REUNIÓN ANUAL

RICORS | TERAV+

2026

RED ESPAÑOLA
DE TERAPIAS
AVANZADAS

ALICANTE

19 Y 20 DE NOVIEMBRE 2026

CUADERNO
DE SESIONES
Y PÓSTERS

SESIONES
HOTEL AC ALICANTE

COORDINAN
MANEL JUAN OTERO
DAMIÁN GARCÍA OLMO
SALVADOR MARTÍNEZ

COMITÉ CIENTÍFICO
PAULA RÍO
FRANCISCO MARTÍN MOLINA
FELIPE PROSPER
CRISTINA EGUIZABAL
MARÍA EUGENIA FERNÁNDEZ SANTOS
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ELENA GARRETA
CRISTINA AVENDAÑO
JOAQUIM VIVES
GLORIA CARMONA
JAVIER GARCÍA CASTRO

EDITADO POR
RED ESPAÑOLA DE TERAPIAS AVANZADAS
DEL INSTITUTO DE SALUD CARLOS III
PROYECTO RICORS/TERAV+ RD24/0014/0001

FINANCIADO POR
INSTITUTO DE SALUD CARLOS III (ISCIII)
COFINANCIADO POR LA UNIÓN EUROPEA.

DEPÓSITO LEGAL
MU-1854-2025

FUNDACIÓ DE RECERCA CLÍNIC BARCELONA
INSTITUT D'INVESTIGACIONS BIOMÈDIQUES AUGUST PI I SUNYER.

REUNIÓN ANUAL

RICORS | TERAV+

2026

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PROGRAMA
DE SESIONES
Y PÓSTERS

PROGRAMA DE SESIONES Y PÓSTERS

TERAPIA GÉNICA
PRIMERA MESA DE PONENCIAS

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 1

TOWARD CLINICAL TRANSLATION OF LENTIVIRAL GENE THERAPY FOR DIAMOND-BLACKFAN ANEMIA: PRECLINICAL STUDIES TARGETING RPS19.

PONENTE: Susana Navarro. Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT-FJD. Madrid). RD24/0014/0023.

RD24/0014/0023. RD24/0014/0035, RD24/0014/0008.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 1

FIRST STEPS TOWARDS RENAL GENE THERAPY: A PILOT STUDY OF ADPKDII TREATMENT IN MICE.

PONENTE: Rafael Aldabe. Universidad de Navarra (CIMA). Pamplona. RD24/0014/0004.

RD24/0014/0004, RD24/0014/0026.

PÓSTER 1 – COMUNICACIÓN ORAL

MODIFICACIÓN SUPERFICIAL DE NANOPARTÍCULAS LIPÍDICAS PARA LA ENTREGA FUNCIONAL DE MATERIAL GENÉTICO EN CÉLULAS HEMATOPOYÉTICAS Y HEPÁTICAS.

PONENTE: Lucía Enríquez. Universidad del País Vasco. RD24/0014/0012.

RD24/0014/0012, RD24/0014/0023.

PÓSTER 2 – COMUNICACIÓN ORAL

ALHAMBRA: AN ACTIVATION-INDUCIBLE PROMOTER FOR SAFER AND MORE CONTROLLABLE NEXT GENERATION CAR-T THERAPIES.

PONENTE: Alberto Tagliaferro. GENYO. (Ibs). Universidad de Granada. RD24/0014/0005.

RD24/0014/0005, RD24/0014/0010.

INMUNOTERAPIA CELULAR ANTITUMORAL
SEGUNDA MESA DE PONENCIAS

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 2

UPDATED SAFETY AND EFFICACY RESULTS OF TRANSPOCART19, A NON-VIRAL SLEEPING BEAUTY TRANSPOSON-ENGINEERED ANTI-CD19 CAR-T CELL THERAPY FOR RELAPSED/REFRACTORY B-CELL LYMPHOMAS.

PONENTE: Lucía López del Corral. Instituto de Investigación Biomédica de Salamanca (IBSAL). RD24/0014/0034.

RD24/0014/0034, RD24/0014/0010, RD24/0014/0027, RD24/0014/0023, RD24/0014/0014.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 2

THE FIRST POINT-OF-CARE CAR ADVANCED THERAPY IN THE BASQUE COUNTRY: A FIRST-IN-HUMAN PHASE I CLINICAL TRIAL OF ALLOGENEIC UMBILICAL CORD BLOOD-DERIVED CAR-NK-CD19 CELLS (NKARI-0001) FOR RELAPSED OR REFRACTORY CD19+ B-CELL LYMPHOID MALIGNANCIES (EU CT NUMBER: 2026-527288-14-00).

PONENTE: Cristina Eguizabal. IIS Biobizkaia-Centro Vasco de Transfusión y Tejidos Humanos (CVTTH). RD24/0014/0025.

RD21/0017/0024, RD21/0017/0029, RD21/0017/0002 y RD24/0014/0025, RD24/0014/0001, RD24/0014/0035.

PÓSTER 1 – COMUNICACIÓN ORAL

DUAL TRAC AND B2M GENOME EDITING PRESERVES ANTILEUKEMIC ACTIVITY OF CLL1-DIRECTED CAR-T CELLS FOR ALLOGENEIC IMMUNOTHERAPY OF AML.

PONENTE: Alejandro Millán. GENYO. (Ibs). Universidad de Granada. RD24/0014/0005.

RD24/0014/0005, RD24/0014/0030.

PÓSTER 2 – COMUNICACIÓN ORAL

NOVEL HER2-SPECIFIC SDAB-CAR-T CELLS EXHIBIT ENHANCED ANTITUMORAL EFFICACY IN HER2-POSITIVE CANCERS.

PONENTE: Carmen Barbero-Jiménez. Clínica Universidad de Navarra (UNAV). Pamplona. RD24/0014/0010.

RD24/0014/0005, RD24/0014/0010.

TERAPIA CELULAR SOMÁTICA Y MEDICINA REGENERATIVA
TERCERA MESA DE PONENCIAS

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 3

A PHASE III, MULTICENTER, RANDOMIZED, CONTROLLED, OPEN-LABEL WITH BLINDED-ENDPOINT, CLINICAL TRIAL TO ASSESS THE EFFICACY OF INTRA-ARTERIAL INFUSION OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS IN PATIENTS WITH ISCHEMIC STROKE.

PONENTE: Gloria Carmona. Red Andaluza de diseño y traslación de Terapias Avanzadas de la Fundación Pública Andaluza Progreso y Salud. Sevilla. RD24/0014/0030.

RD24/0014/0005, RD24/0014/0027, RD24/0014/0030, RD24/0014/0035, RD24/0014/0040.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 3

MSCS FOR ACUTE GRAFT-VERSUS-HOST DISEASE IN SPAIN: A NATIONWIDE MULTICENTER STUDY INTEGRATING CLINICAL OUTCOMES AND PRODUCT CHARACTERISTICS.

PONENTE: Fermín Sánchez-Guijo. Instituto de Investigación Biomédica de Salamanca (IBSAL). RD24/0014/0034.

RD24/0014/0001, RD24/0014/0006, RD24/0014/0008, RD24/0014/0010, RD24/0014/0013, RD24/0014/0014, RD24/0014/0021, RD24/0014/0024, RD24/0014/0027, RD24/0014/0030, RD24/0014/0034, RD24/0014/0035, RD24/0014/0036, RD24/0014/0037, RD24/0014/0040.

PÓSTER 1 – COMUNICACIÓN ORAL

PATHWAY FOR QUALIFICATION AND CLINICAL APPLICATION OF A 3D HUMAN BIOLOGICAL VENTRICULAR ASSIST DEVICE FOR ENDSTAGE HEART FAILURE.

PONENTE: Manuel M. Mazo. Clínica Universidad de Navarra (UNAV). Pamplona. RD24/0014/0010.

RD24/0014/0010, RD24/0014/0035.

PÓSTER 2 – COMUNICACIÓN ORAL

ENSAYO CLÍNICO FASE IIB PARA EVALUAR LA EFICACIA Y SEGURIDAD DEL TRATAMIENTO CON CÉLULAS MESENQUIMALES (MSC) ALOGÉNICAS EN INYECCIÓN OCULAR COMO TERAPIA DE LA ENFERMEDAD DE INJERTO CONTRA RECEPTOR (EICR) REFRACTARIA A TRATAMIENTOS CONVENCIONALES. (TERAV-EYE).

PONENTE: María Eugenia Fernández-Santos. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM). Madrid. RD24/0014/0035.

RD24/0014/0034 y RD24/0014/0035.

INNOVACIONES TECNOLÓGICAS Y BIOMODELOS
CUARTA MESA DE PONENCIAS

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 4

ENGINEERING HUMAN CARDIAC ORGANOIDS AS IN VITRO MODELS TO STUDY CARDIAC DEVELOPMENT AND DISEASE.

PONENTE: Elena Garreta. Instituto De Bioingeniería de Cataluña (IBEC). Barcelona. RD24/0014/0026.

RD24/0014/0026, RD24/0014/0010; RD24/0014/0035.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 4

MATRIZ CARDÍACA DECELULARIZADA Y RECELULARIZADA PARA EL TRATAMIENTO DE INSUFICIENCIA CARDÍACA AVANZADA.

PONENTE: Marcos Vázquez. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM). Madrid. RD24/0014/0035.

RD24/0014/0008, RD24/0014/0010, RD24/0014/0026 y RD24/0014/0035.

PÓSTER 1 – COMUNICACIÓN ORAL

DEVELOPMENT OF LIPID NANOPARTICLES FOR NON-VIRAL CAR-T CELL ENGINEERING.

PONENTE: María Blanco Prieto. Instituto de Investigación Sanitaria de Navarra. (IdISNA). Pamplona. RD24/0014/0041.

RD24/0014/0041 y RD24/0014/0010.

PÓSTER 2 – COMUNICACIÓN ORAL

ADVANCED ELECTROSPUN PCL SCAFFOLDS FUNCTIONALIZED WITH ALLOGENEIC PRP (ALOPRP3D) AS A BIOLOGICAL DRESSING FOR CHRONIC WOUND HEALING.

PONENTE: Cristina Eguizabal. IIS Biobizkaia- Centro Vasco de Transfusion y Tejidos Humanos (CVTTH). RD24/0014/0025.

RD21/0017/0024, RD21/0017/0018; RD24/0017/0025, RD24/0014/0026.

FORMACIÓN Y CIENCIA REGULATORIA
QUINTA MESA DE PONENCIAS

MODERADOR: PROF. JOAQUIM VIVES. COORDINADOR PROGRAMA.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 5

AUTORIZACIÓN DE FABRICANTES ACADÉMICOS DE TA (MIA). REQUISITOS REGULATORIOS DE LOS OGM.

PONENTE: Prof. Joaquim Delgadillo. Banc de Sang i Teixits (BST). Barcelona.

RD24/0014/0037.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 5

ACTUALIZACIÓN CAMBIOS NORMATIVOS EN MARCHA CON IMPACTO EN LAS TERAPIAS AVANZADAS. COMITÉ REGULATORIO DE TERAV+.

PONENTE: Prof. Arantxa Sancho. Hospital Universitario Puerta de Hierro. Madrid. RD24/0014/0021.

PÓSTER 1 – COMUNICACIÓN ORAL

ACTIVIDADES COLABORATIVAS EN FORMACIÓN.

PONENTE: Prof. Joaquim Vives. Banc de Sang i Teixits (BST). Barcelona.

RD24/0014/0037.

CONFERENCIA INVITADA.

TERAPIAS AVANZADAS: PASADO, PRESENTE Y FUTURO DE LAS CÉLULAS EMBRIONARIAS.

PONENTE: Prof. Agustín Zapata. Prof. Emérito de la Universidad Complutense de Madrid.

PROGRAMA DE SESIONES Y PÓSTERS

RELACIONES NACIONALES E INTERNACIONALES SEXTA MESA DE PONENCIAS

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 6

11:35-11:55 H. RELACIONES NACIONALES E INTERNACIONALES DE TERAV+.

PONENTE: Prof. Gloria Carmona. Red Andaluza de diseño y traslación de Terapias Avanzadas de la Fundación Pública Andaluza Progreso y Salud. Sevilla. RD24/0014/0030.

CONFERENCIA COLABORATIVA – LÍNEA DE COLABORACIÓN 6

11:55- 12:15 H. PROGRAMAS INTERNACIONALES ALINEADOS CON LOS OBJETIVOS DE LA RED: ERDERA.

PONENTE: Prof. Salvador Martínez. Instituto de Neurociencias de Alicante (IN-UMH). RD24/0014/0022.

CONFERENCIA INVITADA:

12:15 - 12:35 H. CAN WE ASK CELLS TO BECOME MEDICINES?

PONENTE: Prof. Massimo Dominici. University of Módena and Reggio Emilia, Módena, Italy.

TERAPIA GÉNICA

EDICIÓN GÉNICA DE HAO1 MEDIADA POR AAV COMO TERAPIA DE REDUCCIÓN DE SUSTRATO EN LA HIPEROXALURIA PRIMARIA TIPO 1.
GRUPOS: RD24/0014/0004, RD24/0014/0026.

GENERACIÓN DE MODELOS Y HERRAMIENTAS PARA EL ESTUDIO Y LA INTERVENCIÓN TERAPÉUTICA EN ENFERMEDAD DE POMPE.
GRUPOS: RG24/0014/0004, RD24/0014/0005, RG24/0014/0034.

PRECLINICAL CHARACTERIZATION OF SECOND-GENERATION ANTI-CLL-1 CAR-T CELLS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA.
GRUPOS: RD24/0014/0005, RD24/0014/0010.

CAR-T CELL-DERIVED EXTRACELLULAR VESICLES AS A NOVEL PLATFORM FOR PANCREATIC CANCER IMMUNOTHERAPY.
GRUPOS: RD24/0014/0005, RD24/0014/0001.

MULTI-TOOL IN SILICO IMMUNOGENICITY PROFILING OF CD19/CD22-TARGETING CAR CONSTRUCTS.
GRUPOS: RD24/0014/0005, RD24/0014/0010.

SINGLE-CELL MULTIOMIC PROFILING DEFINES MOLECULAR SIGNATURES OF HUMAN LONG-TERM HEMATOPOIETIC STEM CELLS FOR NEXT GENERATION GENE THERAPY APPLICATIONS.
GRUPOS: RD24/0014/0010, RD24/0014/0023.

OPTIMIZATION OF LENTIVIRAL VECTOR PRODUCTION THROUGH PLASMID MINIATURIZATION AND NANOPLASMID TECHNOLOGY.
GRUPOS: RD24/0014/0016, RD24/0014/0023, RD24/0014/0005.

DESARROLLO COLABORATIVO DE NANOPARTÍCULAS LIPÍDICAS PARA LA EDICIÓN GÉNICA *IN VIVO* DEL FACTOR XI COMO ESTRATEGIA ANTITROMBÓTICA.
GRUPOS: RD24/0014/0023, RD24/0014/0012.

IN VITRO AND IN VIVO FUNCTIONAL RESTORATION OF THE HEMATOPOIETIC LINEAGE DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS CORRECTED FOR THE MUTATION-CAUSING APDS2 SYNDROME.
GRUPOS: RD21/0017/0024, RD21/0017/0029, RD24/0014/0025, RD24/0014/0023 Y RD24/0014/0001.

DISCREPANCIES BETWEEN VECTOR COPY NUMBER AND CAR-T CELL EXPRESSION: THE IMPACT OF FREE VIRAL DNA ON PCR-BASED ASSAY.
GRUPOS: RD24/0014/0030, RD24/0014/0027.

DESARROLLO DE MODELOS NEURONALES DERIVADOS DE PACIENTES Y NUEVAS ESTRATEGIAS DE TERAPIA GÉNICA PARA ENFERMEDADES NEUROLÓGICAS RARAS.
GRUPOS: RD24/0014/0039, RD24/0014/0013 Y RD24/0014/0037.

INMUNOTERAPIA CELULAR ANTITUMORAL

OPTIMIZATION IN LENTIVIRAL VECTOR MANUFACTURING FOR PRODUCTION OF CAR-T THERAPY AND OTHER CELL THERAPIES (LENTI-UP).
GRUPOS: RD24/0014/0001, RD24/0014/0006, RD24/0014/0002.

TERAPIA CAR-T ESPECÍFICA FRENTE A CLAUDINA 18.2 PARA EL CÁNCER GÁSTRICO Y PANCREÁTICO.
GRUPOS: RD24/0014/0002, RD24/0014/0001.

VALIDACIÓN DEL CENTRO DE TERAPIAS AVANZADAS (CTA) COMO CENTRO PRODUCTOR DESCENTRALIZADO (*POINT-OF-CARE*) PARA LA FABRICACIÓN DE LA TERAPIA CAR-T ARI0003 EN GALICIA.
GRUPOS: RD24/0014/0002, RD24/0014/0001.

ENHANCING CAR-T THERAPY FOR HER2+ TUMORS: CONTROLLED CAR AND CYTOKINE EXPRESSIONS FOR IMPROVED EFFICACY AND SAFETY.
GRUPOS: RD24/0014/0005, RD24/0014/0010.

DEVELOPMENT OF NANOTRUCK19-22-IL18I: A NEXT-GENERATION CAR-T CELL COMBINING SDAB-BASED DUAL CD19/CD22 TARGETING AND INDUCIBLE IL-18 DELIVERY TO OVERCOME ANTIGEN ESCAPE AND FUNCTIONAL EXHAUSTION.
GRUPOS: RD24/0014/0005, RD24/0014/0010.

SMART ANTI-EPCAM TRUCKS WITH ANTIGEN-DENSITY-DEPENDENT CYTOTOXICITY AND TUMOR-RESTRICTED IL-18 EXPRESSION.
GRUPOS: RD24/0014/0005, RD24/0014/0010.

HETEROGENEIDAD ANATÓMICA DE LA INFILTRACIÓN LEUCÉMICA DURANTE LA ENFERMEDAD RESIDUAL Y LA RECAÍDA DE LA LLA-B: ESTUDIO PILOTO MEDIANTE CITOMETRÍA DE FLUJO Y MICROSCOPÍA TRIDIMENSIONAL.
GRUPOS: RD24/0014/0008, RD24/0014/0012, RD24CIII/0002/0002.

IDENTIFICACIÓN DE NEOANTÍGENOS CANDIDATOS EN CÁNCER PEDIÁTRICO COMO NUEVAS DIANAS PARA LA INMUNOTERAPIA BASADA EN LINFOCITOS T.
GRUPOS: RD24/0014/0001, RD24/0014/0008.

DESARROLLO DE UN MODELO PREDICTIVO DE RESPUESTA A CAR-T MEDIANTE LA INTEGRACIÓN DE BIOMARCADORES INMUNOINFLAMATORIOS PREINFUSIÓN.
GRUPOS: RD24/0014/0034, RD24/0014/0008.

GENERACIÓN DE LINFOCITOS T ESPECÍFICOS DE ADENOVIRUS (VST) DE DONANTE ALTERNATIVO, GUIADA POR PREDICCIÓN *IN SILICO* DE UNIÓN A HLA PARA EL TRATAMIENTO DE ADENOVIRIASIS REFRACTARIA TRAS TRASPLANTE HAPLOIDÉNTICO DE PROGENITORES HEMATOPOYÉTICOS EN PACIENTE PEDIÁTRICA.
GRUPOS: RD24/0014/0008, RD24/0014/0009.

ALLONANOCART33: NOVEL TRANSPOSON-SDAB-BASED ALLOGENEIC CD33-TARGETED CAR-T CELLS FOR PATIENTS WITH R/R AML OR MDS.
GRUPOS: RD24/0014/0003, RD24/0014/0010, RD24/0014/0034, RD24/0014/0040.

FRAMEWORK-REGION CHARGE OPTIMIZATION ENHANCES THE ANTI-TUMOR POTENCY OF CAR-T CELLS ACROSS MULTIPLE SOLID TUMORS BY CALIBRATING TONIC SIGNAL.
GRUPOS: RD24/0014/0005, RD24/0014/0010RD2, 4/0014/0041.

IDENTIFICACIÓN DE NUEVAS DIANAS DE SUPERFICIE MEDIANTE PROTEÓMICA DE MEMBRANA Y DESARROLLO PRECLÍNICO DE UNA TERAPIA CART DIRIGIDA CONTRA LEUCEMIA LINFOBLÁSTICA AGUDA DE CÉLULAS T (T-ALL).
GRUPOS: RD24/0014/0015, RD24/0014/0024, RD24/0014/0042, RD24/0014/0023.

TARGETING MUTANT CALRETICULIN WITH CAR-T CELLS: FROM PRECLINICAL PROOF OF CONCEPT TO FIRST-IN-HUMAN CLINICAL DEVELOPMENT.
GRUPOS: RD24/0014/0015.

IMPACT: AN INTEGRATED PLATFORM FOR SCALABLE AND PERSONALISED CAR-T CELL MANUFACTURING.
GRUPOS: RD24/0014/0016, RD24/0014/0001.

HAPLOIDENTICAL CD45RA^{-} NKG2D-CAR-T CELLS IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS WITH RELAPSED/REFRACTORY SARCOMA: PRELIMINARY RESULTS OF THE ONGOING PHASE I CAR4SAR TRIAL (NCT06087341).
GRUPOS: RD24/0014/0024, RD24CIII/0002/0002.

PRECLINICAL VALIDATION OF A SECOND GENERATION B7-H3 CAR T-CELL THERAPY FOR PEDIATRIC SOLID TUMORS.
GRUPOS: RD24/0014/0024, RD24/0014/0023.

PRECLINICAL VALIDATION OF A CD7 CAR T-CELL THERAPY FOR PEDIATRIC NON-B-CELL MALIGNANCIES.
GRUPOS: RD24/0014/0024, RD24/0014/0023.

TERAPIA CAR T DUAL CD19/22 EN UNA ADULTA JOVEN CON VASCULITIS ANCA ANTI-MPO EN HEMODIÁLISIS.
GRUPOS: RD24/0014/0023, RD24/0014/0024.

GENERATING NK CELLS IN VITRO FROM UMBILICAL CORD BLOOD UNITS IN A FEEDER FREE GMP SYSTEM.
GRUPOS: RD21/0017/0024, RD21/0017/0029 Y RD24/0014/0025, RD24/0014/0001, RD24/0014/0023.

DUAL-TARGET CAR-NKS WITH ON- AND OFF-TUMOR ACTIVITY MAY OVERRIDE IMMUNE SUPPRESSION IN SOLID CANCERS: AN "OFF THE SHELF" NKG2D-CD19-CAR-NK CELL THERAPY (DUAL-CAR-NK).
GRUPOS: RD21/0017/0024, RD21/0017/0025, RD21/0017/0029 Y RD24/0014/0025, RD24/0014/0024, RD24/0014/0001, RD24/0014/0004.

GENERATING NKG2D-CAR-TRANSDUCED NATURAL KILLER (NK) CELLS FOR TREATING OSTEOSARCOMA.
GRUPOS: RD21/0017/0024, RD21/0017/0025, RD21/0017/0034 Y RD24/0014/0025, RD24/0014/0024, RD24/0014/0004.

EL PERFIL METABÓLICO Y LA PERSISTENCIA FUNCIONAL COMO HERRAMIENTAS PARA MEJORAR LA CARACTERIZACIÓN DE LOS PRODUCTOS CAR-T.
GRUPOS: RD24/0014/0027, RD24/0014/0021, RD24/0014/0001, RD24/0014/0036.

EXOFUCOSYLATION AS A TOOL TO ENHANCE THE ANTITUMOR ACTIVITY OF EXPANDED CAR-T CELLS AGAINST SOLID TUMORS.
GRUPOS: RD24/0014/0005, RD24/0014/0040.

NG2–ITGA4 AXIS REGULATES RHO GTPASES AND LEUKEMIA AGGRESSIVENESS IN KMT2A-R B-ALL AND IS TARGETABLE WITH NATALIZUMAB.
GRUPOS: RD24/0014/0042, RD24/0014/0040.

PTGER3-DIRECTED CAR T-CELL IMMUNOTHERAPY FOR EWING SARCOMA.
GRUPOS: RD24/0014/0006, RD24/0014/0042.

TISA-CEL AND AXI-CEL CAR STRUCTURE INFLUENCES THE DEVELOPMENT OF RESISTANCE TO CD19-CAR-T THERAPY.
GRUPOS: RD24/0014/0024, RD24/0014/0042.

PROGRAMA DE SESIONES Y PÓSTERS

TERAPIA CELULAR SOMÁTICA Y MEDICINA REGENERATIVA

ADVANCED THERAPY USED AS A POTENTIAL TREATMENT WITHIN THE NATIONAL PILOT FERTILITY PRESERVATION PROGRAM FOR PERIPUBERTAL AND PREPUBERTAL CHILDREN WITH CANCER AND GENETIC SYNDROMES IN OSAKIDETZA.

GRUPOS: RD21/0017/0024, RD21/0017/0018 Y RD24/0017/0025, RD24/0014/0026.

TOPICAL USE OF EXOSOMES FROM HUMAN MATERNAL MILK ACCELERATES ACUTE CUTANEOUS WOUND HEALING.

GRUPOS: RD24/0014/0009, RD24/0014/0012.

PROGRAMA DE USOS COMPASIVOS CON ASCS EN CAUSTICACIÓN OCULAR.

GRUPOS: RD24/0014/0014 Y RD24/0014/0035.

INNOVACIONES TECNOLÓGICAS Y BIOMODELOS

EVALUATION OF ABCD1 NANOPLASMID-MEDIATED GENE DELIVERY IN AN IN VITRO MODEL OF X-LINKED ADRENOLEUKODYSTROPHY.

GRUPOS: RD24/0014/0022.

ADVANCED ELECTROSPUN PCL SCAFFOLDS FUNCTIONALIZED WITH ALLOGENEIC PRP (ALOPRP3D) AS A BIOLOGICAL DRESSING FOR CHRONIC WOUND HEALING.

GRUPOS: RD21/0017/0024, RD21/0017/0018 Y RD24/0017/0025, RD24/0014/0026.

ENGINEERING HUMAN CARDIAC ORGANOIDS AS IN VITRO MODELS TO STUDY CARDIAC DEVELOPMENT AND DISEASE.

GRUPOS: RD24/0014/0026, RD24/0014/0010, RD24/0014/0035.

MATRIZ CARDÍACA DECELULARIZADA Y RECELULARIZADA PARA EL TRATAMIENTO DE INSUFICIENCIA CARDÍACA AVANZADA.

GRUPOS: RD24/0014/0008, RD24/0014/0010, RD24/0014/0026, RD24/0014/0035.

DEVELOPMENT OF LIPID NANOPARTICLES FOR NON-VIRAL CAR-T CELL ENGINEERING.

GRUPOS: RD24/0014/0010, RD24/0014/0041.

FORMACIÓN Y CIENCIA REGULATORIA

QUALITY IMPLEMENTATION IN THE TRANSLATION OF RESEARCH INTO ADVANCED THERAPIES: A SURVEY-BASED APPROACH TO DEVELOPING AN INTEGRATED QUALITY FRAMEWORK.

GRUPOS: RD24/0014/0016, RD24/0014/0002, RD24/0014/0001, RD24/0014/0008, RD24/0014/0003, RD24/0014/0030, RD24/0014/0010, RD24/0014/0004, RD24/0014/0006, RD24/0014/0014, RD24/0014/0023, RD24/0014/0040, RD24/0014/0013, RD24/0014/0039, RD24/0014/0037, RD24/0014/0041.

IMPULSO AL TALENTO EN TERAPIAS AVANZADAS: RESULTADOS Y LECCIONES APRENDIDAS TRAS EL PRIMER AÑO DEL PROGRAMA DE MENTORING DE LA RED RICORS/TERAV+.

GRUPOS: RD24/0014/0037, RD24/0014/0021, RD24/0014/0014, RD24/0014/0001.

FLUENTCELLS: ESTRATEGIA MULTIDISCIPLINAR CONTRA LA DESINFORMACIÓN Y LAS MALAS PRÁCTICAS EN TERAPIAS AVANZADAS.

GRUPOS: RD24/0014/0012, RD24/0014/0001, RD24/0014/0037.

PROGRAMA 1. TERAPIA GÉNICA
SESIONES

TOWARD CLINICAL TRANSLATION OF LENTIVIRAL GENE THERAPY FOR DIAMOND-BLACKFAN ANEMIA: PRECLINICAL STUDIES TARGETING RPS19. RD24/0014/0023. RD24/0014/0035, RD24/0014/0008.

Y Giménez[1,2,3], M Palacios[1,2,3], R Sanchez[1,2,3], C Zorbas[4], Jorge Peral[1,2,3], A Puzik[5], L Ugalde[1,2,3], O Alberquilla[1,2,3], M Villanueva[1,2,3], P Río[1,2,3], E Gálvez[6], L Da Costa[7], M Strullu[8], A Catala[9], Anna Ruiz[9], JC Segovia[1,2,3], J Sevilla[6], B Strahm[5], C M. Niemeyer[5], C Beléndez[2,10,11], T Leblanc[8'], D.LJ. Lafontaine[4], J Bueren[1,2,3], **S Navarro[1,2,3]**.

[1]Division of Hematopoietic Innovative Therapies, CIEMAT, Madrid, Spain.

[2]Instituto Nacional de Investigación Biomédica en Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, España.

[3]Advanced Therapies Unit, IIS-Fundación Jimenez Diaz (IIS-FJD, UAM), 28040 Madrid, Spain.

[4]RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S./FNRS), Université libre de Bruxelles (ULB), Biopark campus, B-6041 Gosselies, Belgium.

[5]Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[6]Hospital del Niño Jesús, Madrid, Spain.

[7]AP-HP, Hematology diagnostic laboratory, Hôpital Robert-Debré, F-75019, Paris, France; University of Paris; Hematim, UR4666, UPJV; LABEX GR-EX

[8]AP-HP, service Immuno-Hématologie pédiatique, Hôpital R. Debré, F-75019, Paris, France

[9]Hospital San Joan D´Deu, Barcelona, Spain

[10]Sección de Hematología y Oncología Pediátricas, Hospital General Universitario Gregorio Marañón, Madrid, Spain

[11]Facultad de Medicina, Universidad Complutense de Madrid, España.

[12]Instituto Investigación Sanitaria Gregorio Marañón, Madrid, España.

CENTROS PARTICIPANTES: CIEMAT, CIBERER, Hospital Gregorio Marañon, Hospital Vall D´Hebron, Hospital San Juan D´Deu, CIBERONC/CUN/FIMA.

Diamond-Blackfan anemia syndrome (DBAS; OMIM #105650) is a rare bone marrow failure (BMF) disorder with an estimated prevalence of 5-10 cases per million live births. Its clinical manifestations are primarily erythroblastopenia, accompanied by congenital abnormalities and elevated risk of cancer. Approximately 40% of patients with DBA rely on transfusion support. Hematopoietic stem cell transplantation (HSCT) remains the only potentially curative treatment option available for patients under the age of 10. In older patients, HSCT is associated with high toxicity, leading to significant mortality and long-term morbidity. These challenges underscore an urgent clinical need for a substantial proportion of these patients. Most DBA cases are associated with autosomal dominant mutations in any of 26 genes that encode for ribosomal proteins. *RPS19* is the most frequently mutated, representing 25% of all the DBA genes. This study summarizes the preclinical work conducted to initiate an ex vivo lentiviral mediated gene therapy trial for patients with mutations in *RPS19*.

Initially, two lentiviral vectors (LVs) carrying the codon-optimized *RPS19* gene under different eukaryotic promoters (the phosphoglicerate kinase; *PGK.CoRPS19*, and the elongation factor alpha in its short version; *EF1α. CoRPS19*) were developed and tested. The efficacy proof of concept was demonstrated through the restoration of *RPS19* expression and ribosomal biogenesis in RPS19-deficient K562 cells. Based on its superior performance, the *PGK.CoRPS19* LV, was selected for further evaluation in CD34+ hematopoietic stem and progenitor cells (HSPCs) from patients with DBAS. The transduction these cells with this LV improved the erythroid differentiation and increased BFU-E colony formation in vitro. Subsequently, in vivo experiments in immunodeficient mice showed the engraftment, enhanced erythropoiesis and polyclonal hematopoietic reconstitution and reverted ribosomal biogenesis in the *RPS19* -corrected cells. These results offered compelling evidence suggesting that LV-mediated gene therapy with the *PGK.CoRPS19* LV should offer an effective approach to correct the hematological defects characteristic of RPS19-deficient DBA patients. In vivo toxicity and LV-biodistribution studies following infusion of transduced cells in mice completed the preclinical safety evaluation of this gene therapy approach. In addition, single-cell RNA studies confirmed the transcriptomic effects associated with gene correction of *RPS19*-mutated CD34+ cells. Our preclinical findings allowed us to obtain the Orphan Drug designation (EMA/OD/0000060656) for this LV gene therapy approach, as well as a National grant to initiate a Phase I clinical trial in Spain (DIAMOND). This phase I trial is under development. DIAMOND Phase I will serve to assess the safety and efficacy associated to the infusion of autologous CD34+ cells transduced with the *PGK.CoRPS19* LV in DBA patients with mutations in *RPS19*. Preclinical work and the development to start the trial will be presented in this meeting.

FIRST STEPS TOWARDS RENAL GENE THERAPY: A PILOT STUDY OF ADPKDII TREATMENT IN MICE. RD24/0014/0004, RD24/0014/0026.

Sergio Milagros, Beatriz Carte, Nerea Santana, Daniel Moya, Elena Garreta, Nuria Montserrat, Rafael Aldabe, Gloria González-Aseguinolaza

CIMA (Pamplona), IBEC (Barcelona).

Chronic kidney disease (CKD) represents a major global health and economic burden, and genomic technologies have reshaped our understanding of its causes. Although diabetes and hypertension underlie most adult renal disease, monogenic causes account for 50–70% of children requiring kidney replacement therapy and 10–30% of adult-onset CKD, amounting to roughly 6.5–10% of all cases. Therapeutic progress has not followed: most monogenic kidney diseases still lack a disease-modifying treatment, leaving generic nephroprotection, dialysis and transplantation as the only options. Long-term curative alternatives are urgently needed.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic kidney disease, affecting approximately 1 in 1,000 individuals, and tolvaptan — the only approved disease-modifying therapy — slows rather than halts progression. The disease arises from loss of function in PKD1 (around 75% of cases) or PKD2 (around 15%), and because it is dosage-sensitive, restoring polycystin expression above a critical threshold should be sufficient to alter its course. The PKD1 coding sequence is approximately 13 kb and exceeds AAV packaging capacity, whereas PKD2 at roughly 3 kb is readily accommodated, making ADPKD type 2 the rational entry point for an AAV-based approach.

We characterised three AAV serotypes with renal tropism in mice and confirmed this tropism in human kidney organoids. We then generated a renal AAV expressing human polycystin-2 under a CMV promoter and delivered it by retro-ureteral administration to the left kidney of PKD2 knockout mice at an early stage of disease. Longitudinal microCT showed a clear reduction in growth of the treated left kidney over three months in three of four animals, accompanied by suppression of cyst formation. These findings support the feasibility of treating ADPKD type 2 with an AAV vector.

MODIFICACIÓN SUPERFICIAL DE NANOPARTÍCULAS LIPÍDICAS PARA LA ENTREGA FUNCIONAL DE MATERIAL GENÉTICO EN CÉLULAS HEMATOPOYÉTICAS Y HEPÁTICAS. RD24/0014/0012, RD24/0014/0023.

Lucía Enríquez Rodríguez[1], María Isabel Carbonell-Simón[2], Miruna Giurgiu[2], Idoia Gallego Garrido[1], Aida García-Torralba[2], Andrea Molinos-Vicente[2], Laura Fernández Rosa[2], Laura Sáenz del Burgo Martínez[1], Gustavo Puras Ochoa[1], Óscar Quintana-Bustamante[2], María García Bravo[2], José Carlos Segovia Sanz[2], José Luis Pedraz Muñoz[1].

[1]Grupo NanoBioCel, CIBER-BBN, Universidad del País Vasco, Vitoria-Gasteiz.

[2]División de Tecnología Celular, Unidad de Innovación Biomédica, CIEMAT-CIBERER, Madrid/ Unidad de Terapias Avanzadas, Instituto de Investigación Sanitaria Fundación Jiménez Díaz, UAM, Madrid.

CENTROS PARTICIPANTES: Universidad del País Vasco y CIEMAT.

Las terapias génicas dirigidas a células hematopoyéticas y hepáticas requieren que el material genético llegue a poblaciones concretas, en órganos a los que no se accede con una administración directa no invasiva. Las nanopartículas lipídicas (LNP) son el vehículo de referencia, pero su superficie condiciona a qué células llegan, y con qué eficacia. En este contexto, plantear qué modificaciones superficiales favorecen la entrega en cada población diana es un paso necesario para poder ejecutar la intervención genética efectiva.

Con este objetivo, y en el marco de TERAV+, hemos desarrollado y caracterizado distintas estrategias de funcionalización de la superficie de las LNP en colaboración con el grupo de José Carlos Segovia en el CIEMAT. Partimos de una formulación base (MC3, DSPC, colesterol y DMGPEG2K) cargada con un mRNA de seguimiento, bien luciferasa (FLuc) o proteína verde fluorescente (EGFP). Para desarrollar un tropismo preferencial a hígado, se desarrollaron y compararon la formulación base con la adición de Tween 80 (0,01 % p/v) y con la modificación de parte del PEG conjugado a Manosa o N-acetil-galactosamina. Para facilitar el tropismo a células hematopoyéticas, se funcionalizó la superficie con aptámeros dirigidos al antígeno CD117, o con anticuerpos anti-CD117. Todas las formulaciones se caracterizaron mediante dispersión de luz dinámica (DLS), potencial zeta y eficiencia de encapsulación, así como un análisis de su morfología mediante crio-TEM.

Las formulaciones hepáticas presentaron tamaños de 73 a 91 nm, potenciales zeta próximos a la neutralidad (de −2,6 a −5,0 mV) y una encapsulación del 82 al 91 %, con menor PDI en Tween 80 y manosa (0,07-0,08) que en la base (0,18) y GalNAc (0,24). Las hematopoyéticas midieron entre 79 y 100 nm, con PDI de 0,11 a 0,19 y encapsulación superior al 93 %. La conjugación de aptámeros redujo el potencial zeta de +4,8 mV a valores cercanos a cero, y la de anticuerpos lo hizo de forma más moderada (2,6–2,9 mV), un efecto compatible con el apantallamiento de la carga superficial por el ligando. El análisis morfológico mediante crio-TEM mostró diferencias superficiales entre las LNP sin modificar y las funcionalizadas.

En conjunto, la modificación superficial altera atributos fisicoquímicos y morfológicos medibles, lo que sienta las bases para seleccionar la superficie que permita ejecutar la intervención genética en cada población diana. Esta colaboración ha permitido abordar de forma conjunta el diseño, la caracterización y la evaluación de estas formulaciones.

ALHAMBRA: AN ACTIVATION-INDUCIBLE PROMOTER FOR SAFER AND MORE CONTROLLABLE NEXT GENERATION CAR-T THERAPIES. RD24/0014/0005, RD24/0014/0010.

Tagliaferro-Quiñonero A.[1,2], Fernández-Coca P.[2], Ruiz de Francisco J.[3], Gómez-Báez L.[2], Cortijo-Gutiérrez M.[2], Ayllón V.[1,2], JR Rodriguez-Madoz[5,6], F Prósper[5,6], M Tristán-Manzano[1,2,3,4], F Martin[1,2,3,4,*].

[1]Department of Cellular Biology, Faculty of Sciences, University of Granada, Granada, 18006, Spain;

[2]Gene and Cell Therapy Group, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENyO), PTS, Granada, 18016, Spain

[3]LentiStem Biotech, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Av. de la Ilustración, 114,18007, Granada, Spain

[4]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, 18006, Spain

[5]Biosanitary Research Institute of Granada (ibs.GRANADA), University of Granada, Granada, 18012, Spain.

[5]Hemato-Oncology Program, Cancer Division, Center for Applied Medical Research (CIMA)

[6]University of Navarra. Pamplona, Spain.

Background: Fourth-generation CAR-T cells (TRUCKs) capable of secreting immunomodulatory payloads (such as IL-18) represent a promising strategy to overcome the immunosuppressive microenvironment of solid tumors. However, uncontrolled expression of pro-inflammatory cytokines carries significant risks of systemic toxicity. While NFAT-based activation-inducible promoters have been developed to restrict payload expression to target recognition, current systems suffer from high basal activity (leakiness) in resting conditions or in response to homeostatic cytokines.

Methods: To enhance transcriptional control, we engineered a panel of synthetic promoters combining novel regulatory elements. The top candidate, ALHAMBRA (*Activation-Linked High-expression And Maximal Basal Repression in Absence of tumor cells*), was integrated into lentiviral vectors driving eGFP or IL-18. Primary human T cells and anti-CD19 CAR-T cells were evaluated for basal leakiness, kinetic onset, and antigen specificity under non-activating conditions (IL-2, IL-7/IL-15, $CD19^-$ tumor cells) and antigenic stimulation (anti-CD3/CD28 or $CD19^+$ target cells) in comparison with leading benchmarks (NFATsyn, NFATmIL2).

Results: The ALHAMBRA platform demonstrated an optimal regulatory profile featuring maximal repression off-target and rapid, robust induction upon cognate antigen engagement:

- **Superior Off-Tumor Silencing:** ALHAMBRA achieved 80–90% complete basal silencing in resting CAR-T cells, in the presence of antigen-negative tumor cells, or under IL-2 exposure, compared to only 40–50% observed with the top benchmark (NFATsyn).
- **Robust Tumor-Driven Inducibility:** Upon specific tumor antigen engagement, ALHAMBRA showed >6-fold higher inducibility relative to state-of-the-art competitors (NFATmIL2).
- **Controlled Payload Delivery:** Driving IL-18 expression under ALHAMBRA ensured strict antigen dependence, preventing cytokine leakiness in circulation while delivering maximal localized expression at the tumor site.

Conclusion: The ALHAMBRA promoter overcomes the critical limitation of transgene leakiness in activation-inducible CAR-T systems. By combining unprecedented off-tumor silencing with potent, tumor-driven inducibility, ALHAMBRA provides a robust and safer regulatory platform for next-generation 4th-generation CAR-T therapies.

PROGRAMA 1.
TERAPIA GÉNICA
PÓSTERES

EDICIÓN GÉNICA DE HAO1 MEDIADA POR AAV COMO TERAPIA DE REDUCCIÓN DE SUSTRATO EN LA HIPEROXALURIA PRIMARIA TIPO 1. RD24/0014/0004, RD24/0014/0026.

Andrea Llanos, María Isabel Carbonell-Simón, Laura Torella, Aída García-Torralba, África Vales, Martín Bilbao, José-Carlos Segovia, María García-Bravo, Gloria González-Aseguinolaza.

CIMA (Pamplona); CIEMAT (Madrid)

La hiperoxaluria primaria tipo 1 (PH1) se debe a la deficiencia de alanina-glioxilato aminotransferasa (AGXT). PH1 provoca sobreproducción hepática de oxalato, nefrocalcinosis e insuficiencia renal. La inactivación del gen *HAO1*, que codifica la glicolato oxidasa (GO), una enzima que está aguas arriba de la ruta del glioxilato, disminuye la formación de glioxilato y constituye una estrategia de reducción de sustrato ya validada clínicamente con ARN de interferencia. Nuestro objetivo es lograr esa inactivación de forma permanente mediante edición génica hepática con vectores adenoasociados.

En colaboración con el CIEMAT se generaron modelos celulares HepG2 AGXT-KO que sobreexpresan *HAO1*. Se diseñaron seis construcciones SaCas9 nickasa (D10A) bajo el promotor hepatoespecífico AAT, con pares de ARN guía dirigidos a los exones 2, 4, 5 y 7 de HAO1. Se analizaron la eficiencia de edición (TIDE y secuenciación de amplicones), la expresión de GO (western blot) y la producción de oxalato tras inducción con 10 mM de glicolato. Tras seleccionar las guías con los mejores resultados, se empleó el mismo modelo celular para sustituir la nickasa por una nucleasa y comparar el promotor hepato-específico con un promotor fuerte de expresión ubicua (CMV). Estas construcciones se utilizaron para producir genomas recombinantes de AAV, que se encapsidaron en un serotipo hepatotrópico, con el objetivo de evaluar el efecto de los distintos factores sobre la eficacia de edición y la integración del AAV.

El modelo celular reproduce el exceso de producción de oxalato que se observa en los pacientes. La transducción con AAV (MOI 3×10^5) redujo el oxalato respecto a las células PH1 no tratadas, con el mejor resultado para AAT-D10A. Actualmente estamos analizando la eficacia de edición, el patrón de integración de los AAV y la expresión de la proteína GO.

GENERACIÓN DE MODELOS Y HERRAMIENTAS PARA EL ESTUDIO Y LA INTERVENCIÓN TERAPÉUTICA EN ENFERMEDAD DE POMPE. RG24/0014/0004, RD24/0014/0005, RG24/0014/0034.

Alba Gámez Gámez[1,2,3], Laura Martínez Ruíz[1,4], Laura de Lara-Peña[1,3,5], Eliana Barriocanal Casado[1,3,5], Iris Ramos-Hernández[1,2], Lucía Méndez[6], Patricia Hernández-Carabias[7], Belén Morales[5,8], Jose Manuel Entrena[5,8], Africa Vales[9], Enrique Mesonero[9], Francisco Martín[1,2,10], Manuel A. Sanchez-Martín[6,7], Gloria Gonzalez-Aseguinolaza[7], F. Javier Molina-Estévez[1,2,11*], Pilar Muñoz Fernández[1,2,3*]

[1]Centro Pfizer-Junta de Andalucía-Universidad de Granada de Genómica e investigaciones Oncológicas (Genyo), Granada

[2]Departamento de Biología Celular, Facultad de Ciencias, Universidad de Granada, Granada

[3]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada

[4]Luxembourg Institute of Health (LIH), Luxemburgo, Bélgica

[5]Centro de Investigación Biomédica (CIBM), Universidad de Granada, Granada

[6]Instituto de investigación biomédica de Salamanca (IBSAL) Salamanca, Spain

[7]Servicio de Transgénesis. Universidad de Salamanca (USAL). Salamanca, Spain

[8]Centro de Instrumentación Científica (CIC), Universidad de Granada, Granada, España

[9]Gene Therapy for Rare Diseases Program, DNA & RNA Medicine Division, CIMA, Universidad de Navarra, Navarra

[10]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada

[11]Fundación para la Investigación Biosanitaria de Andalucía Oriental (FIBAO), Granada.

La enfermedad de Pompe (glucogenosis tipo II) es un trastorno neuromuscular hereditario causado por mutaciones en la enzima α-glucosidasa ácida (GAA), lo que provoca una acumulación de glucógeno en tejidos, principalmente en hígado, músculo esquelético y cardíaco. La terapia de reemplazo enzimático es insuficiente, lo cual ha puesto en foco sobre la terapia génica que puede transferir copias funcionales del gen mutado para suministrar enzima GAA de manera continua y autónoma en pacientes. Los vectores adenoasociados de serotipo 8 (AAV8) destacan por su elevada eficiencia de transducción y tropismo preferencial hacia el tejido hepático y muscular. Esto permite convertir al hígado en una fábrica sostenible e inmunotolerada para la secreción de la enzima mejorando el resto de los órganos afectados.

Inicialmente, evaluamos tres proteínas Gaa de ratón, que difieren en el péptido señal: IGF-2 (S2G), IFNB1 (SiFGAA) e IFNB1 + LSP (SiFLGAA). Se clonaron en el plásmido pAAV-MCS con promotor un SFFV y un elemento regulador WPRE, flanqueados por ITRs de AAV2 y señal de poli(A). Se produjeron y titularon vectores AAV8. Esto fue realizado por el laboratorio de la Dra. Gloria González-Aseguinolaza (CIMA, Pamplona). En Granada, estos vectores AAV8 fueron inoculados por vía intravenosa en ratones GAA-/- (B6; 129-Gaatm1Rabn / J) y se analizaron a corto y largo plazo. Recientemente, se han generado 2 variantes GAA humanas fusionando los péptidos de señalización GAA optimizado (ga) y IFG2 (i2) con la porción secretable de GAA (coGAA). Estos se han clonado en vectores lentivirales para poder estudiar su expresión bajo promotores modulados por linaje (CD11b, CD68) en líneas celulares IOPD generadas mediante CRISPR/Cas9 en Granada. Y en colaboración con Manuel A. Sánchez en USAL, hemos generado el primer modelo pompe inmunodeficiciente $GAA^{del\ c.480\ del2C}$ realizado en fondo NSG (NOD.$Prkdc^{scid}$ $IL2RG^{null}$ $Gaa^{delCC480-481/SAL}$ o NSGG), que estamos caracterizando como plataforma para evaluar herramientas de terapia génica. Finalmente, vamos a clonar y producir las nuevas variantes de GAA humana en vectores AAV8.

En paralelo, en relación con la investigación de nuevas tecnologías de transferencia génica, nuestro grupo de investigación en GENYO (Granada) y la Universidad de Granada hemos desarrollado nuevos vectores lentivirales que codifican péptidos de GAA humanos diseñados mediante ingeniería genética. En colaboración con el grupo de la Dra. Gloria González (CIMA, Pamplona) la eficacia terapéutica tanto de los AAV8s como de los lentivirus serán evaluados en nuestros modelos murinos de Pompe.

PRECLINICAL CHARACTERIZATION OF SECOND-GENERATION ANTI-CLL-1 CAR-T CELLS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA. RD24/0014/0005, RD24/0014/0010.

V Ronco[1], P Heredia[1], C Camacho[2,3], A Olaechea[1], K Paulovic[1], A Millán[1,6], SA Navarro[2,3], H Boulaiz[2,3], JA Marchal[2,3], Juan R. Rodriguez-Madoz[4], E Atilla[5], IC Herrera[6,7,*] and K Benabdellah[1,*]

[1]Genomic Editing applied to Advanced Therapies (eGATA) Group. Pfizer-University of Granada-Junta de Andalucía Center for Genomics and Oncological Research (GENYO), Granada, Spain.

[2]Department of Anatomy and Human Embryology, Faculty of Medicine, University of Granada, Granada, Spain.

[3]Biopathology and Regenerative Medicine Institute (IBIMER), Granada, Spain.

[4]Hemato-Oncology Program, Cima Universidad de Navarra. IdiSNA. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[5]Division of Transplantation and Cellular Therapy, Department of Medicine, University of Miami, Miller School of Medicine, Sylvester Comprehensive Cancer Center, Miami, Florida, USA.

[6]Cellular Therapy Group. Maimonides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain.

[7]Reina Sofía University Hospital, Cordoba, Spain.

Background: Acute myeloid leukemia (AML) remains a major challenge for cellular immunotherapy due to the limited availability of suitable target antigens, the difficulty of achieving durable CAR-T cell responses, and the immunosuppressive bone marrow microenvironment. CLL-1 is a promising target for AML-directed CAR-T therapy. However, optimizing CAR architecture and addressing inhibitory signalling and T-cell alloreactivity are important steps towards improving therapeutic performance. Combining rational CAR design with targeted disruption of *TRAC*, encoding the T-cell receptor alpha constant region, and *PDCD1*, encoding the inhibitory receptor PD-1, represents a complementary strategy to develop optimized CAR-T cells with the potential for improved functionality and persistence.

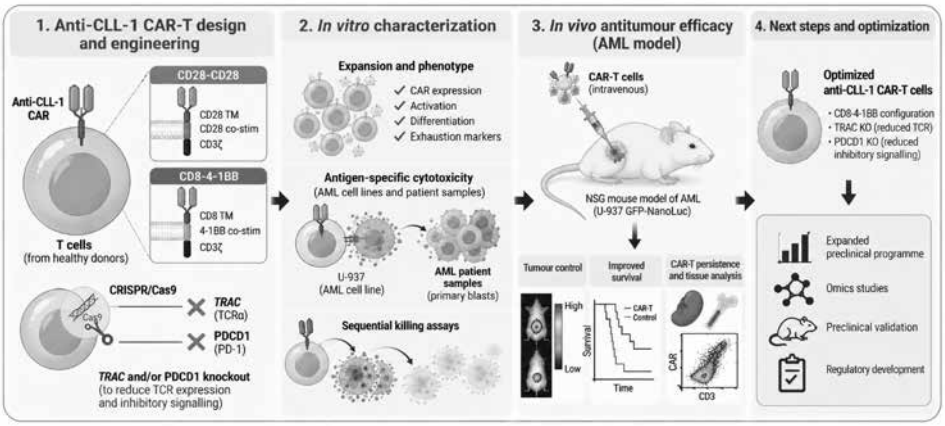
Objective: To compare second-generation anti-CLL-1 CAR-T cells incorporating distinct transmembrane and co-stimulatory domain combinations (CD28-CD28 and CD8-4-1BB), and to investigate CRISPR/Cas9-mediated disruption of *TRAC* and *PDCD1* as an additional strategy to reduce T-cell receptor expression and inhibitory signalling, respectively, towards the development of optimized CAR-T products for the treatment of AML.

Methods: Second-generation anti-CLL-1 CAR constructs incorporating CD28-CD28 or CD8-4-1BB transmembrane and co-stimulatory domain combinations were generated using lentiviral vectors. Human peripheral blood mononuclear cell-derived T cells from healthy donors were transduced and characterized for CAR expression, expansion, activation, differentiation, and exhaustion-associated markers. Antigen-specific cytotoxicity was evaluated against U-937 AML cells engineered to express GFP-NanoLuc, including co-culture and sequential killing assays. Antitumor activity and CAR-T cell persistence were further investigated in an NSG mouse model of AML using longitudinal bioluminescence imaging, survival analysis, and flow cytometry. In parallel, CRISPR/Cas9-mediated disruption of *TRAC* and *PDCD1* was explored as an additional strategy to optimize CAR-T cell functionality.

Results: Both anti-CLL-1 CAR designs were successfully generated and exhibited antigen-specific antileukemic activity. Comparative characterization revealed differences in expansion, immunophenotype, activation, and exhaustion-associated markers according to CAR architecture. In vitro, the CD8-4-1BB design displayed a more favorable functional profile than the CD28-CD28 design, including enhanced expansion and cytotoxic activity, reduced expression of selected exhaustion-associated markers, and improved persistence-related features. In the murine AML model, 4-1BB-based CAR-T cells demonstrated tumor control, prolonged survival compared with non-transduced controls, and detectable CAR-T cell persistence. Longitudinal bioluminescence imaging enabled

the monitoring of disease progression and treatment response, while tissue analyses provided additional information on tumor distribution and CAR-T cell infiltration. Genetic disruption of *TRAC* and *PDCD1* is being investigated as a complementary strategy to further optimize these cells.

Conclusions: This study provides a preclinical foundation for the development of CLL-1-directed CAR-T cells for acute myeloid leukemia (AML), highlighting the importance of CAR architecture in shaping T-cell phenotype, functionality, and antitumor activity. The favorable profile observed with the CD8-4-1BB configuration supports its further development and evaluation. Our next priority is to advance this candidate through an expanded preclinical programme. Collaboration within the TERAV network will be instrumental in accelerating this work, particularly through partnerships in Omics studies preclinical validation, and regulatory.



CAR-T CELL-DERIVED EXTRACELLULAR VESICLES AS A NOVEL PLATFORM FOR PANCREATIC CANCER IMMUNOTHERAPY. RD24/0014/0005, RD24/0014/0001.

Paula Heredia-Velázquez[1,2], Kristina Paulovic[1], Carmen Griñán-Lisón[3,4,5,6], Saúl Navarro-Marchal[3,4,5,6,7,8], María Hernández-Valladares[3,9], André Görgens[10,11,12], Caridad Díaz[13], Lucía Guevara[13], María Tristán-Manzano[1,3], María Dolores Carmona-Luque[14], Manel Juan[15,16,17,18], Concha Herrera[14], Samir El Andaloussi[10,11,12], Juan Antonio Marchal[2,3,4,5,6]*, Karim Benabdellah.

[1]Department of Applied Genome Editing for Advanced Therapies, Pfizer–University of Granada–Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Granada, Spain.

[2]Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain.

[3]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain.

[4]Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain.

[5]BioFab i3D Lab – Biofabrication and 3D (Bio)printing Laboratory, University of Granada, Granada, Spain.

[6]Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Granada, Spain.

[7]Department of Physiology, Faculty of Pharmacy, University of Granada, Granada, Spain.

[8]Clinical Management Unit of Digestive Diseases, San Cecilio University Hospital, Granada, Spain.

[9]Department of Physical Chemistry, School of Sciences, University of Granada, Granada, Spain.

Background: Extracellular vesicles (EVs) derived from chimeric antigen receptor (CAR)-T cells represent a promising cell-free immunotherapeutic strategy, combining antigen recognition with the potential advantages of a cell-free product. However, their therapeutic development is limited by challenges in production yield, product characterization and functional consistency. Engineering EV biogenesis in producer cells may offer a strategy to address these limitations.

Objective: To investigate whether genetic engineering of primary human T cells can modulate EV production and molecular composition while preserving CAR-associated antitumour activity.

Methods: Primary human T cells were engineered to express a CAR and further modified through CD63 overexpression or knockout of Gene X, a regulator of EV biogenesis. EVs were isolated and characterized by nanoparticle tracking analysis, flow cytometry-based surface profiling and proteomic and metabolomic analyses. Their antitumour activity was assessed in vitro and in an orthotopic pancreatic ductal adenocarcinoma model.

Results: Engineered CAR-T cells produced EV preparations displaying surface-associated CAR and characteristic EV markers, with broadly preserved size distributions across conditions. Surface profiling confirmed the presence of T-cell-associated markers, while proteomic and metabolomic analyses revealed molecular differences associated with the distinct engineering strategies. CAR-T-derived EVs exhibited antitumour activity against CD19-positive pancreatic cancer cells in vitro. Following intravenous administration in an orthotopic pancreatic cancer model, CAR-bearing EVs reduced tumour progression and prolonged survival compared with control conditions. These findings support the feasibility of modulating EV biogenesis in CAR-T cells while retaining the functional properties of the resulting vesicles.

Conclusions: Engineering CAR-T cells to modulate EV biogenesis provides a potential route towards cell-free immunotherapies for solid tumours. Beyond their use as standalone therapeutic agents, CAR-T-derived EVs may offer opportunities for combination strategies designed to complement or enhance existing CAR-T cell therapies. Further development will require standardized manufacturing, robust potency assays and systematic evaluation of biodistribution, safety and immunogenicity. Collaboration within the framework of TERAV+ between EV researchers, advanced therapy developers, and clinical teams will

[10]Division of Biomolecular and Cellular Medicine, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.

[11]Department of Cellular Therapy and Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, Huddinge, Sweden.

[12]Karolinska ATMP Center, Karolinska Institutet, ANA Futura, Huddinge, Sweden.

[13]MEDINA Foundation, Granada, Spain.

[14]Cell Therapy Group, Maimonides Institute of Biomedical Research of Córdoba (IMIBIC), Córdoba, Spain.

[15]Department of Immunology, Centre de Diagnòstic Biomèdic (CDB), Hospital Clínic de Barcelona, Barcelona, Spain.

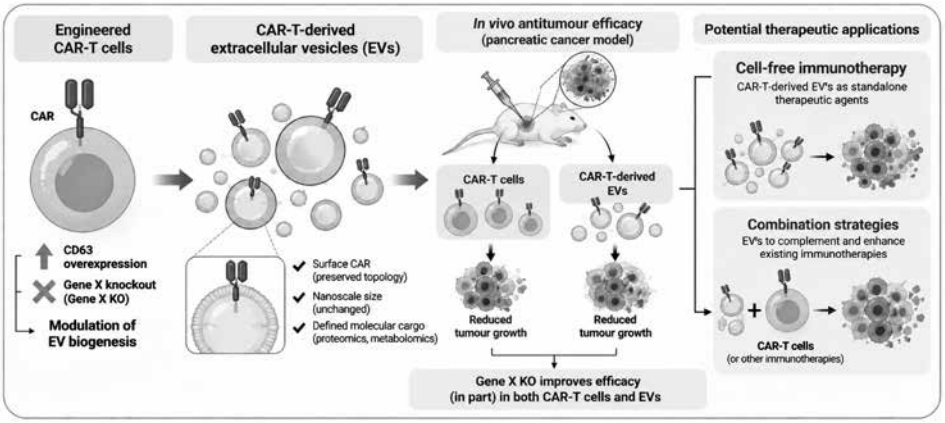
[16]Departments of Biomedical Sciences and Medicine and Health Sciences, University of Barcelona, Barcelona, Spain.

[17]Fundació de Recerca Clínic Barcelona – Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

[18]Immunotherapy Joint Platform of Hospital Sant Joan de Déu and Hospital Clínic de Barcelona, Barcelona, Spain.

[19]Department of Hematology, Reina Sofía University Hospital, Córdoba, Spain.

be essential to define the translational potential of these versatile therapeutic platforms.



MULTI-TOOL IN SILICO IMMUNOGENICITY PROFILING OF CD19/CD22-TARGETING CAR CONSTRUCTS. RD24/0014/0005, RD24/0014/0010.

Antonio Matilla Serrano[1,4], Jon Ruiz de Francisco[2,4], I Molina Pineda[3], JR Rodriguez-Madoz[5,6], F Prósper[5,6], M Tristán-Manzano[1,2,3,4,*], F Martin[1,2,3,4,*].

[1]Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO). Granada.

[2]LentiStem Biotech, GENYO. Granada.

[3]Departamento de Bioquímica, Biología Molecular III e Inmunología, Facultad de Medicina Granada.

[4]ibs.GRANADA Instituto de Investigación Biosanitaria.

[5]Hemato-Oncology Program, Cancer Division, Center for Applied Medical Research (CIMA).

[6]University of Navarra. Pamplona, Spain.

[*] *Share senior authorship*

CAR-T cells targeting CD19 have transformed the treatment of B-cell malignancies, but relapses driven by antigen loss and the limited persistence of the infused cells remain major challenges. Targeting CD19 and CD22 simultaneously is a promising strategy to prevent antigen escape. Classical CARs rely on single-chain variable fragments (scFv) as antigen-binding domains, and combining two of them in a bispecific receptor yields large constructs. NanoCARs replace the scFv with nanobodies (VHH), single-domain fragments derived from camelid heavy-chain antibodies. Their small size (~15 kDa) makes bispecific receptors much more compact, which eases vector packaging and reduces the surface exposed to the immune system, potentially lowering the immunogenic burden. Even so, the extracellular region of any CAR can elicit humoral and cellular responses that neutralize the receptor or eliminate the CAR-T cells, compromising safety and persistence. Anticipating this risk during the design stage, before clinical translation, is therefore essential.

We developed an automated computational workflow in bash that integrates nine complementary tools covering the main axes of immunogenicity. B-cell epitopes were predicted with BepiPred (linear epitopes), Emini (surface accessibility) and Kolaskar & Tongaonkar (antigenicity), and with ElliPro and DiscoTope, which use three-dimensional structures to identify protruding residues and discontinuous epitopes. For T-cell responses, NetMHCpan, MHCflurry and SMM predict peptide binding to a broad panel of HLA class I alleles ($CD8^+$ route), while NetMHCIIpan predicts binding to class II alleles ($CD4^+$ helper route).

The strength of this approach lies in the overlap of results across algorithms, enabling the detection of protein segments where multiple immunogenic criteria converge simultaneously, conferring greater robustness to the predictions compared to any analysis performed by each tool separately. Here, we validated this workflow comparing the extracellular region of four VHHbased nanoCARs vs three ScFv derived sequences. As expected, we corroborated the lower immunogenic potential of the VHH at both humoral and cellular level and we identified consensus positions in ScFv-derived regions that can be implied in a potential immunogenic risk.

We applied the workflow to eight constructs: anti-CD19 and anti-CD22 nanobodies, the anti-CD19 scFv used in Kymriah (tisagenlecleucel), an anti-CD22 scFv, two bispecific CD19/CD22 nanobody constructs differing in their linker, and two bispecific scFv constructs (CD19-CD22 and CD22-CD19). This allowed us to compare formats of different length, identify the linker associated with the lowest predicted immunogenicity, and evaluate the contribution of the binding domains for each target antigen. The consensus positions identified are candidates for targeted protein engineering aimed at reducing immunogenicity without compromising antigen binding, supporting the design of CD19/CD22 nanoCAR-T therapies with improved persistence and safety.

SINGLE-CELL MULTIOMIC PROFILING DEFINES MOLECULAR SIGNATURES OF HUMAN LONG-TERM HEMATOPOIETIC STEM CELLS FOR NEXT GENERATION GENE THERAPY APPLICATIONS. RD24/0014/0010, RD24/0014/0023.

D. Mouzo[1], A.R. Lopez-Perez[2], A. Ortega-Legarreta[3], N. Planell[4], P. San Martin-Uriz[5], P. Aguirre-Ruiz[5], G. Fernandez-Ruz[6], C. Rodriguez-Res[4], M. Garnica[5], D Mazzeo[7], R. Martinez-Turrillas[5], S. Rodriguez-Diaz[5], C. Bezares[5], J. Rifon[8], V. Lagani[9], R. Lehmann[9], J. Tegner[9], T. Ezponda[10], S. Navarro[7], J.A. Bueren[7], P. Rio[7], D. Gomez-Cabrero[11], J.R. Rodriguez-Madoz[10] and F. Prosper[8]

[1]Translational Bioinformatics Unit, Navarrabiomed. Pamplona, Spain. / Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London. London, UK.

[2]Translational Bioinformatics Unit, Navarrabiomed. Pamplona, Spain. / Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[3]Translational Bioinformatics Unit, Navarrabiomed. Pamplona, Spain.

[4]Computational Biology Program, Cima Universidad de Navarra, IdiSNA. Pamplona, Spain.

[5]Hemato-Oncology Program, Cima Universidad de Navarra, IdiSNA. Pamplona, Spain.

[6]Hematology and Cell Therapy. Clinica Universidad de Navarra. IdiSNA. Pamplona, Spain.

[7]Biomedical Innovation Unit, Center for Research on Energy, Environment and Technology (CIEMAT). Madrid, Spain. / Advanced Therapies Unit, Jiménez Díaz Health Research Institute (IIS-FJD, UAM). Madrid, Spain. / Biomedical Network Research Center for Rare Diseases (CIBERER). Madrid, Spain.

[8]Hematology and Cell Therapy. Clinica Universidad de Navarra. IdiSNA. Pamplona, Spain. / Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain. / Biomedical Network Research Center for Cancer (CIBERONC). Madrid, Spain.

Hematopoietic stem and progenitor cells (HSPCs) constitute the basis of gene therapy/editing strategies for different hematologic and even non-hematologic disorders. The long-term efficacy of these approaches depends on the successful engraftment and persistence of long-term repopulating hematopoietic stem cells (LT-HSCs), whose precise identification remains challenging due to the intrinsic heterogeneity of the HSPC compartment. In this work, we aimed at defining the transcriptomic and epigenetic signatures that robustly distinguish human HSPC subpopulations, with particular emphasis on primitive LT-HSCs. The improved molecular characterization of these populations could facilitate the more precise targeting of therapeutically relevant cells, predict the function of genetically modified HSCs, and reduce the number of animals used for evaluating the safety and efficacy in HSC gene therapies.

To this end, we performed single-cell multiome profiling (scRNA-seq + scATAC-seq) of $CD34^+$ bone marrow (BM) cells from five healthy donors, enabling simultaneous analysis of gene expression and chromatin accessibility at single-cell resolution. Cell annotation was achieved through a combined strategy integrating automated SingleR classification and manual refinement using established HSPC markers. Within the BM $CD34^+$ cell compartment, we identified a transcriptionally defined HSC population that could be further resolved into three distinct subtypes based on clustering analyses and StemPublic stemness scoring: (i) quiescent LT-HSCs (qLT-HSCs), enriched for quiescence-associated genes including *EMCN* and *TEK*; (ii) activated LT-HSCs (aLT-HSCs), characterized by *MECOM* and *RUNX1* expression; and (iii) short-term HSCs (ST-HSCs), marked by elevated *CDK6*. These analyses enabled the definition of a novel transcriptional signature (StemFingerprint), that accurately distinguishes LT-HSCs from closely related progenitor cell populations. Given the widespread clinical use of mobilized peripheral blood (mPB) HSCs in gene therapy, we additionally profiled $CD34^+$ cells from mPB samples of three healthy donors. Application of StemnessFingerprint successfully identified LT-HSCs and ST-HSCs in mPB, whereas qLT-HSCs were absent, consistent with previous observations indicating rapid exit from quiescence during mobilization. Comparative analyses across BM and mPB revealed conserved stemness-associated transcriptional programs between HSC sources. Importantly, we identified previously underappreciated LT-HSC-associated genes, including *FTH1*, *RYR3*, and *SRGN*, suggesting potential roles in iron homeostasis, calcium signaling, and niche interactions, respectively. To further characterize the regulatory mechanisms underlying HSC states, we analyzed transcription factor motif accessibility and inferred gene regulatory networks. qLT-HSCs displayed prominent ETS1 regulatory activity, consistent with maintenance of quiescence, whereas aLT-HSCs

[9]Bioscience Program, BESE, KAUST. Thuwal, Saudi Arabia.

[10]Hemato-Oncology Program, Cima Universidad de Navarra, IdiSNA. Pamplona, Spain. / Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain. / Biomedical Network Research Center for Cancer (CIBERONC). Madrid, Spain.

[11]Division of Biomedical Sciences, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia.

showed increased centrality of MYC and KLF6, reflecting activation and proliferative programs. Notably, MYC co-expression with TFEB and TFR1 recapitulated regulatory pathways associated with the transition from quiescence to activation. Finally, we examined the StemnessFingerprint across independent bulk and single-cell datasets to study if it captures biologically relevant variations. Committed progenitors consistently showed lower StemnessFingerprint scores compared to phenotypically defined primitive HSC population ($CD34^+CD38^-$ CD90+CD45RA-Lin-). Moreover, both bulk and single-cell RNA-seq datasets showed significantly higher StemnessFingerprint scores in HSC-enriched populations from older donors than from younger ones. These findings suggest that ageing is associated with a molecular primitive HSC program. When the StemnessFingerprint score is applied to a dataset of MDS progression, we saw a negative correlation of the StemnessFingerprint score with the IPSS-M risk.
Overall, this study provides a comprehensive single-cell molecular map of human HSPCs from BM and mPB, integrating transcriptional and epigenetic landscapes with stemness-associated functional states. By defining robust molecular and regulatory signatures of LT-HSCs, particularly quiescent and activated states, our work provides valuable tools for improved HSC identification and clinical application.

OPTIMIZATION OF LENTIVIRAL VECTOR PRODUCTION THROUGH PLASMID MINIATURIZATION AND NANOPLASMID TECHNOLOGY. RD24/0014/0016, RD24/0014/0023, RD24/0014/0005.

Alvaro Murillo[1,2], Oscar Quintana-Bustamante[3,4,5], Maria Tristan-Manzano[6,7,8], Jose-Carlos Segovia[3,4,5], Francisco Martin-Molina[4,6,7,8,9], Unai Perpiña[1,2], Josep M. Canals[1,2]

[1]Laboratory of Stem Cells and Regenerative Medicine, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences; Institute of Neurosciences; and Creatio, Production and Validation Center of Advanced Therapies, University of Barcelona.

[2]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain.

[3]Cell Technology Division, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT).

[4]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

[5]Unidad Mixta de Terapias Avanzadas. Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM), Madrid, Spain.

[6]GENYO, Centre for Genomics and Oncological Research, Pfizer–University of Granada–Andalusian Regional Government, Granada, Spain.

[7]Instituto de Investigación Biosanitaria ibs., Granada, Spain.

[8]LentiStem Biotech, Granada, Spain.

[9]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain.

Lentiviral vectors are widely used in gene therapy and biomedical research, but manufacturing efficiency remains a significant challenge, often limiting scalability and increasing production costs. Plasmid backbone composition and size are important determinants of transfection efficiency and vector yield. We hypothesize that reducing plasmid size and replacing conventional plasmids with nanoplasmids will enhance lentiviral particles production without compromising vector quality.

In this collaborative project, we are developing and evaluating an optimized lentiviral production platform based on two complementary strategies: (i) rational reduction of non-essential plasmid sequences to generate smaller transfer and packaging plasmids, and (ii) implementation of nanoplasmid technology as an alternative to conventional bacterial plasmids. Plasmid designs were refined through sequence analysis The resulting constructs generation and validation are underway. In parallel, HEK293T knockout clones provided by Dr. Francisco Martin-Molina's lab will be evaluated for their capacity to enhance lentiviral particle production. Proof of concept experiments are expected to provide insight into the relative contribution of plasmid miniaturization and nanoplasmid technology to lentiviral particles manufacturing performance. These data will be evaluated in the context of vector yield, reproducibility, and product quality.

This study aims to establish an optimized plasmid design framework for improving lentiviral particles production. Successful implementation could facilitate more efficient and cost-effective vector manufacturing for both research and therapeutic applications.

DESARROLLO COLABORATIVO DE NANOPARTÍCULAS LIPÍDICAS PARA LA EDICIÓN GÉNICA *IN VIVO* DEL FACTOR XI COMO ESTRATEGIA ANTITROMBÓTICA. RD24/0014/0023, RD24/0014/0012.

García-Bravo, María[1]; Zaragoza-Huesca, David[2]; Quintana-Bustamante, Oscar[1]; Padilla, Jose[2]; Fuentes, Marina[2]; Molinos-Vicente, Andrea[1]; Bravo-Pérez, Carlos[2]; García-Torralba, Aida[1]; Rojo-Carrillo, Juan José[2]; López-Correas, Paloma[2]; Arques, María Consuelo[2]; Vicente, Vicente[2]; Lozano, María Luisa[2]; Enríquez-Rodríguez, Lucía[3]; Pedraz, José Luis[3]; Segovia, José Carlos[1]; Corral, Javier[2]; de la Morena Barrio, María Eugenia[2]

[1]División de Tecnología Celular, Unidad de Innovación Biomédica, CIEMAT-CIBERER, Madrid/ Unidad de Terapias Avanzadas, Instituto de Investigación Sanitaria Fundación Jiménez Díaz, UAM, Madrid.

[2]Servicio de Hematología, Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB, CIBERER-ISCIII, Murcia.

[3]Grupo NanoBioCel, CIBER-BBN, Universidad del País Vasco, Vitoria-Gasteiz.

CENTROS PARTICIPANTES: IIS Fundación Jiménez Díaz (CIEMAT) (FJD), Universidad del País Vasco.

El factor XI (FXI) de la coagulación constituye una diana de especial interés para el desarrollo de nuevas estrategias antitrombóticas, ya que participa en la amplificación de la generación de trombina durante la trombosis, pero desempeña un papel menos relevante en la hemostasia fisiológica. La deficiencia congénita de FXI se asocia con una menor incidencia de eventos trombóticos y con un fenotipo hemorrágico leve. En este contexto, la inactivación del gen *F11* mediante edición génica *in vivo* dirigida específicamente al hígado, órgano responsable de la síntesis de este factor, mediante nanopartículas lipídicas podría constituir una alternativa a los tratamientos anticoagulantes convencionales, permitiendo una reducción prolongada de los niveles de FXI tras una única administración. Esta estrategia ha sido implementada gracias a la colaboración con el grupo de José Luis Pedraz (UPV/EHU), en el marco de TERAV+, centrada en el desarrollo y optimización de nanopartículas lipídicas (LNPs) para la administración *in vivo* de los componentes del sistema CRISPR/Cas9. La experiencia del grupo de la UPV/EHU en el desarrollo de sistemas de administración ha permitido disponer de formulaciones accesibles de LNP, ampliando las posibilidades de desarrollo y aplicación de esta estrategia de edición génica.

A partir de este trabajo colaborativo, se ha optimizado una formulación de LNPs funcionalizadas con N-acetilgalactosamina (GalNAc), capaz de encapsular el ARNm codificante de la nucleasa Cas9 y ARN guías (sgRNA) específicos frente al gen *F11*. Las LNP se administraron por vía intravenosa a ratones WT C57BL/6 para evaluar la eficacia de edición hepática y su capacidad para reducir la expresión y actividad del FXI circulante. La administración de una única dosis permitió alcanzar porcentajes de edición del gen *F11* próximos al 50 % en hígado, con una correlación entre la eficiencia de edición y la reducción de la expresión hepática de *F11*. Esta edición se tradujo en una disminución de los niveles plasmáticos de FXI superior al 90%, confirmada mediante *Western blot* y espectrometría de masas, y en una reducción de su actividad coagulante hasta aproximadamente el 15 % de los valores control. Tanto la edición génica, como la reducción de FXI, se mantuvieron durante al menos cinco meses después de la administración.

Por otro lado, el análisis de la frecuencia de edición en distintos tejidos mostró que el gen *F11* se editó preferentemente en el hígado. Asimismo, no se detectaron modificaciones en los sitios *off-target* evaluados, ni alteraciones relevantes en los niveles de otras proteínas hepáticas secretadas al plasma analizadas.

En conjunto, los resultados demuestran la viabilidad de la administración *in vivo* de sistemas CRISPR/Cas9 mediante LNPs para conseguir una reducción eficaz y sostenida de una diana de interés terapéutico. La colaboración entre los tres grupos ha permitido ampliar las posibilidades de desarrollo terapéutico para la edición génica de *F11*. Asimismo, ha dado lugar a la presentación de una solicitud de patente europea conjunta en mayo de 2026 (EP26382722.2) y a la preparación de un manuscrito científico próximo a su envío.

IN VITRO AND IN VIVO FUNCTIONAL RESTORATION OF THE HEMATOPOIETIC LINEAGE DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS CORRECTED FOR THE MUTATION-CAUSING APDS2 SYNDROME.

RD21/0017/0024, RD21/0017/0029, RD24/0014/0025, RD24/0014/0023 Y RD24/0014/0001.

Romayor I[1-3], Inglés-Ferrándiz M[1,2], Martin-Inaraja M[1-3], Herrera L[1-3], Sáez-de-Cámara A[1-3], Olalla B[4], Río P[3,4], Santos S[1-3], Vesga MA[1-3], Biritxinaga L[1-3], Anguita J[3,5], M Juan[3,5], Allende LM[7], Gonzalez-Granado LI[8], Eguizabal C[1-3]

[1]Cell Therapy, Stem Cells and Tissues Group, Biobizkaia Health Research Institute, Barakaldo, Spain.

[2]Advanced Therapies Unit, Basque Center for Blood Transfusion and Human Tissues (CVTTH), Osakidetza, Usansolo, Spain.

[3]Red Española de Terapias Avanzadas (TERAV, TERAV+), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[4]Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain; Advanced Therapies Unit, Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid, Spain; Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain

[5]Inflammation and Macrophage Plasticity Laboratory, CIC bioGUNE-BRTA (Basque Research and Technology Alliance), Derio 48160, Spain

[6]Servei d´Immunologia. Hospital Clínic de Barcelona. Hospital Sant Joan de Déu. Institut d'Investigacions Biomèdiques August Pi i Sunyer Hospital. Universitat de Barcelona, Barcelona, Spain

[7]Immunology Department, University Hospital 12 de Octubre, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), School of Medicine, University Hospital 12 de Octubre, Complutense University of Madrid, Madrid, Spain

[8]Primary Immunodeficiencies Unit, Pediatrics, Hospital 12 Octubre (imas12), Complutense University School of Medicine, Madrid, Spain.

APDS2 disorder is a human inborn error of immunity caused by mutations in *PIK3R1* gene. Such genetic alterations affect to the correct development, maintenance, regulation and lineage differentiation during hematopoiesis in the bone marrow niche. As a result, APDS2 patients present a dysfunctional immune system characterized by heterogeneous clinical manifestations. To date, the standard supportive treatments against APDS2 disease consist in prophylactic antibiotic therapy, immunoglobulin replacement therapy and hematopoietic stem cells (HSCs) transplantation, bringing poor efficacy and many adverse effects. Giving the lack of efficient therapies, we generated an APDS2-derived induced pluripotent stem cell (iPSC) line (CVTTHi001-A). Then, we corrected the APDS2-caused point mutation in CVTTHi001-A cells using CRISPR/Cas9 molecular tool, and validated their lymphoid differentiation capacity and functional recovery in 2D culture conditions, compared to APDS2-mutated cells. Additionally, taking into account that the bone marrow (BM) sustains the hematopoiesis process, we developed BM organoids from APDS2-corrected and mutated iPSCs, which represents a unique physiologically relevant in vitro model to study pathologies such as APDS2 syndrome. Finally, in order to evaluate the restoration of APDS2-corrected iPSCs in vivo, we are analyzing the engraftment capacity of HSCs differentiated from APDS2-corrected and mutated iPSCs. Altogether, our therapeutic approximation aims to obtain a personal source of healthy transfusable components from the APDS2 patient.

DISCREPANCIES BETWEEN VECTOR COPY NUMBER AND CAR-T CELL EXPRESSION: THE IMPACT OF FREE VIRAL DNA ON PCR-BASED ASSAY. RD24/0014/0030, RD24/0014/0027.

Raquel Muñoz García[1,2], Estefanía García-Guerrero[1,2], Raúl García Lozano[3], Belén Sierro-Martinez[1], Maribel Lara-Chica[1,2], María Dolores de la Rosa-Garrido[1,2], Miguel A. Montiel Aguilera[2], María Bermejo-González[2], Esteban Márquez-Pérez[2], Paola Hernández-Díaz[1,2], Beatriz Guijarro-Albaladejo[1], José Antonio Pérez-Simón[1], Gloria Carmona-Sánchez[2,4], Blanca Arribas-Arribas[2].

[1]Instituto de Biomedicina de Sevilla (IBiS), UGC de Hematología-Hospital Universitario Virgen del Rocío (HUVR), Universidad de Sevilla, Seville, Spain.

[2]Unidad de Producción y Reprogramación Celular de Sevilla (UPRC), Red Andaluza de diseño y traslación de Terapias Avanzadas (RAdytTA), Servicio Andaluz de Salud/Fundación Pública Andaluza Progreso y Salud, Seville M.P., Spain.

[3]Servicio de inmunología, Hospital Universitario Virgen del Rocío (HUVR), Universidad de Sevilla, Seville, Spain.

[4]Red Andaluza de diseño y traslación de Terapias Avanzadas-RAdytTA, Fundación Pública Andaluza Progreso y Salud M.P.-FPS, Seville, Spain.

CENTROS PARTICIPANTES: IBiS (Instituto de Biomedicina de Sevilla), RAdytTA-FPS (Red Andaluza de diseño y traslación de Terapias Avanzadas de la Fundación Progreso y Salud).

The production of CAR-T cells requires the CAR gene to be integrated to ensure long-term expression of the receptor. This gene integration is uncontrolled, both in terms of its genomic location and the number of integration events. As part of the safety assessment of genetically modified cell-base medicinal products, the number of vector copies per cell (Vector Copy Number, VCN) must be characterized and appropriately controlled according to regulatory agencies, since an excessive number of integrated vector copies may increase the risks (European Medicines Agency 2020).

For our product (CARTemis-1, EUCT number 2022-503063-15-00), the Vector Copy Number (VCN) is analyzed by PCR, for which we have established a limit of 10 integrated copies per cell. PCR is a reliable analytical method for detecting CAR DNA integrated into the host cell (integrated CAR gene).

VCN variants exist depending on the type of viral vector and the type of cells. Minor variations may also occur when comparing different batches with the same type of viral vector within the same type of cells. These smaller differences are due to vector variability from batch to batch and variations in the starting cell material within the same production process. In any case, despite these minor differences, the VCN is generally expected to correlate with the percentage of CAR expression, since a higher number of integrated copies of the CAR gene should lead to greater expression of the receptor protein.

During the three CARTemis-1 validation batches, the VCN was measured 8 days after lentiviral vector addition to the leukapheresis material from healthy donors, following the prior selection of $CD8^+$ and $CD4^+$ cell populations. The resulting VCN values were 0.97, 1.51 and 0.87 copies/cell, with corresponding CAR expression levels of 30.3 %, 34.2 % and 36.9 %, respectively, determined by flow cytometry.

In subsequent batches manufactured from patient leukapheresis material, the VCN was measured 6 days after lentiviral vector addition -two days earlier than during the validation stage. This adjustment was made either because the target dose had been achieved earlier or because difficulties in CAR^+ cell growth were anticipated. At this earlier time point, unexpectedly high VCN values were observed; however, the expression percentages were lower. Specifically, VCN results were 37.83, 3.27, 2.67, 32.23 and 6.09 copies/cell, with corresponding

CAR expression percentages of 14.2%, 21.7%, 22.9%, 13.34% and 9.04%, respectively.

We suspected that the PCR assay might also detect non-integrated DNA in other forms, such as cDNA or other forms of free DNA, which are less stable than integrated DNA.

To determine whether the presence of non-integrated DNA could lead to VCN overestimation, VCN was monitored at different time points after the viral vector addition without washing the cell culture. DNA from the second CARTemis-1 validation batch, which had previously yielded a VCN of 1.51 copies/cell, was included as a reference control in the PCR analyses. The reference control results remained stable throughout all assays, demonstrating the day-to-day reliability of the PCR. VCN analyses were performed at Virgen del Rocio University Hospital (HUVR) using custom PCR probes.

The remaining results (summarized in Table 1) indicated that the VCN decreased as more days elapsed since vector administration. This supports the hypothesis that the assay detects free DNA, which is more unstable than integrated DNA. However, further studies are required to confirm this hypothesis.

Table 1

Batch	**Day 5 (once LV has been added) VCN % CAR+**	**Day 6 (once LV has been added) VCN % CAR+**	**Day 7 (once LV has been added) VCN % CAR+**	**Day 8 (once LV has been added) VCN % CAR+**
Patient 1	NA	37.83 copies/cell 14,2%	NA	4.65 copies/cell 30.5%
Patient 2	16.11 copies/cell 24,0%	3.27 copies/cell 21,7%	NA	1.59 copies/cell 24,5%
Patient 3	NA	NA	2.67 copies/cell 22.9%	NA
Patient 4	NA	32.23 copies/cell 13.34%	14.46 copies/cell 26.7%	14.96 copies/cell 19.80%
Patient 5	not calculable	6.09 copies/cell 9.04%	3.43 copies/cell 9.14%	2.02 copies/cell 10.2%

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DESARROLLO DE MODELOS NEURONALES DERIVADOS DE PACIENTES Y NUEVAS ESTRATEGIAS DE TERAPIA GÉNICA PARA ENFERMEDADES NEUROLÓGICAS RARAS. RD24/0014/0039, RD24/0014/0013 Y RD24/0014/0037.

M. Page, L. Rodríguez, I. Rodríguez, A. Behr, J. Sauleda, S. Verdés, A. Brao, J. del Rey, B. Almolda, A. Consiglio, A. Bosch y **M. Chillón**.

Universitat Autònoma Barcelona (UAB); Vall d'Hebron Research Institute (VHIR); Banc de Sang i Teixits (BST-VHIR).

Las enfermedades neurológicas raras representan un importante desafío terapéutico debido a su heterogeneidad genética, complejidad fisiopatológica y limitada disponibilidad de tratamientos eficaces. En el marco de la red TERAV+, la colaboración entre los grupos RG21 y RG13 se centra en el desarrollo de modelos celulares humanos y nuevas estrategias de terapia génica dirigidas a estas patologías.

Se han generado células madre pluripotentes inducidas (iPSC) derivadas de pacientes con paraplejía espástica tipo 52 (SPG52), posteriormente diferenciadas a neuronas corticales, proporcionando una plataforma experimental para investigar los mecanismos patológicos y evaluar futuras intervenciones terapéuticas. Paralelamente, se han obtenido neuronas corticales y organoides de tronco encefálico derivados de pacientes con síndrome de Wolfram, ampliando las posibilidades de estudiar alteraciones neurológicas en sistemas humanos relevantes. Asimismo, se encuentra en desarrollo la generación de neuronas corticales derivadas de pacientes con síndrome GNB1.

Estas aproximaciones se complementan con el desarrollo de vectores virales adenoasociados (AAV) optimizados para aplicaciones de terapia génica *in vivo*, incluyendo nuevas variantes con tropismo selectivo por subpoblaciones neuronales. Adicionalmente, se están investigando estrategias terapéuticas para la esclerosis lateral amiotrófica (ELA) mediante vectores AAV que expresan la forma secretada de Klotho (sKL), una proteína con potencial neuroprotector. Esta aproximación ha obtenido la designación de medicamento huérfano por la FDA, constituyendo un avance relevante hacia su desarrollo clínico.

El programa contempla también estudios preclínicos de eficacia y seguridad en enfermedades neurológicas y neuromusculares, junto con la optimización de procesos de producción de vectores y herramientas de edición génica.

En conjunto, la integración de modelos neuronales derivados de pacientes, organoides y tecnologías avanzadas de transferencia génica establece una plataforma traslacional para investigar mecanismos de enfermedad, explorar nuevas intervenciones terapéuticas y facilitar el desarrollo de tratamientos personalizados para patologías neurológicas raras actualmente sin opciones terapéuticas satisfactorias.

PROGRAMA 2. INMUNOTERAPIA CELULAR ANTITUMORAL
SESIONES

UPDATED SAFETY AND EFFICACY RESULTS OF TRANSPOCART19, A NON-VIRAL SLEEPING BEAUTY TRANSPOSON-ENGINEERED ANTI-CD19 CAR-T CELL THERAPY FOR RELAPSED/REFRACTORY B-CELL LYMPHOMAS. RD24/0014/0034, RD24/0014/0010, RD24/0014/0027, RD24/0014/0023, RD24/0014/0014.

Lucía López-Corral[1,2], Miguel A. Canales[3], A. Martín García-Sancho[1,2], Javier Delgado[4], Begoña Díez[5], Susana Inoges[3], Ascensión López-Díaz de Cerio[3], Juan R. Rodríguez-Madoz[2,3], Rosa M. Yáñez[5], Carlos Grande[3], Ana A. Martín-López[1], Estefanía Pérez-López[1], Miriam López-Parra[1,2], Eva M. Villarón[1], Almudena Navarro-Bailón[1,2], Joaquín Gómez Espuch[6], Alberto Musseti[7], Javier Cornago[8], Juan A. Bueren[2,5], Esperanza López-Franco[1], Jose Antonio Pérez-Simón[4], Fermín Sánchez-Guijo[1,2] and Felipe Prósper[2,3].

[1]IBSAL-University Hospital of Salamanca, Salamanca, Spain.

[2]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Spain.

[3]Clínica Universidad de Navarra, Pamplona, Spain.

[4]University Hospital Virgen del Rocío, Sevilla, Spain.

[5]CIEMAT/CIBERER – IIS Fundación Jiménez Díaz, Madrid, Spain.

[6]Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain.

[7]Catalan Institute of Oncology (ICO) – Hospital Duran i Reynals, Barcelona, Spain.

[8]IIS Fundación Jiménez Díaz – Universidad Autónoma de Madrid, Madrid, Spain.

Background: TranspoCART19 is an academic, non-viral anti-CD19 CAR-T cell therapy generated using the Sleeping Beauty transposon system. It incorporates a second-generation 4-1BB/CD3ζ CAR and a truncated EGFR (hEGFRt) reporter/safety switch. Preclinical development demonstrated a reproducible GMP-compliant manufacturing process, antitumor activity comparable to lentiviral CAR-T cells, and a favorable integration and safety profile, supporting clinical translation [1]. We report updated results from the ongoing multicenter phase I/IIa clinical trial (NCT06378190) in patients with relapsed/refractory (R/R) B-cell lymphomas.

Methods: This is an open-label, multicenter, non-randomized phase I/IIa trial evaluating safety, feasibility, and preliminary efficacy. Phase I followed a 3+3 dose-escalation design with three dose levels (1, 3, and 5 $\times 10^6$ CAR-T cells/kg), followed by a phase IIa expansion cohort at the recommended phase II dose (RP2D). The CAR-T product is infused fractionally (10%, 30%, and 60% on days 0, +1, and +4). Following completion of phase I and an integrated assessment of safety, preliminary efficacy, in-vivo expansion, and manufacturing feasibility, 3 $\times 10^6$ CAR-T cells/kg (DL2) was selected by the Scientific Committee and DSMB as the RP2D.

Results: Ten patients were treated during phase I. Three additional patients with follicular lymphoma have entered the phase IIa expansion cohort at the RP2D; two have completed their first response assessment and the third is receiving TranspoCART19 at the current data cutoff. In addition, two additional patients have provided informed consent and are awaiting leukapheresis. Manufacturing was successfully completed for all treated patients. One patient in the phase IIa expansion cohort experienced an initial manufacturing failure requiring repeat leukapheresis and remanufacturing; a second product was successfully generated and subsequently infused.

Safety: No DLTs were observed during dose escalation, and no new safety signals have emerged during the phase IIa expansion. Among the 12 patients with available post-infusion safety follow-up, CRS occurred in 6/12 (50%): five grade 1 and one grade 2, with no grade ≥3 events. Only two patients required tocilizumab, consisting of a single dose in each case. One patient with rapidly progressive primary CNS lymphoma developed early disease progression and neurological deterioration formally reported as grade 3 ICANS. However, attribution to CAR-T toxicity was considered uncertain given the markedly abnormal neurological status at baseline, concomitant disease progression, and the

absence of detectable CAR-T cell expansion in either peripheral blood or cerebrospinal fluid. The patient did not complete the DLT assessment period and was considered non-evaluable for dose escalation by the DSMB, leading to the enrollment of an additional patient at DL1. No other patient developed ICANS or other neurological toxicity. There were no cases of IEC-HS and hematotoxicity was limited, with no aplastic phenotype or requirement for thrombopoietin receptor agonists; No treatment-related deaths occurred.

Efficacy: Among 12 patients evaluable for response, the best overall response rate was 91.7% (11/12), including 10 complete responses (83.3%), one partial response (8.3%), and one progressive disease (8.3%). The only patient with primary refractory disease had rapidly progressive primary CNS lymphoma with overt disease progression before CAR-T infusion. At a median follow-up of 11.2 months (range, 1.0–27.3), two additional disease progressions have occurred after an initial response: one patient with PR at day +28 progressed around day +100, while one patient with an initial CR developed follicular lymphoma progression 18 months after infusion. Neither has required subsequent lymphoma-directed therapy to date. Both response-evaluable patients treated in the phase IIa expansion achieved CR at day +28.

CAR-T kinetics: In-vivo TranspoCART19 expansion was observed in all phase I patients except the patient with primary CNS lymphoma, with a median time to peak expansion of 14 days (range, 14–21). Median Cmax was 85.2 cells/µL (range, 7.6–560.6) and median AUC0–28 was 1,892 cells/µL × days (range, 85–9,021). No clear dose–response relationship was observed across dose levels.

Conclusions: TranspoCART19 demonstrates overall manufacturing feasibility and a highly favorable safety profile, with no DLTs and predominantly low-grade CRS. High response rates and durable remissions have been observed, together with consistent in-vivo CAR-T expansion. Based on the integrated assessment of safety, efficacy, CAR-T kinetics, and manufacturing feasibility, 3×10^6 CAR-T cells/kg was selected as the RP2D. Phase IIa expansion is ongoing,

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THE FIRST POINT-OF-CARE CAR ADVANCED THERAPY IN THE BASQUE COUNTRY: A FIRST-IN-HUMAN PHASE I CLINICAL TRIAL OF ALLOGENEIC UMBILICAL CORD BLOOD-DERIVED CAR-NK-CD19 CELLS (NKARI-0001) FOR RELAPSED OR REFRACTORY CD19+ B-CELL LYMPHOID MALIGNANCIES (EU CT NUMBER: 2026-527288-14-00). RD21/0017/0024, RD21/0017/0029, RD21/0017/0002 Y RD24/0014/0025, RD24/0014/0001, RD24/0014/0035.

L Herrera[1,2,3,10], A Etxebarria[1,2,3,10], I Romayor[1,2,3], M Alvarez[1,2,3,10], D Barriales[1,2], A Palacios[1,2,3], S Santos[1,2,3], L Biritxinaga[1,2,3], MA Vesga[1,2,3], J Anguita[3,4,5], E Gainza[3,6], L Cuevas[3,6], JC Garcia[3,7], E Amutio[3,7], ME Fernandez[8,3], M Juan[3,9,10], C Eguizabal[1,2,3,10]

[1]Advanced Therapies Unit, Basque Center for Blood Transfusion and Human Tissues, Osakidetza, Galdakao 48960, Spain;

[2]Cell Therapy, Stem Cells and Tissues Group, Biobizkaia Health Research Institute, Barakaldo 48903, Spain;

[3]Red Española de Terapias Avanzadas (TERAV), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[4]Macrophage and Tick Vaccine Laboratory, CIC bioGUNE, Derio, Biscay, Spain.

[5]Ikerbasque, Basque Foundation for Science, Bilbao, Biscay, Spain.

[6]Servicio de Hematología, Hospital Galdakao-Usansolo, 48960, Galdakao (Bizkaia)

[7]Servicio de Hematologia, Hospital Universitario Cruces, Barakaldo (Bizkaia)

[8]Department of Cardiology, Hospital General Universitario Gregorio Marañón, Universidad Complutense de Madrid, Spain; Cell Production Unit, Department of Cardiology, Instituto de Investigación Sanitaria Hospital Gregorio Marañón (IiSGM), Madrid, Spain

[9]Immunotherapy department. Servei d'Immunologia. Hospital Clínic - Sant Joan de Déu, UB, IDIBAPS, 08036 Barcelona

[10]Red de Inmunoterapia del Cancer "REINCA" (RED2022-134831-T)

CENTROS PARTICIPANTES**:** IIS Biobizkaia-CVTTH**,** CIC bioGUNE, Hospital Galdakao-Usánsolo, Hospital Universitario Cruces, Hospital Clinic, Hospital Gregorio Marañon

B-cell hematologic cancers such as leukemia and lymphoma are common forms of pediatric and adult cancers worldwide. Acute Lymphoblastic Leukemia (ALL) is the most common cancer among children with a prevalence of 20-25% of all cases. The survival rate for these patients at 5 years is 79.2%, but it is still an incurable disease in many patients. Chimeric Antigen Receptor (CAR) T cells therapy has arisen as a new alternative to conventional therapies in order to treat advanced refractory cancers. However, this therapy has some undesirable side effects such as cytokine release syndrome (CRS) and neurotoxicity. In addition, despite the good results in clinic, some patients are refractory or relapsed after CART cell treatment. NK cells from cord blood could be a good alternative for CAR based therapy, as they do not cause this kind of side effects, and CD19 CAR NK can be an "off the shelf immunotherapy" to treat patients with refractory or relapse B hematological cancers. Accordingly, our lab has developed preclinical studies from cord blood CD19 CAR-NK cells in order to translate them into a pioneer authorized Phase I clinical trial to treat patients with refractory or relapse B cell haematological cancers (EUCT NUMBER: 2026-527288-14-00). Consequently, all this work lead to GMP compliant manufacturing for cord blood CD19 CAR-NK cells.

DUAL TRAC AND B2M GENOME EDITING PRESERVES ANTILEUKEMIC ACTIVITY OF CLL1-DIRECTED CAR-T CELLS FOR ALLOGENEIC IMMUNOTHERAPY OF AML. RD24/0014/0005, RD24/0014/0030.

Alejandro Millán-López[1,2], María Ortiz-Bueno[2], Antonio Ballesteros-Ribelles[1], Kornel Labun[3], Víctor Ronco-Díaz[2], Raquel Bernardo[4,5], Isabel María Vallejo-Bermúdez[4,5], Alicia Moreno Pantoja[6], Sonia Nogueras[6], Rosario Jiménez[6], Mónica Santos González[1,7], Vanesa Martín[8], Gloria Carmona[7], Erden Atilla[9], Duško Lainšček[10], Juan Antonio Marchal[11,12,13], Concha Herrera[1], Karim Benabdellah[2].

[1]Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Unidad de Terapia Celular, Hospital Universitario Reina Sofía, Servicio de Hematología y Hemoterapia, Córdoba, España.

[2]GENyO, Centro de Genómica e Investigación Oncológica Pfizer–Universidad de Granada–Junta de Andalucía, Granada, España.

[3]Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, 02-106 Warsaw, Poland.

[4]Immunology and Allergy Group (GC01), Maimonides Biomedical Research Institute of Córdoba (IMIBIC), University of Córdoba, Reina Sofía University Hospital, Córdoba, Spain.

[5]Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain.

[6]Cellular Therapy Unit, Reina Sofía University Hospital, Córdoba, Spain.

[7]Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RAdytTA), Servicio Andaluz de Salud / Fundación Pública Andaluza Progreso y Salud M.P. (FPS), Seville, Spain.

Background: Despite recent advances, effective cellular immunotherapies for acute myeloid leukemia (AML) remain limited by the lack of readily available products. CLL1 is a promising target for chimeric antigen receptor (CAR) T-cell therapy in AML. However, autologous manufacturing is constrained by disease-related T-cell dysfunction, manufacturing failures, and delays in product availability. The development of allogeneic CAR T cells could overcome these limitations, although immunological barriers, including host-versus-graft responses and natural killer (NK) cell-mediated rejection, remain major challenges. Simultaneous disruption of TRAC and B2M represents a strategy to reduce alloreactivity and susceptibility to immune rejection while preserving antileukemic activity.

Objective: To develop a preclinical-grade CLL1-directed CAR T-cell product incorporating simultaneous TRAC and B2M disruption (uCAR-AML) for the treatment of AML, and to evaluate its antileukemic activity, allogeneic immune compatibility, and suitability for clinical translation, including the definition of manufacturing and quality-control requirements and the preclinical and regulatory framework needed to support its future clinical development.

Methods: Healthy-donor T cells were transduced with a lentiviral vector encoding a second-generation anti-CLL1 chimeric antigen receptor (CAR) and edited using CRISPR/Cas9 to disrupt TRAC and B2M, generating candidate allogeneic CAR-T products (uCAR-AML). Cells were expanded in G-Rex culture systems and characterized by flow cytometry to assess CAR expression, editing-associated phenotypes, and T-cell differentiation. Antileukemic activity was evaluated against AML cell lines and primary AML samples. T-cell phenotype and exhaustion-associated markers were assessed following repeated antigen exposure, while cryopreservation studies evaluated the retention of CAR expression. Allogeneic immune compatibility was investigated through PBMC coculture assays. Finally, antitumor activity was assessed in a murine leukemia model by longitudinal bioluminescence imaging and survival analysis. These studies were designed to support preclinical product development and inform subsequent manufacturing, quality-control, and regulatory requirements for future clinical translation.

Results: Dual-edited uCAR-AML products were successfully generated and expanded, with mean CAR expression increasing from 29.00% at day 7 to 51.13% at day 12. At day 7, 75.21% of cells displayed a $CD3^{-}B2M^{-}$ phenotype. Although expansion was lower than in conventional CAR-AML products, edited cells retained proliferative capacity during manufacturing, highlighting the importance of further process optimisation and product consistency for clinical translation. Functionally, uCAR-AML cells generated from three independ-

[8]Servicio de Hematología y Hemoterapia, Hospital Universitario Reina Sofía, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Córdoba, España.

[9]University of Miami, Miller School of Medicine, Miami, USA.

[10]National Institute of Chemistry, Ljubljana, Slovenia.

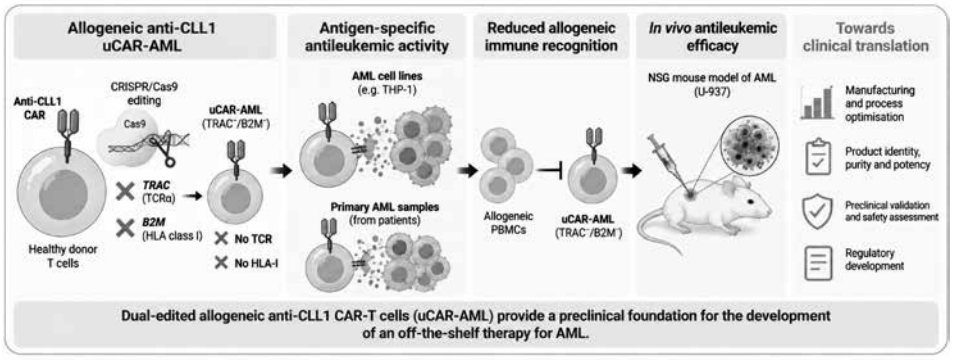
[11]Instituto de Investigación Biosanitaria ibs.GRANADA, University Hospitals of Granada, University of Granada, Granada, Spain.

[12]BioFab i3D Lab – Biofabrication and 3D (Bio)printing Laboratory, Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain.

[13]Excellence Research Unit "Modeling Nature" (MNat), University of Granada, Granada, Spain.

ent donors showed preferential cytotoxicity against CLL1-positive THP-1 cells over CLL1-negative Namalwa cells, achieving mean specific lysis of 82.20% and 86.28% at 48 hours at effector-to-target ratios of 1:5 and 1:10, respectively. Antileukemic activity was also demonstrated against three primary AML samples, with 35.93–62.06% specific lysis at 48 hours at a 1:5 ratio. Following repeated antigen exposure, T-cell differentiation profiles shifted predominantly from central to effector memory phenotypes, while cryopreserved products retained detectable CAR expression. In allogeneic coculture assays, uCAR-AML products were associated with reduced PBMC activation, including a significant product-associated effect on CD25 expression, supporting further investigation of their immunological compatibility. In a murine leukemia model, both conventional and dual-edited CAR-T cells prolonged survival compared with non-transduced controls. Animals receiving CAR-AML and uCAR-AML survived until approximately days 35 and 42, respectively, whereas all control animals reached the survival endpoint between days 14 and 21. Nevertheless, heterogeneous responses and subsequent disease progression in treated animals highlight the need to optimise therapeutic performance and establish robust preclinical evaluation criteria.

Collectively, these findings establish a preclinical proof of concept for CLL1-directed, dual-edited CAR-T cells and provide a foundation for further development towards clinical translation. Key next steps include optimizing and standardizing manufacturing, defining product identity, purity, potency and genetic-editing quality attributes, and establishing the safety and comparability assessments needed to support future regulatory development.



NOVEL HER2-SPECIFIC SDAB-CAR-T CELLS EXHIBIT ENHANCED ANTITUMORAL EFFICACY IN HER2-POSITIVE CANCERS. RD24/0014/0005 AND RD24/0014/0010.

C Barbero-Jimenez[1], P Justicia-Lirio[1], E Molina[2], I Ibañez[2], F Bernasconi-Bisio[2], R Redondo-Frutos[1], M Garnica[1], S Rodriguez-Diaz[1], L Puchades-Carrasco[3], N Gomez-Cebrian[3], A Pineda-Lucena[2], F Martin[4], F. Prosper[5] and JR Rodriguez-Madoz[6]

[1]Hemato-Oncology Program, Cima Universidad de Navarra. IdiSNA. Pamplona, Spain.

[2]Therapeutic Innovation Program, Cima Universidad de Navarra. IdiSNA. Pamplona, Spain.

[3]Drug Discovery Unit, Instituto de Investigación Sanitaria La Fe (IIS La Fe). Valencia, Spain.

[4]Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), Granada, Spain

[5]Hematology and Cell Therapy Department, Clinica Universidad de Navarra. IdiSNA. Cancer Center Clinica Universidad de Navarra (CCUN). Centro de Investigacion Biomedica en Red de Cancer (CIBERONC). Pamplona, Spain.

[6]Hemato-Oncology Program, Cima Universidad de Navarra. IdiSNA. Cancer Center Clinica Universidad de Navarra (CCUN). Centro de Investigacion Biomedica en Red de Cancer (CIBERONC). Pamplona, Spain.

The Human Epidermal Growth Factor Receptor 2 (HER2) is a clinically validated tumor-associated antigen and a well-established target for antibody-based therapies. However, the efficacy of antibody-drug conjugates (ADCs) is frequently compromised by payload-associated resistance mechanisms and mutations affecting antibody-binding domains. Chimeric antigen receptor (CAR) T cells represent a promising alternative, leveraging direct T-cell cytotoxicity while enabling the targeting of distinct HER2 epitopes. Nevertheless, the high affinity of conventional HER2-directed CARs may induce severe on-target, off-tumor toxicity, highlighting the need for affinity-optimized constructs capable of discriminating between physiological and tumor-associated antigen densities. Here, we developed, characterized, and functionally evaluated optimized single-domain antibody (sdAb)-based CAR-T cells targeting HER2.

A panel of sdAbs spanning a broad range of HER2-binding affinities was generated to identify candidates capable of maximizing antitumor activity while minimizing safety risks. Structural modeling using AlphaFold-Multimer predicted distinct HER2-sdAb binding interfaces, providing insights into epitope-dependent functional properties. Selected sdAbs were incorporated into second-generation 4-1BBζ CAR constructs and compared with a conventional FRP5-scFv-based HER2 CAR-T. sdAb-CARs displayed robust antigen-dependent activation across NFAT, NF-κB, and AP-1 signaling pathways, while exhibiting significantly reduced tonic signaling in the absence of antigen, consistent with enhanced specificity and a potentially improved safety profile. sdAb-CAR-T cells derived from healthy donors mediated potent and selective *in vitro* cytotoxicity against HER2-positive tumor cells. Although all candidates showed comparable activity against HER2-high targets, functional differences became evident under low-antigen conditions, where higher-affinity sdAb-CAR-T cells demonstrated superior cytotoxicity, supporting the concept that CAR efficacy becomes increasingly affinity-dependent at low target densities. Finally, *in vivo* efficacy was assessed in NSG xenograft models. In the HER2-high gastric cancer model NCI-N87, all sdAb-CAR-T candidates induced complete tumor remission. Importantly, in HER2-low hepatocellular (PLC/PRF/5) and lung (A549) tumor models, only high-affinity sdAb-CAR-T cells achieved complete tumor regression. Notably, our lead candidate demonstrated selective *in vivo* discrimination in bilateral xenograft models bearing HER2-high NCI-N87 tumors on one flank and HER2-low A549 tumors on the contralateral flank. In this setting, CAR-T cells selectively eradicated the HER2-high tumor while sparing the HER2-low lesion, highlighting the capacity of this affinity-tuned approach to preserve a favorable

therapeutic window by balancing potent antitumor efficacy with reduced risk of off-tumor toxicity.

In conclusion, our study demonstrates that affinity-optimized sdAb-based HER2 CAR-T cells can achieve selective targeting of tumors with high HER2 expression while limiting activity against low-expressing tissues *in vivo*. These findings support the clinical potential of affinity-tuned HER2 CAR-T therapies and provide a strong rationale for further studies aimed at defining the therapeutic window and safety profile of our lead candidate in advanced preclinical models.

PROGRAMA 2. INMUNOTERAPIA CELULAR ANTITUMORAL

PÓSTERES

OPTIMIZATION IN LENTIVIRAL VECTOR MANUFACTURING FOR PRODUCTION OF CAR-T THERAPY AND OTHER CELL THERAPIES (LENTI-UP). RD24/0014/0001, RD24/0014/0006, RD24/0014/0002.

Daniel Parras[1], Guillermo Iruela[2,3], Andrea Macián[1], Mariona Baliu[4], Susana Rives[5], Julio Delgado[1], Manel Juan[1], Cristina Bernadó[1,2,3*], Hugo Calderón[1,2].

[1]RECERCA Clinic Barcelona/(IIS-FRCB-IDIBAPS).

[2]Centro Tecnológico LEITAT, Leitat Medical.

[3]Gene Vector Barcelona, SL.

[4]Complejo Hospitalario y Universitario de Santiago (CHUS-Galaria). Universidad de Vigo. Grupo de Galicia Sur.

[5]Hospital Sant Joan de Déu. Fundación para la Investigación y Docencia Sant Joan de Déu (HSJD/FSJD).

Adoptive cellular immunotherapy of T cells modified to express Chimeric Antigen Receptors (CAR) has been a paradigm shift for cancer treatment, and in particular, has demonstrated outstanding results in hematological cancers. Our academic group at Hospital Clínic Barcelona (FRCB-IDIBAPS) has developed a CART against CD19 (ARI001) for B-cell cancers. It has obtained authorization for use from the Spanish Agency for Medicines and Health Products (AEMPS) and for the first time for an academic product, the PRIME designation from the EMA (European Medicines Agency). The group has also developed CART-BCMA (ARI002) for multiple myeloma (MM) and other mature B-cell neoplasms, for which it has also obtained authorization for use from the AEMPS.

The CAR-Ts manufacturing process uses transduction of T cells with viral vectors containing the CAR sequence. The production protocol currently authorized for the different CARs is carried out under GMP (Good Manufacturing Practices) conditions in clean rooms and consists of obtaining batches of lentiviruses on a small scale using multilayer flasks. Considering future perspectives, due to the success of these therapies, it is necessary to optimize and scale the current lentiviral production process to obtain a product of higher purity and allow the treatment of a greater number of patients.

Within the framework of the LENTIUP collaborative project, the manufacture of lentiviral vectors has been optimized by implementing a production system in bioreactors and optimized purification to be used in CAR-T therapy. The optimized process includes an upstream phase using fixed-bed bioreactors for production with adherent cells and a multi-step downstream phase with the relevant incorporation of chromatography purification. As a result, the obtention of greater quantity of the highest purity lentiviral vector in accordance with the most demanding regulatory requirements of the Spanish and European regulatory agencies (AEMPS/EMA).

In the LENTI-UP collaborative project funded by the Complementary Plan for Biotechnology Applied to Health (C17. I1), the Fundació Clínic per a la Recerca Biomèdica and Hospital Clínic de Barcelona (FCRB-HCB) have participated as coordinators, with the collaboration of the LEITAT Technology Centre, the Sant Joan de Déu Hospital - Sant Joan de Déu Foundation for Research and Teaching (HSJD/FSJD), and the Santiago Hospital and University Complex (CHUS-Galaria). The implementation has been complemented with the obtaining of additional competitive public-private funding (INVECTO, ISCIII-CDTI).

The scaled lentiviral production platform established is applicable to products in current development and future. The capacities that the consortium members have acquired, complemented with CERTERA, allow to have the capacity for pilot-scale optimization and subsequent GMP-scale transfer adaptable to different types of viral vectors for ex vivo and in vivo use. The objective is to allow rapid adaptation to new products and thus accelerate their development and ultimately facilitate access to the most innovative therapies for patients.

TERAPIA CAR-T ESPECÍFICA FRENTE A CLAUDINA 18.2 PARA EL CÁNCER GÁSTRICO Y PANCREÁTICO. RD24/0014/0002 Y RD24/0014/0001.

Joel Verísimo García, Cristian Sánchez Espinel, Raquel Almuiña González, Lorena Boquete Vilariño, Mariona Baliu Piqué, Manel Juan Otero, África González Fernández

CINBIO IN1, GALARIA, HOSPITAL CLINIC BCN.

Los cánceres gástrico y pancreático constituyen, respectivamente, la 5.ª y la 6.ª causa de muerte por cáncer en todo el mundo, así como el 5.º y el 11.º tumor más frecuente. Ambos se asocian a un mal pronóstico, debido en gran medida al diagnóstico tardío y a la limitada eficacia de las opciones terapéuticas actuales. La claudina 18.2 es una proteína transmembrana que forma parte de las uniones estrechas del epitelio gástrico. Sus epítopos son, en su mayoría, inaccesibles en los tejidos sanos, pero se sobreexpresan en determinados tumores, entre ellos los gástricos y pancreáticos. Este patrón de expresión restringido la convierte en una diana prometedora para la inmunoterapia.

En este contexto, nos propusimos desarrollar una terapia con células CAR-T dirigida contra la claudina 18.2. Para ello, inmunizamos ratones con la proteína humana claudina 18.2 y generamos anticuerpos monoclonales (AcMo) murinos mediante tecnología de hibridomas. El AcMo CL6 se seleccionó por su capacidad de reconocer la claudina 18.2 mediante ELISA sin presentar reactividad cruzada con la isoforma claudina 18.1, expresada en pulmón y que difiere en el primer bucle extracelular (ECL1). Mediante citometría de flujo confirmamos que CL6 se une específicamente a una región extracelular de la claudina 18.2 en varias líneas humanas pancreáticas y gástricas y en células transfectadas con claudina 18.2, pero no en líneas de otros tejidos, en células no transfectadas ni en células transfectadas con claudina 18.1. Además, CL6 reconoce tumores pancreáticos claudina 18.2+ mediante inmunohistoquímica.

A partir de su scFv, construimos un receptor de antígeno quimérico (CAR) de segunda generación con los dominios de señalización de CD3ζ y 4-1BB, y evaluamos su actividad *in vitro*. Las células CAR-T anti-claudina 18.2 mostraron una citotoxicidad significativa frente a líneas humanas de cáncer pancreático y gástrico que expresan claudina 18.2, sin actividad frente a las que carecen de esta proteína. Estos resultados demuestran que el scFv derivado de CL6 permite generar células CAR-T con actividad citotóxica específica frente a la claudina 18.2 y respaldan su evaluación *in vivo* en cáncer gástrico y pancreático.

VALIDACIÓN DEL CENTRO DE TERAPIAS AVANZADAS (CTA) COMO CENTRO PRODUCTOR DESCENTRALIZADO (*POINT-OF-CARE*) PARA LA FABRICACIÓN DE LA TERAPIA CAR-T ARI0003 EN GALICIA. RD24/0014/0002 Y RD24/0014/0001.

Marta Español-Rego[1], Leticia Alsewaran[1], Nuria López Lorenzo[2], Marta Varela Eirín[1], Sergio Navarro-Velázquez[2], Karen Tréboles[2], Kelly Rojas[2], Lorena Boquete Vilariño[1], E. Azucena González-Navarro[2], Manel Juan Otero[2], África González Fernández[3]

[1]Centro de fabricación de terapias avanzadas de Galicia, Galaria, EPSS

[2]Hospital Clínic de Barcelona

[3]CINBIO, grupo de inmunología, Universidad de Vigo / Instituto de investigación Sanitaria Galicia Sur (IISGS)

La terapia CAR-T ha supuesto un enorme avance en el tratamiento antitumoral, especialmente en neoplasias hematológicas. Sin embargo, una de sus principales limitaciones es su accesibilidad, debido a los elevados costes y los largos tiempos de espera por de la escasez de slots de producción. El Hospital Clínic de Barcelona (HCB) ha desarrollado un programa de CAR-T académico bajo el modelo de fabricación *Point-of-Care* (PoC) para aproximar el tratamiento al paciente y reducir costes. En este contexto, el Centro de Terapias Avanzadas (CTA) de Galicia se ha incorporado (junto con el Hospital Ramón y Cajal y el Hospital Virgen de la Arrixaca) al proceso de validación para ser designado como centro productor de la terapia dual ARI0003 (anti-CD19/anti-BCMA).

El objetivo de este trabajo es cualificar al CTA como centro productor descentralizado en el modelo PoC del HCB para la fabricación de la terapia ARI0003, promoviendo el acceso equitativo a las terapias avanzadas en Galicia y capacitando al CTA en el desarrollo y producción de futuras terapias.

Para la cualificación del centro se establecieron cuatro requisitos, siguiendo las recomendaciones de la guía ICH (*International Conference on Harmonisation*) Q5E de comparabilidad de productos biotecnológicos/biológicos sometidos a cambios en su proceso de fabricación.

A) Validación de controles de calidad críticos (recuento celular, viabilidad y citometría de flujo para subpoblaciones TBNK y células CAR-T+).

B) Tres procesos consecutivos de llenado aséptico (*media fill*) superados con éxito.

C) Fabricación de un ensayo simulado (*mock run*).

D) Producción en paralelo de 3 lotes de ARI0003 comparables con los fabricados en el centro de referenciaHCB (variabilidad intercentro <25%), partiendo del mismo material de partida (leucoaféresis) divididas al 50% (dos criopreservadas y una en fresco).

Los requerimientos A, B y C se completaron satisfactoriamente, alcanzando en el *mock run* una dosis total de $2103x10^6$ células totales y un porcentaje de transducción del 63% CART+ tras 8 días de cultivo. Las validaciones en paralelo correspondientes al requisito D se encuentran en la fase final de desarrollo, con fecha de finalización el 12 de noviembre. Los resultados definitivos de calidad y producción de los lotes comparativos se presentarán durante la reunión TERAV.

La cualificación técnica del CTA dentro de la red PoC de ARI0003 permitirá descentralizar la producción de CAR-Ts, garantizando un acceso más rápido y equitativo para los pacientes en Galicia y habilitando la infraestructura para la fabricación de nuevas terapias avanzadas.

ENHANCING CAR-T THERAPY FOR HER2+ TUMORS: CONTROLLED CAR AND CYTOKINE EXPRESSIONS FOR IMPROVED EFFICACY AND SAFETY. RD24/0014/0005; RD24/0014/0010.

A Hinckley Boned[1,2], P Justicia-Lirio[3,4], C Barbero-Jiménez[3,4], J Ruiz-de Francisco[4], M Cortijo-Gutiérrez[1], K Pavlovic[1], A Matilla-Serrano[1,5], C Panisello[5], P Menendez[5], C Bueno[5], JR. Rodríguez-Madoz[3], F Prosper[3], A Rosa[6], J Roos[6], P Loskill[6], Tristán-Manzano[1,2,7] and F Martin[1,2,7].

[1]Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO), PTS, Granada, Spain.

[2]Biosanitary Research Institute of Granada (ibs.GRANADA), University of Granada, Granada, Spain.

[3]Cima Universidad de Navarra, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain, Cancer Center Clínica Universidad de Navarra (CUN), Pamplona, Spain.

[4]LentiStem Biotech, (GENYO), PTS, Granada, Andalusia, Spain.

[5]Josep Carreras Leukemia Research Institute, Barcelona, Spain

[6]NMI Natural and Medical Sciences Institute at the University of Tübingen, Markwiesenstr. 55, Reutlingen, 72770, Germany.

[7]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain.

Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionized cancer treatment by enabling T cells to specifically recognize and eliminate tumor cells. However, limited CAR-T cells persistence and the tumor microenvironment present serious barriers to efficiently treat solid tumors. This limited persistence can be caused by uncontrolled CAR expression which also leads to severe toxicities. To solve this, 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 a FRP5-based anti-HER2CAR and was compared to the control of a strong constitutive EF1α promoter. AWHER2 CAR-T cells exhibited lower surface CAR expression, reduced tonic signaling, and an improved phenotype. They demonstrated comparable cytotoxic activity upon tumor cell challenges against MIAPaCa-2 cells, while maintaining a more favorable phenotype and reduced secretion of proinflammatory cytokines (GM-CSF, TNFα, IFNγ, and IL-2). However, when 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. These findings suggest that reducing CAR density can improve baseline T-cell fitness but may impose a signaling ceiling that limits its performance in the demanding solid-tumor environment. This led us to consider combining our low-density CAR expression with an immunomodulatory strategy.

We selected IL-18 because it can promote T-cell activation and IFNγ production, while also activating NK cells and supporting macrophage polarization toward an M1 phenotype. However, constitutive IL-18 secretion could worsen systemic inflammatory toxicities. To control its expression, we used the doxycycline-inducible LOP system, which enables pro-IL-18 expression and IL-18 secretion following doxycycline induction and T-cell activation. AWCAR and LOPIL18 were introduced sequentially to generate AW-LOP18 CAR-T cells. Doxycycline-induced IL-18 enhanced cytotoxicity in both plated and spheroid assays against $HER2^{++}$ MIAPaCa-2 cells. In NSG mice bearing subcutaneous PLC tumors, induction of LOPIL18 improved tumor control, particularly in female mice. We also investigated IL-18 effects on immunosuppressive cells with cytotoxic assays with AWHER2CAR-T cells against spheroids containing PBMC-derived M2 macrophages and PDAC-on-chip models, and also against an Ewing sarcoma model with ARI CAR-T cells. Although LOP18 increased the lytic capacity of both CAR-T cell types, the effect appeared more consistent with direct enhancement of CAR-T activity than with macrophage-mediated polarization. Adding M2 macrophages did not clearly increase tumor lysis of LOP18, and no distinct macrophage polarization was observed. Further work should characterize the amount and timing of IL-18 secretion when combined with lower CAR activation and determine whether macrophage changes arise from IL-18 or from features of the models themselves.

Overall, the combined strategy appears promising, but requires longer-term efficacy assays and safety studies. A more highly secreted IL-18 variant could also be investigated.

DEVELOPMENT OF NANOTRUCK19-22-IL18I: A NEXT-GENERATION CAR-T CELL COMBINING SDAB-BASED DUAL CD19/CD22 TARGETING AND INDUCIBLE IL-18 DELIVERY TO OVERCOME ANTIGEN ESCAPE AND FUNCTIONAL EXHAUSTION. RD24/0014/0005; RD24/0014/0010.

María Tristán-Manzano[1,2,3,*], Juan R. Rodriguez-Madoz[4,8,9,*], Patricia Lara-Vera[5], Iván Gonzalez-Ruiz[5], Pedro Justicia-Lirio[4], Saray Rodriguez-Diaz[4], Rebeca Martinez-Turrillas[4], Susana Inoges[6,8,9], Ascension López-Diaz de Cerio[6,8,9], Antonio Pineda-Lucena[7,8], Felipe Prosper[6,8,9,*], Francisco Martin[1,2,3,*].

[1]GENYO, Centre for Genomics and Oncological Research, Pfizer, University of Granada, Andalusian Regional Government, PTS Granada, Granada, Spain.

[2]Instituto de Investigación Biosanitaria ibs. GRANADA, Granada, Spain.

[3]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada. Granada, Spain.

[4]Hemato-Oncology Program, Cima Universidad de Navarra. IdiSNA. Pamplona, Spain.

[5]LentiStem Biotech, PTS Granada, Granada, Spain

[6]Hematology and Cell Therapy Department, Clinica Universidad de Navarra (CUN). IdiSNA. Pamplona, Spain.

[7]Therapeutic Innovation Program, Cima Universidad de Navarra. Pamplona, Spain.

[8]Cancer Center Clinica Universidad de Navarra (CCUN), Pamplona, Spain.

[9]Centro de Investigacion Biomedica en Red de Cancer (CIBERONC), Madrid, Spain.

Background: Within the CAR-T Andalucía Public Procurement of Innovation initiative, the UTE GRANA CAR-T consortium (LentiStem Biotech, CIMA Universidad de Navarra, and Clínica Universidad de Navarra), in collaboration with Granada University and ibs.GRANADA, is developing next-generation CAR-T therapies to overcome key limitations of current commercial products, including relapse, limited persistence, and treatment-related toxicities. Although CD19-directed CAR-T therapies have transformed the treatment of B-cell malignancies, relapse remains a major challenge due to antigen escape and insufficient CAR-T persistence. Dual CD19/CD22 targeting may address these limitations, but increasingly complex CAR architectures can compromise T-cell fitness. In parallel, strategies based on constitutive cytokine secretion may enhance CAR-T activity but raise concerns regarding long-term toxicity and uncontrolled immune activation. Here, we combined an optimized bispecific CD19/CD22 CAR based on single-domain antibodies (sdAbs) with ALHAMBRA, a novel antigen-inducible gene-expression platform designed to restrict IL-18 production to sites of tumour engagement, generating NanoTRUCK19-22-IL18i cells with tightly regulated cytokine secretion and minimal background activity.

Methods: Four bispecific CD19/CD22 CARs were generated by combining previously validated single-domain antibodies (sdAbs) in tandem configurations differing in antigen orientation and linker composition. Second-generation 4-1BB CARs were expressed in primary human T cells and characterized for expansion, transduction efficiency, differentiation, activation status, exhaustion, and tonic signalling. Antigen-specific cytotoxicity, cytokine secretion, and resistance to antigen escape were evaluated using single-antigen and antigen-knockout leukaemia models, while antitumor efficacy was assessed in xenograft models of systemic B-cell leukaemia. The lead dual-targeting CAR was subsequently combined with ALHAMBRA, an antigen-inducible gene-expression platform designed to restrict cytokine production to sites of CAR engagement. Different IL-18 formats (pro-IL18 and optimized secreted IL-18) were cloned into ALHAMBRA lentiviral vectors and evaluated for inducibility by mRNA, reporter expression, and mature IL-18 secretion using Lumit-18 technology. IL-18 bioactivity was assessed on T cells and M2 macrophages, and NanoTRUCK19-22-IL18i functionality was determined through serial tumour rechallenge assays measuring CAR-T expansion, persistence, and cytotoxic activity.

Results: All four bispecific CARs showed efficient manufacturing, preserved early-memory phenotypes, and displayed minimal tonic signalling or premature exhaustion. Dual-targeting CAR-T cells retained independent recognition of CD19 and CD22, effectively eliminating single-antigen $CD19^{KO}$ or $CD22^{KO}$ leukemia cells demonstrating resistance to single-antigen escape, superior

cytokine production, tumour control, and survival compared with state-of-the-art monospecific CAR-T cells. In heterogeneous leukaemia models, ~30% of treated animals remained tumour-free at day 50. Following selection of the lead construct, NanoTRUCK19-22-IL18i cells were generated using the ALHAMBRA inducible platform. While inducible pro-IL18 showed limited bioactive cytokine release, an optimized secreted IL-18 format enabled tightly regulated antigen-dependent secretion (600-1000 pg/mL) with minimal background activity. Secreted IL-18 was biologically active, promoting T-cell responsiveness and repolarizing M2-like macrophages toward a pro-inflammatory phenotype. Importantly, NanoTRUCK19-22-sIL18i cells demonstrated enhanced CAR-T expansion, persistence, and antitumor activity after multiple tumours rechallenges, including against CD19KO and CD22KO leukaemia cells associated with a higher presence of CAR-T cells. *In vivo* evaluation is currently ongoing.

Conclusions: These results demonstrate the feasibility of combining sdAbs-based dual-antigen targeting and inducible cytokine delivery within a single CAR-T-cell product to address major unmet needs in the field. The NanoTRUCK19-22-sIL18i platform showed improved resistance to antigen escape, enhanced functional persistence, and controlled release of bioactive IL-18 upon tumour recognition, supporting the development of safer and more durable CAR-T therapies. Collectively, these advances represent a significant milestone within the CAR-T Andalucía Public Procurement of Innovation program toward the generation of next-generation CAR-T products with increased clinical impact and broader patient accessibility.

SMART ANTI-EPCAM TRUCKS WITH ANTIGEN-DENSITY-DEPENDENT CYTOTOXICITY AND TUMOR-RESTRICTED IL-18 EXPRESSION. RD24/0014/0005, RD24/0014/0010.

J Ruiz de Francisco[1,2], L Gomez-Baez[1,2], A Hinckley Boned[1,5], C Barbero-Jiménez[3], A Linde-Rodriguez[4], A Matilla[1,5,6], P Justicia-Lirio[7], A Tagliaferro[1], V Gálvez- Pérez, JA Garcia-Salcedo[4], M Tristán-Manzano[1,5*] and F Martín-Molina[1,5,6*]

[1]Gene and cell therapy group, Genyo, Granada, Spain.

[2]Lentistem Biotech, Granada, Spain

[3]Hemato-Oncology Program, CIMA Universidad de Navarra, Navarra, Spain.

[3]Program of Immunology and Immunotherapy, CIMA Universidad de Navarra, Pamplona, Spain.

[4]Biology of non-canonical DNA Structures Group, Genyo, Granada, Spain

[5]Instituto Biosanitario de Granada (ibs.GRANADA), University of Granada, Granada, Spain.

[6]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain

[7]Program of Immunology and Immunotherapy, CIMA Universidad de Navarra, Pamplona, Spain.

Despite the remarkable clinical success of CAR-T cell therapies in hematological malignancies, their efficacy in solid tumors remains limited. Major challenges include the scarcity of truly tumor-specific antigens and the presence of an immunosuppressive tumor microenvironment (TME), which restricts CAR-T cell persistence and functionality. To overcome these barriers, fourth-generation CAR-T strategies capable of locally delivering immunomodulatory payloads have emerged as promising alternatives. In particular, IL18-secreting CAR-T cells have shown significant potential to remodel the TME and enhance antitumor immunity. However, uncontrolled cytokine release remains a major safety concern, highlighting the need for tightly regulated expression systems that minimize basal leakage while maintaining robust inducible production.

EpCAM is an attractive target for epithelial tumors. EpCAM is consistently and strongly overexpressed in a wide range of epithelial-derived solid tumors, while its expression in healthy tissues is limited and largely restricted to basolateral membranes. This expression pattern provides a therapeutic window that could be better exploited using highly specific CAR designs. Single-domain antibody (VHH)-based CAR-T cell therapy offers several advantages over conventional ScFv-based constructs, 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. These VHHs exhibited a broad range of affinities, spanning from high-affinity binders to lower-affinity variants. This diversity in binding strength and epitope recognition provides valuable flexibility for tuning CAR sensitivity and activation thresholds. Each VHH was incorporated into a second-generation CAR construct. Functional assessment using a Jurkat dual-reporter system demonstrated specific NFAT and NFκB activation upon antigen recognition with minimal basal activation.

All four VHH-derived CARs displayed lower tonic signaling compared with conventional ScFv based CAR architectures while maintaining or improving antigen-dependent activation. All VHH-CAR-T variants efficiently lysed $EpCAM^+$ target cells but their cytotoxic activity varied with antigen density. While some constructs retained potent killing capacity independently of EpCAM levels, others preferentially targeted EpCAM-high tumor cells. These differences likely reflect the distinct affinity and epitope-binding properties, providing a therapeutically relevant means to discriminate tumor cells overexpressing EpCAM from healthy tissues with basal antigen expression.

To further enhance therapeutic functionality while improving safety, we engineered an inducible IL18 expression platform based on an optimized activa-

tion-dependent lentiviral vector system (ALHAMBRA) developed by the group. Compared with current state-of-the-art inducible designs, this novel platform demonstrated improved activation-dependent IL18 expression together with markedly reduced basal leakage, enabling tighter control of cytokine release. In addition, we found that the controlled expression of IL-18 in EpCAM CAR-T+ (ALH-TRUCKs) enhanced the proliferation and were able to polarize M2 macrophages to M1 in comparison with CAR-T cells.

In summary, we developed and characterized four EpCAM-targeting VHH-based CAR-T constructs exhibiting antigen-concentration-dependent cytotoxicity and favorable signaling profiles. In parallel, we implemented a next-generation IL18 expression system that improves inducibility while minimizing cytokine leakage. Together, this work presents a smart anti-EpCAM TRUCK configuration capable of discriminating EpCAM-high tumor cells from EpCAM-low healthy tissues, while ensuring IL-18 expression occurs exclusively in the presence of tumor antigen.

HETEROGENEIDAD ANATÓMICA DE LA INFILTRACIÓN LEUCÉMICA DURANTE LA ENFERMEDAD RESIDUAL Y LA RECAÍDA DE LA LLA-B: ESTUDIO PILOTO MEDIANTE CITOMETRÍA DE FLUJO Y MICROSCOPÍA TRIDIMENSIONAL. RD24/0014/0008, RD24/0014/0012, RD24CIII/0002/0002.

[1]Sara González-Soto;[2]Sergio Andrés Pineda Castillo;[3,4]Laura Hidalgo;[1]Alberto Varas;[3]Javier García-Castro;[1]Lidia M Fernández de Sevilla;[2]Amaia Cipitria,[1,5]Ángeles Vicente.

[1]Departamento de Biología Celular e Histología, Universidad Complutense de Madrid-IIS 12 de Octubre

[2]Instituto de Investigación Sanitaria Biogipuzkoa

[3]Unidad de Biotecnología Celular, Instituto de Salud Carlos III, Madrid

[4]Unidad de Innovación Biomédica, CIEMAT/ Unidad de Terapias Avanzadas IIS-FJD.

[5]Instituto de Investigación 12 de Octubre.

La leucemia linfoblástica aguda de precursores B (LLA-B) coloniza diferentes tejidos, donde las interacciones con el microambiente pueden favorecer la persistencia leucémica y la resistencia al tratamiento. Nuestro grupo ha demostrado previamente que los agregados leucémicos leptomeníngeos favorecen la expansión de los blastos y su supervivencia frente al metotrexato (MTX). En este estudio piloto investigamos la heterogeneidad anatómica de la infiltración leucémica en la médula ósea femoral y craneal durante la progresión, la enfermedad residual y la recaída.

Se emplearon modelos xenogénicos de LLA-B establecidos mediante la inoculación intravenosa de células humanas NALM6 o RS4;11 en ratones inmunodeficientes NSG. Se estudiaron animales con enfermedad avanzada no tratada, enfermedad residual tras la administración de metotrexato (MTX) a dosis subóptimas o intensivas, y recaída tras la suspensión del tratamiento. El MTX se administró dos veces por semana desde el día 7 posterior a la inoculación leucémica. La carga tumoral se cuantificó mediante citometría de flujo en médula ósea femoral y craneal, mientras que su distribución anatómica se analizó mediante microscopía de fluorescencia tridimensional Light Sheet en fémures y cráneos completos de animales portadores de NALM6-GFP, conservando en estos últimos la duramadre. Los fémures y cráneos fueron sometidos a aclarado óptico usando etil cinamato como agente clarificante.

La citometría reveló diferencias en la persistencia leucémica entre ambos compartimentos medulares tras el tratamiento. Durante la recaída, la médula craneal presentó una elevada carga leucémica, mientras que la infiltración femoral permanecía relativamente reducida. La microscopía tridimensional mostró una distribución anatómica heterogénea. En el fémur, la enfermedad avanzada se acompañó de una intensa alteración de la cavidad diafisaria, mientras que en la enfermedad residual y, especialmente, la recaída, los focos fluorescentes parecieron concentrarse en las regiones epifisaria y metafisaria distales, asociadas al hueso trabecular. En el cráneo, los agregados mostraron una marcada asociación espacial con las líneas de unión ósea desde fases tempranas. Tras el MTX persistieron focos restringidos, incluidos algunos próximos al territorio pontocerebeloso, mientras que durante la recaída destacaron acumulaciones en los huesos que recubren los bulbos olfatorios, con escasa señal en la bóveda craneal central. La conservación de la duramadre impide atribuir todos los focos exclusivamente al compartimento medular.

En conjunto, este estudio piloto pone de manifiesto una heterogeneidad anatómica de la infiltración leucémica y de su persistencia tras el tratamiento con MTX, tanto entre diferentes compartimentos óseos como dentro de un mismo hueso. La distribución de los focos en regiones epifisarias y metafisarias del

fémur y su asociación con líneas de unión ósea y territorios anatómicos específicos del cráneo sugieren que determinados microambientes podrían contribuir a la localización y persistencia de los blastos. Estas observaciones, junto con nuestros resultados previos sobre la expansión y quimiorresistencia de los agregados leptomeníngeos, justifican investigar la posible participación de los microambientes óseos y de la interfase craneomeníngea en la enfermedad residual y la recaída de la LLA-B.

IDENTIFICACIÓN DE NEOANTÍGENOS CANDIDATOS EN CÁNCER PEDIÁTRICO COMO NUEVAS DIANAS PARA LA INMUNOTERAPIA BASADA EN LINFOCITOS T. RD24/0014/0001, RD24/0014/0008.

Gonzalo García-Aguilera, G Soria-Alcaide, Marta Portasany-Rodríguez, Adriana Ortega, Luis Blasco, Isabel Colmenero, Alba Rubio, Rafael Moreno Olié, Ramón Alemany, Marcel Costa-García, Jorge García-Martínez, Manuel Ramírez-Orellana, África González Murillo.

CENTROS PARTICIPANTES: FIB Hospital Infantil del Niño Jesús, Institut Català d'Oncología en Institut Català d'Oncologia (ICO)/ IDIBELL.

Los neoantígenos son antígenos derivados de alteraciones genéticas somáticas que pueden generar epítopos reconocidos por linfocitos T específicos del tumor. Su identificación y validación funcional pueden contribuir al descubrimiento de linfocitos infiltrantes de tumor (TILs) reactivos frente a células tumorales y al desarrollo de nuevas estrategias de inmunoterapia celular. Esta aproximación resulta especialmente relevante en los tumores sólidos pediátricos, clásicamente considerados fríos, que suelen presentar una baja carga mutacional tumoral (tumor mutational burden, TMB) y, en consecuencia, un repertorio limitado de neoantígenos candidatos.

Por tanto, propusimos un objetivo que era identificar neoantígenos en tumores sólidos pediátricos mediante análisis bioinformáticos integrados de datos genómicos y transcriptómicos, como primer paso para la identificación de nuevas dianas inmunoterapéuticas y el estudio de TILs reactivos frente al tumor.

Para ello se recogieron muestras de tumores sólidos resecados y muestras pareadas de células mononucleares de sangre periférica (PBMCs), obtenidas el día de la cirugía y utilizadas como control de línea germinal, de pacientes intervenidos en el Hospital Infantil Universitario Niño Jesús, previa firma del consentimiento informado. Las muestras se analizaron mediante secuenciación del exoma completo (WES) y secuenciación del transcriptoma (RNA-seq). En colaboración con el grupo RG13, se llevó a cabo la predicción de neoantígenos mediante el pipeline nextNEOpi (v1.5), ejecutado en el entorno Nextflow (v22.10.8). Esta herramienta integra datos de WES tumoral y de línea germinal con datos de RNA-seq del tejido tumoral para predecir neoepítopos potencialmente presentables por moléculas HLA de clase I y II. El pipeline contempla neoantígenos derivados de variantes de nucleótido único (SNV), inserciones y deleciones (indels), así como fusiones génicas.

Se analizaron 11 muestras tumorales pediátricas: un tumor testicular en recaída, dos rabdomiosarcomas, seis neuroblastomas, un sarcoma renal y un tumor de Wilms. La predicción bioinformática no identificó neoantígenos candidatos en algunas muestras. En aquellas con predicción positiva, el número de neoepítopos identificados fue reducido, generalmente inferior a siete por tumor. Estos resultados preliminares ponen de manifiesto las limitaciones de la identificación bioinformática de neoantígenos en determinados tumores pediátricos y plantean la necesidad de explorar estrategias complementarias para mejorar su detección.

Este trabajo constituye un primer paso hacia el establecimiento de un flujo de trabajo para identificar neoantígenos candidatos en tumores sólidos pediátricos, una estrategia especialmente importante en tumores con una tasa mutacional baja, como los tumores pediátricos. La optimización de los métodos de predicción y la posterior validación experimental permitirán evaluar su relevancia inmunológica y explorar la identificación de TILs reactivos frente al tumor, con el objetivo último de desarrollar nuevas inmunoterapias celulares para pacientes pediátricos con tumores sólidos en recaída o refractarios.

DESARROLLO DE UN MODELO PREDICTIVO DE RESPUESTA A CAR-T MEDIANTE LA INTEGRACIÓN DE BIOMARCADORES INMUNOINFLAMATORIOS PREINFUSIÓN. RD24/0014/0034, RD24/0014/0008.

[1]Sandra Muntión,**[2]**Rosa Sacedón;**[1]**Carmen Santos,**[1]**Almudena Navarro-Bailón,**[1]**Lucia López-Corral,**[2]**Ángeles Vicente;**[1]**Fermín Sánchez-Guijo.

[1]Departamento de Medicina de la Universidad de Salamanca y Área de Terapia Celular y Departamento de Hematología del IBSAL-Hospital Universitario de Salamanca, Salamanca, España.

[2]Departamento de Biología Celular e Histología, Facultad de Medicina, Universidad Complutense de Madrid-IIS 12 de Octubre.

Introducción y objetivos. La eficacia de las células CAR-T y el desarrollo de CRS e ICANS presentan una importante heterogeneidad. Investigamos si el estado inmunoinflamatorio previo a la infusión se relaciona con la respuesta clínica y la toxicidad, y su posible utilidad predictiva.

Métodos. Estudio longitudinal exploratorio de 21 pacientes con linfoma no Hodgkin tratados con axi-cel (n=14) o tisa-cel (n=7). Se analizaron 20 citocinas plasmáticas solubles en D0, D+7 y D+28, proteína C reactiva (PCR), recuentos absolutos de neutrófilos (RAN) en D−1 y expansión circulante de CAR-T. Se evaluaron respuesta completa (RC) a D+30 y seis meses, desarrollo de CRS y/o ICANS. Las combinaciones de predictores candidatos se evaluaron mediante regresión logística regularizada y validación cruzada *leave-one-out* (LOOCV).

Resultados. Once pacientes alcanzaron RC en D+30 y diez presentaban RC a seis meses. La progresión a D+30 se asoció con mayores concentraciones basales de IL-8 (p=0,045), IL-18 (p=0,012) y PCR (p=0,032), y menores RAN en D−1 (p=0,007). La IL-2 basal mostró una tendencia a valores superiores en los respondedores a D+30 (p=0,064), alcanzando significación estadística al comparar pacientes con y sin RC a seis meses (p=0,010). Estas observaciones orientaron la evaluación exploratoria comparativa del valor predictivo de RC a D+30 y seis meses de modelos que combinaron IL-8, IL-18, IL-2, RAN y/o PCR. El modelo simplificado PCR/IL-2/RAN alcanzó AUC LOOCV de 0,900 a D+30 y 0,855 a seis meses. La eliminación de IL-2 redujo las AUC a 0,800 y 0,673, respectivamente, sugiriendo una contribución predictiva complementaria de esta citocina. A seis meses, el modelo IL-18/PCR/IL-2/RAN alcanzó una AUC de 0,882, la mayor de las combinaciones evaluadas para ese momento. Se identificaron además dos pacientes con perfiles basales discordantes respecto a la evolución clínica: uno con un fenotipo inflamatorio excepcionalmente elevado que alcanzó RC y otro con un perfil de citocinas marcadamente bajo que presentó progresión. Por otra parte, 17 pacientes desarrollaron CRS, asociado a mayores concentraciones basales de CCL2 e IL-7. Entre los pacientes CRS+, quienes progresaron mostraron mayores niveles basales de IL-8, IL-18, IL-10 y CXCL10 que quienes respondieron. Cinco de estos pacientes desarrollaron ICANS. La evolución entre D7 y D28 de los niveles de FasL difirió entre los pacientes CRS+ que desarrollaban neurotoxicidad frente a los que no (p=0,0061). Los pacientes con RC a seis meses presentaron mayores recuentos circulantes de CAR-T en D+7 (p=0,0128), asociación no observada con el máximo recuento registrado.

Conclusiones. Los resultados sugieren que el estado inmunoinflamatorio previo a la infusión podría condicionar la eficacia antitumoral incluso cuando la activación de las CAR-T desencadena CRS. La contribución predictiva complementaria de los marcadores del estado inflamatorio basal, IL-2 y los recuentos de neutrófilos sugiere que diferentes componentes del contexto inmunológico del paciente podrían influir en la eficacia terapéutica de las CAR-T

GENERACIÓN DE LINFOCITOS T ESPECÍFICOS DE ADENOVIRUS (VST) DE DONANTE ALTERNATIVO, GUIADA POR PREDICCIÓN *IN SILICO* DE UNIÓN A HLA PARA EL TRATAMIENTO DE ADENOVIRIASIS REFRACTARIA TRAS TRASPLANTE HAPLOIDÉNTICO DE PROGENITORES HEMATOPOYÉTICOS EN PACIENTE PEDIÁTRICA. RD24/0014/0008, RD24/0014/0009.

Aguilar-Gallardo C[1], Gonzálεz-Vicent M[2], Gómεz-Seguí I[1], Bonora-Centelles A[1], Cruz Lópεz I[1], Megías-Vericat JE[1], Verdejo de Antonio J[2], Molina B[2], Vinagre S[2], Alarcón Miguεz A[3], Planelles Silvestre MD[4], Guerrero Lópεz RM[4], Ramírεz Orellana M[3], Poveda Andrés JL[1]

[1]Unidad de Terapias Avanzadas (UTA), Hospital Universitari i Politècnic La Fe / Instituto de Investigación Sanitaria La Fe, Valencia.

[2]Unidad de Trasplante Hematopoyético, Hospital Infantil Universitario Niño Jesús, Madrid.

[3]Unidad de Fabricación de Medicamentos de Terapia Avanzada de la Fundación para la Investigación Biomédica del Hospital Infantil Universitario Niño Jesús, Madrid

[4]Centro de Transfusiones de la Comunidad Valenciana.

Introducción

Las infecciones por adenovirus (AdV) constituyen una complicación grave tras el trasplante haploidéntico de progenitores hematopoyéticos (TPH), en el que la reconstitución inmune retardada favorece viremias refractarias al tratamiento antiviral. La transferencia adoptiva de linfocitos T específicos de virus (VST) es una estrategia disponible en algunos centros, pero su generación requiere que el donante presente una restricción HLA compartida con el receptor para asegurar la eficacia de la terapia.

Caso clínico:

Paciente pediátrica de 3 años con leucemia mieloblástica aguda en primera remisión completa sometida a TPH haploidéntico de sangre periférica con depleción TCRαβ/CD19 (donante: la madre) el 23/12/2025, que desarrolló una adenoviremia activa refractaria. Las infusiones previas de linfocitos de memoria (CD45RA+ depletados) del mismo donante habían resultado ineficaces para controlar la adenoviriasis. El tipaje HLA de alta resolución de la receptora incluía alelos infrarrepresentados en población caucásica y no recogidos en la cobertura declarada de los pools de péptidos comerciales. El producto VST-AdV-específico no estaba disponible para su producción en el centro tratante de la paciente.

Metodología:

Análisis *in silico,* selección de donante y producción

La UTA recibió la solicitud de producción de la terapia por parte del centro tratante de la paciente, iniciándose los trámites y alcanzándose los acuerdos necesarios entre ambas instituciones. Ante la ausencia de cobertura HLA validada para los alelos de la receptora en los pools comerciales de epítopos definidos, se realizó un análisis predictivo *in silico* con herramientas públicas como estrategia para superar esa limitación. Se predijo la unión péptido-HLA de la secuencia completa del hexón de AdV5 (UniProt P04133, la misma que cubre el pool AdV5 Hexon según su ficha técnica) a los alelos HLA de la receptora, de clase I (NetMHCpan-4.1) y de clase II (NetMHCIIpan-4.3). El análisis identificó regiones de unión fuerte para alelos de clase I, con C*07:02 como el de mayor número (26 regiones únicas) y A*31:01 como alelo adicional de interés, y definió el criterio de emparejamiento donante-receptora: A*31:01 + C*07:02. Se seleccionó una donante portadora de ambos alelos (clase I) con memoria anti-AdV demostrable (serología IgG positiva y frecuencia neta de linfocitos T CD3+IFN-γ+ tras estímulo peptídico de 0,021%, superior al umbral de ≥0,01%). Se adoptó un protocolo de estimulación combinada con los pools PepTivator AdV Select y AdV5 Hexon, este último constituido por péptidos solapantes que cubren la secuencia completa del hexón, y el producto se generó mediante

enriquecimiento de células secretoras de IFN-γ con el CliniMACS Cytokine Capture System, bajo condiciones GMP. El producto final tenía un volumen de 5,66 mL y un total de 5,9 × 10^5 células $CD3^+IFN\text{-}\gamma^+$. El volumen de infusión se ajustó a 2,65 mL en función de la composición de linfocitos T residuales $CD3^+IFN\text{-}\gamma^-$, para mantener esta población por debajo de 10^4 células/kg, lo que correspondió a una dosis de 24.057 células $CD3^+IFN\text{-}\gamma^+$/kg. El producto presentó una viabilidad del 74%, una pureza $CD3^+IFN\text{-}\gamma^+$ del 70,7% y una composición de $CD4^+$ del 58,3% y $CD8^+$ del 28,7%, y el volumen infundido cumplió todos los criterios de liberación establecidos.

Resultados clínicos:

La paciente desarrolló adenoviremia a los 4 meses del TPH, refractaria a cidofovir semanal e inmunoglobulinas sustitutivas, con una carga viral máxima de 3×10^6 copias/mL y enfermedad diseminada con afectación respiratoria. Las infusiones quincenales de linfocitos de memoria (hasta 5×10^6/kg) no fueron eficaces, por lo que se solicitó a La Fe VST de donante alternativo en uso compasivo. A la semana de la infusión la carga viral descendió por debajo de 1.000 copias/mL, con negativización al mes. No presentó efectos secundarios de la infusión. En la actualidad la paciente se encuentra en remisión completa de la leucemia, con quimerismo completo del donante y sin reactivación por adenovirus.

Conclusiones

Este caso muestra la viabilidad de generar VST anti-AdV en receptores con perfiles HLA infrecuentes mediante una estrategia individualizada de selección de donante guiada por predicción *in silico* y el empleo de pools de péptidos solapantes. La administración del producto se asoció a una respuesta clínica favorable. Asimismo, este caso representa un ejemplo de colaboración e intercambio de medicamentos de terapia avanzada de producción académica entre dos centros del Sistema Nacional de Salud, con relevancia para el modelo de fabricación descentralizada y colaborativa de ATMP en el marco de $TERAV^+$.

ALLONANOCART33: NOVEL TRANSPOSON-SDAB-BASED ALLOGENEIC CD33-TARGETED CAR-T CELLS FOR PATIENTS WITH R/R AML OR MDS. RD24/0014/0003, RD24/0014/0010, RD24/0014/0034, RD24/0014/0040.

A. Alfonso-Pierola[1], S. Inoges[1], A. Lopez-Diaz de Cerio[1], P. Jauregui[1], S. Rodriguez-Diaz[2], R. Martinez-Turrillas[2], F. Bernasconi-Bisio[3], V. Ibarra[3], E. Molina[3], C. Ceballos[4], S. Villar[1], MC. Viguria[4], M. Redondo[4], M. Blaquer[5], J. Martinez-Lopez[6], D. Valcarcel[7], M. Guerreiro[8], P. Montesinos[8], F. Sanchez-Guijo[9], A. Pineda-Lucena[3,10], JR. Rodriguez-Madoz[2,10,11], F. Prosper[1,2,10,11]

[1]Hematology and Cell Therapy Department. Clinica Universidad de Navarra (CUN). IdiSNA.

[2]Hemato-Oncology Program. Cima Universidad de Navarra. IdiSNA.

[3]Therapeutic Innovation Program, Cima Universidad de Navarra.

[4]Hematology Department. Hospital Universitario de Navarra. IdiSNA.

[5]Department of Hematology, Virgen de la Arrixaca University Hospital. IMIB.

[6]Department of Hematology, Hospital Universitario 12 de Octubre, Universidad Complutense, Centro Nacional de Investigaciones Oncológicas

[7]Vall Hebron Institute of Oncology (VHIO), Hematology Department, Vall d'Hebron University Hospital

[8]Hematology Department, Hospital Universitari i Politècnic La Fe

[9]Hematology Department, IBSAL-University Hospital of Salamanca. University of Salamanca.

[10]Cancer Center Clinica Universidad de Navarra (CCUN).

[11]Centro de Investigacion Biomedica en Red de Cancer (CIBERONC).

Patients with relapsed/refractory (R/R) acute myeloid leukemia (AML) or high-risk myelodysplastic syndromes (MDS) face a dismal prognosis, with median overall survival below 8 months and limited therapeutic options. Although chimeric antigen receptor (CAR)-T cell therapies have revolutionized the treatment of several hematological malignancies, their application to AML and MDS remains challenging. Major obstacles include the lack of tumor-specific antigens, impaired autologous CAR-T cell manufacturing due to patients' clinical condition and previous treatments, and rapid disease progression that may preclude timely treatment. Furthermore, conventional viral vector-based genetic engineering presents additional limitations, including potential insertional genotoxicity, risks of malignant transformation, and high manufacturing costs.

The alloNanoCART33 project aims to overcome these limitations by developing an innovative, virus-free, allogeneic CAR-T cell therapy targeting CD33 through the integration of four complementary technological strategies:

(i) single-domain antibody (sdAb)-based CAR constructs to enhance antitumor efficacy (ii) incorporation of an inducible safety switch to enable controlled CAR-T cell elimination (iii) CRISPR/Cas9-mediated gene editing to generate allogeneic CAR-T cells (iv) non-viral genetic engineering using the *Sleeping Beauty* transposon system to facilitate stable CAR integration while reducing manufacturing complexity and costs.

We successfully developed and comprehensively characterized CD33-targeting sdAb-based CAR-T cells that demonstrated enhanced cytotoxicity against primary AML cells and superior *in vivo* antitumor efficacy compared with conventional scFv-based CAR-T cells. Incorporation of a truncated epidermal growth factor receptor (EGFRt) into the CAR construct enabled efficient *in vivo* elimination of CAR-T cells following cetuximab administration, providing a functional safety mechanism. Importantly, the combination of CRISPR/Cas9-mediated gene editing with *Sleeping Beauty* transposon-mediated CAR integration enabled the generation of functional HLA class I-knockout/TCR-knockout (HLA-I^{KO}/TCR^{KO}) allogeneic CAR-T cells without compromising their antitumor activity. Comprehensive preclinical safety assessments, including genotoxicity and integration-site analyses, revealed no detectable adverse effects associated with genetic modification, evidence of genotoxicity, abnormal clonal expansion, or preferential enrichment of specific cellular clones or transposon integration sites over time. These findings support the favorable preclinical safety profile and genomic stability of this combined non-viral genetic engineering strategy.

Collectively, these results establish the preclinical proof of concept for a virus-free, sdAb-based allogeneic CAR-T cell therapy targeting CD33, supporting its clinical translation as a potentially safer, cost-effective, off-the-shelf treat-

ment for patients with R/R AML or high-risk MDS. Importantly, the alloNano-CART33 phase I/II clinical trial has been recently funded through the Independent Clinical Trials Programme of the Instituto de Salud Carlos III (ISCIII, Spain). The study will primarily evaluate safety and tolerability, with preliminary antitumor efficacy as a secondary endpoint, and will include a phase I dose-escalation stage following a conventional 3+3 design, followed by a phase II expansion cohort comprising 20 additional patients.

FRAMEWORK-REGION CHARGE OPTIMIZATION ENHANCES THE ANTI-TUMOR POTENCY OF CAR-T CELLS ACROSS MULTIPLE SOLID TUMORS BY CALIBRATING TONIC SIGNAL. RD24/0014/0005, RD24/0014/0010 AND RD24/0014/0041.

Pedro Justicia-Lirio[1,2], Álvaro Gómez-Morón[3], Beatriz Perucha[1], Rodrigo Redondo-Frutos[2], Marta Gorraiz[1], Maialen Martinez-Tabar[1], Silve Vicent[4], Celia Martín-Otal[1], Carmen Barbero-Jimenez[2], Francisco Martin[5], Maria Blanco-Prieto[6], Hanna Algül[7], Anna Mondino[8], Antonio Pineda-Lucena[9], Noa Martin-Cofreces[3], Teresa Lozano[1], Juan J. Lasarte[1], Felipe Prosper[2,10] and Juan R. Rodriguez-Madoz[2]

[1]Immunology and Immunotherapy Program, Cima Universidad de Navarra. IdISNA. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[2]Hemato-Oncology Program, Cima Universidad de Navarra. IdISNA. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[3]Immunology Unit from Hospital Universitario de la Princesa, Universidad Autónoma de Madrid and Instituto de investigación Sanitaria La Princesa (IIS-IP), Madrid, Spain.

[4]Solid Tumors Program, Cima Universidad de Navarra. IdISNA. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[5]Department of Genomic Medicine, Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research (GENYO).

[6]Department of Pharmaceutical Sciences, School of Pharmacy and Nutrition, Universidad de Navarra, IdISNA. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[7]Comprehensive Cancer Center München, Institute for Tumor Metabolism, Klinikum rechts der Isar, Technical University of Munich, School of Medicine and Health, Munich, Bavaria, Germany.

[8]Lymphocyte activation Unit, Division of Immunology, Transplantation and Infectious Diseases. IRCCS San Raffaele Scientific Institute. Milan, Italy.

[9]Therapeutic Innovation Program, Cima Universidad de Navarra. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

[10]Hematology and Cell Therapy Department, Clinica Universidad de Navarra (CUN). IdiSNA. Cancer Center Clinica Universidad de Navarra (CCUN). Pamplona, Spain.

The therapeutic efficacy of chimeric antigen receptor (CAR)-T cells in solid tumors is frequently compromised by tonic signaling, an antigen-independent, constitutive activation that precipitates premature cellular exhaustion and profound metabolic failure. Although intracellular signaling can be modulated, the intrinsic structural drivers of tonicity within the single-chain variable fragment (scFv) remain poorly defined. In this study, we demonstrate that the spatial distribution of positive charge patches (PCPs) within the framework regions (FR) of the extra domain A (EDA)-targeting F8 scFv is a primary factor driving spontaneous CAR clustering and chronic T cell pseudo-activation. To overcome this, we implemented a rational biophysical engineering strategy focused on optimizing the electrostatic charge balance of the CAR construct. Through systematic neutralization of framework-region positive charge patches, we developed EDA^{mut}, an optimized variant engineered to decouple tonic signaling from antigen binding affinity. Comprehensive evaluation using RNAseq, metabolic profiling, and confocal microscopy demonstrated that this mitigation effectively prevents the metabolic hyper-activation and terminal differentiation programs that chronically afflict wild-type (WT) EDA CAR-T cells. Consequently, EDA^{mut} CAR-T cells maintained a quiescent, memory-like state enriched for stemness-associated transcripts and exhibited significantly reduced expression of inhibitory exhaustion markers prior to antigen encounter and after multiple tumor rechallenges. Furthermore, high-resolution spatial analysis revealed that EDA^{mut} successfully prevented antigen-independent CAR microclustering and basal CD3zeta pre-polarization. Upon antigen encounter, the variant facilitated the formation of a superior immunological synapse characterized by highly precise molecular reorganization, larger CD3zeta clusters, and directed polarization of the Golgi apparatus and microtubule-organizing center (MTOC). This structural stabilization translated into enhanced functional persistence and superior anti-tumor efficacy. EDA^{mut} CAR-T cells induced robust and sustained tumor regression in a human hepatoma xenograft model and significantly delayed tumor progression in an immunocompetent murine pancreatic ductal adenocarcinoma model. Regarding safety, high-dose infusions in healthy mice confirmed a robust *in vivo* safety profile, demonstrating the absence of systemic toxicity or on-target off-tumor reactivity. In conclusion, we showed that framework-region electrostatic charge balancing is a critical biophysical parameter for preventing CAR-T cell exhaustion, providing a universal blueprint for designing calibrated, persistent, and safe synthetic receptors against solid tumors.

IDENTIFICACIÓN DE NUEVAS DIANAS DE SUPERFICIE MEDIANTE PROTEÓMICA DE MEMBRANA Y DESARROLLO PRECLÍNICO DE UNA TERAPIA CART DIRIGIDA CONTRA LEUCEMIA LINFOBLÁSTICA AGUDA DE CÉLULAS T (T-ALL). RD24/0014/0015, RD24/0014/0024, RD24/0014/0042, RD24/0014/0023.

Sofía Blasco Sebastián[1,2], Iñaki Arretxe[1], Andrea Moreno[3], Eduardo Aranda[3], Carmen Mestre-Duran[4,5], Jordi Minguillón[4,6,7], Antonio Pérez-Martínez[4,5,7,8,9], Pablo Velasco[7,10,11,12], Rebeca Sanz[3,13], Carmen Oñate[7,14,15], Eva MªGálvez[7,15,16], Ariel Ramírez-Labrada[7,14,17,18], María Teresa Olave[7,17,18], Julián Pardo[2,7,14,15] & Diego Sánchez-Martínez[1,7,13,15].

[1]Inmunoterapias Celulares Avanzadas, Instituto de Investigación Sanitaria Aragón (IIS Aragón), Centro de Investigación Biomédica de Aragón (CIBA), Zaragoza, España

[2]Departamento de Microbiología, Pediatría, Radiología y Salud Pública, Universidad de Zaragoza, Zaragoza

[3]Cancer Heterogeneity and Immunomics (CHI), Instituto de Investigación Sanitaria Aragón (IISA)

[4]CIBERER-ISCIII, IdiPAZ-CNIO Translational Research Unit in Pediatric Hemato-Oncology, La Paz University Hospital Research Institute, Spanish National Cancer Center, Madrid, Spain

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

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

[7]Red RICORS de Terapias Avanzadas TERAV+, Carlos III Health Institute, Madrid, Spain.

[8]Pediatric Hemato-Oncology Department, La Paz University Hospital, Madrid, Spain

[9]Pediatric Department, Autonomous University of Madrid, Spain

[10]Pediatric Oncology and Hematology Department, Hospital Vall d'Hebrón, Barcelona, Spain.

[11]Departament de Pediatria, Obstetricia, Ginecologia, Medicina Preventiva i Salut Pública. Universitat Autonoma de Barcelona, Barcelona

[12]Childhood Cancer & Blood Disorders Research Group, Vall d'Hebron Research Institute (VHIR). Barcelona

[13]Aragon I+D Foundation for Research & Development (ARAID)

[14]Inmunoterapia, Inflamación, Infección y Cáncer (I3C), Instituto de Investigación Sanitaria Aragón (IIS Aragón), Centro de Investigación Biomédica de Aragón (CIBA), Zaragoza, España

[15]Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III (ISCIII), Zaragoza, Spain

[16]CSIC, Instituto de Carboquímica (ICB), Zaragoza, Spain

[17]Universidad de Zaragoza, Zaragoza

[18]Hematology Service, Lozano Blesa University Clinical Hospital, Zaragoza

La leucemia linfoblástica aguda de células T (T-ALL) es una enfermedad heterogénea en la que proliferan sin control células linfoides inmaduras de tipo T. En concreto, la T-ALL en recaída refractaria presenta un pronóstico desfavorable por la ausencia de terapias dirigidas eficaces y específicas, lo que hace necesario encontrar alternativas de tratamiento. La terapia con linfocitos T con receptor quimérico de antígeno (CAR-T) ha supuesto un gran avance en neoplasias hematológicas de estirpe B. Sin embargo, su traslación a células T supone un desafío por el efecto de toxicidad y fratricidio debido a la expresión compartida del antígeno diana en las células T-ALL y en las células CAR-T. Por ello, resulta prioritario descubrir antígenos tumorales específicos para el desarrollo de inmunoterapias de tumores malignos de células T que amplíen las opciones terapéuticas eficaces.

Con el objetivo de identificar nuevas dianas terapéuticas, se realizó un cribado de proteínas de superficie mediante proteómica de membrana, a partir del cual se seleccionaron tres candidatos antigénicos (A, B y C). Su perfil de seguridad *on-target/off-tumor* se evaluó mediante análisis bioinformático de datos de secuenciación de RNA de célula única (scRNA-seq) procedentes de tejidos sanos. Paralelamente, se caracterizó la expresión de los tres candidatos mediante citometría de flujo en muestras primarias de pacientes con LLA-T. El antígeno C mostró expresión en la mayoría de las muestras analizadas al diagnóstico, manteniéndose en las muestras de recaída disponibles. A partir de estos resultados, se generó un CAR-T de segunda generación dirigido frente a este antígeno (CART-C), cuya producción y viabilidad se caracterizaron. Su capacidad citotóxica se evaluó *in vitro* frente a las líneas celulares de LLA-T MOLT4, MOLT16, JURKAT, LOUCY y HPB-ALL, así como en muestras primarias de pacientes. El CAR-T-C mostró actividad antitumoral frente a las líneas celulares evaluadas, siendo totalmente específico, y fue capaz de eliminar células leucémicas procedentes de muestras primarias, acompañado de una elevada liberación de interferón gamma (IFN). Asimismo, su actividad citotóxica se confirmó *in vivo* en un modelo de xenoinjerto en ratones NSG con la línea celular MOLT4 en el que fue capaz de ralentizar el injerto leucémico.

En conjunto, estos resultados identifican el antígeno C como una potencial diana terapéutica para el desarrollo de una estrategia CAR-T frente a la LLA-T y respaldan la continuación de su evaluación preclínica. No obstante, es necesario profundizar en la caracterización de su expresión en tejidos sanos y en la evaluación del perfil de seguridad del CART-C con el objetivo de su traslación a la clínica.

TARGETING MUTANT CALRETICULIN WITH CAR-T CELLS: FROM PRECLINICAL PROOF OF CONCEPT TO FIRST-IN-HUMAN CLINICAL DEVELOPMENT. RD24/0014/0015.

Cecilia Pesini, Mario Gil-Bellido, Lorena S Millan, Carmen Oñate, Adanays Calvo-Pérez, Llipsy Santiago, Eldris Iglesias, Jorge Paúl Bernal, Miguel Araujo-Voces, Laura Paz Artigas, Laura García-Martínez, Francisco J Roig, Nieves Movilla Meno, José Manuel García-Aznar, Bárbara Menéndez-Jandula, María Teresa Olave, Gemma Azaceta Reinares, Marta Garrote, Alberto Álvarez-Larrán, Eva M Gálvez, Diego Sánchez Martínez, Maykel A Arias, Julián Pardo, Ariel Ramírez-Labrada.

Instituto de Investigación Sanitaria Aragón (IIS Aragón); Universidad de Zaragoza; Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); Hospital Clínic de Barcelona; Universidad de Barcelona; Universidad San Jorge; Instituto de Investigación en Ingeniería de Aragón (I3A); Hospital Universitario Miguel Servet; Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID); Hospital Clínico Universitario Lozano Blesa; Instituto de Carboquímica (ICB-CSIC); Biamics, Servicios de Bioinformática S. L.

Background: The adoptive transfer of T cells engineered to express chimeric antigen receptors (CAR-T) has shown high efficacy in treating various hematologic malignancies. However, many hematologic disorders, such as BCR:ABL1-negative myeloproliferative neoplasms (MPNs), lack effective treatment options. Some of these neoplasms are characterized by recurrent mutations in CALR, resulting in the expression of mutant calreticulin (mCALR), a neoantigen absent from healthy tissues. This makes mCALR a highly specific and attractive target for CAR-T cell therapy.

Methods: Five distinct CARs were designed based on monoclonal antibody sequences targeting mCALR. The most effective construct was selected through functional in vitro assays and subsequently evaluated against established cell lines, patient-derived malignant cells, and xenograft models. Antitumor efficacy, CAR-T cell persistence, tissue infiltration, and animal survival were assessed. Bulk and single-cell RNA sequencing were performed to identify potential resistance mechanisms, followed by functional validation and pharmacological rescue experiments.

Results: Among the five constructs, one demonstrated superior and selective cytotoxicity against mCALR-expressing cells, with no activity against mCALR-negative controls. These CAR-T cells efficiently eliminated patient-derived MPN cells and controlled disease progression in xenograft models, significantly prolonging animal survival. Therapeutic efficacy was associated with CAR-T cell persistence and infiltration into affected tissues. Transcriptomic analyses identified the upregulation of anti-apoptotic proteins as a potential resistance mechanism. Functional experiments demonstrated that elevated BCL-2 expression reduced CAR-T efficacy, which could be restored by combining CAR-T cells with venetoclax.

Clinical translation: Building on these preclinical findings, we are advancing the development of anti-mCALR CAR-T cells toward a first-in-human phase I clinical trial for patients with CALR-mutated MPNs. This translational effort includes the completion of preclinical safety studies and the preparation of the regulatory documentation required for clinical authorization. The proposed trial aims to establish the feasibility and safety of this novel therapeutic approach and provide the first clinical evidence supporting the selective targeting of a driver mutation-derived neoantigen in MPNs.

Conclusions: Our findings demonstrate, for the first time, the feasibility of selectively targeting mutant calreticulin using CAR-T cells, establishing a strong preclinical rationale for a new therapeutic strategy in MPNs. The identification of apoptosis-related resistance mechanisms further supports the development of rational combination approaches. Together, these results provide the foundation for the clinical translation of anti-mCALR CAR-T cell therapy.

IMPACT: AN INTEGRATED PLATFORM FOR SCALABLE AND PERSONALISED CAR-T CELL MANUFACTURING. RD24/0014/0016, RD24/0014/0001.

Josep M. Canals[1,2], Unai Perpiñá[1,2], Cristina Herranz[1,2], Felipe Chiappe[1,2], Irene Porcar[1,2], Maria Camanyes[1,2], Mª José Garcia-Escolano[1,2], Julio Delgado[2,3], Sergio Navarro[2,3,4], Hugo Calderón[2,4], Azucena González-Navarro[2,4]

[1]Laboratory of Stem Cells and Regenerative Medicine, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences; Institute of Neurosciences; and Creatio, Production and Validation Center of Advanced Therapies, University of Barcelona, Barcelona, Spain.

[2]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain.

[3]Hospital Clínic de Barcelona, Barcelona, Spain.

[3]Universitat de Barcelona, Barcelona, Spain.

[4]Department of Immunology, Hospital Clínic de Barcelona, Barcelona, Spain.

Chimeric antigen receptor (CAR)-T cell therapies have transformed the treatment of refractory haematological malignancies, yet their widespread implementation remains constrained by complex manufacturing processes, limited lentiviral vector availability, high costs and variability in patient-derived starting materials. IMPACT (Intelligent Manufacturing Platform for Advanced CAR Therapies) is a five-year Horizon Europe project bringing together 15 entities across nine countries to develop an integrated, scalable and digitally supported manufacturing platform. The project aims to improve manufacturing consistency, accelerate quality assessment and support patient-specific process optimisation.

Coordinated by the University of Barcelona (UB) through Creatio, its Centre for the Production and Validation of Advanced Therapies, IMPACT combines innovations in lentiviral vector production, purification and stabilization with nanomechanical quality control, organ-on-chip models and an artificial intelligence-driven digital twin. UB leads the scale-up of lentiviral vector production using closed fixed-bed bioreactors and serum-free culture conditions, targeting a tenfold increase in production capacity. UB will also integrate and assess novel purification strategies, benchmark emerging quality control technologies against established GMP methods, and manufacture GMP-grade vectors for final process validation. In parallel, UB will develop a blood–brain barrier-on-chip model to investigate mechanisms relevant to CAR-T-associated neurotoxicity, integrated with tumour and bone marrow models within a multiorgan platform.

IDIBAPS and Hospital Clínic de Barcelona (IDIBAPS-HCB) provide the translational link between manufacturing innovation and clinical application. Together with Leiden University Medical Center (LUMC), they will integrate historical manufacturing and clinical data and prospectively characterize 40 GMP CAR-T productions, including 20 at IDIBAPS-HCB. Analyses will relate starting material characteristics, cellular metabolism, phenotype and potency to manufacturing performance and patient outcomes, informing the digital twin. IDIBAPS-HCB will assess CAR-T products generated using the improved vectors and evaluate patient-specific manufacturing adjustments. IDIBAPS will lead final process validation, including harmonization of GMP documentation, two optimized manufacturing runs at each clinical manufacturing center, and preparation of an updated investigational medicinal product dossier for regulatory submission.

IMPACT is expected to deliver a validated framework connecting vector production, functional testing and predictive manufacturing control. By combining UB's bioprocessing expertise with IDIBAPS-HCB's clinical manufacturing capabilities, the project aims to enable more consistent, sustainable and personalized CAR-T production and broaden patient access to advanced therapies.

Funded by the European Union under the Horizon Europe Grant Agreement No. 101286805. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union can be held responsible for them.

HAPLOIDENTICAL CD45RA⁻ NKG2D-CAR-T CELLS IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS WITH RELAPSED/ REFRACTORY SARCOMA: PRELIMINARY RESULTS OF THE ONGOING PHASE I CAR4SAR TRIAL (NCT06087341). RD24/0014/0024, RD24CIII/0002/0002.

Halin Bareke[1], Adrian Ibanez-Navarro[1,2], Pilar Guerra Garcia[3], Isabel Mirones[4], Laura Hidalgo[5,6,7], Patricia Garcia-Rodriguez[8], Estefania Ayala-Jimenez[1], Elisa Izquierdo[9], Jose Juan Pozo-Kreilinger[10], Mar Tapia Viñe[11], Adela Escudero[9], Javier García-Castro[7,12], Antonio Pérez-Martínez[1,3,13]

[1]CIBERER-ISCIII, IdiPAZ-CNIO Translational Research Unit in Pediatric Hemato-Oncology, La Paz University Hospital Research Institute, Spanish National Cancer Center, Madrid, Spain.

[2]Department of Orthopedics, La Paz University Hospital, Madrid, Spain.

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

[4]Advanced Therapy Medicinal Products Production Unit Pediatric Hemato-Oncology Department, La Paz University Hospital, Madrid, Spain

[5]Biomedical Innovation Unit, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT), 28040, Madrid, Spain.

[6]Advanced Therapies Unit., Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD/UAM), 28040, Madrid, Spain.

[7]Cellular Biotechnology Unit, Instituto de Salud Carlos III (ISCIII), 28220, Madrid, Spain.

[8]Cellular Biotechnology Unit, Instituto de Salud Carlos III (ISCIII), 28220, Madrid, Spain.

[9]Institute of Medical and Molecular Genetics INGEMM, La Paz University Hospital, Madrid, Spain.

[10]Pathology Department, La Paz University Hospital, Madrid, Spain

[11]Department of Musculoskeletal Radiology, La Paz University Hospital, Madrid, Spain

[12]Instituto de Investigación de Enfermedades Raras (IIER) & Departamento de Desarrollo de Medicamentos de Terapias Avanzadas (DDMTA), Instituto de Salud Carlos III, 28220, Madrid, Spain.

[13]Pediatrics Department, Autonomous University of Madrid, Madrid, Spain.

CENTROS PARTICIPANTES: La Paz University Hospital Research Institute, Spanish National Cancer Center (IdiPAZ/CNIO), Instituto de Salud Carlos III (ISCIII).

Background. Relapsed and refractory pediatric and young adult sarcomas have poor prognosis and very limited treatment options. Sarcoma cells express ligands of NKG2D (NKG2DL), an activating receptor of natural killer cells, and in preclinical models second-generation CD45RA⁻ NKG2D-CAR-T cells effectively controlled osteosarcoma. CAR4SAR is an academic phase I trial testing donor-derived CD45RA⁻ NKG2D-CAR-T cells in patients aged 0–40 years with advanced NKG2DL⁺ sarcoma.

Methods. After cyclophosphamide/fludarabine lymphodepletion, patients receive three weekly intravenous CAR-T infusions (Arm A), or the same three plus one intratumoral infusion (Arm B). The trial uses 3+3 dose escalation at 1, 2 and 3 × 10^6 cells/kg/infusion. Cells are manufactured from haploidentical donors. Primary endpoints are dose-limiting toxicity (DLT) and maximum tolerated dose. Biological samples are collected to study persistence of the infused cells, cytokine profiles and tumor immune evasion, and to characterize the infusion product and the patients' immune populations.

Results. Nine heavily pretreated patients have been enrolled, aged 12 to 26 (median 17): seven on the trial and two under compassionate use with autologous product. They had osteosarcoma (n=5), rhabdomyosarcoma (n=2) or Ewing sarcoma (n=2), and up to six prior relapses or progressions. Eight have been infused. Cohort 2 of Arm B has been completed with no DLTs. In cohort 2 there were three cases of grade 1 CRS, and most adverse events were related to lymphodepletion. In one patient, the infused cells persisted for two months, with 2% donor chimerism. One patient with multiply relapsed localized osteosarcoma had a partial response at day 60; all other patients had progressive disease. The final product had an effector-memory phenotype (CCR7⁻CD45RA⁻) with low expression of the immune checkpoint molecules PD-1 and TIM-3. Primary cell lines have been established from biopsies of four patients. In vitro, adding the anti-TIGIT antibody tiragolumab increased killing of osteosarcoma cells by the infusion product.

Conclusions. Manufacturing of haploidentical CD45RA⁻ NKG2D-CAR-T cells is feasible and their administration is safe in the doses tested in heavily pretreated sarcoma patients. Clinical activity has so far been limited to one partial response, which points to the need to strengthen CAR-T function in the sarcoma microenvironment. TIGIT blockade increased the in vitro cytotoxicity of the infusion product. Together with patient-derived primary cell lines as a testing platform, these findings support evaluating checkpoint-modulating combinations in future cohorts.

PRECLINICAL VALIDATION OF A SECOND GENERATION B7-H3 CAR T-CELL THERAPY FOR PEDIATRIC SOLID TUMORS. RD24/0014/0024, RD24/0014/0023.

Sara Naharro[1], Esther Díaz-Maroto[1], Alicia Martín-Ayuso[1], Alfonso Alba[1], Lucía Fernández[1], Andrés París[1], Antonio Pérez-Martínez[1,2], Jordi Minguillón[1,3].

[1]CIBERER-ISCIII, IdiPAZ-CNIO Translational Research Unit in Pediatric Hemato-Oncology, La Paz University Hospital Research Institute, Spanish National Cancer Center, Madrid, Spain

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

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

Pediatric solid tumors account for ~60% of childhood cancers, including neuroblastoma, sarcoma, and CNS tumors such as glioblastoma, AT/RT, and DIPG. Despite overall 5-year survival reaching 80%, a subset of patients with refractory or relapsed tumors still lack effective therapies, and certain high-risk tumors carry very poor prognosis. This unmet clinical need has motivated exploration of cellular immunotherapies, including CAR T-cell therapy.

CAR T-cell therapy has achieved remarkable success in pediatric hematologic malignancies, with currently seven FDA/EMA-approved products. In contrast, no CAR T-cell therapy has yet been approved for solid tumors, where efficacy is limited and responses are often short-lived. Key challenges include antigen heterogeneity, the immunosuppressive tumor microenvironment (TME), and poor CAR T-cell infiltration and persistence.

In this project we developed a CAR T-cell approach targeting the pan-tumor-associated antigen B7-H3, broadly expressed in pediatric solid tumors. A second-generation CAR incorporating 4-1BB and CD3ζ domains (B7H3-BBz) was constructed using the scFv from an in-house developed monoclonal antibody against B7-H3. Activated T-cells expanded and were efficiently transduced using B7H3-BBz lentivirus. Cytotoxicity was validated in vitro against different tumor cell lines: SJ-GBM2 (glioblastoma), DAOY (medulloblastoma), A673 (Ewing sarcoma), and three AT/RT cell lines (G401, BT-12, CHLA-226), being the AT/RT cell lines the ones with less efficacy. A pilot *in vivo* was conducted to evaluate the efficacy and safety of B7H3-BBz CAR T-cells in AT/RT xenograft models (BT-12 and G401 cell lines), established in immunodeficient NSG mice. In both studies, this therapy was well tolerated and helped to control tumor growth in the CAR T-cell group.

B7H3-targeted CAR T-cell therapy shows promise activity against pediatric solid tumors with high unmet clinical needs. Our results justify further preclinical optimization and evaluation, in order to move on to translational and clinical validation of this second generation CAR T-cell approach. Reduced initial efficacy in AT/RT supports the development of improved next-generation CARs to overcome TME-mediated suppression.

PRECLINICAL VALIDATION OF A CD7 CAR T-CELL THERAPY FOR PEDIATRIC NON-B-CELL MALIGNANCIES. RD24/0014/0024, RD24/0014/0023.

Sara Naharro[1,2], Alicia Martín-Ayuso[1], Esther Díaz-Maroto[1], Marta Ibáñez-Navarro[1], Lucía Fernández[1], Antonio Pérez-Martínez[1,3], Jordi Minguillón[1,4]

[1]CIBERER-ISCIII, IdiPAZ-CNIO Translational Research Unit in Pediatric Hemato-Oncology, La Paz University Hospital Research Institute, Spanish National Cancer Center, Madrid, Spain.

[2]Molecular Biology Department, Universidad Autónoma de Madrid, Madrid, Spain.

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

[4]Advanced Therapies Mixed Unit, CIEMAT/IIS-FJD, Madrid, Spain.

CAR T-cell therapy is an adoptive treatment where T-cells are modified ex vivo to target tumor-associated antigens, demonstrating promising results in B-cell hematological cancers. However, effective therapies for non-B-cell malignancies are still needed. CAR T-cell therapy in T-cell neoplasms faces challenges due to T-cell fratricide and off-tumor toxicity from shared antigens like CD7 (Chiesa et al., 2025, NEJM; Oh et al., 2024, Nat Med) or CD5 (Pan et al., 2025, Nat Med; Hill et al., 2024, Blood). Our main goal in this project is to develop and validate a CAR T-cell therapy for pediatric relapsed non-B-cell malignancies, addressing the current lack of therapeutic alternatives in Spain for these patients. For this, we selected CD7 as a marker predominantly expressed in T-cells and other leukemias and designed a CAR T-cell therapy against this marker, encoded as K12-BBz, using its natural ligand (K12/SECTM1). T-cells from healthy donor buffy coats were activated with anti-CD3/CD28 for 3 days and subsequently transduced with K12-BBz lentiviral vector. To prevent T-cell fratricide, CAR T-cells were treated with pharmacological inhibitors (PI) ibrutinib and dasatinib during expansion (Watanabe et al, Mol Therap 2023). On day 7, spectral flow cytometry was performed to analyse the CAR T-cell immunophenotype, followed by cytotoxicity assays with pediatric tumor cell lines Jurkat and MOLT4.

In vitro activation, transduction, expansion and cytotoxicity experiments demonstrated that K12-BBz CAR T-cells produced with PI are more effective killing T-ALL cell lines Jurkat and MOLT4 than without PI. Next, the first preliminary in vivo experiment using NSG immunodeficient mice and Jurkat cells showed better tumor control and prolonged survival in mice infused with K12-BBz CAR T-cells. We are currently validating our approach in further in vivo experiments with promising preliminary efficacy, that will be presented in this annual meeting.

Our findings highlight a promising CAR T-cell approach for the treatment of pediatric non-B-cell malignancies. Further in vivo experiments and GMP validation are needed to move on to clinical validation with pediatric patients, which is underway in our laboratory.

TERAPIA CAR T DUAL CD19/22 EN UNA ADULTA JOVEN CON VASCULITIS ANCA ANTI-MPO EN HEMODIÁLISIS. RD24/0014/0023, RD24/0014/0024.

Andrés París Muñoz[1], Begoña Rivas[2], Jordi Minguillón[1,3], Elena Sánchez Zapardiel[2], Lucía del Pino[2], Katia Pieri[1], Juan Martín[1], Alicia Matín[1], Omaira Alberquilla[3], Rebeca Sánchez[3], José Carlos Segovia[3], Isabel Mirones[2], Antonio Pérez Martínez[1,2]

[1]Unidad Mixta IdiPAZ-CNIO

[2]Hospital Universitario La Paz

[3]CIEMAT.

Las terapias CAR T dirigidas contra antígenos propios del linaje B están suponiendo un cambio de paradigma en el tratamiento de las enfermedades autoinmunes reumáticas altamente refractarias: lupus eritematosos sistémico, esclerosis sistémica, miopatías inflamatorias, vasculitis, etc. A través de una depleción profunda del compartimento de linfocitos B, estas terapias tienen la potencialidad de inducir una remisión completa de la enfermedad, durante largos periodos de tiempo, en ausencia de fármacos inmunosupresores.

En el presente trabajo se describe el primer uso de una terapia CAR T anti-CD19/CD22 académica en una adulta joven en hemodiálisis con vasculitis asociada a ANCA anti-MPO refractaria y graves complicaciones que, a pesar de las múltiples líneas terapéuticas convencionales recibidas, presentaba niveles elevados de anticuerpos anti-MPO en sangre. En concreto, la paciente recibió, por uso compasivo, una terapia CAR-T dual anti-CD19/CD22 producida, académicamente, en la Unidad de Terapias Avanzadas del Hospital Universitario La Paz. Se administraron 0,75 × 10^6 células CAR-T/kg en fresco y la misma dosis criopreservada el día +3. Se caracterizó el producto de infusión mediante un panel de citometría espectral de 29 colores optimizado en colaboración con el laboratorio de citometría de flujo del CIEMAT.

El tratamiento fue bien tolerado, con CRS de grado 1. Los anti-MPO se negativizaron el día +65. Tuvo una mejora clínica general según la escala BVAS. Se observó expansión específica de células CAR-T en un contexto de aplasia B periférica, con predominio del fenotipo de memoria central y mayor proporción de células activadas. El producto contenía una subpoblación de linfocitos T CD8+ de memoria efectora proliferativa, correspondiente al 11,53 % de los linfocitos T CD3+ viables.

Esta expansión específica de células CAR-T durante la aplasia B periférica y el descenso sostenido de anti-MPO sugieren una depleción profunda del compartimento B tisular.

GENERATING NK CELLS IN VITRO FROM UMBILICAL CORD BLOOD UNITS IN A FEEDER FREE GMP SYSTEM. RD21/0017/0024, RD21/0017/0029 Y RD24/0014/0025, RD24/0014/0001, RD24/0014/0023.

Andrea Sáez de Cámara[1-3], Lara Herrera[1-4], Irene Romayor[1-3], Silvia Santos[1-3], Miguel Ángel Vesga[1-3], Laura Biritxinaga[1-3], Manel Juan[3,4,6], Beatriz Olalla[3,7], Paula Rio[3,7], Cristina Eguizabal[1-4]

[1]Grupo de Terapia Celular, Células Madre y Tejidos, Centro Vasco de Transfusión y Tejidos Humanos, IIS Biobizkaia, Galdakao, España.

[2]Advanced Therapies Unit, Basque Centre for Blood Transfusion and Human Tissues, Osakidetza, Galdakao, 48960, España.

[3]Red Española de Terapias Avanzadas (TERAV), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, España.

[4]Red de Inmunoterapia del Cancer "REINCA" (RED2022-134831-T).

[5]Grupo de Inmunología e Inmunoterapia del Cáncer, IIS Biobizkaia, Barakaldo, España.

[6]Servei d´Immunologia. Hospital Clínic de Barcelona. Hospital Sant Joan de Déu. Institut d'Investigacions Biomèdiques August Pi i Sunyer Hospital. Universitat de Barcelona, Barcelona, España.

[7]Centro de Investigaciones Energéticas Medioambientales y Tecnológicas and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIEMAT/CIBERER), Madrid, España.

CENTROS PARTICIPANTES: Centro Vasco de Transfusión y Tejidos Humanos (CVTTH), IIS Biobizkaia, Hospital Clínic de Barcelona, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT).

Hematological malignancies represent a major global health challenge. Recent advances in chimeric antigen receptor (CAR) T-cell therapies have demonstrated remarkable efficacy against leukemia, lymphoma, and multiple myeloma, including the development of the CD19-targeted CAR-T product ARI-0001. However, CAR-T therapies are associated with adverse effects, such as cytokine release syndrome and neurotoxicity, highlighting the need for alternative therapeutic approaches. In this context, natural killer (NK) cells have emerged as a promising source for CAR-engineered cell therapies due to their intrinsic antitumor activity, favorable safety profile, and potential for generating allogeneic off-the-shelf products. Nevertheless, their limited ex vivo expansion remains a challenge for clinical applications, emphasizing the need to explore alternative cell sources capable of producing large numbers of functional NK cells. In this regard, NK cells differentiated from umbilical cord blood (UCB)-derived hematopoietic stem and progenitor cells (HSPCs) represent a promising alternative due to their high proliferative capacity. Therefore, our aim is to generate functional NK cells from UCB-HSPCs using GMP-compatible, feeder-free culture systems for their potential application in CAR-NK therapies. We have successfully generated mature and functional NK cells and explored their genetic modification using the ARI-0001 CAR construct, with the aim of developing CAR-NK cell therapies. Furthermore, proteomic analyses revealed that the generated NK cells exhibited protein expression profiles resembling those of adult peripheral blood-derived NK cells. Additionally, in vivo experiments are currently being performed to evaluate the antitumor efficacy of the generated NK cells against the K562 cell line.

DUAL-TARGET CAR-NKS WITH ON- AND OFF-TUMOR ACTIVITY MAY OVERRIDE IMMUNE SUPPRESSION IN SOLID CANCERS: AN "OFF THE SHELF" NKG2D-CD19-CAR-NK CELL THERAPY (DUAL-CAR-NK). RD21/0017/0024, RD21/0017/0025, RD21/0017/0029 Y RD24/0014/0025, RD24/0014/0024, RD24/0014/0001, RD24/0014/0004.

A Etxebarria[1,2,6], L Herrera[1,2,6,8], D Barriales[1,2], Álvarez M[1,2,6,8], S Santos[1,2,6], M Juan[6,7,8], A Pérez-Martínez[3,4,5,6], M Zalacain[6,9], A Patiño[6,9], M Alonso[6,9], Cristina Eguizabal[1,2,6,8]

[1]Advanced Therapies Unit, Basque Center for Blood Transfusion and Human Tissues, Osakidetza, Galdakao, Spain.

[2]Cell Therapy, Stem Cells and Tissues Group, Biobizkaia Health Research Institute, Barakaldo, Spain.

[3]Hospital La Paz Institute for Health Research, IdiPAZ, University Hospital La Paz, Madrid, Spain.

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

[5]Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain.

[6]Red Española de Terapias Avanzadas (TERAV, TERAV+), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[7]Servei d´Immunologia. Hospital Clínic de Barcelona. Hospital Sant Joan de Déu. Institut d'Investigacions Biomèdiques August Pi i Sunyer Hospital. Universitat de Barcelona, Barcelona. Spain.

[8]Red de Inmunoterapia del Cancer "REINCA" (RED2022-134831-T).

[9]Health Research Institute of Navarra (IdiSNA), Pamplona, Spain; Solid Tumor Program, CIMA-Universidad de Navarra, Pamplona, Spain; Department of Pediatrics, Clínica Universidad de Navarra, Pamplona, Spain.

CENTROS PARTICIPANTES: CVTTH-BIOBIZKAIA, Hospital La Paz, Hospital Clinic, CUN.

Cancer is the main cause of death globally, with solid tumors such as lung, breast and colorectum being the most prevalent. Conventional treatments based on surgery, radiation and chemotherapy are ineffective for patients with relapsed or refractory diseases. In the last decade, chimeric antigen receptor (CAR)-based therapy has become one of the most promising immunotherapeutic strategies against cancers. Especially, CAR-T cells have shown outstanding clinical results in the treatment of hematological malignancies. However, in the case of solid tumors there are different challenges that limit the efficacy of this therapy, including antigenic heterogeneity of the tumor, the immunosuppressive tumor microenvironment, and the low capacity of the cells for infiltrating into the tumor. Recently, CAR-NK cells have being proposed as an alternative cell source for being engineered with CARs due to their safety profile, their innate potential to kill malignant cells through different mechanisms and the possibility to use them as an allogeneic product. In this context, the aim of this study is the development of DUAL-CAR-NK cells as an "off-the-shelf" innovative product based on the recognition of two tumor antigens, including NKG2D ligands and CD19, an off-tumor antigen present in normal B cells. The use of DUAL-CAR-NK cells could improve its level of expansion even in presence of immune suppression, increasing its cytotoxic activity against tumor cells. We have generated DUAL-CAR-NK cells from adult peripheral blood samples and their functionality has been tested against different tumor cell lines. Thus, this strategy could become an effective therapeutic approach, paving the way for further pre-clinical studies to evaluate the potential of DUAL-CAR-NK cells against aggressive solid tumors with presence of B cells.

GENERATING NKG2D-CAR-TRANSDUCED NATURAL KILLER (NK) CELLS FOR TREATING OSTEOSARCOMA. RD21/0017/0024, RD21/0017/0025, RD21/0017/0034 Y RD24/0014/0025, RD24/0014/0024, RD24/0014/0004.

A Etxebarria[1,2,6], L Herrera[1,2,6], D Barriales[1,2,6], S Santos[1,2,6], M Juan[6,7], A Pérez-Martínez[3,4,5,6], Zalacain M[6,7], Patiño A[6,7], M Alonso[6,7], Cristina Eguizabal[1,2,6,8]

[1]Advanced Therapies Unit, Basque Center for Blood Transfusion and Human Tissues, Osakidetza, Galdakao, Spain.

[2]Cell Therapy, Stem Cells and Tissues Group, BioBizkaia Health Research Institute, Barakaldo, Spain.

[3]Hospital La Paz Institute for Health Research, IdiPAZ, University Hospital La Paz, Madrid, Spain.

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

[5]Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain.

[6]Red Española de Terapias Avanzadas (TERAV/TERAV+), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[7]Health Research Institute of Navarra (IdiSNA), Pamplona, Spain; Solid Tumor Program, CIMA-Universidad de Navarra, Pamplona, Spain; Department of Pediatrics, Clínica Universidad de Navarra, Pamplona, Spain

[8]Red de Inmunoterapia del Cáncer "REINCA" (RED2022-134831-T)

CENTROS PARTICIPANTES: CVTTH-BIOBIZKAIA, Hospital La Paz, CUN-CIMA.

Osteosarcoma (OS) is the most common primary malignant bone tumor, with an incidence worldwide of approximately one to three cases annually per million. It typically affects children and young adults with a second smaller peak of incidence in those individuals over the age of 50. The current main treatment is based on the combination of surgery and neoadjuvant chemotherapy leading to a long-term survival rate of 70%. However, these strategies are inefficient for the metastatic or relapsed osteosarcoma patients, in who the 5-year survival rate is less than 20%. As a result, in the last decades, alternative novel therapeutical approaches are being developed, including chimeric antigen receptors (CARs)-based immunotherapy, in which immune effector cells such as T cells are genetically engineered to express CARs in their surface membrane for improving the recognition and killing of tumor cells. CAR-transduced T (CAR-T) cells have revolutionized the cancer therapy, however toxicities associated to this autologous therapy such as cytokine release syndrome and immune effector cell-associated neurologic syndrome limit its widespread. Therefore, efforts have been made to find a more appropriate cell type for being genetically engineered with CARs. Among other immune cell types, natural killer (NK) cells have been proposed as a potential allogenic alternative due to their cytolytic capacity and their safety profile. Since NKG2D receptor (NKG2DR) is one of the most important activating receptor expressed in NK and osteosarcoma cells express ligands for NKG2D receptor, the aim of this study is the production of NKG2D-CAR-NK cells in order to target and kill more effectively osteosarcoma cells. The increased expression of NKG2DR on the surface of effector cells is correlated with an increased cytotoxic capacity. We hope that these results will stimulate the development of experimental studies, testing the potential effectiveness of the new concepts of CAR-NK therapies against aggressive solid tumors.

EL PERFIL METABÓLICO Y LA PERSISTENCIA FUNCIONAL COMO HERRAMIENTAS PARA MEJORAR LA CARACTERIZACIÓN DE LOS PRODUCTOS CAR-T. RD24/0014/0027, RD24/0014/0021, RD24/0014/0001, RD24/0014/0036.

Beatriz Guijarro-Albaladejo[1], Belén Sierro-Martínez[1], Inmaculada Carrasco-Brocal[1], Paola Hernández-Díaz[1], María de la Rosa-Garrido[1], Raquel Muñoz-García[1], Jose Antonio Bejarano-García[1], Joan Mañé-Pujol[2], Patricia Alcalde-Mellado[1], Alfonso Rodríguez-Gil[1], Virginia Escamilla-Gómez[1], Maribel Lara-Chica[1], Cristina Aguirre-Portolés[3], Víctor Galán-Gómez[3], Laura Escribà-García[4,5,6], Marta Español-Rego[7], Luis Gerardo Rodríguez-Lobato[2], Javier Briones[4,5,6], Carlos Fernández de Larrea[2], Antonio Pérez-Martínez[3], Ángela Bella[1], José Antonio Pérez-Simón[1*], Estefanía García-Guerrero[1*].

[1]Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, España.

[2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona, España.

[3]Unidad de Investigación Traslacional en Oncohematología Pediátrica, Instituto de Investigación del Hospital Universitario La Paz, Centro Nacional de Investigaciones Oncológicas. CIBERER-ISCIII. Madrid, España.

[4]Servicio de Hematología, Hospital de la Santa Creu i Sant Pau, Barcelona, España.

[5]Laboratorio de Hematología Experimental, Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, España.

[6]Instituto de Investigación contra la Leucemia Josep Carreras, Barcelona, España.

[7]Departamento de Inmunología, Centre de Diagnòstic Biomèdic (CDB), Hospital Clínic de Barcelona, Barcelona, España.

INTRODUCCIÓN

La variabilidad en la eficacia clínica de la terapia CAR-T evidencia la necesidad de identificar características de los productos generados asociadas a su funcionalidad a largo plazo, ya que los métodos convencionales como la citometría de flujo o los análisis de citotoxicidad no reflejan adecuadamente su potencial terapéutico. Este estudio compara las propiedades funcionales y metabólicas de distintos diseños CAR y explora su relación con la evolución clínica de pacientes tratados con terapia CAR-T.

MATERIALES Y MÉTODOS

Se compararon dos versiones del BCMA-CAR académico CARTemis-1, con dominios de coestimulación 4-1BB (CARTemis-1-BB) o CD28 (CARTemis-1-28), mediante análisis de viabilidad, expansión e inmunofenotipo antes y después de la estimulación antigénica mediante citometría de flujo, así como análisis de citotoxicidad tras una ronda de estimulación con el antígeno. Se llevaron a cabo análisis de estimulación repetida con el antígeno, metabolismo mitocondrial y transcriptómica. Además, se caracterizaron 15 productos CAR-T infundidos a pacientes con distintas neoplasias, clasificándolos según la duración de la supervivencia libre de progresión: respuesta prolongada (>18 meses) frente a ausencia de respuesta o recaída antes de los 18 meses.

RESULTADOS

El análisis convencional mediante citometría de flujo no detectó diferencias relevantes entre CARTemis-1-BB y CARTemis-1-28 en viabilidad, expansión o inmunofenotipo, tanto antes como después del estímulo con el antígeno. Sin embargo, la exposición repetida al antígeno demostró una mayor capacidad citotóxica sostenida en CARTemis-1-BB en comparación con CARTemis-1-28, así como una mayor capacidad respiratoria de reserva y respiración máxima tras la estimulación. El análisis transcriptómico mostró, además, un enriquecimiento en firmas relacionadas con la activación de los linfocitos T en CARTemis-1-BB, mientras que CARTemis-1-28 presentó respuestas relacionadas con las vías Wnt/TGF-β.

Dado que estos parámetros metabólicos se han asociado con una mayor persistencia y funcionalidad de las células CAR-T, se exploró su utilidad para identificar productos asociados a una mejor evolución clínica. Para ello, se caracterizaron metabólicamente distintos productos CAR-T infundidos a pacientes en el Hospital Universitario Virgen del Rocío, el Hospital Universitario La Paz, el Hospital Clínic de Barcelona y el Hospital de la Santa Creu i Sant Pau. El análisis identificó diferencias en los perfiles metabólicos de los productos según la

evolución clínica, observándose una mayor actividad respiratoria en aquellos asociados a una supervivencia libre de progresión prolongada frente a los procedentes de pacientes con evolución desfavorable.

CONCLUSIONES

El análisis metabólico de los productos CAR-T permite detectar diferencias funcionales que pueden pasar inadvertidas mediante técnicas convencionales como la citometría de flujo y el análisis de la citotoxicidad. Estas estrategias podrían contribuir a mejorar la selección preclínica de constructos CAR-T y a identificar parámetros complementarios para la evaluación de la calidad de los productos celulares y su potencial relación con los resultados clínicos.

EXOFUCOSYLATION AS A TOOL TO ENHANCE THE ANTITUMOR ACTIVITY OF EXPANDED CAR-T CELLS AGAINST SOLID TUMORS. RD24/0014/0005 AND RD24/0014/0040.

Jesús I. Gil-Chinchilla[1,2], Jon Ruiz de Francisco[3,4], Ramón Hurtado-Guerrero[5], Miguel Blanquer[2], Francisco Martin[3,6,7], David García-Bernal[2], María Tristán-Manzano[3,6,7].

[1]ATMPs Production Unit-GMP Facility, Virgen de la Arrixaca University Clinical Hospital, Biomedical Research Institute of Murcia Pascual Parrilla–IMIB, 30120, Murcia, Spain.

[2]Hematopoietic Transplant and Cellular Therapy Unit, Biomedical Research Institute of Murcia Pascual Parrilla–IMIB, University of Murcia and Virgen de la Arrixaca University Hospital, Murcia, Spain

[3]GENYO, Centre for Genomics and Oncological Research, Pfizer, University of Granada, Andalusian Regional Government, PTS Granada, Granada, Spain.

[4]LentiStem Biotech, PTS Granada, Granada, Spain.

[5]Aragon Health Research Institute/ BIFI, University of Zaragoza, Zaragoza, Spain.

[6]Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Granada, Spain.

[7]Biosanitary Research Institute ibs. GRANADA, Granada, Spain.

Background: CAR-T cells therapies have revolutionized the treatment of hematological malignancies; however, their efficacy in solid tumours remains limited. Among the multiple barriers affecting therapeutic success, inefficient trafficking, extravasation and persistence of transferred lymphocytes within the tumour microenvironment represent major challenges. Physiological leukocyte migration relies on tightly regulated interactions between endothelial selectins and sialofucosylated glycans expressed on the surface of immune cells, including the sialyl Lewis X (sLeX) epitope. These glycan-dependent interactions mediate the initial rolling and adhesion events required for tissue homing, suggesting that modulation of the cellular glycocalyx may represent a promising strategy to improve the biodistribution of adoptively transferred cells. In this collaborative study involving IMIB/University of Murcia, IIS Aragón/University of Zaragoza and GENYO/University of Granada, we investigated whether enzymatic exofucosylation using wild-type or mutant fucosyltransferases (FUTs) could enhance the expression of homing-associated glycan structures on expanded human CAR-T cells while preserving cell viability and potencially improving their antitumour activity against solid tumor models.

Methods: Human EpCAM-targeting CAR-T cells were generated from activated peripheral blood mononuclear cells (PBMCs), expanded during 21 days and subjected to exofucosylation using recombinant FUT6, FUT7 or mutant FUT7 (FUT7P). Glycan remodeling was assessed by HECA-452 staining, a surrogate marker of sialofucosylated epitopes (sLeX), in total CAR-T cells and in specific T-cell subsets. Viability, phenotypic composition and cytotoxic function were evaluated by flow cytometry and 3D tumour spheroid assays of MiaPaca-EpCAMlow cells.

Results: Exofucosylation efficiently increased HECA reactivity in CAR-transduced cells, with all three enzymes producing significant increases compared with non-fucosylated controls, and FUT6 showing the strongest effect. Enhanced HECA expression was maintained for at least 48 h after treatment. Glycan remodeling was observed in both $CD4^+$ and $CD8^+$ CAR-T-cell subsets, while the overall CD4/CD8 composition remained unchanged, indicating that exofucosylation modifies the cell-surface glycome without altering major T-cell lineage distributions. Analysis of CD62L expression revealed an enrichment of HECA-positive cells within the $CD62L^+$ compartment and an increase in the proportion of $CD62L^+$ $HECA^+$ T-cells following exofucosylation, suggesting preferential glycan remodeling of homing-associated T-cell populations. Importantly, cell viability was unaffected by any of the exofucosylation strategies. In preliminary functional studies using a challenging 3D MiaPaCa-EpCAMlow spheroid model and highly expanded CAR-T-cell products displaying approx-

imately 35% CAR expression, exofucosylated CAR-T cells retained cytotoxic activity at 1:5, Effector: Target, whereas non-fucosylated conditions did not exert any cytotoxic lysis at 48h.

Conclusions: Enzymatic exofucosylation rapidly and efficiently remodels the glycocalyx of human CAR-T cells, generating stable HECA-reactive glycan signatures across both $CD4^+$ and $CD8^+$ subsets while preserving viability and antitumour activity. The observed enrichment of glycan remodeling within CD62L-expressing populations supports further investigation of this approach as a strategy to enhance CAR-T-cell trafficking and biodistribution in vivo. Preliminary functional data indicate that glycoengineering is compatible with CAR-T-cell effector activity, warranting evaluation in more physiologically relevant homing and efficacy in vivo models.

NG2–ITGA4 AXIS REGULATES RHO GTPASES AND LEUKEMIA AGGRESSIVENESS IN KMT2A-R B-ALL AND IS TARGETABLE WITH NATALIZUMAB. RD24/0014/0042 Y RD24/0014/0040.

Alba Rubio-Gayarre[1,2,3,4], Meritxell Vinyoles[1,4], Juan Ramon Tejedor[5,6,7], Mercedes Guerrero-Murillo[1,4], Narcis Fernandez-Fuentes[1,4], Rita Caracuel-Peramos[2], María C Plaza-Calonge[2], Alba Martinez-Moreno[1,4], Heleia Roca-Ho[1,4], Paolo Petazzi[1], Alba Panella-Alferez[2], Angela Meseguer-Giron[1,4], Kirsten S. Vrenken[8], Mario F. Fraga[5,6,7], Pilar Leoz-Allegre[9], Carlos Palacio-Garcia[10], Pablo Velasco[11,12,13], Jose Luis Fuster[4,14,15], Ronald W Stam[8], Juan Carlos Rodriguez-Manzaneque[2], Xose R Bustelo[16,17,18], Javier Robles-Valero[16,17,18], Pablo Menendez[1,4,18,19,20,21*], Clara Bueno[1,4,18*] and Belen López-Millan[1,2,3,4*].

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 KMT2A-r B-ALL patients. 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 leukemia progression 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 (PDX) 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.

[1]Stem Cell Biology, Developmental Leukemia and Immunotherapy Group. Josep Carreras Leukemia Research Institute, Barcelona, Spain.

[2]GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Junta de Andalucia, Granada, Spain.

[3]Department of Physiology. University of Granada. Granada, Spain.

[4]RICORS-TERAV+ Network, ISCIII, Madrid, Spain.

[5]Fundacion para la Investigacion Biosanitaria de Asturias (FINBA), Instituto de Investigacion Sanitaria del Principado de Asturias (ISPA), Instituto Universitario de Oncologia de Asturias (IUOPA), Hospital Universitario Central de Asturias (HUCA), Universidad de Oviedo, Asturias, Spain.

[6]Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), ISCIII, Asturias, Spain.

[7]Nanomaterials and Nanotechnology Research Center (CINN-CSIC), University of Oviedo, Asturias, Spain.

[8]Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands.

[9]Hematology Department, La Santa Creu i Sant Pau Hospital, Barcelona, Spain.

[10]Hematology Department, Vall d'Hebron University Hospital, Vall d'Hebron Hospital Campus, Barcelona, Spain.

[11]Pediatric Oncology and Hematology Department, Vall d'Hebron University Hospital, Barcelona, Spain.

[12]Department of Pediatrics, Obstetrics, Gynecology, Preventive Medicine and Public Health. Autonomous University of Barcelona, Barcelona, Spain.

[13]Childhood Cancer & Blood Disorders Research Group, Vall d'Hebron Research Institute (VHIR). Barcelona, Spain.

[14]Department of Pediatric Oncohematology, Virgen de la Arrixaca University Hospital, Murcia, Spain.

[15]Instituto Murciano de Investigacion Biosanitaria, Murcia, Spain.

[16]Centro de Investigacion del Cancer, CSIC-University of Salamanca, Campus Unamuno, 37007 Salamanca, Spain.

[17]Instituto de Biologia Molecular y Celular del Cancer, CSIC-University of Salamanca, Campus Unamuno, 37007 Salamanca, Spain.

[18]Centro de Investigacion Biomedica en Red de Cancer (CIBERONC), ISCIII, Madrid, Spain.

[19]Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain.

[20]Institucio Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Spain.

[21]Institut de Recerca Sant Joan de Deu-Barcelona Pediatric Cancer Centre (IRSJD-PCCB), Barcelona, Spain.

Carla Panisello[1,2,3,4,5], Raúl Mañogil[4,6,7*], Rosario Aschero[4,6*], Carolina Castilla[8*], Gonçalo Rodrigues[4,6*], Meritxell Vinyoles[1,3], Alba Martínez-Moreno[1,3], Heleia Roca-Ho[1,3], Narcis Fernandez-Fuentes[1,3], Aïda Falgàs[1,3,9], Maximilian M L Knott[10,11,12,13], Javier Fernández-Calles[5,14], Mercedes Guerrero-Murillo[1,3,5], Virginia Rodriguez-Cortez[1,3], Mireia Pellicer[1,3,5], Wolfgang Hartmann[15], Uta Dirksen[16], Nazaret Sánchez-Sierra[6], Anna Alonso-Saladrigues[6,17], Susana Rives[3,6,17], Eva Rodríguez[18], Silvia Planas[18], Andrea Greco[4,19], Alicia Castañeda[6], Cristina Larrosa[6], Moira Garraus[4,6], Alessandra Magnani[20], Julio Castaño[20], Pablo Engel[13], Thomas G.P. Grünewald[10,11,12,13], Enrique de Álava[8,9],21#, Clara Bueno[1,3],9#, Ángel M. Carcaboso[4],6#, Jaume Mora[4],16#, Pablo Menendez[1,3,4,9,14],22#

PTGER3-DIRECTED CAR T-CELL IMMUNOTHERAPY FOR EWING SARCOMA. RD24/0014/0006, RD24/0014/0042.

Ewing sarcoma (EwS) is an aggressive bone and soft tissue malignancy affecting children, adolescents, and young adults, for whom relapsed disease remains associated with dismal prognosis. CAR T-cell therapy for solid tumors is hampered by the scarcity of cell surface antigens and immunosuppressive tumor microenvironments. We identified prostaglandin E2 (PGE2) receptor EP3 (PTGER3) as a broadly expressed and functionally relevant therapeutic EwS target with limited expression in normal tissues. CAR-PTGER3 T-cells were generated and demonstrated potent, antigen-dependent activity across multiple cell line/patient-derived *in vitro* and *in vivo* EwS-models. A GMP-grade CAR-PTGER3 T-cell product (SJD-PCCB-001) was generated for first-in-human use in refractory EwS patient. SJD-PCCB-001 administration resulted in rapid and profound antitumor activity confirmed by massive tumor necrosis but revealed toxicities consistent with systemic hyperinflammation, including macrophage activation syndrome/hemophagocytic lymphohistiocytosis (MAS/HLH). This first-in-human experience provides proof-of-concept for PTGER3-directed CAR T-cell therapy in EwS while highlighting refinements for its clinical translation.

[1]Josep Carreras Leukemia Research Institute, Barcelona, Spain.

[2]Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain.

[3]Red Española de Terapias Avanzadas (TERAV)-Instituto de Salud Carlos III (ISCIII). Madrid, Spain.

[4]Pediatric Cancer Program. Institut de Recerca Sant Joan de Déu. Barcelona, Spain.

[5]PhD programme in Biomedicine, University of Barcelona, Barcelona, Spain.

[6]SJD Pediatric Cancer Center Barcelona (PCCB). Hospital Sant Joan De Déu, Barcelona, Spain.

[7]PhD programme in Medicine and Translational Research, University of Barcelona, Barcelona, Spain.

[8]Institute of Biomedicine of Sevilla (IBiS), Virgen del Rocio University Hospital, CSIC, University of Sevilla, Seville, Spain.

[9]Centro Investigación Biomédica en Red en Oncología (CIBERONC), ISCIII, Madrid. Spain.

[10]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany.

[11]Division of Translational Pediatric Sarcoma Research, German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Heidelberg, Germany.

[12]National Center for Tumor Diseases (NCT), a partnership between DKFZ and Heidelberg University Hospital, Germany.

[13]Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany.

[14]Department of Biomedical Sciences, School of Medicine, University of Barcelona, Barcelona, Spain.

[15]Gerhard-Domagk-Institute of Pathology, University of Muenster, Muenster, Germany

[16]Pediatrics III, University Hospital Essen, West German Cancer Center, German Cancer Consortium (DKTK) site Essen, National Center for Tumor Diseases (NCT) West, Essen, Germany.

[17]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain.

[18]Anatomical Pathology Department. Hospital Sant Joan de Déu, Barcelona, Spain.

[19]Arrhythmia, Inherited Cardiac Diseases and Sudden Death Unit, Department of Cardiology, Hospital Sant Joan de Déu, Barcelona, Spain.

[20]Advanced Therapies and Immunotherapy Platform, Sant Joan de Déu Barcelona Children's Hospital, Barcelona, Spain; Study Group for Immune Dysfunction Diseases in Children (GEMDIP), Institut de Recerca Sant Joan de Déu (IRSJD), Barcelona, Spain

[21]Department of Normal and Pathological Cytology and Histology, School of Medicine, University of Seville, Seville, Spain.

[22]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

TISA-CEL AND AXI-CEL CAR STRUCTURE INFLUENCES THE DEVELOPMENT OF RESISTANCE TO CD19-CAR-T THERAPY. RD24/0014/0024, RD24/0014/0042.

Marta Krawczyk[1,2,3], Klaudyna Fidyt[2,4,5*], Narcis Fernandez-Fuentes[4*], Tomasz Winiarski[6], Monika Pepek[2], Pablo Bousquets-Muñoz[6,7], Xose S. Puente[7,8], Ana Gutiérrez-Fernández[7,8], Jacinta Davis[9], Daria Owczarek[10], Agnieszka Graczyk-Jarzynka[1], Pablo Sanz Galarreta[11,12,13], Pablo Menendez[4,5,8,14,15,16], Anna Szumera-Ciecklewicz[17], Emmanuelle Benard[18], Sébastien Wälchli[18], Andrei Thomas-Tikhonenko[9,19], Víctor M. Pérez García[11,12,13], Víctor Galán-Gómez[20,21], Adela Escudero López[21,22], Elisa Izquierdo Delgado[21,22], Jordi Miguillón[21,23], Antonio Pérez-Martínez[20,21,23,24], Magdalena Winiarska[1],2#

CD19-CAR-T cells are a major therapy for relapsed/refractory B-cell malignancies, yet ~50% of patients relapse after infusion. Clinical data suggest that tisa-cel treatment is associated with higher rates of CD19 mutation than axi-cel, though direct comparisons have been lacking. Although both products target the FMC63 epitope, they employ different structures of hinge/transmembrane/costimulatory domains (CD8α/CD8α/4-1BB in tisa-cel, CD28/CD28/CD28 in axi-cel). Here, we show that CAR structure is a critical determinant of CD19 loss in tumor B cells. Specifically, repeated exposure to CD19-4-1BB-based CAR-T cells, but not CD19-CD28-based counterparts, drives FMC63-epitope and total CD19 protein loss. Consistent with clinical observations, resistance to CD19-4-1BB-based treatment is associated with aberrant splicing, loss of heterozygosity, and frameshift/missense mutations in CD19 in exons encoding the FMC63 epitope. Mathematical modeling indicates that the failure of CD19-4-1BB-based CAR-T cells to eliminate CD19low cells, unlike CD28-based CARs, promotes the expansion of resistant populations. These results suggest that optimizing recognition of CD19low cells could prevent the emergence of resistant clones and reduce relapse rates. We also show that commonly used diagnostic anti-CD19 monoclonal antibodies detect epitopes distinct from FMC63 and may therefore give misleading CD19-positive results, underscoring the need for FMC63-specific assessment to guide diagnostic and therapeutic decisions.

[1]Department of Immunology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

[2]Department of Immunology, Medical University of Warsaw, Warsaw, Poland

[3]Doctoral School of Translational Medicine, Mossakowski Medical Research Institute, Polish Academy of Sciences, Centre of Postgraduate Medical Education, Warsaw, Poland

[4]Josep Carreras Leukaemia Research Institute, Barcelona, Spain

[5]Red Española de Terapias Avanzadas (TERAV) - Instituto de Salud Carlos III (ISCIII)

[6]Warsaw University of Technology, Institute of Control and Computation Engineering, Warsaw, Poland

[7]Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Oviedo, Spain

[8]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain

[9]Division of Cancer Pathobiology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

[10]Laboratory of Cancer Metabolism, Department of Experimental Oncology, Laboratory of Cancer Metabolism, Maria Sklodowska-Curie National Research Institute of Oncology Warsaw, Warsaw, Poland

[11]Mathematical Oncology Laboratory (MOLAB), Instituto de Matemática Aplicada a la Ciencia y la Ingeniería, University of Castilla-La Mancha, Ciudad Real, Spain

[12]Department of Mathematics, Escuela Técnica Superior de Ingeniería Industrial, University of Castilla-La Mancha, Talavera de la Reina, Spain

[13]Laboratorio de Oncología Matemática, Instituto de Investigación Sanitaria de Castilla-La Mancha (IDISCAM), Ciudad Real, Spain

[14]Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain

[15]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

[16]Institut de Recerca Hospital Sant Joan de Déu–Pediatric Cancer Center Barcelona (SJD-PCCB), Barcelona, Spain

[17]Biobank, Maria Sklodowska-Curie National Research Institute of Oncology Warsaw Poland

[18]Translational Research Unit, Section of Cellular Therapy, Department of Oncology, Oslo University Hospital, Oslo, Norway

[19]Department of Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA

[20]Pediatric Hemato-Oncology Department, La Paz University Hospital, Madrid, Spain

[21]CIBERER-ISCIII, IdiPAZ-CNIO Translational Research Unit in Pediatric Hemato-Oncology, La Paz University Hospital Research Institute, Spanish National Cancer Center, Madrid, Spain

[22]Institute of Medical and Molecular Genetics (INGEMM), La Paz University Hospital, Madrid, Spain

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

[24]Pediatric Department, School of Medicine, Autonomous University of Madrid, Spain

PROGRAMA 3. TERAPIA CELULAR SOMÁTICA Y MEDICINA REGENERATIVA

SESIONES

A PHASE III, MULTICENTER, RANDOMIZED, CONTROLLED, OPEN-LABEL WITH BLINDED-ENDPOINT, CLINICAL TRIAL TO ASSESS THE EFFICACY OF INTRA-ARTERIAL INFUSION OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS IN PATIENTS WITH ISCHEMIC STROKE. RD24/0014/0005, RD24/0014/0027, RD24/0014/0030, RD24/0014/0035, RD24/0014/0040.

Gloria Carmona-Sánchez[1,2,†], Blanca Quijano-Ruiz[1,†], Olga Fernández-López[1], Rosario Jiménez-Moreno[3], María Martín-López[2], Blanca Arribas-Arribas[2], Mónica Santos-González[3], Miguel Ángel Montiel-Aguilera[2], Sonia Nogueras[3], Elisabet Aguilar-Bohorquez[1], María del Mar Macías-Sánchez[1], Virginia Escamilla[4], Vanesa Martín[5], Nancy Rodríguez Torres[4], María Eugenia Fernández-Sántos[6], Miguel Blanquer-Blanquer[7], Concha Herrera-Arroyo[3,5], Andrés García Pastor[8], Joaquín Zamarro Parra[9], Miriam Fernández Gómez[10], Reyes de Torres[11], Benito Galeano Bilbao[12], María José Álvarez[13], Francisco Moniche[14]

[1]Red Andaluza de diseño y traslación de Terapias Avanzadas-RAdytTA, Fundación Pública Andaluza Progreso y Salud M.P., Sevilla, España

[2]Unidad de Producción y Reprogramación Celular de Sevilla (UPRC)-Planta CTTC Campus Virgen del Rocío de Sevilla, Red Andaluza de diseño y traslación de terapias Avanzadas, Servicio Andaluz de Salud/Fundación Pública Progreso y Salud

[3]Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC), Unidad de Terapia Celular, Hospital Universitario Reina Sofía de Córdoba, Red Andaluza de diseño y traslación de terapias Avanzadas, Servicio Andaluz de Salud/Fundación Pública Andaluza Progreso y Salud M.P.

[4]Departamento de Hematología, Hospital Universitario Virgen del Rocío de Sevilla. Instituto de biomedicina de Sevilla (IBIS)

El ictus isquémico constituye una de las principales causas de discapacidad adquirida en adultos. Aunque la trombólisis intravenosa y la trombectomía mecánica han mejorado significativamente su abordaje en fase aguda, una proporción relevante de pacientes mantiene secuelas funcionales, por lo que resulta necesario desarrollar nuevas estrategias neuroreparadoras. En este contexto, las células mononucleares autólogas de médula ósea representan una alternativa de interés por su potencial para modular la respuesta inflamatoria y favorecer mecanismos asociados a la angiogénesis, la neuroplasticidad y la reparación cerebral. Estudios clínicos previos han demostrado la seguridad y viabilidad de su administración intraarterial durante la fase aguda del ictus y han aportado indicios de un posible beneficio funcional en pacientes con afectación moderada o grave.

IBIS-2, identificado mediante el código de protocolo CMMo-Ictus-2026 y el número EuCT 2026-527126-12-00, es un ensayo clínico académico fase III, multicéntrico, prospectivo, aleatorizado, controlado, abierto y con evaluación ciega de los resultados. El estudio incluirá 168 pacientes adultos con ictus isquémico agudo en el territorio de la arteria cerebral media, con un déficit neurológico de entre 6 y 20 puntos en la escala NIHSS. Los participantes serán asignados en proporción 1:1 al grupo control, que recibirá el tratamiento estándar, o al grupo experimental, que recibirá una dosis de 2×10^6 células mononucleares autólogas de médula ósea por kilogramo de peso corporal mediante infusión intraarterial.

En el grupo experimental se realizará una punción de cresta ilíaca para la obtención de médula ósea. El producto celular se obtendrá mediante centrifugación en gradiente de densidad, sin expansión ni modificación de su función biológica, y será administrado mediante microcatéter en la arteria cerebral media ipsilateral al infarto. La obtención, fabricación y administración deberán completarse dentro de los siete días posteriores al inicio del ictus, lo que exige una coordinación estrecha entre los equipos clínicos, las unidades de producción, el transporte del medicamento y las áreas de neurorradiología intervencionista.

El objetivo principal es evaluar la eficacia del tratamiento sobre la recuperación funcional a los tres meses, medida mediante la proporción de pacientes que alcancen independencia funcional, definida como una puntuación de 0 a 2 en la escala de Rankin modificada. Como objetivos secundarios se evaluarán la seguridad y factibilidad del tratamiento, la evolución funcional y neurológica

a los tres y seis meses, y los posibles cambios asociados a la terapia mediante neuroimagen avanzada y biomarcadores plasmáticos.

Un elemento diferencial del ensayo es su modelo colaborativo de fabricación descentralizada en el marco de la Red TERAV. El protocolo contempla inicialmente la participación de la Unidad de Terapia Celular del Hospital Universitario Reina Sofía de Córdoba y de la Unidad de Producción y Reprogramación Celular de Sevilla, ubicada en la Planta Centro de Transfusión, Tejidos y Células de Sevilla. Debido a la complejidad logística del estudio y a la necesidad de garantizar la fabricación y administración dentro de la ventana terapéutica de siete días, se prevé ampliar la red de fabricantes incorporando también la sala GMP del Hospital General Universitario Gregorio Marañón de Madrid y la sala GMP del Hospital Clínico Universitario Virgen de la Arrixaca de Murcia. Esta organización permitirá aproximar la fabricación al paciente, reforzar la capacidad operativa del estudio y facilitar la incorporación de nuevos hospitales participantes.

IBIS-2 representa, por tanto, un proyecto colaborativo de especial relevancia para TERAV, no solo por su carácter multicéntrico, sino también por la articulación de un modelo coordinado de fabricación descentralizada de un medicamento de terapias avanzadas de origen autólogo, condicionado por una ventana terapéutica limitada. El ensayo permitirá evaluar la eficacia clínica del tratamiento y, al mismo tiempo, generar experiencia sobre la capacidad de la red para abordar estudios académicos complejos que requieren coordinación asistencial, productiva, logística y regulatoria entre diferentes comunidades autónomas**.**

[5]Servicio de Hematología y Hemoterapia. Hospital Reina Sofia. Instituto Maimonides de Investigacion (IMIBIC)

[6]Unidad de Producción de Medicamentos de Terapias Avanzadas (UP-MTAS). Instituto de Investigación Hospital Gregorio Marañon (IISGM). Madrid

[7]Hematología Hospital C.U. Virgen de la Arrixaca. IMIB-Pascual Parrilla. Universidad de Murcia.

[8]Sección de Neurología Vascular - Centro de Ictus, Hospital General Universitario Gregorio Marañón. Instituto de investigación Sanitaria Gregorio Marañón (IISGM), Madrid.

[9]Sección de Neurorradiología Intervencionista, Servicio de Radiología, Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia. Instituto Murciano de Investigación Biosanitaria (IMIB) Pascual Parrilla, Murcia.

[10]Hospital Universitario Torrecárdenas de Almería

[11]Sección de Neurología Vascular - Centro de Ictus, Hospital General Universitario Gregorio Marañón. Instituto de investigación Sanitaria Gregorio Marañón (IISGM), Madrid.

[12]Hospital Universitario Puerta del Mar de Cádiz

[13]Hospital Universitario Reina Sofía de Córdoba

[14]Departamento de Neurología. Hospital Universitario Virgen del Rocío de Sevilla. Laboratorio Neurovascular, Instituto de Biomedicina de Sevilla-IBiS, Sevilla.

LABORATORIOS GMP FABRICANTES

En primera etapa del ensayo:
(1) Unidad de Producción y Reprogramación Celular de Sevilla – Planta Centro de Trasfusión, tejidos y Células de Sevilla y (2) Unidad de Terapia Celular del Hospital Universitario Reina Sofía de Córdoba
En una segunda etapa del ensayo:
(3) Hospital Gregorio Marañón de Madrid y (4) Hospital Clínico Universitario Virgen de la Arrixaca de Murcia.

MSCS FOR ACUTE GRAFT-VERSUS-HOST DISEASE IN SPAIN: A NATIONWIDE MULTICENTER STUDY INTEGRATING CLINICAL OUTCOMES AND PRODUCT CHARACTERISTICS.

RD24/0014/0001, RD24/0014/0006, RD24/0014/0008, RD24/0014/0010, RD24/0014/0013, RD24/0014/0014, RD24/0014/0021, RD24/0014/0024, RD24/0014/0027, RD24/0014/0030, RD24/0014/0034, RD24/0014/0035, RD24/0014/0036, RD24/0014/0037, RD24/0014/0040.

Background: Mesenchymal stromal cells (MSCs) have been used in Spain for acute graft-versus-host disease (aGvHD) for more than two decades, but integrated national data linking clinical outcomes to product characteristics remain limited. This collaborative GETCC/GETH-TC/TERAV+ project aims to evaluate effectiveness and safety and explore clinical and product-related factors associated with treatment outcomes.

Methods: This nationwide, retrospective, multicenter observational study will include adult and pediatric allogeneic hematopoietic cell transplant recipients with grade II–IV steroid-refractory, steroid-dependent or steroid-intolerant aGvHD treated with MSCs between 2010 and 2024. National data on compassionate-use requests provided by the Spanish Agency of Medicines and Medical Devices (AEMPS) were used to identify requesting centers and MSC manufacturing facilities. Patient eligibility will be confirmed through review of local records. Clinical data from participating transplant centers and manufacturing data from TERCEL-TERAV+ GMP facilities will be collected through a standardized REDCap electronic case report form. Product characterization will include tissue source, donor characteristics, culture conditions, passage number, cryopreservation and handling before infusion, cell dose, viability, immunophenotype and available potency assays. These data will be integrated with transplant characteristics, aGvHD severity and organ involvement, previous treatment lines and corticosteroid exposure. The primary endpoint is overall response, defined as complete or partial response, at day +28 after the first MSC infusion. Secondary endpoints include overall response at day +90, organ-specific response at days +28 and +90, corticosteroid sparing, infusion reactions, grade ≥3 infections and serious adverse events through day +90, overall and failure-free survival, and non-relapse mortality. Exploratory analyses will assess associations between clinical and product variables and response, safety and survival. The study has received a favorable opinion from the Salamanca Research Ethics Committee (PI2025122140). A common data collection framework has been defined to integrate information from transplant centers and MSC manufacturing facilities.

Expected contribution: This project aims to integrate clinical experience across Spain with detailed MSC product characterization to generate evidence on effectiveness and safety in aGvHD. Exploring the relationship between patient characteristics, manufacturing variables and clinical outcomes may help refine patient selection, inform manufacturing strategies and guide subsequent collaborative studies within TERAV+, GETCC and GETH-TC. These findings may also contribute to assess the feasibility of future applications for national authorizations of use under the hospital exemption framework for specific MSC products.

PATHWAY FOR QUALIFICATION AND CLINICAL APPLICATION OF A 3D HUMAN BIOLOGICAL VENTRICULAR ASSIST DEVICE FOR ENDSTAGE HEART FAILURE. RD24/0014/0010, RD24/0014/0035.

Manuel M. Mazo Vega, Olalla Iglesias-García, María Eugenia Fernández Santos, Enrique Andreu, Ascensión López, Susana Inogés, Felipe Prósper.

Clínica Universidad de Navarra, Instituto de investigación Sanitaria Gregorio Marañón.

The following presentation aims to provide an overview of a recently funded Horizon Project coordinated by Clínica Universidad de Navarra, centred around performing the qualification of the first 3D printed cardiac engineered tissue for clinical application and the development of a First-in-human trial. The project summary is as follows: Heart failure (HF) is an end-stage condition affecting over a million EU citizens. In spite of intense medical care and medication, advanced HF patients can only be offered a mechanical ventricular support device (LVAD) or heart transplant, making HF an unmet clinical need. The H2020 BRAV3 employed an advanced 3D printing technology, melt electrowriting (MEW) and cutting-edge computational models of the heart to design and fabricate a novel 3D scaffold enabling efficient force transmission. The project developed upscaled (80% reduction in costs) production of human induced pluripotent stem cell (hiPSC)-derived cardiac cells. BRAV3 biofabricated a biological ventricular assist device (BioVAD) by populating the trizonal MEW scaffold with hiPSC-derived cardiomyocytes and cardiac fibroblasts, showing a significant benefit upon cardiac function, in a clinically-relevant model of HF in pig. BRAV3 developed an initial economic framework for the translation of this regenerative therapeutic. PULSE will bring this technology to clinical application within the project's lifetime. We will qualify all procedures to regulatory standards: MEW 3D printing under ISO in clean room, and the hiPSC-cardiac cell and BioVAD production under GMP. We will validate the safety and efficacy of the BioVAD in a GLP-porcine model of HF, exploring 2 ways of dose-scalation, and use digital tools to sustain design improvement and safety screening. Finally, an open label phase 1b study will evaluate safety and feasibility BioVAD implantation as a regenerative therapy in HF patients. PULSE will deploy an intense regulatory oversight to assure the clinical study. A detailed business model-exploitation framework and market analysis will ensure BioVADs economically viable. Patients' views will be analysed through focus groups, feeding into the Dissemination and Communication strategy. PULSE proposes an enterprising, dynamic and technologically advance to break the stalemate in HF treatment.

ENSAYO CLÍNICO FASE IIB PARA EVALUAR LA EFICACIA Y SEGURIDAD DEL TRATAMIENTO CON CÉLULAS MESENQUIMALES (MSC) ALOGÉNICAS EN INYECCIÓN OCULAR COMO TERAPIA DE LA ENFERMEDAD DE INJERTO CONTRA RECEPTOR (EICR) REFRACTARIA A TRATAMIENTOS CONVENCIONALES. (TERAV-EYE). RD24/0014/0034 Y RD24/0014/0035.

María Eugenia Fernández Santos (presentadora)[1], Margarita Calonge[2], Lourdes Vázquez[3], Mi Kwon[1], Esperanza López Franco[3], Marina López Paniagua[2], José María Herreras[2], Lidia Cocho[2], Emiliano Hernández-Galilea[3], Lucía Ibáres[1], Rebeca Bailén[1], Amalia Enríquez de Salamanca[2], Lucía López Corral[3], Susana Suárez Sancho[1], Fermín Sánchez-Guijo[3]

[1]Instituto de Investigación Sanitaria Hospital Gregorio Marañón (IISGM), Hospital General Universitario Gregorio Marañón, Universidad Complutense de Madrid.

[2]Instituto de Oftalmobiología Aplicada (IOBA), Hospital Clínico Universitario de Valladolid, Universidad de Valladolid

[3]Instituto de Investigación Biomédica de Salamanca, Hospital Universitario de Salamanca, Universidad de Salamanca.

La enfermedad del injerto contra el receptor crónica (EICRc) constituye una de las principales complicaciones del trasplante alogénico de células progenitoras hematopoyéticas (aloTPH), con un importante impacto sobre la supervivencia y la calidad de vida de los pacientes. La afectación ocular es frecuente y puede manifestarse como enfermedad de ojo seco (EOS) grave, secundaria al daño inflamatorio e inmunológico de la unidad funcional lagrimal, generando síntomas incapacitantes y riesgo de deterioro visual significativo. La EOS relacionada con EICRc se caracteriza por inflamación crónica, alteración de la homeostasis lagrimal y daño progresivo de los tejidos de la superficie ocular y de las glándulas secretoras de lágrima. Aunque existen múltiples tratamientos disponibles, incluyendo lubricación, oclusión lagrimal, derivados hemáticos, derivados biológicos, terapias antiinflamatorias e inmunomoduladoras tópicas, los casos graves continúan representando un importante desafío clínico. Las MSC han demostrado propiedades inmunorreguladoras, inmunosupresoras, antiinflamatorias y regenerativas. Diversos estudios evidencian su capacidad para inhibir la proliferación y activación de linfocitos T, B y NK, modular macrófagos y células dendríticas, y reducir la producción de citocinas proinflamatorias implicadas en el daño corneal. Asimismo, favorecen la regeneración tisular mediante el estímulo de la proliferación epitelial corneal, la diferenciación hacia células limbo-corneales, la recuperación de células caliciformes conjuntivales y la mejora estructural de las glándulas lagrimales.

Se plantea el EC TERAV-EYE (EudraCT 2024-519772-10-00), que incluirá 30 pacientes randomizados a dos brazos, de 15 pacientes cada uno, tratados con 6,25 x 10^6 y 12,5 x 10^6 MSC de tejido adiposo (ASCs) alogénicas, respectivamente. El objetivo principal es evaluar la eficacia y la seguridad de la inyección subconjuntival de ASCs en pacientes con EICRc y con afectación ocular (EOS grave), mediante la valoración de la mejoría clínica y estadísticamente significativa de los signos y síntomas propios de la enfermedad. Además, se analizarán como objetivos secundarios: los acontecimientos adversos relacionados con la aplicación de la inyección subconjuntival de ASC en pacientes con EICRc y con afectación ocular (EOS grave), establecer nuevos biomarcadores que puedan ser utilizados para valorar de una forma objetiva la evolución de este tipo de pacientes y estudiar la relación de la expresión de marcadores propios de la superficie ocular por parte de las ASCs con su eficacia clínica.

Con este ensayo se pretende demostrar que las MSC provenientes de tejido adiposo representan una alternativa terapéutica prometedora para pacientes con EICRc y afectación ocular severa. Su capacidad para modular la respuesta inmunitaria, reducir la inflamación y promover la regeneración de los tejidos oculares dañados las posiciona como un potencial medicamento de terapia avanzada para el tratamiento de la EOS refractaria asociada a esta enfermedad. Su aplicación clínica podría contribuir a mejorar la función visual, la calidad de vida y el pronóstico ocular de estos pacientes.

Financiación: ISCIII (ICI23/00027).

PROGRAMA 3. TERAPIA CELULAR SOMÁTICA Y MEDICINA REGENERATIVA

PÓSTERES

ADVANCED THERAPY USED AS A POTENTIAL TREATMENT WITHIN THE NATIONAL PILOT FERTILITY PRESERVATION PROGRAM FOR PERIPUBERTAL AND PREPUBERTAL CHILDREN WITH CANCER AND GENETIC SYNDROMES IN OSAKIDETZA. RD21/0017/0024, RD21/0017/0018 Y RD24/0017/0025, RD24/0014/0026.

Martin-Inaraja, Myriam[1]; Herrera, Lara[1,8]; Santos, Silvia[1,8]; Solorzano, Estibaliz[2]; Lopez-Almaraz, Ricardo[2]; Prieto, Maria Begoña[3]; Matorras, Roberto[3]; Chueca, Maria[4]; Somoza, Iván[5]; Perez-Martinez A[6,8], Corral-Sanchez, Dolores[6]; Dominguez-Riscart, Jesús[7]; Eguizabal, Cristina[1,8]

[1]Advanced Therapies Unit, Basque Center for Blood Transfusion and Human Tissues, Osakidetza, Galdakao 48960, Spain;Cell Therapy, Stem Cells and Tissues Group, Biobizkaia Health Research Institute, Barakaldo 48903, Spain;

[2]Biobizkaia Health Research Institute-Cruces University Hospital, Pediatric Department, Barakaldo 48903, Spain.

[3]Biobizkaia Health Research Institute-Cruces University Hospital-Basque Country University-IVI Bilbao, Human Reproduction Unit, Barakaldo 48903, Spain.

[4]Paediatric Endocrinology Unit, Complejo Hospitalario de Navarra. C/Irunlarrea, 3. 31008 Pamplona, Spain.

[5]Pediatric Urology, Department of Pediatric Surgery, Children's Hospital Teresa Herrera, Complexo Hospitalario Juan Canalejo, A Coruna, Spain

[6]Pediatric Onco-Hematology Department, La Paz University Hospital, Madrid, Spain.

[7]Pediatric Endocrinology, Department of Pediatrics, Puerta del Mar University Hospital, 11009 Cádiz, Spain

[8]Red Española de Terapias Avanzadas (TERAV and TERAV+), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain

CENTROS PARTICIPANTES**:** BioBizkaia Health Research Institute-CVTTH, Cruces University Hospital, La Paz University Hospital, Complejo Hospitalario de Navarra, Puerta del Mar University Hospital y Children's Hospital Teresa Herrera, Complexo Hospitalario Juan Canalejo

Some of the cancer treatments for oncologic peripubertal and prepubertal children and children with genetic syndromes, such as Klinefelter Syndrome (KS), can cause fertility problems in adulthood. Adult males have the option to cryopreserve sperm before starting such treatments. However, peripubertal and prepubertal patient (those who have not reached puberty) do not have this option, so to the date, there is no alternative to restore the fertility in the future. Currently, there are three strategies to bring back the fertility of these patients: 1) in vitro expansion and autotransplantation of spermatogonial stem cells (SSCs), 2) autotransplantation of testicular biopsy and 3) in vitro spermatogenesis. We collected and processed testicular biopsies of both adult, peripubertal and prepubertal patients with fertility problems. Each biopsy is divided into 3 fragments: for histological study, for clinical use and for research. This fertility preservation program is centralized at Basque Center for Blood Transfusion and Human Tissues in Osakidetza and participates several hospitals of Spain. Oral and written information is given and all of them signed an informed consent. We have collected and processed 60 adult testicular biopsies from azoospermic patients, 27 of KS patients, 18 oncologic patients and 8 with other fertility related pathologies. We have observed that the majority of adult patients, all oncologic patients and 50% of KS peripubertal and prepubertal patients express both germ cell markers (VASA and MAGEA4) by immunofluorescence. In conclusion, we achieved to set a national fertility preservation program for boys with cancer and genetic syndromes such as Klinefelter Syndrome in Osakidetza. It is important to create guidelines or recommendations so these patients can benefit from these experimental programs for future stem cell therapy to restore fertility.

TOPICAL USE OF EXOSOMES FROM HUMAN MATERNAL MILK ACCELERATES ACUTE CUTANEOUS WOUND HEALING. RD24/0014/0009, RD24/0014/0012.

Leire Gardeazabal[1], Laura Yndriago[1], Alejandra Crespo-Barreda[2], Javier Aldazabal[3], Jacobo Paredes[3], Ander Izeta[1,3*] and Pilar Martin-Duque[2*]

[1]Biodonostia Health Research Institute, San Sebastián, Spain

[2]Instituto de Salud Carlos III, Madrid, Spain

[3]Tecnun, School of Engineering, University of Navarra, San Sebastián, Spain.

*Both authors have contributed equally.

Correspondence to: ANDER. IZETAPERMISAN@osakidetza.eus

Cutaneous wound healing is a complex but highly efficient process. Under certain pathologic conditions healing may be impaired, thus lengthening the process and eventually leading to repeated medical intervention and wound chronicity. Exosomes (secreted extracellular vesicles) are present in biological fluids where they exert a key role in intercellular communication at the tissue, organ and organismal levels. Since human breast milk presents abundant maternal extracellular vesicles (MEVs) with immunomodulatory and pro-regenerative properties, in the present study we aimed to evaluate if topical application of MEVs into open wounds would be beneficial for healing. Excision full-thickness skin wounds of 4-mm diameter were generated in the dorsal skin of C57BL/6 mice, and 20 g human MEVs isolated at breastfeeding weeks 9, 11, 12 and 15 postpartum were topically placed onto the wounds. Control wounds were vehicle-treated. Macroscopic measurements up to 7 days post-wounding revealed that MEV-treated wound area significantly decreased as compared with controls. Histological analyses at day 7 post-wounding showed no differences between groups in the granulation tissue area. However, dermal integrity of the wounds appeared to vary under MEV treatments isolated at different breastfeeding weeks. Our preliminary results suggest that MEV treatment at certain breastfeeding weeks may induce faster wound closure but also granulation tissue aberration.

Acknowledgments: Authors are grateful to the scientific-technical services for their support and to the funding agencies and grants TERAV+ and AESI 2024 PI24CI-II/00046; CNS2023-145442.

PROGRAMA DE USOS COMPASIVOS CON ASCS EN CAUSTICACIÓN OCULAR. RD24/0014/0014 Y RD24/0014/0035.

June Artaechevarria, Ignacio Jiménez Alfaro, María Eugenia Fernández, Damián García Olmo y Mariano García Arranz.

Instituto de Investigación Sanitaria Fundación Jiménez Díaz (FJD)

Instituto de investigación Sanitaria Gregorio Marañón (IiSGM).

El Servicio de Oftalmología de la Fundación Jiménez Díaz en colaboración con la sala de producción celular del Instituto de Investigación Hospital Universitario Gregorio Marañón ha desarrollado, tras el ensayo clínico CELOPHIN (tratamiento de Penfigoide ocular cicatricial) con células troncales mesenquimales derivadas del tejido adiposo alogénicas, un programa de usos compasivos para el tratamiento de la causticación ocular.

Durante este último año se han tratado 2 pacientes diagnosticados de causticación ocular severa, en un caso asociada a sosa caustica y el otro asociado a una quemadura por cemento. Tras la ausencia de respuesta a tratamiento médico estándar e implantes de membrana amniótica, se valoró la opción de inyección de células madre subconjuntival. El tratamiento consistió en ambos casos en inyección subconjuntival de 0.4mL por cuadrante ocular y 0.4mL de instilación tópica sobre la superficie ocular. En el postoperatorio recibieron corticoterapia y antibioterapia tópica, así como suero autólogo. Una vez realizado el tratamiento, de forma ambulatoria, los pacientes fueron revisados en consulta a las 24h, a los 7 días, 1 mes, 6 meses y 1 año.

Durante el postoperatorio se observó una reducción de la inflamación ocular y una detención del crecimiento vascular periférico. Además, se observó una re-epitelización corneal progresiva con resolución del defecto epitelial corneal. Como principales resultados podemos indicar la ausencia de efectos adversos en ambos casos, una reducción significativa de la inflamación ocular que conllevó una mejoría en la visión preservando la estructura de la superficie ocular, y una mejor calidad de vida expuesta por los pacientes.

Conclusión. A pesar del bajo número de pacientes, el programa de usos compasivos de ASCs en causticación ocular se ha demostrado como una terapia segura, y parece que indicar una tendencia a la eficacia, demostrando una mejoría que deberá ser confirmada mediante un ensayo clínico en el futuro.

ABSTRACT

The Ophthalmology Department of the Fundación Jiménez Díaz, in collaboration with the Cell Production Unit of the Gregorio Marañón University Hospital Research Institute, has developed a compassionate use program for the treatment of ocular chemical burns, following the CELOPHIN clinical trial (treatment of ocular cicatricial pemphigoid) using allogeneic adipose-derived mesenchymal stem cells (ASCs).

Over the past year, two patients diagnosed with severe ocular chemical burns were treated: one case involved sodium hydroxide (caustic soda) injury and the other was associated with a cement burn. Following a lack of response to standard medical treatment and amniotic membrane transplantation, subcon-

junctival stem cell injection was considered as a therapeutic option. In both cases, the treatment consisted of a subconjunctival injection of 0.4 mL per ocular quadrant, combined with a 0.4 mL topical instillation onto the ocular surface. Postoperatively, patients received topical corticosteroids and antibiotics, as well as autologous serum. After undergoing the procedure on an outpatient basis, the patients were evaluated in the clinic at 24 hours, 7 days, 1 month, 6 months, and 1 year.

During the postoperative period, a reduction in ocular inflammation and an arrest of peripheral vascular proliferation were observed. Furthermore, progressive corneal re-epithelialization was achieved, with resolution of the corneal epithelial defect. The main outcomes included the absence of adverse effects in both cases, a significant reduction in ocular inflammation—which led to visual improvement while preserving the structure of the ocular surface—and an enhanced quality of life reported by the patients.

Conclusion: Despite the small sample size, the compassionate use program of ASCs for ocular chemical burns has proven to be a safe therapy and appears to indicate a trend toward efficacy, demonstrating an improvement that must be confirmed by a future clinical trial.

PROGRAMA 4. INNOVACIONES TECNOLÓGICAS Y BIOMODELOS

SESIONES

ENGINEERING HUMAN CARDIAC ORGANOIDS AS IN VITRO MODELS TO STUDY CARDIAC DEVELOPMENT AND DISEASE. RD24/0014/0026, RD24/0014/0010, RD24/0014/0035.

Daniel Moya-Rull[1], Maria Gallo[1], Maria Kalil[1], Carolina Tarantino[1], Asier Ullate-Agote[2,3,4], Alberto Centeno[5], Maria Eugenia Fernández-Santos[6], Felipe Prosper[2,3,4], Elena Garreta[1], Nuria Montserrat[1].

[1]Pluripotency for organ regeneration (PR Lab), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

[2]Área de Hemato-Oncología, Centro de Investigación Médica Aplicada, Instituto de Investigación Sanitaria de Navarra (IDISNA), Universidad de Navarra, Pamplona, Spain

[3]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain

[4]Departamento de Hematología, Clínica Universidad de Navarra, Universidad de Navarra, Pamplona, Spain

[5]Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario A Coruña (CHUAC), Sergas, Universidade da Coruña (UDC), As Xubias, A Coruña, Spain.

[6]Department of Cardiology, Hospital General Universitario Gregorio Marañón, Madrid, Spain. ATMPs Production Unit, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain.

The development and use of human pluripotent stem cells (hPSCs) represent an effective tool to recapitulate characteristics related to tissue differentiation, morphogenesis and conversely, human disease. Here, we combined CRISPR/Cas9 genome engineering, hPSC differentiation, organoid technology, and biomimetic extracellular matrix (ECM) approaches to develop novel human cardiac models. Using the inducible iCRISPR2 (iC2) platform, we generated and characterized hPSC reporter lines recapitulating the endogenous expression of the cardiac markers MYH6, MYL2, and SIRPA. These lines have been exploited to define new approaches for the generation of self-assembled cardiac-like organoids which have been characterized at the transcriptomic level by single cell RNA sequencing, conventional molecular biology techniques, and confocal microscopy analysis. Moreover, to improve tissue differentiation and maturation, we developed biomimetic hydrogels derived from decellularized extracellular matrix (dECM) obtained from heart tissues. Cardiac dECM-based hydrogels were incorporated into 2D and 3D cardiac differentiation approaches and supported cardiomyocyte generation while promoting the acquisition of maturation-associated features. Overall, this work shows that exploiting cell-to-cell and cell-to-ECM interactions during the onset of cardiac differentiation allows the establishment of physiologically relevant cardiac organoid models exhibiting enhanced differentiation-features. The new procedures and tools developed here will enlarge the capacity of the IBEC Unit from the Platform for Biobanks and Biomodels from the Institute of Health Carlos III. Furthermore, all the techniques and procedures described here will also help advancing our current understanding on the generation of preclinical models to understand human disease and contribute to the progress of TERAV+ network in the context of Objective 4 entitled “Technological Innovations and Biomodels”.

MATRIZ CARDÍACA DECELULARIZADA Y RECELULARIZADA PARA EL TRATAMIENTO DE INSUFICIENCIA CARDÍACA AVANZADA. RD24/0014/0008, RD24/0014/0010, RD24/0014/0026, RD24/0014/0035.

Vázquez Álvarez, M[1]; Acosta Ocampo, A[1]; Plasencia Martín, V[1]; Suárez Sancho, S[1]; Sanz Ruíz, R[1]; Bermejo, J[1]; Garreta Bahima E[2], Vicente López, MA[3]; Prósper Cardoso, F[4]; Fernández Santos, ME[1]

[1]HGUGM. Departamento de Cardiología, Instituto de Investigación Sanitaria Gregorio Marañón, Hospital General Universitario Gregorio Marañón.

[2]IBEC. Pluripotency for organ regeneration Department. Instituto de Bioingeniería de Cataluña. Barcelona.

[3]UCM. Departamento de Biología Celular, Facultad de Medicina, Universidad Complutense de Madrid.

[4]CIMA. Hematología y Terapia Celular, Clínica Universidad de Navarra, Centro de Investigación Médica Aplicada 2.

Las enfermedades cardiovasculares constituyen la principal causa de muerte a nivel mundial, siendo el infarto de miocardio una de las afecciones con mayor mortalidad. Una gran parte de los pacientes que sufren un infarto de miocardio acaba desarrollando cierto grado de insuficiencia cardíaca. En los casos más avanzados y de peor pronóstico, el único tratamiento efectivo es el trasplante de corazón. Dada la escasez de corazones disponibles, el riesgo de rechazo inmunológico y las limitaciones de los dispositivos de asistencia ventricular, los medicamentos de terapia avanzada (MTA) surgen como una alternativa prometedora para reparar el miocardio dañado en pacientes con insuficiencia cardíaca avanzada.

Este estudio evalúa, como posible medicamento de terapia avanzada frente al daño miocárdico localizado, un hidrogel obtenido de matriz extracelular decelularizada derivada de tejido miocárdico que conserva parte de la composición biológica y estructural del tejido nativo. Este biomaterial permite administrar y retener, gracias a su gelificación a temperatura fisiológica, células, vesículas y/o factores en la zona lesionada para inducir la reparación tisular, además de servir como biotinta para la bioimpresión 3D de parches celulares. Para garantizar su integración funcional es primordial evaluar la distribución y viabilidad celular en su interior. Para ello, en este trabajo se estudia la citotoxicidad *in vitro* del hidrogel utilizando células madre mesenquimales derivadas de tejido adiposo y células cardíacas residentes procedentes de biopsias auriculares. Se evalúa cómo afecta la concentración de hidrogel a la viabilidad celular y, posteriormente, utilizando el hidrogel combinado con células como biotinta, se analiza la viabilidad celular en parches gelificados post bioimpresión 3D, así como la distribución tridimensional y la migración celular en el interior del hidrogel. Todo ello, tras verificar previamente una correcta decelularización con el fin de prevenir el rechazo inmunológico y confirmar el mantenimiento de componentes estructurales clave. El hidrogel muestra cualidades *in vitro* prometedoras para una potencial administración mínimamente invasiva mediante cateterismo, destacando su adecuada gelificación y retención celular, y una alta viabilidad celular a corto y largo plazo.

En conclusión, este posible medicamento de terapia avanzada presenta resultados *in vitro* optimistas para la reparación de lesiones cardíacas localizadas. Con análisis complementarios de composición proteica, microestructura, propiedades reológicas, así como la evaluación de la expresión génica celular en el interior del hidrogel (col. Grupo Dr. Prósper), inmunogenicidad (col. Grupo Dra. Vicente) o, incluso, la incorporación de cardiomiocitos (col. Grupo Dr. Prósper), se pretende obtener un MTA aplicable inicialmente en modelo animal y, a futuro, en pacientes. Dicho MTA podría reducir la dependencia de trasplantes o dispositivos de asistencia ventricular en pacientes con insuficiencia cardíaca avanzada al reparar el daño tisular localizado manteniendo o incrementando el músculo funcional de la pared ventricular.

DEVELOPMENT OF LIPID NANOPARTICLES FOR NON-VIRAL CAR-T CELL ENGINEERING. RD24/0014/0010, RD24/0014/0041.

Amaia Arancón, Blanca Escriche, Elisa Garbayo, Pedro Justicia-Lirio, Saray Rodríguez-Díaz, Rebeca Martínez-Turrillas, Antonio Pineda, Juan Roberto Rodríguez-Madoz, Felipe Prósper, María Blanco-Prieto.

Instituto de Investigación Sanitaria de Navarra, Clínica Universidad de Navarra.

CENTROS PARTICIPANTES: IdisNA, CUN.

Chimeric antigen receptor (CAR)-T cell therapy has transformed the treatment of hematological malignancies, including leukemia, lymphoma and multiple myeloma. However, current manufacturing approaches rely primarily on *ex vivo* genetic modification of T cells using viral vectors, which involves complex, costly and time-consuming procedures.[1,2] Lipid nanoparticles (LNPs) represent a promising non-viral alternative for the delivery of genetic material to T cells, with the potential to enable CAR-T cell generation *in vivo* and simplify current manufacturing processes.[1,3]

This project aims to develop and evaluate LNP-based strategies for transient and stable CAR-T cell generation. Two complementary approaches are being investigated. The first one, evaluating transient CAR expression using mRNA encoding a B-cell maturation antigen (BCMA)-specific CAR, and the second one, testing stable genetic modification using the *Sleeping Beauty* transposon system combining a minicircle DNA encoding a CD19-specific CAR and an mRNA encoding the hyperactive transposase (SB100X).[4-6]

Initial studies have focused on the transient CAR expression strategy. LNPs formulated with the ionizable lipid C14-4 were prepared by microfluidics and characterized in terms of their physicochemical properties. The formulations were subsequently evaluated in primary T cells to optimize LNP dose, administration schedule and cell activation conditions. Preliminary results showed that repeated LNP administration increased CAR expression, reaching approximately 20% CAR-positive cells after four successive administrations in both activated and non-activated primary T cells. These findings demonstrate the ability of the developed LNPs to mediate transient CAR expression in primary T cells.

Ongoing work focuses on functionalizing LNPs with an anti-CD3 nanobody to improve T cell-specific targeting. The functionalized formulations will be evaluated in peripheral blood mononuclear cells to assess transfection efficiency and targeting specificity. In parallel, the *Sleeping Beauty*-based strategy will be investigated to evaluate the potential of LNPs to mediate stable genetic modification of T cells.

Overall, these preliminary findings support the potential of LNPs as a non-viral platform for CAR-T cell engineering. Further optimization of T cell targeting and the development of stable gene integration strategies will contribute to advancing this approach towards *in vivo* CAR-T cell generation.

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ADVANCED ELECTROSPUN PCL SCAFFOLDS FUNCTIONALIZED WITH ALLOGENEIC PRP (ALOPRP3D) AS A BIOLOGICAL DRESSING FOR CHRONIC WOUND HEALING. RD21/0017/0024, RD21/0017/0018 Y RD24/0017/0025, RD24/0014/0026.

Álvarez N[1,2,4], Buruaga L[6], Silván U[3,5], Garreta E[3,7], Santos S[1-3], Jiménez-Rojo L[4], Eguizabal C[1-3]

[1]Cell Therapy, Stem Cells and Tissues Group, Biobizkaia Health Research Institute, Barakaldo, Spain

[2]Advanced Therapies Unit, Basque Center for Blood Transfusion and Human Tissues (CVTTH), Osakidetza, Usansolo, Spain.

[3]Red Española de Terapias Avanzadas (TERAV, TERAV+), Redes de Investigación Cooperativa Orientadas a Resultados en Salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[4]Departamento Biología Celular e Histología, Universidad de País Vasco (UPV/EHU), Leioa, España

[5]Basque Center for Materials, Applications and Nanostructures, Leioa, Spain.

[6]Departamento Mecánica y Producción Industrial, Mondragón Unibertsitatea, Mondragón, España

[7]Pluripotency for organ regeneration (PR Lab), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.

CENTROS PARTICIPANTES: Biobizkaia Health Research Institute, Centro Vasco de Transfusión y Tejidos Humanos (CVTTH), IBEC.

Chronic wounds remain a major clinical challenge, often failing to progress through the normal stages of healing due to persistent inflammation and the insufficient availability of key growth factors. Plasma Rich in Platelets (PRP) provides a concentrated source of bioactive molecules that promote tissue regeneration. However, its clinical use is limited by a short half-life and the lack of an effective delivery system. The main objective of this work is to develop and thoroughly characterize a novel, stable, and ready-to-use three-dimensional (3D) lyophilized biological dressing by combining an electrospun polycaprolactone (PCL) scaffold with allogeneic PRP, as an advanced strategy to accelerate chronic wound closure. PCL scaffolds were fabricated via electrospinning, functionalized with allogeneic PRP, and subsequently lyophilized. The resulting dressings underwent comprehensive physicochemical characterization (FTIR, contact angle), morphological evaluation (SEM), mechanical testing (tensile assay), and functional stability analysis (swelling behavior). Biological performance was assessed through a multi-level in vitro and in ovo approach, including cytocompatibility assays (XTT, Live/Dead) with HFF1 fibroblasts, cell migration (scratch assay), sensibilization assay (h-CLAT) with THP-1 human monocyte cell line, and angiogenic potential using the chorioallantoic membrane (CAM) model. The PCL–PRP scaffolds exhibited excellent cytocompatibility, mechanical robustness suitable for clinical handling, optimal fluid absorption, and significantly enhanced fibroblast proliferation and migration compared to controls. Importantly, the in ovo assay confirmed high biocompatibility and a marked pro-angiogenic effect. Ongoing studies involve the development of a 3D human skin model to evaluate the long-term regenerative efficacy of these scaffolds under physiologically relevant conditions. Altogether, this innovative PCL–PRP dressing represents a promising, scalable, and bioactive platform for advanced wound therapy, effectively combining the structural advantages of PCL with the sustained biological activity of allogeneic PRP to modulate the wound microenvironment and promote healing in chronic, non-resolving wounds.

PROGRAMA 4. INNOVACIONES TECNOLÓGICAS Y BIOMODELOS
PÓSTERES

EVALUATION OF ABCD1 NANOPLASMID-MEDIATED GENE DELIVERY IN AN IN VITRO MODEL OF X-LINKED ADRENOLEUKODYSTROPHY. RD24/0014/0022.

Claudia Pérez-García, Salvador Martínez

Instituto de Neurociencias UMH-CSIC.

X-linked adrenoleukodystrophy (X-ALD) is an inborn error of metabolism caused by mutations in ABCD1 gene, which encodes a peroxisomal transmembrane protein called ALDP, responsible of the transport of very-long chain fatty acid (VLCFA) for peroxisomal degradation. Impaired peroxisomal transport of these fatty acids contributes to the lipid imbalance associated with the disease. Understanding whether these cellular alterations can be modified is relevant for investigating potential therapeutic strategies. Patient-derived cellular models are essential not only to increase knowledge about this complex disease but also to find effective therapies. In this context, we established an in vitro model of X-ALD using human dental pulp-derived mesenchymal stem cells (hDPSCs) from an affected patient, which showed aberrant neutral lipid accumulation, a key characteristic from the disease. Based on this, we aimed to analyse changes in neutral lipid accumulation following the introduction of ABCD1-containing nanoplasmid constructs (A and B).

To evaluate changes in neutral lipid accumulation, BODIPY 493/503 staining and ALDP immunoreactivity were assessed. The analysis revealed differences in neutral lipid-associated fluorescence between basal X-ALD cells and cells associated with the different constructs, indicating that these conditions were accompanied by changes in the cellular lipid phenotype. In parallel, ALDP immunofluorescence was analysed descriptively and suggested a slight increase in protein expression associated with the nanoplasmid constructs.

Overall, these findings suggest that nanoplasmid-associated changes in neutral lipid accumulation and ALDP expression may contribute to the modulation of the diseaseassociated cellular phenotype observed in this patient-derived X-ALD model, providing a basis for further investigation of ABCD1-based therapeutic approaches.

PROGRAMA 5. FORMACIÓN Y CIENCIA REGULATORIA

PÓSTERES

QUALITY IMPLEMENTATION IN THE TRANSLATION OF RESEARCH INTO ADVANCED THERAPIES: A SURVEY-BASED APPROACH TO DEVELOPING AN INTEGRATED QUALITY FRAMEWORK. RD24/0014/0016, RD24/0014/0002, RD24/0014/0001, RD24/0014/0008, RD24/0014/0003, RD24/0014/0030, RD24/0014/0010, RD24/0014/0004, RD24/0014/0006, RD24/0014/0014, RD24/0014/0023, RD24/0014/0040, RD24/0014/0013, RD24/0014/0039, RD24/0014/0037, RD24/0014/0041.

Felipe Chiappe[1], África González-Fernández[2], Armando López-Guillermo[3], María Ángeles Vicente[4], Beatriz Martín[5], Beatriz Fernández[6], David Otaegui[7], Daniel Benítez[3], Diana Sanjurjo[8], Azucena González[3], Eva Gálvez[9], Fernando Torres[10], Froilán Granero[11], Gloria González-Aseguinolaza[12], Gloria Carmona[6], Iolanda Jordan[13], Joaquín Delgadillo[14], José Carlos Segovia[15], Javier García-Castro[5], Juan José Mata[3], José Mª Moraleda[16], Jordi Barquinero[17], Marina I. Garín[15], Álvaro Meana[18], Miguel Chillon[19], Míriam Sánchez-Escamilla[20], María Blanco[21], Manel Juan[3], Marta Alonso[12], Rafael Aldabe[12], Raúl Torres[15], Rosa María Yáñez[15], Rubén Lopez-Vales[19], Ruben Hernandez-Alcoceba[12], Sonia Alonso[7], Unai Perpiñá[1], Josep M. Canals[1]

Advanced therapy medicinal products (ATMPs) offer new therapeutic opportunities; however, their development is subject to complex regulatory and quality requirements that pose challenges to their application. The diversity of products, stakeholders involved, and stages of maturity may lead to heterogeneous quality systems, hindering continuity and compliance with regulatory requirements during transitions between different regulatory frameworks. This study aims to assess the implementation of quality systems and identify the main needs, limitations, and regulatory and compliance challenges perceived by professionals involved in ATMP development.

To this end, an observational study is being conducted through an online survey implemented using the LimeSurvey platform and targeting professionals involved in ATMP research and development, manufacturing, quality, regulation, and clinical application in Spain and other European countries.

The survey results will provide empirical information on reported practices and perceived needs. Together with a documentary and regulatory review of ISO 9001, Good Manufacturing Practice (GMP), and Good Laboratory Practice (GLP), these findings will inform the development of a practical guide intended to facilitate the progressive implementation and integration of, and transition between, the requirements of these quality frameworks. The guide is intended to support professionals involved in ATMP development in designing, implementing, and improving their quality systems.

[1]Laboratory of Stem Cells and Regenerative Medicine, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences; Institute of Neurosciences; and Creatio, Production and Validation Center of Advanced Therapies, University of Barcelona; August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain.

[2]Immunology Group, CINBIO, Universidade de Vigo; Instituto de Investigación Sanitaria Galicia Sur (IIS Galicia Sur), SERGAS–UVIGO, Vigo, Spain.

[3]Hospital Clínic de Barcelona; Fundació de Recerca Clínic Barcelona–Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

[4]Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain.

[5]Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[6]Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RADyTTA), Fundación Pública Andaluza Progreso y Salud, Sevilla, Spain.

[7]Biogipuzkoa Health Research Institute, Donostia–San Sebastián, Spain.

[8]Advanced Therapies Manufacturing Unit, Hospital Universitario 12 de Octubre, Madrid, Spain.

[9]Instituto de Carboquímica, Consejo Superior de Investigaciones Científicas (ICB-CSIC), Zaragoza, Spain.

[10]Medical Oncology Department, Instituto de Investigación Biomédica de A Coruña (INIBIC), Hospital Universitario A Coruña, A Coruña, Spain.

[11]Clínica Universidad de Navarra, University of Navarra; Navarra Institute for Health Research (IdiSNA), Pamplona, Spain.

[12]Centro de Investigación Médica Aplicada (CIMA), University of Navarra, Pamplona, Spain.

[13]Hospital Sant Joan de Déu; Institut de Recerca Sant Joan de Déu, Barcelona, Spain.

[14]Banc de Sang i Teixits (BST), Barcelona, Spain.

[15]Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT); Advanced Therapies Unit, Instituto de Investigación Sanitaria Fundación Jiménez Díaz; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

[16]Instituto Murciano de Investigación Biosanitaria Pascual Parrilla (IMIB); Hospital Clínico Universitario Virgen de la Arrixaca; University of Murcia, Murcia, Spain.

[17]Gene and Cell Therapy Laboratory, Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain.

[18]Tissue Engineering Unit, Centro Comunitario de Sangre y Tejidos de Asturias (CCST), Oviedo, Spain.

[19]Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain.

[20]Hospital Universitario Marqués de Valdecilla–Instituto de Investigación Sanitaria Valdecilla (IDIVAL), Santander, Spain.

[21]Department of Pharmaceutical Sciences, Faculty of Pharmacy and Nutrition, University of Navarra; Navarra Institute for Health Research (IdiSNA), Pamplona, Spain.

IMPULSO AL TALENTO EN TERAPIAS AVANZADAS: RESULTADOS Y LECCIONES APRENDIDAS TRAS EL PRIMER AÑO DEL PROGRAMA DE MENTORING DE LA RED RICORS/TERAV+. RD24/0014/0037, RD24/0014/0021, RD24/0014/0014, RD24/0014/0001.

Joaquim Vives[1], Cristina Avendaño[2], Damian Garcia Olmo[3], Manel Juan[4]

[1]VHIR/Banc de Sang i Teixits, Barcelona

[2]Hospital Universitario Puerta del Hierro

[3]Fundación Jiménez Díaz

[4]IDIBAPS/Hospital Clínic, Barcelona

Introducción: El desarrollo de Medicamentos de Terapia Avanzada (MTAs) requiere un conocimiento multidisciplinar que abarca desde la investigación básica hasta el seguimiento post-comercialización. Para fortalecer la capacitación del personal investigador y favorecer la transferencia de conocimiento dentro de la red RICORS/TERAV+, en septiembre de 2025 se puso en marcha el Programa de Mentoring.

Objetivos: Evaluar el impacto, la cobertura y la efectividad del programa de mentoring, analizando la participación de los grupos de la red, el grado de cumplimiento de los objetivos de desarrollo profesional y la sostenibilidad de la iniciativa.

Metodología: El emparejamiento mentor-mentee se estructuró a partir del mapa de competencias en terapias avanzadas articulado por la red (PMID: 37498257), clasificando los perfiles en 5 dominios clave: investigación básica/descubrimiento temprano, desarrollo no clínico, desarrollo clínico (incluyendo fabricación GMP), regulatoria y monitorización post-comercialización. Se recopilaron datos cuantitativos y cualitativos mediante formularios estructurados en el hito inicial, durante el seguimiento ecuatorial y en la evaluación final previa a la reunión anual.

Resultados: Se expondrá un análisis integral del programa que incluirá: métricas de alcance y representatividad, indicadores de actividad e interacción; grado de satisfacción e impacto percibido junto con la valoración global del programa por parte de los participantes, grado de consecución de metas profesionales individuales y principales hitos alcanzados por los mentees.

Conclusiones y Perspectivas: La presentación ofrecerá una radiografía clara del valor añadido que el mentoring aporta a la cohesión de la red RICORS/TERAV+. Asimismo, se debatirán las lecciones aprendidas para optimizar la estructura organizativa y se sentarán las bases para el lanzamiento de una posible segunda cohorte, promoviendo que los mentees de esta primera edición asuman el rol de mentores para garantizar la continuidad y sostenibilidad de la iniciativa.

FLUENTCELLS: ESTRATEGIA MULTIDISCIPLINAR CONTRA LA DESINFORMACIÓN Y LAS MALAS PRÁCTICAS EN TERAPIAS AVANZADAS. RD24/0014/0012, RD24/0014/0001, RD24/0014/0037.

Raquel Cabrera-Pérez[1], Ander Izeta[2], Manel Juan[3], Joaquim Vives[1].

[1]VHIR/Banc de Sang i Teixits, Barcelona

[2]Biogipuzkoa

[3]IDIBAPS/Hospital Clínic, Barcelona

Introducción: Los medicamentos de terapia avanzada (MTAs) representan uno de los mayores avances de la medicina moderna y ofrecen nuevas oportunidades terapéuticas a pacientes con enfermedades hasta ahora sin opciones de tratamiento eficaces. Sin embargo, su creciente complejidad científica y regulatoria ha favorecido la desinformación, expectativas de curación irreales, turismo médico predatorio y ofertas de intervenciones no autorizadas que dificultan la distinción entre innovación legítima y mala praxis. Este escenario compromete la seguridad de los pacientes, erosiona la confianza pública en la investigación biomédica y plantea importantes desafíos éticos para investigadores, clínicos, fabricantes, reguladores y responsables políticos. FLUENTCELLS es una iniciativa multidisciplinar y colaborativa impulsada desde el Banc de Sang i Teixits para analizar las principales percepciones erróneas sobre estos medicamentos, identificar deficiencias en la comunicación y la regulación, y desarrollar recomendaciones basadas en principios bioéticos que favorezcan una comunicación rigurosa, transparente y adaptada a las necesidades de los diferentes colectivos implicados en el ámbito de los MTAs. La relevancia e innovación de esta propuesta han sido reconocidas recientemente con la concesión del *PRECISEU Best Practice Award* 2026 en la categoría de Cultura y el apoyo de la COST Action CA24114 BTCs4ATMPs para la expansión del proyecto en Europa.

Metodología: FLUENTCELLS se desarrolla mediante un enfoque participativo estructurado en tres fases complementarias. La primera fase, ya completada, consistió en una encuesta dirigida a actores clave del ámbito de las terapias avanzadas con el fin de caracterizar el impacto de la desinformación y recoger propuestas de mejora. Actualmente, el proyecto se encuentra en una segunda fase basada en entrevistas semiestructuradas a investigadores, centros de producción de MTAs, agencias reguladoras, profesionales sanitarios, prescriptores de terapias no autorizadas que operan fuera de los circuitos asistenciales regulados, pacientes, familiares y representantes de asociaciones de pacientes. Los resultados serán analizados mediante una metodología de análisis multicriterio para la toma de decisiones (MCDA, del inglés *Multi-Criteria Decision Analysis*). Para garantizar la robustez y relevancia de los criterios de evaluación, se ha constituido un panel Delphi multidisciplinar integrado por expertos de diferentes ámbitos de las terapias avanzadas, encargado de consensuar y priorizar los criterios empleados en el análisis de las entrevistas. Finalmente, los hallazgos obtenidos se integrarán en un marco de comunicación ética y responsable que

servirá de base para la elaboración de un Libro Blanco de buenas prácticas de comunicación en terapias avanzadas.

Resultados esperados: El proyecto permitirá identificar barreras de comunicación, necesidades formativas, áreas de vulnerabilidad frente a la desinformación y oportunidades de mejora en la interacción entre pacientes, profesionales sanitarios, investigadores, centros de producción y reguladores. Asimismo, facilitará la definición de un marco de referencia común para promover una información basada en la evidencia científica y una toma de decisiones informada y segura.

Conclusiones: FLUENTCELLS constituye una estrategia colaborativa de toda la comunidad de terapias avanzadas que integra perspectivas científicas, clínicas, regulatorias, bioéticas y sociales para abordar uno de los desafíos más relevantes para el sector. Sus resultados se materializarán en una publicación científica y en un Libro Blanco de buenas prácticas de comunicación en terapias avanzadas, concebido como una herramienta de referencia para la Red TERAV y para el conjunto del ecosistema nacional y europeo de terapias avanzadas.

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PROGRAMA
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