SCIENTIFIC RESEARCH REPORT

BANC DE SANG I TEIXITS I 2014

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 Banc of 2014. This was the second year of implementation of the Strategic Plan for R+D+i 2013-2015.

The main research activity focuses on the diagnosis, transfusion medicine and haemostasis, in line with the mission of BST. But we also research in hematopoietic transplantation, immunotherapy and regenerative medicine.

It is worth noting the effort of more than 50 professionals who devote part of their time to research, performing 56 projects, more than half of them in collaboration with other entities.

Among these projects we would like to highlight a clinical trial in high-risk leukemia, funded by the Marató TV3 Foundation, in which 6 pioneer units of hematopoietic transplant of Catalonia are involved, the results of this study may involve a significant improvement in the treatment of these patients.

Year after year the BST maintains its commitment to research and innovation with an expenditure of almost 3 billion euros in 2014. On the other hand, researchers have succeeded in significantly increase incomes from public agencies (through competitive calls) and from agreements with industry.

We are convinced that in the coming years professionals from BST will continue to provide results of their research to society.

Enric Argelagués Vidal

INTRODUCTION BY THE SCIENTIFIC DIRECTOR

We present the Scientific Report of the Banc de Sang i Teixits corresponding to 2014. This year, the report reflects the most important milestones in R+D+i, including for the first time the activity of the Tissue Bank.

Despite the apparent economic recovery, these are still not good times for research. For this reason from the Scientific Direction we want to make an effort to internally finance all research projects of interest to the BST specially those that are hardly financed by public or private agencies.

In 2014, the BST pre-doctoral fellowships program has started. This competitive call has allowed the incorporation of two pre-doctoral researchers who have started their research careers with us. The objective of this program is to promote and retain research talent in addition to providing support to the Principal Investigators for them to develop their projects with continuity.

We are aware of the impact of quality research on the clinical activity of BST in all areas. For this reason, we encourage you all to contribute with original ideas and scientific rigor.

Sílvia Sauleda Oliveras

1. BANC DE SANG I TEIXITS

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

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

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

1.1 GOVERNING BODIES

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

1.1.1 Board of Directors

President: Manel Peiró Posadas
Vice-president: 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	18	5.77
Senior physicians	7	5.17
Junior physicians	20	12.15
Technical staff	9	8.20
TOTAL	54	31.29

1.5.2 Economic data

Breakdown of BST research income for 2014	Euros
Projects funded by public agencies	439,103
Agreements with industry	686,160
Own funds	2,700,408
TOTAL	3,825,671

1.5.3 Organisation of the BST research

The R+D+i Strategic Plan 2013-2015 defines the following 9 Research Programs:

Diagnosis, transfusional medicine & hemostasis	Hematopoietic transplantation & immunotherapy	Reparative & immunomodulatory therapy
PR1 Blood process	PR6 Molecular biology of transplantation	PR8 Substitutive & reparative therapy
PR2 Transfusional safety	PR7 Transplantation of donors & alternative sources	PR9 Large-scale production of cells & tissues
PR3 Therapeutic apheresis		
PR4 Immunohematology		
PR5 Coagulopathies		

In 2014 the Tissue Bank of the Hospital Clinic (Transplant Services Foundation) joined The Blood and Tissue Bank.

1.5.4 Research projects

The following 56 research projects were ongoing during 2014.

ONGOING PROJECTS IN 2014	PRINCIPAL INVESTIGATOR BST	COLLABORATION
PUBLIC AGENCIES		
European Commission	2	1
INSERM, FAPES		1
Carlos III Health Institute	2	5
Spanish Ministry Economy & Competitivity	1	
Spanish Ministry Health Social Service & Equality		6
ONT	1	
Marató TV3	2	1
BST+ Strasbourg University		1
NON-PROFIT PRIVATE AGENCIES		
Mundo Sano		1
BST + International University of Catalonia		1
BST + Anthony Nolan Trust + Nottingham Trent	1	
AGREEMENTS WITH INDUSTRY		
Ablynx		1
Argos		1
Asepeyo		1
Baxter	1	
BSRI	1	
Erythech Pharma		1
Gamida		1
Grífols, S.A.	1	2
Novartis	1	
Pfizer	2	
Progenika	1	
Roche		3
Sanofi		1
StemCyte		1
Therakos		1
OWN FUNDS	10	
TOTAL	56	

1.5.5 Doctoral theses

The following PhD thesis was read in 2014.

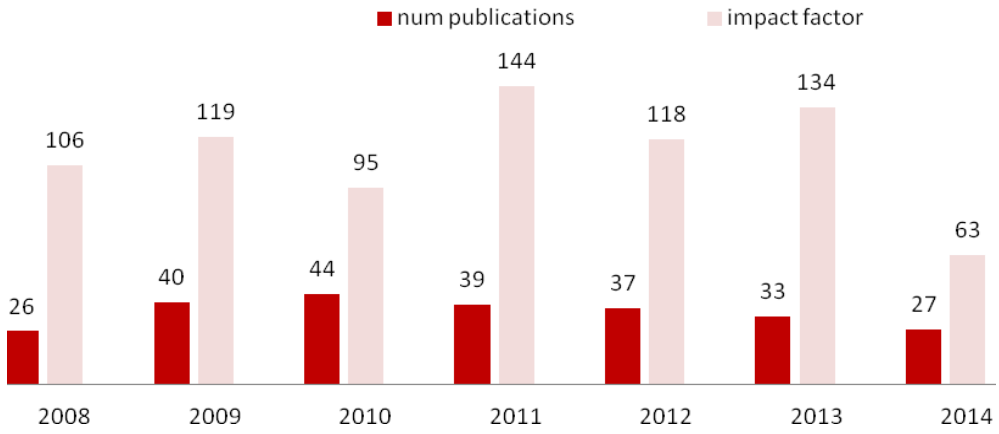
PhD student	Thesis title	Directors	Department	Grade
Marta Caminal Bobet	Tissue engineering for bone regeneration: development in vitro and in vivo tests in sheep	Joaquim Vives Armengol Francesc Gòdia Casablancas	Chemistry Engineering UAB	Excellent cum laude

1.5.6 Publications

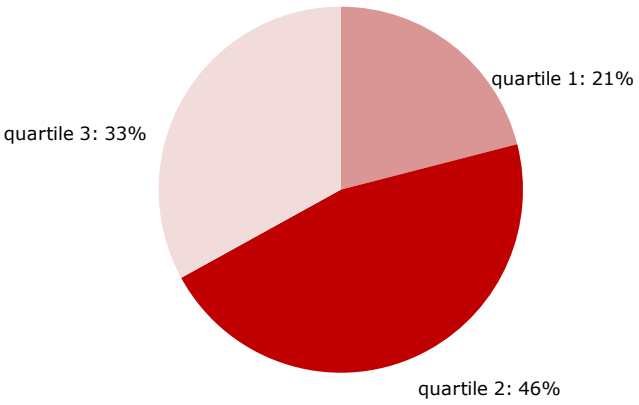
A total of 27 articles were published in scientific magazines by BST investigators in 2014 with an impact factor of 63.

The combined impact factor for 2014 was calculated using Journal Citation Reports (JCR) for 2012. The calculation included original articles, revisions and editorials. Presentations to congresses were excluded.

Evolution of the scientific production of the BST over the last 7 years:



Publications BST 2014:



2013 publications by research areas:

Diagnosis, transfusional medicine & hemostasis	12
Hematopoietic transplantation & immunotherapy	4
Reparative & immunomodulatory therapy	11

1.5.7 Patents

The BST currently has 8 patents in different stages of processing. Seven of them are granted in Spain and 5 are 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: Joan Ramon Grífols Ronda
Phase II clinical trial, single-blind, randomized, placebo controlled, to study the efficacy and security of Nanobody Anti-Factor Von Willebrand administered with adjuvant treatment in patients with Acquired Thrombotic Thrombocytopenic Purpura
Funding organisation: Ablynx
File N°: ALX-0681-2.1/10
Duration: 2012 to 2014

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: 2014 to 2017

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 2014

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

PUBLICATIONS

Grasas A, Pereira A, **Bosch MA, Ortiz P, Puig L**. Feasibility of reducing the maximum shelf life of red blood cells stored in additive solution: a dynamic simulation study involving a large regional blood system. VOX SANG 2014 Dec 4. QUARTILE 2, DECILE 5, IMPACT FACTOR 2.847

BACKGROUND AND OBJECTIVE: Recent studies suggest that transfusion of old red blood cell (RBC)s, mainly those close to the 42-day maximum shelf life (MSL), is associated with increased morbi-mortality. Although there is no formal proof supporting a causal relationship, the precautionary principle asks for corrective interventions whenever they do not bring about other risks or unjustified costs. Here, we investigated the feasibility of reducing the MSL. MATERIALS AND METHODS: A trace simulation model was used to analyse the repercussions of several MSLs on a large regional blood system. The baseline model was fed with real input and output data from years 2009 to 2010 and validated against real inventory data. Shortage and outdate rates and inventory levels for each blood group were derived assuming 42-, 35-, 28-, 21- and 14-day MSLs, as well as several distribution rules and supply shocks (periods without blood collections). RESULTS: The model shows that MSL could be reduced to 28-35 days without major increases in the shortage or outdate rates, even after supply shocks. At the 21-day MSL, the inventory capability to compensate supply shocks was severely reduced and translated into large shortage rates. The later were higher for group O and Rh-negative RBCs as compared to group A and Rh-positive, respectively. CONCLUSION: Reductions of MSL to 28-35 days seem feasible and riskless and do not require major changes in the inventory management policies. Consequently, and giving preponderance to the precautionary principle, the Catalan Blood Agency has decided to reduce the MSL of RBCs from 42 to 35 days.

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 (BARC) 2014 Jun 18. QUARTILE 2, DECILE 5, IMPACT FACTOR 1.399

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 2014 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
Angeles Rico Blázquez

RESEARCH PROJECTS

Principal investigator: Sílvia Sauleda Oliveras

Prevalence of Hepatitis E markers (anti-IgG/IgM and HEV RNA) in Catalonian blood donors
Funding organisation: Novartis
Duration: 2013 to 2014

Principal investigator: Maria Piron

Development of real time protocols for PCRs (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 2014

Principal investigator: Marta Bes Maijo

Association between the haplotypes of the rs12979860 polymorphism of the IL-28B gene and the occult hepatitis B infection
Funding organisation: BST
Duration: 2013 to 2014

Principal investigator: Maria Piron

Cellular immune response to Trypanosoma cruzi: validation of IFN-gamma/IL10 ELISpot as a diagnostic tool in Chagas Disease
Funding organisation: BST
Duration: 2014

Principal investigator: Walter Melchior (Roche), Sílvia Sauleda Oliveras(BST)

PERFORMANCE EVALUATION STUDY Elecsys® HTLV I/II on Elecsys / cobas e
Funding organisation: Roche
File N°: CIM RD001837 / A13P004
Duration: 2014

Principal investigator: Walter Melchior (Roche), Maria Piron (BST)

PILOT STUDY Elecsys® CHAGAS on Elecsys / cobas e
Funding organisation: Roche
File N°: CIM RD001836 / A13P002
Duration: 2014

Principal investigator: Juan Ignacio Esteban Mur (Hospital Vall d'Hebron), Marta Bes Maijo (BST)

CD4 NS3 specific autologous cells functional restoration/expansion to prevent the recurrence of HCV after liver transplantation: optimization of the process for a clinical use
Funding organisation: Carlos III Health Institute
File N°: PI10/01505
Duration: 2011 to 2014

Principal investigator: Michael Busch (BSRI), Sílvia Sauleda Oliveras (BST)

External Quality Assurance Program Oversight Laboratory. Global Surveillance of HIV Diversity and Evaluation of Test Performance Using Viral Panels Derived from Recently Infected Blood Donors
Funding organisation: Blood Systems Research Institute
Duration: 2012 to 2014

Principal investigator: Joaquim Gascón (Hospital Clínic), Maria Piron (BST)
Population pharmacokinetic study of benznidazole in adult patients with Chagas disease. Relation between the benznidazole pharmacokinetics and adverse events
Funding organisation: Fundación Mundo Sano
Duration: 2013 to 2015

Principal investigator: Juan Ignacio Esteban Mur (Hospital Vall d'Hebron), Sílvia Sauleda Oliveras (BST)
Prospective Sample Collection – Evaluation of novel markers for early detection of Hepatocellular Carcinoma
Funding organisation: Roche
Duration: 2014 to 2016

PUBLICATIONS

Sauleda S, Ong E, **Bes M**, Janssen A, Cory R, Babizki M, Shin T, Lindquist A, Hoang A, Vang L, **Piron M, Casamitjana N**, Koppelman M, Danzig L, Linnen JM. Seroprevalence of hepatitis E virus (HEV) and detection of HEV RNA with a transcription-mediated amplification assay in blood donors from Catalonia (Spain). TRANSFUSION 2014 Nov 18. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.526

BACKGROUND: Hepatitis E virus (HEV) is an emerging threat to the safety of blood transfusion. The aim of this study was to determine HEV immunoglobulin (Ig)G and RNA prevalence in Catalan blood donors. STUDY DESIGN AND METHODS: Nearly 10,000 samples were collected from anonymized, unpaid donors at the Banc de Sang i Teixits (Barcelona, Spain) from June to December 2013. For the serology study, a subset of 1082 donations was tested in parallel for HEV IgG using Wantai and Mikrogen enzyme-linked immunosorbent assay tests. Samples were tested individually (individual-donation nucleic acid test [ID-NAT]) for HEV RNA using the Procleix HEV assay (95% limit of detection 7.9 IU/mL). Procleix repeat-reactive donations were confirmed by an in-house real-time polymerase chain reaction (PCR) test. RESULTS: The prevalences of IgG anti-HEV in Catalan blood donors were 19.96% (Wantai assay) and 10.72% (Mikrogen assay). Screening of 9998 samples with the Procleix HEV assay yielded three real-time PCR-confirmed and IgM and IgG anti-HEV-positive donations with viral loads of 250, 564, and 2755 IU/mL. The donation with highest viral load was genotype 3f. HEV RNA positivity rate was one per 3333 donations (0.03%; 95% confidence interval, 0.01%-0.09%). CONCLUSION: The Procleix HEV ID-NAT screening system has provided evidence of HEV RNA presence in Catalan blood donors. Further data are needed to assess the impact of HEV infection in at-risk patients to design the best strategy to increase blood safety.

de Mendoza C, Caballero E, Aguilera A, **Pirón M**, Ortiz de Lejarazu R, Rodríguez C, Cabezas T, González R, Treviño A, Soriano V. HIV-2 and HTLV-1 Infections in Spain, a Non-Endemic Region. AIDS REV. 2014 Jul-Sep;16(3):152-9. QUARTILE 1, DECILE 2, IMPACT FACTOR 4.075

The annual workshop of the Spanish HIV‐2/HTLV Study Group was held at the Instituto de Salud Carlos III in Madrid on December 11, 2013. Nearly 100 experts and researchers in retroviruses other than HIV‐1, the classical AIDS agent, convened for a one‐day meeting devoted to updating knowledge on the epidemiology of HIV‐2 and HTLV-1 infections and discussing new diagnostic and therapeutic strategies, with special attention to non‐endemic regions such as Spain. The Group was funded 25 years ago and since then has been responsible for the national registry of cases, recording all relevant information for each subject and inviting them to enroll in a prospective cohort and biobank. Up to the end of 2013, a total of 297 individuals with HIV‐2 infection were reported in Spain. All but 10 carry HIV‐2 subtype A, with the rest being infected with subtype B. Overall, 71% came from sub‐Saharan Africa. During the last decade, the incidence of new HIV‐2 infections in Spain has remained fairly stable with around 20 cases per year. At the time of diagnosis, plasma HIV‐2 RNA was undetectable in 61% of

individuals and values in viremic subjects tended to be low (2.8 logs on average). To date, only 26% of HIV- 2 individuals have been treated with antiretrovirals. The CD4 counts, however, only increased above 200 cells/mm³ in 42% of them. On the other hand, 74% of non- treated HIV- 2 individuals have > 500 CD4+ T- cells/mm³. As in HIV- 1 infection, X4 tropism in HIV- 2 is associated with lower CD4 counts. A total of 253 individuals with HTLV-1 infection were reported in Spain by the end of 2013. Overall, 58% came from Latin America. HTLV-1- associated myelopathy was diagnosed in 29 patients and adult T- cell leukemia/lymphoma in 18. The highest incidence occurred in 2013, with 34 new HTLV-1 diagnoses, largely as result of expanding HTLV screening in blood banks. Attempts to reduce HTLV-1 proviral load in symptomatic or asymptomatic patients with elevated HTLV-1 DNA using antiretrovirals have produced poor results, although integrase inhibitors could be more successful. Although no cases of HTLV- 3 or - 4 have been identified so far in Spain, 769 individuals have been diagnosed with HTLV- 2 infection. Up to 85% of the latest cases are coinfected with HIV- 1 and are former intravenous drug users.

Quer J, Gregori J, Rodríguez-Frias F, Buti M, Madejon A, Perez-Del-Pulgar S, Garcia-Cehic D, Casillas R, Blasi M, Homs M, Tabernero D, Alvarez-Tejado M, Muñoz JM, Cubero M, Caballero A, delCampo JA, Domingo E, Belmonte I, Nieto L, Lens S, Muñoz-de-Rueda P, Sanz-Cameno P, **Sauleda S, Bes M**, Gomez J, Briones C, Perales C, Sheldon J, Castells L, Viladomiu L, Salmeron J, Ruiz-Extremera A, Quiles-Pérez R, Moreno-Otero R, López-Rodríguez R, Allende H, Romero-Gómez M, Guardia J, Esteban R, Garcia-Samaniego J, Forns X, Esteban JI. High-resolution Hepatitis C virus (HCV) subtyping, using NS5B deep sequencing and phylogeny, an alternative to current methods. J CLIN MICROBIOL. 2014 Nov 5. QUARTILE 1, DECILE 3, IMPACT FACTOR 4.068

BACKGROUND: Hepatitis C virus (HCV) is classified into seven major genotypes and 67 subtypes. Recent studies have shown that in HCV genotype 1-infected patients, response rates to regimens containing direct acting antivirals (DAAs) are subtype-dependent. Currently available genotyping methods have limited subtyping accuracy. METHODOLOGY: We have evaluated the performance of a deep sequencing based HCV subtyping assay, developed for the 454/GS-Junior platform, in comparison with those of two commercial assays (Versant HCV genotype 2.0 and Abbott Real-time HCV genotype II) and using direct NS5B sequencing as a gold standard (direct sequencing), in 114 clinical specimens previously tested by first-generation hybridization assay (82 genotype 1 and 32 with uninterpretable results). RESULTS: Phylogenetic analysis of deep sequencing reads matched subtype 1 calling by population Sanger sequencing (69% 1b, 31% 1a) in 81 specimens and identified a mixed subtype infection (1b/3a/1a) in one sample. Similarly, among the 32 previously indeterminate specimens identical genotype and subtype results were obtained by direct and deep sequencing in all but four samples with dual infection. In contrast, both Versant HCV Genotype 2.0 and Abbott real-time HCV genotype II, failed subtype 1 calling in 13 (16%) samples each, and were unable to identify the HCV genotype and/or subtype in more than half of non-genotype 1 samples. CONCLUSIONS: Deep sequencing is more efficient for HCV subtyping than currently available methods and allows qualitative identification of mixed infections, and may be more helpful to inform treatment strategies with new DAA-containing regimens, across all HCV subtypes.

Campos-Varela I, Esteban JI, **Bes M,** Caralt M, Allende H, Rodríguez-Frías F, Salcedo MT, **Sauleda S**, Charco R, Guardia J, Esteban R, Castells L. Early predictors of antiviral treatment response in liver transplant recipients with recurrent hepatitis C genotype 1. J VIRAL HEPAT 2014 Mar 12. QUARTILE 2, DECILE 3, IMPACT FACTOR 3.082

The success of current antiviral treatment for hepatitis C virus (HCV) recurrence in liver transplant (LT) recipients remains limited. We aimed at evaluating the value of IL28B genotype and early viral kinetics to predict response to standard treatment in the transplant setting. We retrospectively evaluated 104 LT recipients treated for HCV genotype 1 recurrence between 2001 and 2010. Baseline variables, including IL28B

genotype, and early viral kinetics were compared among patients who did or did not achieve a sustained virological response (SVR). Logistic regression analyses of candidate variables were conducted to generate a reliable predictive model based on the minimum set of variables. Twenty-nine (28%) achieved an SVR. On multivariate analysis, the magnitude of HCV RNA decline at 4 weeks (OR: 3.74, 95% CI: 1.64-9.39; P = 0.003) and treatment compliance (OR: 35.27, 95% CI: 3.35-365.54; P = 0.003) were the only independent predictors of SVR. Favourable recipient IL28B genotype significantly correlates with virological response at week 4 (OR 3.23; 95% CI, 1.12-9.15; P = 0.03). By logistic regression analysis, a model including donor age, recipient rs12979860 genotype and viral load at 4 weeks showed the best predictive value for SVR with an area under the receiver operating curve of 0.861. Favourable recipient IL28B genotype strongly correlates with the viral response at week 4 which is the strongest predictor of response. The combination of recipient IL28B genotype and donor age with the week 4 response reliably estimates the probability of SVR early on-treatment and may facilitate therapeutic strategies incorporating new antiviral agents.

Lieberman L, Devine DV, Reesink HW, Panzer S, Wong J, Raison T, Benson S, Pink J, Leitner GC, Horvath M, Compernolle V, Scuracchio PS, Wendel S, Delage G, Nahirniak S, Dongfu X, Krusius T, Juvonen E, Sainio S, Cazenave JP, Guntz P, Kientz D, Andreu G, Morel P, Seifried E, Hourfar K, Lin CK, O'Riordan J, Raspollini E, Villa S, Rebulla P, Flanagan P, Teo D, Lam S, Ang AL, Lozano M, **Sauleda S**, Cid J, Perreira A, Ekermo B, Niederhauser C, Waldvogel S, Fontana S, Desborough MJ, Pawson R, Li M, Kamel H, Busch M, Qu L, Triulzi D. Prevention of transfusion-transmitted cytomegalovirus (CMV) infection: Standards of care. VOX SANG 2014 May 7. QUARTILE 2, DECILE 5, IMPACT FACTOR 2.847

Campos-Varela I, Esteban JI, **Bes M**, Dopazo C, Allende H, Rodríguez-Frías F, Salcedo MT, **Sauleda S**, Charco R, Guardia J, Esteban R, Castells L. Outcome of early vs. deferred antiviral treatment for recurrent hepatitis C in liver transplant recipients. ANN HEPATOL 2014 Mar-Apr;13(2):219-30. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.671

The optimal timing to treat recurrent hepatitis-C virus (HCV) after liver transplantation (LT) remains uncertain. We compared the outcome of early (acute phase) and deferred (chronic phase) antiviral treatment for recurrent HCV infection in this population. Consecutive HCV genotype-1 infected LT patients receiving antiviral therapy between 2001-2010 were retrospectively classified according to histology at treatment start into the early or deferred treatment group. Measured endpoints included sustained virological response (SVR) rates and long-term survival. The study cohort comprised 105 patients: 60 (57%) received early treatment (ET) and 45 (43%) deferred treatment (DT). The median interval from LT to antiviral start was 3 (1-9) and 18 months (11-74) in ET and DT respectively. The SVR rate was similar in both treatment groups (23% ET and 36% DT; p = 0.27). After a median follow-up of 5.8 years, all-cause and liver-related mortality were similar in both groups. Variables independently associated with mortality included pre-treatment bilirubin > 2 mg/dL (HR 6.1, 95%CI: 2.8-13.7; p < 0.001), donor age > 60 (HR 3.1, 95%CI: 1.4-6.7; p = 0.01), and failure to achieve SVR (HR 10.3, 95%CI: 1.3-18.3; p = 0.03). In conclusion, early treatment of recurrent HCV is safe, but does not lead to higher SVR rates. In HCV-infected LT recipients, elevated bilirubin, older donor age, and failure to achieve SVR are independently associated with increased mortality.

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
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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 2014

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 2014

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 2014

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

PUBLICATIONS

Contreras E, de la Rubia J, Del Río-Garma J, Díaz-Ricart M, García-Gala JM, Lozano M; por el Grupo Español de Aféresis. Diagnostic and therapeutic guidelines of thrombotic microangiopathies of the Spanish Apheresis Group. MED CLIN (BARC). 2014 Nov 27. QUARTILE 2, DECILE 5, IMPACT FACTOR 1.399

Thrombotic microangiopathies (TMA) are disorders defined by the presence of a microangiopathic hemolytic anemia (with the characteristic hallmark of schistocytes in the peripheral blood smear), thrombocytopenia and organ malfunction of variable intensity. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome are the most important forms of TMA and, without the adequate treatment, they are associated with high morbimortality. In recent years, significant advances in the knowledge of the pathophysiology of TMA have occurred. Those advances have allowed us to move from a syndromic diagnosis with a similar treatment to all entities to the search of etiologic diagnosis which would lead to a specific treatment, finally leading to a better outcome of the patient. This document pretends to summarize the current status of knowledge of the pathophysiology of TMA and the therapeutic options available, and to offer a diagnostic and therapeutic practical tool to the professionals caring for the patients.

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 2014

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

Muñiz-Diaz E, Puig L. Allergic and anaphylactic reactions to methylene-blue-treated plasma in Catalonia in the period 2008-2013. BLOOD TRANSFUS 2014 Oct;12(4):628-30. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.858

Gómez-Torreiro E, Eiras-Martínez A, Rodríguez-Calvo MI, **Muñiz-Díaz E, Nogués N**, López M, Garaizar A, Ochoa-Garay G. Rh-null phenotype caused by a complete RHAG deletion. TRANSFUSION 2014 Jul 29. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.526

Trucco Boggione C, Luján Brajovich ME, **Tarragó M**, Mattaloni SM, Biondi CS, **Muñiz-Díaz E, Nogués N**, Cotorruelo CM. Molecular structures identified in serologically D- samples of an admixed population. TRANSFUSION 2014 May 12. QUARTILE 2, DECILE 4, IMPACT FACTOR 3.526

BACKGROUND: The D- phenotype is mainly caused by the complete deletion of the RHD gene in Caucasians. However, a plethora of allelic variants have been described among D- individuals from different ethnic groups. STUDY DESIGN AND METHODS: A cohort of 1314 routine serologically D- samples from white Argentineans was studied by molecular methods. RESULTS: Among the 1314 D- samples, 2.1% showed RHD-specific amplifications. One hybrid Rhesus box was detected in all D-/RHD+ samples, suggesting a hemizygous status. The RHDΨ was found in 0.7% of rr samples while DEL and null variants were detected in 16.7% of the D- samples expressing C and/or E antigens. The variants associated with the C antigen were seven RHD-CE-Ds , two RHD(1-2)-CE(2-9)-D(10), two previously unreported RHD(329T>C)-CE(3-9)-D null alleles, one RHD(M295I), and one new RHCE(1-2)-RHD(3361del11 -10) null allele whereas those associated with the E antigen were five RHD(46T>C) and one novel RHD(581insG) null allele responsible for a premature stop codon. CONCLUSIONS: The prevalence of D-/RHD+ samples is higher than that observed in Europeans. More than 50% of the RHD alleles found were represented by RHDψ and RHD-CE-Ds showing the African contribution to the genetic pool of the admixed population analyzed. Interestingly, three new alleles were found, two of them being hybrid structures between previously described RHD variants recombined with RHCE sequences. The knowledge of the RHD allele repertoire in our population allowed the implementation of reliable typing and transfusion strategies for a better management of patients and pregnant women.

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
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INVESTIGATORS
Nina Borràs Agustí
Irene Corrales Insa
Lluis Martorell Cedrés
Rafael Parra López

TECHNICAL STAFF
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 2014

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

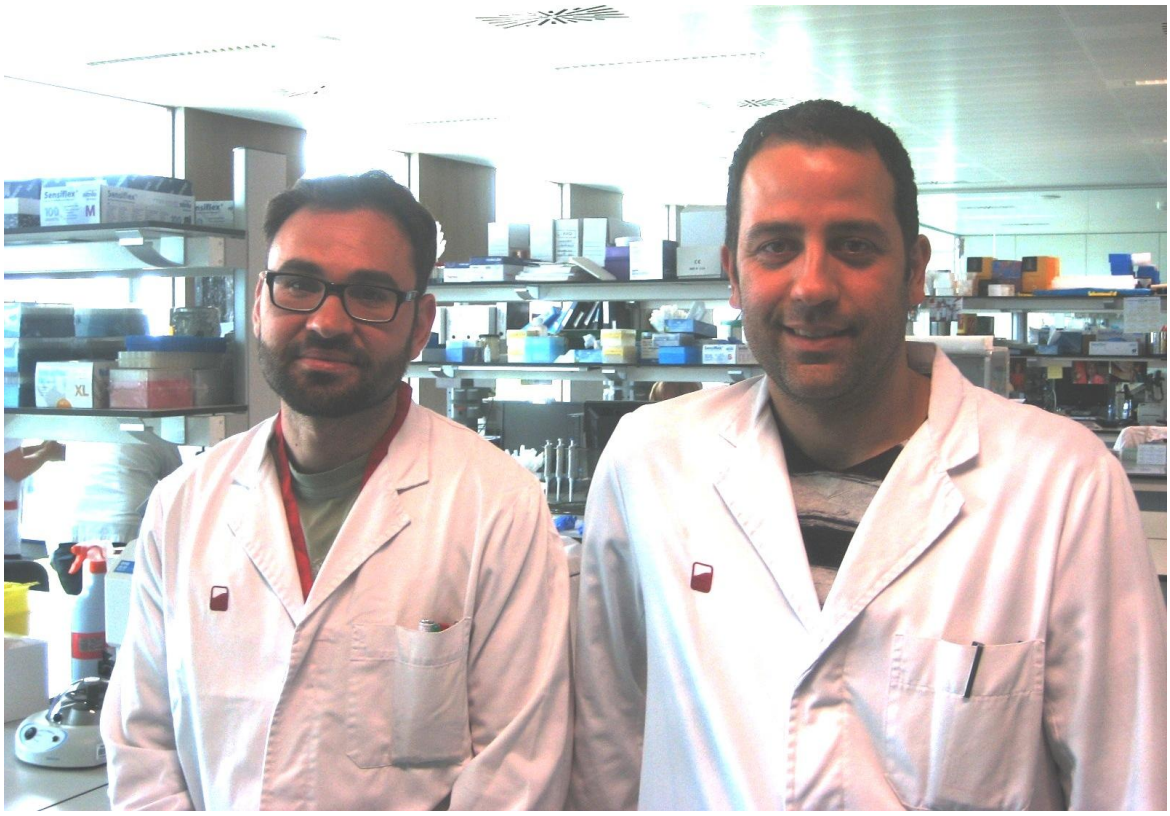
Principal investigator: Rafael Parra López
Non invasive prenatal diagnosis of hemophilia by massive sequencing
Funding organisation: Pfizer
File N°: WS2109762
Duration: 2012 to 2014

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

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: Luisa Ibáñez Mora (Hospital Vall d'Hebron), José Luís Caro Oleas (BST)

Genetic determinants of agranulocytosis associated with the use of drugs: A cases and controls study
Funding organisation: Carlos III Institute of Health
File N°: PI10/02632
Duration: 2012 to 2014

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

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
File N°: 20133230
Duration: 2014 to 2017

Principal investigator: Sergi Querol Giner
Biomarkers of Stem Cell Circulating in Plasma of Cord Blood
Funding organisation: BST, Anthony Nolan Trust and Nottingham Trent University
Duration: 2009 to 2014

Principal investigator: Sergi Querol Giner
Identification of units from the umbilical cord national plan with homozygous CCR5-Δ32 variant
Funding organisation: ONT
Duration: 2014 to 2015

Principal investigator: Sergi Querol Giner, Nerea Castillo Flores
Clinical evaluation of the defrost method of umbilical cord blood previous to the infusion of stem cells and impact on clinical results
Funding organisation: BST
Duration: 2012 to 2014

Principal investigator: Carmen Azqueta Molluna
Implementation, validation and clinical evaluation of a functional cytometry method to predict the clonogenic potency of hematopoietic stem cells based on the determination of apoptopic cells with annexin
Funding organisation: BST
Duration: 2012 to 2014

Principal investigator: Sergi Querol Giner, Dinara Samarkanova
Immunological properties of Platelet Rich Plasma from Cord Blood. Process validation for clinical use
Funding organisation: BST
Duration: 2014

Principal investigator: Marta Torrabadella de Reynoso
Evaluation of the utility of the umbilical cord plasma eye-drops for the treatment of Corneal tropic wounds
Funding organisation: BST
Duration: 2012 to 2014

Principal investigator: Laura Medina Marrero
Proposal for a new consensus for plerixafor administration to decrease the failure rate of mobilization of hematopoietic progenitors
Funding organisation: BST
Duration: 2014 to 2017

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 2015

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°: 2014-000074-19
Duration: 2014 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 2015

Principal investigator: Renato Cunha (Faculty of Medicine of Ribeirâo Preto of Sâo Paulo University, Sergi Querol Giner (BST)
Prognostic association of genetic polymorphisms of drug metabolism and innate immune response on Umbilical Cord Blood Transplantation (UCBT) outcomes
Funding organisation: Institut National de la Santé et la Recherche Médicale, Sâo Paulo State Research Foundation, Brazil
Duration: 2012 to 2014

Principal investigator: Lawrence D. Petz (Stemcyte, International Blood Center), Sergi Querol Giner (BST)
Developing the special inventory of homozygous CCR5 delta32 cord blood units.
Unrelated Cord blood transplantation for patients with advanced AIDS using Δ32CCR5 / Δ32CCR5 single unit or Δ32CCR5 / Δ32CCR5 and CCR5Δ32/CCR5 double cord units.
Funding organisation: Stemcyte, International Blood Center
Duration: 2012 to 2014

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 2014

PUBLICATIONS

Castillo N, García-Cadenas I, García O, Barba P, Heredia CD, 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 2014 Dec 27. QUARTILE 1, DECILE 2, IMPACT FACTOR 3.940

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 × 107/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.

Ruggeri A, Labopin M, Sormani MP, Sanz G, Sanz J, Volt F, Michel G, Locatelli F, Diaz De Heredia C, O' Brien T, Arcese W, Iori AP, **Querol S**, Kogler G, Lecchi L, Pouthier F, Garnier F, Navarrete C, Baudoux E, Fernandes J, Kenzey C, Eapen M, Gluckman E, Rocha V, Saccardi R. Engraftment kinetics and graft failure after single umbilical cord blood transplantation using myeloablative conditioning regimen. HAEMATOLOGICA. 2014 Jun 27. QUARTILE 1, DECILE 2, IMPACT FACTOR 5.935

Umbilical cord blood transplantation recipients are exposed to an increased risk of graft failure, a complication leading to higher transplantation-related mortality. The decision and timing to offer a second transplant after graft failure is challenging. With the aim of addressing this issue, we analyzed engraftment kinetics and outcomes of 1268 patients (73% children) with acute leukemia (64% acute lymphoblastic leukemia, 36% acute myeloid leukemia) in remission who received single-unit umbilical cord blood transplantation after myeloablative conditioning regimen. Median follow-up was 31 months. Overall survival) at 3-year was 47%; 100-day cumulative incidence of transplant related mortality was 16%. Longer time to engraftment was associated with increased transplant related mortality and lower overall survival. Cumulative incidence of neutrophil engraftment at day-60 was 86%, median time 24 days. Probability density analysis showed that the likelihood of engraftment after umbilical cord blood transplantation increased after day+10, peaked on day+21 and slowly decreased to 21% on day+31. Beyond day+31, the probability of engraftment dropped rapidly, and the residual probability to engraft after day+42 was 5%. Graft failure was reported in 166 patients, and 66 of them received a second graft (allogeneic, n=45). Rescue actions, such as the search for another graft, should be considered starting after day+21; diagnosis of Graft failure can be established for patients not achieving neutrophil recovery by day+42. Moreover, subsequent transplants should not be postponed after day+42.

Luevano M, Domogala A, Blundell M, Jackson N, Pedroza-Pacheco I, Derniame S, Escobedo-Cousin M, **Querol S**, Thrasher A, Madrigal A, Saudemont A. Frozen cord blood hematopoietic stem cells differentiate into higher numbers of functional natural killer cells in vitro than mobilized hematopoietic stem cells or freshly isolated cord blood hematopoietic stem cells. PLOS ONE 2014 Jan 29;9(1):e87086. QUARTILE 1, DECILE 2, IMPACT FACTOR 3.730

Adoptive natural killer (NK) cell therapy relies on the acquisition of large numbers of NK cells that are cytotoxic but not exhausted. NK cell differentiation from hematopoietic stem cells (HSC) has become an alluring option for NK cell therapy, with umbilical cord blood (UCB) and mobilized peripheral blood (PBCD34(+)) being the most accessible HSC sources as collection procedures are less invasive. In this study we compared the capacity of frozen or freshly isolated UCB hematopoietic stem cells (CBCD34(+)) and frozen PBCD34(+) to generate NK cells in vitro. By modifying a previously published protocol, we showed that frozen CBCD34(+) cultures generated higher NK cell numbers without loss of function compared to fresh CBCD34(+) cultures. NK cells generated from CBCD34(+) and PBCD34(+) expressed low levels of killer-cell immunoglobulin-like receptors but high levels of activating receptors and of the myeloid marker CD33. However, blocking studies showed that CD33 expression did not impact on the functions of the generated cells. CBCD34(+)-NK cells exhibited increased capacity to secrete IFN-γ and kill K562 in vitro and in vivo as compared to PBCD34(+)-NK cells. Moreover, K562 killing by the generated NK cells could be further enhanced by IL-12 stimulation. Our data indicate that the use of frozen CBCD34(+) for the production of NK cells in vitro results in higher cell numbers than PBCD34(+), without jeopardizing their functionality, rendering them suitable for NK cell immunotherapy. The results presented here provide an optimal strategy to generate NK cells in vitro for immunotherapy that exhibit enhanced effector function when compared to alternate sources of HSC.

Sánchez-Ortega I, **Querol S**, Encuentra M, **Ortega S, Serra A, Sanchez-Villegas JM**, **Grifols J**, **Pujol-Balaguer M, Pujol-Bosch M**, Martí J, Garcia-Cerecedo T, Barba P, Sancho J, Esquirol A, Sierra J, Duarte R. Plerixafor in patients with lymphoma and multiple myeloma: effectiveness in cases with very low circulating CD34+ levels and preemptive intervention versus remobilization. BONE MARROW TRANSPLANTATION 2014 Sep 15. QUARTILE 2, DECILE 3, IMPACT FACTOR 3.541

This retrospective study presents data from 105 consecutive multiple myeloma and lymphoma patients who had PB CD34+ cell counts <10/μL on day 4 of steady-state G-CSF mobilization for autologous hematopoietic cell transplantation. Our results confirm the capacity of plerixafor to improve mobilization outcomes in this clinical setting. In addition, they show that the effectiveness of plerixafor, compared with G-CSF only, translates to patients with very low (<3.5/μL) circulating CD34+ cell counts: overnight CD34+ cell count expansion (5.3- vs 1.7-fold), overall CD34+ cell yield (2.29 vs 0.15 × 106 CD34+ cells per kg) and patients yielding greater than or equal to2 × 106 CD34+ cells per kg (63% vs 3%). Furthermore, our data also show that preemptive plerixafor is significantly more effective and more efficient than in remobilization: CD34+ cell yield in the first apheresis (3.28 vs 2.0 × 106 CD34+ cells per kg) and overall (3.73 vs 2.44 × 106 CD34+ cells per kg), patients yielding greater than or equal to2 × 106 CD34+ cells per kg in the first apheresis (85% vs 44%) and overall (92% vs 64%), all this requiring less days and doses of plerixafor treatment (1.08 vs 1.48). These data would advocate using plerixafor as an early preemptive intervention based on day 4 circulating CD34+ counts, including very high-risk patients with very low circulating levels.

2.3 REPARATIVE & IMMUNOMODULATORY THERAPY

2.3.1 Program 8: Substitutive & reparative therapy & Program 9: Large-scale production of cells & tissues

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.

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RESEARCH PROJECTS

Principal investigator: Joan Garcia Lopez
REDONTAP-Continuous Proliferation & Simultaneous Maturation of Haematopoietic Stem Cells into Blood Cell Lineages
Funding organisation: European Commission
File N°: 229328
Duration: 2012 to 2014

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 2015

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: 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 2014

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 2014

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 2014

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 2014

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 2014

Principal investigator: Marius Aguirre Canyadell (Hospital Vall d'Hebron), Joaquim Vives Armengol i Marta Caminal Bobet (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 2014

Investigador principal: Marius Aguirre Canyadell (Hospital Vall d'Hebron), Arnau Pla Calvet (BST)
Femoral head osteonecrosis treatment with advanced cell therapy and biomaterials in an experimental sheep animal model
Funding organisation: Fundació la Marató de TV3
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: Fundació la Marató de TV3
File N°: 122831
Duration: 2013 to 2015

Principal investigator: Francisco 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: 2014 to 2017

PUBLICATIONS

Caminal M, Moll X, Codina R, Rabanal M, Morist A, Barrachina J, **García J, Pla A, Vives J.** Transitory improvement of articular cartilage characteristics after implantation of polylactide: polyglycolic acid (PLGA) scaffolds seeded with autologous mesenchymal stromal cells in a sheep model of critical-sized chondral defect. BIOTECHNOL LETT 2014 Jun 26. QUARTILE 3, DECILE 6, IMPACT FACTOR 1.853

Clinical translation of emerging technologies aiming at cartilage resurfacing is hindered by neither the appropriate scaffold design nor the optimal cell source having been defined. Here, critical-sized, chondral-only focal defects were created in sheep and treated with clinical-grade, co-polymeric poly-lactide: polyglycolic acid scaffolds either alone or seeded with 3.3 9 106 ± 0.4 9 106 autologous bone marrow- derived mesenchymal stromal cells and studied over 12 month follow-up. An untreated group was included for comparison. Second-look arthroscopy performed at 4 months post-treatment evidenced the generation of neocartilage of better quality in those defects treated with cells. However, macroscopic scores in the cell-treated group declined significantly from 7.5 ± 2.3 at 4 months to 3.1 ± 2.6 ($p = 0.0098$) at 12 months post-treatment, whereas the other two experimental groups remained unaltered during 4–12 month post-treatment. The effectiveness of the cell-based approach proposed in this study is thus restricted to between months 1 and 4 post-treatment.

Caminal M, Fonseca C, Peris D, Moll X, Rabanal RM, Barrachina J, Codina D, García F, Cairó JJ, Gòdia F, **Pla A, Vives J**. Use of a chronic model of articular cartilage and meniscal injury for the assessment of long-term effects after autologous mesenchymal stromal cell treatment in sheep. N BIOTECHNOL 2014 Sep 25;31(5):492-8. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.706

Regenerative therapies using adult stem cells have attracted great interest in the recent years and offer a promising alternative to current surgical practices. In this report, we evaluated the safety and efficacy of an autologous cell-based treatment of osteoarthritis using mesenchymal stromal cells expanded from bone marrow aspirates that were administered intra-articularly. Ten 2-year old ewes were divided in two groups (for analysis at 6 and 12 months, respectively). Full thickness articular cartilage defects of approximately 60mm(2) were created arthroscopically in the medial femorotibial condyles and a meniscal tear in the anterior horn of the medial meniscus in the 20 hind legs. Intra-articular injection of 4mL of either treatment (a suspension of cells) or control (same as treatment, without cells) were applied one month after generating a chronic condition similar to human pathology. Animals were monitored radiographically, by MRI and ultrasound scanning; and macroscopic and histological analyses were conducted at 6 and 12 months. Furthermore a full necropsy was performed at 12 months post-treatment. The intra-articular injection of autologous MSC was safe, as judged by the lack of local or systemic adverse effects during the clinical follow-up and by a full necropsy performed at 12 months post-treatment. Evidence of regeneration of articular cartilage and meniscus was case-dependent but statistically significant improvement was found in specific macroscopic and histological parameters. Such parameters included colour, rigidity, cell distribution and hyaline quality of the refill tissue as well as the structure of subchondral bone.

2.3.2 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: 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: 2014 to 2015

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

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. 2014 Aug 25:1-12. QUARTILE 2, DECILE 5, IMPACT FACTOR 1.710

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.

Casaroli-Marano R, Gallego-Pinazo R, Fernández-Blanco CT, Figueroa MS, Pina Marín B, Fernández-Baca Vaca G, Piñero-Bustamante A, Donate López J, García-Arumí J, Farrés Martí J. Age-Related Macular Degeneration: Clinical Findings following Treatment with Antiangiogenic Drugs. J OPHTHALMOL 2014;2014:346360. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.368

PURPOSE: To survey the management of patients with neovascular age-related macular degeneration (nvAMD) in Spain. METHODS: An observational retrospective multicenter study was conducted. The variables analyzed were sociodemographic characteristics, foveal and macular thickness, visual acuity (VA), type of treatment, number of injections, and the initial administration of a loading dose of an antiangiogenic drug. RESULTS: 208 patients were followed up during 23.4 months in average. During the first and second years, patients received a mean of 4.5 ± 1.8 and 1.6 ± 2.1 injections of antiangiogenic drugs, and 5.4 ± 2.8 and 3.6 ± 2.2 follow-up visits were performed, respectively. The

highest improvement in VA was observed at 3 months of follow-up, followed by a decrease in the response that stabilized above baseline values until the end of the study. Patients who received an initial loading dose presented greater VA gains than those without. CONCLUSIONS: Our results suggest the need for a more standardized approach in the management and diagnosis of nvAMD receiving VEGF inhibitors. To achieve the visual outcomes reported in pivotal trials, an early diagnosis, proactive approach (more treating than follow-up visits), and a close monitoring might be the key to successfully manage nvAMD.

Casaroli-Marano RP, Alforja S, Giralt J, Farah ME. Epimacular brachytherapy for wet AMD: current perspectives. CLIN OPHTHALMOL 2014 Aug 30;8:1661-70.

Age-related macular degeneration (AMD) is considered the most common cause of blindness in the over-60 age group in developed countries. There are basically two forms of presentation: geographic (dry or atrophic) and wet (neovascular or exudative). Geographic atrophy accounts for approximately 85%-90% of ophthalmic frames and leads to a progressive degeneration of the retinal pigment epithelium and the photoreceptors. Wet AMD causes the highest percentage of central vision loss secondary to disease. This neovascular form involves an angiogenic process in which newly formed choroidal vessels invade the macular area. Today, intravitreal anti-angiogenic drugs attempt to block the angiogenic events and represent a major advance in the treatment of wet AMD. Currently, combination therapy for wet AMD includes different forms of radiation delivery. Epimacular brachytherapy (EMBT) seems to be a useful approach to be associated with current anti-vascular endothelial growth factor agents, presenting an acceptable efficacy and safety profile. However, at the present stage of research, the results of the clinical trials carried out to date are insufficient to justify extending routine use of EMBT for the treatment of wet AMD.

Casaroli-Marano RP, Tabera J, Vilarrodona A, Trias E. Regulatory issues in cell-based therapy for clinical purposes. DEV OPHTHALMOL. 2014;53:189-200.

Rapid development in the fields of cellular and molecular biology, biotechnology, and bioengineering medicine has brought new, highly innovative treatments and medicinal products, some of which contain viable cells and tissues associated with scaffolds and devices. These new cell-based therapy approaches in regenerative medicine have great potential for use in the treatment of a number of diseases that at present cannot be managed effectively. Given the unique challenges associated with the development of human cell-based medicinal products, great care is required in the development of procedures, practices, and regulation. In cell therapy, appropriate methodologies in the areas of production, reproducibility, maintenance, and delivery are essential for accurate definition and reliable assurance of the suitability and quality of the final products. Recently, the official European Community agencies (EMA) and the relevant authority in the USA (FDA) have made significant efforts to establish regulatory guidance for use in the application of the cell-based therapies for human patients. The guidelines surrounding cell-based therapy take into account the current legislation, but focus less on the heterogeneity and requirements of individual human cell-based products, including specific combination products and applications. When considering guidelines and regulation, a risk assessment approach is an effective method of identifying priority areas for the development of human cell-based medicinal products. Additionally, effective design and thorough validation of the manufacturing process in line with existing Good Manufacturing Practices (GMPs) and quality control regimes and a program that ensures the traceability and biovigilance of the final products are also all essential elements to consider.

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 2014 Oct 6:1-10. QUARTILE 2, DECILE 5, IMPACT FACTOR 1.710

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 ≥102 and 103 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.

Pinazo-Durán MD, Gómez-Ulla F, Arias L, Araiz J, **Casaroli-Marano R**, Gallego-Pinazo R, García-Medina JJ, López-Gálvez MI, Manzanas L, Salas A, Zapata M, Diaz-Llopis M, García-Layana A. Do nutritional supplements have a role in age macular degeneration prevention? J OPHTHALMOL 2014;2014:901686. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.368

PURPOSE: To review the proposed pathogenic mechanisms of age macular degeneration (AMD), as well as the role of antioxidants (AOX) and omega-3 fatty acids (ω -3) supplements in AMD prevention. MATERIALS AND METHODS: Current knowledge on the cellular/molecular mechanisms of AMD and the epidemiologic/experimental studies on the effects of AOX and ω -3 were addressed all together with the scientific evidence and the personal opinion of professionals involved in the Retina Group of the OFTARED (Spain). RESULTS: High dietary intakes of ω -3 and macular pigments lutein/zeaxanthin are associated with lower risk of prevalence and incidence in AMD. The Age-Related Eye Disease study (AREDS) showed a beneficial effect of high doses of vitamins C, E, beta-carotene, and zinc/copper in reducing the rate of progression to advanced AMD in patients with intermediate AMD or with one-sided late AMD. The AREDS-2 study has shown that lutein and zeaxanthin may substitute beta-carotene because of its potential relationship with increased lung cancer incidence. CONCLUSION: Research has proved that elder people with poor diets, especially with low AOX and ω -3 micronutrients intake and subsequently having low plasmatic levels, are more prone to developing AMD. Micronutrient supplementation enhances antioxidant defense and healthy eyes and might prevent/retard/modify AMD.

van Wijk MJ, Beele H, Brubaker SA, **Navarro A**, Wulff B, Warwick RM. Report of the clinical donor case workshop of the European Association of Tissue Banks Annual Congress 2013. CELL TISSUE BANK 2014 Dec 17. QUARTILE 3, DECILE 6, IMPACT FACTOR 1.171

The European Association of Tissue Banks (EATB) Donor Case Workshop is a forum held within the program of the EATB Annual Congress. The workshop offers an opportunity to discuss and evaluate approaches taken to challenging donor selection and donation ethics, and it strengthens networking between tissue banking professionals. The workshops actively engage participants from a wide array of international expertise, in an informal, secure and enjoyable setting in which learning from peers and finding potential solutions for submitted cases are facilitated. This report reflects some of the discussion at the Donor Case Workshop during the EATB Annual Congress in Brussels in 2013. The

presented cases demonstrate that the findings, their interpretation, the resulting actions and preventive measures in the different tissue facilities are not always predictable. The varied responses from participants and lack of consensus corroborate this and clearly indicate that operating procedures do not comprehensively cover or prepare for all eventualities. For many of the issues raised there is no relevant information in the published literature. By publication of a summary of the discussions we hope to reach a wider audience, to provide information gathered at the workshop and to stimulate individuals and institutions to undertake further literature reviews or to undertake research in order to gather evidence concerning the discussed topics.

Pelegrín L1, **Casaroli-Marano R**, Antón J, García de Vicuña MC, Molina-Prat N, Ignacio Aróstegui J, Yagüe J, Ríos J, Adán A. Predictive value of selected biomarkers, polymorphisms, and clinical features for oligoarticular juvenile idiopathic arthritis-associated uveitis. OCUL IMMUNOL INFLAMM 2014 Jun;22(3):208-12. QUARTILE 3, DECILE 7, IMPACT FACTOR 1.082

PURPOSE: Uveitis is the most common extra-articular manifestation of juvenile idiopathic arthritis (JIA) and is associated with considerable morbidity. The aim of this study was to examine the risk factors associated with uveitis in oligoarticular JIA. METHODS: We conducted a chart review of 86 patients with oligoarticular JIA to assess if antinuclear antibody (ANA) status, gender, and age at JIA onset were associated with the development of uveitis. Biomarkers such as cytokine gene polymorphisms, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level were also assessed. RESULTS: Twenty-seven patients exhibited oligoarticular JIA-associated uveitis. Only the ESR at arthritis onset and the patient's age at arthritis onset were related to uveitis development in our patient sample. CONCLUSIONS: An age-associated risk of uveitis was observed in children younger than 3 years at the time of JIA onset. ESR values at arthritis onset higher than 22 mm/h were also related to uveitis development. KEYWORDS: Age of arthritis onset; chronic anterior uveitis; erythrocyte sedimentation rate; oligoarticular juvenile idiopathic arthritis; polymorphism

Zarbin MA, **Casaroli-Marano RP**, Rosenfeld PJ. Age-related macular degeneration: clinical findings, histopathology and imaging techniques. DEV OPHTHALMOL 2014;53:1-32.

Age-related macular degeneration (AMD) is the most common cause of blindness among people over age 55 years in industrialized countries. Known major risk factors for AMD include: age >55 years, history of smoking, white race, and mutations in various components of the complement system. Early AMD is characterized by the presence of drusen and pigmentary abnormalities. Late AMD is associated with central visual loss and is characterized by the presence of choroidal neovascularization and/or geographic atrophy. Early AMD is associated with a number of biochemical abnormalities including oxidative damage to retinal pigment epithelium (RPE) cells, complement deposition in the RPE-Bruch's membrane-choriocapillaris complex, lipidization of Bruch's membrane, and extracellular matrix abnormalities (e.g. collagen crosslinking, advanced glycation end product formation). Antiangiogenic drugs block the vascular leakage associated with choroidal new vessels, thus reducing retinal edema and stabilizing or restoring vision. At this time, there are no proven effective treatments for the nonexudative complications of AMD. Modern ocular imaging technologies (including spectral domain and phase variance optical coherence tomography, short- and long-wavelength fundus autofluorescence, adaptive optics-scanning laser ophthalmoscopy, and near-infrared reflectance) enable one to follow changes in the RPE, photoreceptors, and choriocapillaris quantitatively as the disease progresses. In addition, one can quantitatively assess the volume of drusen and areas of atrophy. These data, when correlated with the known histopathology of AMD, may provide useful measures of treatment efficacy that are likely to be more sensitive and reproducible than conventional end points such as visual acuity and rate of enlargement of geographic atrophy. As a result, these imaging technologies may be valuable in assessing the effects of cell-based therapy for patients with AMD.

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