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SCIENTIFIC PROPOSAL

TITLE

Preclinical targeting of T-ALL relapse using of a novel immunotherapy with anti-pre-TCR CAR-T cells

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It is imperative to limit the number of pages in the proposal to 28.

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Preclinical targeting of T-ALL relapse using of a novel immunotherapy with anti-pre-TCR CAR-T cells

KEY WORDS:

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SECTIONS TO FILL IN

- Research abstract
- Informative title and abstract of the project: Description of the project in an accessible and understandable language to the non-specialized public. In both Spanish and English
- Background and current status of the topic
- Objectives (3 years)
- Investigation methodology
- Work packages (3 years)
- Work schedule / Calendar (3 years)
- Importance of work in oncology: Relevance of the project in terms of its clinical impact, care and / or technological development
- Social and scientific impact of the proposal
- Plan for the dissemination of research findings to society
- Ethical Implications
- CV Publications of the receiving group

RESEARCH ABSTRACT

T-cell acute lymphoblastic leukemia (T-ALL) is a difficult-to-treat hematopoietic malignancy, which is still accompanied by high rates of relapse and ineffective options for refractory disease, resulting in poor clinical outcomes. Consequently, there is an urgent need to develop novel effective and safe therapeutic strategies to improve life expectancy of T-ALL patients. The last decade has seen important advances in the use of the immune system as a therapeutic agent in cancer treatment. Recent studies showed that T lymphocytes engineered to express chimeric antigen receptors (CAR-T) against tumors represent a powerful new adoptive immunotherapeutic tool for refractory hematopoietic malignancies such as B-ALL and chronic lymphocytic leukemia. Extending the success of CAR-T cells against B-cell leukemias to T-cell malignancies is however problematic, because malignant and normal T cells share a similar profile of surface protein expression, leading to CAR T-cell fratricide. Therefore, a central challenge is the identification of novel T-ALL-associated extracellular target antigens that allow the generation of CAR-T cells with diminished adverse effects. To this end, we considered that T-ALL results from the malignant transformation of T-lymphoid precursors arrested at various early stages of intrathymic development. By taking advantage of a novel and unique model of de novo human T-ALL generation in vivo generated as a result of a project funded by the **Fundación Científica aecc** (**20134201)**, we have extended our knowledge about the successive developmental stages that encompass the physiological generation of human T cells to the pathological situation leading to T-ALL. Our preliminary results provide formal evidence that the pre-TCR, a surface complex transiently expressed during the development of normal T cells, is constantly expressed in a high proportion of human T-ALL and may thus be a suitable T-ALL-specific therapeutic target for a major subgroup of human T-ALL. Given that our group has developed a unique monoclonal antibody (mAb) against the human pTα molecule, we are in a privileged position to generate anti-pre-TCR CAR-T cells. In this study, we will generate CAR-T cells against the pre-TCR complex and will use a preclinical in vivo model to provide proof of concept of the efficacy and safety of this novel immunotherapeutic approach. Our final aim is to bring our laboratory findings to the clinic.

INFORMATIVE TITLE AND ABSTRACT OF THE PROJECT

Description of the project in an accessible and understandable language to the non-specialized public. In both Spanish and English

Novel CAR-T immunotherapy against T-ALL

La leucemia linfoblástica aguda de células T (T-ALL) es un cáncer infantil difícil de tratar, que presenta altas tasas de recaída. Por tanto, existe una necesidad urgente de desarrollar estrategias terapéuticas nuevas, más eficaces y seguras, para mejorar la esperanza de vida de los pacientes. En los últimos años se han producido avances muy importantes en el uso del sistema inmunitario como agente terapéutico en el tratamiento del cáncer. En particular, estudios recientes han demostrado que los linfocitos T de los pacientes se pueden modificar para expresar un receptor que reconoce un antígeno tumoral (CAR-T) y elimina la célula maligna, lo que representa una nueva y poderosa herramienta inmunoterapéutica para varios tumores hematológicos refractarios, como las leucemias linfoblásticas agudas B (B-ALL). Sin embargo, el uso de la terapia CAR-T contra las leucemias T-ALL se ha visto limitado debido al impacto de la terapia sobre los linfocitos T normales del paciente, lo que induciría una inmunodeficiencia severa. La solución consistiría en identificar antígenos de las T-ALL que estén ausentes en las células T sanas. En este estudio, proponemos aprovechar la identificación en nuestro laboratorio de uno de esos antígenos, pre-TCR, y el nuevo enfoque terapéutico CAR-T para tratar un grupo mayoritario de T-ALL que expresan esta molécula ausente en linfocitos T normales. Utilizando modelos preclínicos nuestro objetivo final es demostrar que la administración de linfocitos T que expresan un CAR anti-pre-TCR a ratones inmunodeficientes portadores de una leucemia humana T-ALL, es capaz de erradicar la leucemia y constituye una terapia anti-T-ALL segura y eficaz.

T-cell acute lymphoblastic leukemia (T-ALL) is a difficult-to-treat hematopoietic malignancy with high rates of relapse. Consequently, there is an urgent need to develop novel, effective, and safe therapeutic strategies to improve life expectancy of T-ALL patients. The last decade has seen important advances in the use of the immune system as a therapeutic agent in cancer treatment. In particular, recent breakthrough studies have shown that T cells expressing chimeric antigen receptors (CAR-T) represent an emerging and powerful new adoptive immunotherapeutic tool for a number of refractory hematologic malignancies, including B-cell acute lymphoblastic leukemia (B-ALL). However, CAR-T therapy against T-ALL has been limited due to concerns over the depletion of normal T cells and the potentially higher immunodeficiency impact of T-cell depletion vs B-cell aplasia. The solution would be to identify T-ALL antigens that are absent in healthy T cells. In this study, we propose to take advantage of the identification in our laboratory of one of these antigens, pre-TCR, together with the CAR-T technology, to treat a major group of T-ALL that express this molecule. Using preclinical models, our final objective is to demonstrate that the administration of T lymphocytes that express an anti-preTCR CAR to immunodeficient mice transplanted with a human T-ALL is capable of eradicating the disease and constitutes a safe and efficacious anti-T-ALL therapy.

BACKGROUND AND CURRENT STATUS OF THE TOPIC

BACKGROUND

T-ALL and current therapeutic strategies: the problem

T-cell acute lymphoblastic leukemias (T-ALLs) are highly malignant tumors representing 10% to 15% of pediatric and 25% of adult ALLs in humans (Pui et al., 2004). Despite intensive chemotherapy protocols have markedly improved prognosis in last years, T-ALL is still characterized by high rates of relapse and ineffective options for refractory disease, resulting in poor clinical outcomes. Consequently, there is an urgent need to develop novel, effective and safe therapeutic strategies to improve life expectancy of T-ALL patients. Recent breakthrough studies have shown that chimeric antigen receptors (CARs) represent an emerging and powerful new adoptive immunotherapeutic tool for a number of refractory hematopoietic malignancies (Maus et al., 2014; Wang et al., 2017), as exemplified by the success of CD19-targeting CAR treatment of B-cell ALL and chronic lymphocytic leukemia (Brentjens et al., 2013; Maude et al., 2014). However, reports of targeting T-cell malignancies with CARs are rare despite clear clinical need. CAR therapy utilizes modified patient immune cells, traditionally T cells, to target and eliminate tumours in an antigen-specific and major histocompatibility complex-independent manner. Thus, CAR-T therapy against T-cell antigens has been limited due to concerns over the potentially higher immunodeficiency impact of T-cell depletion vs B-cell aplasia. Recently, the feasibility and efficacy of developing CAR-T therapy has also been demonstrated for T-cell neoplasms (Mamonkin et al., 2015; Pinz et al., 2016), providing proof-of-concept for the development of CAR-T therapy against hematologic malignancies of T cell origin. Still, the central challenge is that malignant and normal T cells share a similar profile of surface protein expression, and the on-target off-tumor cytotoxicity of CAR-T cells may lead to elimination of CAR-T cells as well as normal T cells, which could result in impaired antitumor efficacy and profound immunodeficiency. In general, undesired immune responses generated by CAR-T cells are a consequence of the paucity or lack of definition of truly exclusive tumor antigens, which indicates that there is a need for defining new tumor-associated specific antigens, and generating their corresponding CARs with improved efficacy or tumor-specificity.

T-ALL origin and identification of specific therapeutic targets: the challenge

Identification of T-ALL-specific and sensitive target antigens is a critical step for the development of a safe and efficacious CAR therapy. In this regard, it is important to consider that T-ALLs largely reflect physiologic T-lymphoid development (Asnafi et al., 2003; Soulier et al., 2005). T-ALL cells derive from malignant transformation of T-lymphoid precursors arrested at various early stages of intrathymic development. T-cell precursors that seed the thymus are progressively committed to the T-cell lineages, either αβ or γδ, through sequential maturation stages. Development of the major αβ lineage of T cells involves a highly ordered and tightly regulated maturation program (reviewed by Res and Spits, 1999; Carrasco et al., 2002), which is controlled at successive check-points through two molecular sensors, the pre-T cell receptor (pre-TCR) and the mature TCRαβ, which are sequentially expressed during intrathymic development (Ramiro et al.,

1996; Trigueros et al., 1998). Early pre-T cells that succeed in productive TCRβ rearrangements express a functional TCRβ chain which pairs with an invariant pre-TCRα (pTα) chain (Ramiro et al., 1996) and associates with CD3 subunits to form the pre-TCR (von Boehmer and Fehling, 1997; Carrasco et al., 2002). Surface expression of this pre-TCR complex is necessary for triggering a process, known as β-selection (Hoffman et al., 1996), that induces the expansion and further differentiation of developing pre-T cells into CD4/8 double-positive (DP) thymocytes (Ramiro et al., 1996; Carrasco et al., 1999). This is followed by TCRα rearrangement, TCRδ deletion, and replacement of the pre-TCR by TCR$\alpha\beta$ (Trigueros et al., 1998), resulting in a second step of selection, known as positive or TCRαβ selection. Therefore, pre-TCR expression is restricted to a narrow intrathymic developmental window, but is absent in mature T cells.

Correlation studies of phenotype and T-cell receptor (TCR) status in T-ALL have shown that T-ALLs recapitulate early intrathymic T-cell development stages (Asnafi et al., 2003; Soulier et al., 2005). Notably, half the TCR$\alpha\beta$ lineage T-ALLs analysed in Asnafi's study were reported to express a pre-TCR, as evidenced by RAG-1, pTα, and cytoplasmic TCRβ (cTCRβ) expression, absence of TCRδ deletion, and a surface CD3ε (sCD3ε)$^{-/low}$, CD1a^{+}, CD4/8 DP phenotype, in keeping with a population undergoing β–selection. Also, 40% of TCR$\gamma\delta$ T-ALLs were pTα^{+}, DP, and expressed cTCRβ. Therefore, pre-TCR expression is common in human T-ALL cells. Importantly, events related to the pre-TCR-mediated β–selection process are thought to be important for leukemic transformation in distinct T-ALL subtypes (Chervinsky et al., 2001). In mouse models, it was shown that activating mutations in *NOTCH1*, which are highly prevalent (>65%) in human T-ALLs (Weng et al., 2004), require an intact pre-TCR function for the generation of T-ALL (Allman et al., 2002), or at least have an increased oncogenic potential in collaboration with pre-TCR (Campese et al., 2006), an observation that concurs with the finding that *PTCRA,* the gene encoding pTα, is a direct NOTCH1 target (Reizis et al., 2002). Expression of a constitutively active intracellular domain of Notch3 also favours murine T-cell leukemia in a pre-TCR-dependent manner (Bellavia et al., 2002). Moreover, *NOTCH2*, *NOTCH3*, and *PTCRA* genes are co-expressed in most human T-ALL cases (Bellavia et al., 2002; Soulier et al., 2005). These genes co-cluster in different T-ALL subgroups but not in normal thymic subpopulations, suggesting that their abnormal co-expression could be specifically associated with oncogenesis. Together, these studies indicate that pre-TCR, which is absent in normal T cells, is a functional marker in distinct T-ALL subgroups. Therefore, we have hypothesized that pre-TCR may be a suitable T-ALL-specific target for the development of a safe and efficacious CAR-T therapy. Given that our group has developed a unique monoclonal antibody (mAb) against the human pTα molecule (Ramiro et al., 2001), we are in a privileged position to develop anti-pre-TCR CAR-T cells and to provide proof of concept of the efficacy and safety of this novel immunotherapeutic approach.

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OBJECTIVES

HYPOTHESIS AND SPECIFIC AIMS:

Our preliminary results provide formal support to the hypothesis that pre-TCR may be a suitable T-ALL-specific target for the development of a safe and efficacious CAR-T therapy. Based on this hypothesis, we propose to approach the following specific aims:

1. Defining the functional relevance of pre-TCR as a T-ALL biomarker: prevalence and prognosis.
2. Development of anti-preTCR CAR-engineered T cells and validation of their function in vitro.
3. Preclinical validation *in vivo* of T-ALL-targeted immunotherapy using anti-pre-TCR-specific CAR-T cells: Towards a clinical trial.

PRELIMINARY RESULTS

Functional targeting of pre-TCR on primary human T-ALL cells by the K5G3 anti pTα mAb

To generate pre-TCR-specific CAR-T cells, we will use K5G3, a mouse IgG2a mAb that we raised against the extracellular domain of human pTα, the specific component of the heterodimeric (TCRβ-pTα) pre-TCR (Ramiro et al., 2001). We have shown that K5G3 binds the pre-TCR complex expressed on the surface of developing thymocytes as well as on human leukemic cell lines, such as SupT1, and primary T-ALL cells (**Figure 1**). Pre-TCR expression levels correlate with low surface CD3 expression (Carrasco et al., 2001), and can be distinguished from surface TCRαβ or TCRγδ expression, which associates with higher CD3 expression. While weak pre-TCR surface expression could be considered a drawback for the development of efficient pre-TCR-specific CARs, our preliminary functional studies indicated that it sufficed for activation of primary human pre-TCR+ thymocytes, as measured by calcium mobilization, upon crosslinking with K5G3. Interestingly, activation levels were similar to those induced by the well-known UCHT1 anti-CD3 mAb (**Figure 1**). Moreover, K5G3 is as effective as UCHT1 (or even more) at inducing the activation of PI3K/AKT/mTOR and MAPK signaling pathways in T-ALL human primary pre-TCR+ cells (**Figure2**). Therefore, it is expected that CARs based on our anti-pre-TCR K5G3 mAb will be effective as well.

K5G3-mediated pre-TCR targeting also resulted in the loss of surface CD3 expression on T-ALL cells upon long culture periods (6 days) (**Figure 2**). In vitro treatment of primary T-ALL with a heterogeneous preTCR+/TCRαβ+ phenotype revealed that this effect was specific of cells expressing the pre-TCR and resulted in the selective loss of such cells (**Figure 3**), owing to increased apoptosis (not shown). Therefore, the K5G3 mAb is unable to bind the conventional TCRαβ complex but targets specifically the pre-TCR.

Pre-TCR expression is a functional marker of human T-ALL cells with leukemia-initiating potential

We have assessed the relevance of pre-TCR expression in human T-ALL progression *in vivo*. To this end, we took advantage of a xenotrasplantation mouse model (**Figure 4**), which revealed that serial transplantations of a T-ALL patient sample expressing very low-to-undetectable levels of CD3 resulted in a sequential enrichment in CD3low cells (**Figure 4**). These data indicate such CD3low cells, comprising pre-TCR+ cells, display a growth advantage *in vivo* and thus include the leukemia-initiating cells (LICs). This information is clinically relevant, as molecules involved in LIC activity are promising therapeutic targets against T-ALL relapse. We have confirmed that intact pre-TCR signalling is required for progression of human T-ALL cells *in vivo*. In particular, shRNA-mediated silencing of CMS/CD2AP, a downstream functional adaptor of human pre-TCR, which is functionally associated to a Pro-rich domain in the cytoplasmic tail of human pTα (Navarro et al., 2007 and **Figure 5**) leads to impaired leukemia engraftment and progression in a xenotransplantation assay. These data highlight the beneficial impact that elimination of pre-TCR+ T-ALL cells by anti-pre-TCR CAR-T cells may exert in preventing maintenance and renewal of LICs, thus impairing T-ALL relapse.

Aim 1. Defining the functional relevance of pre-TCR as a T-ALL biomarker

1.1. Analysis of pre-TCR expression frequency in human primary T-ALL

We will perform a retrospective study of the prevalence of pre-TCR expression in a cohort of T-ALL patient samples in collaboration with the Biological Group of Acute Leukemia of the Spanish Society of Hematology and Oncology (SEHOP). The structure formed by the reference laboratories, and the participation of the Servicio de Hemato-Oncología, Hospital Universitario La Paz (Dr. Adela Escudero), which form part of the Group will provide biological material necessary for the project, and, more importantly, will facilitate the exchange of information as well as an integral knowledge of the disease from complementary basic and clinical perspectives, which are mandatory to develop research projects that have a real impact on clinical practice. To this end, our anti-pTα mAb will be distributed among the reference flow cytometry laboratories of the group that centralize the diagnosis and follow-up of all participating Spanish hospitals through Dr. Escudero. This starting point is clinically relevant since it should allow identifying the population of leukemic patients who would benefit from a targeted anti-pre-TCR-specific CAR-T therapy, as example of personalized medicine. We anticipate that around 50% of T-ALL samples at diagnosis express pre-TCR. When available, relapse samples will be also analysed for pre-TCR expression, and compare to diagnosis.

1.2. Prognostic value of pre-TCR expression in T-ALL.

The study variables (positive-negative, and MFI) will be compared in the group of relapses versus no relapses by means of parametric tests (comparison of percentages and comparison of means). Cut-off values will be set to separate patient groups based on the status of a variable (eg, group of T-ALL expressing high levels/proportions of pTα versus low levels). Studies of cumulative incidence of relapse and event-free survival (relapse, death) will be performed according to these groups (Kaplan-Meier model). If the prognostic value is confirmed, (statistically significant association with relapse and / or event free survival) each variable will be included in multivariate analyses (Cox risk model) together with current prognostic factors of the SEHOP-PETHEMA protocol.

1.2. Genetic characterization of Pre-TCR-expressing T-ALL by Next Generation Sequencing (NGS) and prognostic value.

Taking advantage of the expertise of Dr. Escudero in NGS, T-ALL samples shown to express pre-TCR will be analysed for mutations in specific genes associated to T-ALL pathogenesis, focussing on CDKN2A, CDKN2B, FBXW7, FLT3, IL7R, JAK1, NOTCH1, PHF6, PTEN and PTCRA.

This information would be critical to establish correlations with the pre-TCR+ phenotype and would be used in prognosis studies.

Aim 2. Development of anti-pre-TCR CAR-engineered T cells and experimental validation of their function.

2.1. Isolation of the cDNAs for the K5G3 anti-pTα antibody and scFv generation and verification.

PolyA+ RNA will be isolated form the K5G3 hybridoma (IgG2a,κ isotype; Ramiro et al., 2001) and full length heavy and light chain cDNAs will be generated via a commercially available 5'RACE kit (Clontech/Takara) using 3'-gene specific primers complementary to the 3' ends of the coding

sequences of the IgG2a and κ constant domains. These constant domain sequences can be obtained from the Ensembl (http://www.ensembl.org/) and IMGT (http://www.imgt.org/) databases. Full length cDNAs will be sequenced and cloned into a mammalian expression vector. Upon transfection of these constructs into the 293T cell line, antibodies will be purified from these cultures and tested together with K5G3 hybridoma-derived antibodies and the human pre-TCR+ cell line SupT1 in flow cytometry-based competition binding assays for their correct specificity. A DNA sequence encoding the scFv fragments derived from the K5G3 antibody will be synthesized commercially containing the leader and rearranged VJ domains of the Ig light chain coupled via a linker sequence containing 5 repeats encoding a Gly_4Ser motif to the rearranged VDJ sequence of the heavy chain. The resulting scFv will be checked for its correct specificity via the above-mentioned competition assays.

2.2. Generation of a 3rd generation pre-TCR-specific CAR construct and *in vitro* testing

The scFv fragment will be combined with the human CD8α-derived hinge and transmembrane region and the CD28, 4-1BB and CD3ζ intracellular domains into a 3rd generation CAR construct (Carperito et al., 2009, Milone et al., 2009, Tammana et al., 2010), amplifying the individual domains using RT-PCR and combining them via seamless cloning methods into the CAR construct pTA-28BBz]. We will furthermore incorporate a GFP marker, separated from the CAR-coding sequences by a sequence encoding a P2A self-cleaving peptide, in order to allow unequivocal detection of cells expressing the CAR. This construct will be cloned into the lentiviral vector blackbone. Lentiviral vectors (sinLV) overcome most of the limitations of classical gamma retroviral vectors (gRV). sinLVs are highly efficient, can transduce both dividing and nondividing cells. In addition, sinLVs have a lower preference for integration in regions close to the transcription start site of genes (TSS) (Schröder et al., 2002; Wu et al., 2003; Montini et al., 2006; Gonzalez-Murillo et al., 2008). This fact, together with the advanced self-inactivating design of third-generation sinLVs, accounts for the reduced risks of insertional oncogenesis associated with these vectors. Due to the evidence of recent successful in clinical trials for the treatment of monogenetic diseases (Cartier et al., 2009), gene therapy trials, including CAR-T (Qasim et al., 2017), are moving towards the use of lentiviral vectors (sinLVs). For thisn part of the work, we will collaborate with Dr. Hisse van Santen (CBMSO), who is an expert in the field.

The human Jurkat TCRαβ T cell line will be transduced with this lentiviral vector and expression of the CAR construct at the cell surface of the Jurkat T cells will be verified with commercially available polyclonal murine F(ab')2 specific-conjugated antibodies and the function of the activating capacity of the CAR will be verified by analysis of CD69 expression and IL-2 production by the transduced Jurkat T cells upon co-culture with SupT1 cells. Human CD8+ T cells purified from buffy coats (obtained from the Madrid Blood Center under a collaboration agreement with Dr Toribio) will be transduced with the CAR-encoding pTA-28BBz or a control vector and tested in in vitro cytotoxicity assay for their capacity to kill pre-TCR-expressing cell lines (SupT1) and primary T-ALL cells, using Jurkat TCRαβ T cells as controls.

Aim 3. Preclinical validation in vivo of T-ALL-targeted immunotherapy using anti-pre-TCR CAR-engineered T cells: **Towards a clinical trial.**

Next, we will validate the efficacy of pre-TCR CAR-T immunotherapy in vivo in a preclinical assay, in order to provide proof-of-principle for the value of this treatment against human T-ALLs expressing pre-TCR. This is an obligatory step to accomplish the final goal of translating the developed CAR-T therapy to the clinic.

3.1. Quantitative assessment of in vivo T-ALL LIC potential

The preclinical assay is based on a xenotransplantation mouse model previously established in our lab (**Figure 4**), consisting on intravenous injection of human primary T-ALL cells (10^4-10^5), obtained from residual bone marrow samples after final diagnosis of patients, into 6-10 week-old immunodeficient NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ; The Jakcson Laboratory) preconditioned by irradiation (1.5Gys). This model efficiently recapitulates in vivo progression of primary T-ALL human cells expressing pre-TCR and allows for quantitative estimation of T-ALL cells with in vivo LIC activity.

For LIC potential assays, cells of interest will be transplanted into NSG mice under limiting dilution conditions (5 mice/ group) and LIC activity will be calculated based on mice survival using the ELDA software (http://bioinf.wehi.edu.au/software/elda/). We will compare LIC activity of BM cells recovered from mice either subjected to pre-TCR CAR-T immunotherapy or left untreated.

3.2. Therapeutic activity of pre-TCR CAR-T cells in preclinical assays

We will next assess CAR T cell cytotoxicity in NSG mice transplanted with 10^4-10^5 pre-TCR+ T-ALL primary cells (**Figure 6**) transduced with a lentiviral construct developed in our lab encoding the luciferase and GFP genes separated by an IRES (pHR-Luc-IRES-emGFP). We will choose 2 primary T-ALL cell samples using as selection criteria the samples that have the maximal and minimal expression level of pre-TCR. Transduced TCR$\alpha\beta$+ T-ALLs will be transplanted as control. Mice will be monitored by sequential BM aspirates and also by non-invasive whole-body bioluminescence imaging (IVIS) before CAR-T cell infusion to check for engraftment of tumor cells. Mice with established disease (50% of T-ALL cells in BM) will be distributed randomly in 3 groups (at least 5 mice/group) and will be i.v. injected with a single dose of either primary human CD8+ CAR-T cells, or control T cells transduced with GFP only (5×10^6 /each time point), while another group will not receive T cells. CD8+ CAR-T cells will be also transplanted into a group of mice not transplanted with T-ALL. Mice will be given intraperitoneal injection of 1000 UI/ mouse of rhIL-2 once a week and will be monitored visually on a daily basis for their general appearance and bi-weekly for tumor growth by bioluminescence imaging, and T-ALL infiltration in BM aspirates when required. Mice that show clear signs of distress (cachexia) will be sacrificed. Mice that show no signs of distress during the course of the experiment will be sacrificed after two months. BM, spleen, thymus, lymph nodes and brain will be isolated from all sacrificed mice and the number of tumor cells and transduced T cells will be identified and enumerated by flow cytometry using the appropriate markers. We will also assess the expression level of pre-TCR at the cell surface of the tumor cells and the level of CAR

expression by the transduced T cells. This should allow us to provide a quantitative assessment of the capacity of our CAR configuration to endow transduced T cells with control of tumor growth.

3.3. Towards a clinical trial

It is worth mentioning that the final aim of the project is to translate the results into the clinical developmental pathway. Should our results success as proof of concept of the therapeutic strategy, we will extend the preclinical phase on the immunodeficient model to gather information on safety and biodistribution, and then will propose a Phase I trial to the Spanish Medicine Agency. Our clinical collaborator, Dr. Escudero, collaborates has already experience in designing and applying advanced CAR therapies against osteosarcoma in animal models (Fernández et al., 2017) and collaborates with professionals involved in CAR-based clinical trials.

FIGURES

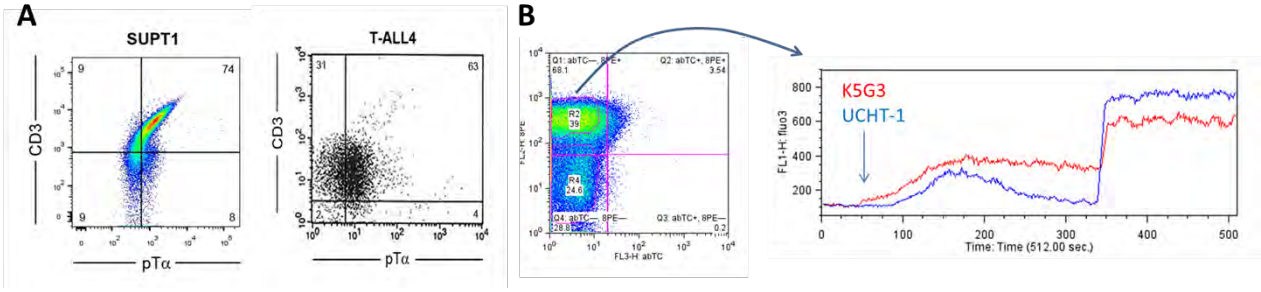

Figure 1. Pre-TCR expression and function of K5G3 anti-pTα mAb. (A) Pre-TCR expression on a human T-ALL cell line (SupT1) and a primary T-ALL cell sample (T-ALL4), as assessed by flow cytometry using K5G3. (B) Calcium mobilization in pre-TCR+ human thymocytes upon pre-TCR crosslingking with either anti-CD3 (UCHT1) or anti-pTα (K5G3) mAbs.

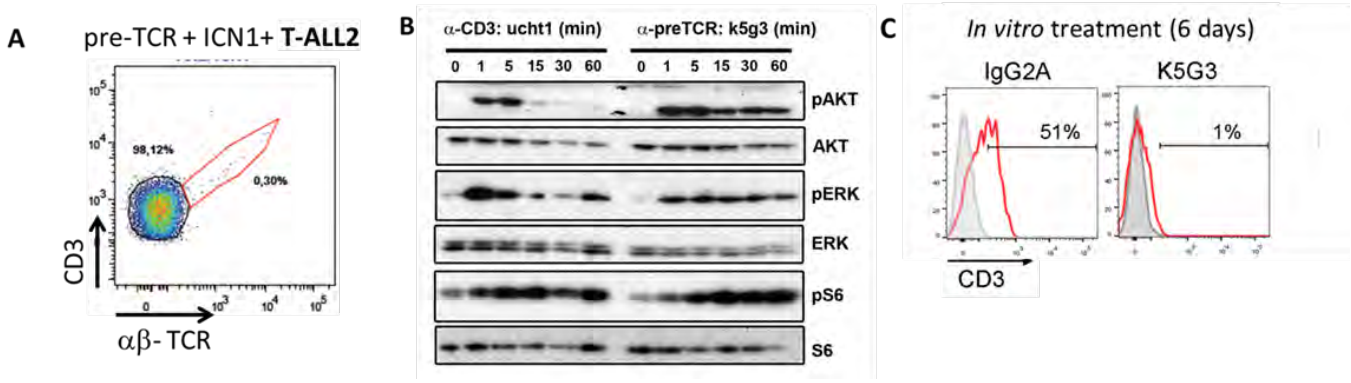

Figure 2. Pre-TCR expression and function in a primary human T-ALL . (A) CD3lo FACS expression associated with pre-TCR in T-ALL2 human primary cells expressing constitutively active Notch1 (ICN1). (B) Activation of the indicated signaling pathways induced upon crosslinking at the indicated times with anti-CD3 (UCHT1) or anti-pre-TCR (K5G3) mAbs, as assessed by western blotting. (C) Loss of surface CD3 expression on T-ALL2 cells upon 6 days of culture in the presence of K5G3 or IgG2a as negative control.

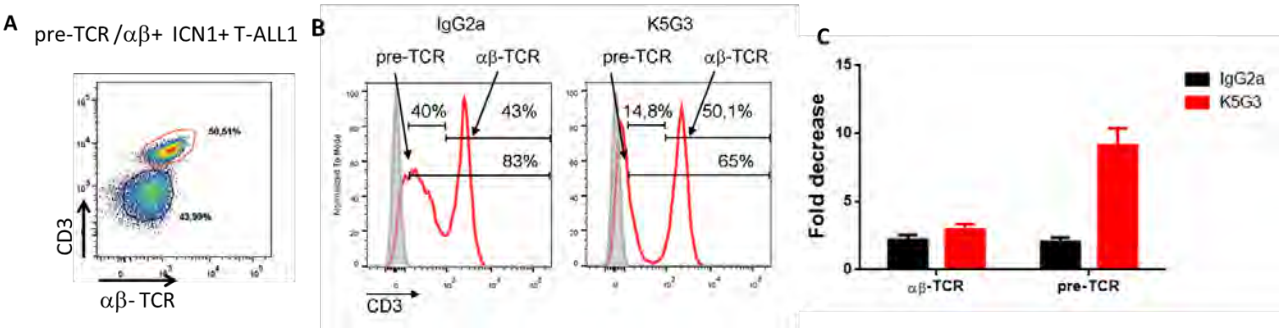

Figure 3. Functional impact of targeting surface pre-TCR expressed on a pre-TCR/TCRαβ+ primary human T-ALL with the K5G3 anti-pTα mAb. (A) FACS analysis of surface CD3 expression levels associated with pre-TCR (low) and TCRαβ (high) on a human primary T-ALL expressing ICN1+ (T-ALL1). (B) Selective loss of low CD3 expression on T-ALL1 cells is induced by K5G3, but not by an isotypic IgG2a control, upon 6 days of culture. (C) Relative decrease of either pre-TCR+ or TCRαβ+ T-ALL1 cell numbers upon 6 days of culture with with anti-CD3 (UCHT1) or anti-pre-TCR (K5G3) mAbs.

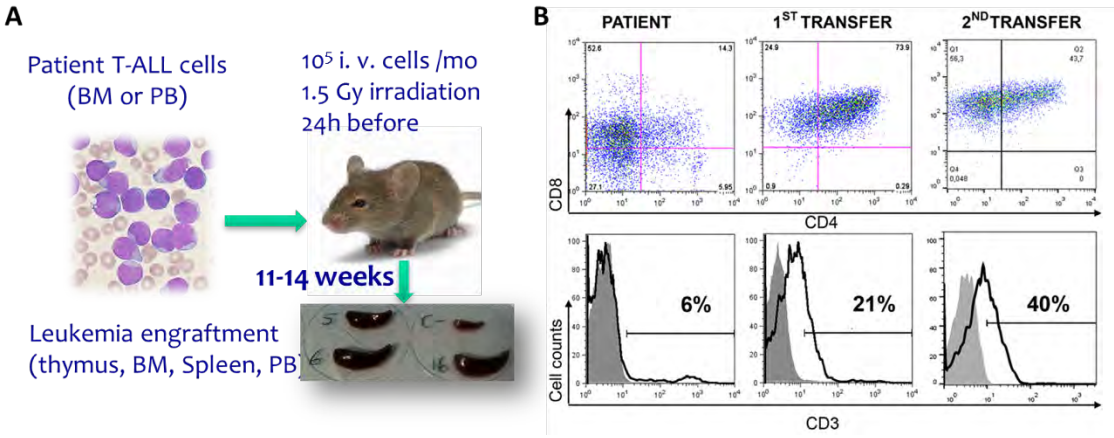

Figure 4. Xenograft mouse model of human primary T-ALL. (A) and in vivo enrichment of primary CD4/8 DP T-ALL cells with a pre-TCR-associated CD3low expression is induced upon serial transplantation (B).

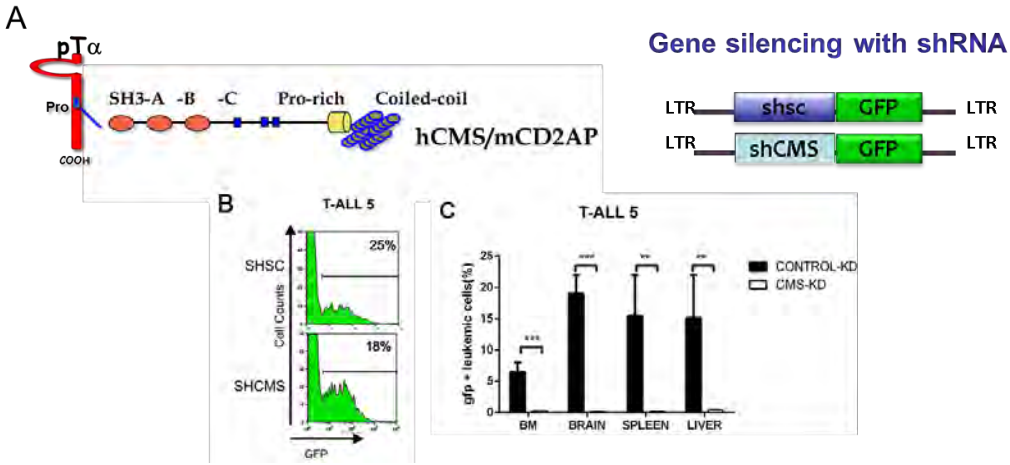

Figure 5. Schematic representation of (A) the binding site CMS/CD2AP to human pTα (left) and (B) the lentiviral (LV) shRNA vector used for silencing of CMS expression. (B) Transduction efficiency of T-ALL5 cells with the LV shown in A and the corresponding control vector. (C) Engraftment of control and silenced T-ALL5 cells in host mice at 10 weeks post-transplant.

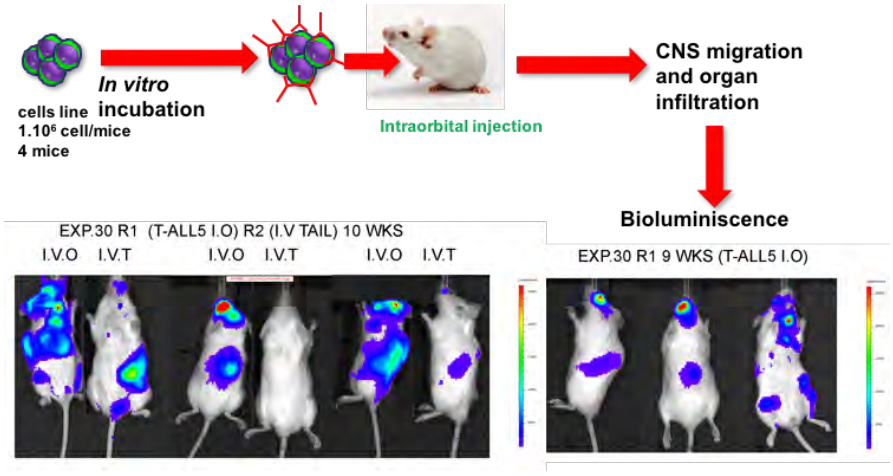

Figure 6. In vivo tracking of T-ALL infiltration by bioluminiscence

WORK PACKAGES

Structure/Organization chart; names and functions of each team member Plan coordination and distribution of tasks

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María Luisa Toribio García: Principal Investigator (PI)

-Develop specific research plans to complete the allocated tasks and produce deliverables.
-Deliver outcomes on time and to high standard.
-Contribute to training, exchange and public engagement activities.
-Direct the work of all personnel allocated to the project and prepare publications.

Adela Escudero López

-Study of the prevalence of pre-TCR expression in a cohort of T-ALL patient samples.

-Prognostic value of pre-TCR expression

-Genetic characterization of Pre-TCR-expressing T-ALL by Next Generation Sequencing (NGS) and prognostic value of gene mutations.

Marina García Peydró

-Generation of preclinical mouse models with pre-TCR+ T-ALL cells.
-Preclinical assays of CAR-T cell immunotherapy in vivo.
-LIC assays of T-ALLs before and after immunotherapy.

Patricia Fuentes Villarejo: Postdoctoral Investigator

-Functional assays about anti-pre-TCR recognition.
-Functional validation of pre-TCR expression and function in T-ALL.
-In vivo tracking of peripheral infiltration by pre-TCR+ T-ALL cells before and after immunotherapy.

Alba Murcia Ceballos: PhD student

-Molecular studies on scFv generation and verification.
-Generation of a 3rd generation pre-TCR-specific CAR construct.
-*In vitro* testing of 3rd generation pre-TCR-specific CARs.

Sara González García: Postdoctoral Investigator

-Generation of anti-preTCR CAR-engineered T cells.

-In vitro assays of functional efficacy of ant-pre-TCR CAR-T cells.

Juan Alcain Sánchez : Technical assistant

-Animal care and breeding.
-Organs' extraction.

WORK PACKAGES

Structure/Organization chart; names and functions of each team member Plan coordination and distribution of tasks

TASKS	Year 1	Year 2	Year 3
Prevalence of pre-TCR expression in T-ALL patients and prognostic value	XXXX	XXXX	XXXX
Genetic characterization by NGS	XXXX	XXXX	XXXX
Generation of preclinical mouse models with pre-TCR+ T-ALL cells	XXXX		
Preclinical assays of CAR-T cell immunotherapy in vivo			XXXX
LIC assays of T-ALLs before and after immunotherapy		XXXX	XXXX
Functional assays about anti-pre-TCR recognition	XXXX		
Functional validation of pre-TCR expression and function in T-ALL	XXXX		
In vivo tracking of T-LL infiltration		XXXX	XXXX
Molecular studies on scFv generation and verification	XXXX		
Generation of a 3rd generation pre-TCR-specific CAR construct	XX	XX	
In vitro testing of 3rd generation pre-TCR-specific CARs		XXXX	
Generation of anti-preTCR CAR-engineered T cells		XXXX	
In vitro assays of functional efficacy of ant-pre-TCR CAR-T cells		XXXX	
Animal care and breeding	XXXX	XXXX	XXXX
Organs' extraction		XXXX	XXXX

IMPORTANCE OF WORK IN ONCOLOGY

Aggressive chemotherapeutic protocols developed during the last years have greatly improved the prognosis of many cancers. However, treated patients have to face devastating secondary effects, which are particularly harmful in the case of children. Moreover, disease relapse is still a major problem and the main cause of decease in many tumors.

As an example, T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive pediatric and adult hematological malignancy, with a high rate of disease relapse and a poor prognosis of relapsed patients. Therefore, searching for specific and more effective anti-tumoral therapies is a general challenge of developed countries. Accordingly, our scientific activity pursues translating research for human health, making sure that basic discoveries have practical benefits and improve the quality of life of patients and will result in more sustainable and efficient public healthcare systems. Focussing on T-ALL, emphasis will be put on translating basic discoveries into clinical applications, including in vivo validation of experimental results, and the development and validation in preclinical models of new immunotherapies of commercial value, as well as on boosting our innovative capacity in collaboration with health-related industries in order to bring our discoveries into public use at the earliest possible time.

SOCIAL AND SCIENTIFIC IMPACT OF THE PROPOSAL

Our scientific activity pursues translating research for human health, making sure that basic discoveries of international scientific and technological relevance have practical benefits and improve the quality of life of patients, and result in more sustainable and efficient public healthcare systems. Focussing on T-ALL, this work will build the preclinical foundations required for development of novel CAR-T immunotherapies of clinical value against T-ALL. The project is therefore positioned to substantially advance the field towards an important therapeutic goal, and, in doing so, to contribute significantly to delivering the promise of relapse inhibition in T-ALL patients. Moreover, we will put our emphasis on translation into clinical applications by boosting our collaborations with clinicians and health-related industries in order to bring our discoveries into public use at the earliest possible time.

New technologies and tools that are developed within this Project will be made available to the international scientific community after obtaining patent or other protection where applicable. Overall, our results will build solid foundations for implementation of new therapeutic strategies forT-ALL treatment. Contacts with Pharma companies will be established based on corresponding R&D agreements with the CSIC.

Distribution through appropriate social networks will be encouraged after intellectual property protection:

Comunidad de Madrid [madri+d] Noticias; Centro de Biología Molecular "Severo Ochoa", CSIC, UAM, Diario Médico; RNE " a hombros de gigantes"; Revista Médica Digital, and Fundación aecc.

http://www.madrimasd.org/informacionidi/noticias/noticia.asp?id=50458

http://www.rtve.es/alacarta/audios/entre-probetas/entre-probetas-leucemia-linfoblastica-aguda-16-11-11/1250428/

http://wwww.investigamedicina.com

http://www.rtve.es/podcast/radio-5/a-hombros-de-gigantes/

http://www.csic.es/web/guest/noticias

http://www.uam.es/ss/Satellite/es/1242649910548/1242658408576/noticia/noticia/Describen_una_nueva_mutacion_implicada_en_la_generacion_de_la_leucemia_linfoblastica_aguda_T.htm

ETHICAL IMPLICATIONS

Report all ethical authorizations and approvals needed to carry out the above proposal, as well as the status. Already approved ethical authorisations are required to be included to the proposal.

All the research will be carried out in compliance with the "Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine: Convention on human rights and biomedicine. Oviedo, 4.IV.1997" and updated versions as approved by the EU. The Project will use peripheral blood/buffy coat units that do not meet criteria for clinical use through an agreement with the CAM Blood Center, Madrid.
The samples will not be removed specifically for the purposes of research. If they are not used for research, they would otherwise be discarded.
Primary T-ALL samples are already available in our lab and were obtained from remaining samples obtained for diagnosis purposes in the context of a previous collaboration with Dr. Manuel Ramírez Orellana (Servicio de Oncología Pediátrica. Hospital Universitario del Niño Jesús. Madrid) and Dr. Purificación García de Miguel (Servivio de Hemato-Oncología. Hospital Universitario La Paz). Samples were made available for research only after consenting by the patient or their legal representative. Remaining samples not used for research would otherwise be discarded. Human samples will be obtained in accordance with procedures approved by our institutional Bioethics Committee, which meets the requirements of the Spanish Law on Biomedical Research about the use and confidentiality of data or samples of human origin.
All animal experiments will conform to national laws approved by the Spanish Research Council (CSIC) Bioethics Committee, and to the EU Directive 86/609 on Animal Rights. Toribio's group has approved animal experimentation protocols for the use of NSG mice in pre-clinical models of tumor growth and anti-tumor therapy by the Comunidad de Madrid. Ethical autorizations include:

-Animal Experimentation. Comunidad de Madrid.
-Normal Blood samples. Centro de Transfusión. Comunidad de Madrid.
-Primary T-ALL samples. Comité Biológico del Grupo de Leucemias Agudas de la Sociedad Española de Hematología y Oncología Pediátrica (SEHOP).

CV PUBLICATIONS OF THE RECEIVING GROUP

Most relevant publications of the group in the last 5 years

- GROUP COMPONENTS
- Most relevant publications of the group in the last 5 years
- Financing of the receiving group (amount, duration, financing entity, financed project)

GROUP COMPONENTS

The project PI, Dr. ML Toribio, is responsible for the area of "lymphohematopoietic human development" and Director of the Department of Cell Biology and Immunology at the CBMSO. Her research interests include the study of the mechanisms regulating the fate and differentiation of human T cell progenitors and of the dysregulation of intrathymic developmental pathways. The group has been pioneer in the identification of human T cell precursors and in characterizing the structure and function of the pre-TCR complex in human thymocytes. This research has contributed in the last years to the current understanding of the cellular processes involved in the physiological development of human T cells, and the identification of the molecular pathways associated with this process whose dysregulation results in T-cell acute lymphoblastic leukemia (T-ALL), such as NOTCH1, IL-7R and Pre-TCR pathways. More recently, the group has pioneered the development of unique models of de novo generation of human T-ALL in vivo and of preclinical models of immunotherapy, as proof-of-concept for further therapeutic developments. (For recent publications and funding see contributions below)

Components of the CBMSO group headed by Dr. Toribio who participate in the proposal:
Dr. Marina García Peydró has extensive experience with studying the generation of human mature T cells in humanized mice transplanted with human HSPCs and ETPs and how Notch signaling can generate T-ALL. Her work has recently contributed to the generation of the first model of human T-ALL pathogenesis in vivo in immunodeficient mice, which recapitulates the human disease and provides a unique tool for addressing the efficiency of novel T-ALL therapies.
Dr. Patricia Fuentes Villarejo is an expert in the study of homing, engrafting and metastasis of human primary T-ALL cells in preclinical animal models that allow tracking organ infiltration of luciferase-labelled primary T-ALL cells in vivo upon transplantation. She has also studied the functional contribution of pre-TCR signaling in normal and T-ALL cells.
Dr. Sara González García is an expert in functionl in vitro assays using normal and T-ALL cells and has extensive experience in molecular biology.
Alba Murcia Ceballos has extensive experience in molecular biology and is an expert in the molecular analisis by biochemical approaches of intracelular signaling pathways triggered by normal and mutant surface receptors.
Juan Alcain is currently in charge of breeding and care of immunodeficient mice.

Dr. Adela Escudero López belongs to the Servicio de Hemato-Oncología Pediátrica Hospital Infantil Universitario La Paz, wich is included in the Comité Biológico del Grupo de Leucemias Agudas de la Sociedad Española de Hematología y Oncología Pediátrica (SEHOP). She is an expert in NGS approaches and personaly handles the required facilities that are available in her lab.

Current research interests of the group include the study of the mechanisms regulating the fate, differentiation of human hematopoietic stem and progenitor cells, with special emphasis on T cell progenitors and the dysregulation of intrathymic developmental pathways, which result in the generation of T-cell acute lymphoblastic leukemia (T-ALL). This research has contributed in the last years to the current understanding of the cellular processes involved in the physiological development of human T cells, and the associated molecular pathways whose dysregulation is critically involved in T-ALL leukemia onset, such as NOTCH1, IL-7R and Pre-TCR pathways. In the context of a previous project funded by the **Fundación Científica aecc** (**20134201**), the group has recently pioneered the development of unique models of de novo generation of human T-ALL in vivo and also of preclinical models of immunotherapy, as proof-of-concept for further therapeutic developments.

The research of the group research has been funded by several National and International Agencies, including the Spanish Ministry of Education and Science and Innovation, the Madrid Regional Government, the Spanish National Health Institute, the 7th European Union Framework Program on Health, and several private foundations, in the last 5 years. The group has maintained a productive scientific activity, with relevant publications in international journals of high impact, national and international collaborations, and a great training activity. Her scientific achievements are summarized as follows:

1. Isolation and characterization of human hematopoietic stem/progenitor cells in pre-thymic locations (cord blood, bone marrow, fetal liver) and human thymus (Sanchez et al, J. Exp Med.1993; Fernandez et al., Blood 1994; Marquez et al, J. Exp Med 1995; Blood 1998).
2. Characterization of new maturation stages in the human thymus (Trigueros et al, J. Exp Med 1998; Carrasco et al, J. Exp Med 1999).
3. Identification of human pre-TCR and regulation of its expression during intrathymic development (Ramiro et al, J. Exp Med 1996; J. Immunol 2001; Carrasco et al, J. Exp. Med. 2001; J Biol Chem 2003; Semin Immunol 2002; Navarro et al, Blood 2007).
4. Characterization of myeloid precursors in human thymus (de Yébenes et al., Blood 2002).
5. Development of methods for genetic modification of human hematopoietic precursors (Ramiro et al., Hum Gene Ther 1998).
6. Characterization of the role of Notch1 in human intrathymic development and regulation of IL-7R (Garcia-Peydró Blood 2003; J. Immunol 2006; Gonzalez et al, J. Exp Med 2009; Curr Top Microbiol Immunol 2012; Tremblay et al., Leukemia 2016).
7. Identification HOXA, IL-7R and PHF6 mutations in T-ALL leukemia (Soulier et al, Blood 2005; Vlierberghe Van et al, Nat Genet 2010; Zenatti et al, Nat Genet 2011).
8. Identification of Notch ligand-specific microenvironments for dendritic cell development in the human thymus (Martín-Gayo et al., J. Exp. Med. 2017).
9. Development of an in vivo model of human T-ALL generation (Garcia-Peydró et al., J. Clin. Invest. under 2nd revision).

Publications of the group in the last 5 years:

Robles-Valero J, Lorenzo-Martín LF, Menacho-Márquez M, Fernández-Pisonero I, Abad A, Camós M, Toribio ML, Espinosa L, Bigas A, Bustelo XR. A Paradoxical Tumor-Suppressor Role for the Rac1 Exchange Factor Vav1 in T Cell Acute Lymphoblastic Leukemia. Cancer Cell. 2017 Nov 13;32(5):608-623.e9.

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Rodriguez RM , Suarez-Alvarez B , Mosén-Ansorena D, García-Peydró, M, Fuentes P, García-León MJ, Gonzalez-Lahera A, Macias-Camara N, Toribio ML, Aransay AM, López-Larrea C. Regulation of the transcriptional program by DNA methylation during human αβ T-cell development. Nucleic Acid Research. 43:760-774.

Cortés JR, Sánchez-Díaz R, Bovolenta ER, Barreiro O, Lasarte S, Matesanz-Marín A, Toribio ML, Sánchez-Madrid F, Martín P. Maintenance of immune tolerance by Foxp3+ regulatory T cells requires CD69 expression. J Autoimmun. 55:51-62. 2014.

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Fernández L, Metais JY, Escudero A, Vela M, Valentín J, Vallcorba I, Leivas A, Torres J, Valeri A, Patiño-García A, Martínez J, Leung W, Pérez-Martínez A.Memory T Cells Expressing an NKG2D-CAR Efficiently Target Osteosarcoma Cells. Clin Cancer Res. 2017 Oct 1;23(19):5824-5835.

García M, Barrio R, García-Lavandeira M, Garcia-Rendueles AR, Escudero A, Díaz-Rodríguez E, Gorbenko Del Blanco D, Fernández A, de Rijke YB, Vallespín E, Nevado J, Lapunzina P, Matre V, Hinkle PM, Hokken-Koelega AC, de Miguel MP, Cameselle-Teijeiro JM, Nistal M, Alvarez CV, Moreno JC. The syndrome of central hypothyroidism and macroorchidism: IGSF1 controls TRHR and FSHB expression by differential modulation of pituitary TGFβ and Activin pathways. Sci Rep. 2017 Mar 6;7:42937.

Fasciani I, Temperán A, Pérez-Atencio LF, Escudero A, Martínez-Montero P, Molano J, Gómez-Hernández JM, Paino CL, González-Nieto D, Barrio LC. Regulation of connexin hemichannel activity by membrane potential and the extracellular calcium in health and disease. Neuropharmacology. 2013 Dec;75:479-90.

Funded grants (in the last 5 years)

MINECO. SAF2016-44857-R

PI: Dr. María Luisa Toribio García

TITLE: Generación de novo de leucemia T linfoblástica aguda (T-ALL) humana in vivo: una herramienta única para el estudio de las bases moleculares de la enfermedad.

2017- 2019. 240.000 €

IV Beca Fundación Uno Entre Cien Mil

PI: Dr. María Luisa Toribio García

TITLE: Inmunoterapia frente a las recaídas en un nuevo modelo de generación *in vivo* de leucemia linfoblástica aguda T humana.

2017-2018. 100.000€

MINECO. SAF2014-62233-EXP

PI: Dr. María Luisa Toribio García

TITLE: Explorando una estrategia de rejuvenecimiento del Sistema Inmunitario envejecido.

2015- 2017. 84.700 €

MINECO. SAF2013-44857-R

PI: Dr. María Luisa Toribio García

TITLE: Moduladores y efectores de la señalización por Notch1 en el desarrollo de los linfocitos T y en leucemia: nuevas terapias dirigidas frente a la T-ALL

2014- 2016.471.900 €

EUROPEAN UNION 7FP. UE-FP7-HEALTH-2013-INNOVATION-1-602587.

PI: Dr. María Luisa Toribio García

TITLE: Development of stem cell based Therapy for Thymic regeneration. THYMISTEM.

2013-2017. 750.000 €

Fundación Científica AECC (20134201)

PI: Dr María Luisa Toribio García

TITLE: Estudio Preclínico del Potencial Terapéutico de Agentes Inhibidores del Receptor de IL-7 (IL-7R) en la prevención y tratamiento de la Leucemia Linfoblástica Aguda (LLA).

2013-2016. 150.000€

MICINN. SAF2010-15106

PI: Dr. María Luisa Toribio García

TITLE: Bases moleculares y celulares de la regeneración hematopoyética y la generación de leucemias: implicaciones fisiológicas y patológicas de Notch y microRNAs.

2010- 2013. 395.000 €

Contracts

Title: "Experimental Plan for detection of preTCR ✔expression in T cells"
Type of contract: I+D
Funding company: Cellectis Therapeutics (CTX) (Dr. Roman Galetto)
Instituition: CBMSO
Duration: 2013-2014
PI: M.L. Toribio. **Amount:** Collaboration
Title : "Preclinical study of the efficacy of TG101348 inhibitor on ex-vivo cells from high-risk BCR-ABL negative childhood acute lymphoblastic leukaemia with JAK2 mutations and assessment of disease reversion in vivo in immunodeficient mice".
Type of contract: I+D
Funding company: SANOFI AVENTIS
Instituition: CBMSO/ Hospital de La Princesa/Hospital Doce de Octubre
Duration: 2013-2014
PI: Elena Fernández-Ruiz. **Amount:** 200.000 euros (30.000 to MLT)

Trained students and postdocs

All trained graduate students have achieved their objectives successfully and have obtained their PhD with the highest grade, including two awards as indicated. All trained students, including previous FPI fellows, are presently working in science as indicated:

*María José Sánchez Sanz. Científico Titular CSIC.

*Edgar Fernández Malavé . PCD. UCM

*Almudena Rodríguez Ramiro. Junior Group Leader CNIC.

*César Trigueros Fernández. Research Scientist Inbiomed. San Sebastián.

*Yolanda Rodríguez Carrasco. Científico Titular CSIC. CNB.

Virginia García de Yébenes. Contrato postdoctoral. CNIC.

Maria Navarro Lobato. Contrato Ramón y Cajal. UAM

Enrique Martín Gayo. Contrato postdoctoral Harvard. USA

CANCER INFANTIL Y CANCERES POCO FRECUENTES 2018

BUDGET

TITLE

Preclinical targeting of T-ALL relapse using of a novel immunotherapy with anti-pre-TCR CAR-T cells

PRINCIPAL INVESTIGATOR

María Luisa Toribio García

RESEARCH CENTRE
Centro de Biología Molecular Severo Ochoa. CSIC-UAM

RESEARCH TEAM

Marina García Peydró. Centro de Biología Moleculas Severo Ochoa. CSIC-UAM.
Patricia Fuentes Villarejo. Centro de Biología Moleculas Severo Ochoa. CSIC-UAM.
Sara González García. Centro de Biología Moleculas Severo Ochoa. CSIC-UAM.
Alba Murcia Ceballos. Centro de Biología Moleculas Severo Ochoa. CSIC-UAM.
Juan Alcain Sánchez. Centro de Biología Moleculas Severo Ochoa. CSIC-UAM.
Adela Escudero López. Servicio de Hemato-Oncología Pediátrica Hospital Infantil Universitario La Paz

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In case of there is more than one group, all budgets must be done in separate charts and combined in one pdf document and uploaded at "Información económica" section.

BUDGET

	YEAR 1	YEAR 2	YEAR 3	TOTAL
PERSONNEL	35.000	35.000	35.000	105.000
EXPENDABLE MATERIAL	33.000	33.000	33.000	99.000
EQUIPMENT	0	0	0	0
TECHNICAL SERVICES	30.000	30.000	30.000	90.000
TRAVEL EXPENSES	1.000	1.000	1.000	3.000
OTHERS	1.000	1.000	1.000	3.000
TOTAL	100.000	100.000	100.000	300.000

The aecc Scientific Foundation grants aid of up to € 300.000 gross to three years, at a maximum of € 100.000 gross per year.
Taking into account the origin of the funds of the aecc Scientific Foundation, the financing granted in no case may be used for indirect costs.
The concepts susceptible to this grant are detailed in Annex I *"COST GUIDANCE Information about eligible costs"* of this document.

PERSONNEL

The budget is required to contract a postdoct with experience in animal models, flow cytometry and molecular biology and functional assays. Total expenses including taxes according to CSIC will be 35.000 euros/ year.

SUBTOTAL: 105.000,00€

EXPENDABLE MATERIAL:

Monoclonal antibodies
Cell culture medium, FCS and plastic material
Cell isolation kits (Miltenyi)
Transduction reagents (Retronectin)
Cytokines
Restriction enzymes
Biochemical reagents

SUBTOTAL: 0,00€

EQUIPMENT:

SUBTOTAL: 0,00€

TRAVEL EXPENSES:

National and International Congresses (1.000 €/year)

SUBTOTAL: 3.000,00€

OTHERS:
Courier services

SUBTOTAL: 3.000,00€

TECHNICAL SERVICES (TS):

Animal Facilities
Flow cytometry Facilities
Microscopy Facilities
NGS

SUBTOTAL:	90.000,00€

TOTAL:	300.000,00€

JUSTIFICATION OF THE BUDGET

PERSONNEL

The budget is required to contract a postdoct with experience in animal models, flow cytometry and molecular biology and functional assays. Total expenses including taxes according to CSIC will be 35.000 euros/ year.

EXPENDABLE MATERIAL
Monoclonal antibodies
Cell culture medium, FCS and plastic material
Cell isolation kits (Miltenyi)
Transduction reagents (Retronectin)
Cytokines
Restriction enzymes

Courier for sample transportation

National and International Congresses for results dissemination

TECHNICAL SERVICES

Animal Facilities
Flow cytometry Facilities
Microscopy Facilities
NGS

En cumplimiento de lo previsto en la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal, el solicitante queda informado tanto de que los datos de carácter personal relativos a los investigadores facilitados junto con las solicitudes para la convocatoria, serán incorporados a un fichero que, debidamente inscrito en el Registro General de Protección de Datos y, siendo responsabilidad de la Fundación ***aecc*** *y con domicilio en C/ Amador de los Ríos, nº 5, Código Postal 28010, Madrid tiene como finalidad la evaluación y valoración de la solicitud presentada así como, en caso de ser seleccionada, para el mantenimiento, seguimiento, control y gestión del programa seleccionado.*

Del mismo modo, los datos personales incluidos en las candidaturas no seleccionadas, serán asimismo conservados y mantenidos en dicho fichero. Estos datos podrán ser utilizados por la Fundación aecc con la finalidad de mantenerles informados sobre las actividades y noticias relacionadas con la propia Fundación y la Asociación Española Contra el Cáncer.

Los interesados podrán ejercitar, en cualquier momento, sus derechos de acceso, rectificación, cancelación y oposición mediante solicitud dirigida a la Fundación aecc, c/ Amador de los Ríos, 5 – 28010 Madrid o fundacion.cientifica@aecc.es, acompañada de una fotocopia del DNI. Del mismo modo, en caso de que la candidatura presentada no sea seleccionada, los interesados podrán revocar en cualquier momento su consentimiento para la conservación de sus datos en los términos previstos en el párrafo anterior.