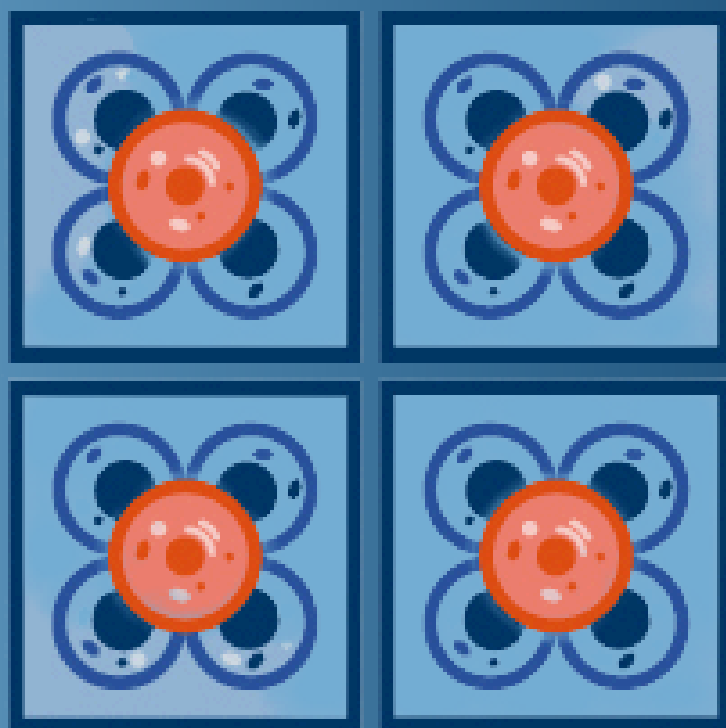




ABSTRACT BOOK

CELL -BASED THERAPIES IN ONCOLOGY

2nd VHIO-BBVA Foundation International Symposium

13-14 November, 2025 | Barcelona, Spain



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We are delighted to welcome you to the 2nd edition of the VHIO–BBVA Foundation International Symposium on Cell-Based Therapies in Oncology, taking place at the Sant Pau Art Nouveau Site in Barcelona.

This meeting will once again convene leading voices in the field to share and discuss the latest breakthroughs in adoptive cell therapies, with a special focus on their applications in solid tumors, pediatric cancers, early clinical development, and the evolving European regulatory landscape.

Over two days, you will have the opportunity to learn from and engage with 22 distinguished international experts, including keynote speakers. The program features plenary lectures, poster sessions, and interactive roundtable discussion, all designed to foster meaningful collaboration and dialogue across disciplines.

We warmly encourage you to take an active role in this unique forum for scientific exchange and networking. Thank you for joining us in Barcelona for what promises to be an inspiring and impactful gathering.

Welcome!

The Organizing Committee



At VHIO, we are deeply committed to sustainability and to reducing our environmental footprint. Through the Green VHIO Initiative, we encourage all participants to join us in taking small but meaningful steps toward a greener future:

🌸 Return your nametag at the end of the conference so we can reuse it in future events and reduce waste.

🌸 Plant your note pad! The small note pads provided are made with seed paper- simply plant the cover in soil, water it, and watch beautiful flowers grow.

🌸 Reuse your VHIO cup. Each attendee will receive a reusable cup — bring it along to your favorite café or use it throughout the event to help us minimize single-use plastics.

🌸 Go paperless! Our program and abstract book are fully digital , helping us avoid unnecessary printing.

Together, let's make this event a little greener and help our planet bloom.



The Vall d'Hebron Institute of Oncology (VHIO), a reference center for personalized medicine in oncology, was created in 2006. Thanks to its pioneering multidisciplinary and translational research model and its participation in consortia and projects with other prestigious centers worldwide, it has positioned itself as one of Europe's most important comprehensive centers. VHIO is capable of transforming, in record time, the latest research discoveries in the laboratory in early clinical trials and, therefore, in new opportunities for cancer patients, including cell therapies.



The BBVA Foundation promotes knowledge through research, artistic endeavours, and cultural creations. In collaboration with VHIO, the Foundation established the pioneering Comprehensive Program of Cancer Immunotherapy & Immunology (CAIMI) in 2017. This program has significantly improved VHIO's research in Immuno-Oncology, as well as advanced the development of cell therapies. As a result of this valuable support, we are delighted to present the inaugural symposium, which aims to disseminate high-quality research findings and foster collaboration among international experts to advance our understanding of cancer.

CAIMI PROGRAM

The Comprehensive Cancer Immunotherapy and Immunology Program (CAIMI) of the VHIO-BBVA Foundation investigates how to harness the immune system to advance cancer immunotherapies. Therapeutic advances in oncologic immunotherapy have rapidly emerged in recent years, reflecting the crucial interaction between the human immune system and cancer. Despite the success of applying immunotherapy in oncology, only a portion of treated patients responds, necessitating urgent customization of these treatments. Other challenges in this field include improving the efficacy of current immunotherapies and developing new treatments. With the CAIMI Program, we aim to address pressing needs to advance cancer immunotherapies by co-funding various translational projects linked to the early clinical development phases of immunotherapy, as well as an investigator-initiated trials.



Program Day 1		
8:15-9:00	Registration	
9:00-9:10	Opening remarks	Alena Gros , VHIO
Session I: Advances in Gene and Cell Therapies		Chairs: Joaquin Arribas & Martí Farrera-Sal
9:15-10:00	Keynote lecture. Towards in vivo CAR-T	Saar Gill , University of Pennsylvania
10:00-10:25	Precision TIL Therapy for Poorly Immunogenic Metastatic Cancers	Udai Kammula , University of Pittsburgh
10:25-10:50	Academic Model for CAR-T Development: Results in Multiple Myeloma with AR1000zh and Beyond	Carlos Fernández de Larrea , IDIBAPS - Hospital Clínic Barcelona
10:50-11:15	Gene Therapy for Rare Liver Disorders: From the Bench to the Patients	Gloria González Aseguinolaza , Clínica Universidad de Navarra
11:15-11:45	Coffee break	
11:45-12:10	Novel Targets for Immune Redirection	Joaquin Arribas , VHIO/ HMRIB
12:10-12:35	A Portfolio of Cellular Immunotherapies for ALK-Driven Cancers	Roberto Chiarle , Harvard Medical School / University of Turin
12:35-13:15	Short talks session	
	Development Of New Cellular Immunotherapies Against Cancer Based on T Lymphocytes Trained with Bacteria	Daniel Gomez , CNB-CSIC
	Identification of Clinically Relevant T-Cell Receptors for Personalized T-Cell Therapy Using Combinatorial Algorithms	Rémy Pétremand , UNIL
	Preclinical Characterization of ARI-007: CD7-Negative T-Cells Engineered with an Academic Anti-CD7 CAR for T-Cell Cancer Treatment	Marta Soria Castellano , IDIBAPS
13:15-14:15	Group picture and Lunch break	
14:15-14:30	Guest Lecture. Enhancing Patient Engagement in Cancer Research: A Focus on Patient-Centric Approaches to Scientific Discovery	Marta Puyol , Asociación Española Contra el Cancer (AECC)
Session II: Towards Clinical Translation		Chair: Elena Garralda
14:30-14:55	Harnessing Peripheral Blood to Create Next-Generation T-Cell Therapies for Solid Cancers	Alena Gros , VHIO
14:55-15:20	Novel Inventions to Improve T-cell Therapy	Sine Reker , Technical University of Denmark
15:20-15:45	Clinical Translation of ATMPs - why Mapping the Hurdles from Preclinical Development to Clinical Use is Important	Annette Kunkle , Charité – Universitätsmedizin Berlin
15:45-16:10	Short talks session	
	Enhancing Anti-BCMA CAR- T Cell Therapy Response in Multiple Myeloma by Combining in Silico Perturbation Predictions and Drug Repurposing	Alessia Buratin , IJC
	Embedding GMP Principles in the Development of Next-Generation Tumor-Resilient TCR-T Cells	Daniel Lycke Kristiansen , Oslo University Hospital
16:20-18:00	Poster session & Networking cocktail	



Program Day 2

Session III: Emerging Concepts in Adoptive Cell Therapy		Chair: Pere Barba
9:00-9:25	Modulating Signaling Activities to Enhance CAR-T Cell Potency	Judith Feucht , University of Tübingen
9:25-9:50	Multi-Modal Preclinical Tools to Advance CAR-T Cell Therapy in Solid Tumors	Martí Farrera-Sal , VHIO
9:50-10:15	Deconstructing Aging with Senolytic CAR-T Cells	Corina Amor , Cold Spring Harbor Laboratory
10:15-10:40	STAb-Therapy: Physiological, Multi-Target, and Multi-Effector Immune Redirection for Cancer	Luis Álvarez-Vallina , Hospital Del Mar Research Institute
10:40-11:15	Coffee break	
11:15-12:00	Short talks session	
	BACH2 Dosing Promotes Stemness Without Compromising Effector Functions to Enhance T Cell Persistence and Anti-Tumor Efficacy	Alberto Conti , University of Cambridge
	In Vivo- Based CRISPR Screen Identifies ZC3H12C as a Mediator of CAR-T Cell Dysfunction in Solid Tumors	Paula Barbao , IDIBAPS
	Unravelling Tumor-Intrinsic Resistance Mechanisms to T-Cell-Mediated Cytotoxicity in Pancreatic Cancer	Judit Díaz Gómez , VHIO
12:00-12:55	Roundtable discussion: Establishing Operational Networks for CGT Products in the EU	
	Chair: Silvia Martín Lluesma , VHIO	
	Manel Juan , IDIBAPS - Hospital Clínic Barcelona	
	Cristina Castilla-Llorente , Gustave Roussy	
Participants	Miquel Salas , Biocat	
	Francisco Martin , SETGYC/ GENYO	
	Johan van Eldere , European University Hospital Alliance	
13:00-14:00	Lunch break	
Session IV: Determinants of Cell Therapy Activity		Chair: Alena Gros
14:00-14:25	Understanding and Exploiting Cytokine and Cellular Activity in the Extracellular Tumor Environment	Suman Mitra , University of Lille
14:25-14:50	Engineering Resilient CAR-T Cells: Strategies to Overcome Resistance	Sonia Guedan , IDIBAPS - Hospital Clínic Barcelona
14:50-15:15	Distinct TCR-Driven Transcriptional Programs Determine the Antitumor Activity of Neoantigen-Specific T Cells	Cristina Puig Saus , University of California, Los Angeles
15:15-16:00	Keynote Lecture. The Immunogenicity of Cancer Neoantigens	Thomas Blankenstein , MDC Berlin
16:00	Closing remarks	Silvia Martín Lluesma , VHIO

SPEAKERS

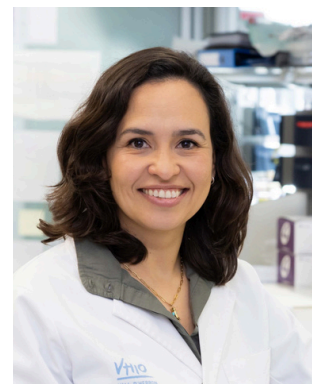


ALENA GROS

Vall d'Hebron Institute of Oncology (VHIO)

Spain

Harnessing Peripheral Blood to Create Next-Generation T-Cell Therapies for Solid Cancers



Ex vivo expansion of tumor-infiltrating lymphocytes can lead to durable tumor regression in patients with metastatic melanoma. Accumulating evidence suggests that TIL frequently recognize neoantigens and that neoantigen reactive TIL play an important role in the antitumor responses of patients treated with immune checkpoint blockade and TIL therapy. These are optimal targets for cancer immunotherapy as their expression is exquisitely confined to the tumor site, and T cells targeting these antigens have not undergone negative selection in the thymus. Despite the promising efficacy of TIL therapy against melanoma tumors, current research in the field focuses on trying to extend this efficacy to other tumor types. We will discuss the existing possibilities to develop personalized T-cell therapies targeting neoantigens for solid cancers, with particular emphasis on minimally invasive T-cell therapies.



ANNETTE KÜNKELE-LANGER

Charité – Universitätsmedizin Berlin

Germany



Clinical Translation of ATMPs - why Mapping the Hurdles from Preclinical Development to Clinical Use is Important

Current EU-wide regulatory and legal frameworks were primarily designed for standard drug production with large patient target groups. The evolving nature of ATMPs resulting from rapid changes in scientific knowledge and technologies requires dynamic adaption of regulatory and legal frameworks better equipped to facilitate development of individualized processes, rapid introduction of ground-breaking technologies and application to small numbers of patients possessing well-defined patient and disease characteristics fitting to the success of the ATMP. This will ensure wide-spread access to ATMPs and an innovative and competitive European GMP landscape.

A collaborative effort is essential to streamline clinical translation of ATMPs developed by academia, pharma, SMEs or not-for-profit organizations. The JOIN4ATMPconsortium unites international experts and stakeholders to create a blueprint for reform across the EU and member states. It brings together all members of the European University Hospital Alliance (includes the largest hospitals in 10 member states) with the existing EU-funded T2Evolve and Restore networks, and with active support from industry partners and patient representatives as 16 core partners. The core partners are joined by contributing partners from the field of ATMP development, production, application and regulation to achieve the most rapid and wholistic assessment of current hurdles and the best way to improve support and regulation of ATMP development and use in the EU and associated countries. JOIN4ATMP leverages the real-world experience of its partners to rigorously map hurdles, then provide recommendations for paths forward with regulatory, economics and policy-making advisors.



CARLOS FERNÁNDEZ DE LARREA

Hospital Clínic | Barcelona

Spain

Academic Model for CAR-T Development: Results in Multiple Myeloma with AR10002h and Beyond



The introduction of B-cell maturation antigen (BCMA) directed chimeric antigen receptor T-cell (CART) therapy to the treatment of relapsed/refractory (R/R) multiple myeloma (MM) is undoubtedly revolutionary. Consequently, the demand for this therapy overcomes availability and, in some countries and health systems, the costs of this therapy are unaffordable. These unmet needs may be improved by academic CARTs.

Our institution developed AR10002h, a second generation 4-1BB-based CAR, with a humanized scFv directed against BCMA. A pivotal, single-arm, open label clinical trial (CARTBCMA-HCB-01) for the treatment of R/R MM patients was performed in 7 Spanish centers across the country, with two CAR-T cell production centers. A booster dose of up to 3×10^6 CAR+cells/kg was planned at least 3 months after the first dose. Results of the first cohort of 30 patients showed an overall response rate (ORR) of 100%, with 67% achieving complete responses (CR). In a more recent update, with 60 patients treated, overall response rate in the first 3 months was 95%. AR10002h administered in a fractioned manner with a booster dose can provide deep and sustained responses in patients with R/R MM, with a relatively low-grade toxicity, especially neurologic events, and the possibility of a point-of-care (PoC) approach.

Beyond the trial, more than 180 patients have been successfully treated with AR10002h through this academic PoC network. AR10002h is reimbursed for patients who meet the treatment indications. An academic European trial with AR10002h and the PoC strategy is currently under development.



CORINA AMOR

Cold Spring Harbor Laboratory (CSHL)

United States of America

Deconstructing aging with senolytic CAR T cells



Most living organisms age, however much remains to be understood about the biology of aging. Cellular senescence, a stress response program characterized by stable cell cycle arrest and a proinflammatory secretome, is a key hallmark of the aging process. While in younger organisms senescent cells are effectively cleared by the immune system; in the elderly they accumulate in tissues and are thought to contribute to aging pathobiology. Yet how do they build up and how do they contribute to aging remains unclear.

Two key limitations that have held the field back in answering these questions have been the lack of: 1) surface markers to isolate and characterize senescent cells from aged tissues and 2) specific somatic approaches to target them without the need to breed and age for years genetically engineered mouse models (GEMMs). To address this, my laboratory harnesses: 1) surface markers to isolate and characterize senescent cells and 2) cellular therapy as a somatic, selective and efficient tool to functionally interrogate the contribution of senescent cells to aging biology. In our research program we intent to shed light into: which aging phenotypes do senescent cells drive and how do they do so; what are the hierarchies governing the interactions between senescence and other hallmarks of aging; how do senescent cells interact with immune cells and other key components of their microenvironment such as the nervous system, and finally, how are these interactions altered in aging resulting in the accumulation of senescent cells in the first place. The long-term goal of our research is to robustly understand the mechanisms whereby senescent cells contribute to aging biology. In so doing we hope to uncover knowledge and develop cell-based therapeutic strategies to treat a diverse range of age-related pathologies.



CRISTINA PUIG SAUS

UCLA Health Jonsson Comprehensive Cancer Center
United States of America

Distinct TCR-Driven Transcriptional Programs Determine the Antitumor Activity of Neoantigen-Specific T Cells



A better understanding of how successful T cell responses induced by immune checkpoint blockade (ICB) eliminate metastatic tumors can guide the design of cellular therapies with enhanced antitumor activity in solid tumors. Toward this goal, we conducted a longitudinal landscape analysis of neoepitope-specific T cell responses induced during treatment in patients with melanoma, both with and without clinical benefit from ICB. Using newly developed technologies to isolate neoepitope-specific T cells, we characterized the targets of the T cell responses and their evolution over time in the peripheral blood and tumors. In these studies, we found that all patients exhibited tumor-reactive, neoantigen-specific T cells targeting a limited number of immunodominant epitopes. Moreover, in patients with clinical benefit from ICB, these responses were polyclonal, with multiple TCR clonotypes specific for the same mutations and recurrently detected over time. Importantly, we identified that distinct TCRs induce different transcriptional and epigenetic programs, which determine the T cell phenotype and its ability to maintain antitumor activity upon chronic antigen stimulation.

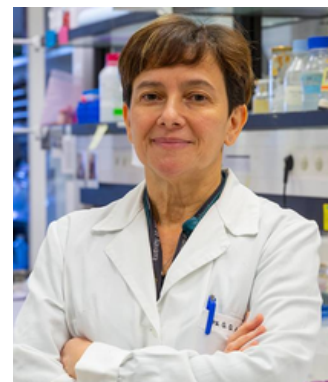


GLORIA GONZÁLEZ ASEGUINOLAZA

Cima Universidad de Navarra (CUN)

Spain

Gene therapy for rare liver disorders: From the bench to the patients



Wilson Disease (WD) is a disorder of Cu homeostasis due to pathogenic variants in the ATP7B copper-transporter gene that impair biliary Cu excretion and cause tissue Cu accumulation and reduced circulating ceruloplasmin (Cp). Limitations of management include treatment side effects, and poor adherence affecting long-term outcomes and survival. VTX-801 is a recombinant adeno-associated viral vector (rAAV) carrying a shortened ATP7B gene with a liver-specific promoter. Previous studies with VTX-801 in 6 to 12-week-old WD mice demonstrated restoration of biliary Cu excretion, increased levels of plasma Cp, reduced liver Cu, improved hepatic histology and 100% long-term survival.

Here we will present the preliminary data of the clinical evaluation of this gene therapy product. GATEWAY is an international phase I/II, open-label, 5-y follow-up, single dose-escalation study of VTX-801 in adults with WD (NCT04537377). This first-in-human study assessed safety, pharmacodynamics and efficacy of VTX-801. VTX-801 response at 3 or 9-month post-infusion was based upon ⁶⁴Cu pharmacokinetic assessments. VTX-801 Cohort 1 (low dose) was completed (N=2 patients), and Cohort 2 (intermediate dose) were treated (N=2). Follow-up liver biopsy at 1 y in a patient from Cohort 1 showed miniATP7B mRNA expression in up to 50% of hepatocytes. Available pharmacodynamic data for Cohort 1 will be presented.



JOAQUIN ARRIBAS

Vall d'Hebron Institute of Oncology (VHIO)

Spain

Novel targets for immune redirection



Immune redirection involves generating artificial contacts between immune effector and target cells through engineered proteins such as chimeric antigen receptors (CARs) or bispecific antibodies.

Our group focuses on identifying tumor-associated and tumor-specific antigens that enable the safe redirection of cytotoxic lymphocytes. We are pursuing several projects at different stages of development:

p95HER2 CAR-T cells. We identified a truncated form of the HER2 tyrosine kinase receptor, p95HER2, as a tumor-specific antigen expressed in approximately 40% of HER2-positive tumors. We have developed CAR-T cells directed against p95HER2 that also secrete a bispecific antibody recognizing target cells with high HER2 expression, while sparing those with physiological levels. These dual-function CAR-T cells show remarkable activity against a variety of HER2-positive tumors and are expected to enter clinical testing shortly.

Mutant tyrosine kinase receptor antibodies. We have generated antibodies specific for a mutant form of a tyrosine kinase receptor expressed in a subset of endometrial cancers. These antibodies do not recognize the wild-type receptor, making them highly tumor-selective and ideal candidates for immune redirection.

MHC-peptide complex targeting. Finally, we are developing antibodies against MHC-peptide complexes to extend immune redirection strategies to intracellular tumor antigens.

Our goal is to accelerate overlapping projects by leveraging the experience gained with the most advanced ones. For example, the regulatory knowledge acquired during the clinical translation of our p95HER2 CAR-T program will allow us to accelerate preclinical development and approval processes for subsequent candidates.



JUDITH FEUCHT
University of Tübingen
Germany

Modulating signaling activities to enhance CAR T cell potency



Chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of patients with certain refractory hematological malignancies. However, insufficient functional T cell persistence impedes the therapeutic success of CAR T cell therapies in a substantial proportion of patients.

We investigate strategies to fine-tune T cell signaling with the goal of enhancing CAR T cell efficacy against hematological and solid tumors. Our efforts include modifications of the CAR design as well as endogenous modulation of signaling pathways using CRISPR base editing. We demonstrate that CAR design-specific calibration of signaling strength enhances CAR T cell efficacy in established preclinical models of hematologic and solid cancers. Refined CAR T cell products exhibit distinct metabolic and transcriptional profiles linked to superior antitumor activity and sustained functionality, without evidence of malignant transformation.

Together, our findings underscore the importance of precise signaling calibration tailored to specific CAR architectures and highlight the potential of precision genome engineering to advance next-generation cellular immunotherapies.



LUIS ALVAREZ VALLINA

Hospital Del Mar Research Institute

Banc de Sang i Teixits

Spanish National Cancer Research Centre (CNIO)

Spain



**STAb-Therapy: Physiological,
Multi-Target, and Multi-Effector
Immune Redirection for Cancer**

T cell redirection strategies are transforming the treatment of hematologic malignancies by recruiting T cells to recognize tumor-associated antigens (TAAs) through engineered receptors such as chimeric antigen receptors (CARs) or bispecific T cell engagers (TCEs). CAR-mediated redirection is TCR-independent and driven by high-affinity interactions that provide both activation and co-stimulatory signals, leading to an atypical immunological synapse. In contrast, TCE-mediated redirection relies on low-affinity, TCR-dependent interactions that promote finely tuned signaling and the formation of canonical synapses, representing a more physiological mode of T cell engagement. Systemically administered TCEs, however, face challenges such as limited tumor penetration and short serum half-life, whereas adoptively transferred CAR-T cells can traffic to tumor sites, persist, and expand in vivo, generating a memory pool that supports long-term antitumor responses.

To integrate the advantages of both modalities, we have engineered T cells to secrete TCEs in vivo (STAb-T cells). This strategy enables the formation of canonical synapses while overcoming limitations of systemic TCE delivery. Tumor trafficking of adoptively transferred T cells ensures localized TCE secretion, achieving effective intratumoral concentrations despite the short serum half-life of soluble TCEs. These properties support the expansion and persistence of STAb-T cells in vivo, leading to durable memory responses and robust therapeutic efficacy in preclinical models of hematologic malignancies. Building on this foundation, next-generation multispecific STAb-T and CAR-STAb platforms expand the concept of immune redirection by combining antigen multiplexing and local TCE delivery. Multispecific STAb-T cells can simultaneously target several TAAs, preventing immune escape and broadening tumor coverage, while CAR-STAb cells integrate direct CAR-mediated cytotoxicity with bystander engagement through secreted TCEs. Together, these modular and synergistic strategies enable coordinated activation of both engineered and endogenous T cells, offering a flexible and physiologically tuned approach to achieve potent, durable, and antigen-heterogeneity-resistant antitumor immunity.



MARTA PUYOL

Spanish Association Against Cancer (AECC)

Spain

Enhancing Patient Engagement in Cancer Research: A Focus on Patient-Centric Approaches to Scientific Discovery



Marta Puyol is the Scientific Director of the Spanish Association Against Cancer and Deputy General Director of the AECC Scientific Foundation, where she carries out tasks related to the funding of research projects, promoting scientific policies that help increase the training and global recognition of Spanish scientists, as well as scientific outreach and research promotion activities. In addition, from this position she has led several high-impact initiatives such as: the creation of the World Cancer Research Day (an initiative launched in 2016 and joined by 350,000 people from around the world); reports on the reality of cancer research in Spain and in various autonomous communities; the promotion of international initiatives to encourage the participation of Spanish researchers in European research networks; and the creation of the patient advocacy program for the involvement of patients in research.

She holds a degree in Molecular Genetics from the University of Sussex (Brighton, England). Upon finishing her studies, she decided to pursue a scientific career, moving to Heidelberg (Germany), where she studied developmental biology at the European Molecular Biology Laboratory (EMBL). In 2003, she earned a degree in Biochemistry and Biotechnology from the Francisco de Vitoria University (Madrid). In 2009, she obtained her PhD from Kingston University (London, England) for her work carried out at the National Cancer Research Center (CNIO) under the supervision of Dr. Mariano Barbacid, where she achieved significant results in describing a new treatment for lung cancer that is now under clinical development at a major pharmaceutical company.

Between 2009 and 2012, she completed her postdoctoral research in Dr. Richard Hynes's laboratory at the prestigious MIT Koch Institute (Cambridge, USA), where she studied breast cancer progression toward metastasis.



MARTÍ FARRERA-SAL

Vall d'Hebron Institute of Oncology (VHIO)
Spain

Multi-Modal Preclinical Tools to Advance CAR-T Cell Therapy in Solid Tumors



While CAR-T cell therapies have demonstrated remarkable success in hematologic malignancies, their efficacy in solid tumors remains limited due to barriers such as tumor heterogeneity, poor infiltration, and immunosuppressive microenvironments. To address these challenges, we present two complementary preclinical platforms focused on advancing CAR-T cell therapy in glioblastoma and non-small cell lung cancer (NSCLC). In the context of glioblastoma, we developed a high-dimensional spectral flow cytometry panel and combined it with imaging-based serial killing assays to characterize CAR-T cell products from the GAIL-B clinical trial. This integrated approach enabled the identification of phenotypic and functional correlates of therapeutic response in pediatric CNS tumors. For NSCLC, we established a matched healthy-tumor lung organoid biobank to evaluate CAR-T cell function in a physiologically relevant system. This organoid platform enables the assessment of patient-specific CAR-T activity and resistance mechanisms, supporting more predictive and personalized immunotherapy development. Together, these tools offer a powerful strategy to dissect and optimize CAR-T cell performance in solid tumors, bridging the gap between preclinical modeling and clinical translation.



ROBERTO CHIARLE
Harvard Medical School
United States of America

A portfolio of cellular immunotherapies for ALK-driven cancers



One major research interest of the Chiarle's lab has been to study the mechanisms and pathways of tumor formation activated by the ALK oncogene, as well as the development of innovative therapies for AK-positive cancers, such as an ALK-specific cancer immunotherapy. Recently, the group has developed an ALK vaccine that instructs the immune system to recognize and eliminate ALK-positive lymphoma and lung cancer cells as well as CAR-T and TCR-T cells to target ALK+ tumors including lung cancer, lymphoma and neuroblastoma. The talk will present the recent developments in this topic.



SAAR GILL

University of Pennsylvania
United States of America

Towards in vivo CART



The in vivo generation of CAR T-cells represents a potentially transformative advancement in adoptive immunotherapy, offering a promising alternative to conventional ex vivo manufacturing. By delivering CAR transgenes directly into circulating T-cells within the patient's body, this approach aims to streamline treatment logistics, reduce production costs, and broaden accessibility.

A variety of gene delivery platforms are under active investigation, including viral vectors such as lentivirus and adeno-associated virus (AAV), as well as non-viral systems like lipid nanoparticles (LNPs). These technologies are being optimized to enhance the specificity, efficiency, and safety of gene transfer. Several challenges will arise as in vivo CAR T-cell generation is translated to the clinic. These include relatively novel risks such as specificity (risk of off-target genetic modification) and efficiency (low number of genetically modified cells), along with more conventional risks such as redirection of T-cells against undesired targets and long-term depletion of healthy cellular counterparts. Strategies under development to mitigate these concerns include the use of transient expression systems (e.g., mRNA), targeted delivery mechanisms to minimize off-target activity, and the incorporation of immune-evasive features to reduce vector clearance.

Additionally, ensuring compatibility with scalable and GMP-compliant manufacturing pipelines is essential for clinical development. Ongoing preclinical research is focused on refining delivery precision, minimizing adverse immune responses, and establishing long-term safety profiles. If these efforts prove successful, in vivo CAR T-cell therapies could overcome many of the current limitations of ex vivo approaches and redefine the landscape of cell-based cancer treatment by offering more accessible treatment options.



SINE REKER HADRUP

Technical University of Denmark (DTU)
Denmark

Novel inventions to improve T cell therapy



Sine Reker Hadrup is a professor and a group leader at the Department of Health Technology, Technical University of Denmark (DTU). Hadrup's lab focuses on the development of multiplex strategies for T cell detection to comprehensively assess antigen-specific tumour reactivity among T cells in cancer patients. Novel technologies developed in her research group has changed the paradigm for T cell detection by introducing the use of molecular tagging systems (DNA barcoding) – this allows simultaneous detection of >1,000 different antigen-responding T cells (published in Nature Biotechnology 2016 and Science Immunology 2019). They are currently using this technology to unravel the immune reactivity towards cancer associate neo- and shared epitopes, published in Cancer Research, Science and Nature), as well as understanding the influence of cancer therapy on the immune recognition fingerprint in individual patients.



SONIA GUEDAN

IDIBAPS | Hospital Clínic Barcelona

Spain

Towards tailored minimally invasive T-cell therapies for solid cancers



CAR-T cell therapy has achieved remarkable success in patients with hematologic malignancies, leading to the approval of seven treatments for B-cell cancers. The next key challenges are to enhance efficacy, extend this approach to solid tumors, and broaden patient access. In this context, the preclinical and clinical development of academic CAR-T cell therapies, delivered through a point-of-care national network—may help accelerate clinical translation.

This presentation will highlight innovative strategies to improve CAR-T cell efficacy in both hematologic and solid tumors, with a focus on reducing T-cell exhaustion and preventing tumor escape through antigen loss. It will also present the preclinical development of novel academic CAR-T products with translational potential, including dual CAR-T cells for lymphoma and HER2-CAR-T cells for breast cancer.



SUMAN MITRA
University of Lille
France

Understanding and Exploiting Cytokine and Cellular Activity in the Extracellular Tumor Environment



Cytokines exert a vast array of immunoregulatory actions critical to human biology and disease. However, the desired immunotherapeutic effects of native cytokines are often mitigated by toxicity or lack of efficacy, both of which result from cytokine receptor pleiotropy and undesired activation of off-target cells. Since cytokines function in the extracellular environment, understanding how physicochemical properties within their operational milieu influence cytokine and cellular activity is not only crucial to understanding their biology but also opens new avenues for therapeutic exploitation.

As a proof of concept, I will discuss how the acidic pH characteristic of the tumor microenvironment modulates cytokine activity and demonstrate how protein engineering tools can be leveraged to allosterically control cytokine function. By designing cytokines that are preferentially activated under tumor-specific conditions, we can improve the therapeutic index of cytokine-based therapies—enhancing their anti-tumor efficacy while reducing systemic toxic effects. This approach represents a paradigm shift in cytokine engineering that exploits the unique properties of the tumor microenvironment for selective therapeutic benefit.



THOMAS BLANKENSTEIN

Max Delbrück Center Berlin (MDC)
Germany

The immunogenicity of cancer neoantigens



We generated mice containing the human TCR α and TCR β gene loci and the human MHC I genes HLA-A*02:01 (HLA-A2). These mice were further modified to be deficient in mouse TCR β and mouse MHC I expression. Thus, these mice, termed ABab-A2, had a humanized T cell recognition system with a diverse human TCR repertoire restricted to HLA-A2. Since human tumor-associated tumor antigens, such as cancer-testis antigens, are foreign to the mice, high-affinity T cell receptors (TCR) can be isolated following immunization. We have several examples, e.g. MAGE-A1 and NY-ESO, that TCR from mice have higher affinity than those isolated from humans. More recently, we established a syngeneic tumor model, which we use as an in vivo model for analyzing the immunogenicity of naturally occurring human cancer neoantigens. The results show a strong immunodominance among different neoantigens. The cellular and molecular mechanisms will be presented.



UDAI KAMMULA

University of Pittsburgh

United States of America

Precision TIL Therapy for Poorly Immunogenic Metastatic Cancers



Metastatic cancer resistance to immune checkpoint inhibition (ICI) is a significant problem in oncology, especially for 'cold' cancers with low tumor mutational burden (TMB).

Thus, our overarching goal is to bioengineer cellular immunotherapies with greater efficacy against these ICI resistant cancers. As initial proof of concept, we began adoptive cell therapy (ACT) studies for metastatic uveal melanoma (UM), a prototypic low TMB and ICI resistant cancer with unmet medical need. Despite being considered an immune 'cold' form of melanoma, our group was the first to report that a subset of surgically resected UM liver metastases naturally harbor tumor infiltrating lymphocytes (TIL) with potent autologous anti-tumor reactivity (Clinical Cancer Research, 2016)¹. Further, we demonstrated ex vivo manufacturing and intravenous infusion of these TIL via ACT could mediate durable cancer regression in metastatic UM patients, including ICI refractory individuals (NCT01814046; Lancet Oncology, 2017)².

These findings establish potent TIL can exist in select ICI resistant metastases and be manufactured ex vivo for ACT. However, a major obstacle is the lack of pre-operative diagnostics to determine which metastases harbor these optimal tumor reactive TIL before a patient undergoes invasive surgical harvest. To address this significant problem, our long-term goal is to develop in situ tumor profiling tools that will guide precision TIL manufacturing to improve potency and ACT outcomes. We recently developed a quantitative transcriptomic metric called Uveal Melanoma Immunogenomic Score (UMIS), assessed using tumor RNA from a single in situ core biopsy sample (Nature Communications, 2024)³. We reported in a pilot study that UMIS level could accurately identify UM metastases that could generate tumor reactive TIL without need for surgery, time consuming ex vivo culture, and labor intensive potency testing. Further, metastasis UMIS level could predict the eventual clinical outcome of UM patients who had undergone TIL-ACT in NCT01814046. Consequently, our group is focused on defining the clinical utility and mechanistic basis of UMIS profiling in guiding precision TIL bioengineering in our new TIL-ACT clinical trial enrolling metastatic UM patients at the University of Pittsburgh (NCT03467516).

Additionally, we have developed a next generation pan-cancer biomarker which is guiding a series of new TIL-ACT trials targeting a large array of poorly immunogenic tumors. Completion of these bioinformatic, bioengineering, and translational studies will define the essential utility of in situ tumor transcriptomics in guiding optimal tumor harvest and precision TIL product manufacturing, thereby improving clinical outcomes of patients with UM and other ICI resistant cancers in the future.

SHORT TALKS



BACH2 Dosing Promotes Stemness Without Compromising Effector Functions to Enhance T Cell Persistence and Anti-Tumor Efficacy

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United Kingdom (UK)

T cell therapies hold promise for treating solid tumors, but their efficacy is limited by poor persistence of transferred cells. Previous strategies to enhance persistence often relied on enforcing constitutively activated, and potentially oncogenic, cell states. In contrast, physiological immune responses are sustained by quiescent, self-renewing progenitor T cells that depend on the transcription factor BACH2. These cells maintain stem-like potential while giving rise to short-lived effectors. Here, we show that quantitative control of BACH2 dosage governs the physiological continuum of stem and effector CD8⁺ T cell states and enables engineering of synthetic states with superior persistence and anti-tumor activity. In CD8⁺ T cells, BACH2 is precisely regulated, with intermediate expression in memory and progenitor-exhausted subsets. Excessive levels of BACH2 locks cells in quiescence, curtailing effector function and tumor control. By contrast, low-dose BACH2 permits effector differentiation while preserving stem-like features, thereby enhancing persistence and therapeutic efficacy. Mechanistically, low-dose BACH2 partially restrains AP-1 occupancy at enhancers, attenuating highly AP-1-dependent genes while maintaining effector programs. This dosage principle extends to other quiescence factors, as low-dose FOXO1 expression augments T cell responses in an analogous manner. Thus, quiescence factor dosage emerges as a key regulator of T cell fate, suggesting that quantitative tuning of gene expression can drive qualitative improvements in cancer immunotherapy.



Enhancing Anti-BCMA CAR- T Cell Therapy Response in Multiple Myeloma by Combining in Silico Perturbation Predictions and Drug Repurposing

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In this retrospective study, we performed longitudinal single-cell RNA and TCR sequencing of CAR⁺ and endogenous T cells from infusion products (IP) and peak expansion samples of 11 relapsed/refractory multiple myeloma patients treated with ARI0002, the academic anti-BCMA CAR T therapy developed at the Hospital Clínic of Barcelona. This analysis generated a unique dataset of over 101,177 T cells spanning the course of CAR T-cell therapy.

After rigorous quality control and integration across patients, we used infusion product and developed an explainable machine learning framework to classify patients with poor vs. long-term remission and identify predictive biomarkers of treatment response. Using both transcriptomic features and TCR diversity metrics, our model accurately predicted patient outcomes from the IP, reaching up to 95% accuracy in cross-validation. Importantly, CAR-negative T cells emerged as the most informative compartment for outcome prediction, suggesting CD4⁺ T-cell effector memory and CD8⁺ cytotoxic T cells, which may serve as powerful resource for patient stratification. Model interpretability revealed candidate surface biomarkers such as SELL and SLAMF7, and highlighted the IL2-STAT5 and TNF-NFκB pathways as key molecular programs associated with response.

Analysis of TCR clonal dynamics added another layer of insight: responders exhibited a modest but consistent enrichment (~1.2-fold) of hyperexpanded CAR-positive highly proliferative T-cell clones compared to non-responders, suggesting that early clonal amplification may mark favorable outcomes.

Finally, we explored therapeutic opportunities by testing drugs in silico on our single-cell data. Using a Compositional Perturbation Autoencoder, we simulated the transcriptional effects of hundreds of compounds and compared them with responder-like profiles on a subtype-specific basis. Barasertib, an Aurora kinase B inhibitor, was predicted to counteract aberrant chemokine receptor signaling that traps non-responder T cells in dysfunctional states, thereby promoting a transcriptional profile resembling functional responder cells.



Development Of New Cellular Immunotherapies Against Cancer Based on T Lymphocytes Trained with Bacteria

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Over the past two decades, novel biological therapies have profoundly reshaped the landscape of cancer treatment (Chen and Mellman 2013; Cole et al. 2023). Immune Checkpoints Inhibitors (ICI) along with Adoptive Cell Therapies, such as CAR-T therapies, have significantly improved overall response rates and enabled long-lasting remissions, particularly in hematologic malignancies. However, the high rates of non-responders and relapses, ranging from 40% to 95% in the case of ICIs (Mao et al., 2023), as well as the limited efficacy of ACTs in solid tumors (Pedrazzoli & Haanen, 2022), remain major challenges to overcome, highlighting the urgent need for more specific and effective therapeutic strategies. In response to this need, our laboratory proposes here a pioneering therapeutic approach based on a previously unreported CD8⁺ T-cell state generated without genetic modification, termed T-Boost. This is achieved through an innovative activation method of CD8⁺ T-cells using bacteria-instructed CD4⁺ T lymphocytes (BacT), which are T CD4⁺ lymphocytes that act as antigen-presenting cells after being instructed with engineered E. coli strains carrying tumor antigens (Cruz-Adalia 2017). T-Boost cells exhibit long-term persistence and sustained effector function within the tumor microenvironment. Moreover, they resist tumor-induced exhaustion, efficiently infiltrate solid tumors, and actively remodel the immune landscape by enhancing cytotoxic responses while reducing exhausted T cells and immunosuppressive populations. Remarkably, T-Boost achieves potent antitumor activity without requiring lymphodepleting chemotherapy, thereby minimizing life-threatening toxicities and paving the way toward safer, more durable ACTs for solid tumors.



Embedding GMP Principles in the Development of Next-Generation Tumor-Resilient TCR-T Cells

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Adoptive T-cell therapy with TCR-T cells provides the unique ability to target intracellular antigens, unlike CAR-T therapies which are restricted to membrane-bound proteins. Despite considerable progress in the field, and the recently FDA-approved TCR-T therapy (MAGE-A4), efficacy in solid tumors is often limited by the immunosuppressive tumor microenvironment. Using single cell transcriptomic data, our collaborators (Zhang et al. Nat Med. 2022) have found genetic signatures for T-cells that show resiliency towards immunosuppressive signals, therefore called resilient T cells (Tres). The presence of these cells in infusion products correlates with a favorable outcome for patients undergoing adoptive T-cell therapy. Among several other markers, these cells are characterized by an enriched CD127 expression and lower expression of the FIBP gene. We hypothesize that by implementing genetic engineering (e.g., knock-out of FIBP) we could recreate the resilient phenotype during the manufacturing process of TCR-T cells. We applied CRISPR knockout of FIBP, a gene associated with poor clinical response, followed by enrichment of positive markers and TCR-engineering to recognize common hotspot mutations in solid tumors. Functionality was assessed by using flow cytometry to measure cytokine release in vitro upon antigen-specific stimulation. Importantly, all procedures were performed using clinical-grade reagents, prioritizing GMP-compatible alternatives from the start to support future translation. CRISPR-mediated FIBP knockout reduced transcript expression by ~60% (qPCR), with loss of protein confirmed by western blot. TIDE analysis showed efficient indel formation. Edited Tres exhibited enhanced cytokine release upon antigen-specific stimulation compared to wild-type controls. We will extend this approach to multiple TCRs recognizing hotspot mutations and further optimize GMP-compliant manufacturing workflows to enable clinical translation of Tres-based therapies.



Unravelling Tumor-Intrinsic Resistance Mechanisms to T- Cell -Mediated Cytotoxicity in Pancreatic Cancer

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Spain

Immunotherapy remains ineffective in pancreatic ductal adenocarcinoma (PDAC). This resistance has been attributed to its low tumor mutational burden, limited immune infiltration, and a desmoplastic, immunosuppressive tumor microenvironment. Challenging this notion, recent studies have shown that neoantigen-specific tumor-infiltrating lymphocytes can be expanded and isolated from pancreatic cancers. Despite this, ex vivo expanded tumor-reactive TILs often fail to recognize and kill the autologous tumor cells, even in the absence of microenvironmental influences. These observations suggest that PDAC cells can evade immune recognition and T-cell-mediated cytotoxicity through cell-intrinsic mechanisms that remain poorly understood. In this study, we sought to identify immune regulators that limit T-cell-mediated killing in pancreatic cancer using a co-culture-based genome-wide CRISPR/Cas9 screen. We employed an in vitro model consisting of a low-passage patient-derived pancreatic cancer cell line that is poorly recognized by TCR-engineered T cells targeting the MMP14p.R158C neoantigen. By conducting a genome-wide CRISPR/Cas9 screen on our in vitro co-culture model, we identified 42 candidate genes whose depletion enhanced susceptibility to T-cell-mediated cytotoxicity. Among the top hits, ablation of the E3 ubiquitin ligase MARCHF5 increased T-cell-mediated killing across three patient-derived in vitro PDAC models, independent of the specific cell line, TCR, and targeted neoantigen. Ongoing mechanistic studies aim to understand the molecular basis by which the loss of MARCHF5 enhances T-cell killing. Preliminary data indicate that MARCHF5 deficiency sensitizes tumors to neoantigen-driven T-cell cytotoxicity through upregulation of the pro-apoptotic protein NOXA. Overall, our work identifies MARCHF5 as a novel target whose immunomodulation holds potential to unleash the immune system's potential to control pancreatic cancer.



Preclinical Characterization of ARI-007: CD7-Negative T-Cells Engineered with an Academic Anti-CD7 CAR for T-Cell Cancer Treatment

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Three in-house CD7-specific scFvs were tested to generate 4-1BB-based CAR-T cells, incorporating CRISPR/Cas9-mediated CD7 knockout to mitigate fratricide. All candidates demonstrated comparable cytotoxicity, cytokine secretion and proliferation against T-ALL cell lines. In a T-ALL mouse model, 124.1D1 and 142.24 CAR-T cells outperformed 142.9 CAR-T cells in tumor control, selecting 142.24-CAR as the final candidate.

We next compared different fratricide-mitigation strategies: i) CD7 knockout, ii) protein expression blocker (PEB) (124.1D1 scFv fused to endoplasmic reticulum retention domain), iii) inhibition of CAR signaling using dasatinib (tyrosine kinase inhibitor), iv) short (3-day) expansion and v) selection of natural CD7neg T-cells. PEB- and CD7KO-CAR-T cells effectively extended survival, in contrast to dasatinib-expanded cells; however, none of these approaches induced long-term responses. CAR-T cells expanded for three days showed enhanced IL-2 production and proliferation. After infusion of these shortly-expanded CAR-T cells to tumor-bearing mice, the CD7neg CAR-T cell population self-selected, improving T-cell persistence and survival. Similarly, CAR-T cells generated from the naturally-occurring CD7neg population achieved durable responses and extended animal survival.

Based on the antitumor potential of CD7neg T-cells, we characterized their phenotype and function. CyTOF analysis of human reactive lymph nodes revealed a heterogeneous population, with approximately 50% CD4⁺ TCM/EM, along with CD8⁺ TEM, Tregs CM and a small fraction of naïve T-cells. During manufacturing, CD7neg T cells showed increased susceptibility to activation-induced death and reduced proliferation than bulk T-cells. Nonetheless, CD7neg T-cells showed increased spare respiratory capacity and polyfunctionality, secreting higher levels of effector and chemoattractive cytokines.

Based on these results and the reduced risk of blast contamination, CD7neg 142.24 CAR-T strategy (ARI-007) was selected for evaluation in a phase I clinical trial in patients with T-ALL/LBL and CD7pos acute myeloid leukemia (AML).



In Vivo- Based CRISPR Screen Identifies ZC3H12C as a Mediator of CAR-T Cell Dysfunction in Solid Tumors

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NSG mice bearing ovarian xenograft tumors were infused with a single dose of anti-HER2 CD28-based CAR-T cells. Tumor-infiltrating T cells (CAR-TILs) were isolated from tumors after infusion (effective phase) and following therapy failure (dysfunctional phase). Bulk RNA-seq and single-cell RNA-seq analysis were performed on dysfunctional CAR-TILs compared to effective CAR-TILs and preinfusion product. Dysfunctional CAR-TILs exhibited hallmarks of exhausted T cells, including impaired ex vivo effector functions and enrichment in exhaustion signatures.

To elucidate mediators of CAR-T cell dysfunction, we selected 300 significantly upregulated genes in dysfunctional CAR-TILs and designed a sgRNA library to genetically modify CAR-T cells using CRISPR/Cas9 technology. We identified 14 significantly enriched gene knockouts in CAR-TILs after in vivo screen, and targeted deletion of four selected candidates enhanced the antitumor effect of CAR-T cells against ovarian xenograft tumors. ZC3H12C emerged as the top the hit in the screen and was confirmed by single-cell RNA-seq to be highly expressed in CAR-TILs undergoing exhaustion in vivo. Genetic ablation of ZC3H12C in HER2-CD28z CAR-T cells mediated enhanced cytotoxicity and cytokine production after repeated stimulations with tumor cells in vitro, compared to control AAVS1-KO CAR-T cells. In vivo, ZC3H12C-KO CAR-T cells showed superior antitumor responses, enhanced T cell persistence and reduced expression of exhaustion markers. Similar results were observed using different in vivo models of lung and pancreatic tumors with Mesothelin-CD28z and CD19-BBz CARs.

By using an in vivo custom CRISPR/Cas9-mediated screen we identified potential gene mediators of CAR-T cell dysfunction. Our findings implicate ZC3H12C in CAR-T cell dysfunction following chronic antigen exposure and demonstrate that ZC3H12C-deficient CAR-T cells exhibit enhanced antitumor activity against solid tumors.



Identification of Clinically Relevant T Cell Receptors for Personalized T Cell Therapy Using Combinatorial Algorithms

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(UNIL)
Switzerland

The adoptive transfer of genetically engineered cells expressing specific T-cell receptors (TCRs) represents a promising personalized cellular therapy. Given the requirement to match both tumor antigens and HLA restriction, only few patients are eligible to these therapies. By exploiting the distinct transcriptomic profile of bone fide tumor-reactive T cells (TRT) relative to bystander cells, we built a personalized in-silico predictor of orphan tumor-reactive TCRs. The predictor, named TRTpred, was benchmarked against several published signatures on 42 tumors, which included 19 melanomas, 17 gastrointestinal cancers, 4 lung cancers, and 2 breast cancers. Using the predictor, we also revealed that the TRT repertoire is consistently richer and more clonal than its bystander counterpart in all tumor indications investigated. Furthermore, in melanoma, we observed that TRTs were predominantly located within tumor islets, while bystanders T-cell clones accumulated in the stroma. Beyond its discovery applications, TRTpred was utilized to identify clinically relevant clones for personalized T-cell therapy. To this end, we developed MixTRTpred by integrating TRTpred with a TCR avidity predictor and a TCR clustering tool (to select panels of relevant TCRs inferred to target different antigens) and benchmarked it in vitro and in vivo. Taking advantage of recent advances in the field of T cell engineering, the accuracy of MixTRTpred indicates that personalized TCR-based therapy is now achievable for most patients with solid tumors.

ROUND TABLE PANELISTS

ESTABLISHING OPERATIONAL
NETWORKS FOR CGT
PRODUCTS IN THE EU



MANEL JUAN

IDIBAPS - Hospital Clínic Barcelona
Spain



Immunology specialist since 1994, currently Head of the Immunology Service at Hospital Clínic de Barcelona and active at Hospital Universitari Germans Trias i Pujol. He leads the Immunotherapy section and platform, focusing on antitumor therapies, particularly CAR-T cells, and has extensive experience in immunodeficiencies, autoimmunity, and cellular immunology. He has coordinated national and European networks, including the Immunodeficiency Group of Catalonia, the Spanish Society of Immunology, and the European Society for Immunodeficiencies, and contributed to organizing major conferences such as ESID 2016 in Barcelona.

He is an accredited resident tutor and associate professor at UAB and UB, coordinating the Official Master's Degree in Advanced Immunology. He has led 22 research projects, published over 200 scientific articles, contributed to reviews and book chapters, and holds four patents. His research focuses on T lymphocyte function, HLA polymorphism, and antitumor immune responses through CAR-T therapy.

His research studies have focused on T lymphocyte function, starting from the molecular mechanisms of adhesion (adhesion molecules and chemokines), evolving in the study of the role of HLA polymorphism in T function (infection, in the Allergy and histocompatibility) and converging on participation in the antitumor response through CARTs. President of the SCI and member and treasurer of the SEI; current president of the national commission of the specialty of Immunology and the Committee of experts of the Ministry of Health for the Advanced Therapies.



CRISTINA CASTILLA-LLORENTE

Gustave Roussy

France



Dr. Cristina CASTILLA LLORENTE, is a hematologist, MD. PhD. in the Department of Hematology at Gustave Roussy Cancer Campus Grand Paris. Since 2022 she is the Head of the JACIE Cell Therapy Programme at Gustave Roussy integrating the clinical activity, the apheresis and cell therapy lab.

This programme performs more than 80 allogeneic transplant per year, more than 140 autologous transplant and more than 50 car T-Cells per year. It is mainly focused on hematological malignancies, but also includes a large activity of high dose chemotherapy and autologous transplant for solid tumors and autoimmune diseases. She is also the head of the Immune effector cells therapy programme again focused mainly on hematological malignancies buy also including the clinical development of new cell therapies for solid tumors and autoimmune diseases.

She is member of the scientific board of the French National Reference Centre for Cell Therapy of Autoimmune Diseases and member of the European Bone Marrow Transplant Group (EBMT) and international Bone Marrow Transplant Group (CIBMTR), being active member on different working parties, the French Bone marrow transplant Society (SFGM-TC) and the Spanish Bone Marrow Transplant Group (GETH). She is also part of the Guidelines Committee of the European Hematologie Association (EHA).

Her scientific contribution includes the implementation of new therapies processes, especially on post-allogeneic transplant complications, acute GI GVHD and others, and more recently, translation research projects on Car T-cells therapy.



FRANCISCO MARTIN
SETGYC/GENYO
Spain



Dr. Francisco Martin Molina is a professor in the Department of Immunology at the University of Granada (GENYO) and founder and scientific advisor of LentiStem Biotech. With over 30 years of experience, he focuses on safer and more efficient gene modification tools for Advanced Therapy Medicinal Products (ATMPs), particularly for cancer and rare diseases. His research includes ex vivo gene therapy using lentiviral vectors and CRISPR-based genome editing for hematopoietic stem cells and T cells, enabling promising strategies for Pompe disease, refractory type B leukemia, and CAR-T therapies for solid tumors.

He has published over 100 articles, with more than 85% in Q1 journals, holds 17 patents (six licensed), and has secured 31 national projects totaling €4.5 million. His pioneering contributions include inducible lentiviral systems, improved CAR-T cells, ATMPs for Wiskott-Aldrich Syndrome, and the identification of GARP, a novel MSC surface marker. Notably, his work led to a licensed patent for safer immunotherapies and supported a Phase I-II clinical trial evaluating an optimized anti-CD19 CAR-T (AWARI), as well as the establishment of Andalusia's first GMP facility for CAR-T production.

Dr. Martin also plays an active leadership role as President-elect of the Spanish Society of Gene and Cell Therapy (SETGyC), coordinator in the TerAv network, and work package coordinator in COST GenHumDi. He teaches and coordinates courses in gene and cell therapy in UGR master's programs and serves as scientific deputy director of GENYO.



MIQUEL SALAS
Business Department at Biocat
Spain



Miquel Salas holds degrees in Pharmacy and Business Administration. For eight years, Miquel has been working in the management and monitoring of onco-hematology clinical trials. Currently, he work as Senior Project Manager for the Innovation and Business Department at Biocat, where he is leading acceleration programs such as CRAASH Barcelona and ATMP Catalyst.

Additionally, he is the Coordinator of ATMP Catalonia, an effort aimed at aligning and enhancing the ecosystem of advanced therapies in Catalonia and positioning the region as a leading hub for the development of these cutting-edge treatments.



JOHAN VAN ELDERE
KU Leuven
Belgium



Johan Van Eldere, MD, PhD, MHM, FESCMID, is emeritus professor of microbiology at KU Leuven, and currently secretary-general of the European University Hospital Alliance (EUHA) and president of the scientific advisory board of Vall d'Hebron Institute of Research.

After his medical degree magna cum laude from KU Leuven in 1983, he pursued his PhD, and completed postdoctoral research at Oxford University, before returning to KU Leuven. He founded the infectious diseases research group, led the bacteriology and mycology lab, and was head of the clinical departments of laboratory medicine and pathology at University Hospitals Leuven. As medical director at UZ Leuven, he guided the hospital through JCI accreditation and established leadership and retention programs for medical staff.

Van Eldere has authored over 150 peer-reviewed publications, focusing on bacterial resistance, biofilm formation, and the development of novel antibiotics. Internationally, he has contributed to ESCMID, EMA, and various pharmaceutical advisory boards and KU Leuven spin-off Aelin Therapeutics to develop a new class of antibiotics.

POSTERS



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3	Alberto Conti	BACH2 dosing promotes stemness without compromising effector functions to enhance T cell persistence and anti-tumor efficacy.
4	Alejandro Ramírez Chacón	CDR3 β repertoire analysis of PD-1 ⁺ TIL products: Identifying potential tumor-reactive TCRs for transgenic TCR therapy
5	Alex Ascunce París	TCoaRse: a structural coarse-grained approach for generalizable TCR-pMHC class I pairing prediction
6	Carla Panisello Aranda	Armoured-CAR T cell-redirecting immunotherapy for metastatic and refractory Ewing Sarcoma
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8	Daniel Gómez	Development of new cellular immunotherapies against cancer based on T lymphocytes trained with bacteria.
9	Daniel Lycke Kristiansen	Embedding GMP Principles in the Development of Next-Generation Tumor-Resilient TCR-T Cells
10	Daniel Parras	Next-generation of clinical-grade lentiviral vector manufacturing for point-of-care CAR-T cell therapy: Towards democratizing CAR-Ts
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#	Name	Title
22	Paula Barbao	In vivo-based CRISPR screen identifies ZC3H12C as a mediator of CAR-T cell dysfunction in solid tumors
23	Rémy Pétremand	Identification of clinically relevant T cell receptors for personalized T cell therapy using combinatorial algorithms
24	Valeria Brunello	ICAM-1-Directed CAR-T Cells as a Novel Therapeutic Strategy for Prostate Cancer
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Self-amplifying RNA vaccines to enhance the efficacy of CAR-T cells in breast cancer.

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Spain

Chimeric antigen receptor (CAR) T (CAR-T) cells represents a revolutionary treatment for hematological tumors but this success has not been extrapolated to solid tumors. The activation and expansion of transferred cells in vivo is key for the success of CAR-T therapy, with both phenomena being highly dependent on the recognition of the target antigen (Ag). In the case of solid tumors, target Ag recognition occurs exclusively in the tumor, but the immunosuppressive microenvironment prevents the efficient activation and expansion of CAR-T cells. Furthermore, CAR-T cells require a relatively high level of Ag expression to be adequately activated and this makes their activation inefficient when tumor cells express low Ag levels. We believe that the promotion of CAR-T cell activation through off-tumor recognition of the target Ag may substantially improve the efficacy of CAR-T therapy in solid tumors. This may be safely achieved using vaccines able to deliver the target Ag. To test our hypothesis, we chose a relevant tumor model based on the mutated version of the epidermal growth factor receptor (EGFRvIII) which is expressed in several cancers. We have genetically modified the murine breast tumor cell line EO771 to express the murine EGFRvIII at low surface density. In an immunocompetent mouse model we studied the role of a Semliki Forest virus (SFV)-based vaccine encoding a the extracellular and transmembrane domains of EGFRvIII (SFVvIII), which does not signalize, to foster the antitumor efficacy of CAR-T cells. We demonstrate that SFVvIII vectors foster the activation, expansion and cytotoxic activity of cognate CAR-T cells in vitro. Moreover, intravenous administration of SFVvIII vaccine strongly increased the number of EGFRvIII-CAR-T cells within the tumor and in both tumor and blood. More importantly, SFVvIII in combination with EGFRvIII-CAR-T cells delayed tumor growth and enhanced overall survival. Overall, our data show that the administration of SFV-based vaccines may increase the therapeutic efficacy of CAR-T therapy in solid tumors.



STAbTAA-1-T cells require costimulatory support for effective targeting of B-ALL cells

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Relapsed B-ALL following CD19-targeted therapies remains a major therapeutic challenge, especially in cases of CD19-negative relapse. TAA-1 is frequently retained and represents a promising alternative pan-B-cell target. However, TAA-1-directed CAR-T cells (CARTAA-1-Ts) have shown limited durable responses. Building on previous studies demonstrating superior efficacy of T cells engineered to secrete bispecific CD19xCD3 or BCMAxCD3 T cell engagers (STAb-T cells) compared with their CAR-T counterparts, we developed TAA-1xCD3-secreting STAb-T cells (STAbTAA-1-Ts). We found that targeting a distal TAA-1 epitope conferred higher binding capacity than targeting a proximal one. However, distal-targeting STAbTAA-1-Ts showed minimal cytotoxicity and weak T cell activation. This likely reflects insufficient costimulation due to the long extracellular domain of TAA-1, a structural limitation not observed with STAb19-Ts, STAbBCMA-Ts, or STAbCD1a-Ts, which all engage proximal epitopes. Co-treatment with TAA-1xco-stimulatory molecule (CSM)-secreting T cells or an agonistic CSM-antibody restored STAbTAA-1-T activity, highlighting the critical role of co-stimulatory signaling in TAA-1-specific STAb-T cell function. Ongoing in vivo studies will assess the efficacy and persistence of this dual TAA-1xCD3+TAA-1xCSM strategy as a next-generation therapeutic approach for B-ALL relapses resistant to CD19-directed immunotherapies.



BACH2 dosing promotes stemness without compromising effector functions to enhance T cell persistence and anti-tumor efficacy.

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T cell therapies hold promise for treating solid tumors, but their efficacy is limited by poor persistence of transferred cells. Previous strategies to enhance persistence often relied on enforcing constitutively activated, and potentially oncogenic, cell states. In contrast, physiological immune responses are sustained by quiescent, self-renewing progenitor T cells that depend on the transcription factor BACH2. These cells maintain stem-like potential while giving rise to short-lived effectors. Here, we show that quantitative control of BACH2 dosage governs the physiological continuum of stem and effector CD8⁺ T cell states and enables engineering of synthetic states with superior persistence and anti-tumor activity. In CD8⁺ T cells, BACH2 is precisely regulated, with intermediate expression in memory and progenitor-exhausted subsets. Excessive levels of BACH2 locks cells in quiescence, curtailing effector function and tumor control. By contrast, low-dose BACH2 permits effector differentiation while preserving stem-like features, thereby enhancing persistence and therapeutic efficacy. Mechanistically, low-dose BACH2 partially restrains AP-1 occupancy at enhancers, attenuating highly AP-1-dependent genes while maintaining effector programs. This dosage principle extends to other quiescence factors, as low-dose FOXO1 expression augments T cell responses in an analogous manner. Thus, quiescence factor dosage emerges as a key regulator of T cell fate, suggesting that quantitative tuning of gene expression can drive qualitative improvements in cancer immunotherapy.



CDR3 β repertoire analysis of PD-1⁺ TIL products: Identifying potential tumor-reactive TCRs for transgenic TCR therapy

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The aim of this project is to track changes in the repertoire of CDR3 β within the TIL product and in the peripheral blood of patients after TIL infusion to identify tumor-specific TCRs. Our final goal is to use these TCRs to generate a second product by either (a) transducing autologous T-cells with transgenic TCRs (t-TCRs) with lentiviral vector or (b) replacing the endogenous TCR with the t-TCR through knock-out knock-in system.

Genomic DNA was extracted from PD-1⁺ TIL products of patients enrolled in the TILS-001 trial (NCT05451784), including two other samples from patient NZ-1014-005: (i) pre-treatment blood (screening), and (ii) blood collected 28 days post-treatment (d28). DNA from unrelated healthy donors and Jurkat cell lines was also included. An in-house multiplex PCR was performed using 23 forward V segment-specific and 13 reverse J segment-specific primers to amplify CDR3 β regions. A second PCR introduced sample-specific barcodes and sequencing adapters. Clonotype analysis was conducted using MIXCR®, Platforma.bio, and ImmuneWatch.

We observed reduced CDR3 β repertoire diversity in TIL products compared to peripheral blood from healthy donors, although variability was noted across individual products. PD-1⁺ selected TILs exhibited higher clonal expansion relative to healthy donor blood. ImmuneWatch enabled annotation of CDR3 β sequences with positive scores for tumor-associated antigens, as well as viral and bacterial antigens. Tracking CDR3 β sequences across the three samples from patient NZ-1014-005 revealed ten unique clonotypes that increased in frequency above 1% at day 28, compared to both the TIL product and pre-treatment blood.

In summary, we have characterized the CDR3 β repertoire of TIL products from the TILS-001 trial, demonstrating reduced diversity and increased clonal expansion. Sequence tracking has identified promising candidates for the development of t-TCR-engineered T cells. Next steps will include analyzing multiple timepoints from all patients enrolled in the clinical trial, performing longitudinal tracking of TCR clonotypes, correlating findings with clinical response, and selecting optimal candidates for the development of transgenic TCR-based therapies.



TCoaRse: a structural coarse-grained approach for generalizable TCR-pMHC class I pairing prediction

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T cells drive the adaptive immune response by recognizing diseased cells through T-cell receptors (TCRs), which bind antigen-derived peptides presented by the Major Histocompatibility Complex (MHC). Predicting TCR-pMHC interactions is crucial for T-cell-directed immunotherapies but remains challenging due to the diversity of TCRs. Existing tools, primarily sequence-based, struggle to generalize to novel (unseen) pMHC targets, limiting broader applicability. To improve this, we have generated the largest synthetic dataset of TCR-pMHC-I structural models to date using AlphaFold3 (33,820 complexes, 169,100 models). These were used to train a structure-based predictor using peptide position-specific energy vectors derived from TCR-peptide and TCR-MHC interactions. To evaluate AlphaFold3 modelling confidence in predictions, we categorized the structural models into quality tiers derived from crystal versus corresponding-model comparisons, which were further adapted for scoring our synthetic data. We obtained 22,181 low-quality, 5,579 acceptable-quality, 11,216 medium-quality, and 1,404 high-quality complexes. Simultaneously, we trained a sequence-based classifier on 34,533 complexes using ESMC embeddings from different TCR regions. We built XGBoost classifiers using structure-only, sequence-only, and combined (bimodal) data. We implemented a SHAP analysis, which identified structural features as the top contributors to predictions, far surpassing sequence-based information. Having assembled and interpreted our predictors, we benchmarked them against state-of-the-art tools on an unseen TCR-pMHC dataset (IMMRE23), ensuring no data leakage. Our results demonstrate improved generalization and top-ranking precision, highlighting the importance of structural features for this task. Overall, this tool has the potential to reliably prioritize bona fide TCR-pMHC-I interactions for immunological and clinical applications.



Armoured-CAR T cell-redirecting immunotherapy for metastatic and refractory Ewing Sarcoma

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Ewing sarcoma (EwS) is the second most common bone and soft tissue sarcoma (BSTS) affecting children and young adults. Limited response rates to current multimodal therapies and the lack of actionable targets contribute to dismal long-term survival in patients with metastatic or refractory disease. This underscores the urgent need for novel therapeutic approaches. Although CAR T-cell therapy holds promise for EwS and BSTS treatment, developing effective and safe CAR T-cells for these solid tumors remains challenging due to i) the scarcity of tumor associated antigens (TAA) that are both specific and safe, and ii) the presence of an immunosuppressive tumor microenvironment (iTME), which hampers CAR T-cell function. Here, we have characterized numerous EwS biopsies and patient-derived xenografts (PDXs) samples through RNAseq, flow cytometry, and immunohistochemistry (IHC), leading to the development of an antibody against TAA-1, an EwS-specific antigen with minimal expression in healthy tissues. TAA-1-directed CAR T cells effectively eliminated EwS cells in vitro and demonstrated potent antitumor activity in localized and metastatic PDX models. Further single cell RNAseq and IHC analysis revealed high expression of TIM-3 ligands in tumor associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) within the EwS-iTME. To counteract this iTME, TAA-CAR T cells were engineered to secrete a molecule blocking the TIM-3 pathway. These armored TIM-3-decoy-secreting TAA-1-CAR T cells outperformed single CARs in tumor killing and induced a pro-inflammatory switch in the iTME. We present a first-in-class CAR T-cell strategy for EwS, potentially extendable to other BSTS, that not only selectively eliminates EwS cells but also secretes a TIM3 blocker to enhance CAR T-cell function and modulate the iTME.



Navigating regulatory challenges in the initiation of clinical trials for advanced therapy medicinal products (ATMPs): a scoping review

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Clinical translation of advanced therapy medicinal products (ATMPs) is complex and entails high costs, as compared to traditional pharmaceuticals. Moreover, ATMPs often target rare diseases or are highly personalized, thus comprising small patient populations and limited market potential. Consequently, ATMP development is primarily conducted in academic institutions and small- and medium sized enterprises (SMEs), institutions which often lack sufficient regulatory expertise. Together with the constantly evolving ATMP regulations, this creates a bottleneck for ATMP developers initiating clinical trials. The present study undertakes a scoping review to identify the regulatory challenges faced by academic and SME ATMP developers when initiating clinical trials. This scoping literature review follows the PRISMA extension for scoping reviews. Three different databases were searched for relevant articles, published between 2014-2024, resulting in 61 articles included for analysis. In addition, websites of EU national regulatory authorities for medicinal products were searched for information regarding available interaction opportunities between ATMP developers and regulatory bodies. The regulatory challenges identified in this scoping review specifically relate to the lack of European as well as national regulatory advice mechanisms and updated regulatory guidance, specifically for clinical trial initiation in ATMPs. We find that developers struggle to navigate the complex data requirements for clinical trial authorization, the GMP-compliant manufacturing of ATMPs and their quality requirements. Moreover, a landscape is built of interaction opportunities between ATMP developers and regulatory bodies in different EU Member States. We hope that this work will be of help when designing new regulatory guidelines and advice mechanisms from regulatory authorities at a European and national level, aimed at boosting clinical translation of innovative ATMPs.



Development of new cellular immunotherapies against cancer based on T lymphocytes trained with bacteria.

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Over the past two decades, novel biological therapies have profoundly reshaped the landscape of cancer treatment (Chen and Mellman 2013; Cole et al. 2023). Immune Checkpoints Inhibitors (ICI) along with Adoptive Cell Therapies, such as CAR-T therapies, have significantly improved overall response rates and enabled long-lasting remissions, particularly in hematologic malignancies. However, the high rates of non-responders and relapses, ranging from 40% to 95% in the case of ICIs (Mao et al., 2023), as well as the limited efficacy of ACTs in solid tumors (Pedrazzoli & Haanen, 2022), remain major challenges to overcome, highlighting the urgent need for more specific and effective therapeutic strategies. In response to this need, our laboratory proposes here a pioneering therapeutic approach based on a previously unreported CD8⁺ T-cell state generated without genetic modification, termed T-Boost. This is achieved through an innovative activation method of CD8⁺ T-cells using bacteria-instructed CD4⁺ T lymphocytes (BacT), which are T CD4⁺ lymphocytes that act as antigen-presenting cells after being instructed with engineered *E. coli* strains carrying tumor antigens (Cruz-Adalia 2017). T-Boost cells exhibit long-term persistence and sustained effector function within the tumor microenvironment. Moreover, they resist tumor-induced exhaustion, efficiently infiltrate solid tumors, and actively remodel the immune landscape by enhancing cytotoxic responses while reducing exhausted T cells and immunosuppressive populations. Remarkably, T-Boost achieves potent antitumor activity without requiring lymphodepleting chemotherapy, thereby minimizing life-threatening toxicities and paving the way toward safer, more durable ACTs for solid tumors.



Embedding GMP Principles in the Development of Next-Generation Tumor-Resilient TCR-T Cells

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Adoptive T-cell therapy with TCR-T cells provides the unique ability to target intracellular antigens, unlike CAR-T therapies which are restricted to membrane-bound proteins. Despite considerable progress in the field, and the recently FDA-approved TCR-T therapy (MAGE-A4), efficacy in solid tumors is often limited by the immunosuppressive tumor microenvironment. Using single cell transcriptomic data, our collaborators (Zhang et al. Nat Med. 2022) have found genetic signatures for T-cells that show resiliency towards immunosuppressive signals, therefore called resilient T cells (Tres). The presence of these cells in infusion products correlates with a favorable outcome for patients undergoing adoptive T-cell therapy. Among several other markers, these cells are characterized by an enriched CD127 expression and lower expression of the FIBP gene. We hypothesize that by implementing genetic engineering (e.g., knock-out of FIBP) we could recreate the resilient phenotype during the manufacturing process of TCR-T cells. We applied CRISPR knockout of FIBP, a gene associated with poor clinical response, followed by enrichment of positive markers and TCR-engineering to recognize common hotspot mutations in solid tumors. Functionality was assessed by using flow cytometry to measure cytokine release in vitro upon antigen-specific stimulation. Importantly, all procedures were performed using clinical-grade reagents, prioritizing GMP-compatible alternatives from the start to support future translation. CRISPR-mediated FIBP knockout reduced transcript expression by ~60% (qPCR), with loss of protein confirmed by western blot. TIDE analysis showed efficient indel formation. Edited Tres exhibited enhanced cytokine release upon antigen-specific stimulation compared to wild-type controls. We will extend this approach to multiple TCRs recognizing hotspot mutations and further optimize GMP-compliant manufacturing workflows to enable clinical translation of Tres-based therapies.



Next-generation of clinical-grade lentiviral vector manufacturing for point-of-care CAR-T cell therapy: Towards democratizing CAR-Ts

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High costs of advanced therapies limit patient access and threaten their long-term sustainability. The Hospital Clínic Barcelona (HCB) is a European leader in developing CAR-T cells against CD19 and B-cell maturation antigen (BCMA) to affordably treat B-cell malignancies (ARI-0001 and ARI-0002h, respectively). Both CAR-T cell therapies were approved for hospital exemption use by the Spanish Agency of Medicines and Medical Devices (AEMPS) in 2021 and 2023, respectively. Indeed, ARI-0001 received the PRiority MEdicine designation by the European Medicines Agency (EMA) in 2022. Access to point-of-care CAR-T cell therapies is critically dependent on the availability of low-cost, high-quality lentiviral vectors (LVVs) to deliver CAR transgene. In HCB, LVVs are currently produced in 10-layer 2D-flasks by HEK-293T cells transfected with the respective plasmid (p)DNAs. The supernatant containing LVVs is harvested after 48 hours, centrifuged, clarified with 0.45µM single-membrane filters and, finally, concentrated/diafiltrated by tangential flow filtration (TFF). These traditional upstream (USP) and downstream (DSP) processes ensure standardized LVVs production. However, scaling-out the USP in a 2D system is inefficient and costly. Additionally, the current DSP does not ensure the removal of process-related impurities to meet regulatory standards for centralized marketing authorization of European Medicine Agency (EMA).

Here, we describe a successful process transfer of our USP into a scalable 3D bioreactor system. We initially achieved a threefold increase in LVV productivity in the 2D-system, from less than 2E6 transducing units (TU) per cm² to ~6E6 TU/cm², by optimizing key parameters of LVV production (i.e.: 293T cell growth, transfection and culture media). This increased LVV yield was then transferred to a 3D fixed-bed bioreactor at low-scale (2.4m²) and subsequently scale-up to a mid-scale bioreactor (10m²) with only minor adjustments. Overall, our optimized and scalable USP in the mid-scale 3D bioreactor yields an average functional LVV concentration of 4.5E7 TU/mL in the raw bulk material, resulting in the collection of 5.5E11 total TU per run. We also optimized our DSP pipeline, achieving an accumulated LVV recovery above 60% and reducing process-related residuals to levels compliant with regulatory requirements. Specifically, after optimizing the clarification and TFF steps, we incorporated a single-step purification using a multimodal CaptoCore700 resin that removed over 99% of major process-related impurities, while maintaining a high functional LVV titer (~2.5E8 TU/mL). Finally, we demonstrate in small-scale comparability studies that these highly pure LVVs can be effectively used in the production of our ARI-0001 product. Remarkably, no differences were observed in the expansion, phenotype or functionality of anti-CD19 CAR-T cells generated with LVVs obtained from our classical versus optimized and scalable LVV manufacturing method.

Overall, the scalability of our optimized USP and DSP ensures a robust supply of high-quality LVVs to support current and future needs of CAR-T cell therapies developed at HCB. Altogether, we present a next-generation LVV manufacturing platform that i) enables a cost-effective model of LVVs production at scale, ii) meets EMA regulatory standards for centralized marketing authorization, and iii) guarantees the long-term sustainability of advanced cell therapies dependent on LVVs.



Tumor-Reactive, Spatially Distinct: Revealing the Landscape of TCRs Within Human Ovarian Tumors Using Spatial Transcriptomics

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Background

Spatial transcriptomics (ST) enables the in situ profiling of gene expression within intact tissues, offering insights into the cellular and molecular landscape of the tumor microenvironment (TME). When combined with spatial TCR sequencing, this approach allows for the analysis of both T-cell clonal distribution and the local transcriptional context. Here, we study the spatial relationships between T-cell clonotypes and tumor regions with distinct immune transcriptional profiles in ovarian cancer. We also explore the potential of this integrative approach to identify tumor-reactive TCRs.

Methods

Four high-grade serous peritoneal carcinoma biopsies were analyzed using the 10X Genomics Visium platform with paired gene expression (GEX) and TCR β libraries. Cell-type deconvolution was performed using RCTD with a scRNA-seq reference atlas. Spatial domains were identified using BayesSpace. TCR clonality and diversity were assessed using Shannon, Gini, and Inverse Simpson indices. TCR reactivity scores were estimated using matched scRNA&TCR-seq data and previously published gene signatures.

Results

BayesSpace identified spatially distinct transcriptional domains within tumor and stromal regions. TCR clonotypes were unevenly distributed across these domains, with some areas showing higher TCR diversity and others dominated by expanded clonotypes. A subset of clonotypes with higher predicted reactivity scores tended to localize in tumor domains enriched in immune signaling pathways, including IFN-I/II responses and HLA gene expression.

Conclusion

These findings suggest that spatial mapping of TCR clonotypes in relation to transcriptional features of the TME may provide insights into localized immune activity in ovarian cancer. While exploratory, this approach could contribute to a better understanding of the spatial dynamics of tumor-infiltrating lymphocytes and their potential to identify tumor-reactive TCRs.



Allele-Specific Regulation of CD33 CAR-T Cells by KIR2DL1 Supports a Strategy for Improved Selectivity in AML immunotherapy

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CAR-T cell therapy has provided patients with hematologic malignancies such as B cell leukemia and lymphoma with potentially lifesaving treatments, but the wider application of this therapy requires overcoming the sometimes life-threatening 'on target – off tumor' responses. For acute myeloid leukemia (AML), a still difficult to treat and deadly disease, strongly tumor-associated antigens have been defined, but their direct targeting by CAR-T cells can cause myelo-suppression and -ablation. Hematopoietic stem cell transplantation, the only treatment with curative attempt available for patients experiencing relapsing or refractory AML, allows for the introduction of HLA-based differences between leukemic cells and donor-derived myeloid lineage cells when performed in clinically relevant haplo-identical donor - patient combinations. Using AML patient samples and in vitro and in vivo assays, we show that by co-expressing a CD33-specific CAR and native, inhibitory KIR2DL1 in T cells these differences can be exploited to obtain strong, MHC allele-dependent and effector - target cell-restricted control of the cytotoxic response of CAR-T cells. Our findings support the development of KIR-gated CAR-T cell strategies in context of clinically relevant haplo-identical hematopoietic stem cell transplants or when tumors undergo loss of homozygosity at the MHC locus.



ImmTAC-secreting T cells as a novel therapeutic platform for melanoma

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ImmTACs (Immune-mobilising monoclonal T cell receptors against cancer) are a novel class of fusion proteins combining a CD8-associated TCR domain with the immune-activating properties of an anti-CD3 ζ single-chain variable fragment (scFv). These molecules trigger anti-tumour responses by targeting peptide-loaded HLA complexes presented by cancer cells. Nevertheless, ImmTAC treatment requires weekly intravenous administration, which results in drug dilution, low intratumoral concentrations, and systemic toxicity, limiting its potential therapeutic efficacy. Based on our experience developing chimeric antigen receptor (CAR)-T cell therapies for haematological malignancies, we propose to genetically engineer tumour-infiltrating lymphocytes (TILs) to secrete ImmTACs (ImmTAC-TILs). By equipping TILs to secrete ImmTACs we aim to achieve T cell homing back to the tumour site and enable a localised delivery of the bispecific molecule.

Here, we report the initial preclinical development of our ImmTAC-TIL therapy. We first investigated the feasibility of genetically modifying T cells from healthy donors to secrete two types of ImmTACs (ImmTAC-T cells): Tebentafusp and IMDA-001 (Immune Mobilising Diversiform Agent), a modified version of Tebentafusp with a proprietary anti-CD3 ζ scFv. ImmTAC-T cells were generated via lentiviral transduction of peripheral blood T cells at a low multiplicity of infection. ImmTAC-T cells expressing IMDA-001 achieved up to 30% transduction, whereas T cells expressing Tebentafusp reached a maximum transduction efficiency of 15%. We did not observe significant differences when tracking T cell subsets (N, EM, CM and TEMRA) throughout expansion. However, Tebentafusp ImmTAC-T cells displayed heightened levels of T cell exhaustion markers and failed to expand as robustly as IMDA-001 ImmTAC-T cells, untransduced (UTD) and CAR-19 T cells. Notably, IMDA-001 ImmTAC-T cells demonstrated potent cytotoxicity against HLA-A2⁺ gp100₂₈₀₋₂₈₈-pulsed targets. Furthermore, ImmTAC secretion by transduced T cells facilitated paracrine coating of non-transduced T cells. Lastly, ImmTAC-mediated cytotoxicity correlated with the upregulation of activation markers in CD8⁺ and CD4⁺ T cells, and the secretion of IFN- γ .

Altogether, our preliminary data suggest that ImmTAC-T cells can be generated and expanded in vitro in a manner comparable to anti-CD19 CAR-T cells, displaying cytotoxic activity through a direct and bystander effect. These findings validate the feasibility and functionality of ImmTAC-T cells and support the further development of ImmTAC-TILs.



Toward TCR Repertoire Sequencing as Predictive and Surrogate Biomarkers in Cancer Immunotherapy

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Over the past decades, cancer care has shifted from conventional modalities to personalized approaches, including immune checkpoint inhibitors (ICIs), adoptive T-cell therapies, and cancer vaccines. Yet durable responses remain limited to a subset of patients, fueling the search for robust biomarkers. Mounting evidence indicates that T-cell receptor (TCR) repertoire features can predict clinical benefit and guide treatment decisions. Immune repertoires span a spectrum: high diversity often reflects immune fitness, while clonal repertoires typically indicate antigen-driven responses. Comprehensive TCR profiling thus emerges as a powerful tool to capture immune status and inform diagnosis, prognosis, and therapeutic monitoring.

TCR profiling has shown potential as a diagnostic and prognostic biomarker, with diversity patterns varying across tumor types and tissues, and higher blood diversity often linked to improved survival. Yet, its most compelling applications lie in predicting and monitoring immunotherapy outcomes. In ICIs, a common tendency links high tumor clonality at baseline with response in melanoma and lung cancer, while early increases in clonality after treatment often correlate with benefit, particularly in melanoma. Still, contrasting results across cohorts and tumor types highlight context-dependent biology and methodological differences. In T-cell therapy, responders' infusion products were enriched for clonotypes already present in the tumor, whereas non-responders predominantly expanded blood-derived clones. Notably, the extent of baseline overlap between tumor and blood repertoires independently predicted clinical outcome, suggesting a path toward blood-based patient stratification.

While evidence linking TCR repertoire features to immunotherapy response is strong, clinical translation has lagged. Current assays lack the resolution, robustness, and predictive modeling capacity needed for real-world use. FDA-approved biomarkers such as PD-L1, TMB, and MSI require tumor biopsies and offer limited predictive value, underscoring the need for better solutions. To address this, we developed SEQTR, the first RNA-based TCR sequencing assay in Switzerland. SEQTR combines unmatched resolution with affordability, scalability, and machine learning-driven modeling. By transforming complex repertoire signals into actionable diagnostics, SEQTR is poised to deliver reliable, non-invasive biomarkers that can unlock the full potential of personalized cancer immunotherapy.



Unravelling tumor-intrinsic resistance mechanisms to T-cell mediated cytotoxicity in pancreatic cancer

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Immunotherapy remains ineffective in pancreatic ductal adenocarcinoma (PDAC). This resistance has been attributed to its low tumor mutational burden, limited immune infiltration, and a desmoplastic, immunosuppressive tumor microenvironment. Challenging this notion, recent studies have shown that neoantigen-specific tumor-infiltrating lymphocytes can be expanded and isolated from pancreatic cancers. Despite this, ex vivo expanded tumor-reactive TILs often fail to recognize and kill the autologous tumor cells, even in the absence of microenvironmental influences. These observations suggest that PDAC cells can evade immune recognition and T-cell-mediated cytotoxicity through cell-intrinsic mechanisms that remain poorly understood. In this study, we sought to identify immune regulators that limit T-cell-mediated killing in pancreatic cancer using a co-culture-based genome-wide CRISPR/Cas9 screen. We employed an in vitro model consisting of a low-passage patient-derived pancreatic cancer cell line that is poorly recognized by TCR-engineered T cells targeting the MMP14p.R158C neoantigen. By conducting a genome-wide CRISPR/Cas9 screen on our in vitro co-culture model, we identified 42 candidate genes whose depletion enhanced susceptibility to T-cell-mediated cytotoxicity. Among the top hits, ablation of the E3 ubiquitin ligase MARCHF5 increased T-cell-mediated killing across three patient-derived in vitro PDAC models, independent of the specific cell line, TCR, and targeted neoantigen. Ongoing mechanistic studies aim to understand the molecular basis by which the loss of MARCHF5 enhances T-cell killing. Preliminary data indicate that MARCHF5 deficiency sensitizes tumors to neoantigen-driven T-cell cytotoxicity through upregulation of the pro-apoptotic protein NOXA. Overall, our work identifies MARCHF5 as a novel target whose immunomodulation holds potential to unleash the immune system's potential to control pancreatic cancer.



Dynamics and relevance of circulating TIL.

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Adoptive cell therapy with tumor-infiltrating lymphocytes (TIL-ACT) is a promising personalized treatment for metastatic epithelial cancers. However, it currently requires a tumor biopsy to isolate and investigate effector cells. Since TIL can circulate from tumors, lymph nodes, and metastases through the bloodstream, and tumor-reactive T cells are detectable at low frequencies in peripheral blood, circulating TILs (cTIL) represent an attractive surrogate. Liquid biopsy could thus provide an accessible source to study the TIL repertoire and isolate tumor-reactive T-cell receptors (TCRs) without requiring invasive procedures, while potentially capturing heterogeneity across lesions.

Here, we aimed to (i) characterize the TCR repertoire overlap between tumor and blood, (ii) identify factors influencing TIL circulation, and (iii) derive a proteo-transcriptomic signature of circulating tumor-reactive T cells (cTTRT). Matched tumor and blood samples (CD45⁺ sorted or CD8⁺ enriched, respectively) were collected before treatment from seven patients with metastatic melanoma undergoing TIL-ACT (3 responders, 4 non-responders). Single-cell RNA sequencing, TCR sequencing, and CITE-seq (132 antibodies) were performed. Using TCRs as barcodes, clonotypes were classified as unique to blood (Bo, n=13,401), unique to tumor (To, n=3,789), or shared (cTIL, n=571). Clonotypes were further annotated as tumor-reactive (TR) or non-tumor-reactive (nonTR) using validated cloning data or an in-house in silico predictor (TRTpred), a tool developed in our lab.

Clinical benefit from TIL-ACT was associated with higher CD8⁺ T-cell repertoire richness and clonality in tumors, but not in blood. Shared TCRs were enriched among abundant clonotypes, suggesting that clonotype frequency facilitates circulation though it is not the sole determinant. Yet a greater fraction of cTIL was detected in the blood of responders. In tumors, they were more cytotoxic and less exhausted than non-circulating T cells; in blood, they displayed stronger effector programs but reduced stemness compared to antigen-experienced cells found exclusively in blood. Tumor-reactive clonotypes were preferentially retained in tumors, with only a small fraction entering circulation, limiting the possibility of defining a robust cTTRT signature in this dataset.



High CD70 Expression in Newly Diagnosed Follicular Lymphoma Is Linked to Relapse and may be exploited through dual CD19-CD70 CAR-T therapy.

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High CD70 Expression in Newly Diagnosed Follicular Lymphoma Is Linked to Relapse and may be exploited through dual CD19-CD70 CAR-T therapy. Follicular lymphoma (FL) is the most common indolent B-cell lymphoma. It remains incurable, with many patients experiencing frequent relapses that increases the risk of transformation to aggressive lymphomas. Although CD19-targeted CAR-T therapies (CART19) are approved for relapsed/refractory (R/R) FL, durable responses remain limited, with a 5-year progression-free survival (PFS) of only 43% (Chong et al., NEJM, 2021). There is a critical need to identify high-risk patients early and develop more effective, personalized therapies.

This study investigated CD70 expression in FL and its potential as a therapeutic target. Immune profiling (nCounter Pan Cancer, Nanostring®) of diagnostic FFPE biopsies taken from FL patients revealed higher levels of CD70 mRNA in patients who later relapsed (n = 20) than in those who achieved long-term remission (n = 12, median follow-up: 14.1 years). This was found to correlate with inferior PFS. Validation at protein level via multiplex immunofluorescence confirmed increased CD70 expression in the relapsed cohort, primarily in tumor cells as well as in subsets of tumor-infiltrating T cells, including CD4⁺, CD8⁺, regulatory T cells and T follicular helper cells. Functional studies showed that CD70⁺ tumor cells had higher proliferation when co-cultured with follicular dendritic cell-derived stimuli. Also, knocking out CD70 (via CRISPR/Cas9) in primary FL cells reduced proliferative responses. To advance towards personalized therapies for these high-risk patients and taking advantage of the academic CAR-T program at our institution, we generated a dual CD19-CD70 CAR-T. A truncated CD27-based construct (CD70 natural ligand) exhibited enhanced anti-lymphoma activity compared to an scFv-based version. The dual CAR-T was successfully generated from healthy donor and FL patient PBMCs, showing robust cytotoxicity in vitro and in patient-derived lymphoma spheroids, particularly in CD19^{low} samples. In vivo, the dual CAR-T demonstrated enhanced tumor control and T-cell expansion compared to CAR-T19 alone and was effective in both CD19⁺ and antigen escape scenario FL models. Overall, this study uncovers the prognosis potential of CD70, its biological role in FL patients and the generation of a novel dual CD19-CD70 CAR-T with therapeutic potential in FL and other B-NHL. Ongoing studies aim to further validate these results and to optimize its clinical application.



Preclinical characterization of ARI-007: CD7-negative T-cells engineered with an academic anti-CD7 CAR for T-cell cancer treatment

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Three in-house CD7-specific scFvs were tested to generate 4-1BB-based CAR-T cells, incorporating CRISPR/Cas9-mediated CD7 knockout to mitigate fratricide. All candidates demonstrated comparable cytotoxicity, cytokine secretion and proliferation against T-ALL cell lines. In a T-ALL mouse model, 124.1D1 and 142.24 CAR-T cells outperformed 142.9 CAR-T cells in tumor control, selecting 142.24-CAR as the final candidate.

We next compared different fratricide-mitigation strategies: i) CD7 knockout, ii) protein expression blocker (PEB) (124.1D1 scFv fused to endoplasmic reticulum retention domain), iii) inhibition of CAR signaling using dasatinib (tyrosine kinase inhibitor), iv) short (3-day) expansion and v) selection of natural CD7neg T-cells. PEB- and CD7KO-CAR-T cells effectively extended survival, in contrast to dasatinib-expanded cells; however, none of these approaches induced long-term responses. CAR-T cells expanded for three days showed enhanced IL-2 production and proliferation. After infusion of these shortly-expanded CAR-T cells to tumor-bearing mice, the CD7neg CAR-T cell population self-selected, improving T-cell persistence and survival. Similarly, CAR-T cells generated from the naturally-occurring CD7neg population achieved durable responses and extended animal survival.

Based on the antitumor potential of CD7neg T-cells, we characterized their phenotype and function. CyTOF analysis of human reactive lymph nodes revealed a heterogeneous population, with approximately 50% CD4⁺ TCM/EM, along with CD8⁺ TEM, Tregs CM and a small fraction of naïve T-cells. During manufacturing, CD7neg T cells showed increased susceptibility to activation-induced death and reduced proliferation than bulk T-cells. Nonetheless, CD7neg T-cells showed increased spare respiratory capacity and polyfunctionality, secreting higher levels of effector and chemoattractive cytokines.

Based on these results and the reduced risk of blast contamination, CD7neg 142.24 CAR-T strategy (ARI-007) was selected for evaluation in a phase I clinical trial in patients with T-ALL/LBL and CD7pos acute myeloid leukemia (AML).



Development of bispecific EGFR/MET-Targeted CAR Macrophage Cell Therapy for Non-Small Cell Lung Cancer

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Chimeric Antigen Receptor (CAR) T cell therapy has revolutionized the treatment of hematologic malignancies, such as leukemia and lymphoma. However, its translation to solid tumors has been restricted by barriers including poor tumor infiltration, antigen heterogeneity, and an immunosuppressive tumor microenvironment. In NSCLC, tumor-associated macrophages contribute substantially to immune evasion, impairing immune surveillance and reducing treatment effectiveness.

CAR-engineered macrophages (CAR-Ms) offer a promising alternative. These cells possess intrinsic phagocytic and cytotoxic activity, enhanced tumor penetration, and a reduced risk of graft-versus-host disease. Collectively, these properties may overcome limitations of CAR-T therapies in solid tumors.

We designed bispecific CAR constructs based on the antibody Amivantamab, which recognizes EGFR and MET—two antigens frequently overexpressed in NSCLC. The constructs include a GM-CSF signal peptide, dual single-chain variable fragments (scFvs) specific for EGFR and MET joined by a flexible linker, CD8 α hinge and transmembrane domains, and a CD3 ζ signaling domain. This design enables dual antigen recognition and functional activation in macrophages.

Protein structures of EGFR and MET extracellular domains were modeled with ESMFold, followed by molecular docking and affinity analysis. The CAR with the highest predicted affinity for EGFR and strong binding to MET was prioritized for subcloning and CAR-M production. Lentiviral packaging and transduction were performed in THP-1 and Jurkat cells as well as human PBMCs. Functional assays using the H1975 NSCLC cell line assessed phagocytosis, immunomodulation, and antitumor efficacy.

In conclusion, our study demonstrates the feasibility of developing a first-generation bispecific EGFR/MET-targeted CAR-M therapy, providing a potential strategy for treatment-resistant NSCLC.



Molecular signature-based approach for identifying tumor-specific TCRs

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Given the limitations of CAR-T cells in the treatment of solid tumors, TCR-T cells emerge as a promising alternative with highly significant advantages. In terms of antigen recognition, TCR-T cells are capable of identifying a greater number of potential target antigens on tumor cells, as MHC presentation is required, which expands the options to thousands of intracellular antigens, including neoantigens. Furthermore, the antigen sensitivity of TCR-T cells is up to 100 times higher than that of CAR-T cells, meaning that they require fewer surface antigens to be activated, significantly increasing the chances of response. Additionally, the physiological regulation in cells with modified TCRs is much better than that found when introducing a CAR, which favors long-term tumor growth control. TCR-T therapies currently available are based on the identification of TCRs through antigen-focused methods, which require costly and time-consuming processes.

To simplify the selection of the most effective TCR-Ts for therapy, we have developed "CIMA_sig", a transcriptomic signature of tumor-reactive lymphocytes based on differential analysis of gene expression, primarily focusing on genes related to activation and exhaustion, and completely independent of the antigen. Thus, by applying this signature to the single-cell RNA and TCR sequencing (scRNA&TCRseq) data obtained from the tumor-infiltrating T lymphocytes (TILs) of the patient, we can predict the TCRs that will perform best in adoptive cell therapy. To empirically validate CIMA_sig, we applied it to scRNA&TCRseq data from TILs of a breast cancer mouse model and selected 20 TCRs, of which 14 scored highly in this signature (TCR-hi), 3 scored low (TCR-lo), and 3 scored intermediate (TCR-i). We cloned them, generated retroviruses, and genetically modified murine T lymphocytes. In vitro assays showed that 12 out of 14 TCR-hi (86%) exhibited reactivity against the corresponding tumor cell line, while none of the TCR-lo and only 1 of the 3 TCR-i were reactive. In vivo, 10 out of 13 TCR-hi evaluated (77%) managed to control tumor growth, whereas all TCRs with intermediate or low scores (6/6) accelerated it. Our data suggest that using molecular signatures to predict tumor-specific TCRs could be an effective strategy for identifying those with therapeutic potential.



Oncolytic Adenovirus armed with a Bispecific T Cell Engager Targeting Folate Receptor Alpha for the Treatment of Ovarian Cancer.

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Oncolytic Adenovirus armed with a Bispecific T Cell Engager Targeting Folate Receptor Alpha for the Treatment of Ovarian Cancer. Oncolytic adenoviruses (OAd)s combine direct tumor cell lysis with the ability to inflame the tumor microenvironment and recruit T cells, a response now recognized as key for therapeutic success. However, these T-cell responses are often suboptimal or skewed toward antiviral rather than antitumoral activity. To address this, we engineered the OAd ICOVIR15K to express a bispecific T-cell engager (BsTE) targeting folate receptor alpha (FR α), a tumor-associated antigen overexpressed in many tumors and especially in ovarian cancer. By linking CD3 on T cells with FR α on tumor cells, the virus exploits the inflammation it induces to redirect recruited T cells against both infected and uninfected tumor cells. ICOVIR15K-FR α BsTE induced strong, antigen-dependent T-cell activation, as shown by upregulation of CD25 and 4-1BB, secretion of IFN γ , TNF α , and IL-2, and robust T-cell proliferation. Activated T cells mediated superior killing of FR α -positive ovarian cancer cells compared to the parental virus in real-time cytotoxicity assays and across multiple cell lines. Notably, the BsTE enabled a bystander effect, eliminating virus-resistant tumor cells when co-cultured with infected cells and T cells. In vivo, ICOVIR15K-FR α BsTE replicated within tumors and, combined with human T-cell transfer, significantly delayed SKOV-3 tumor growth in NSG mice. Altogether, this strategy transforms OAd)s into T-cell-redirecting immunotherapies, amplifying antitumor immunity, extending killing to resistant cells, and confining potent immune activity to the tumor site.



In vivo-based CRISPR screen identifies ZC3H12C as a mediator of CAR-T cell dysfunction in solid tumors

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NSG mice bearing ovarian xenograft tumors were infused with a single dose of anti-HER2 CD28-based CAR-T cells. Tumor-infiltrating T cells (CAR-TILs) were isolated from tumors after infusion (effective phase) and following therapy failure (dysfunctional phase). Bulk RNA-seq and single-cell RNA-seq analysis were performed on dysfunctional CAR-TILs compared to effective CAR-TILs and preinfusion product. Dysfunctional CAR-TILs exhibited hallmarks of exhausted T cells, including impaired ex vivo effector functions and enrichment in exhaustion signatures.

To elucidate mediators of CAR-T cell dysfunction, we selected 300 significantly upregulated genes in dysfunctional CAR-TILs and designed a sgRNA library to genetically modify CAR-T cells using CRISPR/Cas9 technology. We identified 14 significantly enriched gene knockouts in CAR-TILs after in vivo screen, and targeted deletion of four selected candidates enhanced the antitumor effect of CAR-T cells against ovarian xenograft tumors. ZC3H12C emerged as the top the hit in the screen and was confirmed by single-cell RNA-seq to be highly expressed in CAR-TILs undergoing exhaustion in vivo. Genetic ablation of ZC3H12C in HER2-CD28z CAR-T cells mediated enhanced cytotoxicity and cytokine production after repeated stimulations with tumor cells in vitro, compared to control AAVS1-KO CAR-T cells. In vivo, ZC3H12C-KO CAR-T cells showed superior antitumor responses, enhanced T cell persistence and reduced expression of exhaustion markers. Similar results were observed using different in vivo models of lung and pancreatic tumors with Mesothelin-CD28z and CD19-BBz CARs.

By using an in vivo custom CRISPR/Cas9-mediated screen we identified potential gene mediators of CAR-T cell dysfunction. Our findings implicate ZC3H12C in CAR-T cell dysfunction following chronic antigen exposure and demonstrate that ZC3H12C-deficient CAR-T cells exhibit enhanced antitumor activity against solid tumors.



Identification of clinically relevant T cell receptors for personalized T cell therapy using combinatorial algorithms

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The adoptive transfer of genetically engineered cells expressing specific T-cell receptors (TCRs) represents a promising personalized cellular therapy. Given the requirement to match both tumor antigens and HLA restriction, only few patients are eligible to these therapies. By exploiting the distinct transcriptomic profile of bone fide tumor-reactive T cells (TRT) relative to bystander cells, we built a personalized in-silico predictor of orphan tumor-reactive TCRs. The predictor, named TRTpred, was benchmarked against several published signatures on 42 tumors, which included 19 melanomas, 17 gastrointestinal cancers, 4 lung cancers, and 2 breast cancers. Using the predictor, we also revealed that the TRT repertoire is consistently richer and more clonal than its bystander counterpart in all tumor indications investigated. Furthermore, in melanoma, we observed that TRTs were predominantly located within tumor islets, while bystanders T-cell clones accumulated in the stroma. Beyond its discovery applications, TRTpred was utilized to identify clinically relevant clones for personalized T-cell therapy. To this end, we developed MixTRTpred by integrating TRTpred with a TCR avidity predictor and a TCR clustering tool (to select panels of relevant TCRs inferred to target different antigens) and benchmarked it in vitro and in vivo. Taking advantage of recent advances in the field of T cell engineering, the accuracy of MixTRTpred indicates that personalized TCR-based therapy is now achievable for most patients with solid tumors.



ICAM-1–Directed CAR-T Cells as a Novel Therapeutic Strategy for Prostate Cancer

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Prostate cancer is the most prevalent malignancy among men. Statistical analyses of patient-derived clinical samples have revealed a significant increase in intercellular adhesion molecule-1 (ICAM-1) expression in prostate tumors, which further escalates with disease progression. This identifies ICAM-1 as a promising target for CAR-T cell therapy, given its elevated expression on both prostate cancer cells and within the tumor-associated stroma. However, although CAR-T therapies have shown remarkable success in treating hematological malignancies, their application to solid tumors remains a major challenge due to the complexity of the tumor microenvironment and antigen heterogeneity. In solid tumors, CAR-T cells efficacy is hampered by dose-limiting toxicities, “on-target, off-tumor” effects, and the difficulty of sustaining effective immune responses within an immunosuppressive microenvironment. A Dual CAR-T strategy may help to overcome these limitations. The aim of this project is to develop an ICAM-1–directed CAR-T therapy for advanced prostate cancer and to explore its combination with a PSMA- directed CAR in a Dual CAR-T strategy. The goal is to enhance efficacy, safety, and therapeutic durability.

Initial studies validated the binding capacity of ICAM-1 single-chain variable fragments (scFvs) to their antigen and their ability to activate CAR responses in second-generation CAR constructs. The ICAM-1 scFv sequence, derived from the RM3A5 hybridoma cell line developed at our institution, was incorporated into CAR designs. The evaluation included ex vivo T-cell expansion potential and in vitro antitumor activity using patient-derived prostate cancer cell lines.

The second-generation ICAM-1 CAR demonstrated the ability to bind its target antigen, activating intracellular signaling pathways that induced T-cell effector functions. ICAM-1 CAR-T cells showed cytotoxic activity against prostate cancer cell lines in vitro when derived from ICAM-1 knockout T-cells, thereby preventing self-targeting interactions.

ICAM-1–directed CAR-T cells demonstrated effective recognition and killing of prostate cancer cells in vitro, supporting their potential as a therapeutic approach in advanced disease. Furthermore, these findings support the development of Dual PSMA–ICAM-1 CAR-T therapies.



Towards the ultimate recipe for ATMPs: navigating regulatory requirements for GMP-conform manufacturing

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The regulatory framework for ATMPs began with Directive 2004/23/EC and was further reinforced by Regulation (EC) No. 1394/2007. This marked a pivotal shift, classifying tissue- and cell-bank-based products as medicinal products and integrating them into the EU pharmaceutical system. Building on this, the European Commission introduced specific Good Manufacturing Practice (GMP) guidelines for ATMPs (EudraLex Volume 4 – Part IV). Despite this progress, developers continue to face challenges in implementing GMP requirements, which hinders national and international harmonization, the development of transnational manufacturing, and clinical testing of ATMPs within the EU.

Within the EU-funded JOIN4ATMP consortium, which unites leading hospitals from 10 member states, we conducted an open online survey to identify the main barriers to GMP-compliant ATMP manufacturing, including organizational and infrastructural requirements. The survey was completed by 265 experts—developers, manufacturers, and regulatory specialists—from institutions in 20 different countries across Europe. Of these, 84 participants reported direct experience with GMP in the context of ATMP development and completed the specific section of the survey. Their responses highlighted quality control and process validation as the most pressing challenges, largely due to the biological complexity and variability inherent to ATMPs.

Using CAR-T cells as a real-world test case, we observed a strong convergence in release criteria across institutions. Common parameters included viability, purity (% CAR-expressing cells), dosage, vector copy number, endotoxin levels, mycoplasma detection, sterility, and visual appearance. However, potency assays were not consistently included as formal release criteria. Our analysis also uncovered substantial differences in manufacturing practices, particularly in T-cell expansion protocols and logistical workflows, making process validation across centers especially difficult.

These findings underscore both the shared interpretations and local adaptations of GMP requirements. Striking the right balance between harmonization and flexibility will be crucial to enable scale-up and decentralized production across the EU. To deepen these insights, we are planning live interviews with selected survey participants in the coming months.

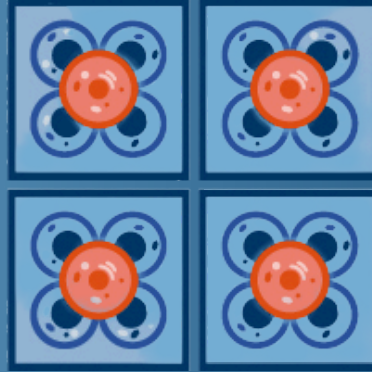


Characterization of BB-NK-001: an academic IL-12/15/18- preactivated NK cell product for the treatment of acute myeloid leukemia

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Short preactivation of natural killer (NK) cells with interleukins (IL)-12, IL-15, and IL-18 induces the development of cytokine-induced memory-like (CIML) NK cells that exhibit memory-like properties and enhanced anti-tumor activity, making them a promising option for treating acute myeloid leukemia (AML) and other cancers. Automated cell processing systems such as the CliniMACS Prodigy® (Miltenyi Biotec) facilitate the cost-effective GMP-compliant production of such cell therapies in academic settings. We have manufactured an academic IL-12/15/18-preactivated NK cell product, named BB-NK-001, using the CliniMACS Prodigy® system. For that, NK cells were isolated from leukapheresis from healthy donors and preactivated for 18h with IL-12/15/18. After incubation, cells were harvested and freshly used. BB-NK-001 cells were phenotypically characterized by flow cytometry. Their effector functions and cytotoxic activity were evaluated against hematological cancer cell lines (K562, 721.221, Jurkat, Ramos) and primary AML blasts, and their proliferation response to IL-2. Non-preactivated NK cells were also used as controls.

Finally, BB-NK-001 cell biodistribution was assessed in immunodeficient NSG mice, and in vivo anti-tumor efficacy of BB-NK-001 cells was evaluated in a K562-GFP/Luc-xenograft NSG mouse model. Flow cytometry analysis revealed that BB-NK-001 products were highly pure in NK cells (99% of CD56⁺CD3^{neg} cells), highly enriched in activation markers (CD25, CD69, and CD137), and had a greater proliferative capacity. Additionally, they showed higher degranulation (CD107a expression), increased production of effector cytokines (IFN- γ and TNF- α), and greater cytotoxic activity against tumor cells compared to control NK cells. Finally, BB-NK-001 cell in vivo biodistribution was detected at 1 and 7 but not at 90 days after cell injection in various organs, including blood, spleen, bone marrow, lungs, and liver, in an organ-specific and dose-dependent manner. Finally, K562-xenograft mice treated with BB-NK-001 demonstrated an increased survival, reduced leukemia burden and reduced presence of tumor cells in liver and lungs. Our data show that IL-12/IL-15/IL-18-preactivated NK cells can be successfully produced using the CliniMACS Prodigy® system, and are effective against hematological malignances supporting their potential as cancer immunotherapy."



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