SCIENTIFIC RESEARCH REPORT

BANC DE SANG I TEIXITS I 2015

Generalitat de Catalunya
Departament de Salut

Banc de Sang i Teixits

INDEX

PRESENTATION OF MANAGING DIRECTOR

We present the Report of Investigation of the Blood and Tissue Bank of 2015 in which we collect the most outstanding projects in this second and final year of implementation of the Strategic Plan for R+D+i 2013-2015.

This report is the result of the research we have carried out in all defined programs: blood and breast milk process, transfusion safety, therapeutic apheresis, immunohematology, coagulopathies, molecular biology of transplantation and transplantion of donors and alternative sources, advanced therapies and tissue bank.

Throughout 2015, it is worth noting the contribution to the research of about 3 million euros from the Blood and Tissue Bank, and nearly achieved a million thanks to external financing. These resources have enabled us to carry out 49 projects, half in collaboration with other entities, and have also enabled us to offer more publications with a higher impact factor.

We begin now a stage where we intend to give impetus to new projects in immunotherapy and regenerative medicine in the fields of blood and tissues, keeping the lines of research in immunology and coagulation disorders, among others. This stage will lead Dr. Joan Garcia, new scientific director of the Blood and Tissue Bank, and the entire team will continue contributing and sharing knowledge to get more and more effective therapies.

Enric Argelagués Vidal

INTRODUCTION BY THE SCIENTIFIC DIRECTOR

Memory 2015 represents the culmination of the Strategic Research Plan initiated in 2013 by Dr. Jordi Sierra and continued by Dra. Silvia Sauleda, my predecessors. We owe them recognition of its momentum, its achievements and, of course, to the illusion that they transmitted.

Overall, R & D + i in the BST is healthy and is in a time of remarkable progression. Proof of this is the number of active projects and greater access to funding that I am sure will improve in future public calls.

The more selective indicator: the number of publications and their impact factor has evolved favorably, with an increase of more than 65% of the two parameters, situating us close to 2010-12 figures, when we still had the scientific production from LIRAD.

All research programs have contributed to this result also having participated in over 50 projects in collaboration with hospital services and companies, mobilizing 45 professionals many of them sharing care tasks.

Finally, note the educational tasks undertaken by the Chair of Transfusion and Cell and Tissue Therapy where professionals from BST teach at national and international official masters for physicians, graduates in health sciences and nurses, which already have hundreds of certificated students.

This year we began another voyage where, again, the self-imposed and search for solutions to diseases that affect our society, will be the nexus of all professionals of BST.

Joan Garcia Lopez

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: Carles Constante Beitia
Secretary: Josep Ramon Arisa Clusella
Members: Francesc Brosa Llinares, Enric Contreras Barbeta, Francesc Gòdia Casablancas, José J. Navas Palacios, Miquel Rutllant Bañeras, Josep Maria Campistol Plana, Emili Sullà Pascual, Roberto Gili Palacios, Pere Soley Bach, Teresa Ribas Algueró, Santiago Suso Vergara, Roser Vallès Navarro and Maria Antònia Viedma Martí.

1.1.2 Commissions of the Board of Directors

Economics and audits: Teresa Ribas Algueró, Francesc Brosa Llinares, Carmen Garcia Jarque and Emili Sullà Pascual
Innovation and Research: Francesc Gòdia Casablancas, José J. Navas Palacios and Miquel Rullant Bañeras
Corporative Development: Roberto Gili Palacios, Roser Vallès Navarro, José J. Navas Palacios, Miquel Rullant Bañeras and Santiago Suso Vergara

1.1.3 Strategic Committe of Tissues

President: Josep Maria Campistol Plana
Members: Santiago Suso Vergara, Maria Antònia Viedma Martí and Francesc Gòdia Casablancas
Guests: Enric Argelagués Vidal, Isabel López Asión, Esteve Trias Adroher, Dolors Heras Ribot and David Font Ferrer

1.2 DIRECTION AND MANAGEMENT BODIES

1.2.1 Direction Committee

Managing Director: Enric Argelagués Vidal
Assistant to Managing Director: Isabel López Asión
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
Marketing Director: Elena Hernandez Ruiz de Salazar
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 Muñiz Díaz
Barcelona. Vall d'Hebron and Clínic: Dolors Castellà Cahíz
Barcelona. Sant Pau: Alba Bosch Llobet
Badalona. Germans Trias i Pujol: Joan Ramon Grífols Ronda
L'Hospitalet. Bellvitge: Lluís Massuet Bosch
Manresa. Fundació Althaia/Terrassa. Mútua de Terrassa: Ramon Salinas Argente
Girona. Dr. Josep Trueta: Joan Profitós Tuset
Lleida. Arnau de Vilanova: Juan Manuel Sánchez Villegas
Tarragona. Joan XXIII/Tortosa. Verge de la Cinta/Reus. Sant Joan: Enric Contreras Barbeta

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 Muñiz 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	16	4.10
Senior physicians	7	6.40
Junior physicians	11	8.40
Technical staff	11	10.20
TOTAL	45	29.10

1.5.2 Economic data

Breakdown of BST research income for 2015	Euros
Projects funded by public agencies	188,284
Agreements with industry	670,687
Own funds	2,860,514
TOTAL	3,719,485

1.5.3 Organisation of the BST research

The R+D+i Strategic Plan 2013-2015 defined 9 Research Programs that were re-estrutured in 2015 as follows:

Diagnosis, transfusional medicine & hemostasis	Hematopoietic transplantation & immunotherapy	Reparative & immunomodulatory therapy
PR1 Blood process	PR6 Molecular biology of transplantation	PR8 Advanced therapies
PR2 Transfusional safety	PR7 Transplantation of donors & alternative sources	PR9 Tissue Bank
PR3 Therapeutic apheresis		
PR4 Immunohematology		
PR5 Coagulopathies		

1.5.4 Research projects

ONGOING PROJECTS IN 2015	PRINCIPAL INVESTIGATOR BST	COLABORATION
PUBLIC AGENCIES		
European Commission	3	1
Carlos III Health Institute	3	4
Spanish Ministry Economy & Competitivity	1	
Spanish Ministry Health Social Service & Equality		7
ONT	1	
Marató TV3	3	1
Brasilian Ministry Science Technology & Innovation	1	
Strasbourg University + BST		1
AGREEMENTS WITH INDUSTRY		
Argos		1
Baxter	1	
Erythech Pharma		1
Gamida		1
Grífols, S.A.	2	2
Pfizer	1	
Progenika	1	
Roche		1
Sanofi		1
Sotio		1
Therakos		1
OWN FUNDS	10	
TOTAL	49	

1.5.5 Doctoral theses

Two doctoral theses were read or directed by BST investigators in 2015.

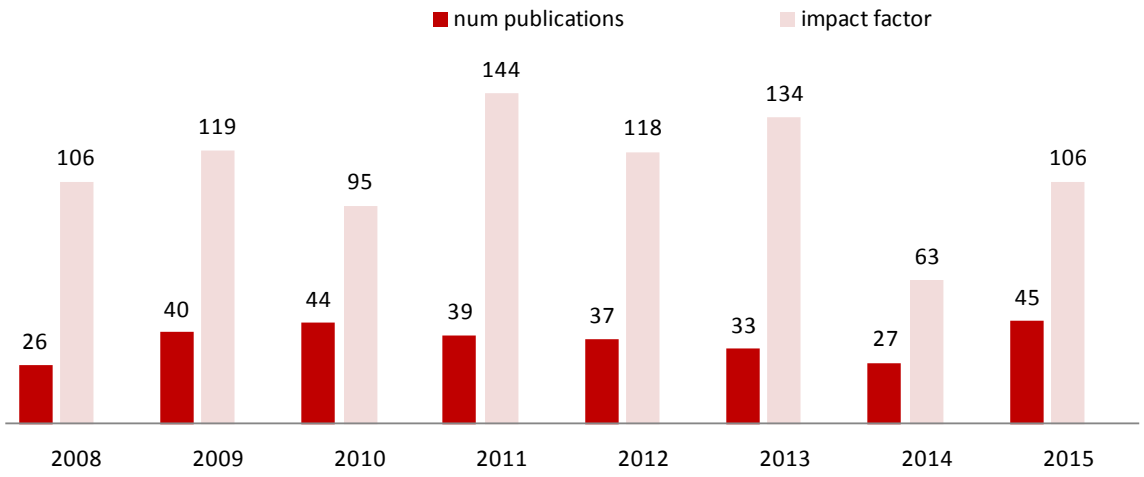
PhD student	Thesis title	Directors	Department	Grade
Laia Ferré	Allergology network: a new model of care for medical specialties	Ramon Salinas	UIC	Excellent cum laude
Marga Codinach	Prenatal myelomeningocele repair using mesenchymal stromal cells of amniotic fluid in a sheep model	César Fontecha and Joaquim Vives	UAB	Excellent cum laude

1.5.6 Publications

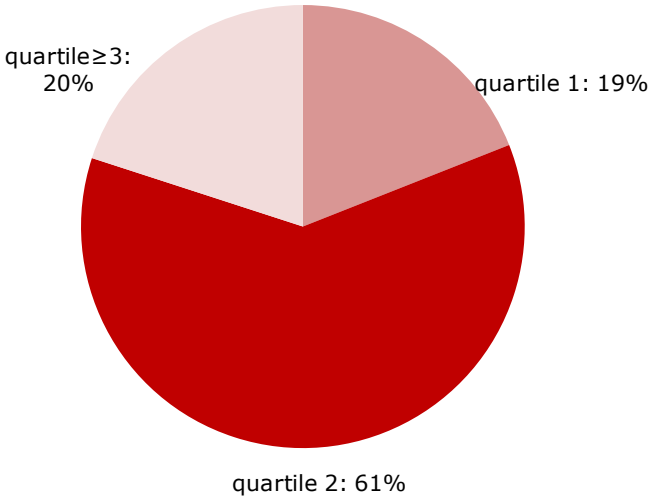
A total of 45 articles were published in scientific magazines by BST investigators in 2015 with an impact factor of 106.

The combined impact factor for 2015 was calculated using Journal Citation Reports (JCR) for 2013. The calculation included original articles, revisions and editorials. Presentations to congresses were excluded.

Evolution of the scientific production of the BST over the last 8 years:



Publications BST 2015:



1.5.7 Patents

The BST currently has 8 patents in different stages of processing. Seven of them are granted in Spain and 3 are granted or in process abroad.

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.

2. RESEARCH ACTIVITY OF THE BST

2.1 DIAGNOSIS, TRANSFUSIONAL MEDICINE & HEMOSTASIS

2.1.1 Program 1: Blood and breast milk process

This program includes projects whose purpose is to improve blood donation, the production of blood components, and their use in transfusions and other applications.

PERSON IN CHARGE
Lluís Puig Rovira

INVESTIGATORS
Joan Ramon Grífols Ronda
Gemma Valeta Juan

RESEARCH PROJECTS
Principal investigator: Gemma Valeta Juan
Toxicological screening of abuse drugs in breast milk from a donor in a breast milk bank
Funding organisation: BST
Duration: 2011 to 2015

Principal investigator: Carmen Rosa Pallás Alonso (Hospital 12 Octubre), Gemma Valeta Juan (BST)
Comparative study of HTST with Holder pasteurization in a human milk bank: microbiological, nutritional, biochemical and immunological parameters
Funding organisation: Carlos III Health Institute
Duration: 2013 to 2015

Principal investigador: Albert Oriol Rocafiguera (ICO Badalona), Joan Ramon Grífols Ronda (BST)

A Multicentre, open, randomized, controlled phase IIb trial evaluating efficacy and tolerability of GRASPA (L-asparaginase encapsulated in red blood cells) plus low-dose cytarabine vs low-dose cytarabine alone, in treatment of newly diagnosed acute myeloid leukemia patients, over 65 years, unfit for intensive chemotherapy.
Funding organisation: ERYTECH Pharma
File N°: GRASPA-AML2012-01
Duration: 2015 to 2017

Investigador principal: Maria Jose Martinez Zapata (H Sant Pau), Alba Bosch Llobet (BST)

Prevention of postoperative bleeding: A multicenter, randomized, parallel, controlled clinical trial, evaluating the efficacy of fibrin glue and tranexamic acid in patients undergoing interventions for sub-capital femoral fracture.
Funding organisation: Ministry of Health, Social Service & Equality
File N°: EC11-341
Duration: 2012 to 2016

PUBLICATIONS

Martinez-Zapata MJ, Orozco L, Balius R, Soler R, **Bosch A**, Rodas G, Til L, Peirau X, Urrútia G, Gich I, Bonfill X; PRP-RICE group. Efficacy of autologous platelet-rich plasma for the treatment of muscle rupture with haematoma: a multicentre, randomised, double-blind, placebo-controlled clinical trial. BLOOD TRANSFUS 2015 Sep 21:1-10. QUARTILE 3, DECILE 8, IMPACT FACTOR 1.901

BACKGROUND: The goals of the treatment of muscle injuries are to shorten the time of healing and to avoid relapses. The aim of this study was to assess the efficacy of autologous platelet-rich plasma (PRP) in the healing of muscle injuries. MATERIALS AND METHODS: A multicentre, randomised, double-blind, parallel, controlled clinical trial was conducted in 71 patients (81.8% males) aged 45.6 (SD=10.0) years with muscle tears in the legs and haematoma. The haematoma was evacuated in all patients. Thirty-three patients were randomised to a single dose of autologous PRP and 38 patients to simulation of PRP administration. The primary end-point was time to complete recovery of muscle injury. Secondary end-points were pain, relapses, ultrasound parameters, and adverse events. The total follow-up per patient was 12 months. RESULTS: Time to complete recovery after the treatment was 31.63 days (SD=15.38) in the PRP group, and 38.43 days (SD=18.58) in the control group (p=0.261). Pain decreased over time in both groups without statistical differences between them. Eight patients relapsed (seven in the control group, and one in the PRP group). There were no adverse effects related to the interventions. DISCUSSION: Autologous PRP did not significantly improve the time to healing compared to that in the control group.

Sorigué M, Xicoy B, **Grifols JR**, Ribera JM. Autoimmune hemolytic anemia refractory to medical treatment after chlorine dioxide intake in a patient with idiopathic thrombocytopenic purpura. MED CLIN 2015 Apr 8;144(7):332-3. QUARTILE 3, DECILE 6, IMPACT FACTOR 1.252

Blasi A, Beltran J, Pereira A, **Puig L.** The cryoprecipitate: that old unknown. REV ESP ANESTESIOL REANIM. 2015 Apr;62(4):204-12.

Cryoprecipitate is a plasma derivative rich in fibrinogen and other procoagulant factors. It has been successfully used for decades in the treatment of the coagulopathy of trauma patients, cardiovascular surgery, liver failure and disseminated intravascular coagulation. Although cryoprecipitate is routinely used in many western countries, most of the Spanish regional blood banks stopped its production in the late 1990's. Moreover, in recent years there is a movement to replace cryoprecipitate with manufactured fibrinogen

concentrate. As a consequence, many of the younger anaesthesiologists did not have any direct experience with cryoprecipitate. This article aims to describe the characteristics of cryoprecipitate since it is a different product from manufactured fibrinogen concentrate, with its own specific indications that deserve to be further studied in clinical trials.

2.1.2 Program 2: 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. In this area one of the most relevant works of 2015 has been the project *Prevalence of Hepatitis E markers in Catalonian blood donors*. 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

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 2016

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: 2015 to 2016

PUBLICATIONS

Treviño A, Caballero E, de Mendoza C, Aguilera A, **Pirón M**, Soriano V; Spanish HIV-2/HTLV Study Group. The Burden of Neglected HIV-2 and HTLV-1 Infections in Spain. AIDS REV 2015 Nov 30;17(4). QUARTILE 1, DECILE 2, IMPACT FACTOR 4.023

HIV-2 and HTLV-1 infections are globally less frequent than those produced by HIV-1, the classical AIDS agent. In Spain and up to the end of 2014, a total of 310 cases of HIV-2, 274 of HTLV-1, and 776 of HTLV-2 infections had been reported. No cases of HTLV-3 or HTLV-4 infections have been identified so far in Spain. Most persons infected with HIV-2 or HTLV-1 acknowledge epidemiological risk factors for contagion, such as originating from or living in endemic regions and/or having had sexual partners from those areas. However, risk factors could not be recognized in up to 20-25% of carriers in Spain. Thus, it seems worth keeping a high level of clinical suspicion in order to identify earlier these neglected human retroviral infections, since diagnostic procedures and antiviral treatment are specific for each of these agents. In this article we summarize the major contributions reported at the meeting of the Spanish Group for HIV-2/HTLV held in Madrid in December 2014.

Bruhn R, Lelie N, Busch M, Kleinman S; International NAT Study Group. Relative efficacy of nucleic acid amplification testing and serologic screening in preventing hepatitis C virus transmission risk in seven international regions. TRANSFUSION 2015 Jun;55(6):1195-205. QUARTILE 2, DECILE 3, IMPACT FACTOR 3.568

BACKGROUND: The relative contribution of serologic screening and nucleic acid testing (NAT) to prevent hepatitis C virus (HCV) transmission has not been rigorously addressed. STUDY DESIGN AND METHODS: Twenty-one blood organizations in seven geographical regions performing individual-donation (ID)-NAT in parallel with anti-HCV screening provided data from 10,897,105 donations to establish HCV infection rates in first-time, lapsed, and repeat donations. Screening efficacy was modeled for: anti-HCV alone, HCV antigen/antibody (combo), minipool (MP)-NAT in pools of 8 and 16 with anti-HCV, ID-NAT and anti-HCV, and ID-NAT alone. Probabilities of infectivity for red blood cell transfusions were estimated as 100% from window period (WP) and concordant HCV RNA/antibody-positive (concordantly positive [CP]) donations and 0.028% from anti-HCV-positive and RNA-negative probable resolved (PR) donations. RESULTS: There were 5146 confirmed infections (30 WP, 3827 CP, and 1289 PR). Infection rates and transmission risks varied substantially across regions and by donation status. Residual risk with ID-NAT and serology screening was estimated at one in 250,000 in Egypt and at one in 10,000,000 in other regions combined; risk would increase to one in 7300 and one in 312,000, respectively, if NAT had not been performed. ID-NAT with or without anti-HCV testing showed higher efficacy than either MP-NAT or combo assays, particularly in

lapsed or repeat donors in whom 99.2, 98.5, and 93.2% of infectious donations were estimated to be interdicted by these respective testing strategies. CONCLUSIONS: The incremental efficacy of anti-HCV testing when ID- NAT screening is performed was minimal, particularly for screening lapsed and repeat donations.

Grabarczyk P, Koppelman M, Boland F, **Sauleda S**, Fabra C, Cambie G, Kopacz A, O'Riordan K, van Drimmelen H, O'Riordan J, Lelie N. Inclusion of human immunodeficiency virus Type 2 (HIV-2) in a multiplex transcription-mediated amplification assay does not affect detection of HIV-1 and hepatitis B and C virus genotypes: a multicenter performance evaluation study. TRANSFUSION 2015 Jun 23. QUARTILE 2, DECILE 3, IMPACT FACTOR 3.568

BACKGROUND: The Ultrio Elite assay (Hologic/Grifols) runs on the Panther blood screening system and is comparable to the Ultrio Plus assay apart from the addition of oligonucleotides for human immunodeficiency virus Type 2 (HIV-2) detection. In this multicenter evaluation study the analytical sensitivity and genotype detection efficiency of the two assay versions were compared. STUDY DESIGN AND METHODS: The analytical sensitivity and genotype detection efficiency were analyzed by replicate (18-303) testing of 27 hepatitis B virus (HBV), hepatitis C virus (HCV), HIV-1, and HIV-2 standard dilution panels calibrated in international units (IUs) and copies/mL. A wider range of subgenotypes was tested at 25 copies/mL. Specificity was evaluated in 30,756 donor samples. RESULTS: The 95% lower limits of detection (LODs) in Ultrio Elite assay on WHO standards were 4.6, 7.3, 23.5, and 23.3 IU/mL for HBV, HCV, HIV-1, and HIV-2, respectively, and ranged from 13 to 44, 7 to 23, 6 to 15, and 9 copies/mL on genotype panels of the respective viruses. Comparable LODs had been previously found on the same panels with the Ultrio Plus assay. The specificity was 99.95% on initial test and 100% in the repeat test algorithm. CONCLUSION: The change in the oligonucleotide design of the Ultrio Elite assay to enable HIV-2 detection has not affected the analytical sensitivity for the other viruses regardless of the genotype. Genotype reference panels are instrumental to compare the sensitivity of nucleic acid test assay versions and could serve as an alternative to seroconversion panels.

Petrik J, Lozano M, Seed CR, Faddy HM, Keller AJ, Prado Scuracchio PS, Wendel S, Andonov A, Fearon M, Delage G, Zhang J, Shih JW, Gallian P, Djoudi R, Tiberghien P, Izopet J, Dreier J, Vollmer T, Knabbe C, Aggarwal R, Goel A, Ciccaglione AR, Matsubayashi K, Satake M, Tadokoro K, Jeong SH, Zaaijer HL, Zhiburt E, Chay J, Teo D, Chua SS, **Piron M, Sauleda S**, Echevarría JM, Dalton H, Stramer SL. Hepatitis E. VOX SANG 2015, Jul 21. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.303

Piron M, Plasencia A, Fleta-Soriano E, Martinez A, Martinez JP, Torner N, **Sauleda S**, Meyerhans A, Escalé J, Trilla A, Pumarola T, Martinez MJ. Low Seroprevalence of West Nile Virus in Blood Donors from Catalonia, Spain. VECTOR BORNE ZOONOTIC DIS 2015 Nov 18. QUARTILE 2, DECILE 3, IMPACT FACTOR 2.531

West Nile virus (WNV) is an emerging arbovirus first recognized in Europe in the 1950s. Since then, outbreaks have been reported in several European countries. In 2010, the first WNV outbreak was recorded in Spain, affecting the southern part of the country. We conducted a seroprevalence study in the Catalonia region (northeastern Spain), an area considered at high risk of arbovirus transmission. A total of 800 serum samples from blood donors were collected and screened for antibodies against WNV by enzyme-linked immunosorbent assay (ELISA) and confirmed by a microneutralization assay. More than 50 samples tested positive by ELISA, but only one sample contained neutralizing antibodies against WNV and was obtained from a donor native of Pakistan. The low seroprevalence detected may serve as reference baseline data for monitoring WNV activity in our region in future years.

Bes M, Vargas V, **Piron M, Casamitjana N**, Esteban JI, Campos-Varela I, **Puig L, Sauleda S.** Doubtful Role of IL28B Polymorphism in Occult Hepatitis B Infection.

INTERVIROLOGY 2015 May 28;58(3):160-165. QUARTILE 4, DECILE 9, IMPACT FACTOR 1.773

AIMS: To investigate the influence of IL28B polymorphism in occult hepatitis B infection (OBI) and whether IL28B genetic variants are associated with hepatitis B virus (HBV)-specific T-cell responses. PATIENTS AND METHODS: The rs12979860 IL28B genotype was determined in 34 OBI blood donors, 22 spontaneous HBV resolvers, 36 inactive HBV carriers and 25 seronegative donors. T-cell responses to HBV recombinant proteins were assessed by interferon-γ enzyme-linked immunospot assay. RESULTS: The frequency of the IL28B CC genotype among OBI patients was similar to that of inactive carriers [41 vs. 39%, respectively, p = 0.961; odds ratio (OR) = 1.10; 95% confidence interval (CI) = 0.42-2.86; p = 0.845]. The IL28B CC genotype was found more frequently in spontaneous resolvers, although the differences were not significant (45 vs. 39%, spontaneous resolvers and inactive carriers, respectively; p = 0.828; OR = 1.31; 95% CI = 0.45-3.83; p = 0.622). HBV-specific T-cell responses were detected in OBIs, and significantly stronger T-cell responses towards hepatitis B envelope antigen were observed in those with the IL28B CC genotype. In spontaneous resolvers and inactive carriers, IL28B CC did not correlate with the magnitude of T-cell responses.
CONCLUSIONS: In OBI donors, IL28B CC correlates with the intensity of HBV-specific T-cell responses. In this study, IL28B CC is not statistically associated with OBI or with HBV clearance, but a larger number of cases is needed before completely ruling out its role in HBV infection.

2.1.3 Program 3: Therapeutic apheresis

Therapeutic apheresis are procedures consisting of the external processing of the blood using a cell separator in order to remove a blood component that is causing a disease, with the return of the remaining components to the body.

The removed component can be blood cell (cytapheresis) or plasma (plasma exchange or selective plasmapheresis).

Although there are some conditions in which therapeutic apheresis are the first-line treatment, since they represent the best option for patients, generally they constitute second-line options or are contributing to other therapies. But the overall weight of this treatment is increasing in recent years, especially from the very studies that increase the scientific evidence that supports this type of procedure.

PERSON IN CHARGE
Enric Contreras Barbeta

INVESTIGATORS
Alba Bosch Llobet
Pilar Ortiz Murillo
Lluís Massuet Bosch
Dolors Castellà Cahiz
Joan Ramon Grífols Ronda

RESEARCH PROJECTS
Principal investigator: Alba Bosch Llobet, Joan Ramon Grífols Ronda i Dolors Castellà Cahiz
An International Phase 3 Randomized Trial of Autologous Dendritic Cell Immunotherapy (AGS 003) Plus Standard Treatment of Advanced Renal Cell Carcinoma
Funding organisation: Argos Therapeutics
File N°: 2012-000871-17
Duration: 2013 to 2016

Principal investigator: Jordi Sierra Gil (Hospital Sant Pau), Alba Bosch Llobet i Dolors Castellà Cahiz (BST)
A Randomized Controlled Study of Extracorporeal Photoapheresis (ECP) Therapy with UVADEXTM for the Treatment of Patients with Moderate to Severe Chronic Graft-versus-Host Disease (cGvHD)
Funding organisation: Therakos Inc
File N°: 10-005, 2010-022780-35
Duration: 2012 to 2015

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°: IG1002
Duration: 2012 to 2016

Principal investigator: Mónica Povedano Panades (Hospital de Bellvitge), Lluís Massuet Bosch (BST)
Pilot study on the effects of plasma exchange in motor dysfunction and cognitive function in patients with Amyotrophic Lateral Sclerosis
Funding organisation: Grífols
File N°: IG1309
Duration: 2014 to 2015

Principal investigator: Gemma Mur (Hospital Vall d'Hebron), Dolors Castellà Cahiz (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°: 2013-002801-71
Duration: 2015 to 2016

Principal investigator: Joan Carles (Hospital Vall d'Hebron), Dolors Castellà Cahiz (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°: 2012-002814-38
Duration: 2015 to 2016

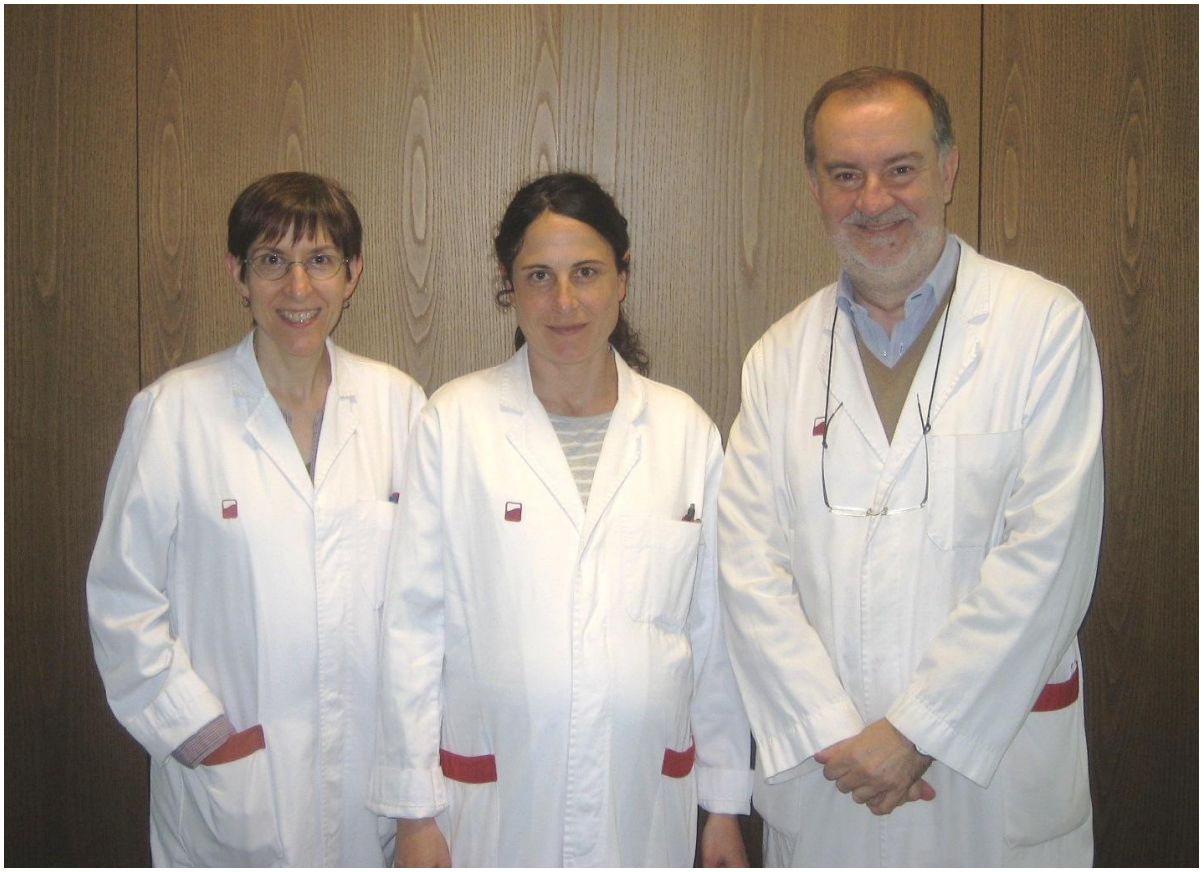
Principal investigator: Susana Rives Solà (Hospital Sant Joan de Déu), Enric Garcia Rey (BST)
Infusion of autologous T cells engineered to express anti-CD19 as therapy for patients with relapsed or refractory CD19+ leukaemia or lymphoma: a pilot study
Funding organisation: Carlos III Health Institute
File N°: ICI14/00224
Duration: 2015 to 2017

PUBLICATIONS
Borras-Novell C, **García Rey E**, Perez Baena LF, Jordan Garcia I, **Castella Cahiz D**, Cambra F. Therapeutic Plasma Exchange in Acute Disseminated Encephalomyelitis in Children. J CLIN APHER 2015 Aug 31. QUARTILE 4, DECILE 8, IMPACT FACTOR 1.579

Acute disseminated encephalomyelitis (ADEM) is an inflammatory demyelinating disease of the central nervous system that is probably due to an autoimmune mechanism with an acute presentation and a monophasic course. The management of patients with ADEM is based on supportive therapy, corticosteroids, and intravenous immunoglobulin, and in selected cases, with therapeutic plasma exchange (TPE). The aim of our study is to evaluate the efficacy of TPE, as adjuvant therapy in pediatric patients with ADEM. We retrospectively reviewed the medical records of children with the diagnosis of ADEM between 2009 and 2011 to which TPE was indicated and were admitted in the ICU of Hospital Sant Joan de Deu (Spain). The diagnosis of ADEM was made by clinical and laboratory criteria and by the presence of compatible lesions on cranio-spinal Magnetic Resonance Imaging (MRI). For signaling TPE, we followed the guidelines established by the American Association of Apheresis (ASFA) in 2010. Five cases were identified. The predominant neurological symptoms in our patients were: altered level of consciousness, seizures, motor deficits, cranial nerve disorders, and aphasia. Most important demyelinating lesions were located in cortical and subcortical white matter of the brain and highlighted brainstream. Patients performed between 4 and 5 sessions, with no reported side effects. Progressive clinical improvement was evident in all patients, with good neurosensory response to stimulation, cessation of seizures, and recovery of limb mobility. Nowadays, one patient's right paresis persists and another suffers epileptic seizures. None of the cases in our series presented new episodes of demyelination. Due to the suggested immune-mediated pathogenesis of ADEM, treatment is based on immunomodulatory agents, being glucocorticoids the most important ones. The treatment can be complemented with intravenous immunoglobulin and plasmapheresis. Available data suggests that plasma exchange is beneficial in children with ADEM who fail these treatments. The good tolerance of the procedure without adverse reactions and the progressive neurological improvement detected in the reviewed cases suggest that the use of TPE in pediatric patients is a good therapeutic option when performed in an experienced center.

Del Rio-Garma J, de la Rubia J, Romon I, **Contreras E**, Garcia-Erce J, Arbona C, Pereira A. Type of virus-securized plasma an treatment refractoriness in thrombotic thrombocytopenic purpura. BLOOD 2015, 125:3860-3867

2.1.4 Program 4: Immunohematology



The Immunohematology 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 Diaz

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 2016

Principal investigator: Núria Nogués Gálvez
BLOOD NGS: Product for the complete typing of ABO and RH systems
Funding organisation: Progenika
Duration: 2014 to 2016

PUBLICATIONS

Finning K, Bhandari R, Sellers F, Revelli N, Villa MA, **Muñiz-Díaz E, Nogués N**. Evaluation of red blood cell and platelet antigen genotyping platforms (ID CORE XT / ID HPA XT) in routine clinical practice. BLOOD TRANSFUS 2015 Oct 29:1-8. QUARTILE 3, DECILE 8, IMPACT FACTOR 1.901

BACKGROUND: High-throughput genotyping platforms enable simultaneous analysis of multiple polymorphisms for blood group typing. BLOODchip® ID is a genotyping platform based on Luminex® xMAP technology for simultaneous determination of 37 red blood cell (RBC) antigens (ID CORE XT) and 18 human platelet antigens (HPA) (ID HPA XT) using the BIDS XT software. MATERIALS AND METHODS: In this international multicentre study, the performance of ID CORE XT and ID HPA XT, using the centres' current genotyping methods as the reference for comparison, and the usability and practicality of these systems, were evaluated under working laboratory conditions. DNA was extracted from whole blood in EDTA with Qiagen methodologies. Ninety-six previously phenotyped/genotyped samples were processed per assay: 87 testing samples plus five positive controls and four negative controls. RESULTS: Results were available for 519 samples: 258 with ID CORE XT and 261 with ID HPA XT. There were three "no calls" that were either caused by human error or resolved after repeating the test. Agreement between the tests and reference methods was 99.94% for ID CORE XT (9,540/9,546 antigens determined) and 100% for ID HPA XT (all 4,698 alleles determined). There were six discrepancies in antigen results in five RBC samples, four of which (in VS, N, S and Doa) could not be investigated due to lack of sufficient sample to perform additional tests and two of which (in S and C) were resolved in favour of ID CORE XT (100% accuracy). The total hands-on time was 28-41 minutes for a batch of 16 samples. Compared with the reference platforms, ID CORE XT and ID HPA XT were considered simpler to use and had shorter processing times. DISCUSSION: ID CORE XT and ID HPA XT genotyping platforms for RBC and platelet systems were accurate and user-friendly in working laboratory settings.

Goldman M, **Nogués N**, Castilho LM. An overview of the Progenika ID CORE XT: an automated genotyping platform based on a fluidic microarray system. IMMUNOHEMATOLOGY 2015;31(2):62-8.

Automated testing platforms facilitate the introduction of red cell genotyping of patients and blood donors. Fluidic microarray systems, such as Luminex XMAP (Austin, TX), are used in many clinical applications, including HLA and HPA typing. The Progenika ID CORE XT (Progenika Biopharma-Grifols, Bizkaia, Spain) uses this platform to analyze 29 polymorphisms determining 37 antigens in 10 blood group systems. Once DNA has been extracted, processing time is approximately 4 hours. The system is highly automated and includes integrated analysis software that produces a file and a report with genotype and predicted phenotype results.

2.1.5 Program 5: 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
Lluis Martorell Cedrés
Rafael Parra López

TECHNICAL STAFF
Natàlia 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°: PI11/03029
Duration: 2012 to 2015

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°: PI12/01494

Duration: 2013 to 2015

Principal investigator: Francisco Vidal Pérez
Design and development of a protocol for HLA very high resolution typing by new generation sequencing technology
Funding organisation: BST
Duration: 2012 to 2015

Principal investigator: Rafael Parra López
Development of an efficient high-throughput platform for hemophilia A. Drug screening and gene correction using patient specific induced pluripotent stem cells (iPSCs).
Funding organisation: Pfizer
Duration: 2013 to 2015

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 2015

PUBLICATIONS

Parra R, Nemes L, Jiménez-Yuste V, Rusen L, Cid AR, Charnigo RJ, Baumann JA, Smith L, Korth-Bradley JM, Rendo P. Prospective surveillance study of haemophilia A patients switching from moroctocog alfa or other factor VIII products to moroctocog alfa albumin-free cell culture (AF-CC) in usual care settings. THROMB HAEMOST 2015 Aug 13;114(4) QUARTILE 1, DECILE 1, IMPACT FACTOR 5.760

This prospective, open-label, postauthorisation safety surveillance study assessed clinically significant inhibitor development in patients with severe haemophilia A transitioning from moroctocog alfa or other factor VIII (FVIII) replacement products to reformulated moroctocog alfa (AF-CC). Males aged ≥ 12 years with severe haemophilia A (FVIII:C) < 1 IU/dl), > 150 exposure days (EDs) to recombinant or plasma-derived FVIII products, and no detectable inhibitor at screening were enrolled. Primary end point was the incidence of clinically significant FVIII inhibitor development. Secondary end points included annualised bleeding rate (ABR), less-than-expected therapeutic effect (LETE), and FVIII recovery. Patients were assigned to one of two cohorts based on whether they were transitioning to moroctocog alfa (AF-CC) from moroctocog alfa (cohort 1; n=146) or from another recombinant or plasma-derived FVIII product (cohort 2; n=62). Mean number of EDs on study was 94 (range, 1-139). Six positive FVIII inhibitor results, as determined by local laboratories, were reported in four patients; none were confirmed by a central laboratory, no inhibitor-related clinical manifestations were reported, and all anti-FVIII antibody assays were negative. Median ABRs were 23.4 and 3.4 in patients categorised at baseline as following on-demand and prophylactic regimens, respectively; 86.5 % of bleeding episodes resolved after one infusion. LETE incidence was 0.06 % and 0.19 % in the on-demand and prophylaxis settings, respectively. FVIII recovery remained constant throughout the study. No new safety concerns were identified. This study found no increased risk of clinically significant FVIII inhibitor development in patients transitioning from moroctocog alfa or other FVIII replacement products to moroctocog alfa (AF-CC).

Batlle J, Pérez-Rodríguez A, **Corrales I**, López-Fernández MF, Rodríguez-Trillo Á, Lourés E, Cid AR, Bonanad S, Cabrera N, Moret A, **Parra R,** Mingot-Castellanos ME, Balda I, Altisent C, Pérez-Montes R, Fisac RM, Iruín G, Herrero S, Soto I, de Rueda B, Jiménez-Yuste V, Alonso N, Vilariño D, Arija O, Campos R, Paloma MJ, Bermejo N, Toll T, Mateo J, Arribalzaga K, Marco P, Palomo Á, Sarmiento L, Iñigo B, Nieto M, Vidal R, Martínez MP, Aguinaco R, César JM, Ferreiro M, García-Frade J, Rodríguez-Huerta AM, Cuesta J, Rodríguez-González R, García-Candel F, Cornudella R, Aguilar C, **Borràs N, Vidal F.** Molecular and clinical profile of von Willebrand disease in Spain (PCM-EVW-ES): Proposal

for a new diagnostic paradigm. THROMB HAEMOST 2015 Aug 6;114(6). QUARTILE 1, DECILE 1, IMPACT FACTOR 5.760

The diagnosis of von Willebrand disease (VWD) remains difficult in a significant proportion of patients. A Spanish multicentre study investigated a cohort of 556 patients from 330 families who were analysed centrally. VWD was confirmed in 480. Next generation sequencing (NGS) of the whole coding VWF was carried out in all recruited patients, compared with the phenotype, and a final diagnosis established. A total of 238 different VWF mutations were found, 154 were not included in the Leiden Open Variation Database (LOVD). Of the patients, 463 were found to have VWF mutation/s. A good phenotypic/genotypic association was estimated in 96.5 % of the patients. One hundred seventy-four patients had two or more mutations. Occasionally a predominant phenotype masked the presence of a second abnormality. One hundred sixteen patients presented with mutations that had previously been associated with increased von Willebrand factor (VWF) clearance. RIPA unavailability, central phenotypic results disagreement and difficult distinction between severe type 1 and type 3 VWD prevented a clear diagnosis in 70 patients. The NGS study facilitated an appropriate classification in 63 of them. The remaining seven patients presented with a VWF novel mutation pending further investigation. In five patients with a type 3 and two with a type 2A or 2B phenotype with no mutation, an acquired von Willebrand syndrome (AVWS) was suspected/confirmed. These data seem to support NGS as a first line efficient and faster paradigm in VWD diagnosis.

Altisent C, Martorell M, Crespo A, Casas L, Torrents C, **Parra R**. Early prophylaxis in children with severe haemophilia A: clinical and ultrasound imaging outcomes. HAEMOPHILIA 2015 Aug 28. QUARTILE 2, DECILE 5, IMPACT FACTOR 2.468

AIM: This observational study was undertaken with the aim to describe the characteristics and evaluate the outcomes of prophylactic treatment in children with severe haemophilia A (HA) treated at our centre. METHODS: Twenty-five patients aged 4-19 years with severe HA, no history of inhibitors and treated with at least two infusions of factor VIII (FVIII) per week were studied. Prophylactic doses and annual joint bleeding rate (AJBR) were retrospectively evaluated over the last 5 years. Current joint status was assessed using the Haemophilia Joint Health Score (HJHS) (136 joints of 23 patients) and the Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) procedure (124 joints of 21 patients). RESULTS: Median AJBR was 0.2 and median prophylaxis dose 65.4 IU^{-1} kg^{-1} $week^{-1}$. Median total HJHS was 0 (range 0-13) and total HEAD-US 1 (0-8). At the joint level, 85.3% of joints were normal on HJHS and 79.0% on US. The ankle was the joint most commonly affected, considering bleeding and ultrasound results. Correlation was found between HEAD-US scores and bleeding scores but not between HEAD-US and HJHS scores. HJHS and HEAD-US scores were concordant in 91/124 (73.4%) joints (86 joints normal and five abnormal). Ultrasound detected minimal changes in 19.6% of joints with normal physical function, whereas 12.2% of joints considered normal on ultrasound showed changes at HJHS. CONCLUSION: A well-preserved joint status was found in our cohort. High-resolution US detected a higher percentage of abnormalities than the physical evaluation, but the clinical implications of these findings still need to be ascertained.

Fischer K, Iorio A, Makris M; all EUHASS collaborators. FVIII inhibitor development according to concentrate: data from the EUHASS registry excluding overlap with other studies. HAEMOPHILIA 2015 Jul 24. QUARTILE 2, DECILE 5, IMPACT FACTOR 2.468

Martorell L, Corrales I, Ramirez L, Parra R, Raya A, Barquinero J, **Vidal F.** Molecular characterization of ten F8 splicing mutations in RNA isolated from patient's leucocytes: assessment of in silico prediction tools accuracy. HAEMOPHILIA 2015 Mar;21(2):249-57. QUARTILE 2, DECILE 5, IMPACT FACTOR 2.468

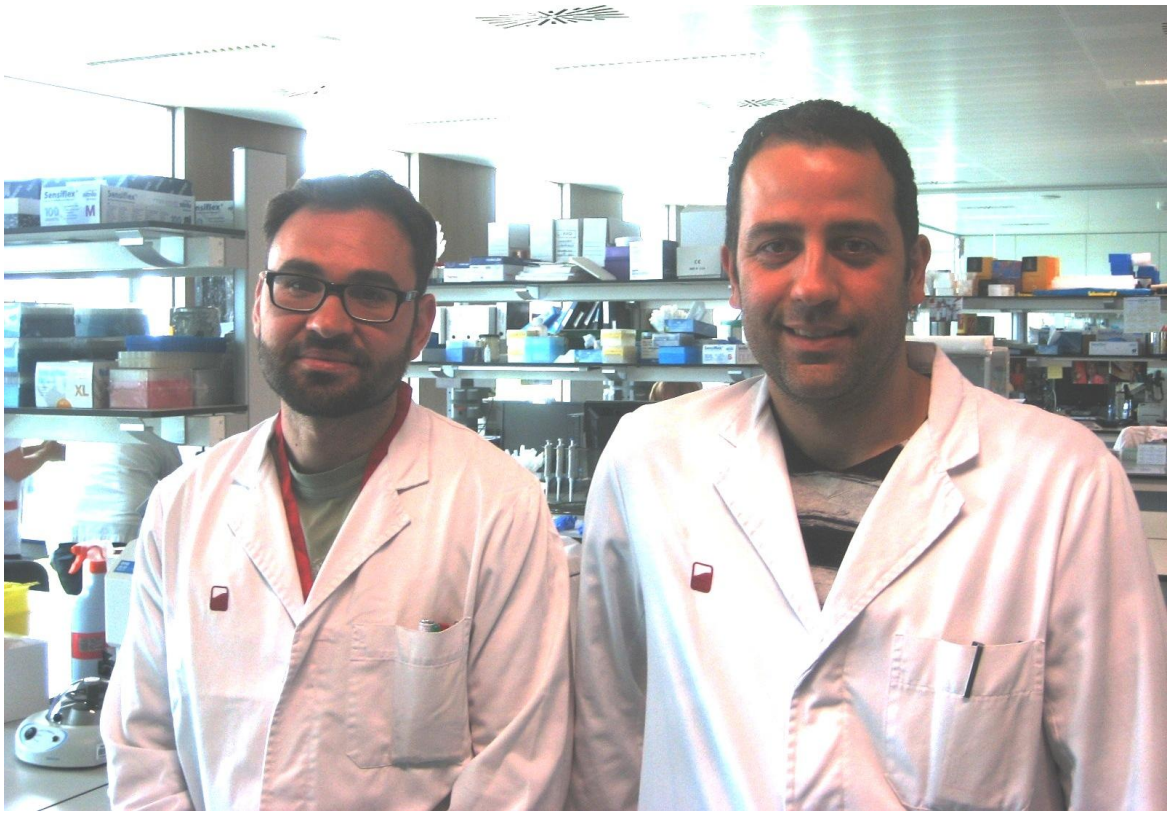
Although 8% of reported FVIII gene (F8) mutations responsible for haemophilia A (HA) affect mRNA processing, very few have been fully characterized at the mRNA level and/or systematically predicted their biological consequences by in silico analysis. This study is aimed to elucidate the effect of potential splice site mutations (PSSM) on the F8 mRNA processing, investigate its correlation with disease severity, and assess their concordance with in silico predictions. We studied the F8 mRNA from 10 HA patient's leucocytes with PSSM by RT-PCR and compared the experimental results with those predicted in silico. The mRNA analysis could explain all the phenotypes observed and demonstrated exon skipping in six cases (c.222G>A, c.601+1delG, c.602-11T>G, c.671-3C>G, c.6115+9C>G and c.6116-1G>A) and activation of cryptic splicing sites, both donor (c.1009+1G>A and c.1009+3A>C) and acceptor sites (c.266-3delC and c.5587-1G>A). In contrast, the in silico analysis was able to predict the score variation of most of the affected splice site, but the precise mechanism could only be correctly determined in two of the 10 mutations analysed. In addition, we have detected aberrant F8 transcripts, even in healthy controls, so this must be taken into account as they could mask the actual contribution of some PSSM. We conclude that F8 mRNA analysis using leucocytes still constitutes an excellent approach to investigate the transcriptional effects of the PSSM in HA, whereas prediction in silico is not always reliable for diagnostic decision-making.

Villarrubia R, Oyagüez I, Álvarez-Román MT, Mingot-Castellano ME, **Parra R**, Casado MA. Cost analysis of prophylaxis with activated prothrombin complex concentrate vs. on-demand therapy with activated factor VII in severe haemophilia A patients with inhibitors, in Spain. HAEMOPHILIA 2015 May;21(3):320-9. QUARTILE 2, DECILE 5, IMPACT FACTOR 2.468

OBJECTIVE: A cost analysis model was developed to compare annual cost of prophylaxis with activated prothrombin complex concentrate (aPCC) vs. on-demand therapy with activated recombinant factor VII (rFVIIa) in severe haemophilia A patients with inhibitors for the Spanish National Health System (NHS). METHODS: Model inputs were drug cost for prophylaxis (aPCC) and for on-demand treatment (rFVIIa or aPCC); bleeding episodes management (excluding bypassing agent cost); surgical costs and disease management (excluding bleeding episodes). Annual bleeding episodes treated on-demand was assumed to be 25, whereas breakthrough bleeds on prophylaxis was 8. Dose for prophylaxis was 75.72 U kg(-1) , three times per week. The total on-demand dose/bleeding episode was 679.66 μg kg(-1) (rFVIIa) and 235.28 U kg(-1) (aPCC). The average bleeding cost (€2998) considered different bleeding sites (62.5% joints, 28.6% muscles and soft tissues, 3.6% mucocutaneous tissues and 5.4% other areas). A 7.5% deduction was applied to ex-factory drug prices. Unitary costs (€2013) derived from local databases. Sensitivity analyses (SA) were performed. RESULTS: Annual cost of aPCC prophylaxis (€524 358) was 16% lower than on-demand treatment with rFVIIa (€627 876). Yearly drug costs were €497 017 for aPCC (€73 166 for on-demand treatment and €423 850 for prophylaxis), and €548 870 for rFVIIa. Disease management cost (€2645 per year) and surgical procedures (€708 per year) were common for both strategies. In the SA prophylactic treatment led to savings between €26 225 and €-1 008 960. CONCLUSION: Prophylaxis with aPCC reduces number of bleeding episodes in severe haemophilia A patients with inhibitors. aPCC prophylaxis resulted in savings in excess of €100 000 per-patient per year, being 16% less costly than on-demand treatment with rFVIIa, for the Spanish NHS.

2.2 HEMATOPOIETIC TRANSPLANTATION & IMMUNOTHERAPY

2.2.1 Program 6: 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 rol as genetic factor of susceptibility and modifier of the phenotype in a Spanish population.
Funding organisation: Carlos III Institute of Health
File N°: PI13-01272
Duration: 2014 to 2016

2.2.2 Program 7: 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 haemapoietic 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 haemapoietic products collected in order to produce a therapeutic product with the expected qualities: safe and functional. The availability of high quality haemapoietic 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

Dr. Sergi Querol was awarded "Scientific Supporter of the Year" from the "Anthony Nolan Supporter Awards" announced in November 2015 at the House of Commons in London.

PERSON IN CHARGE
Sergi Querol Giner

INVESTIGATORS
Carmen Azqueta Molluna
Nerea Castillo Flores
Emma Enrich Randé
Laura Medina Marrero
Dinara Samarkanova
Marta Torrabadella Reynoso

RESEARCH PROJECTS
Principal investigator: Sergi Querol Giner
Prophylactic infusion of donor lymphocites in cord blood transplantation
Funding organisation: La Marató de TV3 Foundation
File N°: 20133230
Duration: 2015 to 2017

Principal investigator: Sergi Querol Giner
Identification of units from the umbilical cord national plan with homozygous CCR5-Δ32 variant
Funding organisation: ONT
Duration: 2015 to 2015

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°: 2015-000510-22
Duration: 2015 to 2016

Principal investigator principal: Siamak Bahram (Strasbourg University), Sergi Querol Giner (BST)
Assessment of the role of non-conventional MHC class I MICA and MICB genes in unrelated hematopoietic stem cell transplantations
Funding organisation: BST and Strasbourg University
Duration: 2014 to 2016

Principal investigator: David Valcárcel Ferreiras (Hospital Vall d'Hebron), Sergi Querol Giner (BST)
NiCord® allogenic 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°: 2015-000074-19
Duration: 2014 to 2016

Principal investigator: Cristina Diaz Heredia (Hospital Vall d'Hebron), Sergi Querol Giner i Dolors Castellà Cahiz (BST)
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°: EC11-559
Duration: 2012 to 2015

Principal investigator: Xinxin Li (H Sant Joan de Deu), Marta Torrabadella (BST)
Improvement in efficiency in cord blood donation programs through prenatal population selectionFunding organisation: BST
Duration: 2015 to 2016

Principal investigator: Cristina Diaz Heredia (Hospital Vall d'Hebron), Dolors Castellà Cahiz (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 2016

PUBLICATIONS

Hough R, Danby R, Russell N, Marks D, Veys P, Shaw B, Wynn R, Vora A, Mackinnon S, Peggs KS, Crawley C, Craddock C, Pagliuca A, Cook G, Snowden JA, Clark A, Marsh J, **Querol S**, Parkes G, Braund H, Rocha V. Recommendations for a standard UK approach to incorporating umbilical cord blood into clinical transplantation practice: an update on cord blood unit selection, donor selection algorithms and conditioning protocols. BR J HAEMATOL 2015 Nov 18. QUARTILE 1, DECILE 2, IMPACT FACTOR 4.959

Allogeneic haemopoietic stem cell transplantation offers a potentially curative treatment option for a wide range of life-threatening malignant and non-malignant disorders of the bone marrow and immune system in patients of all ages. With rapidly emerging advances in the use of alternative donors, such as mismatched unrelated, cord blood and haploidentical donors, it is now possible to find a potential donor for almost all patients in whom an allograft is indicated. Therefore, for any specific patient, the transplant physician may be faced with a myriad of potential choices, including decisions concerning which donor to prioritize where there is more than one, the optimal selection of specific umbilical cord blood units and which conditioning and graft-versus-host disease prophylactic schedule to use. Donor choice may be further complicated by other important factors, such as urgency of transplant, the presence of alloantibodies, the disease status (homozygosity or heterozygosity) of sibling donors affected by inherited disorders and the cytomegalovirus serostatus of patient and donor. We report UK consensus guidelines on the selection of umbilical cord blood units, the hierarchy of donor selection and the preferred conditioning regimens for umbilical cord blood transplantation, with a summary of rationale supporting these recommendations.

Castillo N, García-Cadenas I, Barba P, Martino R, **Azqueta C,** Ferrà C, **Canals C,** Sierra J, Valcárcel D, **Querol S.** Post-thaw viable CD45+ cells and clonogenic efficiency are associated with better engraftment and outcome after single cord blood transplantation in adult patients with malignant diseases. BIOL BLOOD MARROW TRANSPLANT 2015 (21):2167-72. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.348

The quantity of cells is widely accepted as the main factor influencing the outcome after umbilical cord blood transplantation (UCBT) however, the quality of the cord blood units (CBUs) has been less studied. In order to determine the impact of qualitative variables in UCBT outcomes, we conducted a multicenter retrospective study in adult patients with hematological malignancies who underwent single UCBT after a common myeloablative conditioning regimen. One hundred and ten patients from 3 institutions [median age, 35 years (range 18-55)] were included. Quantitative (TNC and total CD34þcells) and qualitative variables [viable CD45þ (vCD45þ), vCD34þ and clonogenic efficiency [(CLONE), quotient of post-thaw colony-forming units (CFU)] and pre-freeze CD34þ cells predicted engraftment in univariate analysis however, only 2 qualitative variables remained significant in the multivariate analysis. Infusion of more than 2 107 post-thaw

vCD45þ cells per kilogram was significantly associated with faster neutrophil (P ¼ .01), platelet engraftment (P ¼ .01), higher disease-free (P ¼ .01) and overall survival (0.02). In addition, CLONE ≥20% predicted a faster neutrophil (P ¼ .005), platelet engraftment (P ¼ .01) and contributed to decrease the non-relapse mortality (P ¼ .02). Our study suggests that the vCD45þ cells dose and CLONE are powerful surrogate markers of graft quality and can potentially help on CBUs selection if tested with representative reference samples.

Castillo N, García-Cadenas I, García O, Barba P, Diaz-Heredia C, Martino R, **Azqueta C**, Ferrà C, **Canals C**, Elorza I, Olivé T, Badell I, Sierra J, Duarte R, Valcárcel D, **Querol S**. Few and nonsevere adverse infusion events using an automated method for diluting and washing before unrelated single cord blood transplantation. BIOL BLOOD MARROW TRANSPLANT 2015 Apr;21(4):682-7. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.348

Graft dilution and DMSO washing before cord blood (CB) administration using an automated system may offer low incidence of adverse infusion events (AIE), ensuring reproducible cell yields. Hence, we analyzed the incidences and significance of immediate AIE, cellular yield, and engraftment after single CB infusion. One hundred and fifty-seven patients (median age, 20 years; range, 1 to 60) received a single CB unit for treatment of hematologic and nonhematologic malignancies with myeloablative conditioning after graft dilution and washing. The median total nucleated cell (TNC) doses was 3.4 × 10(7)/kg (range, 2 to 26) and the median post-thaw recovery was 84% (range, 45 to 178). The cumulative incidence of neutrophil engraftment at 50 days was 84% (95% confidence interval [CI], 83 to 93). A total of 118 immediate AIE were observed in fifty-two (33%) patients. All reported AIE were transient, graded from 1 to 2 by Common Terminology Adverse Events version 4. The most frequent toxicity was cardiovascular but without any life-threatening reaction. Infused TNC, recipient's weight, and rate of infusion per kilogram were risk factors associated with cardiovascular AIE in multivariate analysis (odds ratio [OR], 1.2 (95% CI, 1.1 to 1.4); P < .001; OR, .94 (95% CI, .9 to .97); P < .001; and OR, 1.5 (95% CI, 1.2 to 1.8); P < .001; respectively). In summary, use of an automated method for graft washing before CB administration showed low incidence of AIE without compromising cell yields and engraftment. Infused TNC dose, recipient's weight, and rate of infusion per kilogram were risk factors associated with infusion reactions.

Escobedo-Cousin M, Jackson N, Laza-Briviesca R, Ariza-McNaughton L, Luevano M, Derniame S, **Querol S**, Blundell M, Thrasher A, Soria B, Cooper N, Bonnet D, Madrigal A, Saudemont A. Natural Killer Cells Improve Hematopoietic Stem Cell Engraftment by Increasing Stem Cell Clonogenicity In Vitro and in a Humanized Mouse Model. PLOS ONE 2015 Oct 14;10(10):e0138623. QUARTILE 2, DECILE 2, IMPACT FACTOR 3.534

Cord blood (CB) is increasingly used as a source of hematopoietic stem cells (HSC) for transplantation. Low incidence and severity of graft-versus-host disease (GvHD) and a robust graft-versus-leukemia (GvL) effect are observed following CB transplantation (CBT). However, its main disadvantages are a limited number of HSC per unit, delayed immune reconstitution and a higher incidence of infection. Unmanipulated grafts contain accessory cells that may facilitate HSC engraftment. Therefore, the effects of accessory cells, particularly natural killer (NK) cells, on human CB HSC (CBSC) functions were assessed in vitro and in vivo. CBSC cultured with autologous CB NK cells showed higher levels of CXCR4 expression, a higher migration index and a higher number of colony forming units (CFU) after short-term and long-term cultures. We found that CBSC secreted CXCL9 following interaction with CB NK cells. In addition, recombinant CXCL9 increased CBSC clonogenicity, recapitulating the effect observed of CB NK cells on CBSC. Moreover, the co-infusion of CBSC with CB NK cells led to a higher level of CBSC engraftment in NSG mouse model. The results presented in this work offer the basis for an alternative approach to enhance HSC engraftment that could improve the outcome of CBT.

García-Cadenas I, **Castillo N**, Martino R, Barba P, Esquirol A, Novelli S, Orti G, Garrido A, Saavedra S, Moreno C, Granell M, Briones J, Brunet S, Navarro F, Ruiz I, Rabella N, Valcárcel D, Sierra J. Impact of Epstein Barr virus-related complications after high-risk allo-SCT in the era of pre-emptive rituximab. BONE MARROW TRANSPLANT 2015 Jan 12. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.466

We monitored 133 high-risk allo-SCT recipients for 6 months after transplant for EBV reactivation by quantitative real-time PCR. Rituximab was given as pre-emptive therapy for viremia >1000 copies/mL. The 1-year cumulative incidence of EBV reactivation was 29.4% (95% confidence interval (CI): 18-40) in patients monitored due to initial high-risk characteristics (n=93) and 31.8% (95% CI: 19.7-44) in those followed because of the development of refractory GVHD (n=40). Overall response rate to Rituximab was 83%. Nine patients (9.6%) developed post-transplant lymphoproliferative disorder (PTLD) at a median of +62 days after SCT. Eight of them showed a concomitant CMV reactivation. Second SCT was the only risk factor associated with EBV infection and PTLD in multivariate analysis (hazard ratio (HR) 2.6 (95% CI: 1.1-6.4; P=0.04) and HR 6.4 (95%CI: 1.3-32; P=0.02)). The development of EBV reactivation was not associated with non-relapse mortality or OS (P=0.97 and P=0.84, respectively).

Bitan M, van Walraven SM, Worel N, Ball LM, Styczynski J, **Torrabadella M**, Witt V, Shaw BE, Seber A, Yabe H, Greinix HT, Peters C, Gluckman E, Rocha V, Halter J, Pulsipher MA. Determination of Eligibility in Related Pediatric Hematopoietic Cell Donors: Ethical and Clinical Considerations. Recommendations from a Working Group of the Worldwide Network for Blood and Marrow Transplantation Association. BIOL BLOOD MARROW TRANSPLANT 2015 Aug 22. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.348

Related donors for hematopoietic cell (HC) transplantation are a growing population in recent years because of expanding indications for allogeneic transplantation. The safety and welfare of the donor are major concerns for the transplantation community, especially for related sibling donors of young recipients who are children and, thus, not able to fully consent. Because donation of HC does not improve the donor's own physical health and carries a risk of side effects, careful assessment of medical risks specific to the individual donor, as well as consideration of ethical and legal aspects associated with donation from a child, must be considered. In addition, donor centers must balance the needs of both the donor and the recipient, understanding the inherent conflict parents may have as they can be overly focused on the very sick child receiving a transplant, rather than on the relatively less significant health or emotional problems that a sibling donor may have, which could impact risk with donation. Likewise, consideration must be made regarding the nature of the relationship of the sibling donor to the recipient and also aspects of performing research on pediatric HC donors. In this article, as members of the Donor Issues Committee of the Worldwide Network for Blood and Marrow Transplantation, we review key ethical concerns associated with pediatric donation and then give recommendations for screening potential child donors with underlying health conditions. These recommendations are aimed at protecting the physical and emotional well-being of childhood donors and arise out of the Third International Conference on Health and Safety of Donors sponsored by the Worldwide Network for Blood and Marrow Transplantation.

Fry LJ, **Querol S**, Gomez SG, McArdle S, Rees R, Madrigal JA. Assessing the toxic effects of DMSO on cord blood to determine exposure time limits and the optimum concentration for cryopreservation. VOX SANG 2015 Apr 20. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.303

BACKGROUND AND OBJECTIVES: Advantages of using cord blood (CB) over other sources of haematopoietic progenitor cells, such as bone marrow, include the ability to cryopreserve and bank the samples until requested for a transplant. Cryopreservation requires the addition of a cryoprotectant to prevent the formation of intracellular ice during freezing. Dimethyl sulphoxide (DMSO) is commonly used at a concentration of

10% (v/v); however, there is evidence to suggest this chemical is toxic to cells as well as to patients after infusion. MATERIALS AND METHODS: The toxic effects of DMSO were assessed through cell viability and in vitro functional assays in fresh and post-thaw CB samples before determining the maximum exposure time and optimal concentration for cryopreservation. RESULTS:
A dose-dependent toxicity of DMSO was observed in fresh samples with 40% removing all viable and functional haematopoietic progenitor cells (HPC). In fresh and post-thaw analysis, minimal toxic effect was observed when cryopreservation was delayed for up to 1 h after 10% DMSO addition. After thawing, DMSO washout was superior to dilution or unmanipulated when maintained for long periods (advantage observed 1 h after thawing). Finally, the optimum concentration for cryopreserving CB was found to be 7·5 to 10% with detrimental effects observed outside of this range. CONCLUSION: These results support the use of 7·5-10% as the optimal DMSO concentration and the maximum exposure time should be limited to <1 h prior to freezing and 30 min post-thaw.

Duarte RF, Salgado M, Sánchez-Ortega I, Arnan M, **Canals C**, Domingo-Domenech E, Fernández-de-Sevilla A, González-Barca E, Morón-López S, **Nogues N**, Patiño B, Puertas MC, Clotet B, Petz LD, **Querol S**, Martinez-Picado J. CCR5 Δ32 homozygous cord blood allogeneic transplantation in a patient with HIV: a case report. LANCET HIV 2015 Jun;2(6):e236-42.

BACKGROUND: Allogeneic donor CCR5 Δ32 homozygous haemopoietic cell transplantation (HCT) provides the only evidence to date of long-term control of HIV infection. However, availability of conventional CCR5 Δ32 homozygous donors is insufficient to develop this as a therapeutic strategy further. METHODS: We present a 37-year-old patient with HIV-1 infection and aggressive lymphoma who had disease progression after five lines of radiochemotherapy including an autologous HCT, and in the absence of matched sibling donors, received an allogeneic HCT with four of six HLA-matched CCR5 Δ32 homozygous cord blood cells (StemCyte, Covina, CA), supported with purified CD34+ cells from a haploidentical sibling. Blood or tissue samples were obtained before and weekly after HCT to monitor transplant and HIV infection, including chimerism analysis, CCR5 genotyping and viral tropism, viral isolation and sequence, viral reservoir analysis, immune activation and proliferation, and ex-vivo cell infectivity assays. Combined antiretroviral therapy continued during the procedure. FINDINGS: The patient's HIV was CCR5-tropic by genotypic and phenotypic analyses. Baseline latent reservoir tests showed HIV DNA copies in bulk and resting CD4 T cells and in gut-associated lymphoid tissue, CD4 T-cell-associated HIV RNA, replication competent viral size of 2·1 copies per 10(7) CD4 T cells, and single copy assay of 303 copies per mL. After HCT, plasma HIV DNA load was undetectable by ultrasensitive analyses. Upon cord blood full chimerism, the patient's CCR5 Δ32 homozygous CD4 T cells responded to proliferation and activation stimuli and became resistant to infection by the patient's viral isolate and by laboratory-adapted HIV-1 strains. Death related to lymphoma progression regretfully prevented long-term monitoring of the patient's viral reservoir. INTERPRETATION: CCR5 Δ32 homozygous cord blood reconstitution can successfully eliminate HIV-1 and render the allogeneic graft recipient's T lymphocytes resistant to HIV infection. Thus, they build on the evidence available to strongly support the use of cord blood as a strategic platform for a broader application of non-functional CCR5 transplantation to other infected individuals. FUNDING: Spanish Secretariat of Research, the American Foundation for AIDS Research (amfAR).

Petz L, Burnett J, Li H, Li S, Tonai R, Bakalinskaya M, Shpall E, Armitage S, Kurtzberg J, Regan D, Clark P, **Querol S**, Gutman J, Spellman S, Gragert L, Rossi J. Progress toward curing HIViv infection with hematopoietic cell transplantation. STEM CELLS CLONING 2015:8 109-116.

HIV-1 infection afflicts more than 35 million people worldwide, according to 2014 estimates from the World Health Organization. For those individuals who have access to antiretroviral therapy, these drugs can effectively suppress, but not cure, HIV-1 infection.

Indeed, the only documented case for an HIV/AIDS cure was a patient with HIV-1 and acute myeloid leukemia who received allogeneic hematopoietic cell transplantation (HCT) from a graft that carried the HIV-resistant CCR5-Δ32/Δ32 mutation. Other attempts to establish a cure for HIV/AIDS using HCT in patients with HIV-1 and malignancy have yielded mixed results, as encouraging evidence for virus eradication in a few cases has been offset by poor clinical outcomes due to the underlying cancer or other complications. Such clinical strategies have relied on HIV-resistant hematopoietic stem and progenitor cells that harbor the natural CCR5-Δ32/Δ32 mutation or that have been genetically modified for HIV-resistance. Nevertheless, HCT with HIV-resistant cord blood remains a promising option, particularly with inventories of CCR5-Δ32/Δ32 units or with genetically modified, human leukocyte antigen-matched cord blood.

2.3 REPARATIVE & IMMUNOMODULATORY THERAPY

2.3.1 Program 8: 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.

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.

The Advanced therapies program with B-DEBATE (International Center for Scientific Debate Barcelona), Biocat and La Caixa Foundation organized in February 2015 the

meeting "Advanced Cellular Therapies and Regenerative Medicine. The Promise in the 21st Century " in which 27 experts in the field from Europe and USA participated.

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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°: 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°: RD12/0019/0015
Duration: 2013 to 2016

Principal investigator: Joaquim Vives Armengol
Estudi de les propietats anti-inflamatòries i immunomoduladores dels medicaments de teràpia avançada desenvolupats a Xcelia
Funding organisation: BST
Duration: 2015 to 2018

Principal investigator: Joaquim Vives Armengol
Feasibility study of Grifols' fibrin sealant as scaffold for human mesenchymal stromal cells in traumatological disorders
Funding organisation: Grifols
Duration: 2015 to 2016

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°: EC10-209
Duration: 2012 to 2016

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°: EC11-158
Duration: 2012 to 2016

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°: EC10-266
Duration: 2012 to 2016

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°: EC10-208
Duration: 2012 to 2016

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°: EC11-436
Duration: 2012 to 2016

Principal investigator: Marius Aguirre Canyadell (Hospital Vall d'Hebron), Joaquim Vives Armengol (BST)
Experimental cell therapy study for the treatment of critical size defect with "ex vivo" expanded adult mesenchymal stem cells
Funding organisation: Carlos III Health Institute
File N°: PI11/02231
Duration: 2012 to 2015

Investigador principal: Marius Aguirre Canyadell (Hospital Vall d'Hebron), Joaquim Vives Armengol (BST)
Femoral head osteonecrosis treatment with advanced cell therapy and biomaterials in an experimental sheep animal model
Funding organisation: La Marató de TV3 Foundation
File N°: 61/C/2012
Duration: 2013 to 2015

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°: 122831
Duration: 2013 to 2015

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 allogeneic bone tissue in non hypertrophic pseudoarthrosis of long bones
Funding organisation: ASEPEYO and BST
File N°: 2013-005025-23
Duration: 2015 to 2017

PUBLICATIONS

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 2015 Nov 5. QUARTILE 2, DECILE 3, IMPACT FACTOR 3.1

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 works 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.

Vives J, Oliver I, Pla A. Quality compliance in the shift from cell transplantation to cell therapy in non-pharma environments. CYTOTHERAPY 2015; 0: 1-6. QUARTILE 2, DECILE 3, IMPACT FACTOR 3.1

Along with academic and charitable organizations, transfusion centers have ventured into the stem cell field, with the aim of testing of novel cell-based therapeutics in a clinical setting for future marketing approval. The fact that quality management structures, which are required for compliance with good scientific practice regulations, were originally designed for product development in corporate environments represents a major challenge for many developers. In this Commentary, challenges that non-pharmaceutical institutions must overcome to translate cell-based products into clinical therapies will be discussed from a quality standpoint. Furthermore, our development experience for a mesenchymal stromal cellebased therapy will be shared as a case study.

Milián E, Prats E, Cairó JJ, Gòdia F, **Vives J**. BHRF1 exerts an antiapoptotic effect and cell cycle arrest via Bcl-2 in murine hybridomas. J BIOTECHNOL 2015 Jun 7;209:58-67. QUARTILE 2, DECILE 3, IMPACT FACTOR 2.884

Apoptosis has been widely studied in order to find methods to increase the life-span and production performance in large-scale animal cell cultures. The use of anti-apoptotic genes has emerged as an efficient method to reduce apoptosis in a variety of biotechnological relevant cell lines, including CHO and hybridomas, alternatively to small molecule inhibitors. It is already known that expression of BHRF1, an Epstein-Barr virus-encoded early protein homologous to the anti-apoptotic protein Bcl-2, protects hybridoma cells from apoptosis in batch and continuous operation modes resulting in a delay in the cell death process under glutamine starvation conditions. In the present study, the mechanism of action of BHRF1 was investigated in a murine hybridoma cell line. BHRF1 protein was found in the mitochondrial cell fraction both under normal growing conditions and apoptosis-inducing conditions. Remarkably, the expression of the anti-apoptotic gene bcl2 in BHRF1-expressing cells was up-regulated 25-fold compared to mock-transfected controls under apoptosis triggering conditions and its expression correlated with survival of transgenic cultures and cell cycle arrest in G1. Bcl-2 activity was revealed to be crucial for the BHRF1-mediated effect since the addition of specific inhibitors of Bcl-2 (namely HA14-1 and YC-137) resulted in a loss of function of BHRF1-expressing cells under glutamine starvation conditions. Moreover, the interaction of BHRF1 with the pro-apoptotic BH3-only Bim conferred mitochondrial stability to BHRF1 expressing cells under apoptosis-triggering conditions.

Caminal M, Peris D, Fonseca C, Barrachina J, Codina D, Rabanal RM, Moll X, Morist A, García F, Cairó JJ, Gòdia F, **Pla A, Vives J.** Cartilage resurfacing potential of PLGA scaffolds loaded with autologous cells from cartilage, fat, and bone marrow in an ovine model of osteochondral focal defect. CYTOTECHNOLOGY 2015 Jan 17. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.449

Current developments in tissue engineering strategies for articular cartilage regeneration focus on the design of supportive three-dimensional scaffolds and their use in combination with cells from different sources. The challenge of translating initial successes in small laboratory animals into the clinics involves pilot studies in large animal models, where safety and efficacy should be investigated during prolonged follow-up periods. Here we present, in a single study, the long-term (up to 1 year) effect of biocompatible porous scaffolds non-seeded and seeded with fresh ex vivo expanded autologous progenitor cells that were derived from three different cell sources [cartilage, fat and bone marrow (BM)] in order to evaluate their advantages as cartilage resurfacing agents. An ovine model of critical size osteochondral focal defect was used and the test items were implanted arthroscopically into the knees. Evidence of regeneration of hyaline quality tissue was observed at 6 and 12 months post-treatment with variable success depending on the cell source. Cartilage and BM-derived mesenchymal stromal cells (MSC), but not those derived from fat, resulted in the best quality of new cartilage, as judged qualitatively by magnetic resonance imaging and macroscopic assessment, and by histological quantitative scores. Given the limitations in sourcing cartilage tissue and the risk of donor site morbidity, BM emerges as a preferential source of MSC for novel cartilage resurfacing therapies of osteochondral defects using copolymeric poly-D,L-lactide-co-glycolide scaffolds.

Oliver-Vila I, Coca MI, Grau-Vorster M, Pujals-Fonts N, Caminal M, Pla A, Garcia J, Vives J. Off-the-shelf mesenchymal stromal cells derived from umbilical cord tissue. BMC PROCEEDINGS 2015, 9 (Suppl 9)

Oliver-Vila I, van Deusen AL**, Palau R, Vives J.** Quality compliance in the development of cellbased medicines in non-pharma environments. BMC PROCEEDINGS 2015, 9 (Suppl 9).

Vives J, Blanco M, Caminal C, Coca MI, Codinach M, Coll R, Doral M, Lloret M, Oliver-Vila I, Ortega I, Reales L, Requena-Montero M, Rodriguez L, Torrents S, Garcia J. Development of an advanced cell therapy product indicated for the treatment of gonarthrosis. BMC PROCEEDINGS 2015; 9 (Suppl 9)

Caminal M, Labroza JP, **Oliver-Vila I,** Alzaga-Gragera M**, Marín-Gallén S, Pla A, Garcia J, Vives J.** Ex vivo production of red blood cells from human cord blood. BMC PROCEEDINGS 2015, 9 (Suppl 9).

2.3.2 Program 9: 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.

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RESEARCH PROJECTS

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°: 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°: 120630
Duration: 2013 to 2017

Principal investigator: Ricardo Casaroli Marano

Interferon-γ Release Assay (IGRA), GeneXpert platform and Real-time Polymerase Chain Reaction (RT-PCR): Implications for the diagnosis and management of tuberculosis-related ocular inflammation
Funding organisation: Brasilian Ministry of Science, Technology and Innovation
Duration: 2015 to 2018

Principal investigator: Oscar Fariñas Barbera

Demineralized bone matrix development with human collagen.
Funding organisation: BST
File N°: 1/2014 BTB
Duration: 2015 to 2017

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°: 1/2015 BTB
Duration: 2015 to 2017

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°: PER-ECL-2013-06
Duration: 2015 to 2016

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

ARISE: Aortic Valve Replacement using Individualised Regenerative Allografts: Bridging the Therapeutic Gap
Funding organisation: European Comission
File N°: SEP-210137838
Duration: 2014 to 2018

PUBLICATIONS

Fraccaroli A, Pitter B, Taha A, Seebach J, Huveneers S, Kirsch J, **Casaroli-Marano R**, Zahler S, Pohl U, Gerhardt H, Schnittler HJ, Montanez E. Endothelial Alpha-Parvin

Controls Integrity of Developing Vasculature and is Required for Maintenance of Cell-Cell Junctions. CIRC RES 2015 Apr 29. QUARTILE 1, DECILE 1, IMPACT FACTOR 11.09

RATIONALE: Angiogenesis and vessel integrity depend on the adhesion of endothelial cells (EC) to the extracellular matrix (ECM) and to adjacent ECs. The focal adhesion protein alpha-parvin (α-pv) is essential for vascular development. However, the role of α-pv in ECs in vivo is not known. OBJECTIVE: To determine the function of α-pv in ECs during vascular development in vivo and the underlying mechanisms. METHODS AND RESULTS: We deleted the α-pv gene specifically in ECs of mice to study its role in angiogenesis and vascular development. Here we show that endothelial-specific deletion of α-pv in mice results in late embryonic lethality associated with hemorrhages and reduced vascular density. Postnatal induced EC-specific deletion of α-pv leads to retinal hypovascularization due to reduced vessel sprouting and excessive vessel regression. In the absence of α-pv, blood vessels display impaired VE-cadherin junction morphology. In vitro, α-pv deficient ECs show reduced stable adherens junctions, decreased monolayer formation and impaired motility, associated with reduced formation of integrin-mediated cell-ECM adhesion structures and an altered actin cytoskeleton. CONCLUSIONS: Endothelial α-pv is essential for vessel sprouting and for vessel stability.

Mazoteras P, **Casaroli-Marano RP**. In vitro biofilm distribution on the intraocular lens surface of different biomaterials. J CATARACT REFRACT SURG 2015 Sep;41(9):1980-8. QUARTILE 1, DECILE 3, IMPACT FACTOR 2.552

PURPOSE: To study the disposition of bacterial adhesion to intraocular lens (IOL) biomaterials depending on the material and region of the optic IOL surface: center or peripheral edge. SETTING: School of Medicine, University of Barcelona, Barcelona, Spain. DESIGN: Experimental study. METHODS: For the in vivo study, IOLs were explanted from donor ocular globes without clinical symptoms of endophthalmitis. Biofilm formation was qualitatively studied by scanning electron microscopy (SEM). For the in vitro study, 5 IOL biomaterials (hydrophilic acrylic, hydrophobic acrylic, poly[methyl methacrylate] [PMMA], heparinized PMMA, and silicone) were contaminated with a biofilm-producing strain of Staphylococcus epidermidis. Bacterial densities were quantitatively (colony-forming units per area) compared by SEM and direct counting of viable adherent bacteria, according to the biomaterial, region of the IOL optic surface, and time of incubation. For SEM, bacterial adhesion was also qualitatively classified according to the characteristics of biofilm observed: structure, cocci per cluster, homogeneity of cluster distribution, and extracellular matrix production. RESULTS: At 3 hours of incubation, bacterial counts for hydrophilic acrylic and PMMA IOLs were significantly lower, but at 72 hours there were no statistically significant differences among biomaterials. A higher density of bacteria was observed at the periphery of the IOL's optic of assayed biomaterials for in vitro and in vivo studies. Biofilm formation and the presence of extracellular matrix were predominantly restricted to the edges of IOL optic surface. CONCLUSION: Bacterial adhesion and biofilm development on the IOL optic surface depended on the region and biomaterial of the IOL. FINANCIAL DISCLOSURE: Neither author has a financial or proprietary interest in any material or method mentioned.

Verdaguer P, Gris O, **Casaroli-Marano RP**, Elies D, Muñoz-Gutierrez G, Güell JL. Intraocular Lens Opacification After Endothelial Keratoplasty as Analyzed by Environmental Scanning Electron Microscopy. CORNEA 2015 May 11. QUARTILE 2, DECILE 3, IMPACT FACTOR 2.360

PURPOSE: To describe a case of hydrophilic intraocular lens (IOL) opacification based on IOL analysis after Descemet stripping automated endothelial keratoplasty.
METHODS: A 60-year-old woman had uneventful phacoemulsification after the implantation of a hydrophilic IOL (Akreos-Adapt; Bausch & Lomb) into both eyes. Because of postoperative corneal decompensation in the right eye, 2 Descemet stripping automated endothelial keratoplasty operations were performed within 1 year. After the second procedure, the graft was not well attached, requiring an intracameral injection of

air on day 3. After 1 year, opacification was observed on the superior 2/3 of the anterior surface of the IOL, along with a significant decrease in visual acuity. The IOL was explanted 6 months after the opacification. RESULTS: Environmental scanning electron microscopy followed by x-ray microanalysis revealed an organic biofilm on the surface of the IOL. CONCLUSIONS: To our knowledge, this is the first reported case in which the material deposited on the lens is organic rather than calcific.

Casaroli-Marano RP, Sousa-Martins D, **Martínez-Conesa EM**, Badaró E, Nunes RP, Lima-Filho AA, Rodrigues EB, Belfort R Jr, Maia M. Dye solutions based on lutein and zeaxanthin: in vitro and in vivo analysis of ocular toxicity profiles. CURR EYE RES 2015 Jul;40(7):707-18. QUARTILE 3, DECILE 5, IMPACT FACTOR 1.663

PURPOSE: To study the safety profile of Lutein/Zeaxanthin(L/Z)-based natural dye solutions in in vitro and in vivo models. MATERIAL AND METHODS: In vitro cytotoxicity and cellular growth experiments were carried out on ARPE-19 and human corneal epithelial (HCE) cell lines using different L/Z-based dye solutions, either alone or in association with brilliant blue (BB) or trypan blue (TB). Light and transmission electron microscopy studies were performed seven days after intravitreal injection of dye solutions in rabbits. Electroretinogram (ERG) recordings were taken at baseline and before histopathology. RESULTS: In vitro cytotoxicity assays demonstrated that the different L/Z-based solutions (from 0.3 to 2%), either alone or in association with BB (0.025%) or TB (0.04%), did not significantly alter mitochondrial activity (≤15%) in the cell lines tested. In addition, in vitro cell growth was inhibited by up to 60% depending on the dye solution, and in direct proportion to the concentration assayed. There was no evidence of structural alterations in the neurosensory retina, retinal pigment epithelium (RPE), or choriocapillaris-choroidal complex. b-Wave ERG records showed no significant differences (±15.2%) in comparison with baseline. CONCLUSIONS: L/Z-based dye solutions demonstrated a safe profile in in vitro and in vivo models, and may be a useful tool for staining intraocular structures.

Mazoteras P, Bispo PJ, Höfling-Lima AL, **Casaroli-Marano RP**. DNA extraction methods for panbacterial and panfungal PCR detection in intraocular fluids. CURR EYE RES 2015 Jul;40(7):697-706. QUARTILE 3, DECILE 5, IMPACT FACTOR 1.663

PURPOSE: Three different methods of DNA extraction from intraocular fluids were compared with subsequent detection for bacterial and fungal DNA by universal PCR amplification. MATERIAL AND METHODS: Three DNA extraction methods, from aqueous and vitreous humors, were evaluated to compare their relative efficiency. Bacterial (Gram positive and negative) and fungal strains were used in this study: Escherichia coli, Staphylococcus epidermidis and Candida albicans. The quality, quantification, and detection limit for DNA extraction and PCR amplification were analyzed. Validation procedures for 13 aqueous humor and 14 vitreous samples, from 20 patients with clinically suspected endophthalmitis were carried out. RESULTS: The column-based extraction method was the most time-effective, achieving DNA detection limits ≥10(2) and 10(3)CFU/100 µL for bacteria and fungi, respectively. PCR amplification detected 100 fg, 1 pg and 10 pg of genomic DNA of E. coli, S. epidermidis and C. albicans respectively. PCR detected 90.0% of the causative agents from 27 intraocular samples collected from 20 patients with clinically suspected endophthalmitis, while standard microbiological techniques could detect only 60.0%. The most frequently found organisms were Streptococcus spp. in 38.9% (n = 7) of patients and Staphylococcus spp. found in 22.2% (n = 4). CONCLUSIONS: The column-based extraction method for very small inocula in small volume samples (50-100 µL) of aqueous and/or vitreous humors allowed PCR amplification in all samples with sufficient quality for subsequent sequencing and identification of the microorganism in the majority of them.

Nadal J, Kudsieh B, **Casaroli-Marano RP.** Scleral Fixation of Posteriorly Dislocated Intraocular Lenses by 23-Gauge Vitrectomy without Anterior Segment Approach. J OPHTHALMOL 2015:391619. QUARTILE 2, DECILE 4, IMPACT FACTOR 1.935

BACKGROUND. To evaluate visual outcomes, corneal changes, intraocular lens (IOL) stability, and complications after repositioning posteriorly dislocated IOLs and sulcus fixation with polyester sutures. DESIGN. Prospective consecutive case series. Setting. Institut Universitari Barraquer. Participants. 25 eyes of 25 patients with posteriorly dislocated IOL. METHODS. The patients underwent 23-gauge vitrectomy via the sulcus to rescue dislocated IOLs and fix them to the scleral wall with a previously looped nonabsorbable polyester suture. Main Outcome Measures. Best corrected visual acuity (BCVA) LogMAR, corneal astigmatism, endothelial cell count, IOL stability, and postoperative complications. RESULTS. Mean follow-up time was 18.8 ± 10.9 months. Mean surgery time was 33 ± 2 minutes. Mean BCVA improved from 0.30 ± 0.48 before surgery to 0.18 ± 0.60 ($p = 0.015$) at 1 month, which persisted to 12 months (0.18 ± 0.60). Neither corneal astigmatism nor endothelial cell count showed alterations 1 year after surgery. Complications included IOL subluxation in 1 eye (4%), vitreous hemorrhage in 2 eyes (8%), transient hypotony in 2 eyes (8%), and cystic macular edema in 1 eye (4%). No patients presented retinal detachment. CONCLUSION. This surgical technique proved successful in the management of dislocated IOL. Functional results were good and the complications were easily resolved.

Rodríguez A, Sandiumenge A, Masnou N, Gómez A, Margarit N, Ferrer-Gracia V, Carbonell E, **Navarro A**, Pont T. Medical Students for Tissue Procurement, a 10-Year Experience in a Large University Hospital: An Exportable Model? TRANSPLANT P 2015 Oct;47(8):2314-7. QUARTILE 3, DECILE 7, IMPACT FACTOR 0.984

OBJECTIVE: The objective of this study was to describe tissue procurement activity performed during 10 years (2004-2014) by trained medical students in a large university hospital. METHODS: In this study, third to sixth year medical students were trained as in-hospital Tissue Coordinators (Tc) to perform tissue procurement activity on a 24/7 schedule supervised by an on-call senior Transplant Coordinator (sTC) in a large university hospital. Tc duty consisted of detection, initial evaluation of all hospital deaths, donor's family approach for tissue donation, and retrieval logistics organization, including corneal tissue retrieval after training and certification. They also assist sTC in organ procurement activity. RESULTS: A total of 18,931 deaths were prospectively evaluated, 79% of whom (n = 14,879) presented medical contraindications for tissue donation. Of the remaining 4052 (21%) potential tissue donors (PTD), 2522 (62%) were not converted into real donors, mostly due to family refusal (66%; n = 1650) followed by detection system failure and other logistical issues (34%; n = 872). A total of 2814 corneal units, 225 skin donations, 327 muscleskeletal tissue donations, 91 blood vessels donations, and 177 heart valve donations were obtained from the remaining 1530 (38%) real donors. Tissue potentiality increased from 19% to 43% throughout the study period as a consequence of the fluctuating acceptance criteria used by tissue banks depending on tissue demand. CONCLUSIONS: The tissue donation program performed by trained students was successful in achieving a high and sustainable tissue donation rate in a large university hospital.

Casaroli-Marano RP, Nieto-Nicolau N, **Martínez-Conesa EM**, Edel M, B Álvarez-Palomo A. Potential Role of Induced Pluripotent Stem Cells (IPSCs) for Cell-Based Therapy of the Ocular Surface. J CLIN MED 2015 Feb 12;4(2):318-42.

The integrity and normal function of the corneal epithelium are crucial for maintaining the cornea's transparency and vision. The existence of a cell population with progenitor characteristics in the limbus maintains a dynamic of constant epithelial repair and renewal. Currently, cell-based therapies for bio replacement—cultured limbal epithelial transplantation (CLET) and cultured oral mucosal epithelial transplantation (COMET)—present very encouraging clinical results for treating limbal stem cell deficiency (LSCD) and restoring vision. Another emerging therapeutic approach consists of obtaining and implementing human progenitor cells of different origins in association with tissue engineering methods. The development of cell-based therapies using stem cells, such as

human adult mesenchymal or induced pluripotent stem cells (IPSCs), represent a significant breakthrough in the treatment of certain eye diseases, offering a more rational, less invasive, and better physiological treatment option in regenerative medicine for the ocular surface. This review will focus on the main concepts of cell-based therapies for the ocular surface and the future use of IPSCs to treat LSCD.

Keller J, Giralt J, Alforja S, **Casaroli-Marano RP**. Altering the clinical course of Sorsby fundus dystrophy with the use of anti-vascular endothelial growth factor intraocular therapy. RETIN CASES BRIEF REP 2015 Spring;9(2):104-5.

PURPOSE: Sorsby fundus dystrophy is a rare hereditary condition causing choroidal neovascularization leading to vision loss. Previously, treatment was mostly unsuccessful. Here, we analyze the result of various treatments administered over the years. METHODS: Retrospective case-note review. PATIENTS: Three adults from a Spanish family with Sorsby fundus dystrophy showed a very varied course. The untreated case was blind at presentation. Administration of photodynamic therapy and intravitreal anti-vascular endothelial growth factor agents managed to modify the history of disease, more successfully with the latter. CONCLUSION: The intravitreal administration of anti-vascular endothelial growth factor agents seems to reduce the extent of scarring in Sorsby fundus dystrophy albeit without halting the episodic recurrences. This may lead to improved outcomes even when the visual acuity is compromised.

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