

SCIENTIFIC RESEARCH REPORT

BANC DE SANG I TEIXITS | 2016

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PRESENTATION OF GENERAL DIRECTOR

We present you the memory 2016, a document that gathers the main research projects in which we have worked at the Blood and Tissue Bank. It has been the year in which we have approved the Strategic Plan for research that will take us to the Horizon 2020. This Strategic Plan focuses on five key areas: hemotherapy, tissues, cell therapy, biological safety, and also for the first time in the social aspect of the donation.

Coinciding with the development of the Research Plan, we launched the Catalan Stem Cell Transplantation Alliance, which brings together the main clinical teams of Catalonia with professionals of the Blood and Tissue Bank and aims to bring together knowledge and generate activity of applied research in stem cells. Also highlight the new agreement with the British Institute of research Anthony Nolan, with whom we maintain a close relationship for years. Thanks to this new agreement, we have the advice of Dr. Alejandro Madrigal, scientific director of the Institute, to help contribute and lead very important research projects to advance in the treatment of diseases such as leukemia.

The year that we have left behind is relevant also in the area of tissues, given that we have completed the integration of the two banks in Catalonia, joining all activity in one place, in the central headquarters Frederic Duran i Jordà. Thanks to a commitment to future infrastructure, this 2016 we have inaugurated a surface of clean rooms that will allow us to carry out the daily activity in an optimal manner and also deal with the future projects, in collaboration with clinical institutions as much as possible.

This 2016, we picked up our research in almost fifty research projects. All, with the unique perspective of improving the quality of life of all people requiring treatments we have available.

Enric Argelagués Vidal



INTRODUCTION BY THE SCIENTIFIC DIRECTOR

Once again we bring you the research report of the Blood and Tissue Bank. Now, the one of the year 2016.

If you could somehow summarize what has happened the last year would be using the syllable "re" as a common factor of words such as Reunion, Reflection, Relaunch. But ... that will come later; We will speak now of what we have done and achieved.

As can be seen in the following pages, in a time that has not ceased to be difficult, we have maintained the research activity, with 64 researchers, 70% with partial dedication and 49 active projects, including those that we finance with own funds (14), those who obtain competitive financing (22) and those that result from collaboration with industry (13).

Some of these projects have generated two doctoral theses (15 already in the last 10 years) and two new European patents (out of a total of 7).

On the other hand, the number of publications has been clearly lower but maintaining the overall impact factor and has doubled the number of publications in the first quartile, an indicator of their qualitative improvement.

Another fact that often goes unnoticed is the incorporation of new products or services resulting from research or self-development. Without entering into details, in 2016, the laboratories of Immunohematology, Histocompatibility, Congenital Coagulopathies and the Tissue Bank benefited from the result of research and internal developments.

From the academic point of view, through the chair, we have maintained the pace of the international master's degree "EMTACT" and we have organized two scientific days in collaboration with the European School of Transfusion Medicine, with which we have an agreement.

Now yes: returning to the "re", say that in 2016 we have gathered the divisions of Advanced Therapies and Cell Therapy Services. This will certainly boost research in this area. We have also assembled the tissue bank, now in the headquarters facilities, which will favor all kinds of synergies.

We have also reflected on the past and the future of research in the BST and we have proposed a new strategic plan that has a greater impact on translating the results of research into society and on a greater scientific impact of our activity.

And finally, and this is perhaps the most important aspect of our future, the BST has once again opted for a relaunch of the research, approving a new strategic plan, the PER 2017-20 that we hope will serve as a beacon and guide for addressing the new challenges.

Joan Garcia Lopez

1. BANC DE SANG I TEIXITS

The Banc de Sang i Teixits (Blood and Tissue Bank - BST) is the public company of the Catalan Ministry of Health whose mission is to guarantee the supply of blood of sufficient quality, for all the citizens of Catalonia. The BST manages and administers the donation, transfusion and analysis of blood and blood plasma. It also acts as a centre for obtaining and processing tissues and cord blood units and develops other lines of activity as a centre specialized in immunobiology, molecular analysis, cell therapy and regenerative medicine.

- BST is the backbone of the hemotherapy system in Catalonia
- Its activity extends to all public and private centres in Catalonia as well as others in Spain, providing a proximity service to donors and customers
- BST aims to be a first level centre in management, innovation research on hemotherapy and tissues

The BST participates in its own research projects or in collaboration with all the centres of the Catalan Health Institute, a large part of the Public Hospital Network and Catalan Universities and also promotes strategic alliances with research centres and industry.

1.1 GOVERNING BODIES

The Governing Bodies of the Banc de Sang i Teixits are the Board of Directors, his Commissions and the Strategic Committee of Tissues.

1.1.1 Board of Directors

President: Manel Peiró Posadas

Vice-president: Pilar Magrinyà Rull

Secretary: Rafael Gomáriz Parra

Members: Antoni Castells Garagou, Enric Contreras Barbeta, Francesc Gòdia Casablanças, Miquel Rutllant Bañeras, Emili Sullà Pascual, Roberto Gili Palacios, Vicenç Martínez Ibáñez, Ivan Planas Miret, Santiago Suso Vergara and Maria Antònia Viedma Martí.

1.1.2 Commissions of the Board of Directors

Economics and audits: Ivan Planas Miret, Carmen Garcia Jarque and Emili Sullà Pascual

Innovation and Research: Francesc Gòdia Casablanças and Miquel Rutllant Bañeres

Corporate Development: Roberto Gili Palacios, Miquel Rutllant Bañeres and Santiago Suso Vergara

1.1.3 Strategic Committee of Tissues

President: Antoni Castells Garagou

Members: Santiago Suso Vergara, Maria Antònia Viedma Martí and Francesc Gòdia Casablanças

Guests: Enric Argelagués Vidal, Isabel López Asi6n and Esteve Trias Adroher

1.2 DIRECTION AND MANAGEMENT BODIES

1.2.1 Direction Committee

Managing Director: Enric Argelagués Vidal

Assistant to Managing Director: Isabel López Asi6n

Director of People and Values: Esther Solà Saplana
Communication Director: Aurora Masip Treig
General Services Director: Joan Ovejo Cortes
Director of the Blood Division: Lluís Puig Rovira
Information and Communications Technology Director: Albert Herrero Espinet
Coordinator of the Territorial Centres: Enric Contreras Barbeta

1.2.2 Territorial Centres Committee

Managing Director: Enric Argelagués Vidal
Assistant to Managing Director: Isabel López Asión
Director of the Blood Division: Lluís Puig Rovira
Director of the Immunohematology Division: Eduardo Muñoz Díaz
Barcelona. Vall d'Hebron and Clínic: Rafael Parra Lopez
Barcelona. Sant Pau: Alba Bosch Llobet
Badalona. Germans Trias i Pujol: Joan Ramon Grífols Ronda
L'Hospitalet. Bellvitge: Isabel Gonzalez Medina
Manresa. Fundació Althaia/Terrassa. Mútua de Terrassa: Ramon Salinas Argente
Girona. Dr. Josep Trueta: Anna Millan Alvarez
Lleida. Arnau de Vilanova: Juan Manuel Sánchez Villegas
Tarragona. Joan XXIII/Tortosa. Verge de la Cinta/Reus. Sant Joan: Virginia Callao Molina

1.3 ADVISORY BODIES

1.3.1 Internal Scientific Committee

The Internal Scientific Committee is the advisory body in charge of watch over the realization of those tasks linked with the promotion and development of the R+D+I in the organization.

Between the tasks that this committee has to perform we highlight:

- Reviews the R+D+i policy and assures its diffusion and knowledge
- Coordinates the development of the Strategic Plan for R+D+I and evaluates its degree of attainment
- Ensures the achievement of the annual objectives for R+D+I
- Leads the activities associated with the Technology Watch (vigilance, prospective, analysis...)
- Periodically reviews the scientific production, the economic aspects and the personnel of the Research Area
- Takes part, as responsible unit of the programs, of the research activities and evaluates the improvement of the projects (foreseeing deviations and problems)
- Review the methodology of the process for continuous improvement

Composition:

- BST Scientific Director
- Coordinators of the R+D+i programmes: Lluís Puig Rovira, Sílvia Sauleda Oliveras, Enric Contreras Barbeta, Eduard Muñoz Díaz, Francisco Vidal Pérez, José Luis Caro Oleas, Sergi Querol Giner, Joan Garcia López i Arnau Pla Calvet
- Members of the Area of Innovation and Projects
- Manager of the Information and Communication Technologies, General Services, Marketing and communication Divisions (when appropriate)

1.3.2 External Scientific Committee

The new Strategic Research Plan for R+D+i has restored the External Scientific Committee.

Between the tasks that this committee would have to perform we highlight:

- Evaluates annually the activity of R+D+I developed in the BST
- Gives opinion and suggestions on the adequacy and the monitoring of the Strategic Research Plan for R+D+i
- Makes recommendations on the lines of research and programs (foster, auditing, redirect...)
- Provides guidance on how to increase the external resources for research and on possible partnerships to establish
- Performs functions of external technology watch

Composition:

- Prof. Alejandro Madrigal, London (President)
- Prof. Miguel López Botet, IMIM UPF
- Prof. Juan Ignacio Esteban, HVH UAB
- Prof. Herman Einsele, Univ. Würzburg
- Prof. Ellen van der Schoot, Sanquin
- Dr. Jose Antonio Pérez Simón, IBIS, Sevilla
- Dr. Juan Antonio Bueren, CIEMAT
- Jordi Martí Pi-Figueras, Celgene

1.4 LOCATION

The corporate headquarters of the Banc de Sang i Teixits are located on the corner of Passeig Taulat and Lope De Vega, in the 22@ technological district of Barcelona. The building centralises the various lines of activity and a large part of the 600 professionals of the organisation. The BST has also headquarters in major hospitals of Catalonia.

1.5 SUMMARY OF RESEARCH ACTIVITY

1.5.1 Research and technical staff

	Number	FDA
Principal investigators	3	1.43
Senior investigators	20	9.69
Investigators	32	16.78
Technical staff	9	5.40
TOTAL	64	33.30

1.5.2 Economic data

Breakdown of BST research income for 2016	Euros
Projects funded by public agencies	234,264
Agreements with industry	521,982
Own funds	2,860,514
TOTAL	3,616,760

1.5.3 Organisation of the BST research

The R+D+i Strategic Plan 2013-2015 defined 8 Research Programs that were re-structured in 2016 as follows:

Diagnosis, transfusional medicine & hemostasis	Hematopoietic transplantation & immunotherapy	Reparative & immunomodulatory therapy
PR1 Transfusional safety	PR5 Molecular biology of transplantation	PR7 Advanced therapies
PR2 Therapeutic apheresis	PR6 Transplantation of donors & alternative sources	PR8 Tissue Bank
PR3 Immunoematology		
PR4 Coagulopathies		

1.5.4 Research projects

ONGOING PROJECTS IN 2016		
	PRINCIPAL INVESTIGATOR BST	COLABORATION
PUBLIC AGENCIES		
ACCIÓ		1
European Commission	2	2
Carlos III Health Institute	4	3
Spanish Ministry Economy & Competitvity	1	
Spanish Ministry Health Social Service & Equality		6
Marató TV3	3	
AGREEMENTS WITH INDUSTRY		
Abbott		1
Baxalta	1	
Baxter	1	
Gamida		1
Grífols, S.A.	1	1
Immunocellular Therapeutics LTD		1
Novartis		1
Progenika	1	
Roche		1
Sanofi		1
Sotio		1
Therakos		1
OWN FUNDS	13	
TOTAL		48

1.5.5 Doctoral theses

Two doctoral theses were read or directed by BST investigators in 2016.

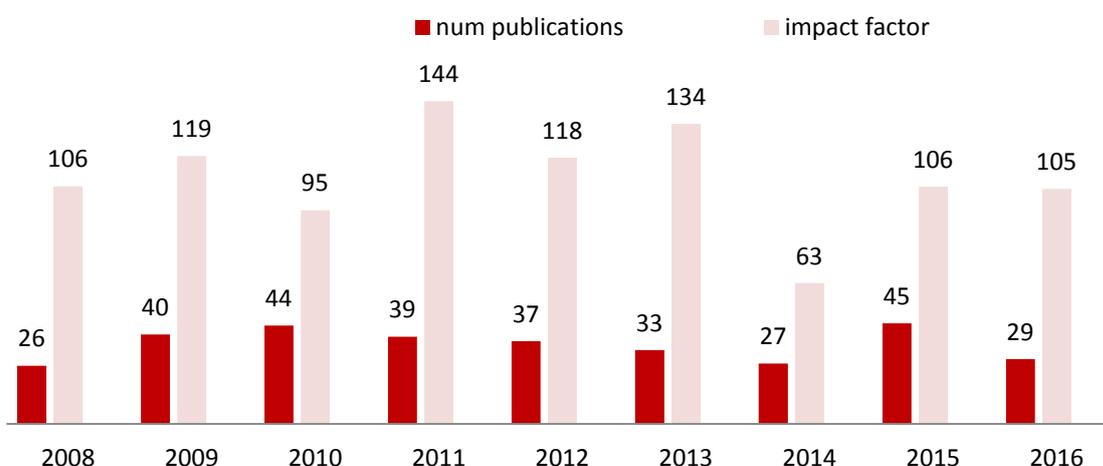
PhD student	Thesis title	Directors	Department	Grade
Maricel Subirà	Improving the functioning of a polyvalent medical day hospital in a district hospital based on the analysis of specific indicators and costs of the activities it performed	Ramon Salinas, Andres Lopez	UAB, Department of Medicine	Excellent
Bachar Kudsieh	Repositioning and transescleral fixation in the sulcus with polyester sutures, from intraocular lenses dislocated to vitreous cavity by vitrectomy 23-gauge: Evaluation of a surgical technique	Ricardo Casaroli, Jeroni Nadal	UB, Department of Surgery	Excellent Cum Laude unanimously

1.5.6 Publications

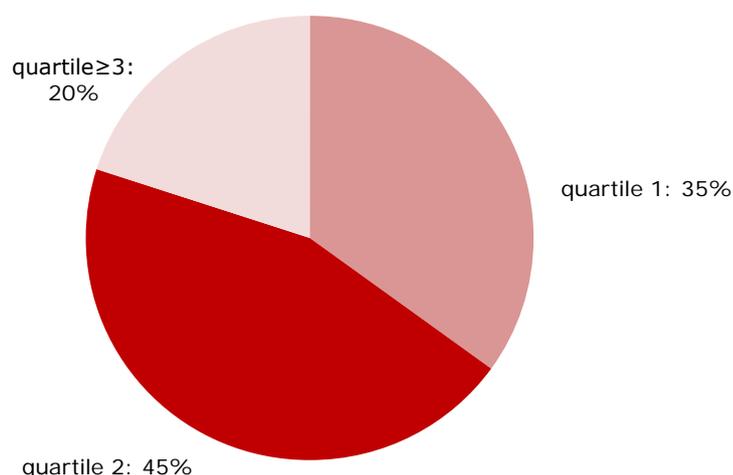
A total of 29 articles were published in scientific magazines by BST investigators in 2016 with an impact factor of 104.83.

The combined impact factor for 2016 was calculated using Journal Citation Reports (JCR) for 2014. The calculation included original articles, revisions and editorials. Presentations to congresses were excluded.

Evolution of the scientific production of the BST over the last 9 years:



Publications BST 2016:



1.5.7 Patents

The BST currently has 7 patents in different stages of processing. Six of them are granted in Spain and several in USA, Colombia, Mexico, Belgium, Germany, France, United Kingdom, Italy and Holland.

1.6 TEACHING IN RESEARCH

The central element of teaching at the BST is the master of Transfusion Medicine and Cell Therapy, organised through the Autonomous University of Barcelona (UAB) with the support of the Doctor Robert Foundation. Even though this master is not research oriented, some students become interested in pursuing their doctoral studies. The master degree, begun in 2003, has improved in format and internationalisation. Its purpose is specialised training in all processes that take place in a blood bank (donation, processing, transfusion, immunohematology, management and certification) and a tissue bank with a far-reaching cell therapy program. The master for nurses in blood transfusion and cellular and tissue therapy has started in 2012.

The BST participates in directing professionals who are writing dissertations and doctoral theses. Also collaborates in the training of different degrees (Nursing, Medicine, Biology, Pedagogy, Economy and Pharmacy) with agreements with UB, UAB, UPF, UPC, UIC and URV.

The BST organizes stays of training for diverse professional through collaboration agreements with most Latin American countries (Argentina, Uruguay, Colombia, Mexico...) and other European countries like the United Kingdom, Portugal, Sweden, Italy, etc.

Since October 2012, BST has the accreditation as Teaching Unit (BOE law 495/2010 30th of April), with the responsibility of teaching the residents of haematology and hemotherapy of Catalonia.

Other related projects

Chair of Transfusion Medicine and Cell and Tissue Therapy

The Autonomous University of Barcelona, the Blood and Tissue Bank and the Doctor Robert Foundation, created in 2008, the Chair of Transfusion Medicine and Cell and Tissue Therapy (CMT3).

The Mission of the Chair is to promote, assist and strengthen the training, research and consultancy in the field of Transfusion Medicine and Cell and Tissue Therapy, promoting collaboration between researchers and teachers of biomedical, health and welfare.

Since its inception, the CMT3 has led a project included in the sub-European Erasmus Education, Audiovisual & Culture Executive Agency. It has also participated in the project Eurocord-ED, within the subprogram Leonardo da Vinci.

On the other hand, in terms of postgraduate training, the first edition of EMTACT (European Master in Transfusion Medicine and Advanced Cell Therapies) and the first edition of "Master for nurses in blood transfusion and cellular and tissue therapy" have been finished. The first edition of "Master's degree in transfusion medicine and advanced cell therapies" and the second edition of "Master for nurses in blood transfusion and cellular and tissue therapy" have successfully started.

DoHeCa Project. Donor Health Care

The DoHeCa project, funded by the European Commission (file: 538986-LLP-1-2013-1-ERASMUS-EQR) led by the Dutch Blood Bank Sanquin, began by the end of 2013. This 3 years duration project, aims to implement a European Master in Donation, Transfusion and Transplantation of Blood, Cells, Tissues and Organs. Our Tissue Bank is one of the 15 partners of this project where prestigious Universities, Hospitals and Blood and Tissue Banks from 8 countries of the European Union participate.

1.7 THE BANC DE SANG I TEIXITS WEB SITE

The Blood and Tissue Bank has two web sites: www.bancsang.net and www.donarsang.gencat.cat. Both have versions in Catalan, Spanish and English.

www.bancsang.net has information throughout the organization. The contents are divided into six contents blocks (corporate information, donors, receivers, professionals, R+D+i and teaching).

The page is regularly updated with news and has an application for managing online orders. It includes documentation in PDF and video.

www.donarsang.gencat.cat is a website aimed for donors and potential donors and aims to disclose the donation as an act of solidarity, civic engagement and citizen participation.

It offers all the information on the need to donate blood, its uses and the state of the reserves. Also allows searching by town or zip code of upcoming mobile donation campaigns. It also features a news section about donating blood.

In the private area of this site, the donor can modify his own contact details; view his history of donations and blood type.

The blog bancsang.net/blog contains information on the corporate, welfare and scientific activity of the Banc de Sang i Teixits and is addressed to the whole of the citizenship. It has an electronic newsletter to which anyone can subscribe to receive e-mail content updates.

The blog moltesgracies.net contains stories of people who have needed blood and tissues for their treatments. It has a form so that any receiver can explain its history. In this way it is intended to visualize the importance of the donations, putting face to the people who benefit from them directly.

2. RESEARCH ACTIVITY OF THE BST

2.1 DIAGNOSIS, TRANSFUSIONAL MEDICINE & HEMOSTASIS

2.1.1 Program 1: Transfusional safety



The Transfusion Safety Laboratory (LST) is comprised of the Healthcare Unit and the R&D&I Unit for transmissible agents. The R&D&I activity of the LST can be classified in the following main lines:

- A. Viral hepatitis (HBV, HCV and HEV) and co-infection with HIV.
- B. Epidemiological research and development of new tools for the detection of emerging infectious agents (Chagas disease, HTLV-I/II, Chikungunya virus, malaria, XMRV).

The final end-point of these lines is to improve physiopathological and epidemiological knowledge and the detection of infectious agents relevant to the safety of blood products, cord blood and tissues.

It is also important to highlight the activity undertaken to improve knowledge of the presence of pathogens coming from other countries among the BST Catalan reference population. The objectives of studies performed along these lines is to plan and establish strategies to guarantee the safety of blood products based on the correct selection of blood donors and the application of diagnostic tests. It must be born in mind that the BST is the only centre that distributes blood products in Catalonia and is directly responsible for maintaining and promoting research along these lines.

PERSON IN CHARGE

Sílvia Sauleda Oliveras

INVESTIGATORS

Marta Bes Maijo

Natàlia Casamitjana Ponces

Maria Piron

Carmen De la Torre-Monmany Rial

TECHNICAL STAFF

Mireia Parés Guerrero
Angeles Rico Blázquez

RESEARCH PROJECTS

Principal investigator: Maria Piron

Development of real time protocols for PCRs (ZIKA, Dengue, Chikungunya, HTLV-I, HTLV-II, etc) as screening tools or supplementary analyses of emerging infectious pathogens and a field study of emerging pathogens in high-risk travellers and immigrant donors

Funding organisation: BST
Duration: 2009 to 2017

Principal investigator: Sílvia Sauleda Oliveras

Hepatitis E and transfusion safety: Validation of an in-house method of HEV RNA screening in blood donations and prevalence study in oncohematology patients

Funding organisation: BST
Duration: 2016 to 2017

Principal investigator: Sílvia Sauleda Oliveras

Surveillance of Strain Diversity, Viral Genome Characterization, and New Virus Discovery in Spanish Blood Donors

Funding organisation: Abbott
Duration: 2016 to 2019

Principal investigator: Rafael Esteban (Hospital Vall d'Hebron), Marta Bes (BST)

HCV resistance mutations to new direct acting antivirals represents a key factor to optimize cost-effective treatments

Funding organisation: Carlos III Health Institute
Duration: 2016 to 2018

Principal investigator: Juan Ignacio Esteban Mur (Hospital Vall d'Hebron), Sílvia Sauleda Oliveras (BST)

Prospective Sample Collection – Evaluation of novel markers for early detection of Hepatocellular Carcinoma

Funding organisation: Roche
Duration: 2014 to 2016

PUBLICATIONS

Riveiro-Barciela M, **Sauleda S**, Quer J, Salvador F, Gregori J, **Pirón M**, Rodríguez-Frías F, Buti M. Red blood cell transfusion-transmitted acute hepatitis E in an immunocompetent subject in Europe: a case report. TRANSFUSION 2016 Oct 26. QUARTILE 2, IMPACT FACTOR 3.225

BACKGROUND: Acute hepatitis E in industrialized countries is usually related to intake or manipulation of undercooked or raw meat. Cases of transfusion-transmitted hepatitis E have rarely been documented in immunosuppressed patients, mainly after receiving frozen plasma. **STUDY DESIGN AND METHODS:** A 61-year-old man was admitted to hospital for jaundice. His personal history included disseminated bacillus Calmette-Guerin infection treated with antituberculous drugs. He had received red blood cell (RBC) transfusion 2 months previously, during admission for mycotic aneurysm surgery. Since liver function tests worsened despite stopping antituberculous drugs, other causes of acute hepatitis were explored. **RESULTS:** Acute hepatitis E was diagnosed by the presence of both immunoglobulin M and hepatitis E virus (HEV) RNA. Traceback procedure for the 8 RBC units was carried out, and one of the eight archive plasma samples tested positive for HEV RNA, with an estimated viral load of 75,000 IU/mL.

Phylogenetic analysis revealed the same HEV strain Genotype 3 in one of the transfused RBC products and in the patient's serum sample. **CONCLUSION:** Transfusion of RBCs with detectable HEV RNA is a risk factor for acute hepatitis E in immunocompetent patients in Europe.

RESEARCH PROJECTS

Principal investigator: Mercè Boada Rovira (Fundació ACE), Pilar Ortiz Murillo (BST)

A multicenter, randomized, controlled study to evaluate the efficacy and safety of short-term plasma exchange followed by long-term plasmapheresis with infusion of human albumin combined with intravenous immunoglobulin in patients with mild-moderate Alzheimer's disease

Funding organisation: Grífols

File N^o: IG1002

Duration: 2012 to 2017

Principal investigator: Gemma Mur (Hospital Vall d'Hebron), Rafael Parra Lopez (BST)

A phase I trial of actively personalized peptide vaccinations plus immunomodulators in patients with newly diagnosed glioblastoma concurrent to first line temozolomide maintenance therapy

Funding organisation: European Commission

File N^o: 2013-002801-71

Duration: 2015 to 2017

Principal investigator: Joan Carles (Hospital Vall d'Hebron), Rafael Parra Lopez (BST)

A Randomized, Double Blind, Multicenter, Parallel-Group, Phase III Study to Evaluate Efficacy and Safety of DCVAC/PCa Versus Placebo in Men with Metastatic Castration Resistant Prostate Cancer Eligible for 1st Line Chemotherapy

Funding organisation: Sotio

File N^o: 2012-002814-38

Duration: 2016 to 2017

Principal investigator: Susana Rives Solà (Hospital Sant Joan de Déu), Enric Garcia Rey (BST)

Infusion of autologous T cells engineered to express anti-CD19 as therapy for patients with relapsed or refractory CD19+ leukaemia or lymphoma: a pilot study

Funding organisation: Carlos III Health Institute

File N^o: ICI14/00224

Duration: 2016 to 2017

Principal investigator: Cristina Diaz Heredia (Hospital Vall d'Hebron), Rafael Parra Lopez (BST)

Single-Arm Study to Assess the Efficacy of UVADEX® (methoxsalen) Sterile Solution in Conjunction With the THERAKOS® CELLEX® Photopheresis System in Pediatric Patients With Steroid-Refractory Acute Graft Versus Host Disease (aGvHD)

Funding organisation: Therakos

File N^o: 2014-004806-14

Duration: 2016 to 2017

Principal investigator: Juan Manuel Gil Gil (ICO Duran i Reynals), Isabel Gonzalez Medina (BST)

STING (Study of Immunotherapy in Newly Diagnosed Glioblastoma): A Phase III randomized double-blind, controlled study of ICT-107 with maintenance temozolomide (TMZ) in newly diagnosed glioblastoma following resection and concomitant TMZ chemoradiotherapy

Funding organisation: Immunocellular Therapeutics, LTD

File N^o: ICT-107-301

Duration: 2016 to 2017

PUBLICATIONS

Boada M, Anaya F, **Ortiz P**, Olazarán J, Shua-Haim JR, Obisesan TO, Hernández I, **Muñoz J**, Buendia M, Alegret M, Lafuente A, Tárraga L, Núñez L, Torres M, **Grifols JR**, Ferrer I, Lopez OL, Páez A. Efficacy and Safety of Plasma Exchange with 5% Albumin to Modify Cerebrospinal Fluid and Plasma Amyloid- β Concentrations and Cognition Outcomes in Alzheimer's Disease Patients: A Multicenter, Randomized, Controlled Clinical Trial. J ALZHEIMERS DIS 2016 Nov 28. QUARTILE 1, IMPACT FACTOR 4.151

BACKGROUND: Studies conducted in animal models and humans suggest the presence of a dynamic equilibrium of amyloid- β ($A\beta$) peptide between cerebrospinal fluid (CSF) and plasma compartments. **OBJECTIVE:** To determine whether plasma exchange (PE) with albumin replacement was able to modify $A\beta$ concentrations in CSF and plasma as well as to improve cognition in patients with mild-moderate Alzheimer's disease (AD). **METHODS:** In a multicenter, randomized, patient- and rater-blind, controlled, parallel-group, phase II study, 42 AD patients were assigned (1:1) to PE treatment or control (sham) groups. Treated patients received a maximum of 18 PE with 5% albumin (Albutein®, Grifols) with three different schedules: two PE/weekly (three weeks), one PE/weekly (six weeks), and one PE/bi-weekly (12 weeks), plus a six-month follow-up period. Plasma and CSF $A\beta$ 1-40 and $A\beta$ 1-42 levels, as well as cognitive, functional, and behavioral measures were determined. **RESULTS:** CSF $A\beta$ 1-42 levels after the last PE compared to baseline were marginally higher in PE-treated group versus controls (adjusted means of variation: 75.3 versus -45.5pg/mL; 95% CI: -19.8, 170.5 versus 135.1, 44.2; $p=0.072$). Plasma $A\beta$ 1-42 levels were lower in the PE-treated group after each treatment period ($p<0.05$). Plasma $A\beta$ 1-40 levels showed a saw-tooth pattern variation associated with PE. PE-treated patients scored better in the Boston Naming Test and Semantic Verbal Fluency ($p<0.05$) throughout the study. Neuropsychiatric Inventory scores were higher in controls during the PE phase ($p<0.05$). **CONCLUSION:** PE with human albumin modified CSF and plasma $A\beta$ 1-42 levels. Patients treated with PE showed improvement in memory and language functions, which persisted after PE was discontinued.

2.1.3 Program 3: Immunoematology



The Immunoematology laboratory is a national and international reference in the diagnosis of immune cytopenia and the typing and characterisation of blood groups.

PERSON IN CHARGE

Eduardo Muñiz Díaz

INVESTIGATORS

Núria Nogués Galvez
Cecilia González Santesteban

RESEARCH PROJECTS

Principal investigator: Núria Nogués Gálvez

Expression of the recombinant Miltemberger III or GP Mur antigen

Funding organisation: Diagnòstic Grífols

Duration: 2013 to 2017

Principal investigator: Núria Nogués Gálvez

BLOOD NGS: Product for the complete typing of ABO and RH systems

Funding organisation: Progenika

Duration: 2014 to 2017

PUBLICATIONS

González C, Esteban R, Canals C, Muñiz-Díaz E, Nogués N. Stabilization of Transfected Cells Expressing Low-Incidence Blood Group Antigens: Novel Methods Facilitating Their Use as Reagent-Cells. PLOS ONE 2016 Sep 7;11(9):e0161968. QUARTILE 1, IMPACT FACTOR 3.898

BACKGROUND: The identification of erythrocyte antibodies in the serum of patients rely on panels of human red blood cells (RBCs), which coexpress many antigens and are not easily available for low-incidence blood group phenotypes. These problems have been addressed by generating cell lines expressing unique blood group antigens, which may be used as an alternative to human RBCs. However, the use of cell lines implies several drawbacks, like the requirement of cell culture facilities and the high cost of cryopreservation. The application of cell stabilization methods could facilitate their use as reagent cells in clinical laboratories. **METHODS:** We generated stably-transfected cells expressing low-incidence blood group antigens (Dia and Lua). High-expresser clones were used to assess the effect of TransFix® treatment and lyophilization as cell preservation methods. Cells were kept at 4°C and cell morphology, membrane permeability and antigenic properties were evaluated at several time-points after treatment. **RESULTS:** TransFix® addition to cell suspensions allows cell stabilization and proper antigen detection for at least 120 days, despite an increase in membrane permeability and a reduction in antigen expression levels. Lyophilized cells showed minor morphological changes and antigen expression levels were rather conserved at days 1, 15 and 120, indicating a high stability of the freeze-dried product. These stabilized cells have been proved to react specifically with human sera containing alloantibodies. **CONCLUSIONS:** Both stabilization methods allow long-term preservation of the transfected cells antigenic properties and may facilitate their distribution and use as reagent-cells expressing low-incidence antigens, overcoming the limited availability of such rare RBCs.

Flesch BK, Morar B, Comas D, **Muñiz-Diaz E**, **Nogués N**, Kalaydjieva L. The AQP1 del601G mutation in different European Romani (Gypsy) populations. *BLOOD TRANSFUS* 2016 May 11: 1-2. QUARTILE 3, IMPACT FACTOR 2.239

Meler E, Porta R, **Canals C**, Serra B, Lozano M. Fatal alloimmune thrombocytopenia due to anti-HLA alloimmunization in a twin pregnancy: A very infrequent complication of assisted reproduction. *TRANSFUS APHER SCI* 2016 Nov 2. QUARTILE 4, IMPACT FACTOR 0.768

The most frequently involved antigen in severe fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the human platelet antigen 1a. Platelets express the HLA-A and B antigens on their membrane and some studies report that maternal anti-HLA class I antibody can also cause FNAIT. We report here a very unusual case of a first twin pregnancy produced in vitro by oocyte and semen donation where the mother developed markedly elevated HLA antibodies, in the absence of anti-platelet or anti-neutrophil antibodies, that provoked in one of the twins a profound thrombocytopenia and intracranial hemorrhage and a mild thrombocytopenia and neutropenia in the second twin lasting until the fourth month of life. In addition, anti-D alloimmunization provoked hemolytic disease of the newborn with intrauterus anemia detected in the first twin and post-natal anemia in the second twin that required red blood cell transfusion and phototherapy. We hypothesize that the complete HLA-incompatible twin pregnancy due to the oocyte donation might have contributed to the severity of the clinical manifestations.

2.1.4 Program 4: Coagulopathies



The program of research into congenital coagulopathies of the Banc de Sang i Teixits has had a dual character since its foundation in 1998: support for the diagnosis of congenital coagulation disorders and other hereditary diseases; and the investigation and development of new perspectives in the diagnosis and therapeutic field. A large part of the current objectives is innovation of technological tools and their transfer into laboratory routine.

The main lines are centred on the study of hereditary diseases or blood defects of enormous clinical, economic and social relevance such as haemophilia or von Willebrand's disease, as well as other aspects derived from these, and other, coagulopathies. In detail, the research objectives of the unit can be described as:

- A. Identification of the mutations responsible for haemophilia A and B in the Spanish population.
- B. Applications to therapeutic orientation, genetic advice, prenatal and pre-implantation diagnosis.
- C. Molecular diagnosis of von Willebrand's disease: study of genotype-phenotype relationship and their application to clinical diagnosis.
- D. Establishment of protocols and the genetic study of rare monogenic bleeding disorders: FXI deficit, FXIII deficit, combined FV and FVIII deficit, FVII deficit, Glanzmann's thrombasthenia, etc...
- E. Collection and use of stem cells with patient-specific induced pluripotency to improve diagnosis and treatment of hemophilia.

- F. In-depth studies of the molecular events found in some affected individuals and the genotype-phenotype relationship constituting the most basic area of the team's objectives.
- G. Clinical epidemiological studies aimed at the exhaustive identification of the clinical characteristics of patients with congenital coagulopathies and their response to different therapeutic options. These studies often entail the creation of different types of registers.

It is important to emphasise that epidemiological studies are reflected on the Hemobase web site (<http://www.hemobase.com>), dedicated to haemophilia and von Willebrand's disease. It includes the first register of characterised mutations of haemophilia patients in the Spanish population. It is a dynamic register with permanent updates. It includes general information on haemophilia, its classification, clinical characteristics and diagnosis difficulties, as well as the biochemical and molecular characteristics of the genes. Hemobase is recognised by the NCBI and Orphanet as a specific database of mutations of the FVIII, FIX and VWF loci.

The research activity is associated with the commitment of the Haemophilia Unit of Vall d'Hebron Hospital (reference centre for congenital coagulopathies in Catalonia) to the development of molecular protocols, applicable genetic advice and prenatal diagnosis. The Haemophilia Unit offers specialised healthcare to patients with hemorrhagic congenital coagulopathies such as haemophilia, von Willebrand's disease, thrombopathies and other coagulation factor deficits. Congenital coagulopathies, and especially haemophilia, are rare complex diseases. Achieving effective treatment requires a program of integral therapy. The Haemophilia Unit has an experienced multidisciplinary team that develops integral patient care, carries out daily healthcare control through clinical sessions, and has become a reference centre for congenital coagulopathies on a national and international level. Equally outstanding is the participation of the unit in numerous international multicentre studies (ITI, RODIN, HIGS and EUHASS).

PERSON IN CHARGE

Francisco Vidal Pérez

INVESTIGATORS

Nina Borràs Agustí
Irene Corrales Insa
Lluís Martorell Cedrés
Rafael Parra López

TECHNICAL STAFF

Nàtalia Comes Fernandez
Lorena Ramírez Orihuela

RESEARCH PROJECTS

Principal investigator: Francisco Vidal Pérez

Use of patient-specific induced pluripotent stem cells to improve diagnosis and treatment of hemophilia A

Funding organisation: European Commission

File N^o: PI11/03029

Duration: 2012 to 2016

Principal investigator: Francisco Vidal Pérez

Application of the new next generation sequencing technologies to the molecular diagnosis of congenital coagulopathies

Funding organisation: Carlos III Institute of Health

File N^o: PI12/01494
Duration: 2013 to 2016

Principal investigator: Francisco Vidal Pérez

Clinical and molecular profile of patients with von Willebrand disease (PCM-EVW-ES): Spanish registry
Funding organisation: Baxter
Duration: 2014 to 2016

Principal investigator: Francisco Vidal Pérez

Development and implementation of new molecular massive analysis tools for global approach to diagnosis and research of congenital coagulopathies
Funding organisation: Carlos III Health Institute
File N^o: PI15/01643
Duration: 2016 to 2019

Principal investigator: Francisco Vidal Pérez

Study of the molecular and clinical profile of VWD: extension of the Spanish VWD cohort (pcm-evw.es) and diagnosis improvement through new technologies
Funding organisation: Baxalta
File N^o: H16-32544
Duration: 2016 to 2019

Principal investigator: Francisco Vidal Pérez

Diagnóstico molecular de la hemofilia en Cuba. Estudio de la variabilidad genética y epidemiología poblacional.
Funding organisation: BST
Duration: 2016 to 2017

PUBLICATIONS

Martin-Fernandez L, Marco P, **Corrales I**, Pérez R, **Ramírez L**, López S, **Vidal F**, Soria JM. The Unravelling of the Genetic Architecture of Plasminogen Deficiency and its Relation to Thrombotic Disease. SCI REP 2016 Dec 15;6:39255. QUARTILE 1, IMPACT FACTOR 5.578

Although plasminogen is a key protein in fibrinolysis and several mutations in the plasminogen gene (PLG) have been identified that result in plasminogen deficiency, there are conflicting reports to associate it with the risk of thrombosis. Our aim was to unravel the genetic architecture of PLG in families with plasminogen deficiency and its relationship with spontaneous thrombotic events in these families. A total of 13 individuals from 4 families were recruited. Their genetic risk profile of thromboembolism was characterized using the Thrombo inCode kit. Only one family presented genetic risk of thromboembolism (homozygous carrier of F12 rs1801020 and F13A1 rs5985). The whole PLG was tested using Next Generation Sequencing (NGS) and 5 putative pathogenic mutations were found (after in silico predictions) and associated with plasminogen deficiency. Although we can not find genetic risk factors of thrombosis in 3 of 4 families, even the mutations associated with plasminogen deficiency do not cosegregate with thrombosis, we can not exclude plasminogen deficiency as a susceptibility risk factor for thrombosis, since thrombosis is a multifactorial and complex disease where unknown genetic risk factors, in addition to plasminogen deficiency, within these families may explain the thrombotic tendency.

Ferrán B, Martí-Pàmies I, Alonso J, Rodríguez-Calvo R, Aguiló S, **Vidal F**, Rodríguez C, Martínez-González J. The nuclear receptor NOR-1 regulates the small muscle protein, X-linked (SMPX) and myotube differentiation. SCI REP 2016 May 16;6:25944. QUARTILE 1, IMPACT FACTOR 5.578

Recent works have highlighted the role of NOR-1 in both smooth and skeletal muscle, and have proposed this nuclear receptor as a nexus that coordinates muscle performance and metabolic capacity. However, no muscle specific genes regulated by NOR-1 have been identified so far. To identify NOR-1 target genes, we over-expressed NOR-1 in human vascular smooth muscle cells (VSMC). These cells subjected to sustained over-expression of supraphysiological levels of NOR-1 experienced marked phenotypic changes and up-regulated the skeletal muscle protein X-linked (SMPX), a protein typically expressed in striated muscle and associated to cell shape. By transcriptional studies and DNA-protein binding assays, we identified a non-consensus NBRE site in human SMPX promoter, critical for NOR-1 responsiveness. The expression of SMPX was higher in human skeletal muscle myoblasts (HSMM) than in human VSMC, and further increased in HSMM differentiated to myotubes. NOR-1 silencing prevented SMPX expression in HSMM, as well as their differentiation to myotubes, but the up-regulation of SMPX was dispensable for HSMM differentiation. Our results indicate that NOR-1 regulate SMPX in human muscle cells and acts as a muscle regulatory factor, but further studies are required to unravel its role in muscle differentiation and hypertrophy.

Fidalgo T, Salvado R, **Corrales I**, Pinto SC, **Borràs N**, Oliveira A, Martinho P, Ferreira G, Almeida H, Oliveira C, Marques D, Gonçalves E, Diniz M, Antunes M, Tavares A, Caetano G, Kjällerström P, Maia R, Sevivas T, **Vidal F**, Ribeiro L. Genotype-phenotype correlation in a cohort of Portuguese patients comprising the entire spectrum of VWD types: impact of NGS. *THROMB HAEMOST* 2016 Mar 17;116(1). QUARTILE 1, IMPACTE FACTOR 4.984

The diagnosis of von Willebrand disease (VWD), the most common inherited bleeding disorder, is characterised by a variable bleeding tendency and heterogeneous laboratory phenotype. The sequencing of the entire VWF coding region has not yet become a routine practice in diagnostic laboratories owing to its high costs. Nevertheless, next-generation sequencing (NGS) has emerged as an alternative to overcome this limitation. We aimed to determine the correlation of genotype and phenotype in 92 Portuguese individuals from 60 unrelated families with VWD; therefore, we directly sequenced VWF. We compared the classical Sanger sequencing approach and NGS to assess the value-added effect on the analysis of the mutation distribution in different types of VWD. Sixty-two different VWF mutations were identified, 27 of which had not been previously described. NGS detected 26 additional mutations, contributing to a broad overview of the mutant alleles present in each VWD type. Twenty-nine probands (48.3%) had two or more mutations; in addition, mutations with pleiotropic effects were detected, and NGS allowed an appropriate classification for seven of them. Furthermore, the differential diagnosis between VWD 2B and platelet type VWD ($n = 1$), Bernard-Soulier syndrome and VWD 2B ($n = 1$), and mild haemophilia A and VWD 2N ($n = 2$) was possible. NGS provided an efficient laboratory workflow for analysing VWF. These findings in our cohort of Portuguese patients support the proposal that improving VWD diagnosis strategies will enhance clinical and laboratory approaches, allowing to establish the most appropriate treatment for each patient.

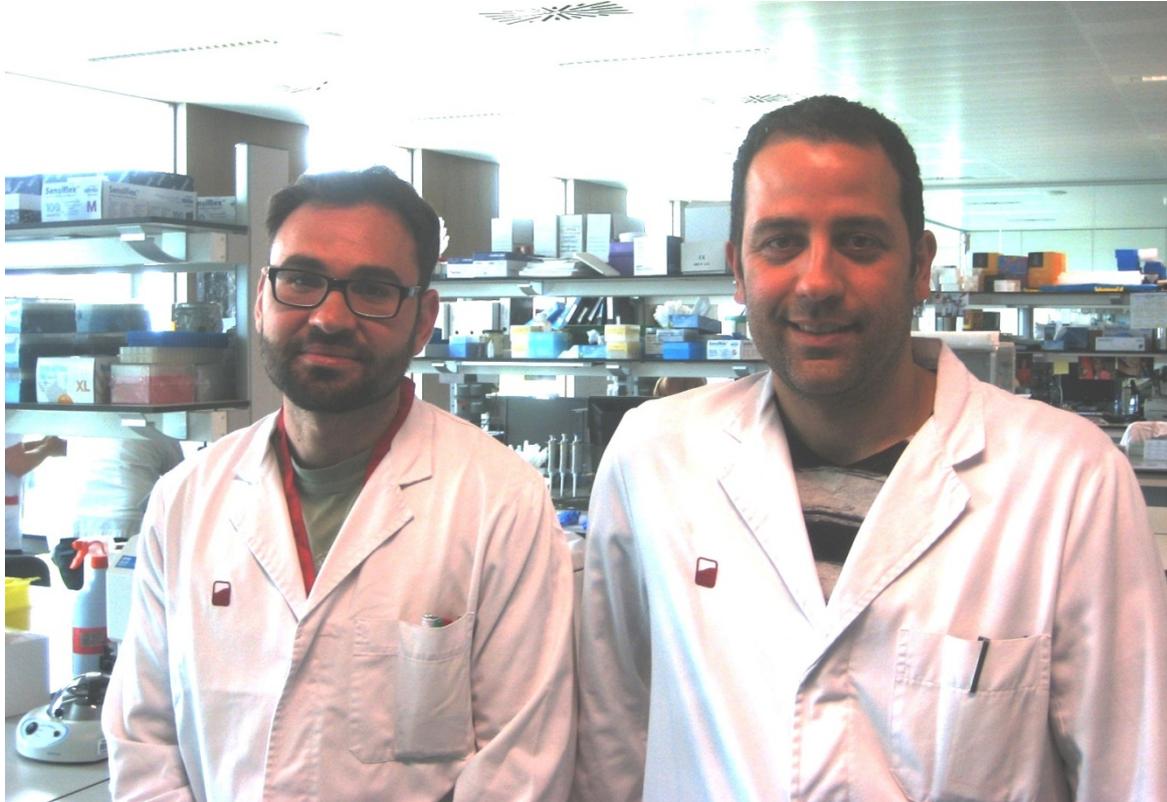
Iavecchia L, Safiya A, Salat D, Sabaté M, Bosch M, Biarnés A, Camps A, **Castellà D**, Lalueza P, Pons V, Teixidor J, Villar MM, Agustí A. Impact of implementing a protocol on the perioperative management in patients treated with anti-thrombotics admitted for hip fracture surgery: an observational study. *BASIC CLIN PHARMACOL TOXICOL* 2016 May 6. QUARTILE 2, IMPACT FACTOR 2.288

This study aimed to describe the impact of implementing a protocol on the perioperative management of patients admitted for hip fracture treated with anti-thrombotics. A protocol was designed based on recommendations from the American College of Chest Physicians (ACCP). After its implementation (May 2012), information on anti-thrombotic management was collected from admission to three months after surgery in retrospective (October 2011-March 2012) and prospective (October 2012-March 2013) cohorts. Patients' thromboembolic risk was classified into high, moderate or low according to the

ACCP categories. A total of 113 and 101 cases were included in the retrospective and prospective cohorts, respectively. No differences in age, gender, American Society of Anaesthesiology score or thrombotic risk categories were observed between cohorts. Most patients were treated with aspirin or triflusal (55.1 and 48.1% in each cohort, respectively), clopidogrel (24.5 and 26.6%) or acenocoumarol (16.3 and 20.2%). In moderate-high thromboembolic risk patients, a higher rate of bridging therapy with full doses of enoxaparin (18.5% and 50%, $p=0.04$ before and 9.1% and 43.7%, $p=0.02$ after surgery) and a lower rate of aspirin discontinuation (76% and 55.3%, $p=0.03$) were observed in the prospective cohort. Both cohorts had a similar percentage of cases with bleeding (68.1 and 68.3%) and thrombotic events (11.5% and 13%). No differences in the timing between surgery and the discontinuation or resumption of anti-thrombotics were noted. After protocol implementation, aspirin was less often stopped and bridging therapy with therapeutic doses of enoxaparin was used more often. However, interruption and resumption times of anti-thrombotics remained almost unchanged. In order to achieve these goals, more efforts should be made to implement the protocol in clinical practice.

2.2 HEMATOPOIETIC TRANSPLANTATION & IMMUNOTHERAPY

2.2.1 Program 5: Molecular biology of transplantation



The main lines of research are:

- A. Clinical Immunology
- B. Technological Development

Our professionals have teaching, healthcare, and research obligations in the area of Immunology and Immunogenetics.

Our laboratory is actively involved in various research projects with clinical groups of the hospitals that we give support to, as well as the cord blood bank of the BST. All these studies are grouped in the section of Clinical Immunology.

We highlight the development of own protocols for HLA typing, especially in applications for diagnosis of diseases of autoimmune origin, which have been conducted in recent years. Some of these protocols have already reached the stage of commercialization in collaboration with an external company. Currently the development has been directed towards the use of new technologies, such as next-generation sequencing, in the HLA high-resolution typing. These examples demonstrate our ability to go all the way from the study of basic mechanisms and knowledge generation, until the application of the results in the laboratory and its extension to a commercial application.

PERSON IN CHARGE

José Luis Caro Oleas

INVESTIGATORS

Francesc Rudilla Salvador

RESEARCH PROJECTS

Principal investigator: Josep Gámez Carbonell (Hospital Vall d'Hebron), José Luís Caro Oleas (BST)

Study of HLA-DR/DQ haplotypes in sporadic and familial forms of autoimmune MG. Analysis of their role as genetic factor of susceptibility and modifier of the phenotype in a Spanish population.

Funding organisation: Carlos III Institute of Health

File N°: P113-01272

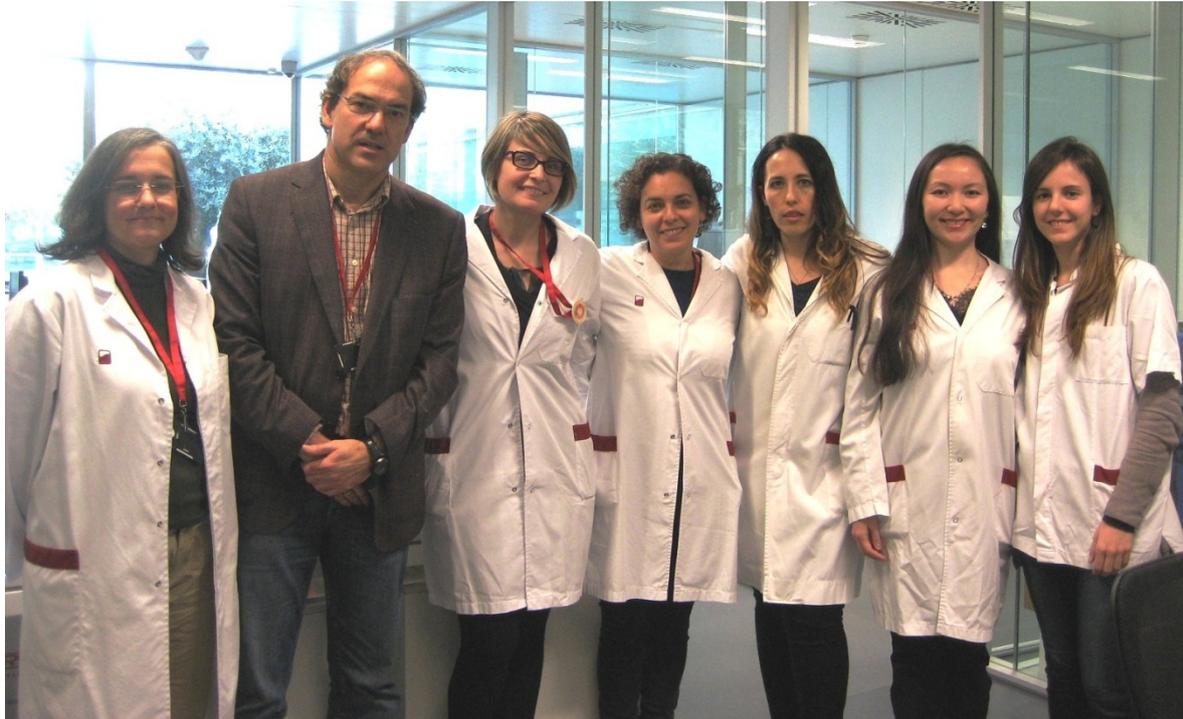
Duration: 2014 to 2016

PUBLICATIONS

Planelles D, Vilches C, González-Escribano F, Muro M, González-Fernández R, Sánchez F, Gonzalo Ocejo J, Eiras A, **Caro JL**, Palou E, Campillo JA, de Juan MD, Montes O, Balas A, Marín L, Torío A, Fernández-Arquero M, González-Roiz C, López-Vázquez A, Cisneros E, Abad-Molina C, López R, Abad-Alastruey ML, Serra C, García-Alonso AM, Vicario JL. Report From the First and Second Spanish Killer Immunoglobulin-Like Receptor Genotyping Workshops: External Quality Control for Natural Killer Alloreactive Donor Selection in Haploidentical Stem Cell Transplantation. *TRANSPLANT PROC* 2016 Nov; 48(9):3043-3045. QUARTILE 3, IMPACT FACTOR 0.982

An important factor affecting the success in the setting of related haploidentical hematopoietic stem cell transplantation (HSCT) is the graft-versus-leukemia effect mediated by natural killer (NK) cells when the donor displays NK alloreactivity versus the recipient. NK cell function is regulated by killer immunoglobulin-like receptors (KIR) and it has been described that donor KIR genotype influences transplantation outcome. This has led to a requirement of laboratories to have a quality assurance program for validation and control of their KIR genotyping methods. The goal of the 1st and 2nd Spanish KIR Genotyping Workshops was to provide an external proficiency testing program in KIR genotyping for Spanish immunology and transplant laboratories. These workshops were conducted during the years 2014-2016 and consisted of 17 participating laboratories typing a set of 20 samples. The presence/absence of 16 mandatory KIR loci (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DP1, 3DL1, 3DL2, 3DL3, 3DS1, and 3DP1) was evaluated per sample. Methods for KIR genotyping included polymerase chain reaction with the use of sequence-specific primers and sequence-specific oligoprobes. Consensus typing was reached in all samples, and the performance of laboratories in external proficiency testing was satisfactory in all cases. The polymorphism detected in the small sample studied in both workshops is indicative of an ample variety of KIR gene profiles in the Spanish population.

2.2.2 Program 6: Transplantation of donors & alternative sources



Hematopoietic stem cells are used in clinical situations to reconstitute bone marrow function. These cells can be obtained from bone marrow or mobilised peripheral blood of an adult, but also from the umbilical cord blood after giving birth. The administration of these cells to a patient regenerates haemopoietic and immune functions, contributing to the saving of many lives of patients suffering from cancer or acquired or genetic medullar insufficiency. The mission of the cell processing area of the Banc de Sang i Teixits is to transform the haemopoietic products collected in order to produce a therapeutic product with the expected qualities: safe and functional. The availability of high quality haemopoietic tissue is an essential factor for transplant and therefore investigating its improvement could contribute to therapeutic success.

All this is performed in BST laboratories using techniques for volume reduction, cell selection, cryopreservation and storage, and assays of product quality based on cell cultures and cytometric analysis. In addition, collaboration agreements have been established with centres of excellence that complement our own tools, including the Hospital del Mar Medical Research Institute, the Anthony Nolan Research Institute in the United Kingdom, as well as transplant centres of Catalonia to evaluate application of the products at a clinical level.

- A. Collection and processing of high quality hemopoietic progenitor cells to enhance their graft
- B. Selecting the best allogeneic donor
- C. Mobilization and apheresis
- D. Non-hematologic use of cord blood

PERSON IN CHARGE

Sergi Querol Giner

INVESTIGATORS

Carmen Azqueta Molluna
Nerea Castillo Flores
Emma Enrich Randé
Susana Garcia Gomez
Núria Martinez Llonch
Laura Medina Marrero
Dinara Samarkanova
Marta Torrabadella Reynoso
Elena Valdivia Garcia

RESEARCH PROJECTS

Principal investigator: Sergi Querol Giner

Prophylactic infusion of donor lymphocytes in cord blood transplantation
Funding organisation: La Marató de TV3 Foundation
File N^o: 20133230
Duration: 2016 to 2017

Principal investigator: Sergi Querol Giner

Clinical efficacy of platelet gel from cord blood for the treatment of diabetic foot ulcers
Funding organisation: BST
File N^o: 2015-000510-22
Duration: 2015 to 2017

Principal investigator: Marta Torrabadella Reynoso

Study of the influence of previous donor pregnancies on the outcome of unrelated allogeneic cord blood transplants
Funding organisation: BST
File N^o: PR(BST)370/2016
Duration: 2016 to 2017

Principal investigator: David Valcárcel Ferreiras (Hospital Vall d'Hebron), Sergi Querol Giner (BST)

NiCord® allogeneic trasplant of stem and progenitor cells derived from umbilical cord blood ex vivo expanded in adolescent and adult patients with malignant hematologic malignancies
Funding organisation: Gamida
File N^o: 2016-000074-19
Duration: 2014 to 2016

Principal investigator: Cristina Diaz Heredia (Hospital Vall d'Hebron), Sergi Querol Giner (BST)

FANCOSTEM: Phase I/II clinical trial to evaluate the safety and effectiveness of the mobilization and collection of CD34+ cells after treatment with mozobil and filgrastim in Fanconi anemia patients for subsequent use in gene therapy trials
Funding organisation: Spanish Ministry Health Social Service & Equality
File N^o: EC11-559
Duration: 2012 to 2017

Investigador principal: Susana Rives Solà (H Sant Joan de Déu), Sergi Querol Giner (BST)

A Phase II, single arm, multicenter trial to determine the efficacy and safety of CTL019 in pediatric patients with relapsed and refractory B-cell acute lymphoblastic leukemia CAR: Chimeric Antigen Receptor
Funding organisation: Novartis
File N^o: 2013-003205-25
Duration: 2016 to 2017

Principal investigator: Xinxin Li (H Sant Joan de Deu), Marta Torrabadella (BST)

Improvement in efficiency in cord blood donation programs through prenatal population selection

Funding organisation: BST

Duration: 2016 to 2017

Principal investigator: Cristina Diaz Heredia (Hospital Vall d'Hebron), Rafael Parra Lopez (BST)

Phase 1/2 combination study of dose-finding and comparative, open, randomized to evaluate the efficacy and safety of plerixafor in conjunction with standard regimens for mobilizing hematopoietic stem cells into peripheral blood and subsequent collection by apheresis, versus only standard regimens for mobilization in pediatric patients 2 to <18 years with solid tumors who are eligible for autologous transplants

Funding organisation: Sanofi

File N°: 2010-019340-40

Duration: 2014 to 2017

PUBLICATIONS

Castillo N, García-Cadenas I, Barba P, **Canals C**, Díaz-Heredia C, Martino R, Ferrà C, Badell I, Elorza I, Sierra J, Valcárcel D, **Querol S**. Early and Long-Term Impaired T-Lymphocyte Immune Reconstitution after Cord Blood Transplantation with Antithymocyte Globulin. BIOL BLOOD MARROW TRANSPLANT 2016 Nov 22. QUARTILE 2, IMPACT FACTOR 3.404

Immune reconstitution is crucial to the success of allogeneic hematopoietic stem cell transplantation. Umbilical cord blood transplantation (UCBT) has been associated with delayed immune reconstitution. We characterized the kinetics and investigated the risk variables affecting the main lymphocyte subsets recovery in 225 consecutive pediatric and adult patients [males, n=126, median age=15, range 0.3-60, interquartile range, 4-35] who underwent myeloablative single-UCBT between 2005 and 2015 for malignant and non-malignant disorders. Low CD4+ and CD8+ T-cell counts were observed up to 12 months after UCBT. In contrast, B and NK cells recovered rapidly early after transplantation. In a multivariate regression model, factors favoring CD4+ T-cell recovery ≥ 200 cells/ μ L were: lower dose antithymocyte globulin [(ATG) [HR=3.93 (95%CI; 2.3-5.83), P=0.001], negative recipient CMV serostatus [HR=3.76 (95%CI; 1.9-5.74), P=0.001] and younger age [HR=2.61 (95%CI; 1.01-3.47), P=0.03]. Factors favoring CD8+-T cell recovery ≥ 200 cells/ μ L were: lower dose ATG [HR=3.03 (95%CI; 1.4-5.1), P=0.03] and negative recipient CMV serostatus [HR=1.9 (95%CI; 1.63-2.15), P=0.01]. Our results demonstrate the significant negative impact of ATG on lymphocyte recovery. The reduction of the dose or omission of ATG could improve immune reconstitution and perhaps reduce opportunistic infections after UCBT.

Castillo N, García-Cadenas I, Díaz Heredia C, Martino R, Barba P, Ferrà C, **Canals C**, Elorza I, Olivé T, Badell I, Sierra J, Valcárcel D, **Querol S**. Cord blood units with high CD3+ cell counts predict early lymphocyte recovery after in-vivo T cell depleted single cord blood transplantation. BIOL BLOOD MARROW TRANSPLANT 2016 Mar 30. QUARTILE 2, IMPACT FACTOR 3.404

Although high absolute lymphocyte count (ALC) early after transplantation is a simple surrogate for immune reconstitution, few studies to date have established the predictive factors for ALC after umbilical cord blood transplantation (UCBT). We retrospectively studied the factors associated with early lymphocyte recovery and the impact of the ALC on day +42 (ALC42) of $\geq 300 \times 10^6/L$ on outcomes in 210 consecutive pediatric and adult patients (112 males; median age, 15 years; range, 0.3 to 60 years; interquartile range, 4 to 36 years) who underwent myeloablative in vivo T cell-depleted single UCBT between 2005 and 2014 for malignant and nonmalignant disorders. In a logistic multivariate regression model, factors favoring a higher ALC42 were higher infused

CD3(+) cell dose (odds ratio [OR], 2.7; 95% CI, 1.4 to 5.2; $P = .004$), lower antithymocyte globulin dose (OR, 2.3; 95% CI, 1.2 to 4.5; $P = .01$), and better HLA match (OR, 2.1; 95% CI, 1.1 to 4.1; $P = .03$). In multivariate analysis, lower ALC42 was associated with higher nonrelapse mortality (hazard ratio [HR], 1.76; 95% CI, 1.34 to 2.32; $P = .001$), whereas a higher ALC42 was associated with better disease-free survival (HR, 2.03; 95% CI, 1.15 to 3.6; $P < .001$) and overall survival (HR, 2.03; 95% CI, 1.17 to 3.6; $P < .001$). Our study suggests that the selection of better HLA-matched cord blood units containing higher CD3(+) cell counts and the use of conditioning regimens with lower ATG doses could improve immune reconstitution after UCBT.

Cunha R, Zago MA, **Querol S**, Volt F, Ruggeri A, Sanz G, Pouthier F, Kogler G, Vicario JL, Bergamaschi P, Saccardi R, Lamas CH, Díaz-de-Heredia C, Michel G, Bittencourt H, Tavella M, Panepucci RA, Fernandes F, Pavan J, Gluckman E, Rocha V. Impact of CTLA4 genotype and other immune response gene polymorphisms on outcomes after single umbilical cord blood transplantation. *BLOOD* 2016 Nov 3. QUARTILE 1, IMPACT FACTOR 10.452

We evaluated the impact of recipient and cord blood unit (CBU) genetic polymorphisms related to immune response on outcomes after unrelated CB transplants (CBT). Pre-transplant DNA samples from 696 CBU with malignant diseases were genotyped for NLRP1, NLRP2, NLRP3, TIRAP/Mal, IL10, REL, TNFRSF1B and CTLA4. HLA compatibility was 6/6 in 10%, 5/6 in 39%, and $\geq 4/6$ in 51% of transplants. Myeloablative conditioning was used in 80% and in vivo T-cell depletion in 81% of cases. The median number of total nucleated cells infused was $3.4 \times 10^7/\text{kg}$. In multivariable analysis, Patients receiving CBU with GG-CTLA4 genotype had a poorer neutrophil recovery (HR: 1.33; $p=0.02$), increased non-relapse mortality (HR: 1.50; $p<0.01$) and inferior disease-free survival (HR: 1.41; $p=0.02$). We performed the same analysis in a more homogeneous subset of cohort 1 (cohort 2, $n=305$) of patients transplanted for acute leukemia, all given a myeloablative conditioning regimen and with available allele HLA typing (HLA-A, -B, -C and -DRB1). In this more homogeneous, but smaller cohort, we were able to demonstrate that GG-CTLA4-CBU was associated with increased NRM (HR: 1.85; $p=0.01$). Use of GG-CTLA4-CBU was associated with higher mortality after CBT, which may be useful criterion for CBU selection, when multiple CBUs are available.

Carapito R, Jung N, Kwemou M, Untrau M, Michel S, Pichot A, Giacometti G, Macquin C, Ilias W, Morlon A, Kotova I, Apostolova P, Schmitt-Graeff A, Cesbron A, Gagne K, Oudshoorn M, van der Holt B, Labalette M, Spierings E, Picard C, Loiseau P, Tamouza R, Toubert A, Parissiadis A, Dubois V, Lafarge X, Maumy-Bertrand M, Bertrand F, Vago L, Ciceri F, Paillard C, **Querol S**, Sierra J, Fleischhauer K, Nagler A, Labopin M, Inoko H, von dem Borne PA, Kuball JH, Ota M, Katsuyama Y, Michallet M, Lioure B, Peffault de Latour R, Blaise D, Cornelissen JJ, Yakoub-Agha I, Claas F, Moreau P, Milpied N, Charron D, Mohty M, Zeiser R, Socié G, Bahram S. Matching for the non-conventional MHC-I MICA gene significantly reduces the incidence of acute and chronic GVHD. *BLOOD* 2016 Oct 13;128(15):1979-1986. QUARTILE 1, IMPACT FACTOR 10.452

Graft-versus-host disease (GVHD) is among the most challenging complications in unrelated donor hematopoietic cell transplantation (HCT). The highly polymorphic "MHC class I chain-related gene A", MICA, encodes a stress-induced glycoprotein expressed primarily on epithelia. MICA interacts with the invariant activating receptor NKG2D; expressed by cytotoxic lymphocytes. The MICA gene is located in the MHC, next to HLA-B; hence MICA has the requisite attributes of a bona fide transplantation antigen. Using high-resolution sequence-based genotyping of MICA, we retrospectively analyzed the clinical impact of MICA mismatches in a multicenter cohort of 922 unrelated donor HLA-A, -B, -C, -DRB1, and -DQB1 10/10 allele-matched HCT. Among the 922 pairs, 113 (12.3%) were mismatched in MICA. MICA mismatches were significantly associated with an increased incidence of grade III-IV acute GVHD (HR, 1.83; 95% CI, 1.50 to 2.23; $P<0.001$), chronic GVHD (HR, 1.50; 95% CI, 1.45 to 1.55; $P<0.001$) and non-relapse

mortality (HR, 1.35; 95% CI, 1.24 to 1.46; $P < 0.001$). The increased risk of GVHD was mirrored by a lower risk of relapse (HR, 0.50; 95% CI, 0.43 to 0.59; $P < 0.001$), indicating a possible graft-versus-leukemia effect. In conclusion, when possible, selecting a MICA-matched donor significantly influences key clinical outcomes of HCT in which a marked reduction of GVHD is paramount. The tight linkage disequilibrium between MICA and HLA-B renders identifying a MICA-matched donor readily feasible in clinical practice.

Saccardi R, Tucunduva L, Ruggeri A, Ionescu I, Koegler G, **Querol S**, Grazzini G, Lecchi L, Nanni Costa A, Navarrete C, Pouthiers F, Larghero J, Regan D, Freeman T, Bittencourt H, Kenzey C, Labopin M, Baudoux E, Rocha V, Gluckman E. Impact of cord blood banking technologies on clinical outcome: a Eurocord/Cord Blood Committee (CTIWP), European Society for Blood and Marrow Transplantation and NetCord retrospective analysis. *TRANSFUSION* 2016 May 31. QUARTILE 2, IMPACT FACTOR 3.225

BACKGROUND: Techniques for banking cord blood units (CBUs) as source for hematopoietic stem cell transplantation have been developed over the past 20 years, aimed to improve laboratory efficiency without altering the biologic properties of the graft. A large-scale, registry-based assessment of the impact of the banking variables on the clinical outcome is currently missing. **STUDY DESIGN AND METHODS:** A total of 677 single cord blood transplants (CBTs) carried out for acute leukemia in complete remission in centers affiliated with the European Society for Blood and Marrow Transplantation were selected. An extensive set of data concerning CBU banking were collected and correlations with clinical outcome were assessed. Clinical endpoints were transplant-related mortality, engraftment, and graft-versus-host disease (GVHD). **RESULTS:** The median time between collection and CBT was 4.1 years (range, 0.2-16.3 years). Volume reduction (VR) of CBUs before freezing was performed in 59.2% of available reports; in half of these the frozen volume was less than 30 mL. Cumulative incidences of neutrophil engraftment on Day 60, 100-day acute GVHD (II-IV), and 4-year chronic GVHD were 87, 29, and $21 \pm 2\%$. The cumulative incidence of nonrelapse mortality (NRM) at 100 days and 4-year NRM were, respectively, 16 ± 2 and $30 \pm 2\%$. Neither the variables related to banking procedures nor the interval between collection and CBT influenced the clinical outcome. **CONCLUSION:** These findings indicate a satisfactory validation of the techniques associated with CBU VR across the banks. Cell viability assessment varied among the banks, suggesting that efforts to improve the standardization of CBU quality controls are needed.

Sorigue M, Sancho JM, Morgades M, Moreno M, **Grífols JR**, **Alonso E**, Juncà J, Ferrà C, Batlle M, Vives S, Motlló C, García-Caro M, Navarro JT, Millà F, Feliu E, Ribera JM. Relapse risk after autologous stem cell transplantation in patients with lymphoma based on CD34+ cell dose. *LEUK LYMPHOMA* 2016 Aug 26:1-7. QUARTILE 2, IMPACT FACTOR 2.891

It is unclear whether higher CD34 + cell doses infused for ASCT have any influence on survival or relapse in patients with lymphoma. We analyzed the correlation of infused CD34 + cell dose with relapse, survival, and hematopoietic recovery in 146 consecutive patients undergoing ASCT for lymphoma. Higher doses ($>5 \times 10^6/\text{kg}$) were significantly correlated with earlier hematopoietic recovery, fewer infectious episodes, lower transfusion needs. No differences were observed in lymphoma outcomes (4-year relapse incidence of 38% [95%CI: 29%-48%] in the lower dose group versus 51% [95%CI: 30%-69%] in the higher dose group, 10-year OS probabilities of 58% [95%CI: 48%-68%] versus 75% [95%CI: 59%-91%], 10-year DFS probabilities of 47% [95%CI: 37%-57%] versus 42% [95%CI: 23%-61%], $p = \text{NS}$ for all outcomes). In this series, a higher infused CD34 + cell dose did not correlate with survival or relapse but correlated with earlier hematopoietic recovery and lower resource consumption

Sancho JM, Duarte R, **Medina L**, **Querol S**, Marín P, Sureda A; en representación del Grupo de Trabajo de Movilización de la Sociedad Catalana de Hematología y Hemoterapia

y de la Sociedad Catalano-Balear de Transfusión Sanguínea. Mobilization of peripheral blood stem cells with plerixafor in poor mobilizer patients MED CLIN (BARC) 2016 Jun 30. pii: S0025-7753(16)30167-1. QUARTILE 2, IMPACT FACTOR 1.417

BACKGROUND AND OBJECTIVE: Poor mobilization of peripheral blood stem cells (CD34+ cells) from bone marrow is a frequent reason for not reaching the autologous stem cell transplantation (SCT) procedure in patients diagnosed with lymphoma or myeloma. Plerixafor, a reversible inhibitor of the binding of stromal cell-derived factor 1 to its cognate receptor CXCR4, has demonstrated a higher capacity for the mobilization of peripheral blood stem cells in combination with granulocyte colony stimulating factor (G-CSF) compared with G-CSF alone. For this reason, plerixafor is now indicated for poor mobilizer myeloma or lymphoma patients. Some studies have recently indicated that a pre-emptive strategy of plerixafor use during first mobilization, according to the number of CD34+ mobilized cells in peripheral blood or to the harvested CD34+ cells after first apheresis, could avoid mobilization failures and re-mobilizations, as well as the delay of autologous SCT. The aim of this consensus was to perform a review of published studies on pre-emptive strategy and to establish common recommendations for hospitals in Catalonia and Balearics on the use of pre-emptive plerixafor. **METHODS:** For the Consensus, physicians from participant hospitals met to review previous studies as well as previous own data about plerixafor use. The GRADE system was used to qualify the available evidence and to establish recommendations on the use of pre-emptive plerixafor. **RESULTS AND CONCLUSIONS:** After a review of the literature, the expert consensus recommended the administration of pre-emptive plerixafor for multiple myeloma or lymphoma patients with a CD34+ cell count lower than 10 cells/ μ L in peripheral blood (measured in the morning of day 4 of mobilization with G-CSF or after haematopoietic recovery in the case of mobilization with chemotherapy plus G-CSF).

2.3 REPARATIVE & IMMUNOMODULATORY THERAPY

2.3.1 Program 7: Advanced therapies



Based on the conviction that cell therapies will be one of the main exponents of medicine in the future, the Banc de Sang i Teixits created its Advanced Cell Therapy Division under the name of Xcelia in 2009. The purpose of this division is to develop personalised, safe and effective cell medicines and tissue engineering to improve people's health. In accordance with this purpose and taking into account that the products of advanced cell therapy are considered drugs and should be developed and manufactured under pharmaceutical standards, Xcelia research focuses on four basic lines:

- A. The research and development of candidates for cell drugs and biomarkers.
- B. The design and validation of bioprocesses under GMP standards.
- C. The performance of non-clinical studies under GLP regulations.
- D. The performance of clinical trials under GCP regulations.

Initially, the "MEDCEL" and "FACTOCEL" projects were the driving forces behind this research and development activity. Xcelia currently has a pipeline of 6 products with 10 different therapeutic indications ranging from musculoskeletal disorders to immunotherapy. These research products are in different stages of development ranging from non-clinical studies to clinical phases I/II.

PERSON IN CHARGE

Joan Garcia Lopez

INVESTIGATORS

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RESEARCH PROJECTS

Principal investigator: Joan Garcia Lopez

Injectable bone matching last generation hydrogels and bioactive allogenic products for fractures treatment

Funding organisation: Spanish Ministry of Economy and Competitivity

File N^o: IPT-2012-0745-300000

Duration: 2013 to 2016

Principal investigator: Joan Garcia Lopez

Incorporation to the TERCEL network (Cell Therapy) of the RETICS

Funding organisation: Carlos III Health Institute

File N^o: RD12/0019/0015

Duration: 2013 to 2017

Principal investigator: Joaquim Vives Armengol

Study of the anti-inflammatory and immunomodulatory properties of the advanced therapy drugs developed by Xcelia

Funding organisation: BST

Duration: 2016 to 2018

Principal investigator: Josep Maria Canals Coll (Barcelona University), Joan Garcia Lopez (BST)

ADVANCE(CAT) Accelerator for the development of advanced therapies in Catalonia

File N^o: COMRDI15-1-0013

Funding organisation: ACCIÓ

Duration: 2016 to 2019

Principal investigator: Joan Bagó Granell (Hospital Vall d'Hebron), Joan Garcia Lopez (BST)

Prospective randomized clinical trial comparing the spinal fusion in patients with degenerative pathology of lumbar spine, using autologous mesenchymal stem cells immobilized in human bone particles versus autologous iliac crest bone graft of the own patient

Funding organisation: Spanish Ministry of Health Social Service & Equality

File N^o: EC10-209

Duration: 2012 to 2017

Principal investigator: Josep Maria Segur Vilalta (Hospital Clínic), Joan Garcia Lopez (BST)

Allogenic cell therapy pilot clinical trial of ex-vivo expanded adult stem cells conjugated with allogenic bone scaffold for the hip fracture treatment in elderly.

Funding organisation: Spanish Ministry of Health Social Service & Equality

File N^o: EC11-158

Duration: 2012 to 2017

Principal investigator: Xavier Montalbán Gairin (Hospital Vall d'Hebron), Joan Garcia Lopez (BST)

Transplantation of autologous mesenchymal stem cells from bone marrow as a potential therapeutic strategy for the treatment of multiple sclerosis

Funding organisation: Spanish Ministry of Health Social Service & Equality

File N^o: EC10-266

Duration: 2012 to 2017

Principal investigator: Marius Aguirre Canyadell (Hospital Vall d'Hebron), Joan Garcia Lopez (BST)

Autologous mesenchymal stem cell therapy applied to the osteonecrosis of the femoral head

Funding organisation: Spanish Ministry of Health Social Service & Equality

File N^o: EC10-208

Duration: 2012 to 2017

Principal investigator: Joan Carles Monllau Garcia (ICATME), Joan Garcia López (BST)

A safety and efficacy phase I/IIa pilot clinical trial for the meniscus lesion healing by means of autologous mesenchymal stem cells infiltration

Funding organisation: Spanish Ministry of Health Social Service & Equality

File N^o: EC11-436

Duration: 2012 to 2017

Principal investigator: Joan Vidal Samsó (Institut Guttmann), Joan Garcia Lopez (BST)

A prospective, open-label, Intrathecal injection single-dose, phase I/IIa pilot study to assess the safety and to obtain preliminary efficacy results of allogenic stem cells from umbilical cord transplantation in patients with complete chronic traumatic spinal cord injury

Funding organisation: La Marató de TV3 Foundation

File N^o: 122831

Duration: 2013 to 2017

Principal investigator: Fernando Granell Escobar (Hospital ASEPEYO), Joan Garcia Lopez (BST)

A phase IIa, unicenter, prospective, randomized, parallel, two-arms, single-dose, open-label with blinded assessor pilot clinical trial to assess ex vivo expanded adult autologous mesenchymal stromal cells fixed in allogenic bone tissue in non hypertrophic pseudoarthrosis of long bones

Funding organisation: ASEPEYO and BST

File N^o: 2013-005025-23

Duration: 2016 to 2017

PUBLICATIONS

Caminal M, Vélez R, Rabanal RM, Vivas D, Batlle-Morera L, Aguirre M, Barquinero J, García J, Vives J. A reproducible method for the isolation and expansion of ovine

mesenchymal stromal cells from bone marrow for use in regenerative medicine preclinical studies. J TISSUE ENG REGEN MED 2016 Nov 18. QUARTILE 1, IMPACT FACTOR 5.199

The use of multipotent mesenchymal stromal cells (MSCs) as candidate medicines for treating a variety of pathologies is based on their qualities as either progenitors for the regeneration of damaged tissue or producers of a number of molecules with pharmacological properties. Preclinical product development programmes include the use of well characterized cell populations for proof of efficacy and safety studies before testing in humans. In the field of orthopaedics, an increasing number of translational studies use sheep as an in vivo test system because of the similarities with humans in size and musculoskeletal architecture. However, robust and reproducible methods for the isolation, expansion, manipulation and characterization of ovine MSCs have not yet been standardised. The present study describes a method for isolation and expansion of fibroblastic-like, adherent ovine MSCs that express CD44, CD90, CD140a, CD105 and CD166, and display trilineage differentiation potential. The 3-week bioprocess proposed here typically yielded cell densities of 1.4×10^4 MSCs/cm² at passage 2, with an expansion factor of 37.8 and approximately eight cumulative population doublings. The osteogenic potential of MSCs derived following this methodology was further evaluated in vivo in a translational model of osteonecrosis of the femoral head, in which the persistence of grafted cells in the host tissue and their lineage commitment into osteoblasts and osteocytes was demonstrated by tracking enhanced green fluorescent protein-labelled cells.

Oliver-Vila I, Coca MI, Grau-Vorster M, Pujals-Fonts N, Caminal M, Casamayor-Genescà A, Ortega I, Reales L, Pla A, Blanco M, García J, Vives J. Evaluation of a cell-banking strategy for the production of clinical grade mesenchymal stromal cells from Wharton's jelly. CYTOTHERAPY 2016 Jan;18(1):25-35. QUARTILE 2, IMPACT FACTOR 3.293

BACKGROUND AIMS: Umbilical cord (UC) has been proposed as a source of mesenchymal stromal cells (MSCs) for use in experimental cell-based therapies provided that its collection does not raise any risk to the donor, and, similar to bone marrow and lipoaspirates, UC-MSCs are multipotent cells with immuno-modulative properties. However, some of the challenges that make a broader use of UC-MSCs difficult include the limited availability of fresh starting tissue, time-consuming processing for successful derivation of cell lines, and the lack of information on identity, potency and genetic stability in extensively expanded UC-MSCs, which are necessary for banking relevant cell numbers for preclinical and clinical studies. **METHODS:** Factors affecting the success of the derivation process (namely, time elapsed from birth to processing and weight of fragments), and methods for establishing a two-tiered system of Master Cell Bank and Working Cell Bank of UC-MSCs were analyzed. **RESULTS:** Efficient derivation of UC-MSCs was achieved by using UC fragments larger than 7 g that were processed within 80 h from birth. Cells maintained their immunophenotype (being highly positive for CD105, CD90 and CD73 markers), multi-potentiality and immuno-modulative properties beyond 40 cumulative population doublings. No genetic abnormalities were found, as determined by G-banding karyotype, human telomerase reverse transcriptase activity was undetectable and no toxicity was observed in vivo after intravenous administration of UC-MSCs in athymic rats. **DISCUSSION:** This work demonstrates the feasibility of the derivation and large-scale expansion of UC-MSCs from small and relatively old fragments of UC typically discarded from public cord blood banking programs.

Del Mazo-Barbara A, Nieto V, Mirabel C, Reyes B, Garcia-Lopez J, Oliver-Vila I, Vives J. Streamlining the qualification of computerized systems in GxP-compliant academic cell therapy facilities. CYTOTHERAPY 2016 Sep;18(9):1237-9. QUARTILE 2, IMPACT FACTOR 3.293

Codinach M, Blanco M, Ortega I, Lloret M, Reales L, Coca MI, Torrents S, Doral M, Oliver-Vila I, Requena-Montero M, Vives J, Garcia-Lopez J. Design and validation of a consistent and reproducible manufacture process for the production of clinical-grade bone marrow-derived multipotent mesenchymal stromal cells. *CYTOTHERAPY* 2016 Sep;18(9):1197-208. QUARTILE 2, IMPACT FACTOR 3.293

BACKGROUND: Multipotent mesenchymal stromal cells (MSC) have achieved a notable prominence in the field of regenerative medicine, despite the lack of common standards in the production processes and suitable quality controls compatible with Good Manufacturing Practice (GMP). Herein we describe the design of a bioprocess for bone marrow (BM)-derived MSC isolation and expansion, its validation and production of 48 consecutive batches for clinical use. **METHODS:** BM samples were collected from the iliac crest of patients for autologous therapy. Manufacturing procedures included: (i) isolation of nucleated cells (NC) by automated density-gradient centrifugation and plating; (ii) trypsinization and expansion of secondary cultures; and (iii) harvest and formulation of a suspension containing $40 \pm 10 \times 10^6$ viable cells. Quality controls were defined as: (i) cell count and viability assessment; (ii) immunophenotype; and (iii) sterility tests, Mycoplasma detection, endotoxin test and Gram staining. **RESULTS:** A 3-week manufacturing bioprocess was first designed and then validated in 3 consecutive mock productions, prior to producing 48 batches of BM-MSC for clinical use. Validation included the assessment of MSC identity and genetic stability. Regarding production, 139.0 ± 17.8 mL of BM containing $2.53 \pm 0.92 \times 10^9$ viable NC were used as starting material, yielding $38.8 \pm 5.3 \times 10^6$ viable cells in the final product. Surface antigen expression was consistent with the expected phenotype for MSC, displaying high levels of CD73, CD90 and CD105, lack of expression of CD31 and CD45 and low levels of HLA-DR. Tests for sterility, Mycoplasma, Gram staining and endotoxin had negative results in all cases. **DISCUSSION:** Herein we demonstrated the establishment of a feasible, consistent and reproducible bioprocess for the production of safe BM-derived MSC for clinical use.

Casamayor-Genescà A, Pla A, Oliver-Vila I, Pujals-Fonts N, Marín-Gallén S, Caminal M, Pujol-Autonell I, Carrascal J, Vives-Pi M, Garcia J, Vives J. Clinical-scale expansion of CD34+ cord blood cells amplifies committed progenitors and rapid scid repopulation cells. *N BIOTECHNOL* 2016 Oct 31. QUARTILE 2, IMPACT FACTOR 2.898

Umbilical cord blood (UCB) transplantation is associated with long periods of aplastic anaemia. This undesirable situation is due to the low cell dose available per unit of UCB and the immaturity of its progenitors. To overcome this, we present a cell culture strategy aimed at the expansion of the CD34⁺ population and the generation of granulocyte lineage-committed progenitors. Two culture products were produced after either 6 or 14 days of in vitro expansion, and their characteristics compared to non-expanded UCB CD34⁺ controls in terms of phenotype, colony-forming activity and multilineage repopulation potential in NOD-scid IL2Ry^{null} mice. Both expanded cell products maintained rapid SCID repopulation activity similar to the non-expanded control, but 14-day cultured cells showed impaired long term SCID repopulation activity. The process was successfully scaled up to clinically relevant doses of 89×10^6 CD34⁺ cells committed to the granulocytic lineage and 3.9×10^9 neutrophil precursors in different maturation stages. Cell yields and biological properties presented by the cell product obtained after 14 days in culture were superior and therefore this is proposed as the preferred production setup in a new type of dual transplant strategy to reduce aplastic periods, producing a transient repopulation before the definitive engraftment of the non-cultured UCB unit. Importantly, human telomerase reverse transcriptase activity was undetectable, c-myc expression levels were low and no genetic abnormalities were found, as determined by G-banding karyotype, further confirming the safety of the expanded product.

Del Mazo-Barbara A, Mirabel C, Nieto V, Reyes B, García-López J, Oliver-Vila I, Vives J. Qualification of computerized monitoring systems in a cell therapy facility

compliant with the good manufacturing practices. *REGEN MED* 2016 Sep;11(6):521-8. QUARTILE 2, IMPACT FACTOR 2.786

AIM: Computerized systems (CS) are essential in the development and manufacture of cell-based medicines and must comply with good manufacturing practice, thus pushing academic developers to implement methods that are typically found within pharmaceutical industry environments. **MATERIALS & METHODS:** Qualitative and quantitative risk analyses were performed by Ishikawa and Failure Mode and Effects Analysis, respectively. **RESULTS:** A process for qualification of a CS that keeps track of environmental conditions was designed and executed. The simplicity of the Ishikawa analysis permitted to identify critical parameters that were subsequently quantified by Failure Mode Effects Analysis, resulting in a list of test included in the qualification protocols. **CONCLUSION:** The approach presented here contributes to simplify and streamline the qualification of CS in compliance with pharmaceutical quality standards.

Prat S, Gallardo-Villares S, Vives M, Carreño A, **Caminal M, Oliver-Vila I, Chaverri D, Blanco M, Codinach M**, Huguet P, Ramírez J, Pinto JA, Aguirre M, **Coll R, Garcia-López J**, Granell-Escobar F, **Vives J**. Clinical translation of a Mesenchymal Stromal Cell-based therapy developed in a large animal model and two case studies of the treatment of atrophic pseudoarthrosis. *J TISSUE ENG REGEN MED* 2016 Sep 29. QUARTILE 1, IMPACT FACTOR 5.199

Pseudoarthrosis is a relatively frequent complication of fractures, in which the lack of mechanical stability and biological stimuli results in the failure of bone union, most frequently in humerus and tibia. Treatment of recalcitrant pseudoarthrosis relies on the achievement of satisfactory mechanical stability combined with adequate local biology. Herein we present two cases of atrophic pseudoarthrosis that received a Tissue Engineering Product (TEP) composed of autologous Bone Marrow-derived Mesenchymal Stromal Cells (BM-MSC) combined with deantigenised trabecular bone particles from tissue bank. Feasibility of the treatment and osteogenic potential of the cell-based medicine was first demonstrated in an ovine model of critical size segmental tibial defect. Clinical grade autologous BM-MSC were produced following a Good Manufacturing Practice-compliant bioprocess. Results were successful in one case, with pseudoarthrosis resolution, and inconclusive in the other one. The first patient presented atrophic pseudoarthrosis of the humeral diaphysis and was treated with osteosynthesis and TEP resulting in satisfactory consolidation at month 6. The second case presented a recalcitrant pseudoarthrosis of the proximal tibia and the Masquelet technique was followed before filling the defect with the TEP. This patient presented a neuropathic pain syndrome unrelated to the treatment that forced the amputation of the extremity three months later. In this case, the histological analysis of the tissue formed at the defect site evidenced neovascularisation but no overt bone remodelling activity. We conclude that the use of expanded autologous BM-MSC to treat pseudoarthrosis was demonstrated feasible and safe, provided that no clinical complications were reported, and early signs of effectiveness were observed.

Soler R, Orozco L, Munar A, Huguet M, López R, **Vives J, Coll R, Codinach M, Garcia-Lopez J**. Final results of a phase I–II trial using ex vivo expanded autologous Mesenchymal Stromal Cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. *KNEE* 2016 Jan 9. QUARTILE 2, IMPACT FACTOR 1.936.

BACKGROUND: Cellular therapies have shown encouraging results in the treatment of chronic osteoarthritis (OA). Herein, we present the final results of a phase I-II clinical trial assessing the feasibility, safety and efficacy of ex vivo expanded autologous bone marrow Mesenchymal Stromal Cells (MSC, XCEL-M-ALPHA), infused intra-articularly, in patients with knee OA. **METHODS:** Fifteen patients (median age=52years) with grade II(9) or III(6) gonarthrosis (Kellgren & Lawrence classification) and chronic pain were

treated with an intra-articular infusion of $40.9 \times 10^6 \pm 0.4 \times 10^6$ MSC in a phase I-II prospective, open-label, single-dose, single-arm clinical trial. Endpoints were safety and tolerability. Efficacy was measured by the Visual Analogue Scale for pain, algofunctional Health Assessment Questionnaire, Quality of Life (QoL) SF-36 questionnaire, Lequesne functional index and WOMAC score. Cartilage integrity was assessed by Magnetic Resonance Imaging and quantitative T2-mapping at 0, 6 and 12 months. **RESULTS:** The cell-based product was well tolerated with few reported Adverse Events (mild arthralgia and low back pain). There was a relevant decrease in the intensity of pain since day 8 after the infusion, that was maintained after 12 months. The SF-36 QoL test showed improvement of parameters including bodily pain, role physical and physical functioning at month 12. The health assessment questionnaire revealed a significant decrease of incapacity. Moreover, T2 mapping showed signs of cartilage regeneration in all patients at 12 months post-treatment. **CONCLUSIONS:** Single intra-articular infusion of XCEL-M-ALPHA is a safe and well-tolerated cell-based product, associated with a long-lasting amelioration of pain, improvement of QoL (up to four years), and signs of cartilage repair.

2.3.2 Program 8: Tissue bank



The program of R&D of the Tissue Bank is focused on translational research as well as development, optimization and innovation of procedures and techniques for improving the usefulness, quality and safety of human tissues and cells, for therapeutic or bio substitutive purposes. Likewise, researchers also coordinate their projects, analyze their feasibility and, where possible, raise funds for development through competitive public calls (Spanish and European Community), private entities, foundations and with business area related to the sector. Our research program enhances self-sustainability and innovation on the basis of collaboration with the business sector in coordination with clinical translational research groups of reference in the national and international context. Translational research is a tool for continuous improvement and answer to the therapeutic indications, through the use of effective and appropriate approaches and procedures. The strategy of our program of R&D promotes the different lines of research considered strategic for the organization, taking into consideration other aspects such as the fact that our first priority is the patient. And as fundamental pillars of all we have the ethical and regulatory framework, quality and excellence, in addition to the commitment to sustainability.

PERSON IN CHARGE

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Andres Savio Lopez
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RESEARCH PROJECTS

Principal investigator: Esteve Trias Adroher

Euro-GTP-II: Good Practices for demonstrating safety and quality through recipient follow-up

Funding organisation: European Commission

File N^o: 709567

Duration: 2016 to 2019

Principal investigator: Ricardo Casaroli Marano

Therapeutic potential of induced pluripotent stem cells and mesenchymal stromal stem cells from bone marrow nestin positive for the regeneration of the ocular surface

Funding organisation: Carlos III Health Institute

File N^o: PI14/00196

Duration: 2015 to 2017

Principal investigator: Ricardo Casaroli Marano

Cell therapy in ocular surface: Role and biosubstitutive applications of human adult mesenchymal stem cells (ADS and BMDS) for corneal regeneration

Funding organisation: La Marató de TV3 Foundation

File N^o: 120630

Duration: 2013 to 2017

Principal investigator: Ricardo Casaroli Marano

Ex vivo culture and expansion of human corneal endothelial cells in biomimetic biocompatible substrates: Functional characterization and clinical applicability

Funding organisation: BST

Duration: 2016 to 2019

Principal investigator: Esteve Trias Adroher

Clinical research extract amniotic membrane. Study on the efficacy and safety of a new form of presentation of the amniotic membrane for topical use on the eye surface

Funding organisation: BST

File N^o: 1/2015 BTB

Duration: 2015 to 2017

Principal investigator: Oscar Fariñas Barbera

DeminerIALIZED bone matrix development with human collagen.

Funding organisation: BST

File N^o: 1/2014 BTB

Duration: 2015 to 2018

Principal investigator: Nausica Otero Areitio

DMEK: Development of the technique for obtaining and improving the quality of ocular tissue for DMEK -Descemet's Membrane Endothelial keratoplasty

Funding organisation: BST

File N^o: I.2016.036

Duration: 2015 to 2018

Principal investigator: Marisa Perez Rodriguez

Development of a dermal matrix derived from cutaneous bank tissue. Subproject 3: Study of the biological properties of a dermal matrix of human origin for its application in breast reconstruction surgeries.

Funding organisation: BST
File N^o: I.2017.014
Duration: 2016 to 2018

Principal investigator: Josep Nart Molina (International University of Catalonia), Anna Villarrodona Serrat (BST)

Comparative histological and volumetric changes in Guided Bone Regeneration (GBR) technique using two different graft materials (xenograft Bio-Oss® - Geistlich vs Cortical Particulate Allograft–BST) and the same resorbable membrane (Pericardium-BST): a double blind trial

Funding organisation: International University of Catalonia and BST
File N^o: PER-ECL-2013-06
Duration: 2014 to 2017

Principal investigator: Samir Sarikouch (Universitat de Hannover), José Luís Pomar Moya-Prats (H Clínica), Esteve Trias Adroher (BST)

ARISE: Aortic Valve Replacement using Individualised Regenerative Allografts: Bridging the Therapeutic Gap

Funding organisation: European Commission
File N^o: SEP-210137838
Duration: 2014 to 2018

PUBLICATIONS

Mazoterias P, Quiles MG, Bispo PJ, Höfling-Lima AL, Pignatari AC, **Casaroli-Marano RP**. Analysis of intraocular lens biofilms and fluids after long-term uncomplicated cataract surgery. AM J OPHTHALMOL 2016 Jun 15. pii: S0002-9394(16)30276-8. QUARTILE 1, IMPACT FACTOR 3.871

PURPOSE: Postoperative endophthalmitis is a potentially sight-threatening complication of cataract surgery. However, the pathophysiological mechanisms are not completely understood. To study and evaluate the intraocular environment (aqueous and vitreous humors), the capsular tissue, and the intraocular lens (IOL) surfaces of normal eyes after long-term uncomplicated cataract surgery. **DESIGN:** Experimental laboratory investigation. **METHODS:** We studied 69 eyes donated for transplantation that had previously undergone cataract surgery with posterior chamber IOL implantation, and that had no recorded clinical history of postoperative inflammation. We assessed the intraocular environment (DNA traces and biofilm formation) by microbiological evaluation of intraocular fluids using conventional microbiology and molecular techniques, including assessment for the presence of microbes (biofilm formation) on the IOL surface by scanning electron microscopy and ultrastructural capsular remnants by transmission electron microscopy. **RESULTS:** Isolated or aggregated cocci were probable in 18.8% of IOL optic surfaces (n = 13) studied by scanning electron microscopy, suggesting the presence of bacterial biofilm. In three intraocular fluid samples for IOLs with biofilm, we identified 16S rDNA by polymerase chain reaction and sequencing. No microbial contamination was found in intraocular fluids by conventional microbiological methods. **CONCLUSIONS:** Our data suggest the possibility of bacterial biofilm formation on the optic surface of IOLs in normal eyes after long-term uncomplicated cataract surgery even in the absence of clinical or sub-clinical symptomatology.

Nieto-Nicolau, N, **Martínez-Conesa E, Casaroli-Marano R**. Limbal Stem Cells from Aged Donors Are a Suitable Source for Clinical Application. STEM CELLS INTERNATIONAL Volume 2016 (2016), QUARTILE 3, IMPACT FACTOR 2.813

Limbal stem cells (LSC) are the progenitor cells that maintain the transparency of the cornea. Limbal stem cell deficiency (LSCD) leads to corneal opacity, inflammation, scarring, and blindness. A clinical approach to treat this condition consists in LSC transplantation (LSCT) after ex vivo expansion of LSC. In unilateral LSCD, an autologous

transplant is possible, but cases of bilateral LSCD require allogenic LSCT. Cadaveric donors represent the most important source of LSC allografts for treatment of bilateral LSCD when living relative donors are not available. To evaluate the suitability of aged cadaveric donors for LSCT, we compared three pools of LSC from donors of different ages (<60 years, 60–75 years, and >75 years). We evaluated graft quality in terms of percent of p63-positive (p63+) cells by immunofluorescence, colony forming efficiency, and mRNA and protein expression of p63, PAX6, Wnt7a, E-cadherin, and cytokeratin (CK) 12, CK3, and CK19. The results showed that LSC cultures from aged donors can express $\geq 3\%$ of p63+ cells—considered as the minimum value for predicting favorable clinical outcomes after LSCT—suggesting that these cells could be a suitable source of LSC for transplantation. Our results also indicate the need to evaluate LSC graft quality criteria for each donor.

Beele H, van Wijk MJ, Wulff B, Holsboer N, de Bruijn M, Segerström C, Trias E. Report of the clinical donor case workshop of the European Association of Tissue Banks annual meeting 2014. CELL TISSUE BANK 2016 Sep;17(3):353-60. QUARTILE 3, IMPACT FACTOR 1.245

The European Association of Tissue Banks (EATB) donor case workshop is a forum held within the program of the EATB annual congress. The workshop offers an opportunity to discuss and evaluate possible approaches taken to challenging situations regarding donor selection. Donor case workshops actively engage participants with diverging background and experience in an informal, secure and enjoyable setting. The resulting discussion with peers promotes consensus development in deciding tissue donor acceptability, especially when donor health issues are not conclusively addressed in standards and regulations. Finally the workshop serves to strengthen the professional tissue banking networks across Europe and beyond. This report reflects some of the discussion at the workshop during the annual congress in Lund, Sweden, in 2014. The cases presented demonstrate that the implications of various donor illnesses, physical findings and behaviours on the safety of tissue transplantation, may be interpreted in a different way by medical directors and other professionals of different tissue facilities. This will also result in diverging preventive measures and decisions taken by the tissue facilities. Some of the donor cases illustrate varied responses from participants and demonstrate that operating procedures, regulations and standards cannot comprehensively cover all tissue donor illnesses, medical histories and circumstances surrounding the cause of death. For many of the issues raised, there is a lack of published scientific evidence. In those cases, tissue bank medical director judgement is critical to guarantee transplantation safety. This judgement should be based on a proper and documented risk assessment case by case. Conditions or parameters taken into account for risk assessment are amongst others, the type of tissue, the type of processing, the characteristics of the final product, and the availability of an adequate sterilisation methodology. By publishing these difficult donor suitability cases, and the resulting discussions, we provide information for future similar cases and we identify needs for future literature review and scientific research. In this way the donor case workshops play a role in optimizing the quality and security of tissue donation.

Navarro Martínez-Cantullera A, Calatayud Pínuaga M. Obtaining corneal tissue for keratoplasty. ARCH SOC ESP OFTALMOL 2016 May 2.

Cornea transplant is the most common tissue transplant in the world. In Spain, tissue donation activities depend upon transplant coordinator activities and the well-known Spanish model for organ and tissue donation. Tissue donor detection system and tissue donor evaluation is performed mainly by transplant coordinators using the Spanish model on donation. The evaluation of a potential tissue donor from detection until recovery is based on an exhaustive review of the medical and social history, physical examination, family interview to determine will of the deceased, and a laboratory screening test. Corneal acceptance criteria for transplantation have a wider spectrum than other tissues,

as donors with active malignancies and infections are accepted for kearatoplasty in most tissue banks. Corneal evaluation during the whole process is performed to ensure the safety of the donor and the recipient, as well as an effective transplant. Last step before processing, corneal recovery, must be performed under standard operating procedures and in a correct environment.

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