in the immune populations, including an increase in NK cells, CD8+ T cells (particularly non-naïve phenotypes), and DCs with "conventional" phenotype. There was also a decrease in Tregs, compared to anti-PD1 alone and to the control group.

Discussion. ICIs are a promising treatment for cancer patients, but therapy resistance is a major challenge. Effective combination therapies are urgently needed to augment ICIs' potential. Our study explored the potential of combining ICIs with imILT in a poorly immunogenic melanoma mice model. We found that increasing the dose of anti-PD1 led to positive immunomodulation within the tumor environment. Combining imILT with anti-PD1 induced a more effective response than anti-PD1 alone. These results suggest that this combination may overcome the ICIs' limitations and improve clinical outcomes. Although our study has some limitations, our findings provide a promising direction for future studies to explore this combination in immunogenic cell models and other tumor types. Additionally, investigating the combination of imILT with other ICIs may further improve treatment efficacy. Overall, our study highlights the potential of combining imILT with ICIs as a novel and promising approach to cancer therapy.

Dr. Rebeca Sanz-Pamplona

Institute for Health Research Aragón (IISA), Spain

Adoptive NK cell therapy as a therapeutic opportunity for colorectal cancer lung metastasis

Introduction. Colorectal cancer (CRC) metastases are most found in the liver, although lung is the second one. However, therapeutic strategies and underlying biology must be elucidated. We have identified a cluster of metastases from different tumor types that exhibit an inflammatory phenotype. Interestingly, most of these high immune metastases are lung metastases. On the contrary, liver metastases were mainly classified as low immune (Garcia-Mulero et al, JITC, 2020). More recently, we have demonstrated that NK cells control CRC development (Lanuza et al. Front Immun, 2022). Thus, we hypothesize that CRC lung metastases are more likely to benefit from immunotherapy. We aim to compare the immune microenvironment of lung and liver metastasis from CRC and to evaluate the efficacy of adoptive NK cell transfer in an orthoxenograft model.

Methodology. Gene expression data of lung metastases (n=35) and liver metastases (n=153) from CRC were downloaded from open repository GEO. Expression data was used to make a functional analysis and to perform an immune characterization of the samples. Also, mutational data from 624 liver metastases and 146 lung metastases were downloaded from cBioPortal. Fresh surgical specimens of lung metastasis from CRC patients were obtained after surgical resection and orthotopically implanted. Engrafted tumors at early mouse passages are cut and stored in liquid nitrogen for subsequent implantation. As a proof-of-concept (PoC), one of these models was used to test NK treatment. Three groups of mice treated with vehicle (n=3), 5-fluorouracil + irinotecan (n=3), and vehicle + NK92 (n=3) were included in the experiment. Two doses of NK92 were administered by the tail vein (10-12M cells). Mice were sacrificed and tumor size and weight were compared among groups.

Results. First, a molecular characterization comparing lung and liver metastases was done. Lung metastases showed higher immunogenic scores than liver metastases: immunophenoscore (p=2e-10); Antigen presentation score (p=1.7e-10); Cytotoxic T cells, p=1.9e-7; Regulatory T cells, p=9.2e-6; B cells, p=0.001; Dendritic cells, p=1.5e-5; NK cells, p=5.3e-8. Gene set enrichment analysis showed IL6-JAK/STAT signaling, Epithelial Mesenchymal Transition, Interferon Alpha, and Signaling by Interleukins gene sets enriched in lung metastases. At genomic level, no differences were found in total number of mutations but KRAS and FBXW7 mutations were more frequent in lung metastases. Second, the PoC study demonstrated the NK cells treatment feasibility in ortoxenografts models and the efficacy in tumor control. All treated animals showed a significant reduction in tumor weight and tumor size in comparison with animals treated with standard chemotherapy (mean of 0.4 vs. 1.5 and 55.7 vs. 12.12.6, respectively).

Discussion. CRC lung metastases have a favorable immunophenotype that makes them a promising target to NK cell treatment.

Madeleine Benguigui

Technion, Israel

A multi-model preclinical approach identifies interferon-stimulated neutrophils as a biomarker for immunotherapy response in human

Introduction. Despite the success of immune checkpoint inhibitors (ICIs), only ~20-30% of patients display a beneficial response - requiring the discovery of new biomarkers to maximize clinical outcome. While mice are mostly used and cost-effective models to study human disease, translating preclinical biomarkers into clinical practice faces significant obstacles – in part due to the lack of diverse or appropriate models. In this study, we aimed to identify a cellular biomarker for predicting response to anti-PD1 therapy, using various mouse models, and testing its applicability in humans.

Methodology. We generated mutated clones from resistant tumors to ICI, therefore making them sensitive to anti-PD1 therapy. Subsequently, we used single cell RNA sequencing and mass cytometry to search for subset of immune cells with differential frequency between responsive and non-responsive mice. After cell subset identification, a preclinical validation was performed on additional mouse strains and tumor models. Lastly, we used datasets of non-small cell lung cancer patients in order to search for an equivalent immune cell type found in mice, in human samples. This potential biomarker was then tested on cohorts of patients with non-small cell lung cancer and melanoma patients as well as validated in additional datasets of different human cancers.

Results. We identified interferon-stimulated, Ly6Ehi neutrophils as a pre-treatment, blood-borne biomarker for anti-PD1 response in mice. Specifically, high frequency of Ly6Ehi neutrophils, at baseline, correlates with improved outcome. These results were then validated in additional mouse strains and tumor models (renal cell and lung carcinomas). Using equivalent functional cell type with IFN signature genes, we then validated Ly6Ehi neutrophils in cohorts of immunotherapy-treated non-small cell lung cancer (NSCLC) and melanoma patients, where the abundance of Ly6Ehi neutrophils predicts anti-PD1 response. Our main conclusions were further supported by the analysis of publicly available, bulk RNA-seq and scRNA-seq datasets taken from ~1500 cancer patients who underwent ICI therapy. Functionally, these cells sensitize otherwise resistant tumors to anti-PD1 therapy in part by directly activating cytotoxic T cell activity and increasing tumor cell killing mediated by the secretion of IL12.

Discussion. Our study highlights the potential of Ly6Ehi neutrophils as a predictive and immunomodulatory blood borne biomarker for anti-PD1 therapy. Further studies should be conducted to confirm these findings and assess their utilization in an independent cohort of patients. Our findings also highlight the importance of IFN stimulation in supporting anti-tumor immunity, and the potential of Ly6Ehi neutrophils as a therapeutic target.

Dr. Syed Mian

The Francis Crick Institute, United Kingdom

Myelodysplastic Syndrome bone marrow cells are highly dependent on their niches but also can play an instructive role in modelling their microenvironment

Introduction. Myelodysplastic syndrome (MDS) are clonal stem cell diseases characterized mainly by ineffective hematopoiesis. Attempts to generate patient-derived xenograft mouse models for MDS have yielded little or no success. This prompted us to develop an in vivo system that uses gelatin-based scaffolds to generate niches in immunodeficient mice which mimics the human microenvironment.

Methodology. Bone marrow (BM) cells from 48 patients (43 MDS cases, 2 CMML, 2 MDS/MPD and 1 sAML) were studied. Mesenchymal stromal cells (MSCs) were isolated from the BM of the patient or healthy donor samples. Three immunodeficient mouse models (i.e. NSG, NSG-SGM3 and NBSGW) were used. We also used our 2D in vitro co-culture system to culture MDS CD34+ cells (or Healthy donor, HD) with the MDS MSCs or HD MSCs, followed by RNA-Sequencing.

Results. Persistent long-term engraftment of hCD45+ cells ranging from 0.05% to 86% was observed in humanized niches, with 74% of cases having >20% hCD45+ cells. The xenotransplanted cells maintained their genotypic characteristics, as demonstrated by the presence of the gene mutations. Interestingly, hCD45+ cells were not detected in the mouse BM. However, the MDS-ICs do migrate but 'home' only into a supplementary humanized niche. Next, using RNA sequencing of the MDS-MSCs, HD-MSCs, after being co-cultured for 5-7 days with CD34+ cells from MDS, we were able to demonstrate an active cross-talk between the cell types. Some of the affected pathways included osteoclast differentiation, transcriptional mis-regulation in cancer and cell-cell receptor interaction. Interestingly, we revealed that MDS hematopoietic cells were able to switch the healthy transcriptomic profile of the HD-MSCs to an MDS phenotype and that led to the identification of the MDS interactome network between the cell types.

Discussion. Our model provides first evidence that MDS initiating cells are highly dependent on humanized MSC niche(s) that is not provided by mouse BM. We also demonstrate that MDS initiating cells actively alter/modulate the phenotypic changes in the BM-MSC. Altogether, our analysis of MDS BM provides novel insight into the intrinsic altered pathways of MDS MSCs, and has enabled the identification of dysregulated putative novel ligand-receptor pairs. It also points towards a potential complex network of interactions, in which both positive and negative signals, mainly driven by HSPCs, are balanced to maintain the function of the BM niche MSCs, which in-turn maintains the MDS HSPCs.

Dr. Nuno Rodrigues dos Santos

Institute for Research and Innovation in Health i3S - University of Porto, Portugal

Antibody blockade of the P-selectin glycoprotein ligand-1 immune checkpoint enhances human T cell activation against lymphoma cells

Introduction. Lymphomas represent a diverse collection of malignancies, most of which were shown to be refractory to immune checkpoint therapies (e.g. anti-PD-1). Although P-selectin glycoprotein ligand-1 (PSGL-1) was found to be an immune checkpoint protein promoting mouse T cell exhaustion in the context of cancer and infection, an immune regulatory function in human T cells has so far remained to be demonstrated. In this study, we aimed to evaluate PSGL-1 expression dynamics on resting vs activated human T cells and test the potential of PSGL-1 antibody targeting to stimulate T cell responses against lymphoma.

Methodology. Human healthy donor T cells were cultured with irradiated Raji B cell lymphoma line. T cell immunological synapses were analyzed by CD3 and PSGL-1 immunofluorescence staining. T cell activation was assessed by flow cytometry detection of CD25, CD69, IL-2 and IFNγ. In vitro exhausted T cells were generated by repeated CD3/CD28 stimulation and flow cytometry detection of PD-1, TIM-3 and LAG-3. Patient lymphoma cells were obtained from excised lymph nodes. The PSGL-1 PL-1 mAb was used in cocultures.

Results. We found that PSGL-1 expression decreased in healthy donor CD4+ and CD8+ T cells upon CD3/CD28 stimulation. While resting T cells showed PSGL-1 distributed across the cell membrane, PSGL-1 polarized to the opposite pole of the immunological synapse established between T and Raji antigen-presenting cell. When Raji-primed T cells were cocultured again with Raji cells, the percentage of CD69+ and CD25+ activated cells increased but to a larger extent with PL-1 treatment. Furthermore, pre-activated T cells upregulated CD69 and CD25 and increased IL-2 production upon coculture with Raji cells, but in a more sustained manner upon PSGL-1 antibody blockade. The PL1 mAb increased the percentage of CD4+CD69+ T cells and IFN-γ and IL-2 levels after coculture of in vitro exhausted-like T cells with Raji cells. Finally, we cultured unsorted patient mantle cell lymphoma cell suspensions, and found that the PSGL-1 antibody enhanced CD69 expression in T cells.

Discussion. Our results indicate not only that PSGL-1 expression decreases upon activation, but that it also relocalizes upon T cell encounter with a foreign antigen. With our in vitro approach, we demonstrate for the first time that PSGL-1 antibody blockade enhances human T cell activation against lymphoma cells. Therefore, these findings support the notion that PSGL-1 can be a target for future immunotherapeutic options.

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Assessment of T cell responses in preclinical tumor models with undefined target antigens

Introduction. Assessment of functional tumor-specific T cell responses in preclinical tumor models is an important tool for successful translation of new immunotherapies to clinics. Several methods have been introduced to identify tumor-specific T cells. These include antigen specific tetramer staining assays and interferon γ (IFNγ) immunospot assays that provide information on the frequency and functionality of antigen specific T cells, but not their cytotoxic potential. Immunospot assays that simultaneously detect both IFNγ and granzyme B (GrB) are more relevant, as GrB is the key mediator of target cell death by cytotoxic T and natural killer (NK) cells. All of the mentioned tests require known tumor antigen targets which are often unknown in preclinical tumor models. Therefore, assays based on recognition of native tumor cells may be more clinically relevant. In this presentation, we will describe our method to assess tumor-specific responses after immunotherapies without a known antigen.

Methodology. Lymph node immune cells are isolated from treated mice and stimulated by coculturing with relevant tumor cells ex vivo. Immunospot analysis of GrB and IFNγ positive T cells is then performed according to the instructions provided with the used kit. In addition to lymph node immune cells, the method can also be employed for other sources of immune cells, such as splenocytes and peripheral blood mononuclear cells (PBMCs). Additionally, isolated immune cells can be labelled with fluorescent cell tracers ex vivo and used for adoptive transfer into recipient animals, where they can be tracked by intravital imaging. They can also be used in various T cell killing assays to directly prove their cytotoxic activity.

Results. The number of dual spot-forming cells corresponds directly to tumor-reactive lymphocytes. In our previous studies, we successfully used this method to determine the tumor-specific lymphocytes after in situ vaccination with a gene therapy approach (Kamensek et al. Antitumor in situ vaccination effect of TNFα and IL-12 plasmid DNA electrotransfer in a murine melanoma model. Cancer Immunol Immunother. 2018) and whole tumor cell vaccination (Remic et al. Tumor cell-based vaccine contributes to local tumor irradiation by eliciting a tumor model-dependent systemic immune response. Front. Immunol. 2022).

Discussion. The desired outcome of many new immunotherapies tested in preclinical cancer models is to induce a potent T cell response, which specifically recognizes and eliminates tumor cells. Conventional methods to assess specific T cell responses, like tetramer and immunospot techniques, are antigen-specific, meaning they require defined antigen targets, which are often unknown in preclinical tumor models. Although surrogate transgenes like ovalbumin (OVA) can be stably introduced to tumor cell lines, this can alter the immunogenicity of the tumor model. As tumor cells used to generate preclinical tumor models already contain relevant tumor antigens, we reckon they can serve as the antigen source for detecting tumor-specific T cell responses without the need to predefine a target tumor antigen. The described method is well-suited to study tumor-specific T cell responses, particularly after antigen-agnostic immunotherapies, where antigens are not pre-defined or known.

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Bioengineered interleukin-12 enhances immunity in a metastatic PDAC model

Introduction. The desmoplastic collagen-rich tumor microenvironment is a hallmark of pancreatic ductal adenocarcinoma (PDAC), which sequesters cytotoxic lymphocytes and antigen-presenting cells from targeting malignant neoplastic cells. To drive the infiltration of immune cells against cancer cells, one of the powerful strategies is to use cytokine therapies, like interleukin-12 (IL-12). The conjugation of the collagen-binding domain (CBD) augments the ability of IL-12 to induce inflammation by using resident collagen as a scaffold while minimizing cytokine toxicity. Here, we hypothesize that the immune composition and collagen content of the Tumor in mice determines their responsiveness to CBD-IL12 therapy or its combination immunotherapy.

Methodology. Three (7947-immune cold/high collagen, 7784- immune cold/low collagen and 2334- hot/high collagen) of 12 syngeneic orthotopic models of PDAC were classified into immune hot or cold with collagen high/low content using different statistical inferences. Mice were treated with vehicle, CBD-IL-12, or CBD-IL-12 and anti-PD1. Tumor growth was assessed by in vivo bioluminescent imaging (IVIS) in interventional and survival studies. Transcriptome profiling was performed on harvested Tumors.

Results. As predicted by in silico analyses, the combination of CBD-IL-12 and anti-PD1 therapy significantly improved the survival of previously immune-resistant- immune cold, high collagen in vivo model 7947 ($p = 0.00022$) while reducing Tumor ($p = 0.0096$) and metastatic burden in the liver. A multi-parametric flow cytometric analysis of the immune repertoire showed increased Cd3 T cells while reducing immunosuppressive T regulatory cells ($p = 0.03$). Follow-up RNA sequencing analysis revealed an increase in antigen presentation. However, these results were not observed in the other two PDAC models. In the same 7947 model, CBD-IL-12 and anti-PD1 combination immunotherapy in T cell receptor delta chain knock-out mice and nude mice suggest the effect is partially mediated by gamma-delta T and Natural Killer cells, respectively.

Discussion. CBD-IL12/anti-PD-1 targeted immunotherapy was capable of remodeling the tumor immune microenvironment of a subset of PDAC models, highlighting the need for patient selection prior to immunotherapy. Further studies are required to understand the mechanism of action behind the observed results.

Dr. Ondrej Vanek

Charles University - Faculty of Science, Czech Republic

Engineered cytokine/antibody fusion proteins improve delivery of IL-2 to pro-inflammatory cells and promote antitumor activity

Introduction. Progress in cytokine engineering is driving therapeutic translation by overcoming these proteins' inherent limitations as drugs. The interleukin-2 (IL-2) cytokine harbors great promise as an immune stimulant for cancer treatment. However, the cytokine's concurrent activation of both pro-inflammatory immune effector cells and anti-inflammatory regulatory T cells, toxicity at high doses, and short serum half-life limit clinical application. One promising approach to improve the selectivity, safety, and longevity of IL-2 is complexation with anti-IL-2 antibodies that bias the cytokine towards activation of immune effector cells. Although this strategy shows therapeutic potential in preclinical cancer models, clinical translation of a cytokine/antibody complex is complicated by challenges in formulating a multi-protein drug and concerns about complex stability.

Methodology. Tethering IL-2 to the antibody stabilizes the cytokine/antibody complex, reducing off-target activation, and we show that this tethering alone resulted in a dramatic improvement in biased stimulation of Effs. Moreover, fusion of IL-2 to the antibody improves the pharmacokinetics of the therapy by preventing cytokine clearance through covalent linkage to the antibody. Furthermore, as a single agent therapeutic, our IC eliminates questions of IL-2/antibody complex formulation and stoichiometric optimization, mitigating regulatory obstacles to clinical translation. We applied molecular evolution approaches to isolate a variant of the antibody that enhanced the biased expansion of effector over regulatory T cells, and we showed that our evolved variant further accentuated immune effector cell bias both in vitro and in vivo, leading to robust inhibition of tumor growth in mouse models of cancer.

Results. We introduced a versatile approach to designing intramolecularly assembled single-agent fusion proteins (immunocytokines, ICs) comprising IL-2 and a biasing anti-IL-2 antibody that directs the cytokine's activities towards immune effector cells. We establish the optimal IC construction and further engineer the cytokine/antibody affinity to improve immune biasing function. We demonstrate that our IC preferentially activates and expands immune effector cells, leading to superior antitumor activity compared to natural IL-2 without inducing toxicity. This work presents a roadmap for the design and translation of cytokine/antibody fusion proteins. Altogether, this work constitutes a major step forward in advancing IL-2-biasing antibodies as viable candidates for clinical translation.

Discussion. Reports involving other IL-2 muteins, cytokine/antibody complexes, and fusion proteins that act by blocking the IL-2/IL-2R-alpha interaction and enhancing IL-2 interaction with IL-2R-beta and gamma-C demonstrate that combining our designed ICs with additional cancer therapeutics, including immune checkpoint inhibitor antibodies, cancer vaccines, or adoptive cell transfer will only further enhance tumor suppression. Given the numerous active clinical programs involving engineered IL-2 proteins, the stability, selectivity, safety, and antitumor efficacy of our ICs make it appealing for clinical translation. Moreover, the modularity of its single-molecule construction readily invites adaptation to additional formats that will enable greater homing to and retention in the tumor microenvironment.

Poster presenters

Dr. Margareta Correia
Portuguese Oncology Institute of Porto (IPO Porto), Portugal
Modulating PCa cell immunogenicity with EZH2i to increase NK cell-mediated killing

Eduardo Garvín Jiménez
Spanish National Cancer Center (CNIO), Spain
Alveolar macrophages modulate cancer-associated fibroblasts in a preclinical model of NSCLC

Lucie Janstová
Institute of Hematology and Blood Transfusion (ÚHKT), Czech Republic
Modeling complex leukemia microenvironment in vitro for advanced pre-clinical cytotoxicity testing of novel NK cell-based immunotherapy

Dr. José Rodrigo Magaña
Chemical Institute of Sarrià (IQS) - Ramon Llull University (URL), Spain
pBAE polymers: a new delivery platform of biologicals for tumor immunotherapy and targeted therapies application

Dr. Marta Maleszewska
Warsaw University, Poland
HDAC inhibitors as potential agents modulating immune response of microglia in glioma

Dr. Giulia Miglietta
University of Bologna, Italy
New insights in the G-Quadruplex-mediated genome instability to promote an innate immune response in cancer cells

Sara Orehek
National Institute of Chemistry, Slovenia
Antitumor immunity boosted by Gasdermin D induced necrosis

Rubén Prieto-Díaz
University of Santiago de Compostela, Spain
Validation of A2BAR antagonism as an emergent approach for cancer (immuno)therapy: immune, antiproliferative, and antimetastatic Effects

Dr. Daniela Sousa
Institute for Research and Innovation in Health i3S, Portugal
Exploring an in vivo stress model on breast cancer bone metastasis

Poster presenters

Veronika Švubová
Institute of Hematology and Blood Transfusion (ÚHKT), Czech Republic
Immunomodulatory environment of bone marrow niche of myeloid leukemia

Dr. Can Türk
Lokman Hekim University, Turkey
Staphylococcal enterotoxin B dependent immune gene dysregulation and development of resistance to idarubicin in acute myeloid leukemia

Dr. Seyhan Türk
Hacettepe University, Turkey
NK cell dysfunction of acute myeloid leukemia in relation to the RAS

Dr. Mar Valés Gómez
Spanish National Centre for Biotechnology (CNB-CSIC), Spain
Human models to understand innate immunity against cancer

Dr. Eleni Zografos
National and Kapodistrian University of Athens, Greece
Immunotherapy for the neoadjuvant treatment of a clinic-based series of patients with triple-negative breast cancer

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