CD200 GLYCOPROTEIN GENOTYPE'S ROLE IN THE IMMUNE RESPONSE TO HLA-IDENTICAL SIBLING HEMATOPOIETIC STEM CELL TRANSPLANT

A multicenter retrospective cohort study

FINAL DEGREE PROJECT

MARINA CARRACEDO ANDRÉS

CLINICAL TUTOR: DR. DAVID GALLARDO GIRALT

METHODOLOGICAL TUTOR: DRA. RUTH MARTÍ LLUCH

Universitat de Girona
Facultat de Medicina

A Girona y su gente, por todo lo que me ha dado y me hecho crecer.

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Lo voy consiguiendo.

¡Leña lobera!

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1. ABBREVIATIONS

ADAM28: a disintegrin and metalloprotease domain

ALL: acute lymphoblastic leukemia

AML: acute myeloid leukemia

APC: Antigen-Presenting cell

BL: B lymphocytes

C/EBP-β: CCAAT / Enhancer Binding Protein Beta

CLL: chronic lymphocytic leukemia

CTL: cytotoxic T lymphocytes

DC: dendritic cells

DFS: disease-free survival

G-CSF: Granulocyte Colony-Stimulating Factor

GVHD: graft-versus-host disease

GVT: graft-versus-tumor

HSC: Hematopoietic stem cell

HSCT: Hematopoietic stem cell transplantation

IDIBGI: Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta

IDO: Indoleamine-2,3-dioxygenase

IFN γ: interferon gamma

IgSF: immunoglobulin supergene family

LECs: lymphatic endothelial cells

MDSCs: Myeloid-derived suppressor cells.

MM: multiple myeloma

MMP: matrix metalloproteinase

NK: NK lymphocytes

NLRs: NOD-Like Receptors

OS: overall survival

PBPCs: peripheral blood progenitor cells

SF2/ASF: Splicing Factor 2/ Alternative Splicing Factor

TAMCs: tumor-associated myeloid cells

TL: T lymphocytes

TLRs: Toll-Like Receptors

TNFα: Tumor Necrosis Factor Alpha

Treg: regulatory T cells.

TRM: transplant- related mortality

UCB: umbilical cord blood

2. ABSTRACT

BACKGROUND: Hematopoietic stem cell transplantation is the preferred therapeutic approach with curative potential for several hematological and non-hematological pathologies. CD200 is a glycoprotein part of the immunoglobulin supergene family (IgSF) and its binding to its receptor results in a downregulation of the immune system. There is very little information regarding CD200 polymorphisms, and their biological implications remain unclear. It has been suggested that rs1131199 polymorphism could influence the extent of regulation of the CD200 protein's function. CD200 polymorphisms association with complications after allogeneic hematopoietic stem cell transplantations for any cause has not been addressed.

HYPOTHESIS AND OBJECTIVE: The purpose of this study is to evaluate the association of donor's CD200 genotype with overall survival in receptors of HLA-identical sibling donor allogeneic hematopoietic cell transplantation during the years 1991-2015 in hospitals where the "Grupo Español de Trasplante Hematopoyético (GETH)" works.

Secondary objectives include analyzing the association of donor's CD200 genotype with acute GVHD development, relapse, transplant-related mortality (TRM) and disease-free survival (DFS) in receptors of HLA-identical sibling donor allogeneic hematopoietic cell transplantation during the same years in the same hospitals.

METHODS: For this aim, multicenter retrospective cohort study is designed in which 1091 will be included. Their donors' CD200 polymorphism rs1131199 (C or G) will be determined via Allelic discrimination. Overall survival and other clinical outcomes will be compared in a group of patients whose donor was homozygous for allele G against a group of patients whose donor was homozygous for the allele C or heterozygous C/G.

KEYWORDS:

CD200, rs1131199 polymorphism, allogeneic HSCT, HLA-identical sibling donor, acute GVHD, leukemia, myelodysplastic syndromes, myeloablative regimen, T depletion.

3. INTRODUCTION

3.1. HEMATOPOIESIS

We refer to hematopoiesis as a dynamic process in which starting from a common undifferentiated progenitor -the stem cell-, different lineages of circulating blood cells are produced (1). It takes place in several organs: bone marrow, spleen, liver, thymus and lymphatic nodes (2).

Stem cells (which express CD34) have two main properties, which are (2,3):

A. **Differentiation** into specific cell lines, each of them with functions and phenotypes that differ from the progenitor.

   At the beginning of the process, there are two principal lines:

   - **Lymphoid**: the common lymphoid progenitor, which later differentiates into B lymphocytes (BL), T lymphocytes (TL) and spontaneous cytolytic lymphocytes (NK).
     - BL take part in the production of immunoglobulins.
     - TL CD4, also called TL helpers, take part in the activation of BL and macrophages by releasing cytokines. TL CD8, also called cytotoxic, take part in the defense against intracellular pathogens.
     - NK take part in the defense against cells recognized as foreign, either by direct action or through antibody-mediated cellular cytotoxicity.
   - **Myeloid**: the common myeloid progenitor, which later differentiates into monocytes, neutrophils, basophiles, eosinophiles, megakaryocytes (later fragmented in platelets) and erythrocytes (2).

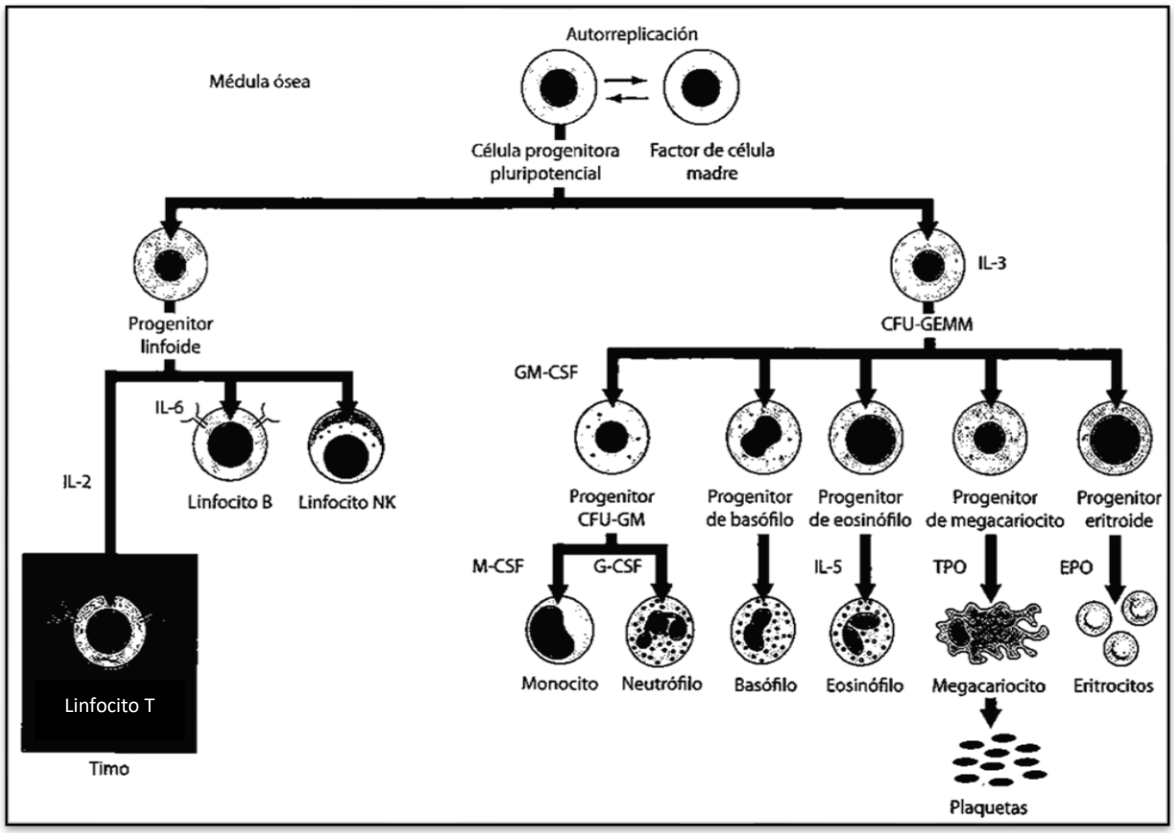

Figure 1. Hematopoiesis process. Adapted from (2)

B. **Self-renewal**. Stem cells have a high proliferating (also called self-renewal) capacity. There are several types of stem cells, with also different potency abilities:

- Pluripotent cells, originated from the embryos, can develop into any of the three germ layers- endoderm, mesoderm and ectoderm.
- Multipotent cells can only differentiate into tissues from one of the previously mentioned germ layers.
- Oligopotent cells can only develop into cells of one specific tissue (4).

3.2. HEMATOPOIETIC STEM CELL TRANSPLANTATION

Hematopoietic Stem Cell Transplantation (HSCT) is a treatment strategy consisting of infusing hematopoietic stem cells (HSC) into patients suffering from hematological and chemotherapy-treated solid cancers or other non-

hematological disorders (such us immunodeficiencies, metabolism innate errors or autoimmune diseases) as a potentially curative treatment, by the rebuild of hematopoiesis and the replacement of damaged (cancerous or not) hematopoietic cells (5,6).

During the year 2022, 46.143 HSCT were documented by 689 European transplantation centers. The 58,8% (27.132) of them were autologous transplantations and the remaining 41,2%, were allogeneic (19.011) (7).

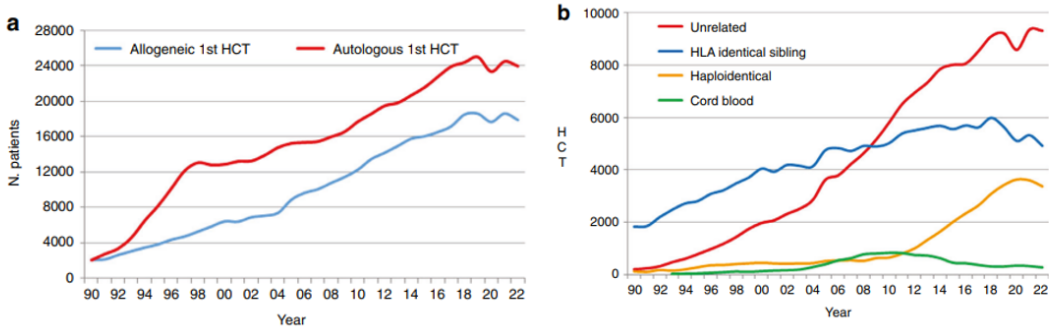

Figure 2. Hematopoietic stem cell transplantation. Extracted from (7).

"A" represents absolute numbers of conducted HSCT, and "B" represents the HSC source chosen between years 1990 and 2022.

As we see in graph B, in allogeneic hematopoietic stem cell transplantations, unrelated donors are the most frequent ones, followed by HLA-identical siblings, haploidentical and umbilical cord blood. The year 2022 was the last one analyzed and published (7).

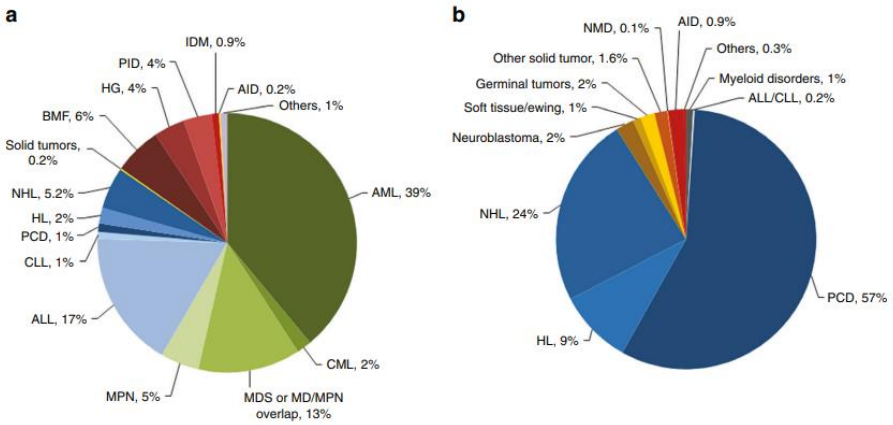

Figure 3. Disease indications for Hematopoietic Stem Cell Transplantation (7)

Myeloid malignancies are represented in green, lymphoid malignancies in blue, solid tumors in brown and non-malignant pathologies, in red. "A" represents allogeneic 1º HSCT and "B", autologous 1º HSCT.

3.2.1. SOURCES OF HEMATOPOIETIC STEM CELLS

- **Bone marrow**

Marrow has historically served as the stem cell source for HSCT. The collection consists of 3-5 aspirations placing wide-gauge needles bilaterally into the patient's posterior iliac crests or the sternum while the patient is sedated with local or general anesthesia. Trocars or drivers are used to penetrate the bone and soft tissues before inserting the aspirating needle. Anesthesia and trocar placement are performed with more precision when fluoroscopy or ultrasound are applied to guide (8,9).

The minimum acceptable dose is 2 x 10^8 nucleated cells/ kg of the receptor's body weight and a safe procedure extracts 20mL/kg of the donor's body weight. Adverse reactions are infrequent (9).

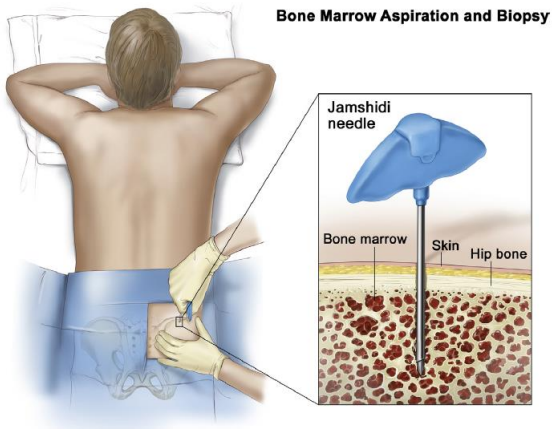

Figure 4. Representation of patient's positioning lying in prone and the needle used for a bone marrow aspiration (8)

- **Peripheral blood**

After a stimulus -generally G-CSF (with the possibility of adding chemotherapy in case of an autologous HSCT)-, mobilization of hematopoietic stem cells to peripheral blood is produced, where they are known by "peripheral blood progenitor cells (PBPCs)" and extracted by leukapheresis.

The minimum acceptable dose is 2 x 10^6 CD34+ cells/ kg of the receptor's body weight and increased HSC concentrations are correlated with a quicker

engraftment. Therefore, 4 x 10^6 CD34+ cells/ kg is seen as optimal, but with higher doses than those, GVHD risk significantly increases due to higher T cell (more regulatory T cells (Treg) and Th2 response) recollection, so they are not recommended.

A frequent adverse reaction is G-CSF- associated bone pain (9).

- **Umbilical cord blood (UCB)**

UCB is obtained from the umbilical vessels right after parturition. It contains high amounts of stem cells lacking from prior immunological exposures, and thus immature which allows its infusion to an HLA- differing patient (9).

Even though UCB is abundant in HSC, the volume of a sample obtained from a newborn size cannot supply the HSC needs of an adult receptor and thus, 2 UCB units are usually infused (9). These low levels of HSC trigger an extended time for neutrophil engraftment and therefore, more infective complications (10).

The 1 UCB unit minimum acceptable dose is 2,5 x 10^7 total nucleated cells or CD34+ cells/ kg of or 2 x 10^5 CD34+ cells/ kg of the receptor's body weight (9).

In the context of autologous transplantation, PBPCs are preferred over a marrow source, whereas when an allogeneic HSCT is performed, other data (such as the disease, the preparative regimen) are taken into account to choose among the two sources.

Here is a table that summarizes the previous information:

Table 1. HSC sources. Adapted from (5,9–11)

	LIMITATIONS	STRENGTHS	USES
BONE MARROW	- Slower and wicker engraftment. - More graft failure - Requirement of anesthesia. - More transplant-related mortality (TRM)	- Less GVHD and adverse reactions	- Average- risk hematological cancers - Non- malignant diseases - Reduced- intensity conditioning (RIC)
PERIPHERAL BLOOD (PBPCs)	- More GVHD (due to higher T cells) - Possible bone pain after G-CSF - More immunosuppression	- Quicker and stronger engraftment (less neutropenia) -Lower costs - Possibly lower recurrence rates. - Less TRM - No anesthesia required	- Autologous HSCT - Advanced or high-risk hematological cancers
UMBILICAL CORD BLOOD (UCB)	- Small sample compared to adult size (requirement of 2 UCB units) -More infections	- Could be used in mismatched HLA receptors.	- Alternative source when HLA related or unrelated donors lack.

3.2.2. TYPES OF TRANSPLANTATIONS

Autologous HSCT consists of the infusion of the patient's endogenous cells, whereas in allogeneic transplantation, the cells proceed from an unrelated, an HLA-identical sibling (who shares both of the haplotypes with the patient) or an haploidentical donor (who shares only one HLA haplotype) (5,7,12).

- **Autologous HSCT**

Its main purpose is to **allow myeloablative regimens** with elevated doses of chemotherapy by then restarting the hematopoiesis in the damaged bone marrow (13).

HSC are extracted from the patient before the chemotherapy (from a peripheral blood source as explained before) and later administered back after the myeloablation. Post-HSCT **immunosuppression is not required** (5).

The phases of the procedure would be the following ones:

Table 2. Autologous HSCT phases. Adapted from (13)

1. HSCs mobilization
2. **PBPCs leukapheresis**
3. **Conditioning chemotherapy**
4. **Reinfusion of HSC**
5. Support until immunity recovery

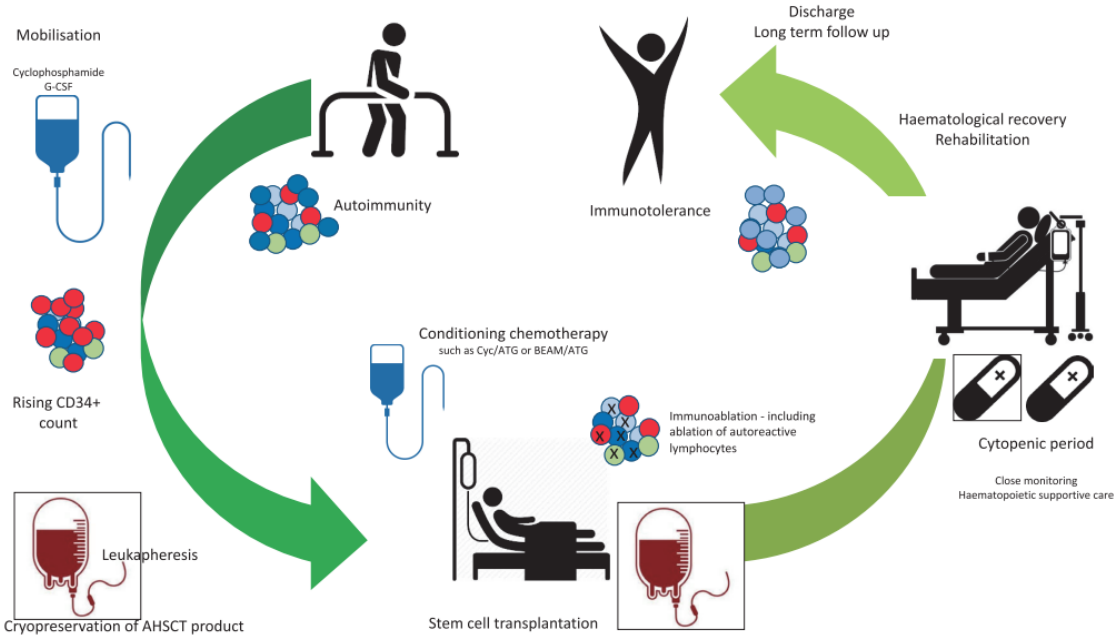

Figure 5. Autologous hematopoietic stem cell transplantation phases (13)

Some solid and hematological malignancies benefiting from this elevated-dose chemotherapy are: recurrent or refractory Hodgkin or not Hodgkin lymphoma, MM, central nervous system and testicular tumors, and to consolidate some acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (5).

Autologous HSCT have shown decreased infections and transplant-related mortality, but an increased disease relapse (5).

- **Allogeneic HSCT**

Unlike autologous HSCT, when an allogeneic donor is involved, a conditioning regimen and post-infusion treatments which cause the **receptor's immunosuppression** are mandatory to successfully achieve the donor's engraftment (and to prevent rejection mediated by T cells and NK which did not respond to previous conditioning) (5,9).

The key process through which allogeneic HSCT performs its function is by the beneficial **graft-versus-tumor (GVT) effects**, which consist of donor cells identifying and eliminating host's remnant malignant cells. T cells play a key role in GVT, but as they are involved, there are more chances to induce a certain level of alloreactivity and generate GVHD (9).

Some hematological malignancies benefiting from this elevated-dose chemotherapy are: acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), myelofibrosis and myelodysplastic syndromes (MDS) (5).

Allogeneic HSCT are associated with higher complications and TRM rates and require preparative regimens and an induced downregulation of the immune system after the procedure to prevent GVHD (9).

- **Syngeneic HSCT**

Donors and receptors share the exact same genotype, as they are identical twins. They are recognized as the most optimal donors for transplantation. A decreased TRM but an augmented tumor relapse rate have been associated (as there is less alloreactivity and thus less GVT effects) (9,14,15).

3.2.3. HSCT PREPARATIVE REGIMENS

Conditioning regimens are implemented before the hematopoietic cell transplantation targeting two main objectives:

- Decrease tumor burden.
- Avoid graft rejection by inducing medullary aplasia and thereby myelosuppression (16).

- ***Myeloablative (high-dose conditioning regimens)***

Myeloablative regimens, which are composed of chemotherapeutic alkylators with or without total-body irradiation (TBI), suppress the bone marrow and therefore, hematopoiesis, generating a deep aplasia involving all cell lineages (16).

Autologous transplantations widely use this regimen because, as explained, the reinfusion of HSC after high-dose conditioning restarts hematopoiesis in the damaged bone marrow, so this allows to maximize the dose-response relationship (9,13).

- ***Total-body irradiation (TBI)***

TBI can enter sanctuary sites, such as the central nervous system and gonads. It has highly immune system downregulation attributes. Most preparation regimens use 12 or 13,2Gy. Higher doses are correlated with more toxicity, TRM and GVHD but less relapse rates (9).

- ***Chemotherapy***

There are several different options. One of them is cytarabine combined with TBI or with other chemotherapeutic agents, such as busulfan (chemotherapy- only conditioning) (9).

- ***Non-myeloablative regimens and Reduced-intensity conditioning (RIC)***

Non-myeloablative preparations trigger slight cytopenias without the need for a posterior HSCT (16).

Allogeneic transplantations could use RIC (resulting in less toxicity and myeloablation) considering the key role of the alloimmune effects the donor's HSC would exert against malignancies (GVT response) (9). RIC regimens do not cause severe cytopenias, but need a posterior HSC infusion due to the **prolonged cytopenias** it triggers (16).

Reduced-intensity conditioning has remarkable strengths, such as lower treatment-associated-toxicities and lower late effects, lower GVHD rates and the treatment enablement of more **fragile and older people** who could not benefit from myeloablative regimens (9,17).

Nevertheless, it also has some limitations, like the demand of increased numbers of HSC to achieve engraftment and immune recovery, the need of a robust GVT response and the weakened tumor burden eliminating response (9,18).

As lower chemotherapy doses are administered, part of the patient's hematopoiesis remains, which leads to a **"mixed donor/host chimerism (5-95% are donor's T cells)"** and thus more relapse rates because of a decreased GVT effect exert by the donor's cells. Immunosuppressive medications are required after RIC to reach a complete donor's HSC engraftment (≥95% are the donor's T cells) (9,19).

3.2.4. COMPLICATIONS OF HSCT

A table is provided below listing the main complications of the hematopoietic stem cell transplantation:

Table 3. Main hematopoietic stem cell transplantation complications. Adapted from (9)

Acute graft-versus-host disease	**Hepatic complications** -Sinusoidal obstructive syndrome -Hepatitis: infectious or noninfectious
Blood group incompatibilities and hemolytic complications	**Infectious complications** (bacterial, fungal, cytomegalovirus, herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, adenovirus, respiratory viruses, human herpesvirus- 6, 7 or 8 and other viruses)
Chronic graft-versus-host disease	**Kidney and bladder complications**
Drug-drug interactions	**Late onset nonmalignant complications** (osteoporosis/osteopenia, avascular necrosis, dental problems, cataracts, chronic fatigue, psychosocial effects and rehabilitation)
Endocrine complications	**Lung injury** (interstitial pneumonitis, diffuse alveolar hemorrhage, engraftment syndrome, bronchiolitis obliterans)
Gastrointestinal complications -Mucosal ulceration/bleeding -Nutritional support	**Neurologic complications** (infectious, transplant conditioning and immune suppression medication toxicities)
Graft failure	**Subsequent malignancies**
Growth and development	**Vascular access complications**

- **Graft failure**

Graft failure consists on an insufficient engraftment of the infused donor's HSC in the host's bone marrow, which results on high morbimortality (owing to the immune system downregulation and prolonged cytopenias) and higher relapse rates (as a consequence of less graft-versus-tumor effect) (20).

Primary graft failure is characterized as the lack of accomplishment during the 28 days after a HSCT of all the listed below items specific to the administered conditioning regimen (9).

Table 4. Primary graft failure characterization items. Adapted from (9)

Myeloablative conditioning	Reduced- intensity conditioning
a. Neutrophiles ≥ 0,5 x 10^9 / L during 3 consecutive days (usually achieved the 21 first days after transplantation)	**Donor chimerism <5%** (less than 5% are the donor's T cells).
b. Platelet ≥ 20, 50 or 100 x 10^9 /L during 7 days without transfusions.	
c. Hemoglobin ≥ 80g / L without transfusions.	

Secondary graft failure, more associated with allogeneic HSCT, consists of the diminishment of donor's cells or its function (of at least 2 of the lines represented in the table above) in a previously engrafted transplantation (9,20).

Graft failure has different therapy options, encompassing growth factors, autologous reconstitution, supplementary hematopoietic progenitor cells infusion or a second HSCT with its corresponding new preparative regimen (21).

▪ Graft-versus-host-disease (GVHD)

Graft-versus-host disease is one of the most common complications of allogeneic HSC transplantations and the main morbimortality reason not associated with relapse (6).

It happens because the donor's immunologically competent cells (mainly mature T lymphocytes) identify the host's tissues as non-self. Therefore, bigger HLA differences (which is the main risk factor) result in higher GVHD rates (6). In the context of matching donor-receptor HLA, hundreds of minor histocompatibility antigens are involved in the development of GVHD. They are peptides displayed in the binding sites of major histocompatibility complex (MHC) antigens, which can be HLA type 1 (displayed in every cell) or HLA type 2 (displayed only on antigen-presenting cells "APC ": DC, macrophages and stimulated T lymphocytes) (9).

GVHD can be labelled as acute or chronic. In the past, this difference was made based on whether the clinic started throughout the first 100 days after the transplantation (considered acute) or after 100 days (named chronic). Nevertheless, the actual diagnoses and classification takes into consideration the clinical and histological aspects,

without the 100 days limit, and an overlap subclassification is also added if chronic and late acute features appear simultaneously. Acute GVHD usually only impacts skin, gastrointestinal tract and liver, whereas the chronic type can be observed in any body part (6,15).

A. ACUTE GRAFT-VERSUS-HOST DISEASE

Its incidence after allogeneic HSCT ranges from 40% to 60% depending mainly on donor-receptor HLA mismatches, but also in other factors such as the patient's age, stage disease, HSC source or the preparation regimen administered (6,9).

Table 5. Acute GVHD phases. Adapted from (9,15)

Pathophysiology of Acute GVHD
1. **Initiation:** The conditioning regimen administered before the allogeneic transplantation harms the receptor's tissues, which results in more exposure of histocompatibility antigens and an elevated pro-inflammatory cytokine secretion (TNFα and IL-1) and the consequent APC stimulation.
2. T cell activation: Donor or receptor's APC present the histocompatibility alloantigens to the donor's mature T lymphocytes, which become activated and start a pro-inflammatory Th1 response involving INF γ and IL-2.
3. Effector phase: Cytokine and cellular effectors (mainly CD4 and CD8 but macrophages and neutrophils too) resulting from the activation explained in the previous phase, facilitate T lymphocytes cytokine-mediated migration to several tissues (typically skin, gastrointestinal tract and liver) harming them through an apoptosis pathway.

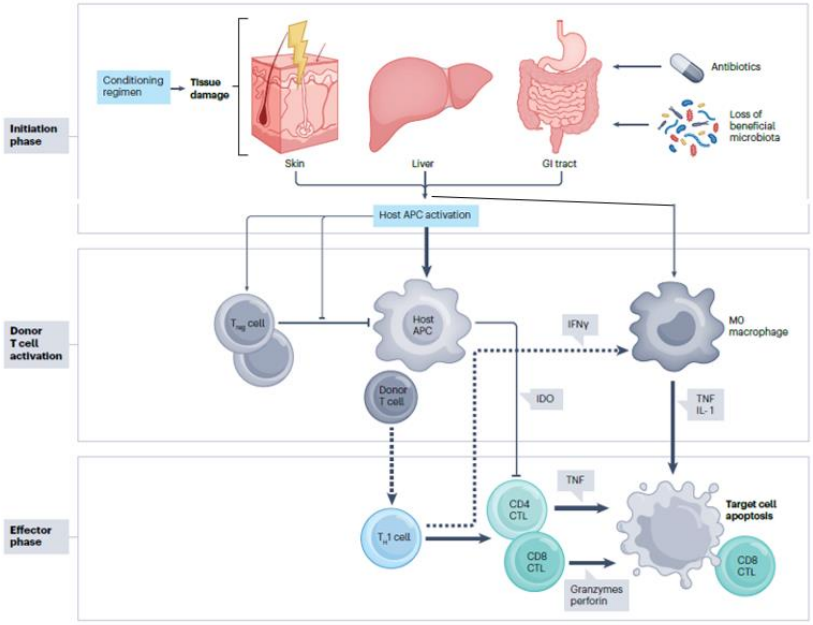

Figure 6. Acute GVHD phases. Adapted from (15)

Its severity can be classified from I to IV depending on organ involvement (considering **skin, bilirubin increase and diarrhea quantity**) (6). ANNEX 1

After an allogeneic HSCT, **immunosuppressive medicines** are necessary to **avoid** an acute graft-versus-host disease. Otherwise, the majority of the patients would suffer it. Several prophylactic regimens have demonstrated advantageous outcomes, being some of the most commonly utilized (9):

- Tacrolimus + methotrexate (MTX) on days 1,3,6, and 11 post-transplantation (9,22).
- Antithymocyte globulin (ATG) + cyclosporine A (CsA) + MTX (22).
- Cyclophosphamide + tacrolimus + mycophenolate mofetil (23).

Strategies involving the **depletion of the donor's T cells** (whether performing ex vivo manipulation of the sample -by filtering it before the HSC infusion- or in vivo, administering for example ATG) have proven **decreased GVHD rates**, but in contrast, graft rejection, relapse and infection rates have augmented (9).

Once it develops, **glucocorticoids** are the selected first-line **treatment**: either oral prednisone or intravenous methylprednisolone (depending on the severity and the organs affected). As a second line, Ruloxitinib is chosen (15).

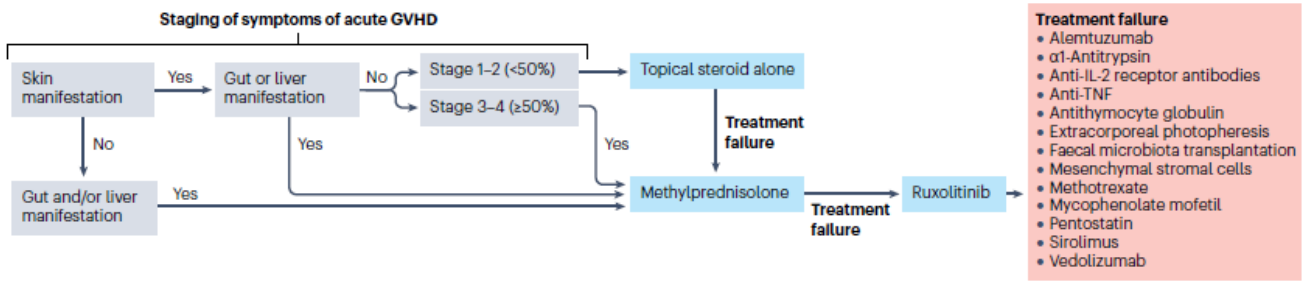

Figure 7. Acute GVHD treatment (15)

B. CHRONIC GRAFT-VERSUS-HOST DISEASE

After an allogeneic HSCT, chronic GVHD may develop due to immune dysfunction and inflammation- fibrosis phenomena, leading to organ damage (24).

There are no established diagnostic biomarkers, so most chronic GVHD assessments rely on **clinical assessments and patient reviews** (24). To determine the chronic GVHD severity, a validated score including general and organ-specific items has been applied (9).

Virtually **every organ can be affected**, highlighting the following findings: lichenoid skin lesions which may evolve to generalized scleroderma, oral lichenoid reactions, vaginal and esophageal stenosis, keratoconjunctivitis sicca, gastrointestinal changes, chronic hepatic disorders and obliterative bronchiolitis (9).

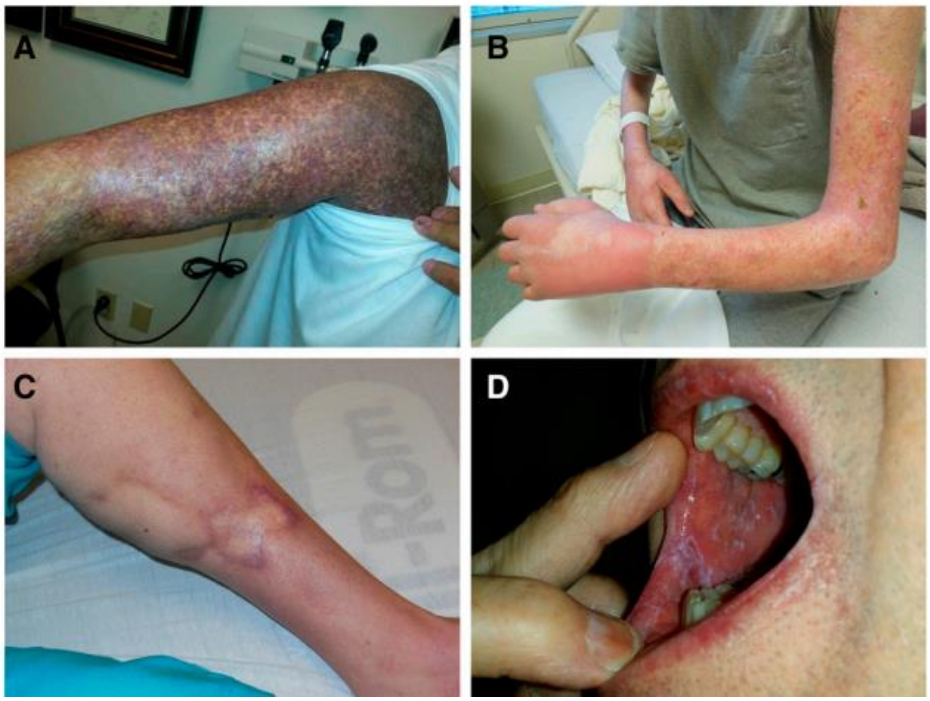

Figure 8. Clinical findings in chronic GVHD. Extracted from (24)

"A" represents lichen planus and poikiloderma, "B" fasciitis and sclerosis, "C" sclerosis and "D" oral lichen planus.

Chronic GVHD has been correlated with higher GVT effects and less relapse rates and is the main complication in long-term allogeneic HSCT survivals. However, its negative effect on TRM (it is the first cause of non-relapse mortality) might surpass the positive reduction of relapse rates (9,24).

90%-95% of the patients develop chronic GVHD during the **first year post-transplantation**, and only 30% have not had a previous acute GVHD (24).

These patients usually need longer immunosuppressive medicines administration (2 years as a median time). Its standard treatment consists on oral **prednisone 1mg/kg/day** accompanied or not with a **calcineurin inhibitor** (such as tacrolimus or cyclosporine), even though 50% of the patients do not achieve an optimal response, and need second-line treatment (such as the frequently used Ibrutinib) (9).

3.2.5. HSCT PHASES

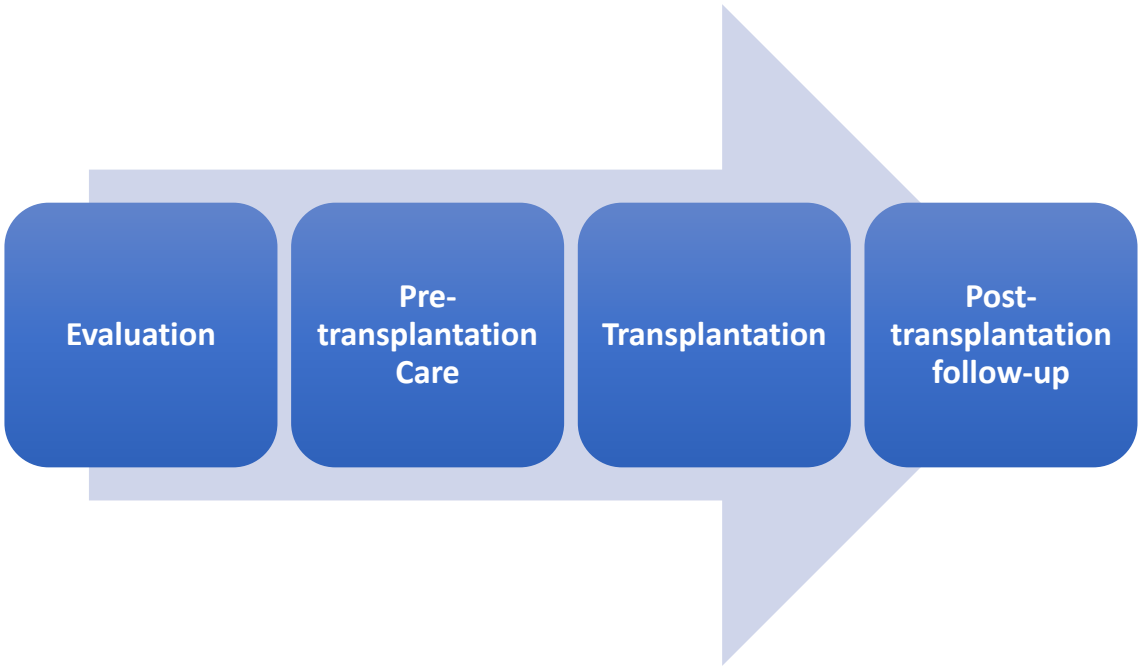

Figure 9. HSCT phases. Adapted from (25)

- ***Evaluation***

This phase determines if a patient (and its donor if it is not an autologous transplantation), meets criteria for the HSCT procedure and if the transplantation is the best therapeutic approach for the patient. It includes:

- HSCT consultation
- Typification of HLA from related or unrelated donors (25).

- ***Pre-transplantation Care***

After the patient is recognized as potentially eligible to receive a HSCT, preparation prior to the initiation of the conditioning regimen begins. It includes:

- Disease severity determination (using CT scans, bone marrow biopsies, serum protein analysis via electrophoresis…).
- Functional test to assess organ performance (lungs, heart, liver, kidneys…).
- Psychosocial evaluation.
- Additional evaluations to determine the donor and patient's suitableness and eligibility (25).

▪ *Transplantation event*

This phase includes from the start of the conditioning regimen until 30-120 days post-transplantation. It covers HSC infusion and its associated hospitalization or outpatient visits. It includes:

- HSC mobilization and collection.
- Administration of the conditioning regimen.
- Conditioning regimen-hospitalization or outpatient visits.
- HSC infusion.
- Post-transplantation-hospitalization or outpatient supportive care.
- Handling complications related to the transplantation (25).

▪ *Post-transplantation follow-up*

Once the transplantation event concludes, this phase begins until the patient is released from regular follow-up care at the transplantation center. Its aim is to ensure the patient's long-term recovery.

- Hospitalization or outpatient supportive care.
- Handling complications related to the transplantation (management of chronic GVHD, vaccinations…)(25).

There is **no standardized consensus** or guidelines for eligibility criteria, so the decision is taken by the transplant center and team through a complicated multidisciplinary evaluation. Parameters taken into account are **disease-related factors** (diagnosis, remission status and Disease risk index) **and patient-related factors**, such as physiologic (age and comorbidities), psychosocial, and economic (26).

- Some examples of scales applied to assess the comorbid condition are Karnofsky Performance Status (KPS), Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) and the frailty score (26).
- **Most hospitals do not consider a strict age limit**. High-dose conditioning was a limitation due to its more pronounced toxicities less well-tolerated in elderly people, but with RIC regimens before allogeneic HSCT this problem has diminished. However, potentially eligible patients for an autologous HSCT must be capable of coping with high chemotherapy doses (as its benefits against tumors are based on the conditioning regimen administered), so age restrictions are frequently more rigid.

  RIC has effectively been carried out in 90-year- old patients, whereas high-dose conditioning is initially contemplated as an option for age 60-65 or younger (but the current supportive care has allowed to perform myeloablative regimens in older than 70-year-old patients with other favorable disease and comorbid conditions) (9). ANNEX 2

3.3. CD200 PROTEIN

3.3.1. CD200 AND CD200R'S STRUCTURE

CD200 and its receptor, CD200R, are both part of the immunoglobulin supergene family (IgSF). They are transmembrane glycoproteins with the following structure:

- Two **Ig-like extracellular domains** that consist of a variable (V) and a constant (C) region. Through these territories, more specifically through the antiparallel β-sheets named as GFCC' in their NH2-terminal domain, CD200 and CD200R are able to interact with each other. (27)
- **Transmembrane Region**
- **Cytoplasmic tail**. This is where CD200 differs more structurally from CD200R. Human CD200R has a long 67 amino acid tail with two tyrosine residues that are part of a NPxY amino acid region shared by

many transmembrane proteins in its intracellular region. It contains asparagine (N), proline (P), a variable amino acid (x) and tyrosine (Y) (28–30).

Tyrosine residues' phosphorylation start an intracellular signaling cascade by generating a binding location -Y286 and Y297 in this case- for proteins with SH2 or phosphotyrosine-binding domain (27).

CD200's cytoplasmic short 19 aa domain was previously thought not to have any signaling relevance, but it has been discovered to be a binding motif for γ secretase. The interaction causes the fragmented-intracellular tail's translocation and binding to DNA, resulting in an augmented transcription of pro-malignant genes (31).

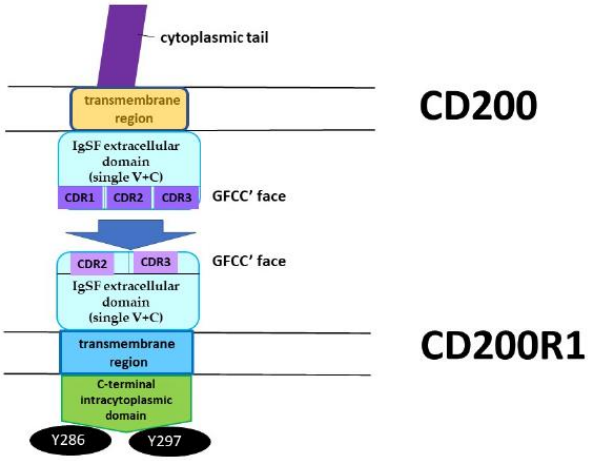

Figure 10. CD200 and CD200R structure (27)

Human CD200, formed by 278 amino acids codified on the chromosome 3 (3q12-q13), is expressed in myeloid and lymphoid cells. It is **constitutively regulated by C/ EPB β** (which is the main transcription regulator, even though there are other molecules such us Sp1, Oct1 and Tst-1)'s interaction with a core promoter region composed by two positive regulatory domains next to the transcriptional initiation site (169bp away), with interaction sites for C/ EPB β. **IFNγ and TNFα** have also been proven to be necessary to regulate CD200's **inducible expression** (27,31).

Its receptor, CD200R, formed by 348 amino acids is encoded by 9 exons at 3q12-13 very near to the CD200 region. There are several isoforms of this receptor (CD200R2, CD200R3, CD200R4 and CD200R5) found in rodent species, but the best distinguished

one and the only one expressed in humans is CD200R1. It can be found in myeloid cells and some T lymphocytes (27).

3.3.2. CD200-CD200R1 INTERACTION

CD200 and its receptor bind at 37ºC with an equilibrium dissociation constant (Kd) of 0,5 µM through their Ig-like extracellular domains (32).

As explained before, there is a specific region, named GFCC' in their NH2-terminal IgSF domain, that has residues with a crucial role: E44, I71, T73, E75 and I133 (27).

Once they bind, CD200R1 tyrosine motifs get phosphorylated and an intracellular signaling cascade causing the inhibition of RAS/MAPK pathway starts. First, the inhibition proteins with phosphotyrosine-binding domains called Dok1 (or Dok-R) and Dok2 (or FRIP) bind to Y297 and Y286. Later, this produces RasGAP and SHIP respectively coupled with the previous molecules, which inhibits Ras pathway, leading to a decreased activation of MAPKs ERK, p38 and JNK (27).

This explanation has been proven in rodent species. Humans follow the same process, except for the Dok1 effects, which are none or indirect, being the signaling cascade mediated mainly by the Dok2 and therefore RasGAP (27).

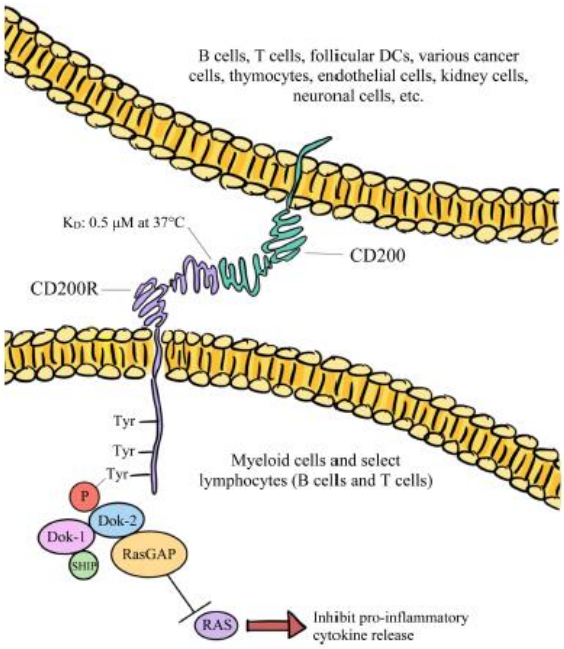

Figure 11. CD200: CD200R interaction and pathway (30)

3.3.3. IMMUNE CELL RESPONSE TO CD200

- **LYMPHOCYTES**

CD200:CD200R1 interaction results in a transition from a mediated Th1 cytokine production to a Th2 cytokine pattern. This leads to a diminished production of pro-inflammatory cytokines (TNFα and INF γ).

It also causes downregulated cytotoxic TL response, which could be explained by a direct suppression due to the presence of CD200R1 in some T cells, or by an indirect inhibition through CD200R's expression in other cells -for example macrophages or DC- (27).

- **MACROPHAGES**

In M1 activated macrophages -through TNFα and INF γ- when CD200:CD200R1 bind, the suppressing Ras pathway -involving Dok2 and RasGAP as explained above- leads to the reduction of its function and the blockade of autoaggressive T cell responses.

We can also see the shift to Th2 immune responses here, because high numbers of CD200R are related to alternatively M2 activated macrophages (through anti-inflammatory cytokines IL4 and IL13), inducing this an augmented Th2 immune response (27).

- **BASOPHILS AND NK CELLS**

CD200:200R1 interaction also inhibited NK and basophil's activity though the suppressed Ras pathway (27).

NK's diminished activity causes an interruption in INF γ secretion, reinforcing the inhibition of Th1 cell response. CD200's expression has also been proven to induce NK cells' apoptosis (30).

- **DENDRITIC CELLS**

CD200's expression in dendritic cells is regulated by different pathways:

- During apoptosis, p53 levels increase, and through the p53 Responding Elements (p53RE) placed in intron 1 of the human CD200 codifying sequency, this causes an overexpression of CD200 in dendritic cells.
- There is evidence that caspases also influence its expression.

These high levels of CD200 in dendritic cells modifies TL activity, which causes a lower production of IFN γ and TNFα, leading to a decreased macrophage activation too (27).

- **MYELO-DERIVED SUPPRESIVE CELLS (MDSCs):**

Like many myeloid lineage cells, MDSCs express CD200R1, and their function is to inhibit immune reactions during diseases, for instance infections, tumors, TPH, autoimmune diseases… They are involved in the downregulation of T cells, the regulation of macrophage and DC's release of cytokines and the upregulation of regulatory T cells (Treg) (27).

Treg consist of CD4 lymphocytes that express Foxp3 transcription factor, and through IL-10, IL-35 and TGF-β cytokines, and other mechanisms, like cytolytic granzymes and perforins, they participate in the suppression of exaggerated immune responses and induce tolerance (33,34).

In the context of the tumor microenvironment, cancerous cells expressing CD200 promote MDSCs proliferation, and through its C200R1, enhances the inhibitory Ras response, perpetuating immunosuppression and favoring cancer growth (27,30).

- **OTHER MOLECULES RELATED TO CD200:**

  - **Indoleamine-2,3-dioxygenase (IDO)**

IDO, present in DC and T cells, catalyzes the transformation of tryptophan to N-formylo-kynurenin. Its overexpression is being studied to be a way in which CD200:CD200R dysregulates the immune system and induces tolerance, mainly after a pro-inflammatory (TNFα and INF γ) cytokine exposure (27).

  - **CD200R's paired receptor "CD200RL".**

The concept "Paired receptor" means that inhibiting receptor CD200R1 is linked to an activating receptor, which is called CD200R1-L (CD200R-like). It can be found in peripheral blood neutrophils and its structure is similar to the CD200R1, but we find differences, like its inability to bind to CD200 or that its cytoplasmic tail does not have the tyrosine residues, although some tyrosine motifs implicated in activating signaling are found in the transmembrane domain. Its activation results in the release of ROS and IL-8 (implicated in neutrophils chemotaxis), which supports the "counterbalancing

theory" that explains how these receptors could participate in immunoregulation through defending against the downregulation of the defenses caused by the pathogens who interact with CD200R1 (30).

- **CD200's splice variant "CD200tr"**

CD200tr is a natural functional variant resulting from CD200's transcription without exon 2 and with a 30 aa shorter N-terminal chain. The splice variant works as the entire glycoprotein's competitive inhibitor, compensating CD200's CTL and IL-2 suppression. As the full-length protein, CD200tr can be found in most human tissues, excluding bones and muscles.

CD200 or CD200tr expression is constitutively regulated by ESE (exonic splicing enhancer) - located in the exon 2 of CD200 – which exerts its function through SF2/ASF protein. A mutation or dysregulation of ESE: SF2/ASF results in more CD200tr and less CD200 expression (27).

- **Toll-Like Receptors (TLRs) and NOD-Like Receptors (NLRs)**

Pathogens' presence activates TLRs and NLRs, which start the innate immune system response and increase CD200 synthesis (and consequently through its pathway, the suppression of macrophage's action). This counteracting feedback prevents bacterial sepsis (27).

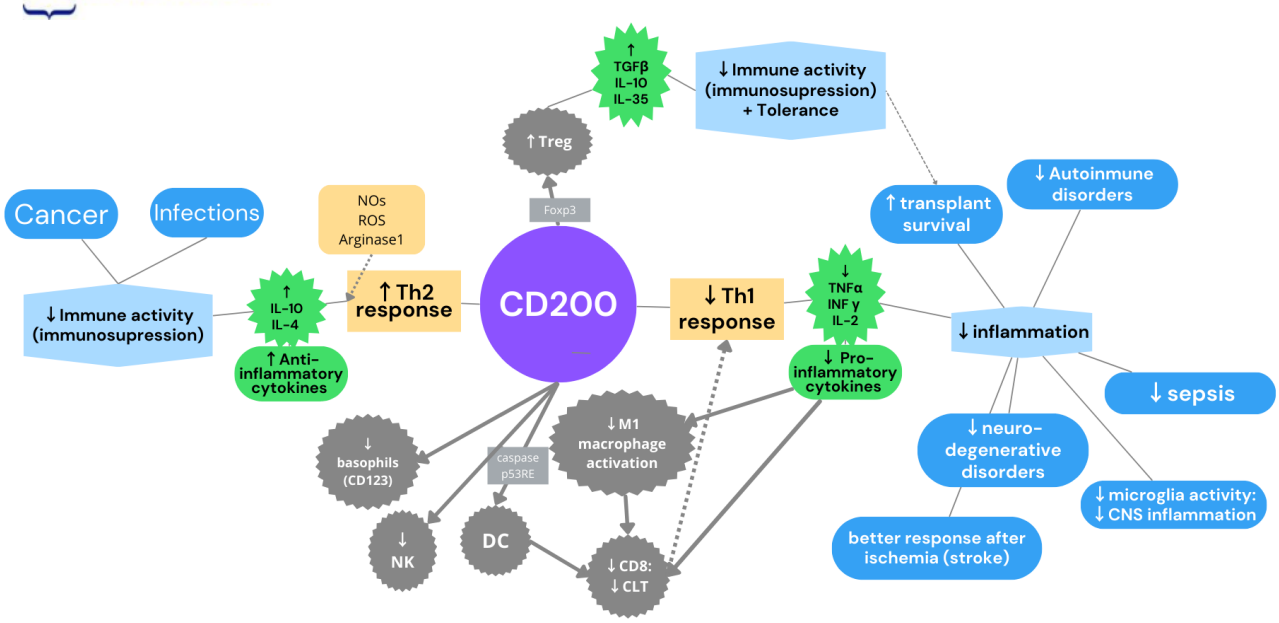

Figure 12. Representation of CD200 responses involving several immune cells, and its biological consequences. Adapted from (27,30)

3.3.4. BIOLOGICAL IMPORTANCE OF CD200

- **RELATED TO TRANSPLANTATIONS:**

CD200 has been shown to perpetuate survival after a transplant, by the previously explained **shift of cytokine release**. Th1 response, involving pro-inflammatory cytokines such as TNFα, IL-2 and INF γ, is inhibited and is polarized towards both a **Th2 immune response** (which involves anti-inflammatory cytokines like IL-4, IL-10) and a TGFβ and IL-10 response that activates **Treg**. These different signaling pathways lead to "tolerogenic" DC and suppression of CTL, and thus a longer allograft survival (27,35).

When an allograft has just been performed, an overexpression of CD200 has been demonstrated necessary to induce **tolerance** to the transplant. Once this is reached, Treg cells can prolongate this tolerance without CD200's action, as long as there is not a secondary severe inflammatory stimulus caused by other circumstances, which breaks this balance and can cause rejection within a previously tolerated graft. Although CD200 presence has shown not to be necessary in the maintenance stage, its pathway activation was proven to be essential for Treg activity and persistent survival in the maintenance period, showing the importance of the CD200:CD200R axis in both induction and maintenance (27,35).

Treg action is elaborated. Investigations suggested they are involved (through IDO and heme oxygenase-1) in modifications of the goal tissue to support tolerance (35).

Other molecules, like the immunoadhesin CD200Fc have been proven to downregulate alloimmunity, which also prolongates graft survival. Immunoadhesin CD200Fc consists of an IgFc domain artificially joined to the extracellular CD200 territory (27).

Some research about human renal transplants concluded that an increased CD200R1/CD200 levels before the procedure anticipate immunocompetence in the host and thus later acute rejection to the transplant. However, these same increased levels after the transplant, anticipate in a non-invasive way, immunosuppression and CMV reactivation and longer time of infection. Post- transplantation monitoring could be adjusted to the numbers of CD200 / CD200R1, performing biopsies (the gold standard acute rejection diagnosis tool), where in cause of rejection, we could see monocyte infiltration. In CMV infections, activated macrophages are also involved, as explained in the next chapters (36).

▪ RELATED TO CANCER

CD200 participates in the tumor microenvironment promoting its development in different ways:

- Tumorigenic signaling pathways: being the main one, initiated by CD200: CD200R1 binding.
- Angiogenesis of new blood vessels supplying the tumor.
- Cancer-associated inflammation: it is regulated by TAMCs (tumor-associated myeloid cells). DC, Tumor-associated macrophages (TAMs) and MDSCs are part of the TAMCs (30,37).

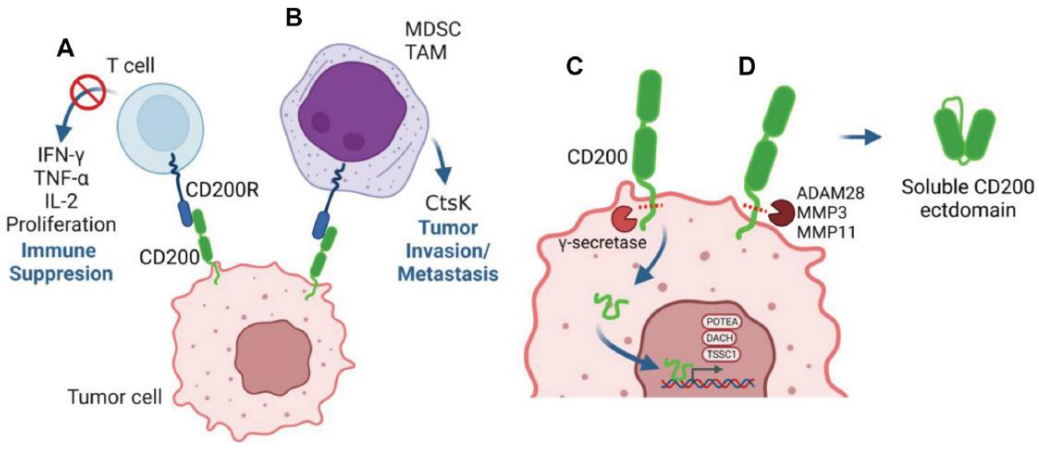

Figure 13. Different vias CD200 exerts its pro-malignant function (31)

As we see in the picture above, CD200 has different vias to exert its pro-malignant function:

a. **CD200:CD200R1 interaction**, through the polarization of the cytokine release with more Th2 anti-inflammatory response (and inhibition of the Th1 response and its pro-inflammatory TNFα, IL-2 and INF γ release) - caused by the suppression of the Ras pathway involving Dok2 and RasGAP- , perpetuates the downregulation of the immune system and facilitates cancerous cells growth (30).
b. As many myeloid cells, **MDSCs** (which are augmented in number in several tumors), express CD200 and release substances such as IDO, NOs, arginase 1, ROS and inhibitory cytokines (IL-10, IL-13 and TGF), causing the consequent suppression of Ras pathway, which leads to the downregulation of T cells, the regulation of macrophage and DC's release of cytokines, the upregulation of Treg, as well as the alteration in other cells explained in previous chapters. This decreases inflammation and therefore, the capacity to defend against tumors, with a poor response to immunotherapy (27).

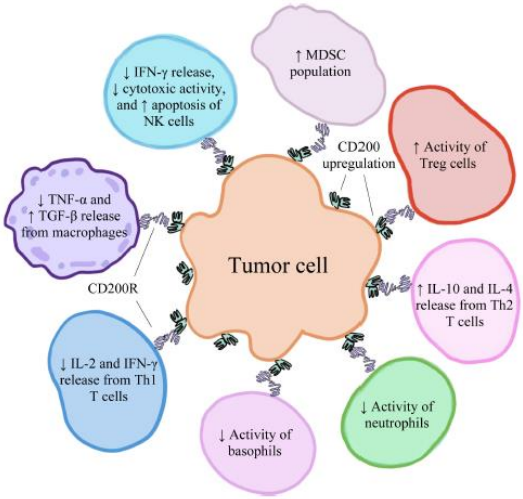

Figure 14. Tumor cells overexpressing CD200 to enhance immunosuppression (30)

c. **γ secretase's** interaction with CD200's cytoplasmic domain causes the fragmented-intracellular tail's translocation and binding to DNA, resulting in an augmented transcription of pro-malignant genes for leukemia (31).

d. **Extracellular** disintegrin and metalloprotease domain (**ADAM28**) **and** matrix metalloproteinase (**MMP**) mediate CD200's excision into a soluble CD200 ectodomain present in the tumor microenvironment and in systemic blood. ADAM28 has been researched in CLL, and MMP, in basal cell carcinoma (31,38).

   The soluble type called sCD200 has been investigated in glioblastoma as well, resulting its presence in a higher immunosuppression, which brings a poor prognostic to cancers (more aggressive diseases and advanced stages rate) but a better response to allografts (30).

These multifunctional pathways through which CD200 acts, makes its therapeutic blockade with an specific monoclonal antibody complicated (31).

Therapies targeting CD200 have been tested. For instance, a recombinant monoclonal antibody named "Samalizumab", which prevents the binding of CD200 to its receptor, was investigated in a clinical trial. It showed unclear results, as tumor burden was reduced in CLL but not in MM patients (39).

CD200 is broadly expressed on different tissues: kidneys, neurons, thymocytes, endothelial cells, DC, T and B cells. Therefore, its overexpression can be found in various tumors, including hematologic malignancies and solid ones, such as AML, CLL, hairy cell leukemia, MM, renal or colon carcinoma, malignant melanoma, glioblastoma, pancreatic or bladder cancer (30,31).

Table 6. Tissues expressing CD200 and CD200R1 (36)

"-" represents no labeling, "+" represents some or all cells are labeled, " +/- " represents weak labeling and "ND" represents not determined.

Tissue distribution of CD200 and CD200R1.

Cell type	Membrane protein	
	CD200	CD200R1
Thymocyte	+	–
T-cell	+/–	–
Activated T-cell	+	–
B-cell	+	–
Activated B-cell	+/–	–
Dendritic cell	+/–	+
Monocyte/macrophage	–	+
Endothelium	+	–
Neuron	+	–
Platelets	–	ND
Erythrocyte	–	–

Its overexpression is generally associated with poor prognosis and lower event-free survival rates. In **CLL**, CD200 has been correlated with circulating tumor cells suppression and with certain chromosome alterations, such as the trisomy of chromosome 12 (which already brings the disease an intermediate risk). In **melanoma**, a polarized Th2 response and suppressed and TNFα and INF γ release was found; and in **AML**, it inhibits Th1 response and NK and T cells' actions. AML patients expressing CD200 hardly ever achieve CR after the induction chemotherapy because high Treg numbers are associated with a poor response to this therapy (27,31).

The last paragraph gives us an idea that **CD200 expressing tumors act in diverse ways**. Some express the protein in the illness' first stages (like melanoma) and some do in advanced phases (like Cutaneous Squamous Cell Carcinoma), and the modulators of its expression are different in dissimilar kinds of tumors (for example, being ERK the regulator of melanoma or FMNL2 of colorectal carcinoma) (31).

CD200 has also been studied in the designated as "**cancer stem cells**". These cells have a high self- renewal and differentiating capacity, and their presence in the tumor's microenvironment enhances cancer's progression and has been associated with a higher recurrence rate and a higher therapy resistance (40).

In a similar manner, lymphatic endothelial cells (**LECs**) regulate immune cells' pass and their interactions with each other. LECs have shown regional specificity and thus different responses, especially during inflammation, so investigators researched paying attention to psoriasis-related skin inflammation. When inflammation is absent, CD200's presence is inconsistent in both the lymph node subcapsular sinus' ceiling and floor. In contrast, when there is inflammation, CD200 increases in the floor population, where we find DC and macrophages. Researchers concluded that CD200 overexpression in the lymph node subcapsular sinus' floor's purpose is to suppress DC and macrophage-dependent inflammation on the first defense line (30).

Tumor- nourishing blood vessels are essential for metastasis, because malignant cells must cross the vessel endothelium to access the circulatory system. In the same way, blood's immune cells need to pass through the endothelium to infiltrate the tumor. As CD200:CD200R1 interaction has been demonstrated to inhibit immune responses in the

regional endothelial cells, many tumors promote CD200's overexpression in order to promote metastasis (30).

▪ RELATED TO INFECTIONS

Several pathogens have added the CD200, CD200R or a CD200's mimic encoding gene to their genome, to induce immunosuppression in their host (shifting from a Th1 proinflammatory cytokine response to a Th2 anti-inflammatory one) and therefore, permitting the pathogens' survival (27).

The CD200 human herpes virus-8 (HHV-8)'s analogue to the human CD200 is the most alike to the host one. It is known as K14 and does not share around 60% of the sequence of CD200, but conserves the cytoplasmic and transmembrane domains, and most importantly, the two extracellular Ig-like regions which allow K14 to interact with CD200R1 with equivalent dynamics kinetics to the human glycoprotein (27).

Kaposi sarcoma needs the host's HHV-8's infection to grow. It usually develops after a latency phase with basal viral-dependent inflammation. The down regulated immune system resulting from the K14: CD200R1 interaction promotes Kaposi Sarcoma development (30).

Some viral infections change SF2/ASF activity through the ESE: SF2/ASF interaction, and thus they cause an increase in CD200tr (to try to competitively inhibit CD200's action) (27).

As the airway is a common microorganism's entry point, its macrophages have an increased CD200R1 presence, which is thought to prevent infection-related inflammation. This was researched with the influenza virus, where the CD200:CD200R1 axis reduces the macrophage population and thus inflammatory lung disease (27).

▪ RELATED TO AUTOIMMUNE DISORDERS

A common investigation about **rheumatoid arthritis** – an autoimmune disease- in rodent species is Collagen-induced arthritis, with associated T and B cell immune defense against type II collagen. When CD200Fc was dispensed to susceptible mice, the Th1 pro-inflammatory immune response typical from Collagen-induced arthritis with high TNFα and INF γ was reduced, and it decreased collagen-sensibilization and thus,

avoided the pathology's development. Th2 immune response also takes place during this Collagen-induced arthritis (27).

CD200:CD200R1 axis, with its polarized Th2 response, has also been proven to reduce **hair-follicle inflammation** in rodent species studies. The skin's keratinocytes in the outer root sheath express elevated CD200 numbers, and through their interactions with CD200R1 immune cells inactivate resident leukocytes and therefore, inflammation (27).

Table 7. CD200: CD200R1 axis' positive and negative effects (27)

Positive Effects of CD200:CD200R Interactions	Negative Effects of CD200:CD200R Interactions
Protects against the development of neurodegenerative disorders (e.g., Alzheimer disease, Parkinson disease) [128,129]	Supports the spread of viral, bacterial, parasite and helminth infections caused by certain viruses (e.g., HHV-8/KSHV, influenza) (see above)
Decreases the development of autoimmune disorders (e.g., CIA, RA, inflammatory retinal diseases etc.) (see above)	Promotes the growth of tumor cells expressing CD200 (e.g., melanoma, breast cancer, prostate cancer, lung cancer, multiple myeloma, acute myeloid leukemia etc.) (see above)
Prolong the survival of allografts, what could be a useful tool in transplantology (see above)	
Reduces the risk of bacterial sepsis [72]	
Promotes recovery after ischemic stroke [130]	
Limits the activity of microglia, and thus, the extent of inflammation in the central nervous system (CNS) (e.g., spinal cord injury) [131]	

3.3.5. CD200'S GENETIC POLYMORPHISMS

There is very little information regarding CD200 polymorphisms. Several of them have been determined, such as rs1131199 or rs2272022. The rs1131199 polymorphism results in an amino acid substitution at codon 11 of the leader peptide, replacing serine with cysteine (Ser11Cys). The biological implications remain unclear. It has been suggested that as rs1131199 involves changes in the leader peptide, it could impact the degree of regulation of the CD200 protein's function, either enhancing or diminishing it (41).

3.4. HISTOCOMPATIBILITY ANTIGENS. HLA.

Donor and recipient immunogenetic compatibility is a crucial factor in the success of HSCT, having more impact than it does in solid organ transplantation. Histocompatibility antigens trigger immune responses (such as GVHD and graft failure) to tissue allografts (42).

The human **major histocompatibility complex (MHC)** encompasses genes (situated in chromosome 6) which code human leukocyte antigen (HLA), a cell surface glycoprotein with extensive genetic variability. There are two HLA (and MHC) variants: HLA class 1 and HLA class 2 antigens, with different characteristics, as listed in the table below (42).

Table 8. Features of Class I and Class II MHC Molecules. Extracted from (43)

Feature	Class I MHC	Class II MHC
Polypeptide chains	α and β_2-microglobulin	α and β
Locations of polymorphic residues	α1 and α2 domains	β1 > α1 domains
Binding site for T cell coreceptor	CD8 binds mainly to the α3 domain	CD4 binds to a pocket created by parts of α2 and β2 domains
Size of peptide-binding cleft	Accommodates peptides of 8–11 residues	Accommodates peptides of 10–30 residues or more
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP

Class I and II MHC composition is made up of 2 α helices arranged in an antiparallel orientation (creating a groove between them) and 8 β chains (42).

In immune reactions, T lymphocytes identify as non-self antigens displayed as peptide fragments attached to MHC molecules. These peptides bind to the groove between the 2 α chains and arrive in vesicles to the cell surface in different ways (44):

- MHC Class I: Proteins are degraded in the **cytosol** by proteasomes and the resulting peptides join the MHC molecules in the endoplasmic reticulum.
- MHC Class II: Proteins are degraded in lysosomes and the resulting peptides join the MHC molecules when vesicles enclosing them combine with **endosomes** (42).

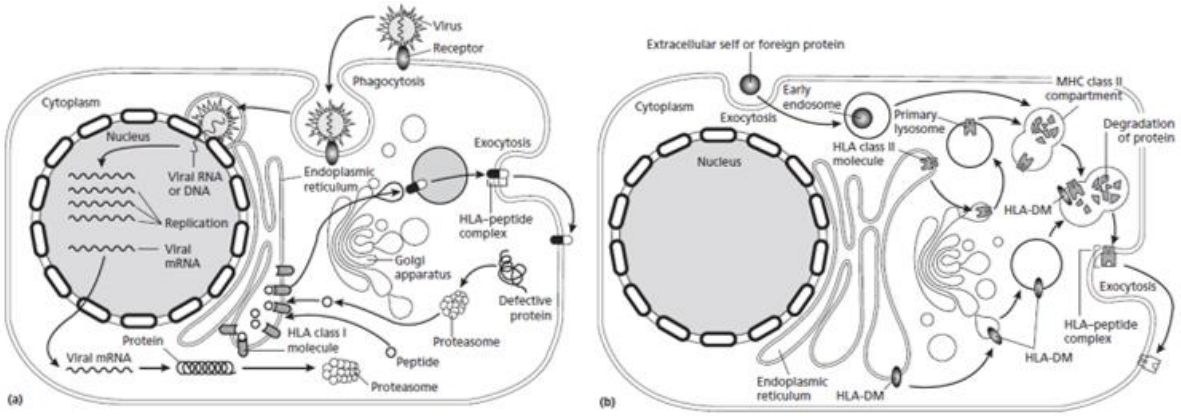

Figure 15. Figure 16. Mechanisms of peptide presentation MHC Class I (a) and MHC Class II (b) molecules. Extracted from (42)

Minor histocompatibility antigens (mHAs) result from non- MHC polymorphisms spread throughout the whole genome and bind to Class I or II MHC after undergoing their respective processing pathways. They are strongly associated with the development of GVHD in HLA-matched HSCT (42).

After an allogeneic transplantation is performed, alloantigens elicit both innate and adaptative immune responses. Antigen-presenting cells (APC), mainly dendritic cells, located at peripheral tissues capture alloantigens and travel to lymph nodes, where they present the MHC-peptide complex to T and B cells, and it binds to the receptors placed in T lymphocytes (the cells most significantly involved in this process). This results in the initiation of an **alloimmune reaction** (42).

Alloantigens can be **directly** recognized by T cells (which means T lymphocytes join to an unaltered allogeneic MHC) or **indirectly** presented (when the alloantigen has been degraded by the hosts' APC and its residues join MHC molecules) (43).

Once the alloimmune reaction is triggered, activated B lymphocytes generate germinal centers for antibody production, while activated T lymphocytes migrate to the periphery in the onset of a **T-cell immune reaction**. Through direct recognition:

- CD8 cells are stimulated by dendritic cells that display MHC class I alloantigens and mHAs attached to self-MHC class I molecules.

- CD4 cells are stimulated by dendritic cells that display MHC class II alloantigens and mHAs attached to self-MHC class II molecules. Costimulation by molecules such as CD86 and its binding CD28 plays a crucial role in this process (42).

Histocompatibility testing after an allogeneic HSCT is a key step to decrease the risk of complications from HLA mismatches (such as GVHD). As HLA alleles are transmitted through generations as a **haplotype**, family studies are crucial to try to find -if possible- the suitable HLA- identical sibling donor (who shares both of the haplotypes with the patient) (44).

4. JUSTIFICATION

Hematopoietic stem cell transplantation is the **chosen potentially curative therapeutic approach** for many hematological (including leukemias or myelodysplastic syndromes) and non-hematological pathologies. Despite the huge recent advances regarding transplantation, complications (such as graft failure, infections or GVHD) and relapse are not infrequent after the procedure. (9)

Therefore, identifying **potential genetic markers** capable of predicting patients' response to HSCT and their overall survival is a key step to lately personalize the treatment applied in each patient (using specific donors, conditioning regimens, HSC sources, immunosuppressive medicines after the procedure…) to improve these rates.

In this context, CD200 glycoprotein has an important role. As explained in the introduction, CD200's interaction with its receptor alters different pathways causing a downregulation of the immune system. This leads us to consider that the expression of CD200 in donors could impact the ability of donors' T lymphocytes to identify the host's or micro-organisms' cells as non-self and respond accordingly, thus **altering the development of post-HSCT complications and survival rates.**

Several studies have been conducted regarding how donor's genotypes of similar proteins which also downregulate the immune system (CTLA-4, PD1, LAG3…) affect clinical outcomes after an HLA-identical sibling donors allogeneic HSCT, but there is **no data available** concerning CD200 (just about specifically multiple myeloma but not including other diseases).

Studying CD200's different genetic polymorphisms could be useful to understand better the pathways through which the protein exerts its function (filling this way a gap in existing scientific literature). In addition, it could also be useful to estimate each patient's response to the HSCT and thus, to **personalize therapeutic approaches** to each patient to **improve clinical outcomes**, **optimizing** this way **resources and healthcare expenses.**

5. HYPOTHESES AND OBJECTIVES

5.1. HYPOTHESES

The **main study hypothesis** is that the donor's CD200 genotype is associated with differences in overall survival in receptors of allogeneic hematopoietic cell transplantation.

Secondary hypotheses are:

- donor's CD200 genotype is associated with differences in the development of acute graft-versus-host disease (GVHD) in receptors of allogeneic hematopoietic cell transplantation.
- donor's CD200 genotype is associated with differences in transplant-related mortality (TRM) in receptors of allogeneic hematopoietic cell transplantation.
- donor's CD200 genotype is associated with differences in relapse rates in receptors of allogeneic hematopoietic cell transplantation

5.2. OBJECTIVES

5.2.1. MAIN OBJECTIVE

- To evaluate how donor's CD200 genotype is associated with overall survival in receptors of HLA-identical sibling donor allogeneic hematopoietic cell transplant.

5.2.2. SECONDARY OBJECTIVES

- To evaluate how donor's CD200 genotype is associated with acute graft-versus-host disease (GVHD) in receptors of HLA identical sibling donor allogeneic hematopoietic cell transplant.
- To evaluate how donor's CD200 genotype is associated with transplant-related mortality (TRM) in receptors of HLA identical sibling donor allogeneic hematopoietic cell transplant.

- To evaluate how donor's CD200 genotype is associated with relapsed malignancies in receptors of HLA identical sibling donor allogeneic hematopoietic cell transplant.

6. METHODOLOGY

6.1. STUDY DESIGN

This study is a multicenter retrospective cohort study.

6.2. STUDY POPULATION

The study population consists of patients treated with a matching-HLA allogeneic HSCT from an HLA-identical sibling donor.

6.2.1. INCLUSION CRITERIA

- Patients treated with an **HLA-identical sibling allogeneic HSCT** in hospitals where the "Grupo Español de Trasplante Hematopoyético (**GETH**)" works during the years **1991-2015**. ANNEX 3
- Signed written **informed consent for the "Red Nacional de Biobancos"** by donors and receptors.
- **Available patient-donor pairs** of DNA samples.

6.2.2. EXCLUSION CRITERIA

As it is a retrospective study, the exclusion criteria are restricted to not fulfilling the previously described inclusion criteria. Therefore, the following population will be excluded from the study:

- Matched unrelated donors
- Mismatched HLA donors

6.3. SAMPLE

Accepting an alpha risk of 0.05 and a power of 0.8 in a two-tailed test DNA samples from **839** patient-donor pairs are required in the group that includes patients treated with homozygous donors for allele C and heterozygous C/G CD200 rs1131199 genotype; and DNA samples from **252** patient-donor pairs in the group only including patients treated with homozygous donors for allele G CD200 rs1131199 genotype, resulting in a total of **1091 patient-donor pairs of DNA samples** to find as statistically significant a proportion

difference, expected to be a 50% in the homozygous for allele C and heterozygous C/G group and a 40% in homozygous for allele G group. As it is a retrospective cohort study, a drop-out rate of 0% has been anticipated. Sample size has been calculated with GRANMO.

Therefore, the study population consists of 1091 patient-donor pairs of DNA samples from patients treated with an HLA- identical sibling allogeneic HSCT in hospitals where the "Grupo Español de Trasplante Hematopoyético (GETH)" works during the years 1991-2015. Previously to DNA preservation, written informed consent was signed by the entire study population -including donors and receptors-. Samples and data for this study will be obtained from the Biobank of the "Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI)", joined to the "Plataforma Red Nacional de Biobancos". All samples will be processed in accordance with standard operating procedures after the authorization of the Ethics and Scientific Committees and adhering to the principles outlined in the Helsinki Declaration.

6.4. VARIABLES

6.4.1. INDEPENDENT VARIABLE

- **Donor's CD200 genotype** in patients receiving an HLA-identical sibling allogeneic HSCT, analyzed as a dichotomous categorical nominal variable: "having a donor who was **homozygous for the G allele** of rs1131199 CD200 genotype" or "having a donor who was **homozygous for the C allele or heterozygous C/G** of rs1131199 CD200 genotype".

  Allele determination (G or C) will be performed using the following equipment and materials on the samples provided by the biobank:

  - *QIAamp DNA Blood Mini Kit (Qiagen, GmbH, Hilden, Germany)*
  - *Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System*
  - *TaqMan® SNP Genotyping Assays real time PCR*

6.4.2. DEPENDENT VARIABLE

- **Overall survival (OS) in months,** analyzed as a quantitative continuous variable.

  "Overall survival" is defined as the period of time from the neoplasia diagnosis until death due to any reason.

- **Transplant-related mortality (TRM),** analyzed as a dichotomous categorical nominal variable: transplant-related death or no transplant-related death.

  "TRM" is defined as patient death resulting from transplant complications. Complications are understood *as the development of any of the events listed below, related with the transplantation procedure:*

Table 9. Main hematopoietic stem cell transplantation complications. Adapted from (9)

Acute graft-versus-host disease	**Hepatic complications** -Sinusoidal obstructive syndrome -Hepatitis: infectious or noninfectious
Blood group incompatibilities and hemolytic complications	**Infectious complications** (bacterial, fungal, cytomegalovirus, herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, adenovirus, respiratory viruses, human herpesvirus- 6, 7 or 8 and other viruses)
Chronic graft-versus-host disease	**Kidney and bladder complications**
Drug-drug interactions	**Late onset nonmalignant complications** (osteoporosis/osteopenia, avascular necrosis, dental problems, cataracts, chronic fatigue, psychosocial effects and rehabilitation)
Endocrine complications	**Lung injury** (interstitial pneumonitis, diffuse alveolar hemorrhage, engraftment syndrome, bronchiolitis obliterans)
Gastrointestinal complications -Mucosal ulceration/bleeding -Nutritional support	**Neurologic complications** (infectious, transplant conditioning and immune suppression medication toxicities)
Graft failure	**Subsequent malignancies**
Growth and development	**Vascular access complications**

- **Acute graft-versus-host disease (GVHD) diagnosis**, analyzed as a dichotomous nominal categorical variable: developing the acute GVHD disease after the allogeneic HSCT or not.

  "Acute GVHD" is defined by fulfilling clinical and histological criteria, as explained in the introduction.
- **Disease-free survival (DFS)** in months, analyzed as a quantitative continuous variable.

  "Disease-free survival" is defined as the period of time starting from the day a curative disease treatment successfully ends, where the patient does not show any disease sign or evidence.
- **Diagnosed condition relapse,** analyzed as a dichotomous categorical nominal variable: relapsed condition or not.

  "Diagnosed condition relapse" is defined as the recurrence of a previous malignancy (at the primary site, contiguous regions or distant metastases in other areas of the body) after being treated initially and reaching complete or partial remission.

6.4.3. CO-VARIABLES

- **Patient's age** in years, analyzed as a quantitative continuous variable.
- **Patient's sex,** analyzed as a dichotomous categorical nominal variable: male or female.
- **Sex mismatch,** analyzed as a dichotomous categorical nominal variable: having sex mismatch or not.

  "Sex mismatch" is defined as male patient receiving HSC from a female donor.
- **Diagnosed condition,** analyzed as a dichotomous categorical nominal variable: "having Acute leukemia or a myelodysplastic syndrome" or "other diagnoses".
- **Disease severity,** analyzed as a dichotomous categorical ordinal variable: having an advanced disease or not.

  Patients with "Advanced disease" are defined as:

  - *Acute leukemia patients surpassing the first complete remission**.***
  - *Chronic myeloid leukemia patients surpassing the first chronic phase.*
  - *Multiple myeloma, lymphoma or myelodysplastic syndrome patients with progressive disease.*

- **Type of induction therapy,** analyzed as a dichotomous categorical nominal variable: using TBI or others.
- **Type of HSCT conditioning,** analyzed as a dichotomous categorical nominal variable: myeloablative or not.
- **Source of stem cells,** analyzed as a dichotomous categorical nominal variable: peripheral blood or bone marrow
- **T cell depletion**, analyzed as a dichotomous categorical nominal variable: T cell depletion or not.

  "T depletion" is defined as either an "ex vivo" (subjecting the graft to a process to eliminate T cells during the collection process of hematopoietic stem cells) or an "in vivo" (administering immunosuppressive medicines to the receptor) procedure which eliminates T lymphocytes to prevent GVHD.

Table 10. Summary of variables, its categories and its measurement

		VARIABLE	DESCRIPTION	CATEGORIES	MEASURED
INDEPENDENT		**Donor's CD200 genotype**	Dichotomous categorical nominal	-*"GG"* -*"GC or CC"* donor's rs1131199CD200 genotype	Using the following equipment and materials on the samples provided by the biobank: -QIAamp DNA Blood Mini Kit (Qiagen, GmbH, Hilden, Germany) -Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System -TaqMan® SNP Genotyping Assays real time PCR
DEPENDENT	*Primary*	***Overall survival (OS)***	Quantitative continuous		*Months, from biobank data*
	Secondary	***Transplant-related mortality (TRM)***	Dichotomous categorical nominal	-*Transplant-related death* -*No transplant-related death*	*Biobank data*
		Acute GVHD	Dichotomous categorical nominal	-*Having acute GVHD* -*Not having acute GVHD*	*Biobank data, following clinical and histological criteria*
		Disease-free survival (DFS)	Quantitative continuous		*Months, from biobank data*
		Diagnosed condition relapse	Dichotomous categorical nominal	-*Relapsed* -*Not relapsed*	*Biobank data, following clinical criteria*
CO-VARIABLES		**Patient's age**	Quantitative continuous		*Years, from biobank data*
		Patient's sex	Dichotomous categorical nominal	-*Male* -*Female*	*Biobank data*
		Sex mismatch	Dichotomous categorical nominal	-*Sex mismatch* -*No sex mismatch*	*Biobank data*
		Diagnosed condition	Dichotomous categorical nominal	-*Acute leukemia or myelodysplastic syndrome* -*Other diagnoses*	*Biobank data, following clinical criteria*
		Disease severity	Dichotomous categorical ordinal	-*No advanced disease* -*Advanced disease*	*Biobank data, following clinical criteria*
		Induction therapy	Dichotomous categorical nominal	-*TBI* -*Others*	*Biobank data*
		Conditioning regimen	Dichotomous categorical nominal	-*Myeloablative* -*No myeloablative*	*Biobank data*
		HSC source	Dichotomous categorical nominal	-*Peripheral blood* -*Bone marrow*	*Biobank data*
		T depletion	Dichotomous categorical nominal	-*T depletion* -*No T depletion*	*Biobank data*

6.5. MEASURING METHODS AND DATA COLLECTION

QIAamp DNA Blood Mini Kit (Qiagen, GmbH, Hilden, Germany) will be used following the manufacturer's instructions to collect DNA from the 200 µl blood samples provided by the IDIBGI Biobank. The DNA will be preserved at -80°C until use.

The rs1131199 polymorphism of the CD200 gene was chosen based on bibliographic research. To identify C or G rs1131199 polymorphism of CD200 gene, allelic discrimination plots will be performed using the Applied Biosystems 7500 Fast Real-Time PCR system with TaqMan real-time PCR primers and probes obtained as commercially available AB Assay-on-Demand reagents (Life Technologies, Carlsbad, CA). The Assay on Demand reagents includes both the necessary primers and fluorescently labeled TaqMan MGB probes (FAM and VIC) to amplify and detect the polymorphism. The PCR cycling conditions and interpretation of the results obtained will be performed according to the manufacturer's instructions. See *ANNEX 4 for deeper information about laboratory techniques.*

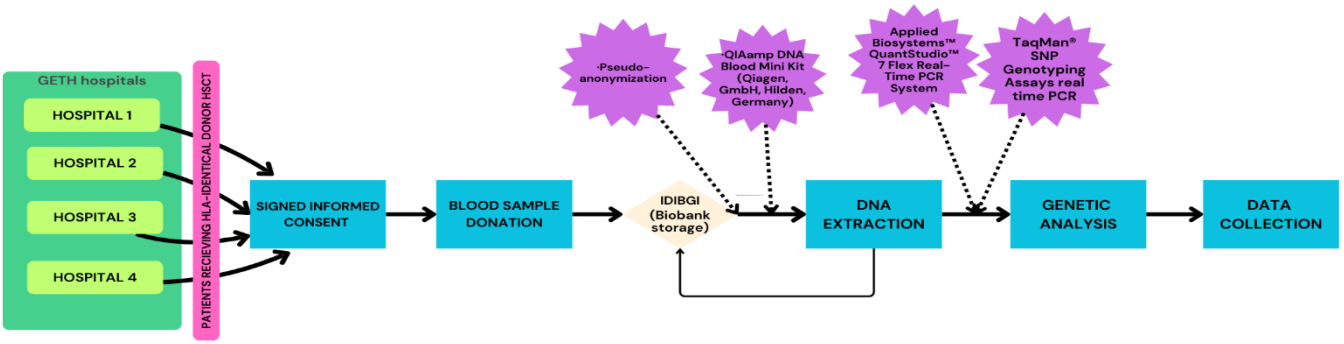

Figure 16. Sample collection representation

Participants' identity will be protected by the creation of an anonymized data base (pseudo- anonymization process) correlating each participant's sample and information provided by the biobank to a code number, eliminating this way all personal information allowing their identification.

7. STATISTICAL ANALYSIS

The statistical analysis will be conducted by the statistician using the SPSS Statistics 30.0.0 and RStudio-4.4.2 softwares. A two-sided p value ≤ 0,05 was determined as statistically significant.

7.1. DESCRIPTIVE ANALYSES

Continuous quantitative variables (including overall survival, disease-free survival and patients' age) will be expressed as **a mean +/- standard deviations** if they follow a normal distribution or as a **median (interquartile range)** if they do not.

On the other hand, **qualitative variables** (including transplant-related mortality, acute GVHD diagnosis, diagnosed condition relapse, patients' sex, sex mismatch, diagnosed condition, disease severity, induction therapy, conditioning regimen, HSC source and T depletion) will be summarized using **frequencies and percentages**.

Regarding the independent variable (rs1131199 CD200 genotype), allele and genotype frequencies will be assigned by direct counting, without using estimations or indirect calculations.

7.2. BIVARIATE ANALYSES

Homogeneity among genotype groups will be assessed utilizing the **T- Student test or ANOVA** for OS and DFS, as they are continuous variables; and **χ2 test** for the qualitative variables TRM, acute GVHD diagnosis and diagnosed condition relapse.

To analyze OS and DFS, the **Kaplan-Meier** technique will be applied, and the curves will be compared using the **log-rank test.**

Variations in TRM, acute GVHD diagnosis and diagnosed condition relapse will be investigated with the use of **Cumulative incidence estimates.**

Relapse analysis will exclude patients with pathologies considered non-malignant.

7.3. MULTIVARIATE ANALYSES

To address possible confounding effects of co-variables, multivariate analyses will be performed including variables that showed significant differences in the bivariate analysis.

Cox proportional hazard models will be carried out to do a multivariate analysis of overall survival and disease-free survival.

Logistic regression models will be applied to analyze the correlation of donor's rs1131199 CD200 genotype and co-variables with TRM, acute GVHD diagnosis and diagnosed condition relapse.

8. ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the Principles of Biomedical Ethics from Beauchamp and Childress (autonomy, beneficence, non-maleficence and justice) and following the ethical principles regarding research with human subjects defined by the World Medical Association in the Helsinki Declaration. Standards outlined in the Oviedo Convention, the Nuremberg Code and the Belmont Report will be compiled as well.

The pertinent project will be submitted for approval by the Clinical Research Ethical Committee (CEIC) of Hospital Universitari Josep Trueta before commencement of the study.

Donors and receptors (or their legal guardians) voluntarily signed the informed consents upon receiving the necessary information for both the HSCT procedure and the inclusion of their DNA samples to the biobank of the "Red Nacional de Biobancos". *See ANNEX 5 for further information about Biobank documents.*

Participants' identity will be protected by the creation of an anonymized database (pseudo- anonymization process) correlating each participant's sample and information provided by the biobank to a code number, eliminating this way all personal information allowing their identification.

There are no conflicts of interest or financial ties to declare associated with this research.

This study will be carried out following the ongoing Spanish and European legislation, which includes:

- "Ley 14/2007", "Real decreto 1716/2011" and "Real Decreto-ley 9/2014", regarding the use of biological samples.
- "Real decreto 1090/2015", regarding the use of sanitary products.
- "Reglamento (UE) 2016/679 del Parlamento y del Consejo Europeo, de abril de 2016" and "Disposición Adicional 17.2 de la Ley Orgánica 3/2018 de Protección de Datos Personales y Garantía de los Derechos Digitales (LOPD-GDD)", regarding protection of personal data.
- "Artículo 3 de la Ley 14/2007", regarding ethical bases for observational studies.

9. STUDY LIMITATIONS AND STRENGTHS

9.1. LIMITATIONS

- HSCT donors or patients who did **not sign the informed consent** for their blood samples to be preserved in the **"Plataforma Red Nacional de Biobancos"** and used in future research (or donors / patients who signed it but their DNA samples are **not available**) have not been included in this study. This might imply that the study sample is less representative of the general population.
- **Only HLA-identical sibling donors** have been included in the study and not others, such as unrelated (which moreover is the most frequent donor type), haploidentical or UCB. This fact should be kept in mind when extrapolating our results.
- Since **diverse hospitals** where the "Grupo Español de Trasplante Hematopoyético (GETH)" operates have been involved in all HSCT phases (including the collection of blood samples which are later submitted to the biobank that provides us the samples to analyze them); **bias** may have occurred **regarding data collection or candidates' selection** (because there is no standardized consensus or guidelines for eligibility criteria, so the decision is taken by the transplant center). To prevent bias introduction, standardized procedures and instruments should be used whenever possible: for example, processing all the samples using laboratory instruments from the same manufacturer, such as the QIAamp DNA Blood Mini Kit (Qiagen, GmbH, Hilden, Germany).
- Data collection for the selected population began many years ago and the study covers a broad range of years, so there may be **advances and differences regarding the procedure** (for example, changes in the conditioning regimens used, with different combinations of chemotherapy medications or chemotherapy combined with TBI; which this study only reflects as "Other regimens that are not TBI", or changes in the profile of patient who can be eligible for transplantation).

This could potentially have confounding effects in this study, for what further investigation of this novel research line is suggested in new studies to account the impact of different conditioning regimens in the response of patients with different CD200 polymorphisms to HSCT.

- The study has been conducted at the **Spanish level**, where a **predominantly Caucasian population** with **specific characteristics** and exposure to specific environmental conditions prevails. Further investigation in other countries and among different ethnic groups is suggested to validate and extrapolate these findings.

9.2. STRENGTHS

- Since the study design is a retrospective cohort, there is **no loss to patient follow-up** and it entails a lower cost and a quicker analysis than carrying out a prospective study.
- To address possible confounding effects, statistical multivariate analyses will be performed **including co-variables**, so the direct impact of the primary variable is isolated, minimizing this way bias and permitting a more accurate assessment of the causal relationship.
- Since diverse hospitals where the "Grupo Español de Trasplante Hematopoyético (GETH)" operates have been included in the study, **populations from different Spanish territories** have been represented as well, which allows us to extrapolate the results at a national level.
- It is the **first study investigating** the association between the donor's **CD200 polymorphisms** and the patient's response **after receiving a HSCT** regardless of the underlying cause.

10. WORK PLAN

10.1. RESEARCH WORKFLOW

- **PHASE 0: STUDY DESIGN**

  After an in-depth review of available literature and publications, the research project protocol will be drafted, including the design, hypotheses, objectives, population and methodology.

  No additional instruction will be needed for laboratory professionals, as they are IdiBGi members with experience in genotyping procedures.

  - Responsible: Principal investigator.
  - Duration: November 2024- January 2025.

- **PHASE 1: ETHICAL EVALUATION**

  The study protocol will be presented to the Clinical Research Ethical Committee (CEIC) of Hospital Universitari Josep Trueta before commencement of the study. After evaluating the project, a detailed report specifying whether it is approved, denied or requires modifications. In case of modifications requirement, another protocol with the demanded adjustments will be presented to the CEIC.

  - Responsible: Principal investigator and CEIC workers.
  - Duration: February 2025- March 2025.

- **PHASE 2: BIOBANK APPLICATION**

  The request for authorization to the biobank for access to samples and associated data will be drafted and presented, along with the required forms and documentation (including the study protocol and CEIC approval). Selection of the most suitable biobank from the "Red Nacional de Biobancos" is made, which, in this case, is determined to be the Biobank of the "Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI)." Upon evaluation of the request, if approved, the Material Transfer Agreement (MTA) will be signed, and the samples will be received.

  - Responsible: Principal investigator and Biobank workers.
  - Duration: April 2025- June 2025

- **PHASE 3: LABORATORY PROCESSING AND DATA COLLECTION**

  Following its use protocols and manufacturer's instructions, laboratory techniques were performed to extract DNA (using the QIAamp DNA Blood Mini Kit) and then genotype it (using Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System and TaqMan® SNP Genotyping Assays real time PCR).

  - Responsible: Laboratory professionals.
  - Duration: First and second weeks of July 2025.

- **PHASE 4: STATISTICAL ANALYSIS**

  Genotyped samples are included in an anonymized database correlating each participant's sample and information provided by the biobank to a code number, maintaining participants' privacy. Statistical analysis is performed as explained in its section above by the statistical.

  Discussion and conclusions of the results will be made in a meeting including the whole research team.

  - Responsible: Statistical analyst and research team.
  - Duration: Third and fourth weeks of July 2025.

- **PHASE 5: RESULTS PUBLICATION, AND REPORT AND SAMPLES SUBMISSION TO THE BIOBANK**

  The study findings will be assembled -with an English editor's help- into a manuscript (including introduction, methodology, results, discussion…) and submitted to Open Access hematology journals. After selecting and registering with the journal, the manuscript will be reviewed, and any required revisions will be made. Once the study is accepted and the applicable publication fee is paid, it will be published.

  The manuscript and the remaining samples will be sent back to IDIBGI.

  - Responsible: Principal investigator and English Editor.
  - Duration: August 2025 – October 2025.

10.2. CHRONOGRAM

Figure 17. Chronogram of the study.

10.3. ESTIMATED BUDGET

The development of this study will entail this estimated budget:

- PERSONNEL COSTS:
  - Statistician 3.000€
  - English editor 800€
- MATERIAL COSTS:
  - Laboratory plasticware 6.000€
  - DNA extraction kits 5.000€
  - Real-time PCR reactive 20.000€
  - Allelic discrimination assays 20.000€
- SERVICES COSTS:
  - Sample delivery service 25.000€
  - Publication fees 3.000€

TOTAL ESTIMATED BUDGET: 82.800€

10.4. WORK TEAM:

This study will be carried out by several professionals:

- **One principal investigator**, who leads the study throughout all its phases. His responsibilities include study design, resource management, work team coordination, results graft and contact with the CEIC, the Biobank and the Open Access hematology journal.
- **Three IdIBGi experienced research technicians**, who are responsible for preparing the samples received from the Biobank, processing them, and conducting DNA genotyping
- **One statistician**, who will perform the statistical analysis.
- **One English editor**, who will translate the study findings manuscript before its submission to the Open Access hematology journal.

11. FEASIBILITY

Work team: Regarding the research team, the principal investigator is a hematologist with expertise knowledge regarding hematopoietic stem cell transplantation and its complications (including acute GVHD), and regarding immune checkpoint inhibitor molecules, such as the CD200. Research technicians are IdiBGi members with experience in genotyping procedures. Therefore, no additional pre-study instruction will be needed. To guarantee data quality, an English editor and a statistician will be hired.

Resources and infrastructure: This study will leverage the well-equipped IdIBGi laboratory, which has the necessary facilities for DNA extraction, genotyping and statistical analysis. Additional instruments and standardized kits (such as laboratory plasticware, DNA extraction kits or Real-time PCR reactive) will be acquired to carry out analysis of genetic polymorphisms. Access to computational tools and bioinformatics software has also been guaranteed for the genetic and statistical analyses required. IdIBGi bears personnel expenses, except for the English editor and the statistician. The study cost is primarily associated with laboratory materials and services (such as publication fees and the sample delivery service). The only extra team members who will be hired are the English editor and the statistician.

Budget and chronogram: The research budget is designed to be realistic and attainable. By effectively managing expenses such as data handling, statistical analysis and having members with big expertise in the field to avoid their pre-study training costs, the study will ensure the optimal use of financial resources. As the samples have already been extracted and will be obtained from a pre-existing biobank, and as the work team is greatly experienced, the estimated time for the study to be completed is 12 months.

Sample availability. Since this study has a retrospective cohort design, no drop out is expected and participants will not go through any further procedures for this study, nor will they actively participate, as information is obtained from DNA samples and information provided by the IdIBGi biobank of the “Plataforma Red Nacional de Biobancos”.

Ethical Considerations. This study adheres to all the ethical principles and to the Spanish and European legislation. Informed consent was voluntarily signed for the transplantation procedure and the posterior storage of all biobank samples, and no new patient recruitment is necessary. Data will go through a pseudo-anonymization process to ensure participants' identity is protected. The study will be developed after the CEIC of Hospital Universitari Josep Trueta and the IdIBGi biobank approval.

To conclude, based on the information provided, **we are confident on the feasibility of this study**, which furthermore fulfills the fundamental requirements for its successful application and is expected to deliver benefits that surpass any potential complications.

12. HEALTH IMPACT

Hematopoietic stem cell transplantation has increased significantly during the last decades as hematological and non-hematological diseases treatment. In addition, it is the preferred potentially curative therapy for many of them.

Hence, discovering genetic markers (in this case, the CD200 glycoprotein, which as explained, is involved in immunosuppression) capable of **predicting patients' response** post-stem cell transplantation is crucial to **anticipate complications** and to individually tailor the therapy to improve clinical outcomes, reducing complication rates and improving survival rates.

Personalizing the treatment each patient receives as much as possible in order to maximize results include improving the selection of donors and stem cell sources, optimizing the use of immunosuppressive treatments post-HSCT or choosing the best conditioning option pre-HSCT.

These advances could be translated into a **longer survival, less relapse rates and less complications development** - for example, infections, graft failure, toxicities or GVHD-, which would suppose a better life quality and less physical and psychological impact.

13. BIBLIOGRAPHY

1. Yoder MC. Blood cell progenitors: Insights into the properties of stem cells. Anat Rec A Discov Mol Cell Evol Biol [Internet]. 2004 [cited 2024 Nov 22];276A(1):66–74. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ar.a.10133

2. Gargani Y. Capítulo 1. Principios de hematología. In: Lo esencial en Hematología e inmunología. 4th ed. Barcelona, España: Elsevier; 2013. p. 1–7.

3. Morel F, Szilvassy SJ, Travis M, Chen B, Galy A. Primitive hematopoietic cells in murine bone marrow express the CD34 antigen. Blood. 1996 Nov 15;88(10):3774–84.

4. Kolios G, Moodley Y. Introduction to Stem Cells and Regenerative Medicine. Respiration [Internet]. 2012 Dec 13 [cited 2024 Nov 22];85(1):3–10. Available from: https://doi.org/10.1159/000345615

5. Saad A, de Lima M, Anand S, Bhatt VR, Bookout R, Chen G, et al. Hematopoietic Cell Transplantation, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw JNCCN. 2020 May 1;18(5):599–634.

6. Hymes SR, Alousi AM, Cowen EW. Graft-versus-host disease. J Am Acad Dermatol [Internet]. 2012 Apr [cited 2024 Dec 9];66(4):515.e1-534. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7360170/

7. Passweg JR, Baldomero H, Ciceri F, de la Cámara R, Glass B, Greco R, et al. Hematopoietic cell transplantation and cellular therapies in Europe 2022. CAR-T activity continues to grow; transplant activity has slowed: a report from the EBMT. Bone Marrow Transplant [Internet]. 2024 [cited 2024 Dec 12];59(6):803–12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11161408/

8. Friedlis MF, Centeno CJ. Performing a Better Bone Marrow Aspiration. Phys Med Rehabil Clin N Am. 2016 Nov;27(4):919–39.

9. Kaushansky K, Lichtman MA, Prchal JT, Levi M, Burns LJ, Linch D. CHAPTER 29. HEMATOPOIETIC STEM CELL TRANSPLANTATION. In: Williams Hematology, 10th Edition. 10th ed. Nueva York: McGraw-Hill Interamericana de España; 2023. p. 439–67. (Williams Hematology; vol. 1).

10. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol. 2016;7:1–15.

11. Anasetti C, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, et al. Peripheral-Blood Stem Cells versus Bone Marrow from Unrelated Donors. N Engl J Med [Internet]. 2012 Oct 18 [cited 2025 Jan 8];367(16):1–16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3816375/

12. Thomas ED, Blume KG, Forman SJ, Negrin RS. Chapter 46. Hematopoietic Cell Transplantation from Human Leukocyte Antigen Partially Matched Related Donors. In: Thomas' Hematopoietic Cell Transplantation. 4th ed. Oxford: Wiley-Blackwell; 2019. p. 657–74.

13. Massey, Jennifer C., Moore, John J., Milliken, Samuel T., Ma, David D. F. Autologous hematopoietic stem cell transplant for autoimmune diseases: evolution,evidence of efficacy, and real-world economics. Blood Cell Ther [Internet]. 2019 Apr 1 [cited 2025 Jan 8];2(2):1–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10599833/

14. Gerull S, Stern M, Apperley J, Beelen D, Brinch L, Bunjes D, et al. Syngeneic transplantation in aplastic anemia: pre-transplant conditioning and peripheral blood are associated with improved engraftment: an observational study on behalf of the Severe Aplastic Anemia and Pediatric Diseases Working Parties of the European Group for Blood and Marrow Transplantation. Haematologica [Internet]. 2013 Nov 1 [cited 2024 Dec 6];98(11):1804–9. Available from: https://haematologica.org/article/view/6845

15. Malard F, Holler E, Sandmaier BM, Huang H, Mohty M. Acute graft-versus-host disease. Nat Rev Dis Primer. 2023 Jun 8;9(1):1–18.

16. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood [Internet]. 2014 Jul 17 [cited 2024 Dec 6];124(3):344–53. Available from: https://doi.org/10.1182/blood-2014-02-514778

17. Fitch T, Lane A, McDonnell J, Bleesing J, Jordan M, Kumar A, et al. Age Impacts Risk of Mixed Chimerism Following Reduced-Intensity Conditioning Hematopoietic Cell Transplantation for Non-Severe Combined Immune Deficiency Inborn Errors of Immunity. Transplant Cell Ther [Internet]. 2024 Jan [cited 2024 Dec 9];30(1):1–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2666636723015774

18. Xu J, Miao W, Yuan H, Liu Y, Chen G, Wang H, et al. Unique Reduced-Intensity Conditioning Haploidentical Peripheral Blood Stem Cell Transplantation Protocol for Patients with Hematologic Malignancy. Transplant Cell Ther [Internet]. 2023 May [cited 2024 Dec 9];29(5):1–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2666636723010734

19. Radford M, Garcia-Horton A, Badami R, Jin E, Usmani N, Grafodatskaya D, et al. Early Mixed Donor Chimerism is a Strong Negative Prognostic Indicator in Allogeneic Stem Cell Transplant for AML and MDS. Transplant Cell Ther [Internet]. 2024 Nov 14 [cited 2025 Jan 17];1–20. Available from: https://www.sciencedirect.com/science/article/pii/S2666636724007590

20. Mata JR, Zahurak M, Rosen N, DeZern AE, Jones RJ, Ambinder AJ. Graft Failure Incidence, Risk Factors, and Outcomes in Patients Undergoing Non-Myeloablative Allogeneic Hematopoietic Cell Transplantation Using Post-Transplant Cyclophosphamide. Transplant Cell Ther. 2024 Jun;30(6):588–96.

21. Rostami T, Rostami MR, Mirhosseini AH, Mohammadi S, Nikbakht M, Alemi H, et al. Graft failure after allogeneic hematopoietic stem cell transplantation in pediatric patients with acute leukemia: autologous reconstitution or second transplant? Stem Cell Res Ther [Internet]. 2024 Apr 22 [cited 2024 Dec 23];15:1–15. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11034046/

22. Ziakas PD, Zervou FN, Zacharioudakis IM, Mylonakis E. Graft-Versus-Host Disease Prophylaxis after Transplantation: A Network Meta-Analysis. PLoS ONE [Internet]. 2014 Dec 8 [cited 2024 Dec 10];9(12):1–18. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4259365/

23. Choi SW, Reddy P. Current and emerging strategies for the prevention of graft versus host disease. Nat Rev Clin Oncol [Internet]. 2014 Sep [cited 2024 Dec 10];11(9):536–47. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4151470/

24. Lee SJ. Classification systems for chronic graft-versus-host disease. Blood [Internet]. 2017 Jan 5 [cited 2024 Dec 11];129(1):30–7. Available from: https://doi.org/10.1182/blood-2016-07-686642

25. Majhail NS, Giralt S, Bonagura A, Crawford S, Farnia S, Omel JL, et al. Guidelines for Defining and Implementing Standard Episode of Care for Hematopoietic Stem Cell Transplantation within the Context of Clinical Trials. Biol Blood Marrow Transplant [Internet]. 2015 Apr [cited 2024 Dec 10];21(4):583–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879114014748

26. Kanate AS, Perales MA, Hamadani M. Eligibility Criteria for Patients Undergoing Allogeneic Hematopoietic Cell Transplantation. J Natl Compr Canc Netw [Internet]. 2020 May 1 [cited 2024 Dec 11];18(5):635–43. Available from: https://jnccn.org/view/journals/jnccn/18/5/article-p635.xml

27. Kotwica-Mojzych K, Jodłowska-Jędrych B, Mojzych M. CD200:CD200R Interactions and Their Importance in Immunoregulation. Int J Mol Sci. 2021 Feb 5;22(4):1–21.

28. Bansal A, Gierasch LM. The NPXY internalization signal of the LDL receptor adopts a reverse-turn conformation. Cell [Internet]. 1991 Dec 20 [cited 2024 Dec 23];67(6):1195–201. Available from: https://www.cell.com/cell/abstract/0092-8674(91)90295-A

29. He R, Browning DD, Ye RD. Differential roles of the NPXXY motif in formyl peptide receptor signaling. J Immunol Baltim Md 1950. 2001 Mar 15;166(6):4099–105.

30. Choe D, Choi D. Cancel cancer: The immunotherapeutic potential of CD200/CD200R blockade. Front Oncol. 2023;13:1–10.

31. Shao A, Owens DM. The immunoregulatory protein CD200 as a potentially lucrative yet elusive target for cancer therapy. Oncotarget [Internet]. 2023 Jan 12

[cited 2024 Dec 2];14:96–103. Available from: https://www.oncotarget.com/article/28354/text/

32. Wright GJ, Cherwinski H, Foster-Cuevas M, Brooke G, Puklavec MJ, Bigler M, et al. Characterization of the CD200 Receptor Family in Mice and Humans and Their Interactions with CD200. J Immunol [Internet]. 2003 Sep 15 [cited 2024 Dec 9];171(6):3034–46. Available from: https://journals.aai.org/jimmunol/article/171/6/3034/71431/Characterization-of-the-CD200-Receptor-Family-in

33. Grover P, Goel PN, Greene MI. Regulatory T Cells: Regulation of Identity and Function. Front Immunol [Internet]. 2021 Oct 5 [cited 2025 Jan 8];12:1–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8524049/

34. Principles of regulatory T cell function. Immunity [Internet]. 2023 Feb 14 [cited 2024 Nov 29];56(2):240–55. Available from: https://www.sciencedirect.com/science/article/pii/S1074761323000146

35. Gorczynski RM, Chen Z, He W, Khatri I, Sun Y, Yu K, et al. Expression of a CD200 Transgene Is Necessary for Induction but Not Maintenance of Tolerance to Cardiac and Skin Allografts. J Immunol [Internet]. 2009 Aug 1 [cited 2024 Nov 30];183(3):1560–8. Available from: https://journals.aai.org/jimmunol/article/183/3/1560/108493/Expression-of-a-CD200-Transgene-Is-Necessary-for

36. Oweira H, Khajeh E, Mohammadi S, Ghamarnejad O, Daniel V, Schnitzler P, et al. Pre-transplant CD200 and CD200R1 concentrations are associated with post-transplant events in kidney transplant recipients. Medicine (Baltimore) [Internet]. 2019 Sep 13 [cited 2024 Nov 30];98(37):1–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6750316/

37. Wang L, Liu JQ, Talebian F, El-Omrani HY, Khattabi M, Yu L, et al. Tumor Expression of CD200 Inhibits IL-10 Production by Tumor-Associated Myeloid Cells and Prevents Tumor Immune Evasion of CTL Therapy. Eur J Immunol [Internet]. 2010 Sep [cited 2024 Dec 9];40(9):2569–79. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3003298/

38. Twito T, Chen Z, Khatri I, Wong K, Spaner D, Gorczynski R. Ectodomain shedding of CD200 from the B-CLL cell surface is regulated by ADAM28 expression. Leuk Res [Internet]. 2013 Jul [cited 2024 Dec 23];37(7):816–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0145212613001240

39. Mahadevan D, Lanasa MC, Farber C, Pandey M, Whelden M, Faas SJ, et al. Phase I study of samalizumab in chronic lymphocytic leukemia and multiple myeloma: blockade of the immune checkpoint CD200. J Immunother Cancer. 2019 Aug 23;7(1):227.

40. Najafi M, Farhood B, Mortezaee K. Cancer stem cells (CSCs) in cancer progression and therapy. J Cell Physiol. 2019 Jun;234(6):8381–95.

41. Gonzalez-Montes Y, Osca-Gelis G, Rodriguez-Romanos R, Villavicencio A, González-Bártulos M, Llopis F, et al. CD200 genotype is associated with clinical outcome of patients with multiple myeloma. Front Immunol. 2024;15:1–11.

42. Thomas ED, Blume KG, Forman SJ, Negrin RS. Chapter 11. Overview of Hematopoietic Cell Transplantation Immunology. In: Thomas' Hematopoietic Cell Transplantation. 4th ed. Oxford: Wiley-Blackwell; 2019. p. 145–60.

43. Abbas, A. K., Lichtman, A. H., Pillai, S. Chapter 6. Antigen Presentation to T Lymphocytes and the Function of Major Histocompatibility Complex Molecules. In: Cellular and Molecular Immunology. 10th ed. Philadelphia, PA: Elsevier; 2021. p. 251–306.

44. Thomas ED, Blume KG, Forman SJ, Negrin RS. Chapter 12. Histocompatibility. In: Thomas' Hematopoietic Cell Transplantation. 4th ed. Oxford: Wiley-Blackwell; 2019. p. 145–60.

45. QIAamp DNA Blood Kits: Blood DNA Extraction. In: QIAGEN [Internet]. Barcelona: QIAGEN; 2024 [cited 2024 Dec 23]. Available from: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-dna-blood-kits?catno=51104

14. ANNEXES

14.1. ANNEX 1: ACUTE GRAFT-VERSUS-HOST DISEASE STAGES

Table 11. Acute graft-versus-host disease stages (15)

Stage	Skin (active erythema only)	Liver (bilirubin (mg/dl))	Upper GI	Lower GI (stool output/ day)
0	No active (erythematous) GVHD rash	<2	No or intermittent nausea, vomiting or anorexia	Adult: <500 ml/day or <3 episodes/day
				Child: <10 ml/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2–3	Persistent nausea, vomiting or anorexia	Adult: 500–999 ml/day or 3 or 4 episodes/day
				Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25–50% BSA	3.1–6	NA	Adult: 1,000–1,500 ml/ day or 5–7 episodes/day
				Child: 20–30 ml/kg/day or 7–10 episodes/day
3	Maculopapular rash >25% BSA	6.1–15	NA	Adult: >1,500 ml/day or >7 episodes/day
				Child: >30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation >5% BSA	>15	NA	Adult and child: severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

BSA, body surface area; GI, gastrointestinal; GVHD, graft-versus-host disease; NA, not applicable. Reprinted with permission from ref. 9, Elsevier.

14.2. ANNEX 2: SUGGESTED PRE-HEMATOPOIETIC STEM CELL TRANSPLANTATION EVALUATION FOR ELIGIBILITY ASSESSMENT

Table 12. Suggested pretransplant evaluation for eligibility assessment. Extracted from (26)

Clinical evaluation - History and physical examination (medical, infectious, transfusion, sexual and mental history and review of concomitant medications) - Assessment of functional status (KPS, ECOG, performance score, Lansky score…) - Hematopoietic cell transplantation-comorbidity index/fragility score	**Laboratory evaluation** - Confirmation of histologic diagnosis (pathology review at the transplant center). - Disease remission status (including biopsies with cytogenetics/molecular panel, imaging studies…) - Human leukocyte antigen typing - Donor specific antibody screen (when indicated) - Donor search (identifying suitable donor and graft type) - ABO typing and antibody screen - Complete blood count with differential / complete metabolic profile
End-organ assessments - Left ventricular ejection fraction (2-dimensional echocardiography or MUGA scan)/ electrocardiogram. - Chest radiograph/ CT scan (when indicated) / pulmonary function testing - Pregnancy test (female patients) - Lumbar punction (high-risk patients and those with history of central nervous system involvement) - Viral serologies (CMV, herpes simplex, VEB, varicella, HIV, hepatitis B/C, human T-lymphotropic virus I/II) - Other infectious serologies (for example, toxoplasma screen, rapid plasma regain test (syphilis), QuantiFERON test	**Consultative services** - Nutritional assessment and dietary consult (if indicated) - Dental evaluation - Psychosocial and supportive care valuation - Caregiver (availability of reliable caregivers is necessary for minimum of 90-100 days posttransplant) - Temporary housing arrangements (usually within 30-60 minutes from the transplant center)

14.3. ANNEX 3: GETH CENTERS PARTICIPATING

Table 13. GETH centers participating in the study

H. Universitario La Princesa (Madrid)	H. Universitario Regional de Málaga (Málaga)	H. Universitario Son Dureta (Palma)
H. de la Santa Creu i Sant Pau (Barcelona)	H. Clínico Universitario de Valencia (Valencia)	H. Clínico Universitario Virgen de la Arrixaca (Murcia)
H. General Universitario Morales Meseguer (Murcia)	H. Universitario y Politécnico La Fe (Valencia)	H. Universitario 12 de Octubre (Madrid)
H. Universitario Reina Sofía (Córdoba)	H. Universitario Virgen del Rocío (Sevilla)	H. Universitario Puerta de Hierro Majadahonda (Majadahonda, Madrid)
H. Clínic de Barcelona (Barcelona)	Institut Català d'Oncología – H. Germans Trias i Pujol (Badalona, Barcelona)	H. Nuestra Señora de Aranzazu (San Sebastián)
H. Universitario Central de Asturias (Oviedo)	H. Universitari Vall d'Hebrón (Barcelona)	H. Universitario La Paz (Madrid)
H. General Universitario Gregorio Marañón (Madrid)	H. Universitario Ramón y Cajal (Madrid)	H. Clínico Universitario de Santiago (Santiago de Compostela)
H. Universitario de Salamanca (Salamanca)	H. Universitario Marqués de Valdecilla (Santander)	Clínica Universidad de Navarra (Pamplona)
Institut Català d'Oncología – H. Duran i Reynals (L'Hospitalet de Llobregat, Barcelona)	H. Universitario de Gran Canaria Doctor Negrín (Las Palmas)	

14.4. ANNEX 4: DNA EXTRACTION (QIAamp DNA Blood Mini Kits)

DNA extraction protocol using QIAamp DNA Blood Mini Kits follows these steps:

- Ensure a proper temperature: bring the samples to room temperature (15 25ºC) and the heating block to 56ºC.
- Introduce 20 µL of proteinase into the microcentrifuge tube.
- Introduce 200 µL of blood sample.
- Introduce 200 µL of Buffer AL.
- Vortex for 15 seconds.
- Incubate at 56ºC for 10 minutes.
- Centrifuge the tube in a brief manner.
- Introduce 200 µL of ethanol (96-100%)
- Vortex for 15 seconds.
- Transfer the tube content to the QIAamp Mini spin column
- Centrifuge at 8000 rpm for 1 minute.
- Place the column into a new tube.
- Introduce 500 µL of Buffer AW1.
- Centrifuge at 8000 rpm for 1 minute.
- Place the column into a new tube.
- Introduce 500 µL of Buffer AW2.
- Centrifuge at 14000 rpm (the maximum speed) for 3 minutes.
- Place the column into a new tube.
- Centrifuge at 14000 rpm (the maximum speed) for 1 minute.
- Place the column into a new tube.
- Introduce 200 µL of Buffer AE or distilled water.
- Incubate at room temperature for 1 minute.
- Centrifuge at 8000 rpm for 1 minute.
- Dispose of the spin column and keep the eluate by transferring it to another tube (45).

14.5. ANNEX 5: INFORMATION SHEET AND INFORMED CONSENT

IDIBGI	Consentimiento Informado Biobanco del Instituto de Investigación Biomédica de Girona	BioBanc

BIO-FOR-060_rev01 (08/05/2020) GET_v4 (Agosto 2022)

HOJA DE INFORMACIÓN AL PACIENTE / DONANTE

En la mayoría de hospitales, además de la tarea asistencial, también se realiza investigación biomédica. Las muestras y datos clínicos obtenidos para el diagnóstico o control de las enfermedades, una vez utilizadas con este fin, resultan útiles y necesarias para la investigación. Por este motivo, 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 en participar en esta propuesta.

¿Qué es un Biobanco? Un Biobanco es una plataforma de apoyo a la investigación que trabaja para obtener, almacenar, gestionar y distribuir muestras biológicas humanas con el fin de fomentar la investigación biomédica de excelencia. Nuestro objetivo es contribuir en la mejora del conocimiento, prevención, diagnóstico, pronóstico y/o tratamiento de las enfermedades. Las muestras y/o datos incluidos en el Biobanco podrán ser cedidos para realizar estudios de investigación, siempre con la previa evaluación de un comité científico y de un comité de ética. Toda la actividad del Biobanco se realiza en cumplimiento de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y el Real Decreto 1716/2011, de 18 de noviembre, de regulación de los Biobancos.

Muestras biológicas e información asociada. Dependiendo de la situación, las muestras podrán proceder de excedentes de pruebas y/o intervenciones quirúrgicas que se le han realizado o se le realizarán a su centro de salud o, aprovechando el proceso asistencial por el que acudió al centro de salud, se le recogerá una muestra adicional. Estas muestras podrán recogerse en los diferentes centros de salud que integra el IDIBGI: el Hospital Universitario de Girona Dr. Josep Trueta (Instituto Catalán de la Salud), el Instituto de Asistencia Sanitaria, el Instituto Catalán de Oncología, el Instituto de Diagnóstico por la Imagen y los centros de Atención Primaria del Instituto Catalán de la Salud en Girona.

En el caso de una extracción de sangre, el riesgo para su salud es muy pequeño, pero puede incluir las molestias habituales de una extracción: dolor de muy poca importancia, piel contusionada, sangrado por donde entra la aguja o la ansiedad ante las agujas. Se tomarán precauciones para evitar estos inconvenientes. En el caso de una muestra de orina y/o de heces, la obtención se realizará por métodos naturales no invasivos, por lo que no existen riesgos asociados. En el caso de una muestra de tejido o de otros fluidos biológicos, la extracción se realizará en el contexto asistencial, por lo que no se añadirá ningún riesgo adicional para usted. Nunca se realizará una intervención exclusivamente para la obtención de muestras para investigación.

Una vez obtenido, el material biológico pasará a formar parte del Biobanco hasta que se agote, sin que ello comprometa el proceso asistencial habitual. Sin embargo, usted podrá disponer de sus muestras cuando sea necesario por motivos de salud, siempre que aún estén disponibles. La identificación de las muestras biológicas y la información asociada será codificada. Únicamente el personal debidamente autorizado del Biobanco podrá acceder a los datos personales, al historial clínico y a los resultados de las pruebas, cuando sea necesario. Para completar la información clínica relacionada con la enfermedad COVID, es posible que los investigadores tengan que recopilar datos accediendo a su historial clínico electrónico con la previa aprobación del proyecto de investigación por parte del Comité de Ética de Investigación Clínica.

Protección de datos y confidencialidad. En cumplimiento del Reglamento (UE) 2016/679, del Parlamento Europeo y del Consejo de 27 de abril de 2016, reglamento general de protección de datos, y de la Ley Orgánica 3/2018, de 5 de diciembre, de protección de datos personales y garantía de los derechos digitales (LOPDGDD), le informamos que el tratamiento de sus datos pasará a formar parte de los tratamientos del IDIBGI, y se le aplicarán las medidas de control y seguridad aplicadas a las categorías especiales de datos. El IDIBGI, como responsable del tratamiento, le informa que podrá ejercer sus derechos de acceso, rectificación, supresión, oposición, objeción y, en su caso, portabilidad y limitación, dirigiéndose al correo electrónico transparencia@idibgi.org o al Parc Hospitalari Martí i Julià, c/Dr. Castany, s/n (Salt). Puede consultar más información sobre la protección de datos en: www.idibgi.org.

Donación de carácter altruista. La donación que usted realiza es gratuita y altruista, por lo que no obtendrá ninguna retribución económica. No se prevé que obtenga beneficios directos para su salud, ya que los resultados que se obtendrán serán con fines de investigación, pero puede ayudar a obtener información que puede beneficiar a la sociedad en un futuro.

Participación voluntaria. Su participación es totalmente voluntaria. Si firma el consentimiento informado confirmará que desea participar. Puede negarse o retirarlo en cualquier momento posterior a la firma, sin que ello repercuta negativamente en su asistencia médica, presente o futura. Podrá revocar el consentimiento en cualquier momento sin necesidad de indicar los motivos y dirigiéndose al Biobanco personalmente, por teléfono, carta o correo electrónico.

Información sobre los resultados de la investigación y el destino de las muestras. En caso de requerirlo, el Biobanco podrá proporcionarle información sobre cuáles son las investigaciones en que se han utilizado sus muestras. Si de estas investigaciones se obtuviera información relevante para su salud o la de sus familiares, ésta le sería comunicada si así lo estima oportuno. En este caso, utilizaríamos los datos de contacto disponibles en su historial clínico.

En caso de eventual cierre del Biobanco, la información sobre el destino de sus muestras estará a su disposición en el Registro Nacional de Biobancos para Investigación Biomédica. Por favor, consulte al Biobanco cualquier duda que tenga ahora o en el futuro sobre este documento.

IDIBGi	Consentimiento Informado Biobanco del Instituto de Investigación Biomédica de Girona	Bio Banc

BIO-FOR-060_rev01 (08/05/2020) GET_v4 (Agosto 2022)

CONSENTIMIENTO INFORMADO

Ejemplar Paciente / Donante

Consentimiento informado para la obtención y utilización de muestras biológicas y/o datos clínicos para investigación biomédica y su conservación en el Biobanco

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 Biobanco en los términos antes explicados, por favor, lea y firme a esta hoja.

Otorgo mi consentimiento informado explícito de forma libre, específica e informada para obtener y conservar mi material biológico y la información clínica asociada en el Biobanco IDIBGI. Este podrá almacenar y utilizar las muestras biológicas obtenidas, los datos clínicos y las pruebas de imagen, con el fin de desarrollar proyectos de investigación biomédica, siempre que estos cuenten con la aprobación de un comité de ética de investigación competente.

Señalar una opción:

☐ **PACIENTE**: persona receptora del trasplante de médula ósea (R).
☐ **DONANTE**: persona donante de médula ósea (D).

Otorgo mi consentimiento explícito para:	Sí	No
• Utilizar el material biológico excedente y/o datos asociados para investigación biomédica.		
• Obtener y utilizar material biológico adicional y/o datos asociados para investigación biomédica.		
• Utilizar las muestras biológicas almacenadas previamente en el hospital.		
• Recibir información derivada de la investigación, si es relevante para mi salud o la de mis familiares.		
• Ser contactado en el caso de necesitar más información o muestras adicionales. Teléfono o email de contacto:		
Deseo incluir las siguientes restricciones sobre el uso de mis muestras y/o datos:		
....		

PACIENTE ☐ / DONANTE ☐	PROFESIONAL QUE INFORMA	REPRESENTANTE LEGAL (TUTOR)* ☐ / TESTIMONIO ☐
Nombre:	*Nombre:*	*Nombre:* .....
Apellidos:	*Apellidos:*	*Apellidos:* .....
DNI:	*DNI:*	*DNI:* .....
Edad:		*Relación con el donante:* .....
NHC:		
Firma:	*Firma:*	*Firma:*

En ....., a ..... de ..... de .....

** Llegada la mayoría de edad, el donante tendrá derecho a revocar o modificar este consentimiento, por lo que deberá estar debidamente informado. En caso de que no ejerza este derecho, se considerará que el actual documento de consentimiento informado sigue vigente.*

Le agradecemos su desinteresada colaboración con el avance de la ciencia y la medicina.

IDIBGi	Consentimiento Informado Biobanco del Instituto de Investigación Biomédica de Girona	Bio Banc

BIO-FOR-060_rev01 (08/05/2020) GET_v4 (Agosto 2022)

CONSENTIMIENTO INFORMADO

Ejemplar Hospital

Consentimiento informado para la obtención y utilización de muestras biológicas y/o datos clínicos para investigación biomédica y su conservación en el Biobanco

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 Biobanco en los términos antes explicados, por favor, lea y firme a esta hoja.

Otorgo mi consentimiento informado explícito de forma libre, específica e informada para obtener y conservar mi material biológico y la información clínica asociada en el Biobanco IDIBGI. Este podrá almacenar y utilizar las muestras biológicas obtenidas, los datos clínicos y las pruebas de imagen, con el fin de desarrollar proyectos de investigación biomédica, siempre que estos cuenten con la aprobación de un comité de ética de investigación competente.

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• Utilizar el material biológico excedente y/o datos asociados para investigación biomédica.		
• Obtener y utilizar material biológico adicional y/o datos asociados para investigación biomédica.		
• Utilizar las muestras biológicas almacenadas previamente en el hospital.		
• Recibir información derivada de la investigación, si es relevante para mi salud o la de mis familiares.		
• Ser contactado en el caso de necesitar más información o muestras adicionales. Teléfono o email de contacto:		
Deseo incluir las siguientes restricciones sobre el uso de mis muestras y/o datos:		
....		

PACIENTE ☐ / DONANTE ☐	PROFESIONAL QUE INFORMA	REPRESENTANTE LEGAL (TUTOR)* ☐ / TESTIMONIO ☐
Nombre:	*Nombre:*	*Nombre:* .....
Apellidos:	*Apellidos:*	*Apellidos:* .....
DNI:	*DNI:*	*DNI:* .....
Edad:		*Relación con el donante:* .....
NHC:		
Firma:	*Firma:*	*Firma:*

En ....., a ..... de ..... de .....

**Llegada la mayoría de edad, el donante tendrá derecho a revocar o modificar este consentimiento, por lo que deberá estar debidamente informado. En caso de que no ejerza este derecho, se considerará que el actual documento de consentimiento informado sigue vigente.*

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CONSENTIMIENTO INFORMADO

Ejemplar Biobanco

Consentimiento informado para la obtención y utilización de muestras biológicas y/o datos clínicos para investigación biomédica y su conservación en el Biobanco

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 Biobanco en los términos antes explicados, por favor, lea y firme a esta hoja.

Otorgo mi consentimiento informado explícito de forma libre, específica e informada para obtener y conservar mi material biológico y la información clínica asociada en el Biobanco IDIBGI. Este podrá almacenar y utilizar las muestras biológicas obtenidas, los datos clínicos y las pruebas de imagen, con el fin de desarrollar proyectos de investigación biomédica, siempre que estos cuenten con la aprobación de un comité de ética de investigación competente.

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• Utilizar el material biológico excedente y/o datos asociados para investigación biomédica.		
• Obtener y utilizar material biológico adicional y/o datos asociados para investigación biomédica.		
• Utilizar las muestras biológicas almacenadas previamente en el hospital.		
• Recibir información derivada de la investigación, si es relevante para mi salud o la de mis familiares.		
• Ser contactado en el caso de necesitar más información o muestras adicionales. Teléfono o email de contacto:		
Deseo incluir las siguientes restricciones sobre el uso de mis muestras y/o datos:		
....		

PACIENTE ☐ / DONANTE ☐	PROFESIONAL QUE INFORMA	REPRESENTANTE LEGAL (TUTOR)* ☐ / TESTIMONIO ☐
Nombre:	*Nombre:*	*Nombre:* .....
Apellidos:	*Apellidos:*	*Apellidos:* .....
DNI:	*DNI:*	*DNI:* .....
Edad:		*Relación con el donante:* .....
NHC:		
Firma:	*Firma:*	*Firma:*

En ....., a ..... de ..... de .....

** Llegada la mayoría de edad, el donante tendrá derecho a revocar o modificar este consentimiento, por lo que deberá estar debidamente informado. En caso de que no ejerza este derecho, se considerará que el actual documento de consentimiento informado sigue vigente.*

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