Genetic predisposition of acute graft-versus-host disease after hematopoietic stem cell transplantation from an HLA-identical sibling donor

A retrospective and multicenter cohort study

FINAL DEGREE PROJECT

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"The good physician treats the disease; the great physician treats the patient who has the disease"

William Osler

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1. ABSTRACT

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a very used treatment for several hematological disorders, especially for acute leukemia. But this type of HSCT is associated with a high incidence of morbidity and mortality. The main complication of HSCT is the graft-versus-host disease (GVHD), an immunological disorder that affects many organs systems. The frequency of GVHD is directly related to the degree of mismatch between patient-donor HLA proteins. The ideal donor has to be HLA-identical with the patient to avoid GVHD. But 40% of recipients of HLA-identical grafts develop systemic GVHD. Thus, there are other disparities that can be associated with GVHD, such as hidden HLA disparities or minor histocompatibility antigens mismatches.

Objectives

To determine the incidence of acute GVHD and overall survival (OS) and disease-free survival (DFS) after allogeneic HSCT from an HLA-identical sibling donor with:

- HLA-DPB1 mismatch
- mHag HA-1 mismatch
- Male-specific HY-antigens mismatch

Methods

A longitudinal retrospective and multicenter cohort composed by 1377 patients receiving an allogeneic HSCT from an HLA-identical sibling donor between 1992 and 2014. All the data are already collected in the Biobank of IDIBGI.

Results

Our preliminary results showed that mHag HA-1 and HY-antigens disparities are significant risk factors of grades II-IV acute GVHD after HLA-identical sibling donor HSCT. Nevertheless, only HY-antigens mismatch influences OS and DFS at 10 years. We did not find association between HLA-DPB1 mismatch and grades II-IV acute GVHD.

Keywords

Allogeneic hematopoietic stem cell transplantation, acute graft versus host disease, HLA-DPB1 mismatch, mHag HA-1 mismatch, HY-antigens mismatch

2. ABBREVIATIONS

APC	antigen presenting cell
BM	bone marrow
CEIC	clinical research ethical committee
CNS	central nervous system
DC	dendritic cell
DFS	disease-free survival
G-CSF	granulocyte colony stimulating factor
GETH	Spanish group of hematopoietic transplant
GVHD	graft-versus-host disease
GVL	graft versus leukemia
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
IDIBGI	biomedical research institute of Girona
mHags	minor histocompatibility antigens
MHC	major histocompatibility complex
MUD	matched unrelated donor
OS	overall survival
PB	peripheral blood
PBHSCT	peripheral blood hematopoietic stem cell transplantation
SNP	single nucleotide polymorphisms
TCR	T-cell receptor

3. INTRODUCTION

3.1 Background

3.1.1 Hematopoietic stem cells

Mature blood cells are produced continuously by less-differentiated precursors that are in turn descended from more primitive progenitors and, originally, from hematopoietic stem cells (HSCs).

Stem cells, including HSCs, have two capacities:

- Self-renewing: to produce some daughter cells that retain stem-cell properties; they do not become specialized.
- Multipotency: the ability to differentiate into all functional blood cells (they can give rise to all lymphoid, myeloid and erythroid cell lineages). In fact, a single HSC can restore the entire lymphohematopoietic system through hematopoiesis (1,2)

HSCs represent approximately 1 in 10.000 cells of the bone marrow (BM) and 1 in 100.000 cells in the peripheral blood and are morphologically very similar to white blood cells. Thus, techniques designed for isolation and identification of HSCs are often dependent on the detection of cell surface markers and cluster of differentiation antigens. HSCs are commonly characterized by the absence of lineage-specific marker expression and are determined as $CD34^{+}$, $CD59^{+}$, $CD90/Thy1^{+}$, $CD38^{low/-}$, c-$Kit^{-/low}$, and Lin^{-}. Detecting the expression of these marker panels allows separation of specific cell populations via techniques like fluorescence-activated cell sorting (3).

3.1.2 Hematopoietic stem cell transplantation: Definition and types

Hematopoietic stem cell transplantation (HSCT) achieves reconstitution of hematopoiesis by the intravenous infusion of hematopoietic stem cells.

The aim of HSCT is to permit hematopoietic reconstitution after potentially curative but myeloablative doses of chemotherapy or chemoradiotherapy (high dose therapy) in the treatment of malignant disease or to replace congenital or acquired life threatening abnormal BM or immune function with a normal hematopoietic and immune system.
Stem cells for the HSCT may be obtained from:

- Bone marrow, by multiple aspirations under general anesthesia (BM harvest)
- Peripheral blood, after ‘mobilization’ by granulocyte colony-stimulating factor (G-CSF) and collection by apheresis (peripheral blood HSCT; PBHSCT)
- Placental cord blood, that has become a useful source of stem cells for pediatric transplants.

And depending on the donor, the HSCT can be:

- Allogeneic: stem cells are obtained from a donor e.g. a matched sibling or normal volunteer (matched unrelated donor; MUD).
- Autologous: the patient acts as his/her own source of stem cells
- Syngeneic: the donor is a monozygotic (identical) twin (4)

3.1.3 HSCT: indications

The indications for HSCT depend on the patient's medical condition, the therapeutic objectives, and the availability and source of stem cells.

Table 1: Diseases commonly treated with hematopoietic stem cell transplantation (5)

Autologous HSCT	Allogeneic HSCT
Cancers - Multiple myeloma - Non-Hodkin's lymphoma - Hodkin's disease - Acute myeloid leukemia - Neuroblastoma - Ovarian cancer - Germ-cell tumors	Cancers - Acute myeloid leukemia - Acute lymphoblastic leukemia - Chronic myeloid leukemia - Myelodysplasic syndromes - Myeloproliferative disorders - Non-Hodkin's lymphoma - Hodkin's disease - Chornic lymphocytic leukemia - Multiple myeloma - Juvenile chronic myeloid leukemia -
Other diseases - Autoimmune disorders - Amyloidosis	Other diseases - Aplastic anemia - Paroxysmal nocturnal hemoglobinuria - Fanconi's anemia - Blackfan-Diamond anemia - Thalassemia major - Sickle cell anemia - Severe combined immunodeficiency - Inborn errors of metabolism - Wiskott-Aldrich syndrome

Allogeneic HSCT following myeloablative conventional conditioning regimen is associated with a higher incidence of transplant-related morbidity and mortality than autogeneic HSCT (in allogeneic HSCT, the 100 day mortality rate ranges between 10 and 40% and; in autogeneic HSCT, the 100 day mortality ranges between 5 and 20%), limiting its applicability to younger patients without significant co-morbidities and with hematological malignancies with high mortality.

Thus, acute leukemia is the most common indication for allogeneic HSCT (49%); acute myeloid leukemia accounts for 33% and acute lymphoblastic leukemia accounts for 16% (6,7).

3.1.4 Allogeneic HSCT: how it works?

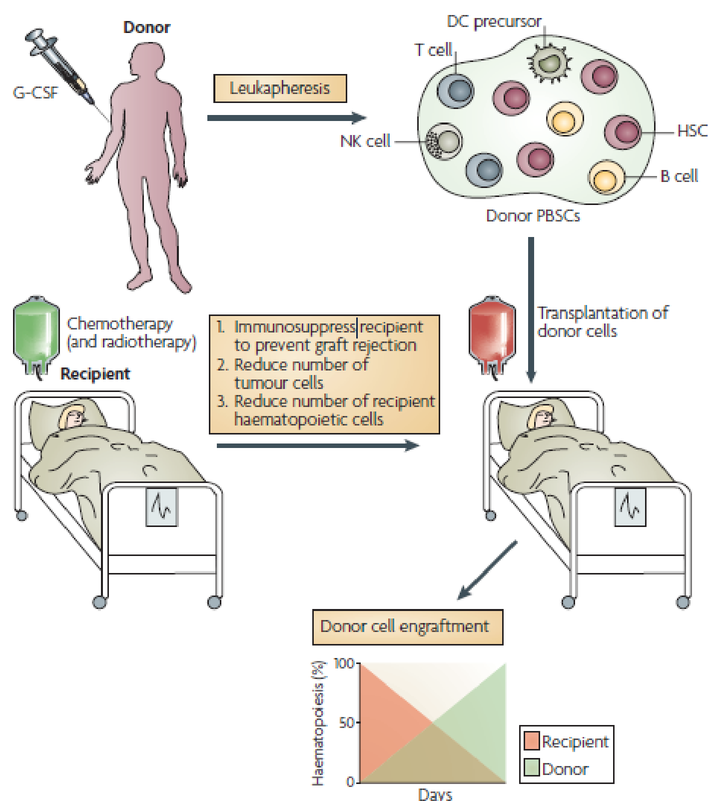

Figure 1. Allogeneic peripheral-blood HSCT (8)

In allogeneic PBHSCT (the most common way to obtain the HSC from the donor):

1. Donors receive G-CSF to mobilize HSCs, which are collected by leukapheresis.
2. Patients (recipients) receive chemotherapy (and radiotherapy), which is designed to prevent immunological graft rejection, reduce the number of tumor cells (when the allogeneic HSCT is used to treat cancer) and to create niches for HSCs engraftment.
3. The leukapheresis product (peripheral-blood HSC is then infused intravenously into the recipient.
4. Engraftment of donor neutrophils and platelets typically occurs between 10 and 20 days post-transplantation, but engraftment of other cell lineages, such as T cells, B cells, macrophages, dendritic cells (DCs), Langerhans cells and erythroid cells, may take longer.

G-CSF-mobilized PBHSCs are enriched for hematopoietic progenitors; they also contain mature CD4+ and CD8+ αβ T cells. These T cells:

- Promote hematopoietic engraftment
- Reconstitute T-cell immunity (particularly in adults with reduced thymic function)
- Mediate a potent beneficial antitumor effect, known as graft versus leukemia (GVL).

Unfortunately, these donor T-cell subsets also cause graft-versus-host disease (GVHD), the broad attack against host tissues by donor T cells2- (8).

3.1.5 Complications of allogeneic HSCT

The risk and the type of complications depend on the preparative regimen received prior to HSCT, the age of the patient at time of HSCT, the presence of comorbid conditions, and the time between the treatment and follow-up. With the exception of immunologic aspects, complications of HSCT are similar to those of intensive antineoplastic therapy.

Specific complications tend to within well-defined periods of immunologic alternation after intensive conditioning regimens: (7)

- Pre-engraftment (0-30 days)
- Early post-transplant (30-100 days)
- Late post-transplant (>100 days)

Complications:

- Infections. The risks for infections are the use of central venous access lines, prolonged neutropenia and immunosuppression. In each period the most prevalent pathogens causing infection are different.

Table 2: Pathogens causing the infection in each period (adapted from 5)

Pre-engraftment	Early post-transplant	Late post-transplant
- Bacteria - *Candida* - *Aspergillus* (if the neutropenia persists)	- CMV - *Pneumocystis jiroveci* - *Aspergillus*	- CMV - Community-acquired respiratory virus - Encapsulated bacterial infections

- Conditioning-related toxicity
    - Mucositis. Generally occurs in pre-engraftment period and increase the risk of pulmonary aspiration.
    - Myopericarditis (rare). It is associated with cyclophosphamide and anthracycline.
    - Central nervous system (CNS) complications may be seen with specific drug treatments (eg. Guillain–Barre´ syndrome after cytarabine treatment)
    - Others. Hepatitis, idiopathic pneumonia syndrome, hemorrhagic cystitis due to cyclophosphamide…
- Relapse of malignancy. Recurrence of malignancy after HSCT is seen in approximately:
    - 20% of patients who undergo transplantation while in early-stage leukemia (in a first remission or a chronic phase).
    - 50-70% of patients who undergo HSCT while in advanced leukemia (a relapse or blast crisis).
- Rejection of marrow graft. Immune-mediated graft rejection is uncommon.
  Its incidence rises with increasing degree of HLA disparity and is also elevated in previously poly-transfused patients with aplastic anemia.
- GVHD. The major complication of HSCT is acute or chronic GVHD.
  It is an immunologically mediated disease that contributes significantly to transplant-related morbidity and mortality and affects many organ systems, including the gastrointestinal tract, liver, skin, and lungs… (5,7).

3.1.6 Allogeneic HSCT: immune response from the graft (GVHD and GVL)

The transplantation of HSC that is not genetically identical (allogeneic) to that of the recipient resulted in an immunologic reaction by the donor lymphocytes against the recipient. This immune reaction is related to histocompatibility; the strongest transplant reactions occur when the human leukocyte antigens (HLA) of the donor and of the recipient are incompatible (5).
There are three requirements for development of GVHD:

1. The graft must contain immunologically competent T cells
2. The recipient must express tissue antigens that are not present in the transplant donor
3. The patient must be incapable of mounting an effective response to eliminate the transplanted cells

The ability of donor T lymphocytes to detect non–self-antigens can contribute to relapse prevention through recognition and subsequent elimination of minimal residual disease (GVL) or lead to GVHD. Thus, the graft versus-leukemia (GVL) beneficial effect is limited by GVHD and depletion of T cells abrogates GVHD and GVL effects with an increased incidence of relapse after transplantation.

Thus, patients surviving chronic GVHD benefited from a reduced tumor relapse rate (5,8–10).

- *GVHD*

The GVHD is an immune response mounted against the recipient of an allograft by mature donor αβ T cells contained in the graft. This immunological disorder affects many organ systems, including the gastrointestinal tract, liver, skin. The number of patients with this complication continues to grow, and many return home from transplant centers after HCT requiring continued treatment with immunosuppressive drugs that increases their risks for serious infections and other complications (8,11)

GVHD is categorized as acute or chronic according to time of presentation (more or less than 100 days), organ involvement, and histology. However, the distinction is not always clear as acute GVHD often evolves into chronic GVHD. In about 2 ⁄ 3 of patients, chronic GVHD was preceded by acute GVHD

- Acute GVHD is characterized by damage to the:
  - Skin (81% of the patients)

    It is frequently the first clinical manifestation. The rash initially involves the nape of the neck, ears, shoulders, the palms of the hands and the soles of the feet. From these initial areas of presentation, the rash may spread to involve the whole integument, eventually becoming confluent. In severe GVHD, the maculopapular rash forms bullous lesions with toxic epidermal necrolysis mimicking Stevens-Johnson syndrome
  - Liver (50% of the patients)

    Liver involvement usually presents in patients with signs of cutaneous and/or gastrointestinal acute GVHD. Rarely, patients have moderate to severe hepatic GVHD without evidence of other organ involvement.

    Hepatic involvement is manifested by abnormal liver function tests (cholestatic hyperbilirubinemia)
  - The gastrointestinal tract (54% of the patients)

    Acute GVHD frequently involves both the upper and lower gastrointestinal tract. Gastrointestinal involvement usually presents with watery diarrhea (>500 mL), abdominal pain and positive histological findings, but may also manifest as nausea, vomiting, and anorexia.

  The grade (I-IV) depends on the stage of acute GVHD in the skin, liver, and gut. Severe GVHD has poor prognosis, with 25% long-term survival (5 years) for grade III disease and 5% for grade IV (7,11).

Overall, acute GVHD occurs in 20–50% of allograft recipients and in up to 80% of recipients of HLA-nonidentical grafts.

- Chronic GVHD has more diverse manifestations and can resemble autoimmune syndromes with, for example, eosinophilic fasciitis, scleroderma-like skin disease and salivary and lacrimal gland involvement It can also causes esophageal stenosis, jaundice, transaminitis, nail dystrophy or loss, fasciitis, vaginal sclerosis, restrictive or obstructive defects on pulmonary function tests… (7,8,11).

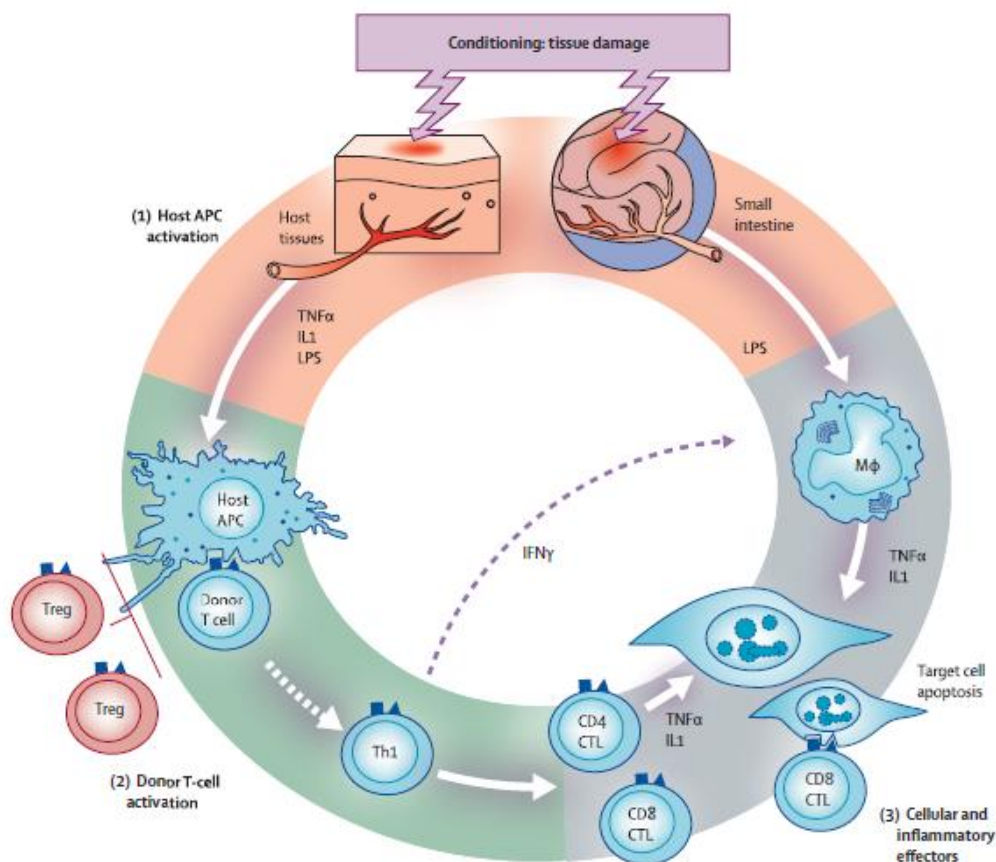

Figure 2: Pathophysiology of acute GVHD (11)

Progression of acute GVHD can be summarized in three sequential steps or phases:

1. Activation of antigen presenting cells (APCs) by the underlying disease and the HSCT conditioning regimen.

   Damaged host tissues respond by producing so-called danger signals, including proinflammatory cytokines (eg, TNFα and interleukins 1 and 6), chemokines, and amplified expression of adhesion molecules, MHC antigens, and costimulatory molecules on host APCs
2. Donor T-cell activation, proliferation, differentiation, and migration in response to host APCs. This second step is the core of the GVHD reaction.

Activation of immune cells results in rapid intracellular biochemical cascades that induce transcription of genes for many proteins, including cytokines and their receptors. T-helper 1 cytokines (interleukin 2, interferon γ and TNFα) are released in large amounts during acute GVHD.

- Interleukin 2 has an important role in the generation and maintenance of CD4+CD25+ regulatory T cells.
- Interferon γ has many functions and can either amplify or reduce GVHD:
  - It might amplify GVHD by direct damage to epithelium in the gastrointestinal tract and skin by induction of immunosuppression by generation of nitric oxide.
  - It could suppress GVHD by hastening apoptosis of activated donor T cells.
- TNFα can be produced by both donor and host cells and it acts in three different ways:
  - It activates APCs and enhances alloantigen presentation
  - It recruits effector cells to target organs via induction of inflammatory chemokines
  - It directly cause tissue necrosis

3. Target tissue destruction.
   This third effector phase of graft-versus-host process is a complex cascade of cellular mediators (such as cytotoxic T lymphocytes and natural killer cells) and soluble inflammatory agents (eg, TNFα, interferon γ, interleukin 1 and nitric oxide). These molecules work synergetically to amplify local tissue injury and further promote inflammation and target tissue destruction (11).

- *GVL*

Some malignant stem cells survive even lethal doses of total-body irradiation and chemotherapy given in preparation for hematopoietic stem-cell transplantation. However, such cells may be eliminated by immunologically active donor cells through an alloimmune attack (mediated by αβ T cells contained in the donor allograft) against recipient hematopoietic neoplasms through the GVL effect (5,8).

3.1.7 HLA matched donor

GVHD arises when donor T cells respond to genetically defined proteins on host cells. The most important proteins are human leucocyte antigens (HLAs) which are highly polymorphic and are encoded by the major histocompatibility complex (MHC) situated in chromosome 6 (9,11).

There are two types of HLAs:

- Class I HLA (A, B, and C) proteins are expressed on almost all nucleated cells of the body at various densities.
- Class II proteins (DR, DQ, and DP) are mainly expressed on haemopoietic cells (B cells, dendritic cells, and monocytes), but their expression can be induced on many other cell types after inflammation or injury (11).

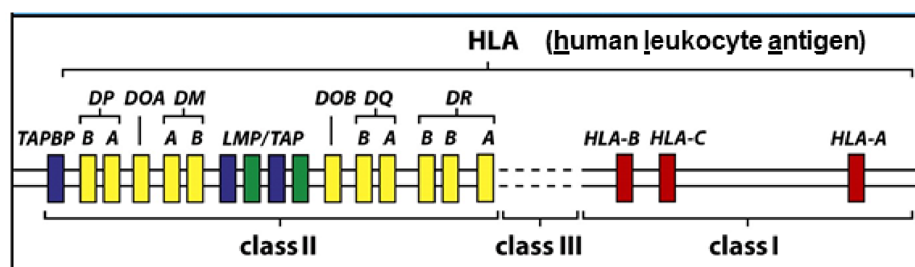

Figure 3. Gene structure of the human MHC (chromosome 6) (15)

The frequency of acute GVHD is directly related to the degree of mismatch between HLA proteins. The ideal donor has to be matched at HLA-A, B, C DRB1 and DQB1 with the recipient if it is a MUD or just HLA-A, B and DRB1 if it is a matched sibling donor with high-resolution DNA typing of HLA genes with PCR techniques.

HLA-DPB1 is not usually typified for donor election, due to the high rate of recombination of the gene with other HLA complex, which results in a higher incidence of disparities (>50%) between unrelated individuals identical to the rest HLA locus.

In fact, even a 6% of HLA-A-B-DRB1 identical siblings have differences in HLA-DPB1, which has been associated with an increased incidence of GVHD (11,12).

3.1.8 Other disparities

Despite HLA identity between a patient and donor, about 40% of recipients of HLA-identical grafts develop systemic acute GVHD. This disorder is due to genetic differences that lie outside the HLA loci and that encode proteins referred to as minor histocompatibility antigens (mHags) (11).

The mHags are peptides derived from polymorphic intracellular proteins that can be presented by HLA class I or class II molecules on the cell membrane, and they can be recognized as “foreign” antigens by T cells from HLA-matched individuals. Throughout the human genome, a huge number of single nucleotide polymorphisms (SNPs) exists within the human population.

If these specific peptides presented in HLA class I or class II molecules can be recognized by HLA-identical $CD8^+$ or $CD4^+$ T cells, respectively, and if these antigen complexes are not recognized as self-antigens by this individual, the peptides are considered to be mHags.

Examples of such mHags that have been shown to play a clinically significant role in human transplantation are male-specific HY-antigens and the mHag HA-1 (10).

- Male-specific HY-antigens.
  In general, gender mismatching significantly affects HSCT outcome; the highest risk for GVHD has been observed in male recipients of female stem cells.
- mHag HA-1.
  The effect of mismatching for the mHag HA-1 on GVHD has been studied by several investigators reporting different outcomes. Whereas some studies observed an association between HA-1 mismatching and the developing of GVHD and GVL, others did not.
  HA-1 mismatch is defined by the presence of $HA\text{-}1^H$ allele in the host but not in the donor (13,14).

3.2 Justification

As it has been demonstrated, GVHD is the main complication of patients treated with allogeneic HSCT, a very used treatment for several hematological malignances, because of its high prevalence and its morbidity and mortality associated.

Thus, we want to study some genetic mechanisms that cause acute GVHD in patients treated with an allogeneic HSCT from an HLA-identical sibling donor. Specifically we will study three disparities between donor and recipient that can be associated with acute GVHD and are not usually typified for donor election:

- HLA-DPB1mismatch (a class II proteins of HLA)
- mHag HA-1 mismatch
- Male-specific HY-antigens (female donor and male recipient)

Previous data of the association of these disparities with acute GVHD from other studies are not definitive. It would be interesting and relevant to know if this association is significant in order to introduce the typing of these disparities in the donor election to decrease acute GVHD and, thus, mortality and morbidity of allogeneic HSCT.

We also want to study a possible association between these three disparities, to know if the risk of acute GVHD is additive or not. We want to figure out if the patient has more risk of acute GVHD and if it is more severe if he has more than one of these disparities with the donor.

4. HYPOTHESIS

The presence of HLA-DPB1, male-specific HY-Ag and/or mHag HA-1 mismatches increases the risk of acute graft versus host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (HSTC) from an HLA-identical sibling donor.

5. OBJECTIVE

The main objective of the study is to determine the association of acute GVHD after allogeneic HSCT from an HLA-identical sibling donor with:

- HLA-DPB1 mismatch
- mHag HA-1 mismatch
- Male-specific HY-antigens mismatch

The secondary objective is to determine overall survival (OS) and disease-free survival (DFS) after allogeneic HSCT from an HLA-identical sibling donor with the presence of HLA-DPB1, male-specific HY-Ag and/or mHag HA-1 mismatches.

6. METHODOLOGY

6.1 Study design

Longitudinal retrospective and multicenter cohort.

6.2 Study population

The target population is composed by patients (more than 15 years old) treated with an HSCT from an HLA-identical sibling donor after a myeloablative conditioning regimen.

Inclusion criteria:

- Patients treated with an allogeneic HSCT from an HLA-identical sibling donor with available DNA sample
- Patients aged more than 15 years old

Exclusion criteria

- Patients received a T-cell depleted graft
- Patients transplanted from an unrelated donor

6.3 Sample selection

The study will include all the DNA samples from 1377 patient-donor pairs collected by 21 Spanish transplant teams of the Spanish Group of Hematopoietic Transplant (GETH) between 1992 and 2014 and included in the Bio Bank of the biomedical research institute of Girona (IDIBGI).

Written informed consent was obtained from patients and donors before DNA storage.

6.4 Sample size

Sample size calculation would be based on the free application GRANMO and the POISSON approximation.

We calculate the sample size twice, because the disparities that we are studying have a different incidence:

- Male-specific HY-antigens mismatch has an incidence of 40%.
  Given this incidence and accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 34 exposed subjects and 51 in the non-exposed would be necessary to recognize as statistically significant a relative risk greater than or equal to 2. A proportion in the non-

exposed group has been estimated to be 0.33. It has been anticipated a drop-out rate of 0% because it is a retrospective study and all the data are already collected.

- HLA-DPB1 mismatch and mHag HA-1 mismatch have an incidence of 6%.
  Given this incidence and accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 21 exposed subjects and 327 in the non-exposed would be necessary to recognize as statistically significant a relative risk greater than or equal to 2. A proportion in the non-exposed group has been estimated to be 0.33. It has been anticipated a drop-out rate of 0%.

Anyway, in our study our sample is larger than what is needed, and it is composed by 1377 patient-donor pairs. Thus, the results will be more significant.

6.5 Study variables

Independent variable

The independent variable is the presence or absence of one or more mismatches between donor and recipient:

- HLA-DPB1 mismatch
- mHag HA-1 mismatch
- Male-specific HY-antigens mismatch

Dependent variable

Dependent variables include:

- Acute GVHD stages II-IV
- OS and DFS after the allogeneic HSCT

Covariates

- Patient’s and donor’s age
- Patient’s and donor’s sex
- Diagnoses
- Source of HSC
- Disease stage
- Patient’s CMV status
- Conditioning regimen
- Pharmacological GVHD prophylaxis

6.6 Measurements

HLA-DP, mHag HA-1 and male-specific HY-antigens mismatches (independent variable)

DNA extraction and genotyping are carried out from the patient's and donor's PB received from 21 Spanish Hospitals of the GETH.

All the DNA samples are stored in the IDIBGI's bio-bank.

DNA extraction

DNA samples were obtained from patient's and donor's peripheral blood using the QIamp DNA Blood Mini Kit (Qiagen protocol), according to the manufacturer's instructions (Annex 1) and stored at -80ºC until use.

HLA-DPB1 and HA-1 genotyping

HLA-DPB1 and HA-1 genotype was determined for both patients and donors by PCR-SSP with allele specific primers (Annex 2).

The amplified products were analyzed by electrophoresis in 3% agarose gel (Annex 3). Samples demonstrating the expected band were considered positive, whereas the absence of this band in the presence of positive control amplification was considered as negative.

The presentation of mHag HA-1 to the T cells is restricted by the HLA-A*02:01 allele. Thus, to assess mHag HA-1 mismatch the patient-donor pair has to be positive for this allele.

Male-specific HY-antigens mismatch assessment

The mismatch is defined by a male recipient and female donor.

Acute GVHD stages II-IV (dependent variable)

Glucksberg scale (I-IV) (table 3) is used to grading acute GVHD. The grade is determined by an assessment of the degree of involvement of the skin, liver, and gastrointestinal tract. The stages of individual organ involvement are combined with the patient's performance status to produce an overall grade.

In our study we define the presence of acute GVHD if the patient is affected by grades II-IV of Glucksberg scale and it is measured by the medical team of the Hospital where is carried out the allogeneic HSCT.

Table 3: Glucksberg scale of acute GVHD

Grade	Skin	Gastrointestinal	Liver	Karnofsky performance
I	1-2	0	0	90-100%
II	3 or	1 or	1	70-80%
III	-	2-3 or	2-4	50-60%
IV	4 or	4	-	30-40%

Skin stages:

- Stage 0: No rash
- Stage 1: Maculopapular rash <25% of body surface area
- Stage 2: Maculopapular rash on 25-50% of BS area
- Stage 3: Generalized erythroderma
- Stage 4: Generalized erythroderma with bullous formation and desquamation

Liver stages:

- Stage 0: Bilirubin <2 mg/dL
- Stage 1: Bilirubin 2-3 mg/dL
- Stage 2: Bilirubin 3.01-6 mg/dL
- Stage 3: Bilirubin 6.01-15 mg/dL
- Stage 4: Bilirubin >15 mg/dL

Gastrointestinal stages:

- Stage 0: No diarrhea, or diarrhea <500 mL/day
- Stage 1: Diarrhea 500-999 mL/day
- Stage 2: Diarrhea 1000-1499 mL/day
- Stage 3: Diarrhea >1500 mL/day
- Stage 4: Severe abdominal pain, with or without ileus

Overall survival and disease-free survival (dependent variables) and covariates

These variables and all the covariates are collected by the medical team of the Hospital where is carried out the allogeneic HSCT.

- OS is defined as time from graft infusion (day 0) to the last follow-up, including death from any cause.
- DFS is defined as the time from graft infusion to recurrence of the original disease or the last follow-up.

7. STATISTICAL ANALYSIS

Statistical analysis will be performed using IBM Statistical Package for Social Sciences (SPSS) for *Windows*®.

Microsoft Access tool will be used to manage computed data.

Univariate analysis

First, we will define variables as categorical or quantitative.

- Independent variable:
  - Mismatches will be considered a categorical dichotomic variable (presence / absence of one or more disparities)
- Dependent variables:
  - Acute GVHD (II-IV) will be considered a time-dependent categorical dichotomic variable (presence/absence).

    Cumulative incidence estimates, considering competitive risks, will be used to explore differences in acute GVHD. Death without signs of GVHD will be considered as a competitive risk for acute GVHD.

    Gray test will be used to determine differences between curves.
  - OS and DFS will be considered time-dependent categorical dichotomic variables.

    Kaplan-Meier curves will be derived to determine OS and DFS and curves will be compared by means of the log-rank test.
- Covariates:
  - Patient's and donor's age will be considered quantitative variables (years)
  - Patient's and donor's sex (male /female), source of HSC (PB/BM), disease stage (early /advanced), patient's CMV status (positive / negative), donor with previous pregnancies (yes / not), conditioning regimen (myeloablative / reduced intensity conditioning regimen), diagnoses (acute leukemia and myelodysplastic syndrome/ other diseases) and pharmacological GVHD prophylaxis (cyclosporine A and methotrexate/other combinations) will be considered categorical dichotomic variables.

Categorical variables will be expressed as relative frequencies and percentages. Quantitative variables will be described by median and interquartile range.

Homogeneity between study groups

Homogeneity between mismatched donor-patient group and non-mismatched donor-patient group will be evaluated using:

- Chi-squared for qualitative variables.
- Student-t test for continuous variables.

A two-sided p value of 0.05 or lower will be considered as statistically significant and it will confirm that the two groups are not homogeneous for that specific variable.

Multivariate analysis

Multivariate analysis will be performed using the Cox regression model. All the variables with a p value at or below 0.2 in the univariate analysis will be included in the multivariate analysis.
Results will be expressed as hazard ratios, and 95% confidence intervals (95% CI).

8. PRELIMINARY RESULTS

HLA-DPB1 mismatch

The homogeneity study demonstrated no clinical significant differences between mismatched and non-mismatched group; all variables were comparable between them.

The cumulative incidence of grades II-IV acute GVHD was compared between the patients receiving an HLA-DPB1 mismatched graft and those without this mismatch (62.5% vs 38.2%; P: 0.173) and no significant differences were found. Moreover, statistical analysis demonstrated no significant differences between the two groups in the 10 years-OS (55% vs 52%; P: 0.718) and in the 10 years-DFS (47.1% vs 44.2%; P: 0.882) (Figure 4).

mHag HA-1 mismatch

The comparison between the pairs carrying the HA-1 antigen mismatch and those non-carriers detected statistical differences with the age. Patients of the mismatched group were younger than the patients of the non-mismatched group (median 37 years; range 15-68 vs 41; 15-72; P: 0.023) and donors of the mismatched group were also younger than donors of the non-mismatched group (median 34 years; range 6-75 vs 41; 3-79; P: 0.008).

Patients of the mismatched group had a higher cumulative incidence of grades II-IV acute GVHD than patients of the non-mismatched group (44.4% vs 34.1%; P: 0.024). But we did not detect significant differences in the 10 years-OS (38.4% vs 48.2%; P: 0.352) and in the 10 years-DFS (40.8% vs 41.3%; P: 0.383) between the two groups (Figure 5).

Male-specific HY-antigens mismatch

We did not detect clinical significant differences between the two groups; all variables were comparable between them.

As we expected, the cumulative incidence of grades II-IV acute GVHD was higher in the mismatched group (43.8% vs 33.5%; P: 0.003). Similarly, we also detected statistical differences between the mismatched and non-mismatched group in the 10 years-OS (37.3% vs 50.2%; P: 0.001) and 10 years-DFS (30.7% vs 45%; P: 0.025) (Figure 6).

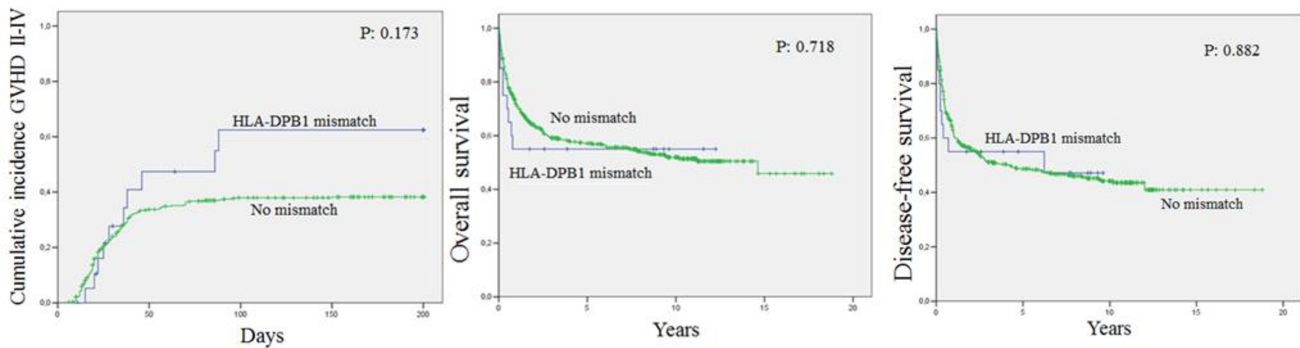

Figure 4. Cumulative incidence of II-IV acute GVHD according to the presence or absence of HLA-DPB1 mismatch (**A**). Overall survival according to the presence or absence of HLA-DPB1 mismatch (**B**). Disease free survival according to the presence or absence of HLA-DPB1 mismatch (**C**).

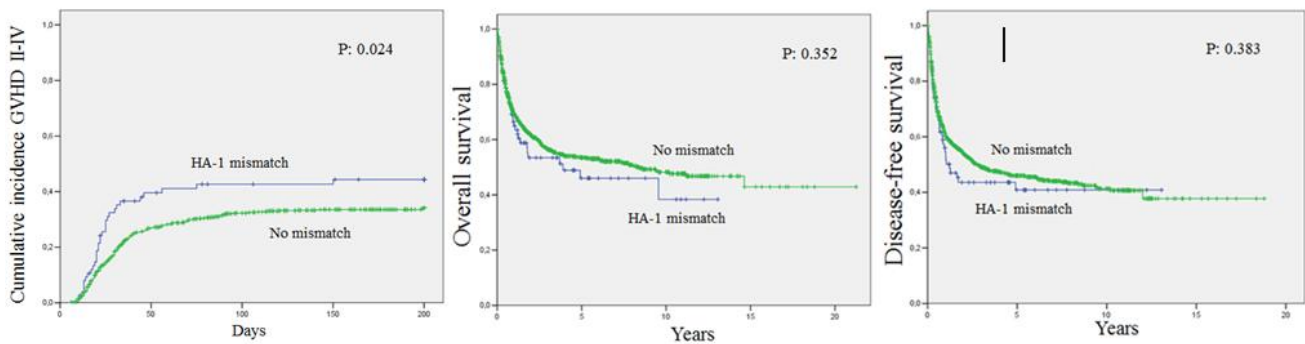

Figure 5. Cumulative incidence of II-IV acute GVHD according to the presence or absence of mHag HA-1 mismatch (**A**). Overall survival according to the presence or absence of mHag HA-1 mismatch (**B**). Disease free survival according to the presence or absence of mHag HA-1 mismatch (**C**).

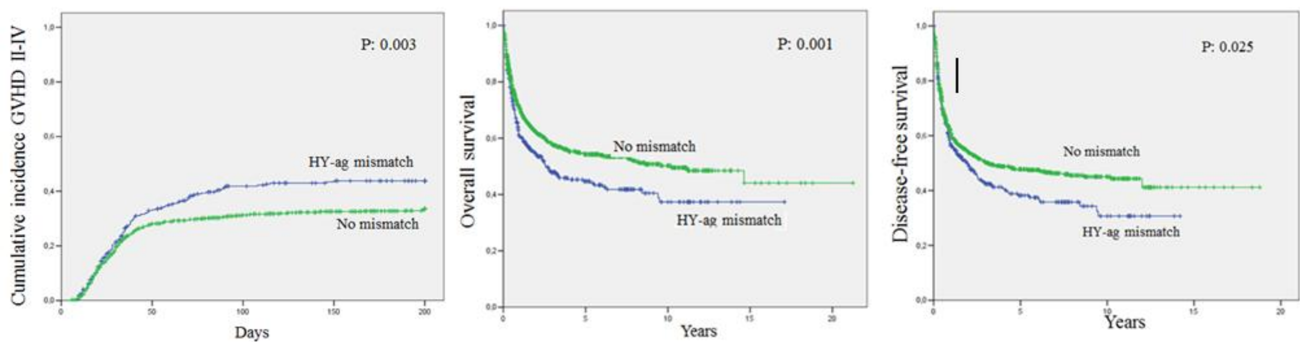

Figure 6. Cumulative incidence of II-IV acute GVHD according to the presence or absence of male-specific HY-antigens mismatch (**A**). Overall survival according to the presence or absence of male-specific HY-antigen mismatch (**B**). Disease free survival according to the presence or absence of male-specific HY-antigens mismatch (**C**).

9. DISCUSSION

We retrospectively studied a cohort of 1377 patients receiving an allogeneic HSCT from an HLA-identical sibling donor and investigated the effect of male-specific HY-antigen, mHag HA-1 and HLA-DPB1 mismatching on HSCT outcome.

Despite some studies demonstrated that HLA-DPB1 mismatch increases the risk of acute GVHD in sibling donor HSCT (16, 17), we found no difference in the cumulative incidence of grades II-IV acute GVHD, OS and DFS between HLA-DPB1 mismatched group and non-mismatched group. These findings can be explained because the HLA-DBP1 cohort is smaller (not all the samples are typified yet), we would have to increase our cohort in order to try to find significant differences between the two groups.

On the other hand, and as we expected, our data confirmed the results from previous studies about the relationship between grades II-IV acute GVHD with HA-1 antigen disparity (15) and HY-antigens mismatch (18). Thus, we can consider these disparities as significant risk factors of acute GVHD (II-IV).

Nevertheless we found no difference in OS and DFS at 10 years between patients carrying the mHag HA-1 mismatch and those non-mismatched. These findings suggest us that there is an alloreactive response against m-Hag HA-1 but it maybe is not so severe to cause an increase of mortality. We should assess the impact of GVHD caused by the mHag HA-1 mismatch on life quality in order to study the relevance of its typing in donor's selection.

Only HY-antigens mismatch decreases significantly OS and DFS at 10 years. This information can be useful to select sibling donors for allogeneic HSCT to improve its outcomes and reduce the HSCT's mortality associated.

These results are just preliminaries, we have to continue our statistical analysis and perform the multivariate analysis in order to detect confounding factors. We also have to study a possible additive effect between the three disparities that we are analyzing on the occurrence of acute GVHD in order to assess if a patient has more risk of acute GVHD if he has more than one of these disparities and if the alloreactive response is more severe.

Further studies are needed to confirm our results.

10. ETHICAL ASPECTS

This research protocol will be presented to the Clinical Research Ethical Committee (CEIC) of Hospital Universitari Dr. Josep Trueta in Girona for its assessment and approval. Moreover, the recommendations given by the committee will be taken into account to carry out the study.

This study will be conducted in accordance to the human rights and to the ethical principles established by World Medical Association in the *Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects* (last actualization, October 2013).

Since it is a retrospective study, we will depart from a previously constructed database. Thus, this is an investigation without risk; there will be no changes on the biological, psychological, physiological or social individuals participating in the study variables performed.
Participants were duly informed and gave signed consent (annex 4) at the time of inclusion, authorizing maintenance of a secure computerized database with their personal data and the maintenance of their DNA in the Biobank of IDIBGI for further investigations.

Data will be taken from the database anonymously according to the article from Spanish Organic Law 15/1999, 13th December, Protección de Datos de Carácter Personal. Therefore, this study guarantees the confidentiality of the patient. All data will be only used for the purpose of the research.

All the investigators will have to declare no conflict of interest.

11. STRENGTHS AND LIMITATIONS

Limitations

In regard to the limitations of this study:

- It should be considered that this study has an observational design.
- There are different covariates than can modify our results. This limitation (confounding bias) will be minimized by the use of a multivariate analysis to adjust results for the confusing factors.
- It is a multicenter study, and all the variables (except DNA typing) are collected by the hospitals where the allogeneic HSCT is carried out. Thus, it can cause an information bias.
  In order to reduce this limitation, trained professionals collected all the information using standardized instruments (like Glucksberg scale to assess acute GVHD).
- The results are only generalized to allogeneic HSCT from an HLA-identical sibling donor.

Strengths

The major strengths of this study are:

- Since it is a retrospective study, all the data are already collected and we won't have loss of patients. Furthermore, the duration of the study will be short and the budget won't be very high.
- Since it is a multicenter study (with more than 20 Spanish Hospitals), our results will be generalized to the Spanish population.
- We already have all the data and our sample size is larger than the one that it is necessary to have a statistically significant result. Thus, our results will be more significant.
- We have the largest cohort of allogeneic HSCT of Europe.

12. WORK PLAN

Stage 0 **Protocol approval** (2 months: September – October 2016)

- Bibliography research and protocol elaboration.
  All the members of the study have to agree with the procedures.
- Ethical committee.
  Protocol's presentation to the CEIC of Hospital Universitari Dr. Josep Trueta in Girona for its assessment and approval.

Stage 1 **Data collection** (1 month: October 2016)

All the data are already collected from the Spanish Hospitals of the GETH.

Patients' selection from the IDIBGI's database with the inclusion/exclusion criteria described before (in the methodology).

Stage 2 **Data analysis** (2 months: October – November 2016)

It includes data analysis using the appropriate statistical test.

Firstly, a descriptive and bivariate analysis will be conducted and, secondly, a multivariate analysis using a Cox regression model to examine the contribution of the confounding variables will be performed.

Stage 3 **Final report** (2 months: December 2016 – January 2017)

- Interpretation of the results
  The investigators will meet to interpret and analyze the results in order to perform the final discussion and conclusions of the study.
- Final report elaboration

Stage 4 **Publication and dissemination of the results** (3 months: February – April 2017)

The final results of this study will be published and disseminated in:

- Scientific publications
- Conference presentations and meetings

13. CHRONOGRAM

PHASES	September 2016	October 2016	November 2016	December 2016	January 2017	February 2017	March 2017	April 2017
Bibliography research								
Protocol elaboration								
Ethical committee								
Data collection								
Data analysis								
Interpretation of the results								
Final report elaboration								
Publication / dissemination								

14. BUDGET

Before starting the study it is necessary to ensure its' financing.

- Human resources:
  - No human resources are considered in the budget. The investigators and the doctors working for the program will not receive a compensation for their work in this study.
- Materials:
  - PCR-SSP reactive (2.000 €)
  - Other laboratory material (500 €)
- Travels, publication and dissemination
  - We assume 1.000 € for possible costs related with transport and accommodation for attending the meetings for two members of the investigator team.
  - We assume costs for the study publication of 500€
  - We assume costs for national and international journey for diffusion of the data of 2.500€

EXPENSES	COSTS
Personal costs	
- Research team and doctors	0€
Material costs	
- PCR-SSP material - Other laboratory material	2.000€ 500€
Travels, publication and dissemination	
- Travel meetings	1.000€
- Publication costs	500€
- Diffusion of the data	2.500€
TOTAL	**6.500€**

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16. ANNEXES

Annex 1: DNA extraction protocol (kit Qiagen) (given by IDIBGI)

Extracció de DNA (kit Qiagen)

- Engegar termobloc a 56 ºC
- Posar en un criotub nou 20 □l de proteïnasa (que s'ha de descongelar prèviament)
- Afegir 200 □l de mostra (150ul si es tracta de MO)
- Afegir 200 □l de buffer AL (150 ul si es tracta de MO)
- Vortejar 15 segons
- Incubar a 56ºC durant 10 minuts
- Pols de centrífuga
- Afegir 200 □l d'etanol absolut (150 ul si es tracta de MO)
- Vòrtex uns 15''
- Pols de centrífuga
- Transvasar-ho tot a l'spincolumn, en un tub col·lector net i centrifugar 1 min a 8000 rpm
- Posar la columna en un nou tub collector i afegir 500 □l de buffer AW1
- Centrifugar 1 min a 8000 rpm
- Canviar la columna a un nou tub col·lector i afegir 500□□l de buffer AW2.
- Centrifugar 3 min a 8000 rpm + 0.30 min a 14000 rpm
- Canviar la columna a un col·lector nou, i afegir 100 □l de buffer AE. Deixar 1 (5) minut a Room Temperature, i centrifugar 1 (2-3) min a 8000rpm.
- Sense canviar la columna a un col·lector nou, afegir 100 □l de buffer AE. Deixar 1 (5) minut a Room Temperature, i centrifugar 1 (2-3) min a 8000rpm
- Llençar spincolumn, i quedar-nos amb l'eluït. Canviar-ho a un criotub, etiquetat correctament.

→ Guardar tubs en caixa de DNATeca i omplir registre

→ La concentració esperada en els 200 ul d'eluït és de 30ng/ul

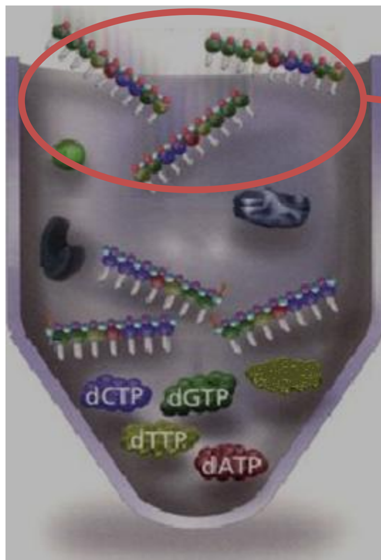
Annex 2: PCR-SSP protocol (given by IDIBGI)

Reacció en cadena de la polimerasa (PCR) amb primers-polimerases específiques (SSP)

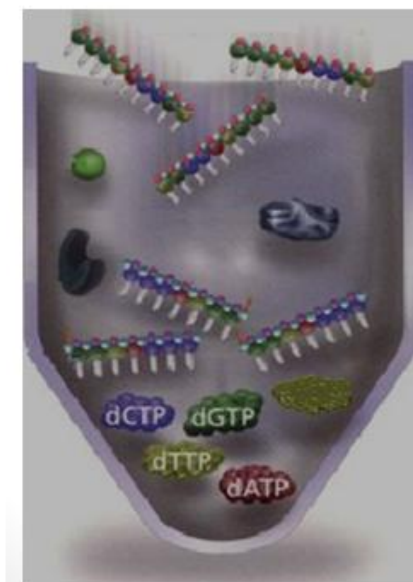
Tècnica qualitativa *in vitro* que té com a objectiu l'amplificació directa o indirecta d'un fragment de DNA.

Requisit: conèixer part d'una regió de DNA o RNA que es vol amplificar (on s'enganxaran els primers específics).

Metodologia:



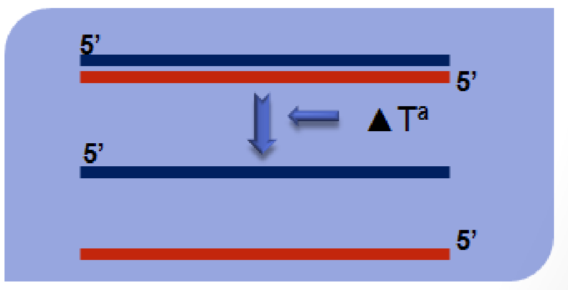
	Primers
Tm	58-60ºC
Dímers	Evitar-los
Contingut C-G	30-80% (màx 2 cada 5 nt)
Longitud	15-30 nt



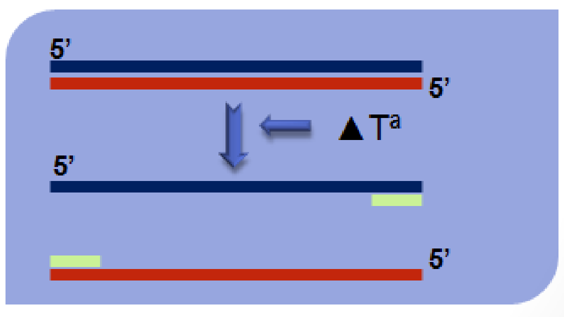
Mètode que consta de tres passos (desnaturalització, anellament i elongació) que formen un cicle, el qual es repeteix un número determinat de vegades (n= 30-40 cicles).

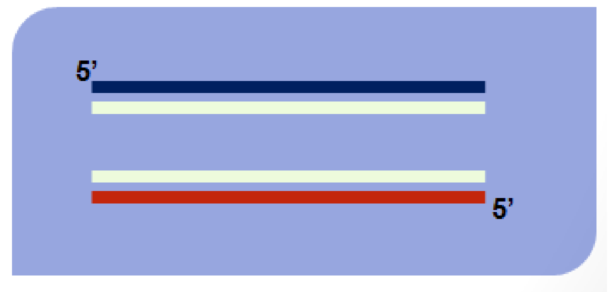
La producció de cadenes de DNA en una PCR contínua és exponencial, per cada molècula de DNA s'obtenen 2 còpies → 2n (n=número de còpies)

- Desnaturalització

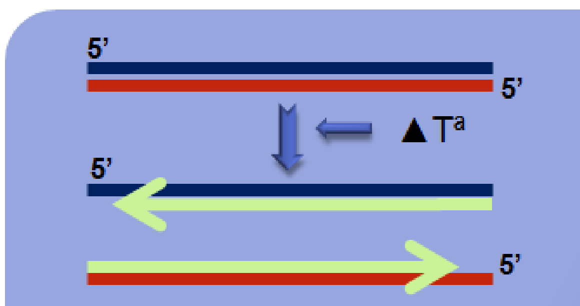


- Anellament

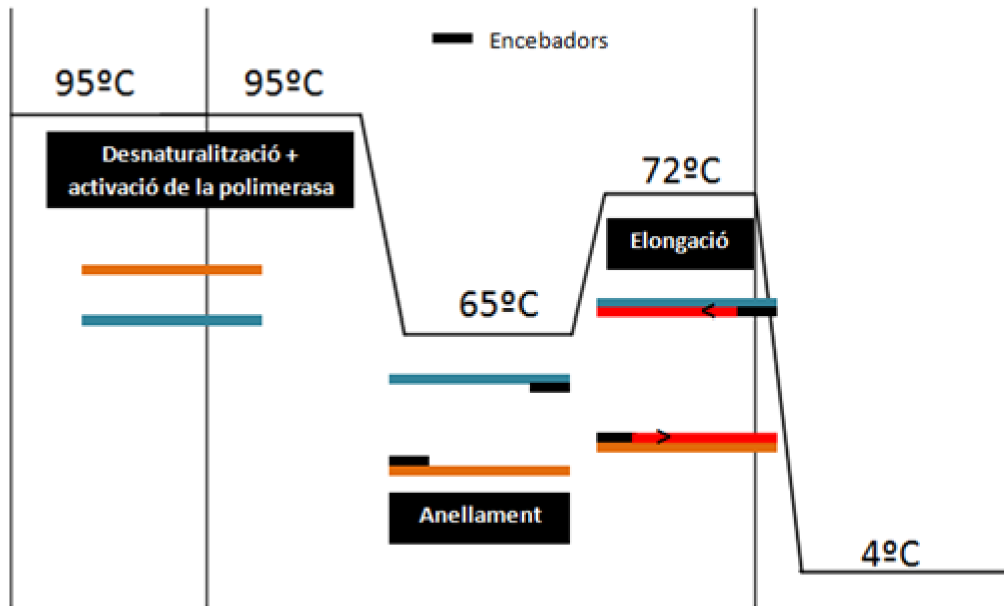




- Elongació



Temperatura a la que es realitza cada part del cicle:



Els resultats s'avaluen amb el gel d'agarosa (Annex 3)

Annex 3: Electrophoresis in 3% agarose gel protocol (given by IDIBGI)

Material i Reactius

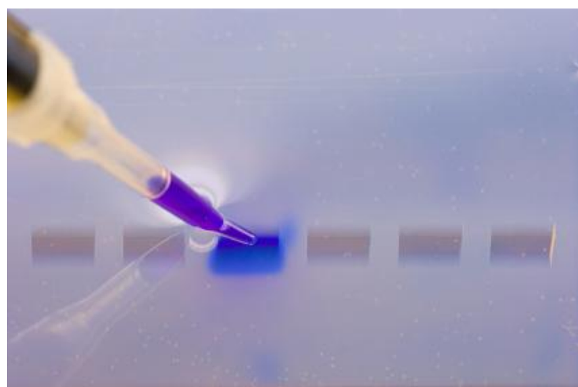
- ✓ Agarosa
- ✓ Midori Green
- ✓ Tampó de càrrega TBE 1x
- ✓ Microones
- ✓ Cubeta gels i motlle per la solidificació del gel
- ✓ Proveta
- ✓ Erlenmeyer
- ✓ Balança

Mètodes

1. Pesar la quantitat de pols d'Agarosa que es necessita. Afegir els ml necessaris per fer el % de concentració del gel.
2. Passar-ho a l'erlenmeyer i escalfar-ho al microones fins que es posi transparent.
3. Refredar sota l'aixeta.
4. Afegir Midori Green. Remenar i abocar-ho al motlle de la cubeta.
5. Col·loquem les pintes i deixem que es refredi.
6. Omplir la cubeta amb Buffer 1xTBE.
7. Carregar les mostres de la PCR amplificada.
8. Connectem a la font i ho deixem córrer.
9. Es desconnectarà la font i posarem el gel al lector d'ultraviolat per veure les bandes.

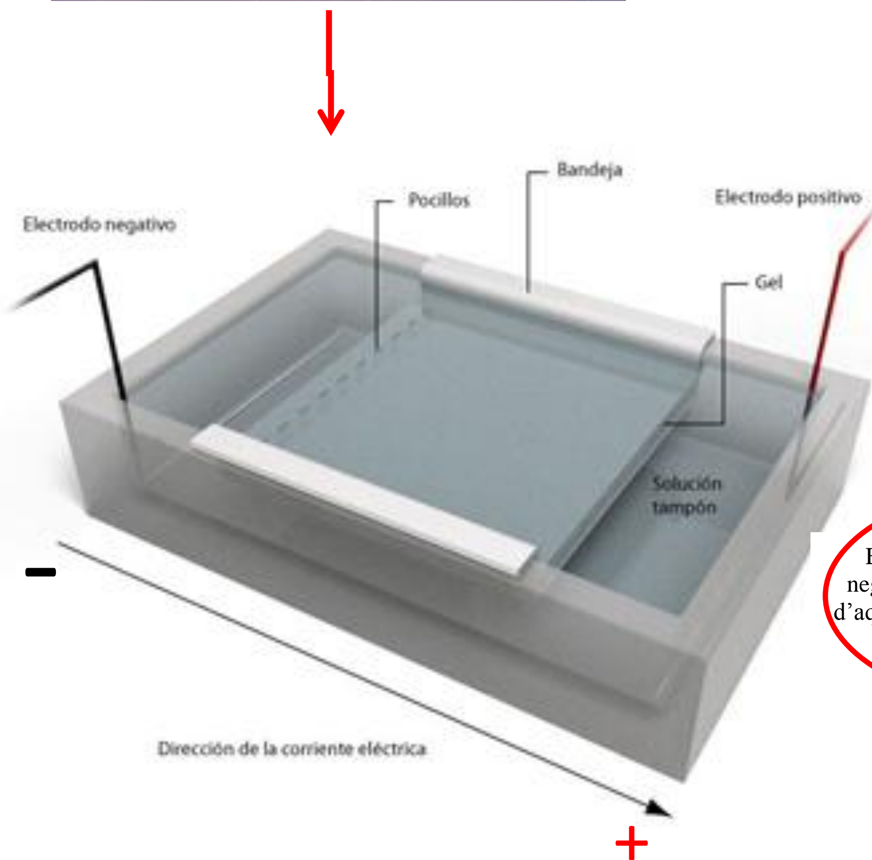
El Midori Green va ser creat per oferir un medi de contrast no cancerigen i menys mutagènic per la electroforesis d'agarosa. A més s'ha demostrat l'alta sensibilitat per fragments petits de DNA amplificat.

Mesurar la quantitat d'agarosa dependrà de la concentració que es vulgui fer del gel, a més concentració, millor resolució. Un % elevat d'agarosa en permetrà veure bandes de DNA amplificades a pes baix molecular.

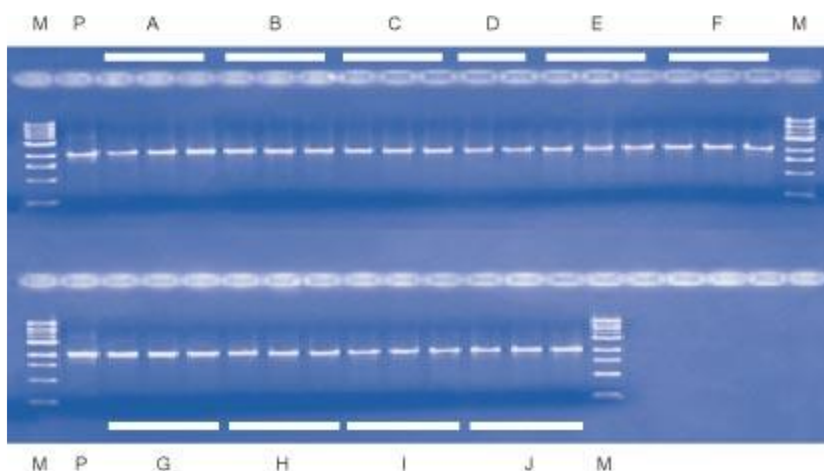


Carregarem les mostres als pous del gel.

Prèviament haurem barrejat tampó de càrrega (normalment blau) i la PCR “amplificada”.



EL DNA és una molècula amb càrrega negativa. Amb un camp elèctric, qualsevol d’aquestes molècules es desplaçaran cap a un pol positiu.



Resultat final d’una PCR amplificada

Annex 4: Signed consent

HOJA DE INFORMACIÓN AL PACIENTE

OBTENCIÓN Y UTILITZACIÓN DE MUESTRAS BIOLÓGICAS Y DATOS CLÍNICOS PARA INVESTIGACIÓN MÉDICA Y CONSERVACIÓN EN EL BIOBANC IDIBGI

En la mayoría de hospitales, además de la asistencia a los pacientes, se realiza investigación biomédica. La finalidad de esta investigación es progresar en el conocimiento de las enfermedades y en su prevención, diagnóstico y tratamiento. Esta investigación biomédica requiere recoger datos clínicos y muestras biológicas de pacientes y donantes sanos para analizarlos y obtener conclusiones con el objetivo de conocer mejor las enfermedades y avanzar hacia su diagnóstico y/o tratamiento.
Las muestras y datos clínicos obtenidos para el diagnóstico o control de las enfermedades, una vez utilizadas para dicha finalidad, resultan también útiles y necesarias para la investigación. De hecho, muchos de los avances científicos obtenidos en estos últimos años en medicina son fruto de este tipo de estudios.

Solicitamos su autorización para la obtención de una muestra biológica adicional que se le extraerá en su hospital de referencia, con el fin de depositarla en la colección GET (DNAteca del Grupo Español de Trasplante Hemotopoyético) del Biobanc IDIBGI, así como su autorización para utilizar la información clínica asociada a este material biológico para proseguir con la investigación biomédica.

Siguiendo lo que establece la Ley 14/2007, de Investigación Biomédica, la Ley Orgánica 15/1999, de Protección de Datos Personales, y sus normas de desarrollo, le solicitamos que lea detenidamente este documento de información y el consentimiento informado que se le adjunta al final para su firma, si está de acuerdo al participar en esta propuesta.

FINALIDAD DE LA INVESTIGACIÓN: progresar en el conocimiento de las enfermedades.

La finalidad de la investigación es mejorar nuestro conocimiento de las enfermedades. Las muestras, los datos clínicos y analíticos y las pruebas de imagen se utilizarán para la investigación biomédica.

CONSIDERACIONES ESPECÍFICAS DE LA COLECCIÓN GET:

El ADN es una molécula que contiene toda la información genética necesaria para las funciones celulares. El código genético da lugar a las características heredadas individualmente, tales como el color de los ojos y el grupo sanguíneo. El motivo por el cual el **Grupo Español de Trasplante Hematopoyético** quiere desarrollar un banco de ADN de receptores y donantes es para identificar cuáles son las diferencias genéticas que se asocian a complicaciones post-trasplante. Una vez identificadas, esta información puede ser muy valiosa para la selección de futuros donantes.
Esta colección contribuirá a los avances en el conocimiento del trasplante de células madre hematopoyéticas, así como a mejorar el éxito de tales trasplantes en el futuro. Se pretende identificar diferencias genéticas entre pacientes y donantes que supongan un riesgo aumentado para desarrollar complicaciones post-trasplante, de tal manera que puedan desarrollarse estrategias individualizadas para cada paciente según su riesgo individual.
Para participar en esta colección se requiere una única donación de una muestra de sangre, que será extraída en el mismo centro donde se realizará el trasplante, por personal cualificado. Los riesgos de participación en dicha colección son exclusivamente las inherentes a la propia extracción de sangre.
La **colección GET** incluye la participación de todos los centros españoles donde se realizan trasplantes alogénicos (a partir de un donante) que así lo deseen. Una vez obtenida la sangre de paciente y donante, ésta será transportada hasta Girona, dónde se halla el nodo responsable de extraer el ADN para almacenarlo en el Biobanco IDIBGI. Los datos clínicos en cuanto al desarrollo del trasplante y sus complicaciones (si es que aparecieran) serán recogidos por el equipo que realiza el trasplante y reportados al centro de referencia.

MUESTRAS BIOLÓGICAS E INFORMACIÓN ASOCIADA: les muestras obtenidas se custodiaran y conservaran en el Biobanc IDIBGI hasta su extinción.

Se guardará y dispondrá de la muestra biológica adicional de *sangre de aproximadamente 6ml*, para realizar estudios de investigación biomédica, sin que este hecho le cause molestias adicionales. De forma paralela a la recogida de muestras se procederá a la recogida de datos antropométricos y clínicos, que se asociarán a sus muestras biológicas.

CI obtención muestra colección GET_v2 mayo 2015
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El GETH cuenta con distintos centros que participan en el reclutamiento de muestras. Una vez obtenida la sangre, se envía a Girona para su procesamiento y almacenamiento en el Biobanc IDIBGI. Los datos clínicos recogidos son introducidos a la aplicación informática del BIOBANC IDIBGI.
Los riesgos de participación con la colección son exclusivamente los asociados a la propia extracción de sangre.
La donación de estas muestras cedidas al Biobanc IDIBGI no impedirá que usted o su familia puedan usarlas, cuando sea necesario por motivos de salud. Las muestras y la información asociada a estas se custodiarán y conservarán en el Biobanc (banco de muestras biológicas) IDIBGI, hasta su extinción.
Este Biobanco es un establecimiento sin ánimo de lucro e inscrito en el *Registro Nacional de Biobancos* dependiente del *Instituto de Salud Carlos III* con la referencia B.0000872, que acoge colecciones organizadas de muestras biológicas e información asociada en las condiciones y garantías de seguridad que exige la legislación anteriormente referida y los códigos de conducta aprobados por los Comités de Ética. Las mencionadas muestras y su información asociada quedan disponibles por aquellos investigadores que lo soliciten al Biobanco.
Cualquier estudio de investigación para el cual se solicite la utilización de estos datos o muestras tendrá que disponer siempre de la aprobación del Comité de Ética de la Investigación Clínica (CEIC) competente, que velará para que los investigadores desarrollen sus estudios siguiendo siempre las más estrictas normas éticas y legales. Además, el comité científico del Biobanco garantizará que los proyectos sean de excelencia científica. La investigación biomédica es actualmente un fenómeno global, de forma que ocasionalmente estas muestras podrán ser cedidas a grupos de investigación fuera de España, siempre que cumplan los requisitos de la legislación española y lo aprueben los correspondientes comités.
A partir de las muestras dadas, en los casos en que la investigación lo requiera, se realizarán estudios genéticos, y a partir de ellos se puede obtener información sobre su salud y la de sus familiares. Siempre se actuará velando por la protección de esta información (apartado protección de datos).
En el caso de ser necesaria alguna muestra adicional, la institución sanitaria se podría poner en contacto con usted para solicitarle nuevamente su colaboración. En este caso se le informará de los motivos y se le solicitará de nuevo su consentimiento.

PROTECCIÓN DE DATOS Y CONFIDENCIALIDAD: las muestras se conservaran codificadas.

Los datos personales que se recojan serán obtenidos, tratados y almacenados cumpliendo en todo momento el deber del secreto, de acuerdo con la legislación vigente en materia de protección de datos de carácter personal. La identificación de las muestras biológicas del Biobanco será sometida a un proceso de codificación. A cada muestra se le asigna un código de identificación, que será el utilizado por los investigadores. Sólo el personal autorizado por el Biobanco podrá relacionar su identidad con los citados códigos. Mediante este proceso los investigadores que soliciten muestras al Biobanco no podrán conocer ningún dato que revele su identidad. Del mismo modo, aunque los resultados obtenidos de la investigación realizada con sus muestras se publiquen en revistas científicas, su identidad no será facilitada. En aquellos estudios en los cuales no se prevean resultados potencialmente útiles para su salud, y de acuerdo con el correspondiente Comité de Ética, las muestras y datos podrán ser anonimizadas, es decir, no habrá ninguna posibilidad de volver a asociar la muestra con su identidad.
Sus muestras y datos clínicos asociados a las mismas pasarán a formar parte del fichero del Biobanco, inscrito en la Agencia de Protección de Datos bajo la responsabilidad del Instituto de Investigación Biomédica de Girona (IDIBGI).
Usted podrá ejercer sus derechos de acceso, rectificación, cancelación y objeción, así como obtener información sobre el uso de sus muestras y datos asociados, dirigiéndose a:

DIRECCIÓ DEL BIOBANC IDIBGI	Avinguda de França s/n
Hospital Universitari de Girona Dr Josep Trueta	17007 Girona
biobanc@idibgi.org	Tfn 972 940 282

CARÁCTER ALTRUISTA DE LA DONACIÓN: La cesión de muestras biológicas que usted realiza al Biobanc IDIBGI es gratuita.

La donación tiene por disposición legal carácter altruista, por lo cual usted no obtendrá ni ahora ni en el futuro ningún beneficio económico de la misma, ni tendrá derechos sobre posibles beneficios comerciales de los descubrimientos que se puedan conseguir como resultados de la investigación biomédica.

PARTICIPACIÓN VOLUNTARIA: Su negativa NO repercutirá en su asistencia médica, presente o futura.

Su participación es totalmente voluntaria. Si firma el consentimiento informado, confirmará que desea participar. Puede negarse a participar o retirar su consentimiento en cualquier momento posterior a la firma sin tener que explicar los motivos y que esto repercuta en su asistencia médica, presente o futura.

REVOCACIÓN DEL CONSENTIMIENTO: si usted decide firmar este consentimiento podrá también cancelarlo libremente. Esto comportará la destrucción de sus muestras.

Si en un futuro usted quisiera anular su consentimiento, sus muestras biológicas serían destruidas y los datos asociados a las mismas serán retirados del Biobanco. También podría solicitar la anonimización de las muestras, de forma que en este caso se eliminaría la relación entre sus datos personales (que revelan su identidad) y sus muestras biológicas y datos clínicos asociadas.

Los efectos de esta cancelación o anonimización no se podrían extender a la investigación que ya se haya realizado. Si deseara cancelar el consentimiento, lo tendría que solicitar por escrito a la Dirección del Biobanc IDIBGI, a la dirección anteriormente mencionada.

INFORMACIÓN SOBRE LOS RESULTADOS DE LA INVESTIGACIÓN: se le proporcionará información si usted la desea recibir.

En el supuesto de que usted lo pida expresamente, el Biobanco podrá proporcionar información sobre cuáles son las investigaciones en que se han utilizado sus muestras y de los resultados globales de estas investigaciones, excepto en el caso de cancelación o anonimización.
Los métodos utilizados en investigación biomédica suelen ser diferentes de los aprobados para la práctica clínica, por el que no tienen que ser considerados con valor clínico para usted. A pesar de esto, en el supuesto de que estas investigaciones proporcionen datos que pudieran ser clínica o genéticamente relevantes para usted e interesar a su salud o a su familia, le serán comunicados si así lo estima oportuno. Así mismo, podría darse el caso de obtenerse información relevante para su familia. En este supuesto, le corresponderá a usted decidir si quiere o no que esta información le sea comunicada. En caso afirmativo, tiene que consignarlo a la casilla que aparece al final de este documento.
Si usted no desea esta información, tenga en cuenta que la ley establece que, cuando la información obtenida sea necesaria para evitar un grave perjuicio para la salud de sus familiares biológicos, un Comité de expertos estudiará el caso y tendrá que decidir si es conveniente informar a los afectados o en sus representantes legales.

Por favor, pregunte al personal sanitario que le ha comunicado esta información sobre cualquier duda que pueda tener, ahora o en el futuro, en relación a este consentimiento. Así mismo, puede comentar sus dudas a su médico, que le pondrá en contacto con el personal sanitario autorizado.

Muchas gracias por su colaboración.

BIOBANC IDIBGI

Le agradecemos su desinteresada colaboración con el avance de la ciencia y la medicina.
De esta manera está colaborando para vencer las enfermedades y
ayudar a multitud de enfermos actuales y futuros.

Ejemplar DONANTE

CONSENTIMENTO INFORMADO

OBTENCIÓN Y UTILIZACIÓN DE MUESTRAS BIOLÓGICAS Y DATOS CLÍNICOS PARA INVESTIGACIÓN MÉDICA Y CONSERVACIÓN EN EL BIOBANC IDIBGI

Si ha comprendido la información que se le ha proporcionado en el documento informativo, resuelto cualquier duda que pudiera tener y decide colaborar con el Biobanc IDIBGI en los términos antes explicados, por favor, lea y firme a continuación esta hoja:

Quién firma el presente documento autoriza a su centro hospitalario de referencia (...*indicar centro*) a obtener la muestra biológica adicional de ....*6 ml de sangre*......... para que puedan ser incorporada a la **colección GET** del Biobanc IDIBGI, el cual podrá almacenar y utilizar científicamente tanto la información clínica y asistencial de su historial médico como las pruebas de imagen y las muestras biológicas obtenidas, con el fin de desarrollar proyectos de investigación biomédica, siempre que estos cuenten con la obligada aprobación del Comité de Ética de Investigación competente.

Confirmo que:

1. Autorizo que la muestra biológica cedida y la información clínica asociada se utilice en investigaciones:
   Nacionales: ☐ SI ☐ NO Internacionales: ☐ SI ☐ NO
2. Deseo que se me comunique la información derivada de la investigación que realmente sea relevante y aplicable para mi salud o la de mi familia:
   ☐ SI ☐ NO Teléfono o email de contacto...................................
3. Autorizo a ser contactado en el caso de necesitar más información o muestras biológicas adicionales:
   ☐ SI ☐ NO Teléfono o email de contacto...................................
4. He expresado mi deseo de que se me respeten las siguientes excepciones respecto al objetivo y métodos de las investigaciones:...
   ....

DONANTE DE MUESTRA	PERSONA QUE INFORMA	☐ TESTIGO [(1)] / ☐ TUTOR [(2)]
Nombre *Apellidos* *DNI* *Edad* *Firma*	*Nombre* *Apellidos* *DNI* *Edad* *Firma*	*Nombre* *Apellidos* *DNI* *Relación con el donante:* *Firma*

(1) Autorizado por el donante
(2) Representante legal

A rellenar en el hospital de origen

☐ PACIENTE
☐ DONANTE

A ...., a ....de.................de.........

Llegada la mayoría de edad, el donante tiene derecho a la anulación del consentimiento. En caso de que no lo ejerza, se considerará que el actual documento de consentimiento continúa vigente.

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Ejemplar HOSPITAL

CONSENTIMENTO INFORMADO

OBTENCIÓN Y UTILIZACIÓN DE MUESTRAS BIOLÓGICAS Y DATOS CLÍNICOS PARA INVESTIGACIÓN MÉDICA Y CONSERVACIÓN EN EL BIOBANC IDIBGI

Si ha comprendido la información que se le ha proporcionado en el documento informativo, resuelto cualquier duda que pudiera tener y decide colaborar con el Biobanc IDIBGI en los términos antes explicados, por favor, lea y firme a continuación esta hoja:

Quién firma el presente documento autoriza a su centro hospitalario de referencia (...*indicar centro*) a obtener la muestra biológica adicional de .... *6 ml de sangre*.................. para que puedan ser incorporada a la **colección GET** del Biobanc IDIBGI, el cual podrá almacenar y utilizar científicamente tanto la información clínica y asistencial de su historial médico como las pruebas de imagen y las muestras biológicas obtenidas, con el fin de desarrollar proyectos de investigación biomédica, siempre que estos cuenten con la obligada aprobación del Comité de Ética de Investigación competente.

Confirmo que:

1. Autorizo que la muestra biológica cedida y la información clínica asociada se utilice en investigaciones:
   Nacionales: ☐ SI ☐ NO Internacionales: ☐ SI ☐ NO
2. Deseo que se me comunique la información derivada de la investigación que realmente sea relevante y aplicable para mi salud o la de mi familia:
   ☐ SI ☐ NO Teléfono o email de contacto...................................
3. Autorizo a ser contactado en el caso de necesitar más información o muestras biológicas adicionales:
   ☐ SI ☐ NO Teléfono o email de contacto...................................
4. He expresado mi deseo de que se me respeten las siguientes excepciones respecto al objetivo y métodos de las investigaciones:...
   ....

DONANTE DE MUESTRA	PERSONA QUE INFORMA	☐ TESTIGO [1] / ☐ TUTOR [2]
Nombre *Apellidos* *DNI* *Edad* *Firma*	*Nombre* *Apellidos* *DNI* *Edad* *Firma*	*Nombre* *Apellidos* *DNI* *Relación con el donante:* *Firma*

[1] Autorizado por el donante
[2] Representante legal

A rellenar en el hospital de origen

☐ PACIENTE
☐ DONANTE

A ...., a ....de..................de.........

Llegada la mayoría de edad, el donante tiene derecho a la anulación del consentimiento. En caso de que no lo ejerza, se considerará que el actual documento de consentimiento continúa vigente.

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