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Libro de abstract – Comunicación oral

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Rewiring Leukemia Aggressiveness by Breaking the ITGA4-NG2 Axis in KMT2A-rearranged B-ALL.

KMT2A-rearranged B-cell acute lymphoblastic leukemia (KMT2A-r B-ALL) is an aggressive subtype of leukemia, characterized by high relapse rates, therapy resistance, and poor prognosis. Although CD19-targeted immunotherapies have significantly benefited patients with relapsed/refractory (R/R) disease, relapses remain common and long-term survival is especially poor in those with KMT2A-r B-ALL. We recently identified the membrane-bound proteoglycan NG2 (CSPG4) as direct transcriptional target of KMT2A fusions, with its expression associated with poor prognosis, early relapse, and glucocorticoid resistance in KMT2A-r B-ALL. However, the molecular mechanism underlying the aggressiveness of KMT2A-r B-ALL remains poorly understood. Here, we identify the α4 integrin subunit (ITGA4) and NG2 as a key biological axis contributing to leukemic aggressiveness. NG2 expression promotes proliferation and migration of KMT2A-r B-ALL cells and it is associated with Rho GTPase activity in an ITGA4-dependent manner. In vivo studies using immunodeficient mice demonstrated that ITGA4 and NG2 cooperate to promote leukemogenesis as combined genetic ablation of both genes significantly delayed disease onset and prolonged survival. Notably, Natalizumab (NTZ)— an FDA/EMA-approved monoclonal antibody targeting ITGA4— delayed leukemia progression and potentiated the efficacy of standard-of-care chemotherapy in KMT2A-r B-ALL patient-derived xenograft models. Collectively, our findings define a novel ITGA4-NG2 signaling axis that drives the aggressiveness of KMT2A-r B-ALL and support the repurpose of NTZ as an adjuvant therapeutic strategy for this high-risk leukemia subtype.

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Development of anti-CLL-1 CAR-T cells from peripheral blood and comparison with anti-CLL-1 CAR-T cells from umbilical cord blood for the treatment of Acute Myeloblastic Leukemia

Acute myeloid leukemia (AML) remains one of the hematologic malignancies with the poorest prognosis, with 5-year survival rates still limited despite intensive chemotherapy and hematopoietic stem cell transplantation. While CAR-T cell therapies have transformed the treatment landscape of lymphoid malignancies, no CAR-T product has yet been approved for AML, largely due to the challenge of identifying safe targets that spare healthy hematopoietic stem cells. CLL-1 (C-type lectin-like molecule-1) is a promising antigen expressed in most AML blasts but absent in healthy hematopoietic stem cells, making it an attractive and safer therapeutic target.

This project focuses on generating and characterizing anti-CLL1 CAR-T cells derived from peripheral blood (PB) and comparing them with CAR-T cells produced from umbilical cord blood (UCB), a source with unique immunological advantages for allogeneic and universal CAR-T manufacturing. CD3+ T cells were isolated, activated, and transduced with a second-generation lentiviral anti-CLL1 CAR containing a 4-1BB co-stimulatory domain. Transduction efficiency, phenotype, exhaustion status, and cytotoxic potential were evaluated. The antitumor activity of PB-CAR-T and UCB-CAR-T was assessed against AML cell lines (THP1, HL60) and primary AML samples.

Both CAR-T products demonstrated specific and robust cytotoxicity against CLL-1–expressing AML cells. However, UCB-CAR-T cells exhibited superior antitumor efficacy, reduced exhaustion, enhanced immune persistence, and a more favorable safety profile compared with PB-CAR-T cells. These findings highlight the potential of UCB-derived CAR-T cells as an optimal platform for the development of universal, off-the-shelf CAR-T therapies for AML. This work supports further in vivo studies and provides a strong foundation for advancing universal anti-CLL1 CAR-T therapies, addressing a critical unmet need for innovative and safer treatments in AML.

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When do we start aging?

Although the circadian rhythm itself is not active in the embryo, components of the circadian cycle, such as the protein BMAL1, are present from early embryonic stages and regulate key biological processes that guide development. This work explores how BMAL1 helps protect the organism from premature aging by ensuring proper epigenome formation, emphasizing the connection between early molecular regulation and the mechanisms that control aging.

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Specific molecular pathways define distinct disease activity based trajectories in Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic, autoimmune and multisystemic disease affecting mainly women and associated with high morbidity and mortality. Its clinical and molecular heterogeneity partially explains the limited success of standard treatments and approved biologics, complicating optimal therapeutic decisions. Currently, therapeutic decisions in SLE are trial-and-error based, until symptoms remit. However, by the time the correct combination of medications is found, significant damage may have occurred, sometimes leading to irreversible tissue and cellular dysfunction, being lupus nephritis one of the most severe manifestations. In such cases, patients may become refractory and unresponsive to any known medication. Within this context, stratifying patients according to their response magnitude, regardless of the treatment initiated, could facilitate the development of personalized medicine approaches in SLE.

An observational cohort of 168 flaring SLE patients initiating a range of commonly prescribed therapies was analyzed. Patients were followed and sampled up to 52 weeks after treatment initiation. Blood transcriptomic profiling was performed using RNA sequencing. Patients were clustered according to their disease activity trajectory, based on a normalized measure of change in the SLEDAI-2K index over time. Interaction models were conducted to assess temporal changes within each trajectory, followed by functional enrichment analyses to characterize the underlying biology. Additionally, predictive models were developed to forecast treatment response within each trajectory.

Preliminary results identified four distinct trajectories, including a subset of "Super Responders", patients who exhibited a marked reduction in disease activity within six weeks of treatment initiation. This group was characterized by a pronounced downregulation of genes related to inflammation and neutrophil activity. Stratifying patients based on these trajectories could inform more precise therapeutic decisions, minimizing exposure to ineffective treatments and ultimately improving prognosis and quality of life for individuals with SLE.

Diego de Miguel Perez

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Extracellular vesicle proteomics as biomarkers of resistance to immune-checkpoint inhibitors in advanced tumors

Background: Patients with advanced solid tumors often develop resistance to immune checkpoint inhibitors (ICIs), highlighting the need to understand this complex mechanism and find predictive biomarkers that guide treatment stratification. Plasma-derived extracellular vesicles (EVs) have emerged as promising minimally invasive immunotherapy biomarkers. We hypothesize that plasma EV protein cargos associate with resistance and outcomes in patients with advanced cancers receiving ICI.

Methods: We analyzed plasma samples from a cohort of patients from the prospective Immune Resistance Interrogation Study (NCT04243720) collected at the time of progression on ICI therapy. EVs were isolated using serial ultracentrifugation, characterized following ISEV guidelines, and analyzed using OLink Immuno-Oncology proteomics panel of 92 proteins.

Results: A total of 57 samples were analyzed, including 37 patients with primary resistance (PR) and 20 with acquired resistance (AR) who received anti-PD-(L)1 in monotherapy or combinations. Approximately, two thirds were melanoma and one third HNSCC patients. Across all samples, we found overexpression of EV levels of ADGRG1, CD28, FGF2, IL10, IL12RB1, IL2, IL33, IL4, MCP3, PD-L2, PTN in AR compared to PR ($p<0.05$). These proteins were enriched in pathways involved in immune regulation and anti-tumor immune responses such as JAK-STAT signaling, T cell activation, and cytokine receptor interactions. Moreover, high levels of these 11 EV proteins were associated with better outcomes including durable response and longer overall survival (OS), independent of adjuvant or metastatic treatment settings.

Conclusions: EV-derived proteins associated with AR with high accuracy, revealed a distinct mechanism of ICI escape, and were an independent predictive biomarker of clinical outcomes including OS. These results underscore the potential of EV proteins as minimally-invasive predictive biomarkers for ICI resistance in solid tumors and to guide clinical decisions or design novel strategies for the treatment of resistant tumors.

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DGCR8 controls retrotransposon-derived double-stranded RNA accumulation and interferon activation

The type I interferon (IFN) response is the main innate immune pathway against viruses in mammals. This pathway must be tightly regulated to prevent viral spread while avoiding excessive immune responses. Here, we show that inactivation of the double-stranded RNA (dsRNA)-binding protein DGCR8 unleashes the IFN response in human cells. We demonstrate that, independently of its function in miRNA biogenesis, DGCR8 restricts the accumulation of endogenous dsRNA. These dsRNAs originate from protein-coding mRNAs that harbour transposable elements (TEs), primarily LINE and SINE elements. We propose that DGCR8 binding to TE-rich mRNAs is essential to resolve dsRNA structures, and in its absence, accumulated dsRNA signals through MDA5 signalling pathway triggering the IFN response. This mechanism may be particularly relevant to conditions where DGCR8 expression levels are altered, such as the 22q11.2 deletion syndrome (22qDS). Supporting this, we show that cells derived from 22qDS patients exhibit an exacerbated type I IFN response, highlighting the importance of suppressing endogenous dsRNA accumulation to prevent unwanted immune activation.

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Improving single-nucleotide variant detection with a novel Dual-Guide CRISPR/Cas13 strategy

The emergence of CRISPR-Cas systems has transformed nucleic acid detection and manipulation. Cas13, a type VI CRISPR effector, targets RNA with high sensitivity through both cis (target RNA) and trans (collateral RNA) cleavage. This property enables the use of fluorescent reporters for sensitive diagnostics. However, Cas13's heightened sensitivity also leads to reduced specificity due to its susceptibility to single-nucleotide mismatches, potentially causing off-target effects. To overcome this limitation, we developed the first dual-guide RNA system for Cas13 that enhances mismatch discrimination and improves target specificity. This system employs two distinct RNAs—dcrRNA and dtracrRNA—which hybridise to refine target recognition and activation. In vitro experiments demonstrated robust cis- and trans-RNase activity, indicating efficient and specific cleavage. The system accurately detected SARS-CoV-2 RNA, demonstrating its potential for pathogen diagnostics, and successfully discriminated between KRAS G12D and G12C mutations—clinically relevant single-nucleotide variants in cancer diagnosis. These results highlight the dual-guide Cas13 platform's potential for precise, rapid, and reliable RNA detection. Overall, this approach represents a significant advance over conventional Cas13 systems, offering improved specificity without compromising sensitivity. Its versatility makes it a promising tool for next-generation molecular diagnostics and precision gene editing applications.

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FIBROMARK: Identification and Validation of Biomarkers for Early Diagnosis of Pulmonary Fibrosis

FIBROMARK is a study combining clinical research and technological development to identify and validate biomarkers for the diagnosis and progression of pulmonary fibrosis (PF). PF is a chronic disease characterised by progressive lung scarring, reduced respiratory capacity, reduced quality of life, and increased mortality. The FP represents ≈40% of ILD, ≈20% of ILD is undetermined, and ≈18-30% of non-fibrotic ILD will develop FP. The identification of consistent and specific biomarkers may facilitate early and acute diagnosis of PF and help discriminate between PF, other ILD and lung diseases.
Currently, the diagnosis of PF relies on pulmonary function tests (PFTs), standard imaging techniques such as high-resolution computed tomography (HRCT), and nonspecific blood tests to detect biochemical markers that help distinguish it from other diseases, including autoimmune disorders. In this context, we propose the use of liquid biopsy techniques as a minimally invasive, precise, and feasible alternative or complementary diagnostic tool.
Using a multi-omic and translational approach that combines quantitative proteomics (extraction, isolation, and characterisation of extracellular vesicles [EVs] and OLINK analysis), ELISA-based validation, and predictive models, our team has identified two potential biomarkers associated with PF development and diagnosis. The biomarkers in question have been described to successfully discriminate Idiopathic pulmonary fibrosis (IPF) patients (these subjects were selected because they present fibrotic-specific phenotype and decrease or avoid biases) from those with COPD and healthy controls, demonstrating a high predictive value (AUC = 0.94).
Preliminary results suggest that these biomarkers may reflect common fibrogenic mechanisms in PF. Future work will involve developing a multiplexed Luminex-type assay as a 'liquid biopsy' for the early diagnosis and personalised management of IPF, supporting the use of precision medicine to distinguish it from other pulmonary or autoimmune-related disorders.

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Integrated CRISPR-Based Engineering and Scalable Manufacturing of Next-Generation CAR-T and TCRKO Cell Therapies

With seven CAR-T therapies already approved by the European Medicines Agency and the U.S. Food and Drug Administration, CAR-T cells have become an established part of the therapeutic arsenal against hematological cancers, achieving unprecedented success in patients with refractory B-cell malignancies. Nevertheless, significant room for improvement remains, as clear therapeutic benefits are still lacking in several groups of leukemia and lymphoma patients.

The limited efficacy observed in non-responders is attributed to multiple factors, including premature exhaustion or death of infused CAR-T cells, the immunosuppressive nature of the tumor microenvironment, and the emergence of treatment-resistant tumor cells. Overall, between 30% and 70% of patients with B-cell lymphomas treated with anti-CD19 CAR-T cells either fail to respond or eventually relapse. Furthermore, many patients are unable to access CAR-T therapy because they do not meet the strict eligibility criteria currently required.

In this context, one of the goal of the TGyC group is to develop universal CAR-T cells derived from healthy donors as an alternative to autologous (patient-specific) CAR-T products for the treatment of B-cell malignancies and their clinical translation. My presentation will focus on two main aspects: (1) a novel strategy to overcome several limitations of conventional allogeneic CAR-T cells by applying genome-editing techniques to delete PD-1 while simultaneously enabling controlled expression of IL-15; and (2) the progress made toward establishing the first GMP-compliant facility in Andalusia dedicated to producing allogeneic CAR-T cells for patient treatment.

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Effect Size-Driven Pathway Meta-Analysis for Gene Expression Data

The steady increase in open data resources that share omics datasets has become a great advantage for studies aiming to integrate or identify common results for a given health disorder. Therefore, data integration techniques have become a key research focus to allow researchers to properly exploit this valuable source of information.

Meta-analysis is a methodology that enables the joint analysis of heterogeneous datasets and has been widely applied in transcriptomic studies. In this context, the most common approach is to perform meta-analysis at the gene level, which may lead to loss of information due to missing values or data heterogeneity. To address these limitations, we propose a novel methodology that leverages single-sample enrichment scoring techniques to aggregate gene expression data into pathway-level matrices. Working with pathways instead of individual genes preserves information even when some genes are missing in certain studies, and leads to more consistent biological patterns.

GSEMA is implemented as an R package available in the CRAN repository: [https://cran.r-project.org/web/packages/GSEMA/index.html].

In this talk, we will provide an overview of the problem, describe the proposed approach, and present use cases that demonstrate its application.

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Tumor-Derived Vesicles Orchestrate Platelet Activation in PDAC: Toward Non-Invasive Biomarker Development.

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is characterized by the absence of specific symptoms and, despite its relatively low incidence, presents a mortality rate of 80% and an overall 5-year survival of only 13.3% (2025) [1]. A persistent hypercoagulable state is a distinctive feature of PDAC and is associated with continuous interactions between platelets (PLTs) and pancreatic tumor cells (TCs) [2]. This indirect, bidirectional communication relies on extracellular vesicles (EVs), including microvesicles (MVs) and exosomes (EXOs), which act as carriers of biomolecules enabling early cancer signaling and promoting hypercoagulation, tumor progression, angiogenesis, and metastasis [3–5]. Our objective is to study EV-mediated communication between PLTs and TCs to understand its contribution to carcinogenesis and to evaluate EVs as a potential non-invasive source of biomarkers for early PDAC diagnosis, treatment, and monitoring [6].
Methodology: EVs will be characterized by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and zeta potential measurements. In vitro cocultures will assess communication between EVs derived from tumor and non-tumor cell lines (HPDE, BxPC-3, PANC-1) and PLTs from healthy donors. Biomolecule transfer and platelet activation will be analyzed by flow cytometry, adhesion by xCELLigence, protein expression by Western blot, and proteomic alterations by LC/MS.
Results and discussion: EVs from TCs induced differential PLT activation and adhesion depending on the vesicle subtype (MVs vs EXOs) and the cell line of origin. EVs also transferred lipid and protein biomolecules to PLTs, identifying this as a key communication mechanism. Proteomic analyses revealed major pathway alterations in PLTs exposed to TC-derived EVs and identified a futured protein biomarker signature for early PDAC detection that must be validated in vitro.
Conclusions: EVs are active mediators of PLT–TC communication and show strong potential as non-invasive biomarkers for PDAC diagnosis and monitoring, as well as promising therapeutic targets.

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Targeting the SWI/SNF Complex: A Promising Therapeutic Strategy for NUT Carcinoma

NUT Carcinoma (NC) is an exceptionally aggressive squamous carcinoma with a median survival of less than 7 months, making it one of the most aggressive solid tumor in humans. NC is driven by specific NUT fusion oncoproteins resulting from chromosomal translocations. These fusion proteins drastically alter the epigenetic landscape of chromatin, characterized by the formation of large regions of acetylated histones known as ""megadomains"". This chromatin dysregulation leads to the overexpression of NUT-associated oncogenes, such as MYC, SOX2, and TP63, preventing cellular differentiation and promoting rapid tumor growth. In this study, we identified the SWI/SNF chromatin remodeling complex (specifically its ATPase activity) as critical to NC biology. We discovered that SWI/SNF is heavily recruited to NC "megadomains" and that the pharmacological degradation or inhibition of SMARCA4 (SWI/SNF ATPase subunit) led to a rapid decrease in chromatin accessibility and expression of NUT-related oncogenes. RNA-seq further confirmed a significant downregulation of MYC target genes and other oncogenic pathways within hours of SMARCA4 degradation.
The inhibition of SWI/SNF activity resulted in cell cycle arrest, reduced cell proliferation and induced epithelial differentiation in NC cells. Notably, we observed increased membrane levels of β-catenin and E-cadherin, which play key roles in promoting cell adhesion in epithelial tissues. Consistent with this, the inhibition of SWI/SNF impaired the migratory capacity of NC cells assessed by wound healing assays.
The effect of this therapeutic strategy was further evaluated in vivo using cell-derived xenografts in murine models, where we observed a significant reduction in NC tumor growth when treating the mice with SWI/SNF inhibitors. Overall, these findings suggest that targeting the SWI/SNF complex represents a promising therapeutic strategy for NUT carcinoma patients.

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Causative treatment strategies for NEDAMSS, a rare pediatric monogenic neurodegenerative disease

Pediatric monogenetic neurodegenerative diseases (NDDs) affect approximately 1% of newborns worldwide, causing significant health threats and care burdens. Here, we look for causative treatment strategies for NEDAMSS (NEurodevelopmental Disorder with regression, Abnormal Movements, loss of Speech and Seizures), a rare NDD affecting 8-year-old Elenita from Granada and others. Heterozygous mutations in the IRF2BPL gene upregulate mitochondrial respiration & WNT signaling in patient brains, as well as in animal models like Drosophila and zebrafish, leading to fast progressive neuronal loss, regression and complete dependency. Further molecular disease mechanisms are unknown and there is no cure. As safe brain gene therapy is still far away, we will develop here a multidisciplinary therapy approach:
1) Prediction of therapy targets with AI tools. 3D structural modeling of IRF2BPL WT-WT and WT-mutant protein complexes with Alphafold3 & Diffdock will allow us to find drugs that directly prevent the formation of the WT-mut complex.
2) Molecular disease mechanism characterization. Differential expression analysis (RNAseq, protein) of patient samples (cerebrospinal fluid, fibroblasts, induced astrocytes/neurons (iA/iN), pull-down of IRF2BPL WT-mut complexes) will shed light on IRF2BPL protein interactors and downstream altered genes, in order to find new pharmacological targets. Proteins will be measured by liquid chromatography coupled to mass spectrometry (LC-MS/MS).
3) FDA-approved drug candidate evaluation, including WNT inhibitors and mitochondrial modulators, on (co-)cultures of patient-derived iA/iN, as well as NEDAMSS zebrafish models carrying patient-mutations. Successful drugs will rescue normal mitochondrial respiration & neuronal survival in iA/iN, lower WNT signaling (iA/iN & zebrafish), & restore motility in zebrafish behavioral tests.
As rare NDDs share common disease mechanisms, successful treatment strategies likely benefit other NDDs without cure.

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Identification of genetic variants associated with response to androgen deprivation therapy in prostate cancer

Prostate cancer (PC) is characterized not only by being one of the most prevalent cancers in men, but also by exhibiting significant clinical heterogeneity, particularly in response to androgen deprivation therapy (ADT), the standard treatment for advanced disease. However, despite its initial effectiveness, over half of ADT-treated patients develop castration-resistant PC within 2-3 years, although this timing varies with tumor stage and other clinical factors. Identifying genetic determinants that underlie differential therapeutic responses may improve patient stratification and guide personalized treatment strategies.

In this study, we designed a targeted gene panel encompassing variants in genes previously associated with PC and drug metabolism. Using this panel, we analyzed a cohort of 220 PC patients and 109 healthy controls to investigate genetic differences related to ADT response. Patients were classified according to their clinical course as good responders (≥5 years of sensitivity to ADT, n=131) or poor responders (<5 years to castration resistance, n=89). Comparative analyses identified 292 variants in 153 genes significantly associated with treatment outcome, including MDM4, DRD2, COL4A1, KIAA1324, and LNPEP, among others. These genes are involved in pathways linked to cellular stress responses, neuroendocrine signaling, extracellular matrix remodeling, hormone-dependent tumor progression and peptide hormone regulation, processes that have been associated with metabolic reprogramming, tumor adaptability and the emergence of resistance to ADT.

The development of a predictive machine learning model based on our targeted panel aims to establish a genomic framework capable of anticipating ADT response, with future applications extending to clinical stratification. This approach may ultimately enable more precise and individualized management of PC patients.

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Biomimetic Nanovaccine for Personalized Immunotherapy in Triple-Negative Breast Cancer

Although immunotherapy has advanced considerably in recent years, major obstacles remain in counteracting the immunosuppressive tumor microenvironment, especially in triple-negative breast cancer (TNBC). For this reason, current efforts are focused on identifying new costimulatory pathways that can enhance immune responses for the development of effective cancer vaccines. One promising example involves the interactions between CCL5, CD40L, and CD40, which together represent a highly relevant co-stimulatory axis in the field of immunotherapy.To replicate this pathway for future application as a personalized cancer vaccine, we developed PLGA nanoparticles (NPs) biomimeticized with T cell membranes derived from TNBC patients, enriched with CD40L, and loaded with CCL5 (referred to as NT40L). These NPs were designed to mimic synthetic T lymphocytes capable of interacting with paired dendritic cells. Flow cytometry analysis revealed that 94.3% of the nanoparticles were positive for surface CD40L, confirming the correct outward orientation of the ligand, while achieving an encapsulation efficiency of 54.86% for CCL5 in PLGA NPs and 65.83% in NT40L. In vitro assays further demonstrated that dendritic cells reached a semi-mature state, as evidenced by morphological changes toward a more adherent phenotype.In conclusion, the designed NPs exhibited suitable physicochemical properties for use as a nanosystem in biomedical applications and provide promising perspectives for the development of personalized therapies that are more effective and less invasive against TNBC. Nevertheless, further in vitro assays (including dual co-stimulation with NT40L and tumor antigens) as well as in vivo studies will be required to fully elucidate their validity and safety as a cancer vaccine.

Libro de abstract – Comunicación poster

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Enhancing CAR-T therapy for HER2+ tumors: controlled CAR and cytokine expressions for improved efficacy and safety

Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionized cancer treatment by enabling T cells to specifically recognize and eliminate tumor cells. However, uncontrolled CAR expression can lead to severe toxicities and reduced therapeutic efficacy. Therefore, precise regulation of CAR expression is crucial to enhance both safety and clinical outcomes. In our laboratory, we engineered a lentiviral promoter based on the regulatory regions of the WAS gene, designed to mimic the expression kinetics of the endogenous T-cell receptor (TCR). This promoter was used to drive the expression of an anti-HER2 CAR and was compared to a control CAR driven by the strong constitutive EF1α promoter. T cells expressing the CAR under the AW promoter exhibited lower surface CAR expression, reduced tonic signaling, and an improved phenotype. AWHER2 CAR-T cells demonstrated comparable cytotoxic activity upon repeated tumor cell challenges against plated MIA PaCa-2 cells, while maintaining a more favorable phenotype and reduced secretion of proinflammatory cytokines (GM-CSF, TNFα, IFNγ, and IL-2). However, when EF1α- and AW-driven CAR-T cells were tested against HER2$^+$ spheroids at a restrictive effector-to-target ratio, EF1α-driven CAR-T cells showed higher lytic activity but a less favorable phenotype. The spheroid model displayed increased HER2 expression and elevated levels of soluble HER2, which partially inhibited the activation of AWHER2 Jurkat TPR cells. In conclusion, TCR-like regulation of CAR expression through the AW promoter represents a promising strategy to balance potency and safety in CAR-T cell therapy under non-restrictive conditions. Nevertheless, additional factors—such as soluble antigen levels and the CAR-T cell–to–tumor cell ratio—must be carefully considered for the successful application of this approach in solid tumor settings.

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Rewiring the cancer epigenome to change cancer chemosensitivity

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Endogenous Retrovirus plays an essential role during morphogenesis

Endogenous retroviruses (ERVs) have shaped the genomes of vertebrates; however, the extent of their influence on evolution, functionality, and disease remains understood. Although most ERVs have lost the ability to mobilize they are transcribed at stage- and tissue-specific manners during embryonic development raising important questions about how their expression is regulated and whether they are functionally important during development. Most co-opted ERVs play important roles during pluripotency, raising the idea that ERVs cooperate with their host in essential biological processes such as embryonic development.

To explore the role of ERVs in morphogenesis, we studied ERV1-3, a recently active ERV subfamily that is specifically expressed in paraxial mesoderm and somites during morphogenesis in zebrafish. We have found that this expression is driven by three recently inserted, full-length copies with only a fragmented pol gene and whose LTRs harbour a unique combination of binding sites for specific mesodermal transcription factors. Among these is tbx16, a Nodal-induced gene, which we found to directly regulates ERV1-3 transcription. This suggests that ERV1-3 expression is integrated into the Nodal signaling pathway, a key regulator of early embryonic development.

Strikingly, knockdown of ERV1-3 RNA using CRISPR-RxCas13d resulted in early defects in axial and paraxial mesoderm development and later produced somite and midline phenotypes closely resembling those observed in Nodal mutants. These phenotypes were partially rescued by injection of a transcript derived from one of the copies, demonstrating that ERV1-3 RNA is functionally required for proper mesoderm development. Moreover, this same ERV1-3 RNA mitigated the severity of defects caused by Nodal inhibition, further supporting its function. Taken together, our findings reveal that a relatively young endogenous retrovirus is transcriptionally regulated by T-box factors and functions downstream of Nodal signalling, contributing to morphogenesis through one of the most conserved signalling pathways in vertebrate evolution.

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CircRNAs as potential biomarkers and therapeutic targets in RBM10-deficient LUAD

Circular RNAs (circRNAs) are non-coding RNAs increasingly implicated in cancer, yet their biogenesis mechanisms are unclear. The splicing regulator RBM10 is frequently mutated in lung adenocarcinoma (LUAD), but its possible role in circRNA regulation has not been explored.

We performed transcriptomic analysis in LUAD cell lines, identifying circHIPK3 and circSMARCA5 as significantly dysregulated upon RBM10 restoration. Subcellular fractionation confirmed RBM10's nuclear localization and circRNAs' cytoplasmic enrichment, suggesting RBM10 controls circRNA biogenesis. PAR-CLIP and RNA pulldown assays showed RBM10 directly binds to the flanking intronic sequences of these circRNAs. Mechanistically, using a splicing reporter system, we found that RBM10 binding to the 3' flanking region is highly effective at promoting exon skipping and subsequent circularization, thereby altering circRNA levels.

Functionally, altering circHIPK3 (overexpression) or circSMARCA5 (silencing) mirrored the tumor-suppressive effects of RBM10 restoration in vitro and in xenograft models. Remarkably, analysis of two independent LUAD cohorts demonstrated that circHIPK3 expression is reduced in tumors and correlates positively with RBM10 expression. Besides, preliminary survival data suggests a worse outcome for patients with dual impairment of RBM10 and circHIPK3.

These findings reveal a novel regulatory mechanism where RBM10 acts as a tumor suppressor by controlling circRNA biogenesis. These circRNAs serve as functional biomarkers, providing potential utility for LUAD stratification and therapy.

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Generation and validation of a doxycycline-inducible RBM15/MKL1 expression vector

Acute Megakaryoblastic Leukemia (AMKL) is a rare subtype of acute myeloid leukemia characterized by the accumulation of immature, morphologically aberrant megakaryoblasts. It accounts for 5% to 15% of pediatric cases, where detecting chromosomal aberrations is of vital relevance. Specifically, the t(1;22) translocation, which generates the RBM15-MKL1 fusion gene, drives an aggressive type of leukemia. In cases not associated with Down Syndrome, this subtype is characterized by a poor prognosis, with a 50% mortality rate and a median survival of only two years. Furthermore, as no secondary mutations have been identified in humans, this gene fusion appears sufficient to manifest the leukemia. Given the embryonic onset of this disease, human pluripotent stem cells (hPSCs) represent an ideal model for study. The aim of this study is to modify and validate a doxycycline-inducible RBM15-MKL1 expression system. To this end, a functional pTRE3G plasmid with doxycycline-inducible expression of the RBM15-MKL1 fusion gene was generated. Its integrity was verified using molecular tools and HEK293T cell cultures. Results confirmed an increase in expression upon doxycycline addition, although a slight leaky expression was observed.

These data demonstrate the relevance of validating a vector before its use in more complex cellular models like hPSCs. The study of RBM15-MKL1 effects will constitute a key tool for understanding AMKL, allowing for the future development of more effective treatments to reduce its aggressiveness.

Keywords: Acute Megakaryoblastic Leukemia, pediatric, t(1;22), RBM15-MKL1, human embryonic cells, pTRE3G.

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Modulating the epigenome of cancer cells as an innovative therapeutic approach.

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Development of a therapy based on nanobody against the SARS-CoV-2 virus.

COVID-19 is a viral disease that represents a global threat to public health due to its high infection rate and its ability to cause epidemic outbreaks. None of the current therapies are completely effective due to the continued emergence of variants, so the development of new treatment methods remains a matter of vital importance.
Nanobodies are small single-domain antibody fragments that have unique properties, such as the ability to recognize particular epitopes (not recognized by conventional antibodies) and their enhanced stability. They have been successfully used in therapeutic approaches as blockers of receptors associated with pathogenic diseases or viruses.
All of these factors make nanoantibodies a promising option for the treatment of COVID-19 and could lead to the development of more effective and affordable therapies.
In this work, therapeutic nanoantibodies have been generated against the SARS-CoV-2 virus. From a library we have identified 18 nanoantibodies that have affinity for the RBD antigen. Of those 18 candidates, 9 have been expressed and purified by high-speed protein liquid chromatography (FPLC). Furthermore, performing a Western Blot, we have observed that 4 nanoantibodies recognize linear epitopes while the other five recognize conformational epitopes.
With the aim of conducting future neutralization tests, we are working on the amplification and titration of current SARS-CoV-2 virus strains from samples of infected patients identified at the Clinical Management Unit of Microbiology of the Virgen de las Nieves University Hospital, the reference laboratory for viruses in Andalusia. This work is being carried out in a level 3 biosafety room, designated for these viruses.

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A genome-wide association study identifies new loci associated with response to SARS-CoV-2 mRNA-1273 vaccine in a cohort of healthy healthcare workers

Introduction: The COVID-19 pandemic had significant global public health consequences, affecting over 200 countries and regions by 2020. The development and efficacy of specific vaccines, such as the mRNA-1273 (Spikevax®) vaccine developed by Moderna Inc., have substantially reduced the impact of the pandemic and mitigated its consequences. This study aims to identify novel genetic loci associated with the effectiveness of the mRNA-1273 vaccine, as measured by elevated anti-Spike (anti-S) IgG levels at multiple time points post-vaccination.

Materials and methods: We conducted three genome-wide association studies (GWAS) in a cohort of Spanish healthcare workers, analyzing anti-S IgG levels at one-month post-vaccination (n=567), at three months post-vaccination (n=447), and the difference in circulating anti-S IgG levels between these two time points (n=447).

Results: We identified fourteen novel loci associated with increasing concentrations of anti-S IgG post-vaccination (p=5.01×10-13 and p=2.81×10-8). Functional results showed that some of the novel risk alleles influence the absolute counts of specific B cell subsets (p=2.57×10-5-8.82×10-3), which are involved in immune signaling pathways and metabolic processes. Furthermore, these variants co-localize with multiple QTLs and epigenetic marks, suggesting that the GWAS hits may affect regulatory activity in promoters, enhancers, and transcriptional regions, thereby modulating gene expression relevant to the humoral immune response.

Discussion: In conclusion, this study highlights the complex interplay of genetic factors influencing the immune response to vaccination, particularly through modulation of B cell activity, immune signaling pathways, and metabolic processes. The identification of genetic variants could inform future strategies to enhance vaccine efficacy and provide a deeper understanding of individual variability in vaccine responses, especially for COVID-19 and other viral infections.

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Comparison of methods for the isolation and characterization of extracellular vesicles and microRNAs from small volume of urine

Background: Urinary extracellular vesicles (uEVs) are a promising source of non-invasive biomarkers for renal diseases, but clinical application is limited by the lack of standardized isolation protocols for downstream digital PCR, flow cytometry and other analytical methods.

Objective: To compare three uEV isolation methods, polyethylene glycol (PEG) precipitation, differential ultracentrifugation and a commercial kit A (Urine Exosome Isolation and RNA purification MiniKit, Norgen Biotek Corp.) to build a complete protocol for characterizing and investigating uEVs from small volume of urine.

Methods: uEVs were isolated from 1 mL urine samples from healthy donors using ultracentrifugation (16,000g/100,000g), PEG 8000 precipitation, and the commercial kit A. EV recovery and tetraspanin (CD9, CD63, CD81) expression were assessed by nanocytometry, and conventional flow cytometry. Particle size and concentration were evaluated by NTA (Horiba ViewSizer-6000, and Particle Metrix Zetaview). A complete protocol was developed for the quantification of miR-423-5p, mir-196-5p miRNAs and their expression were quantified by Absolute Q digital PCR using TaqMan assays.

Results: PEG precipitation method yielded the highest exosome/tetraspanin signal by flow cytometry and the highest miR-423-5p copies/µL, followed by ultracentrifugation and lastly the commercial kit A. NTA allowed the size profiling of the uEVs obtained by all methods; PEG and commercial kit A enriched smaller particles, whereas ultracentrifugation retained a higher proportion of larger particles. PEG isolation, however, showed more nonspecific binding of the uEVs with PEG particles, potentially introducing analytical artifacts. However, this binding was lessened when a further heating step was added following the PEG precipitation, which disassociated the EVs.

Conclusions: PEG-based precipitation is the most efficient approach for isolating uEVs from small urine volumes for flow cytometry and digital PCR, albeit with higher nonspecific background. These findings support PEG-isolated uEVs as a feasible platform for validating miRNA biomarkers and warrant further optimization and application in a clinical setting.

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DECODING NEDAMSS: BIOINFORMATIC INSIGHTS INTO TRANSCRIPTOMIC SIGNATURES AND PATHWAYS ALTERATIONS USING PATIENT-DERIVED SAMPLES

NEDAMSS is an extremely rare and severe neurodegenerative disorder that appears in infancy or early childhood and progresses with worsening neurological symptoms. It is caused by de novo heterozygous mutations in IRF2BPL, an intronless gene at 14q24.3 that encodes a ubiquitously expressed transcriptional regulator. In healthy neurons, full-length IRF2BPL localizes to the nucleus; however, truncated variants mislocalize the wild-type protein to the cytoplasm, promoting aggregation and impaired cellular function. Although its precise role remains unclear, studies suggest involvement in transcriptional regulation, ubiquitin–proteasome pathway, and neuronal development.

The consequences of IRF2BPL mutations have not been thoroughly investigated in human patient-derived cells. In this study, a human in vitro cellular model was generated by reprogramming patient fibroblasts into induced neuronal progenitor cells (iNPCs), followed by differentiation into induced astrocytes (iAs). These samples underwent RNA sequencing and subsequent bioinformatic analysis. Additionally, previously published datasets from four NEDAMSS patient-derived astrocytes and healthy controls, as well as patient and healthy blood samples were integrated.

Our analyses demonstrated clear transcriptomic differences between NEDAMSS patient-derived astrocytes and controls. Differential gene expression and enrichment analyses revealed altered immune-related and neuronal pathways in NEDAMSS, highlighting the potential role of neuroinflammation, vesicle trafficking impairment, and increased Wnt signaling, possibly due to IRF2BPL mislocalization. GSEA results reinforced neuroinflammation and autophagy as potential disease mechanisms. STRING protein-protein interaction mapping of IRF2BPL suggested potentially affected additional regulatory routes. Future research will focus on dissecting underlying molecular mechanisms expanding to data from more patient-derived cellular models, in order to reduce genetic background variability. Advanced pathway inference, transcription factor activity profiling, and eventually single-cell RNA-seq analysis from optimized neuron–astrocyte–microglia co-culture models will be pursued. These analyses will shed light on affected cell types and molecular pathways, making predictions for future treatment interventions in NEDAMSS.

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Gold-Iron Oxide Nanohybrids Embedded in Colloidal Gels for Enhanced Tumor Therapy

Current cancer therapies often lack the capability to selectively target tumor tissues without demaging healthy cells. Additionally, many drug delivery systems exhibit poor control over release kinetics and biodistribution. Furthermore, monotherapy approaches frequently fail to address the heterogeneity and adaptability of tumor microenvironments. Therefore, there is a critical need for integrative solutions that combine diagnostic and therapeutic functions within a single, controllable system.

Our project, CoCoGel, aims to develop a nanotechnology-based platform to overcome current cancer therapy challenges. Our first approach is focused on the synthesis of hybrid nanoparticles composed of gold (Au) anchored on magnetic iron oxide (Fe_3O_4) cores, encapsulated within a silica (SiO_2) shell. Gold offers excellent biocompatibility and strong optical properties (plasmonic resonance), useful for photothermal therapies. Magnetite (Fe_3O_4) is superparamagnetic at the nanoscale, allowing magnetic guidance and heat generation under alternating magnetic fields (magnetic hyperthermia). The silica shell ensures nanoparticle stability and provides a versatile surface for functionalization with tumor-targeting ligands. A second approach utilizes gold nanorods (AuNRs) coated with a silica (SiO_2) shell and with magnetite (Fe_3O_4) nanoparticles functionalized on the external surface. This configuration maintains the synergistic optical and magnetic properties while offering a different surface topology for tunable functionalization and interaction with the biological environment. These particles will be embedded in biocompatible hydrogels to form magnetically controlled colloidal gels (MCCGs), enabling localized administration, magnetic guidance, and synergistic thermal therapies. The application of external magnetic fields allows spatial control over gel positioning and activates hyperthermia effects. Currently, we have developed several hybrid nanosystems that have been thoroughly characterized. They exhibit good biocompatibility and can be effectively activated using magnetic fields and near-infrared (NIR) radiation.

By integrating multiple therapeutic modalities into a single platform, this work establishes a basis for the development of next-generation smart biomaterials for localized, effective, and minimally invasive cancer treatment. This integrative system holds promise for improved precision and efficacy in cancer treatment.

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Identification of Novel Biomarkers in SMARCA4-Deficient Undifferentiated Tumors

Introduction: SMARCA4-Deficient Undifferentiated Tumors (SMARCA4-UT) are rare, aggressive thoracic malignancies driven by the disruption of the SWI/SNF chromatin remodeling complex. Accurate diagnosis remains a significant clinical challenge because SMARCA4 loss is not unique to this entity, and current diagnostic markers of undifferentiation, such as SOX2, exhibit inconsistent expression and low specificity, frequently resulting in misclassification.
Methods: This study used public transcriptomic datasets to identify precise molecular markers. We analyzed expression profiles to reclassify cell lines and compared patient data of SMARCA4-UT against SMARCA4-mutated lung carcinomas and undifferentiated thoracic sarcomas.
Results: Differential expression analysis identified FAM9C as a top-ranking candidate. As a cancer/testis antigen, FAM9C demonstrated superior diagnostic performance compared to SOX2. Unlike SOX2, FAM9C expression was found to be exclusive to SMARCA4-UT samples, offering a distinct advantage in differential diagnosis.
Conclusions: We conclude that FAM9C represents a highly valuable and specific biomarker for SMARCA4-UT. Its exclusive expression pattern allows for better discrimination from other thoracic tumors compared to traditional markers.

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Fluorescent Nanocatalyst for Tracking Copper-Catalyzed Azide–Alkyne Cycloadditions in Cells

Since the introduction of click chemistry by Sharpless and Meldal and its expansion to living systems through Bertozzi's bioorthogonal chemistry, these reactions have transformed chemical biology. Among them, the copper-catalyzed azide–alkyne cycloaddition (CuAAC) is the most prominent example, recognized with the 2022 Nobel Prize in Chemistry for its efficiency and selectivity in forming stable triazoles.

Despite its potential, the biological use of CuAAC is hindered by copper toxicity, oxidation state instability, and reactive oxygen species (ROS) formation, which limit its biocompatibility. Although copper-free click reactions exist, many alkyne-containing precursors would still benefit from CuAAC chemistry if non-toxic copper systems were available.

Here, we report the design and synthesis of a tracker nanocatalyst, Cu@BTTAA-Cy5-NPs, composed of copper metallofluorescent nanoparticles stabilized by the BTTAA ligand and functionalized with the fluorescent dye Cy5. These monodisperse nanoparticles display robust CuAAC catalytic activity and intrinsic fluorescence, enabling real-time monitoring of nanoparticle localization and catalytic progress within living cells.

A comprehensive study revealed that Cu@BTTAA-Cy5-NPs exhibit high catalytic efficiency under mild conditions, excellent recyclability, and broad substrate scope with high yields and short reaction times. Moreover, these nanocatalysts demonstrate outstanding biocompatibility, efficient cellular uptake, and negligible toxicity, representing a new generation of non-biotoxic heterogeneous copper catalysts that combine superior catalytic performance with fluorescence tracking for intracellular and bioorthogonal CuAAC applications.

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A Cell-Free DNA–Based Model for Diagnostic and Prognostic Assessment of Hepatocellular Carcinoma in Cirrhosis

Background & aims: Hepatocellular carcinoma (HCC) remains a substantial global health burden, with early detection being a critical for patient outcomes. Current screening in high-risk populations, especially liver cirrhosis (LC) patients, often lacks sensitivity. This study investigates the potential of circulating-free DNA (cfDNA) analysis as a novel approach for HCC assessment.

Methods: Plasma samples from 39 HCC and 46 LC patients were analyzed for cfDNA concentration and fragment patterns. Associations with clinical features and liver function markers were assessed. A multivariate diagnostic model combining cfDNA, alpha-fetoprotein (AFP), and C-reactive protein (CRP), was developed. Prognostic value was evaluated via survival analysis.

Results: Concentration of cfDNA was significantly higher ($p<0.0001$) in HCC than in LC and correlated with advanced HCC stage ($p<0.05$), lymphovascular invasion ($p<0.005$), and liver dysfunction markers ($p<0.0001$). Fragments of cfDNA were longer ($p<0.05$) in HCC cases. A diagnostic model (CMAC) combining cfDNA, alpha-fetoprotein (AFP) and C-reactive protein (CRP) was constructed using multivariate logistic regression. The CMAC model yielded high diagnostic performance (AUC 0.949) for differentiating HCC from LC, outperforming individual markers (AUROCAFP = 0.777, AUROCCRP = 0.833). High CMAC index was associated with shorter overall survival (OS) in the HCC cohort ($p=0.0089$). Among advanced-stage HCC, cfDNA concentration was the only independent predictor of OS (HR= 1.031, 95%CI=1.010-1.052, $p=0.004$).

Conclusions: Integration of cfDNA analysis with established clinical markers in the CMAC model shows promise as a complementary tool for the detection of HCC in LC patients. Validation in larger, multicenter cohorts will be necessary to confirm these findings and their clinical applicability.

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A Pd-modified porphyrinic MOF for combined bioorthogonal catalysis and photodynamic therapy

Standard chemotherapeutics have several well-known limitations, including systemic adverse effects, poor bioavailability, and short half-lives. To overcome these drawbacks, improved anticancer therapies are needed. One promising strategy involves the use of abiotic transition-metal catalysts (e.g., Pd, Au, Ru, Pt, Cu) to convert inactive prodrugs into cytotoxic drugs, opening the possibility of "synthesizing" drugs at the tumor site. In parallel, light-triggered therapies such as photodynamic therapy (PDT) offer spatial and temporal control through the generation of reactive oxygen species (ROS) in the target tissue by means of photosensitizers, with porphyrin-based structures standing out as some of the most extensively explored. Among these, porphyrinic metal–organic frameworks (MOFs) have recently emerged as highly attractive platforms for PDT, owing to their intrinsic photosensitizing capability, tunable porosity for drug encapsulation, and potential for synergistic therapies.
In this communication, we present the development of a nanoscale Pd-functionalised porphyrin-based MOF that combines both approaches, enabling the in situ depropargylation of an inactive derivative of 5-fluorouracil (Pro-5FU) and PDT under visible light irradiation.

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Revealing new molecular pathways involved in retrotransposon represión

Transposable elements (TEs) constitute a substantial fraction of mammalian genomes and represent a potential source of genomic instability. Their activity is tightly regulated by epigenetic mechanisms, notably DNA methylation and trimethylation of lysine 9 in histone H3, shaping a repressive epigenetic landscape. However, emerging evidence suggests that TE regulation is more complex and dynamic than previously believed, involving additional pathways that may complement or interact with classical silencing systems. In this context, understanding how different layers of epigenetic control cooperate to modulate TE expression across cellular states is essential. Our study aims to identify and characterize novel pathways involved in TE repression, thereby contributing to a deeper understanding of the mechanisms that safeguard genomic integrity in mammals.

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Development of a Novel VHH-Based CAR-T Cells Targeting EpCAM-Positive Tumors

Among potential tumor-associated antigens, epithelial cell adhesion molecule (EpCAM) has gained increasing attention as a therapeutic target. EpCAM is consistently and strongly overexpressed in a wide range of epithelial-derived solid tumors. At the same time, its expression in healthy tissues is limited and largely restricted to basolateral membranes, where accessibility to circulating T cells is reduced. This pattern provides a therapeutic window that could be better exploited with highly specific Nb-based CAR designs. By combining strong tumor recognition with improved safety, EpCAM-directed Nb-CAR-T therapy has the potential to overcome some of the key limitations faced by current CAR-T strategies in solid tumors.

Single-domain antibody (VHH)-based CAR-T cell therapy offers several potential advantages over conventional ScFv-based designs, including smaller size, improved tumor penetration, high antigen specificity, and reduced tonic signaling. In this study, we generated a panel of EpCAM-specific VHHs and selected four lead candidates that were characterized in terms of affinity and binding kinetics. All VHHs bound specifically to the extracellular domain of EpCAM, albeit with varying affinities.

Each VHH was incorporates into a second-generation CAR construct (EF1α promoter, 4-1BB costimulatory domain), designed to co-express a truncated epidermal growth factor receptor (EGFRt) as a safety switch. Functional assessment of the VHH-CAR constructs in a Jurkat dual-reporter system demonstrated varying specific activation of NFAT and NFκB pathways with minimal tonic signaling.

Following construct validation, we generated VHH-CAR-T cells and evaluated their CAR surface expression, basal activation status, and exhaustion marker profiles under resting conditions. In cytotoxicity assays against MIAPaCa2 cell lines expressing varying levels of EpCAM, all VHH-CAR-T variants efficiently lysed EpCAM$^+$ targets, showing potent antigen-dependent cytotoxic activity. Notably, differences in cytotoxic capacity were observed among the individual VHH-CAR constructs, reflecting their distinct binding characteristics.

In summary, we have developed four distinct VHH-based CAR-T constructs targeting EpCAM, each exhibiting unique affinity, cytotoxic capacity and epitope-binding properties. Future studies will determine which construct offers the best tumor-to-healthy tissue discrimination for therapeutic applications.

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Dissecting epigenetic memory mediated by the H3K9 system

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PathMED: An R/Bioconductor package for precision medicine based on single sample molecular scoring

Omics technologies have significantly contributed to precision medicine by enabling outcome prediction based on molecular profiles. However, diagnostic and prognostic applications remain limited due to variability across platforms, protocols, and data processing, which affect the generalizability of machine learning (ML) models trained on single datasets. Single-sample molecular scoring has been proposed as a strategy to address this issue by summarizing molecular data (e.g., gene expression) into gene set or pathway activity scores. This approach enhances biological interpretability and supports ML models that generalize across heterogeneous datasets. Several R packages, such as GSVA and decoupleR, offer such scoring methods, but lack a unified and user-friendly framework. pathMED addresses these gaps by integrating multiple scoring methods under a standardized framework. Furthermore, it includes M-scores, a novel scoring approach that use reference datasets to improve disease characterization and prediction accuracy. To overcome the limitation of heterogeneous gene sets, pathMED includes tools to refine pathways by clustering genes with coordinated activity, revealing more granular and biologically meaningful subpathways. In addition to scoring, pathMED facilitates robust ML model development. It simplifies training, validation, and prediction with molecular scores. We tested pathMED with lung cancer multi-omics data, demonstrating that molecular scores are interoperable across omics.

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Scalable and Clinically-Relevant Expansion of Gene-Edited Allogeneic CAR T Cells Using G-Rex Bioreactors

In the field of CAR-T cell therapies, approaches based on allogeneic T cells represent a promising alternative to overcome the limitations associated with autologous products, such as inter-patient variability, elevated costs, and long manufacturing timelines. By using T cells derived from healthy donors, it is possible to generate "off-the-shelf" allogeneic CAR T cell batches, enabling immediate therapeutic availability, reduced response variability, and improved scalability. However, the use of allogeneic CAR T cells can trigger immune responses in the recipient, primarily due to HLA mismatch and T cell receptor (TCR) mediated alloreactivity. To mitigate these risks, gene editing strategies targeting key molecules such as HLA class I and the TCR have been proposed and already evaluated to reduce immunological rejection and enhance the persistence of allogeneic products. These requirements highlight the importance of developing a robust approach for their manufacture.

Transition from preclinical development to clinical manufacturing of gene-edited T cell-based therapies requires scalable and reproducible platforms that allow high cell yields while maintaining their functionality. In this context, we have implemented the use of G-Rex bioreactors to optimize the expansion of gene edited CAR T cells, with the aim of proving that we can generate sufficient cell numbers under culture conditions more representative of the clinical setting, thus increasing the translational relevance of preclinical models.

Regardless of the type of genetic modification applied to the T cells, culture in G-Rex allowed good overall cell performance and viability, without compromising key phenotypic markers such as those associated with memory or depletion. In addition, no adverse effects on transduction efficiency or gene editing rates were observed.

Considering that the G-Rex system is a suitable candidate for large-scale cell manufacturing, we have initiated the optimization of clinical-grade electroporation protocols in combination with G-Rex expansion for the production of TCR-knockout T cells. Preliminary results show editing efficiencies of approximately 80%, along with robust expansion capacity and preservation of a memory-enriched phenotype. These findings support the potential of this integrated approach for the efficient and scalable generation of TCRKO cells under conditions compatible with clinical translation.

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What it takes to CyTOF-profile 2,500 clinical samples across four immune-mediated diseases

Background: 3TR is a multicenter consortium studying autoimmune, allergic and inflammatory diseases to find common molecular signatures across them. Among various OMICs technologies, mass cytometry (CyTOF) is employed to comprehensively profile immune cell populations.
Objective: Establish a standardized staining and antibody-validation protocol to enable immune monitoring of over 2,500 blood samples from 3TR clinical studies.
Methods: To ensure robust site recruitment, whole blood was fixed with Proteomic Stabilizer and stored at -80°C until processing. Antibody panel development included clone selection in fresh blood, antigen stability testing and titration in fixed/frozen samples from healthy donors and patients, using flow or mass cytometry. Large-scale metal conjugation was performed for higher yields. To improve technical robustness and batch consistency during staining, two protocols were tested. Cells from 3 donors were thawed, lysed, aliquoted and either refrozen immediately or barcoded, pooled and refrozen at -80°C. Depending on the workflow, samples were subsequently barcoded and pooled or immediately stained with the frozen antibody cocktail.
Results: Of the 12 antibody clones evaluated by flow cytometry, 8 met the criteria for inclusion. In total, 55 markers were titrated in CyTOF, and 50 were approved. Then, 35 antibodies were conjugated in-house, with 21 produced at large scale. The final panel includes 42 common markers and 4 disease-specific markers, comprising lineage and functional markers. Panel was tested following the described workflow. A few markers (BAFF-R, CD7 and CD177) displayed shifts in signal intensity between 1-week and 1-month storage. Consequently, a maximum 10-day storage period before staining was established to ensure optimal marker performance.
Conclusions: Standardized CyTOF protocol and 46-plex antibody panel enable robust and reproducible immune profiling of over 2,500 samples from patients, supporting biomarker discovery and personalized therapies.
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Anisotropic Nanostructures Bridging Bioorthogonal Chemistry and Photothermal Cancer Therapy

The site-specific activation of chemotherapeutic agents through bioorthogonal catalysis offers a compelling approach to minimize off-target effects and protect healthy tissues. However, achieving precise spatial and temporal control over transition-metal-catalyzed prodrug activation remains a major challenge. In this work, we introduce anisotropic nanorods composed of palladium and gold (Pd/Au), designed to mediate dealkylation reactions upon near-infrared (NIR) light stimulation. This external trigger enables on-demand regulation of catalytic activity at the tumor location. Surface modification of these nanostructures with PEGylated phospholipids via Au–S linkages improve their colloidal stability and biocompatibility, enhancing their performance in biological environments. In parallel, we developed a tailored library of caged fluorescent probes and prodrugs bearing novel protective groups compatible with this catalytic system. Upon NIR irradiation, the Pd/Au nanorods effectively activate the therapeutic compounds while simultaneously generating localized heat, resulting in potent photothermal ablation of cancer cells both in vitro and in xenografted zebrafish models. Altogether, this work highlights a synergistic therapeutic platform that integrates precise catalytic drug activation with photothermal effects, advancing the development of targeted combination therapies.

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Enhancing CAR-T therapy through engineered EVs targeting AML-specific antigens

CAR-T cell therapy has emerged as a breakthrough in the treatment of hematological malignancies, offering a personalized and targeted approach to cancer treatment. Robust clinical outcomes in B-cell leukemias and lymphomas underscore the potential of CAR-T cells to achieve significant remission rates. However, extending this therapy to other malignancies such as acute myeloid leukemia (AML), presents substantial challenges- most notably, the absence of a specific tumor antigen and the impact of the tumor microenvironment (TME). Recent research has explored extracellular vesicles (EVs), including exosomes and microvesicles, as innovative carriers of bioactive molecules. These EVs can be engineered to target specific cells within the TME, thereby modulating the immune response and enhancing CAR-T cell functionality. In this study, we propose combining EVs engineered to target a frequently upregulated surface antigen in AML with a CAR-T therapy targeting CLL-1, an overexpressed marker in AML blasts and leukemic stem cells (LSCs). These EVs will deliver specific short hairpin RNA (shRNA) specifically targeting CD73, an ectoenzyme involved in adenosine production that promotes local immunosuppression within the TME. Preliminary findings indicate that targeted EVs are effectively home to their intended cellular targets. Furthermore, ongoing studies are evaluating the impact of these targeted EVs on reducing immunosuppressive molecules, improving CAR-T cell proliferation, and enhancing persistence. This targeted immunomodulatory strategy holds promise for overcoming current limitations of CAR-T therapy and advancing more effective and durable treatments for hematological malignancies. Integrating these approaches provides a strong framework for studying exosome-cells interactions and supports their potential applications in next-generation immunotherapies, including CAR-T cell therapy.

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CATALASE SINGLE-ENZYME NANOGELS FOR NOSE-TO-BRAIN DELIVERY IN NEUROLOGICAL DISORDERS

In recent years, the incidence of neurological disorders—including Alzheimer's disease, stroke, and Parkinson's disease—has increased significantly. Among the underlying mechanisms, oxidative stress is widely recognized as a major pathological contributor to various neurovascular conditions.[1] Therapeutic biomacromolecules, particularly enzymes, have emerged as promising candidates for treating these disorders. However, their clinical application remains limited due to rapid degradation or denaturation under physiological conditions, as well as their poor ability to cross the blood–brain barrier (BBB). In this context, single-enzyme nanogels (SENs) have emerged as an innovative strategy to enhance the delivery of therapeutic enzymes to the brain.[2]
This study reports the development and characterization of catalase-based nanogels for nose-to-brain delivery, aimed at mitigating oxidative stress and improving the efficacy of current neurological therapies. Catalase, a key antioxidant enzyme, is encapsulated within nanogels engineered to enhance its stability and bioavailability in the brain following intranasal administration.
In vitro and in vivo studies indicate that the nose-to-brain route facilitates effective penetration into the central nervous system, minimizes systemic side effects, and represents a promising therapeutic alternative. This strategy has the potential to transform neurological treatment, opening new avenues in personalized medicine and enzyme-based therapies for neurodegenerative diseases.

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Identification of new overlapping and disease-specific genetic risk factors for rheumatoid arthritis and radiographic axial spondyloarthritis: a meta-analysis of three large European populations and functional characterization

This study conducted a meta-analysis across three large European cohorts (UKBB, FinnGen, and REPAIR), including 12,660 rheumatoid arthritis (RA) cases, 2,446 radiographic axial spondyloarthritis (r-axSpA) cases, and over 530,000 shared controls. Ten independent SNPs in CARMIL1, GRM4, ITPR3, PRSS16, ZNF322, HTT, IKZF1, MANEA, and MGAM2 were significantly associated with both RA and r-axSpA. Risk alleles included HTTrs363075A, IKZF1rs12718261A, MANEArs72920280T, and MGAM2rs73158426G, while CARMIL1rs72831267C, GRM4rs2495964G, ITPR3rs77601296A, ITPR3rs9469540T, PRSS16rs72843633T, and ZNF322rs6901425G had protective effects. Functional analysis showed GRM4rs2495964G was linked to decreased CCL25 levels (p=0.00030), and ITPR3rs9469540T to reduced IL10 production after LPS stimulation (p=1.3×10^4). The ZNF322rs6901425G allele was associated with reduced TNFB and increased TGM2 levels (p=9.60×10^4 and p=3.00×10^4), both involved in immune signaling and tissue remodeling. Disease-specific associations were found in BTN2A1, BTN3A2, and H2BC11. The BTN2A1rs1977199A allele was protective in RA (OR=0.93) but increased r-axSpA risk (OR=1.23), associated with reduced IL22 (p=0.00016) and elevated HO-1 in obese individuals (p=6.73×10?6). In contrast, BTN3A2rs9393716G and H2BC11rs66462181C increased RA risk but were protective in r-axSpA, linked to decreased HO-1 and IL6 (p=2.43×10^5, 3.28×10^4, 1.18×10^4). These SNPs also acted as eQTLs for immune-related genes such as BTN3A2, HMGN4, and TRIM38. Our findings highlight novel shared and disease-specific variants and key immunoregulatory mediators—IL10, IL22, IL6, CCL25, and HO-1—offering insights for disease stratification and therapeutic targeting.

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Deciphering a New G-Quadruplex-Related Mechanism of Action of Vitamin B3

G-quadruplexes (G4s) are non-canonical secondary DNA structures formed by guanine-rich sequences, involved in transcriptional regulation, replication, and genomic stability (1). Their stability and biological functions can be modulated by interactions with small ligands (2) (3), yet the potential role of endogenous molecules as G4 modulators remains largely unexplored.
Nicotinamide, a key vitamin B3 vitamer, has gained increasing interest in recent years due to its well-established roles as a precursor of NAD^+ and a cofactor for enzymes such as sirtuins and PARPs (4). However, these known functions do not fully explain all the biological effects observed, and some findings remain controversial (5).
In this study, we investigated the interaction of vitamin B3 vitamers, focusing on nicotinamide, with G4 structures. Initial screening of different vitamers identified nicotinamide as the most promising candidate, showing preliminary evidence of G4 binding while preserving the natural context of vitamin B3 biosynthesis and high bioavailability.
Using doses determined by IC50 cytotoxicity assays, we confirmed that cell viability remains largely unaffected, as verified by propidium iodide exclusion. Notably, nicotinamide treatment led to relocalization of nucleolar proteins nucleolin and fibrillarin to the nucleoplasm, increase and stabilization of G4s as observed by BG4 immunofluorescence, cell cycle arrest in G2, and an increase in γH2AX signal, showing cellular effects consistent with G4 stabilization.
Current work includes RNA-seq analysis to identify differentially expressed genes potentially regulated via G4s, and future studies aim to characterize nicotinamide–G4 interactions at the molecular level using biophysical techniques such as NMR and circular dichroism.
Together, these results point to a novel molecular mechanism of action for vitamin B3, expanding its known biological roles beyond classical metabolic and redox functions.

KEYWORDS: G-quadruplex (G4), ligands, vitamins, nicotinamide

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Uncoupling Tumor Suppressor Functions of the BCL7A N-terminal Domain in Germinal Center Lymphomagenesis

The germinal center (GC) reaction is tightly controlled by transcriptional and epigenetic mechanisms, and its dysregulation can drive GC-derived lymphomas. Epigenetic alterations, notably in SWI/SNF chromatin remodeling complexes, have emerged as critical drivers of these malignancies. BCL7A, an accessory SWI/SNF subunit highly expressed in GC B cells, is implicated as a tumor suppressor, but its role in GC biology and lymphomagenesis remains unclear. We used Bcl7a-knockout mice, targeted BCL7A N-terminal domain mutants, and integrated ATAC-seq/RNA-seq analyses to define BCL7A's function. Loss of Bcl7a impaired GC formation and accelerated GC-derived lymphoma development, accompanied by transcriptomic changes including an epithelial–mesenchymal transition (EMT) gene signature. Mechanistic dissection of the BCL7A N-terminus revealed that its tumor suppressor activity requires two distinct features: incorporation into the SWI/SNF complex and direct chromatin binding. Notably, BCL7A mutants deficient in chromatin binding acted in a dominant-negative manner. Integrative ATAC-seq and RNA-seq showed that BCL7A loss reduced chromatin accessibility at key B-cell regulatory loci enriched for master transcription factor motifs. In summary, BCL7A is a critical GC regulator that prevents malignant transformation by maintaining chromatin accessibility and gene expression programs.

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Bioinformatic analysis and directed mutagenesis of the interaction of anti-CD19 CAR.

Chimeric Antigen Receptors (CARs) represent a groundbreaking approach in immunotherapy, offering promising alternatives for the treatment of various cancers. Despite their clinical success, further improvements in both efficacy and safety are still required. In this study, we performed a comprehensive bioinformatic analysis of ARI-0001, a CAR targeting the CD19 antigen, designed at the Hospital Clínic of Barcelona and approved under hospital exemption.

To evaluate the interaction between ARI-0001 and CD19, we applied a previously optimized bioinformatics workflow developed by our group. This workflow integrates advanced tools, including protein-protein complex structure prediction models (AlphaFold2), molecular visualization software (PyMOL and ChimeraX), and methodologies to identify interacting residues (Prodigy, PyDockEneRes, and PDBePISA). Using this approach, we systematically identified the key amino acids responsible for the CAR-antigen interaction, providing detailed insights into the structural determinants of binding.

Based on these findings, we proposed mutated versions of ARI-0001 with potentially altered binding affinities. These modifications were designed to either increase or decrease the strength of CAR-antigen interactions, depending on the therapeutic strategy. By predicting changes in both the affinity and the number of molecular bonds in the mutated complexes compared to the native CAR, this study demonstrates a predictive framework for rational CAR optimization.

Ultimately, this work provides a generalizable methodology that can be applied to other CAR molecules, supporting the rational design of next-generation CARs with improved specificity, safety, and efficacy. The integration of structural bioinformatics and mutational modeling offers a powerful approach for guiding CAR engineering and accelerating the development of more effective immunotherapies.

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Center Harmonization for Multicenter CyTOF Immune Monitoring

Background: 3TR is a multicenter consortium aiming to identify shared molecular signatures across seven chronic immune-mediated diseases. As part of this consortium, we will generate immunophenotyping data for over 2,500 samples, which needs a multicenter approach. To ensure reliable results, these multicenter CyTOF runs and data must be carefully optimized and harmonized.
Objectives: Optimize the sample acquisition protocol for multicenter CyTOF studies and determine whether data acquired across different instruments can be aligned.
Methods: A pilot experiment was performed to optimize acquisition using 46-plex panel together with barcoded whole-blood samples from three donors and compensation beads. The samples were prepared, frozen, and later acquired on one three HELIOS, and one CYTOF XT instrument across three acquisition batches. Sample acquisition scheme varied by batch: Batch 1 used one long run per aliquot, while Batches 2–3 used two shorts runs. PCA was applied using median signal intensities (MSI) to evaluate the effect of acquisition time and aliquot used within and across centers. Main cell populations were manually gated and MSI, coefficients of variations (CV) were calculated. Center enrichment and batch effects were assessed..
Results: The compensation matrix generated using the brightest cytometer (Center1), demonstrated the most effective spillover correction. Flow rate instability contributed to signal drifts in several markers, affecting background-related intensities and cell-associated signals The PCA analysis showed that restricting the analysis to the first 65 minutes of acquisition improved clustering for all centers. Bead-based normalization reduced center-associated variability, resulting in CV values of MSI and cell frequencies below 20% for most cell populations. Samples clustered according to the donors, but center effect was observed, thus requiring reference-based normalization.
Conclusions: Multicenter CyTOF experiments are feasible, however special sample acquisition protocol and additional reference-based normalization are required to align the data.
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Epigenetic memory: how do cells remember past events?

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Dual-Functional Nanocatalyst for Intracellular Screening of CuAAC Reactions

Intracellular copper-catalyzed azide-alkyne cycloaddition (CuAAC) offers immense potential for bioorthogonal chemistry, but its application is severely hindered by copper toxicity and the challenge of controlling catalysis within the complex cellular environment. Heterogeneous copper catalysts can reduce toxicity by minimizing free copper exposure and enabling localized activity, yet optimizing their performance in situ within living cells remains a significant hurdle.

Here, the development of a novel dual-functional nanocatalyst, Cu@BTTAA-Cy5-NPs, that combines robust heterogeneous CuAAC catalytic activity with intrinsic fluorescence tracking is reported. The successful synthesis and characterization of these monodispersed nanoparticles is demonstrated, confirming efficient copper loading stabilized by BTTAA and the nanoparticle matrix, and critically, the retention of Cy5 fluorescence for tracking. This unique dual functionality allows for real-time monitoring of nanoparticle localization and correlation with catalytic product formation via distinct fluorescence channels, enabling, for the first time to our knowledge, comprehensive in situ screening and optimization of CuAAC reaction conditions directly within living cells using fluorescence feedback. The nanoparticles exhibit excellent biocompatibility and cellular uptake, showing no significant toxicity, apoptosis, or oxidative stress at active concentrations.

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Genome-wide DNA methylation and its influence on chromosome X inactivation in female lupus

Objective: Lupus, an autoimmune disease primarily affecting women, is influenced by genetics and the environment. Recent research suggests that epigenetic changes play a role in connecting these factors. In females, a process called X chromosome inactivation (XCI) helps balance X chromosome dosage. However, some X-linked genes escape this process, which is associated with aging and immune-related conditions. This study proposes that DNA methylation changes on the X chromosome may disrupt XCI control, leading to lupus in women by affecting the regulation of immune genes and accelerating aging.
Methods: We used DNAm data obtained from Illumina EPIC and 450K arrays on 310 SLE and 358 CTRLS. Firstly, we ran epigenome-wide association studies separately on females (N=556) and males (N= 112) to identify lupus associated DNAm differential positions on chrX (lupus chrX-DMPs). Secondly, we estimated epigenetic age acceleration using machine-learning algorithms such as Horvath, Hannum, and Levine's epigenetic clocks and studied their associations with DNAm. Finally, we ran trans methylation quantitative methylation loci mapping to identify genetic variants influencing lupus DNAm at lupus chrX-DMP.

Results: Our preliminary results show vast alteration of chrX DNAm in lupus females (N=298 DMPs at FDR < 5%), many of them were not present in men (P > 0.05) and were enriched in genes known to escape XCI (Chi-square, P = 5x10E-5). Some of the greatest DNAm changes were observed in relevant genes such as BCOR, AP1S2 and IQSEC2. Although we discovered fewer alterations in males, DNAm differences were greater between cases and controls, probably due to men only carrying one chromosome X. Interestingly, a high proportion of female lupus chrX DMPs do also show strong associations with epigenetic age acceleration measurements and a strong autosomic genetic control.

Conclusion: Most EWAS ignore chrX DNAm changes between sexes, leaving the genetic and epigenetic factors of diseases like lupus in women unexplored. Our findings show that chrX epigenetic alterations contribute to aging and female lupus by impacting X chromosome inactivation and immune-related gene dysregulation.

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Enhancing CAR-T cell immunotherapy for cancer through optimized production of extracellular vesicles

Chimeric Antigen Receptor (CAR) T cell therapy has achieved remarkable success in hematologic cancers but remains largely ineffective against solid tumors due to antigen heterogeneity, immunosuppressive tumor microenvironments, and limited T-cell infiltration and persistence. Additionally, reliance on autologous cells and complex manufacturing processes hinders scalability and broader clinical use.

Extracellular vesicles (EVs) have emerged as a promising cell-free alternative. These nanosized, biocompatible vesicles can transport diverse bioactive molecules, cross biological barriers, and be engineered for targeted delivery. Importantly, EVs derived from CAR-T cells (EV-CARs) retain functional CARs on their surface and can exert cytotoxic activity without the drawbacks of cell exhaustion or limited persistence.

This study aimed to enhance CAR-T-based immunotherapy by optimizing EV production and evaluating their therapeutic potential. EV-CARs displayed specific antitumor activity in preclinical models, inducing apoptosis through granzyme B and perforin release and effectively inhibiting tumor growth. To overcome current production bottlenecks, we targeted genes involved in exosome biogenesis and secretion. Genetic modifications resulted in a substantial increase in EV yield while maintaining cargo integrity and function, representing a scalable strategy for manufacturing EV-based therapies.

To enable off-the-shelf applications, we also generated EVs from T cells lacking HLA-I molecules. These HLA-I-deficient EVs showed reduced immunogenicity in vitro, suggesting improved persistence and lower risk of immune rejection.

Overall, our findings demonstrate that engineered EV-CARs combine the specificity of CAR-T therapy with the advantages of a cell-free platform. Optimizing their production and immunogenic profile offers a compelling path toward safe, scalable, and effective next-generation cancer immunotherapies, particularly for solid tumors.

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NOVEL FAM133B::CDK6 FUSION GENE DRIVES ABERRANT CELL CYCLE PROGRESION

Fusion genes play a significant role in biomedicine, serving as potential diagnostic markers and therapeutic targets. In non-small cell lung cancer (NSCLC), several fusion genes have been identified, among them ALK, ROS1, NTRK and RET.

In this study, through an integrative bioinformatic analysis of CRISPR and RNAi based vulnerability datasets, we identified previously unreported fusion candidates in lung adenocarcinoma (LUAD) cell lines. Among these, we validated the presence of a FAM133B::CDK6 fusion transcript by RT-qPCR and Sanger sequencing. The fusion produces an in-frame chimeric protein composed of an N-terminal region of FAM133B fused to a truncated CDK6 lacking the ATP-binding pocket and key residues required for interaction with p16.

Functional analyses revealed that FAM133B::CDK6 exerts a clear oncogenic effect. Transcriptomic profiling indicated enrichment of pathways related to apoptosis and alternative splicing, suggesting broad molecular rewiring driven by the fusion. Moreover, the fusion event induced exon skipping alterations affecting several regulators of cell-cycle control. At the protein level, expression of the chimeric isoforms facilitated accelerated re-entry into the cell cycle, consistent with enhanced phosphorylation of the retinoblastoma (Rb) protein.

In summary, these findings uncover FAM133B::CDK6 as a biologically relevant and previously undescribed fusion gene in LUAD. Its impact on alternative splicing dynamics, cell-cycle progression, and Rb signaling supports a mechanistic role in tumorigenesis. This study not only expands the catalog of actionable fusion genes in NSCLC but also highlights FAM133B::CDK6 as a potential biomarker and candidate for targeted therapeutic strategies.

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Bioinformatics approaches for understanding the role of DGCR8-mediated interferon response in 22q deletion síndrome

22q11.2 deletion syndrome (22qDS) is the most frequent microdeletion syndrome, often characterized by congenital heart defects, immune dysregulation, autoimmunity and increased risk of neuropsychiatric disorders such as schizophrenia. It is caused by a heterozygous deletion at chromosome 22 affecting fifty genes including DGCR8, a double-stranded RNA-binding protein required for microRNA biogenesis and repression of transposable element (TE)-derived RNAs. Using DGCR8 knockout in vitro cell models, our group has shown that loss of DGCR8 leads to cytoplasmic accumulation of TE-derived double-stranded RNAs and basal activation of interferon-stimulated genes (ISGs), even in the absence of infection. Most of these TE-dsRNAs derive from gene-dependent rather than self-expressed TEs, suggesting DGCR8 normally resolves TE-rich mRNAs to prevent innate immune recognition. Heterozygous cells showed an intermediate ISG response, while 22qDS patient-derived fibroblasts exhibited heightened ISG induction, indicating that DGCR8 haploinsufficiency primes interferon activation.

Here, we investigated whether this DGCR8-dependent interferon activation also occurs in 22qDS patient-derived transcriptomic datasets from blood cells and induced pluripotent stem cell (iPSC)-derived neuronal models. Both sample types showed significant ISG upregulation and activation of the innate immune response via the JAK/STAT pathway, indicating a basal interferon response. Consistent with our DGCR8 haploinsufficiency model, DGCR8 was among the least downregulated 22q11.2 genes in patient-derived samples, particularly in neuronal models. Correlation analyses further identified DGCR8 as the deleted gene most strongly and inversely associated with ISG expression among 22q11.2 genes. Finally, drug repurposing using Connectivity Map identified compounds capable of reversing the 22qDS interferon signature, including JAK/STAT inhibitors.

These findings provide the first in vivo evidence that a basal interferon response may be a systemic feature of 22qDS. Together with prior results, they support the novel idea that 22qDS might represent a previously unrecognized interferonopathy, with DGCR8 haploinsufficiency contributing to chronic immune activation and possibly to the psychiatric manifestations observed in patients.

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The ADAMTS1–NID1 Axis Shapes Tumor Progression Through Macrophage Reprogramming

Cancer is now recognized as a complex and dynamic ecosystem composed of tumor cells and non-tumor cells, all embedded within a dysregulated extracellular matrix (ECM). Beyond serving as a structural scaffold, the ECM actively participates in tumorigenesis, undergoing constant remodeling that can halted or promote tumor progression depending on the context. Among the main regulators of ECM dynamics are ADAMTS proteases, increasingly associated with immunomodulatory roles within the tumor microenvironment. To elucidate the function of ADAMTS1 in tumorigenesis, we conducted in vivo studies using melanoma and mammary tumor models. The absence of Adamts1 resulted in impaired tumor growth, which correlated with increased vascular deposition of its substrate, the basement membrane glycoprotein NIDOGEN-1 (NID1). Remarkably, NID1 overexpression reproduced this phenotype, inhibiting tumor growth and altering immune cell infiltration, thus mirroring the effects of ADAMTS1 loss.

Characterization of NID1-overexpressing tumors revealed an enrichment of antitumorigenic (M1-like) macrophages. In vitro, macrophage polarization assays corroborated that full-length NID1, but not its proteolytic fragments, induced M1-like polarization via αvβ3 integrin signaling. Transcriptomic integration of murine NID1-overexpressing tumor transcriptomic data with two large human melanoma cohorts identified a conserved gene signature associated with favorable prognosis and high M1-like macrophage abundance, mirroring the phenotype observed in the murine NID1-overexpressing model. These findings postulate NID1 as a tumor-suppressive and immunomodulatory ECM component and highlight the ADAMTS1–NID1 axis as a key driver of tumorigenesis in the extracellular scenario.

Ongoing research of our laboratory is focus on uveal melanoma, where ADAMTS1 appears to support tumor initiation by promoting stem-like and endothelial traits. In this model, the absence of ADAMTS1 also leads to dysregulated NID1 levels, reinforcing the relevance of the ADAMTS1–NID1 axis across different tumor contexts. Understanding how modulation of this pathway influences tumor–immune interactions may open new opportunities to enhance the efficacy of current immunotherapeutic approaches.

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Bioorthogonal Nanodevices for Enhanced CAR-T Cell Therapy Against Solid Tumors

Chimeric antigen receptor (CAR)-T cell therapy has revolutionized cancer treatment by harnessing patients' own immune cells to recognize and eliminate tumor-associated antigens through localized cytotoxic responses. Although highly effective against B-cell malignancies, its efficacy against solid tumors—such as breast cancer—is limited by challenges including antigen loss, an immunosuppressive tumor microenvironment and T-cell exhaustion. To overcome these hurdles, innovative strategies are urgently needed to enhance the antitumor activity of CAR-T cells directly at the tumor site.
Our project integrates nanotechnology, bioorthogonal catalysis, and chemical biology to engineer precise biomedical solutions. Key objectives include designing and characterizing functionalized metal-based nanosystems for robust binding to CAR-T cells. The characterization and confirmation of this nano-decoration process were performed using flow cytometry and confocal microscopy imaging. Concurrently, a library of prodyes designed for rapid activation via nanosystem-mediated bioorthogonal catalysis was developed. We have successfully synthesized first-generation nanosystems and demonstrated their stable and selective surface binding to CAR-T cells without impairing viability. Confocal imaging and flow cytometry confirm efficient immune cell decoration, while preliminary prodye activation assays validate the catalytic activity of the platform.
This work introduces a novel approach to augment CAR-T cell immunotherapy by imparting catalytic functions, enabling in situ drug activation. This strategy offers a promising means to overcome therapeutic resistance in solid tumors, advancing targeted drug delivery towards clinical translation and establishing a new paradigm for CAR-T-based smart nanomedicines.

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Heterochromatin as the guardian of youthful epigenetic memory

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ALHAMBRA: A Novel Promoter for Activation-Dependent Expression in Fourth-Generation CAR-T Cells

Despite being a major breakthrough for hematological cancers, CAR-T therapy remains limited in solid tumors, due to factors such as an immunosuppressive tumor microenvironment. Fourth-generation CAR-T cells, engineered to secrete therapeutic molecules, offer a potential solution, but their secretion must be tightly regulated to avoid off-tumor toxicity. To achieve controlled secretion, several systems use activation-inducible promoters containing multiple NFAT transcription factor binding sites (TFBS) followed by either a synthetic TATA box (pNFATsyn) or the IL-2 minimal promoter (NFATmIL2). However, these promoters still show basal activation-independent expression known as leakiness.

To reduce this leakiness, we designed new NFAT-based promoters incorporating additional TFBS that improve regulation and minimize basal expression. We have named this platform ALHAMBRA (Activation Linked High-expression And Minimal Basal Regulation in Activated T-cells). All ALHAMBRA promoters were incorporated into LVs to express eGFP, and these LVs were used to transduce T cells and CAR-T cells to assess eGFP expression levels at different stages of activation. As controls, the same cells were transduced with NFATmIL2-eGFP and NFATsyn-eGFP LVs. LV-transduced T cells and CAR-T cells were exposed to different stimuli, including pro-inflammatory cytokines and antigen-negative target cells. We also have evaluated he effect of removing each TFBS on the promoter's expression profile.

Compared with LVs carrying NFATmIL2 or NFATsyn promoters, all ALHAMBRA-based LVs exhibit lower gene expression levels in non-activated T cells and CAR-T cells. In summary, in this study we described the ALHAMBRA-based LVs designed to reduce leaking of transgene expression in non-activated CAR-T cells. We demonstrate reduced off-target activation while maintaining or enhancing expression upon T cell stimulation. We propose the ALHAMBRA platform for the development of safer 4th generation CAR/T cells.

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From Bench to Health System: Early Outcomes of LargePanel cfDNA Testing in the Andalusian LOLA Project

Introduction. Tumor heterogeneity in metastatic disease requires multiple tissue biopsies for complete genotyping, unfeasible in clinical practice. Liquid biopsy based on cell-free DNA (cfDNA) offers a minimally invasive alternative. The LOLA Project evaluates the integration of cfDNA NGS assays for advanced non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) into the Andalusian Public Health System in Spain (NCT06997458).
Methods. A prospective, observational, and multicenter study across 12 hospitals (Grant CI24/014). This interim analysis included the first 250 patients recruited between 2024 and 2025. Of 244 samples, 235 yielded valid variant reports after quality control. A total of 134 NSCLC and 101 CRC cases were processed with the TEMPUS xF 150-gene panel and downstream analyses.
Results. Turnaround time was < 10 days in most cases. Over 86 million reads were obtained across all samples, achieving a sequencing quality with a Phred score ≥ 35, paired-read alignment efficiency ≥ 99.9% with Phred > 53, and a median depth ≥ 4375. Among the 1,559 unique variants, 96% had a variant allele frequency ≥0.001; patients had an average of 8 variants for NSCLC and 10 for CRC. The most frequently altered genes were KRAS (12 codon substitutions), EGFR, ARID1A, and ATM. A total of 50 NSCLC and 59 CRC patients harboring at least one actionable variant supported by OncoKB evidence. Preliminary Random Forest analysis identified KRAS, EGFR, and treatment-change variables as key drivers in NSCLC and KRAS, BRAF, and NRAS as key drivers in CRC. Further consensus clustering and silhouette analysis confirmed ten and six cluster partitions for NSCLC and CRC, respectively.
Conclusion. These interim findings confirm the feasibility, robustness, and clinical utility of ctDNA-based NGS in Andalusia, supporting integration into routine oncology practice. Continued recruitment and follow-up will refine biomarker-guided strategies and inform healthcare policy.

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Combined effects of urinary microbiota isolated from UTI patients in a human urothelial microtissue model

Urinary tract infection (UTI) is one of the most common bacterial infections in humans, affecting 400 million cases annually. Its current diagnosis and treatment have become challenging due to the inability to diagnose all patients using traditional routine techniques and the use of broad-spectrum antibiotics that promote the development of antimicrobial resistance. The identification of commensal urinary microbiota has opened up a new field for the study of new therapies and diagnostic methods for UTIs. This study explores the role of the urobiome—the microbial community of the urinary tract—in patients with UTI symptoms, focusing on both positive and negative uroculture cases. Using advanced sequencing techniques, we have characterized the diversity and microbial composition of the urobiome in symptomatic individuals, identifying distinctive taxonomic and functional signatures associated with UTI patients. This analysis has enabled the identification of some bacterial genes with diagnostic potential for these patients. In addition, we have evaluated the host-microbiome interactions by co-culturing the whole urobiome from UTI patients and healthy controls in a 3D urine-tolerant human urothelial model. The urothelial response has been evaluated using different techniques such as microscopy, barrier function analysis and cytokine measurements showing a potential effect of the urobiome isolated from UTI patients on the urothelium. All these results present the urobiome as an important agent in the pathogenesis of UTI, as well as a potential tool for the development of new diagnostic and therapeutic strategies.

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