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Role of B-cell memory on CD38-targeting Human Leukocyte Antigen desensitization therapy in Highly sensitized kidney transplant candidates

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Role of B-cell memory on CD38-targeting Human Leukocyte Antigen desensitization therapy in Highly sensitized kidney transplant candidates

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ABBREVIATIONS

ABMR: antibody-mediated rejection

ADCC: antibody-dependent cell-mediated cytotoxicity

ADPC: antibody-directed cellular phagocytosis

AM: Acceptable mismatch

ASCs: antibody-secreting cells

BAFF: B cell activating factor

BCR: B-cell receptor

Bregs: regulatory B cells

CDC: complement-dependent cytotoxicity

CTLA4: Cytotoxic T-lymphocyte associated protein 4

cPRA: calculated panel reacted antibody

DSA: donor-specific antibodies

ELISA: enzyme-linked immunosorbent assay

ELISPOT: enzyme-linked immunospot

ESRD: end-stage renal disease

FcR: Fc receptor

GC: germinal center

HLA: human leukocyte antigen

HLA-sp: HLA-specific

HR: High responder

IDES: IgG endopeptidase

IL-10: interleukin-10

IL-15: interleukin-15

IL-2: interleukin-2

IL-21: interleukin-21

IL-6: interleukin-6

IL-6R: interleukin-6 receptor

Ig: immunoglobulin

IgG: immunoglobulin-G

IRF4: interferon regulatory factor 4

IVIg: intravenous immunoglobulin

KAS: Kidney Allocation system

LLPCs: long-lived plasma cells

LR: Low responder

MBCs: memory B cells

MFI: mean fluorescence intensity

MDSC: myeloid-derived suppressor cells

MHC: major histocompatibility complex

MM: multiple myeloma

mAbs: monoclonal antibodies

NK: natural killer

NR: Non-responder

PBMCs: peripheral blood mononuclear cells

PATHI: prioritization kidney allocation system

PLA2R: phospholipase A2 receptor

PKE: kidney paired exchange

SFU: spot forming unit

SHM: somatic hypermutation

SLOs: secondary lymphoid organs

SoR: suboptimal responders

TCR: T-cell receptor

TFH: follicular helper T cells

TLR: toll-like receptor

TNF: tumor necrosis factor

Tregs: regulatory T cells

UNOS: united network from organ sharing

I. INTRODUCTION

I. INTRODUCTION

1. Kidney Transplantation as the hallmark of best treatment for all patients with end-stage renal disease

Kidney transplantation stands as the best treatment for end-stage renal disease (ESRD), offering superior outcomes in comparison to any type of dialysis therapy[1,2]. The benefits of kidney transplantation include increased life expectancy, improved quality of life by eliminating the need for regular dialysis treatments and lower risk of complications, such as reduction of cardiovascular diseases, which are a major cause of mortality in ESRD patients. Overall, kidney transplantation not only offers a better quality of life but also reduces medical costs associated with long-term dialysis treatments.

Unfortunately, only a small percentage of patients with ESKD receive a kidney transplant, being most barriers to access kidney transplantation patient-related, physician/provider-related, and system-related[3]. Still, beyond these healthcare system barriers, transplantation also faces some important limitations including the shortage of organs for all patients waitlisted and the lack of significant improvement of long-term graft survival rates, mainly due to activation of the alloimmune response leading to chronic (B-cell mediated) allograft rejection, calcineurin-inhibitors(CNI)-related nephrotoxicity, relapse of primary ESRD as well as the death of the patients with a functioning graft because of adverse events directly associated to chronic immunosuppression such as cancer and opportunistic infections and off-target effects related side effects inducing diabetes, hypertension, dyslipidemia that significantly increase the risk of fatal cardiovascular events[4].

Nevertheless, while advancements in transplant procedures and novel immunosuppressive therapies have led to a slightly improvement of graft and patient survival in the last two decades[5], yet, a great number of transplant organs will fail and patients return to the waiting list, thereby further increasing the pool of kidney transplant candidates[4]. In addition, these patients, generally become highly sensitized against HLA antigens due to the previous antigen exposure, and have important difficulties to find a HLA compatible donor due to a preformed serological memory and remain for very long periods of time, if not for ever in some cases, on chronic dialysis

therapy [6,7]. Therefore, an important field of research has been the study of novel strategies and therapies that could safely overcome this HLA barrier and thus, allow access to kidney transplantation to all patients with ESRD, including those highly sensitized against HLA antigens.

2. The issue of HLA sensitization in human transplantation

Sensitization against HLA antigens is one of the main barriers to overcome for a successful kidney transplantation. Sensitization to allogeneic HLA occurs after a previous exposure to foreign tissue thus, especially after previous transplants but also after pregnancy and blood transfusions, which may all lead to the development of an antigen-specific adaptive immune memory response against HLA antigens. If undergoing transplantation with allografts sharing same HLA molecules as those harbored in previously recognized tissues, such preformed immune memory may lead to a rapid and highly effective recall immune response driving accelerated allograft rejection and graft loss [8,9].

2.1. Major histocompatibility complex HLA

The major histocompatibility complex or MHC is a transmembrane protein responsible for initiating adaptive immune responses by presenting antigens to T cells. In humans, MHC molecules are known as Human Leukocyte Antigens (HLA), since they were first discovered through antigenic differences between white blood cells from different individuals[10].

In the human genome, the HLA system is encoded in a highly polymorphic region on the short arm of human chromosome 6. HLA molecules are highly polygenic, containing several different MHC class I and class II genes conferring each individual a specific set of MHC molecules, and polymorphic, having multiple variants or alleles of each gene within the population as a whole[10]. In humans, there are three class I alpha chain genes called HLA-A, HLA-B and HLA-C, and three pairs of MHC class II alpha and beta chain genes called HLA-DR, HLA-DQ and HLA-DP. For several MHC class I and class II genes there are over 1000 alleles identified in the human population, far more than the allele count of other genes located within the MHC region[10]. Currently, 38.909 HLA and related alleles

are described by the HLA nomenclature and included in the IPD-IMGT/HLA Database[11]. Besides, the total count of distinct HLA alleles within the human genome is highly dynamic, continuously expanding as genetic sequencing technologies advance and ongoing research reveals new alleles.

The expression of HLA molecules is diverse, being HLA class I proteins expressed on nucleated cells, platelets, and sometimes (in about 15% of the population) on red cells and MHC class II more restricted on specialized antigen presenting cells such as B-cells, macrophages, and dendritic cells[12]. Also, under conditions of inflammatory stress, MHC-II expression can be upregulated on a large variety of cells that typically don't present class II antigens[13].

HLA molecules play a complex role in immune surveillance, autoimmune and infectious diseases and also in transplantation, since differences in donor and recipient HLA expression provide a continuous source of antigenic stimulation to the immune system and the strong immune response triggered by the detection of non-self HLAs by adaptive immune cells is considered as the main cause of transplant rejection[14,15].

2.2. Biology of HLA sensitization

Elucidating the immune mechanisms underlying HLA sensitization is crucial to understand the complexities of organ rejection and transplant outcomes. HLA sensitization occurs from any exposure to non-self HLA antigens in events like pregnancies, transfusions or previous transplants, and leads to the development of a specific immune response against HLAs and the production of HLA-sp antibodies and alloreactive T cells that may ultimately limit both access to transplantation and successful allograft outcomes[16]. While HLA sensitization also leads to strong T-cell sensitization and induce the development of highly specific alloreactive memory T cells that may also impact on allograft outcomes[17–19], in this thesis we will focus on the B-cell subset counterpart. In this regard, **Figure 1** shows schematically the natural history of development of B-cell immune memory within different biological compartments after subsequent alloantigen encounters.

2.2.1 First HLA antigen encounter: development of memory B cells and long-lived plasma cells

Naïve B cells first encounter HLA antigens in the B-cell follicles of Secondary lymphoid organs (SLO). B-cell receptor (BCR) binding to a specific HLA result in BCR signaling, activation of naïve B cells and processing and presentation of this HLA on MHC-II molecules in the B-cell surface. Then, naive B cells enhance their metabolic activity and express chemoattractant receptors such as CC-chemokine receptor 7 (CCR7) and EBI2 that guide them to the border of the T cell zone of SLOs, where they engage with cognate antigen-specific TFH that will provide them with the costimulation signals via CD40/CD40L generally necessary for its activation [16,20,21].

After the first naïve B cell – TFH interaction, B cells have at least three different cell fates; They can differentiate into short-lived plasma cells that rapidly secrete low affinity HLA-sp antibodies. Short-lived plasma cells accumulate in the red pulp regions of the spleen and medullary cords of lymph nodes, and their lifespans are generally limited to the course of the infection. They can also enter germinal center (GC) to subsequently differentiate into long-lived plasma cells (LLPCs) and memory B cells (MBC) or they can differentiate into GC-independent MBC[22]. The parameters that determine B-cell fate remain not fully understood, although the affinity of the antigen-BCR interaction and TFH costimulatory signal through CD40L-CD40 seem to play a key role in this process[23]. A low-affinity Ag-BCR interaction induces weakly the interferon regulatory factor 4 (IRF4) and initiates GC B-cell differentiation, whereas a high affinity interaction induces high levels of IRF4 and promotes PC differentiation. Also, CD40-CD40L interaction seem to direct B cells into GC reaction, however, chronic CD40 signaling promotes plasma cell differentiation[23]. Moreover, the nature and structure of antigens also impacts the outcome of immunization[23].

Those naïve B cells that have formed GCs, proliferate and undergo somatic hypermutation (SHM) in the dark zone to enhance their antigen affinity. In this phase, B-cell undergo a selection process where the affinity of the BCR for the antigen is crucial, as higher affinity interactions are more successful at internalizing antigens for presentation to TFHs in the GCs[23].

After SHM, B cells enter the light zone where they can, again, undergo tree different cell fates; they can differentiate into LLPCs and relocate to the bone marrow (BM) where they will constitutively secrete large quantities of high affinity HLA-sp antibodies and persist there potentially a lifetime. They can also differentiate into long-lived MBC that will reside in SLOs and other tissues awaiting to re-encounter cognate antigen, or they can re-enter the GC dark zone to undergo again SHM and subsequent selection to become higher affinity plasma cells and MBC[23]. Therefore, even naive B cells with relatively low-affinity antigen specificity can re-enter GCs and differentiate into high-affinity, LLPCs and MBCs in this second phase of the B-cell memory process.

2.2.2 HLA antigen re-exposure

HLA-sp MBC are distributed recirculating in strategic tissues to maximize the probability of antigen re-encounter including peripheral blood, spleen and the lymph nodes, where it has been demonstrated that rapidly form proliferative focis after antigen recall[24]. In addition to spleen and lymph nodes, MBC can also be found in other tissues like bone marrow, peyer's patches, gingiva, mucosal epithelium of tonsils or the gastro-intestinal tract[25]. Although whether those MBC residing in other tissues are functional remain uncertain[26].

Also, while MBCs remain in SLOs waiting for recall responses, LLPCs stay in the bone marrow continuously secreting HLA-specific (HLA-sp) antibodies. The long survival of LLPCs in the bone marrow niche is thanks to the BM microenvironment that provide factors that support its survival such as the tumor necrosis factor (TNF) family, interleukin-6 (IL-6) family and CXCL12, among others[27].

By cause of all these preformed humoral memory against HLA antigens, the memory recall response upon antigen re-exposure is faster, stronger and more specific than the primary naïve response. After a second HLA antigen exposure, circulating high-affinity antibodies produced by LLPCs are the first to initiate an adaptive immune response, followed by specific MBC, which quickly proliferate and differentiate into plasma cells or, alternatively, re-enter GCs for further affinity maturation and SHM[24] **(Figure 1).** Although the mechanisms that determine this secondary fate decision remain unclear, it has been

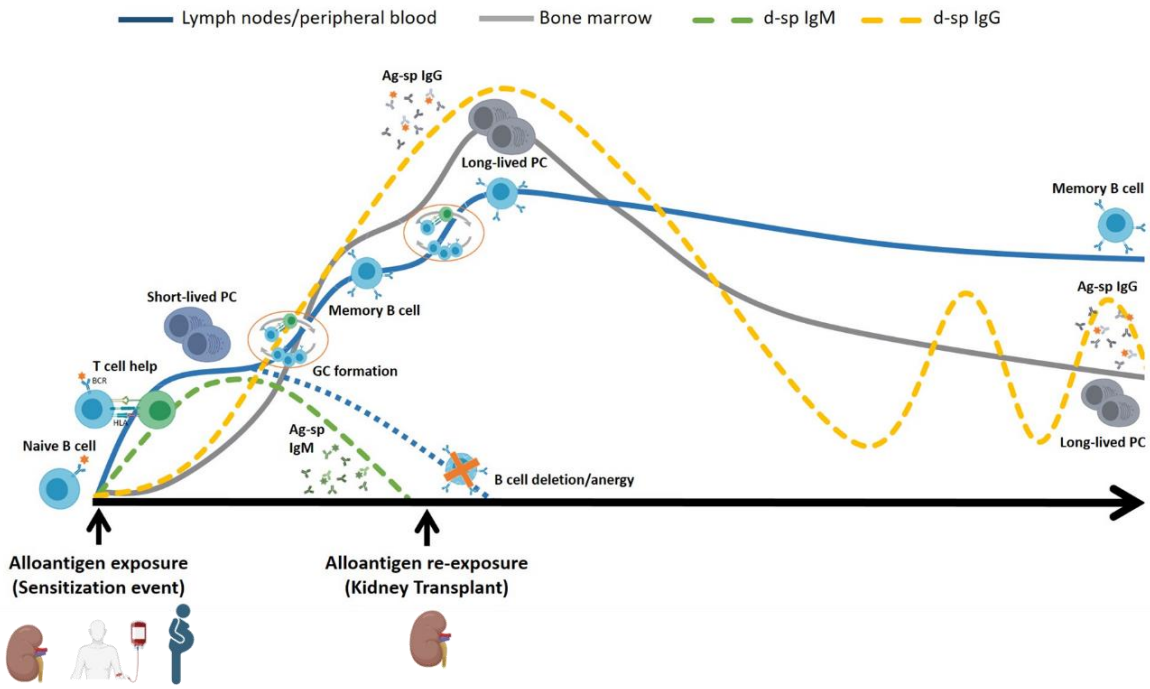

Figure 1. Natural history of humoral immune memory compartments through primary and secondary antigen encounters. Adapted from *Torija et al 2020*[16].

proved that it relies on the strength of signaling via the BCR, CD40 and BAFF receptors, as well as BCR affinity and isotype and expression of CD80 and PDL2 markers [28–30].

2.2.3 Nomenclature and ontology of B-cell subsets

The high diversity present within the B-cell population, including MBC and plasma cells, presents a significant challenge for their systematic classification. As mentioned previously, B cells undergo a complex process of differentiation and maturation that results in a vast array of phenotypic and functional subsets. All these subsets exhibit diverse surface markers, effector functions and tissue localization, depending on its specialized role in the immune response[31]. Therefore, efforts have been made to categorize B-cell subsets despite the lack of universally accepted classification and often contradictory studies.

Peripheral B cells are a highly diverse population and there is a growing difficulty in finding a consistent B-cell classification. In general, peripheral B cells are classified into transitional and mature B cells, with this last group including naïve B cells, MBC and antibody secreting cells (ASCs). The majority of B-cell populations can be identified using CD19, CD20, IgD, CD27, CD38 and CD24 markers[32]. CD20 and CD19 markers follow an

overlapping pattern of expression with the exception of plasma cells, which are CD19+ and CD20-, being CD19 the hallmark of the B-cell lineage. IgD defines the isotype-switching of the B cell and CD27 the memory-like state, being able to identify with these markers isotype-switched MBCs (CD27+ IgD-), unswitched MBCs (CD27+ IgD+), double negative or atypical B cells (CD27- IgD-) and naïve B cells (CD27- IgD+). Also, CD38 and CD24 expression help identify transitional B cells (CD24++, CD38++) and ASCs (CD24-, CD38++). Specially CD38, is highly expressed in the early stages of B-cell development and later is a relevant marker for B-cell differentiation into ASCs[33]. In the BM, B-cell precursors can be identified with CD10 marker and further stratified depending on the expression of CD21, CD24 and CD38. Moreover, CD138 marker may be also added in this compartment to identify LLPCs.

2.3 Assessment of HLA sensitization in kidney transplant candidates.

2.3.1 Serological memory

A. Cellular-based assays. Complement-dependent cytotoxicity (CDC) and Flow-cytometry based assays

Historically, screening for HLA antibodies and determining unacceptable antigens was rather black and white[8]. The first method developed to detect anti-HLA antibodies were cell-based assays, such as the complement dependent cytotoxicity (CDC) assay that determined circulating anti-HLA antibodies using CDC to test the reactivity of patient serum against a panel of healthy blood donors representing the HLA makeup of the local donor population. In this assay, serum is mixed with either potential donor's lymphocytes or commercially available T and B lymphocytes. Complement fixing antibodies present in serum bind to the lymphocytes triggering complement-mediated damage that results in cell death[34,35]. Although this technique was used routinely to predict humoral hyperacute rejection, it has poor reproducibility, high variability and inability to detect non-complement fixing HLA antibodies[36,37]. Another cell-based method to detect anti-HLA antibodies is the crossmatch assay, that is also performed with flow cytometry incubating donor T and/or B lymphocytes from blood, spleen or lymph nodes with the recipient's serum and detect recipient's antibodies binding to donor's cells[16].

B. Single antigen bead assays

At present, solid-phase assays are the more commonly used for the detection of anti-HLA antibodies. These assays rely on solid phase matrices like plates or beads coated with single or multiple HLA antigens that, when the patient's serum is added to this matrix, bound antibodies are detected by means of enzyme-linked immunosorbent assay (ELISA), flow cytometer or Luminex[34] . Currently, the most commonly used platform for solid-phase assays is the Luminex multiplex bead-based system.

In the Luminex platform, each bead is coated with a single HLA antigen and has a distinct color that can be detected independently. Single antigen beads (SAB) are incubated with patient's serum and bound antibodies are detected with a fluorescent-labeled anti-IgG antibody. By using a dual laser this assay identifies both the bound antibody and the SAB. The results are given as the intensity of the mean fluorescent signal (MFI) that is often considered as the measure of the level of each HLA antibody [38–40]. The number of beads with bound antibodies relative to the number tested is the % calculated PRA (cPRA)[15,41]. The calculated PRA (cPRA) score was developed in 2007 by the united network from Organ sharing (UNOS) histocompatibility and is based on identifying HLA specificities considered unacceptable for a sensitized individual using data from a historical donor population[42,43]. Therefore, cPRA represents the percentage of donors who are expected to have unacceptable HLA antigens to which the transplant candidate is sensitized, thus, a cPRA of 95% would be provided to be incompatible with 95% of available donors.

Even if Luminex SAB assay allows a better distinction in anti-HLA antibodies positivity, enables virtual crossmatch, provides semi-quantitative results and provides a quick completion as compared to cell-based assays, it has its limitations. Luminex SAB assay demonstrates a high technical variation in MFI values that can vary up to 20%. Also, the determination of standardized MFI cutoff values is still a topic of discussion due to inconsistencies in MFI readings, double dye equivalent unit molecules, antibody titers, crossmatch outcomes, and clinical implications[44]. Consequently, there exists considerable variation among transplant centers in establishing MFI limit values to identify unacceptable HLAs[45]. Also, even if a strict cutoff value is enforced, which is a contentious practice due to the variability in MFI read-out, other factors such as bead

saturation, potential inhibition and shared epitope phenomena contribute to the risk of drawing incorrect conclusions regarding antibody detection[46]. Importantly, not all alleles are covered by the current SAB assays, leading to quite significant gaps in the ability to identify specificities of HLA antibodies, especially in ethnic minority populations[47].

Moreover, SAB also allow the detection of complement-fixing anti-HLA Abs through complement components C1q and C3d, providing valuable immunological information beyond just the antibody presence[48]. The C1q assay determines if the detected anti-HLA antibodies can bind the first component of the complement system, the C1q, and activate the complement via the classical pathway[49]. In this assay, the SAB with bound anti-HLA antibodies are further incubated with purified C1q that is subsequently detected by an anti-C1q fluorescent Ab and quantified by flow cytometry. A positive fluorescence signal indicates that the anti-HLA antibody can bind C1q and therefore has the potential to activate complement. On the other hand, the C3d assay detects DSA that can activate the complement cascade up to the C3 component[50,51]. In this assay, SAB bound with anti-HLA antibodies are incubated with normal serum as a source of complement. If the anti-HLA antibodies activate the complement, C3 is cleaved and deposited on the beads, where it can be detected through flow cytometry.

2.3.2 Assessment of peripheral B-cell memory against HLA antigens

Current evaluation of sensitization to HLA antigens primary focus on measuring circulating HLA antibodies. These antibodies are indicative predominantly of antibody production by BM-residing LLPCs and/or ongoing generation of short-lived plasma cells (PC). However, these assays may fail to identify humoral sensitization present within the memory B-cell compartment, that have the capability to trigger a rapid and robust humoral immune response post-transplantation leading to acute AMR[52–54]. Developing reliable methods for the detection of HLA-sp MBC in transplantation holds significant promise in revolutionizing our capability to better stratify patient risk and potentially individualize immunosuppressive therapies.

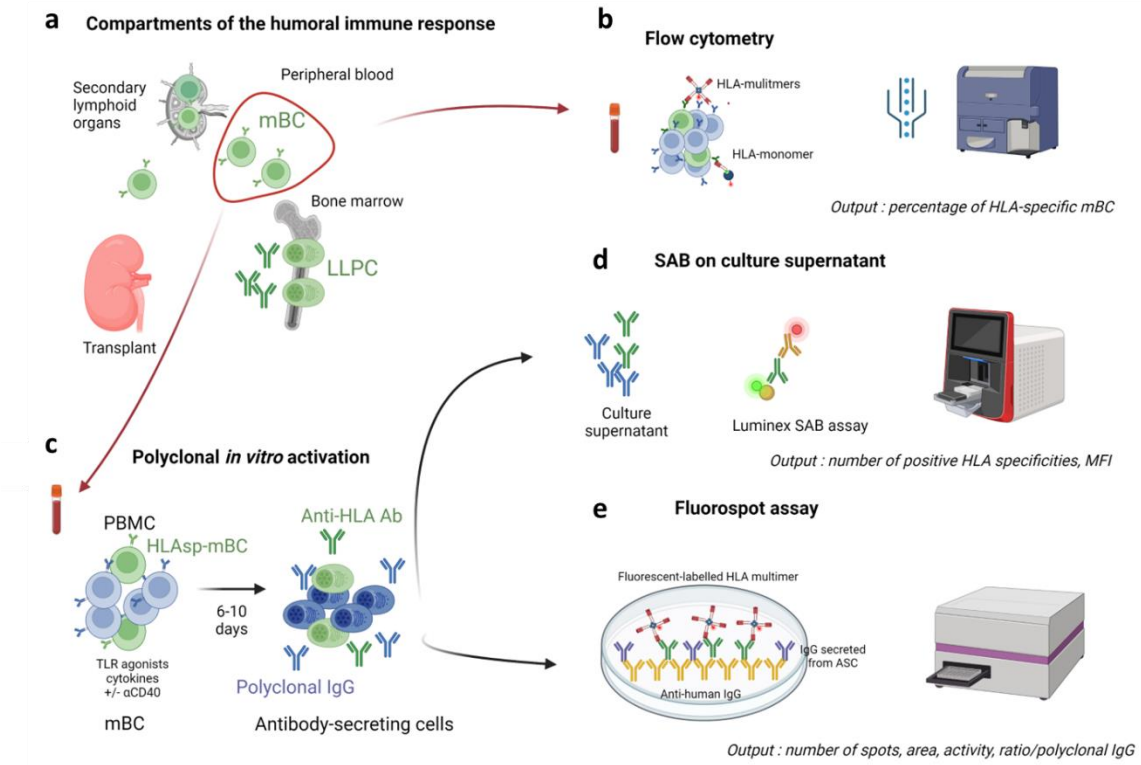

Figure 2. Techniques to detect HLA-specific memory B cells. a) Current technologies evaluating HLA-specific MBC explore the peripheral blood compartment b) Direct cell staining from peripheral blood with distinct fluorochrome-labeled HLA monomers or multimers analyzed by flow cytometry c) Polyclonal activation of PBMC induces the differentiation of MBC into antibody-secreting cells d) HLA-sp MBCs can be either detected by measuring HLA-specific antibodies in culture supernatant using single-antigen beads assays. e) HLA-sp MBCs can be detected using fluorescent-labeled multimerized monomers and detected using a FluoroSpot reader after seeding expanded MBC. *Adapted from Kervella et al 2023*[55].

A. Detection of HLA-sp memory B cells and Plasma cells

Numerous assays are currently being investigated to detect HLA-sp MBC and PC in the context of solid organ transplantation (**Figure 2**). Most methodologies used to detect HLA-sp MBCs consist in an in vitro differentiation of MBCs into ASCs (**Figure 2c**). After MBC expansion, HLA-sp MBCs can be quantified by either using ELISPOT-base assays or HLA-sp IgG antibodies of expanded cellular supernatants may be detected using SAB assay[56] (**Figure 2d-e**). Other approaches based on flow cytometry to phenotype circulating HLA-sp MBC have also been described.

A1. HLA-sp B-cell ELISPOT/FLUORSPOT assays

The principle behind the enzyme-linked immunospot (ELISPOT) assay involves capturing secreted antibodies in close proximity of each seeded cell (**Figure 2e**). In B-cell ELISPOT, ASCs present in a sample are seeded to plates coated with the antigen of interest and

the antigen-specific (Ag-sp) antibodies released by each ASC bond in close proximity to the producing cell. Enzyme or fluorescent-labeled secondary antibodies are used to visualize spots of antibody secretion at the location of each ASCs. Therefore, each spot corresponds to the antibody produced from a single antigen-specific B cell[57,58]. The main characteristic of B-cell ELISPOT is the fact that can only detect a subset of B cells that can functionally secrete immunoglobulins. Therefore, this assay is able to also detect PC that constitutively secrete antibodies or alternatively, MBCs that have been previously polyclonally stimulated in vitro to differentiate into ASCs[59].

Several protocols have been described for the differentiation of MBCs into ASCs, including the use of toll-like receptor (TLR)-agonist such as R848 in combination interleukin-2 (IL-2) or anti-CD40 agonist together with different cytokines such as IL-2, IL-10 and/or interleukin-21 (IL-21), interleukin-15 (IL-15) and IL-6 sometimes adding also a TLR agonist such as ODN/CPG or B-cell activating factor (BAFF)[56,59–61]. This differentiation can be performed using whole peripheral blood mononuclear cells (PBMCs) or with previously isolated B-cells, none of them showing differences in specific B-cell frequencies[62].

The ELISPOT platform is characterized by its sensitivity, making it well-suited for the detection of low-frequency of IgG-producing MBC such as HLA-sp B cells. Fan et all. was the first reporting the application of ELISPOT assay in the transplantation setting to detect blood group tolerance using ABO-specific ELISPOT assay[63]. Later, the first HLA-sp B cell ELISPOT was described by Perry et al. for the quantification of BM-residing HLA-sp PC[64]. In this assay, ELISPOT plates were coated with purified HLA molecules and after PC incubation, bound HLA-sp IgG was detected through an anti-human IgG-biotin followed by streptavidin-peroxidase. Detection of HLA-sp MBC from peripheral blood through HLA-sp B cell ELISPOT assay was first reported by Heidt et al. in 2012[65], seeding previously activated peripheral B cells into Elispot plates coated with streptavidin followed by biotinylated HLA molecules and detecting released HLA-sp antibodies with an anti-human IgG-HRP and substrate. Over the years, this assay has been improved for the detection of simultaneous HLA-sp IgG-secreting MBC. As described in Luque et all[56], for the detection of various HLA specificities, previously differentiated MBCs are seeded

into an anti-IgG-coated Fluorospot plate thanks to the use of fluorochrome-labelled HLA monomers. Released polyclonal IgG antibodies bind to the coated plate and antibodies specific to different HLAs may be detected using multimerized fluorescent-labeled HLA monomers. Multimerized HLA monomers significantly enhances the detection signal of each spot-forming unit (SFU), with higher sensitivity than using only biotinylated monomers[56]. A main advantage of this assay is that it allows the simultaneous detection of different HLA specificities in the same fluorospot well, while enhancing the detection sensitivity of low-frequency HLA-sp mBCs[55]. Another HLA-sp ELISPOT technique, reported by Karahan et al. enabled detection of HLA class II-sp MBCs, coating the elispot plate with anti-human IgG and detecting HLA-sp antibodies released by seeded ASCs through biotinylated HLA-II molecules followed by streptavidin-alkaline phosphatase and BCIP/NBT substrate[66].

In all these ELISPOT-based assays, total polyclonal IgG is also assessed and used as positive control, to confirm ASC viability for each evaluated subject. In addition, a self-HLA monomer should also be evaluated to dismiss any possible unspecific interaction and thus, use as a negative control. Additionally, the total polyclonal IgG assessment allows to provide a functional readout of the frequency of antigen-specific MBC, as the number of HLA-sp IgG-secreting B cells over the global IgG-ASC population[54,56].

Importantly, Fluorospot readouts have improved significantly over the last years, and currently it includes other parameters besides counting spot-forming units (SFU), such as the MFI intensity of each well or the total activity, a parameter that summarizes the number of SFUs of each well and amount of secreted analyte, providing a result with not only the number of HLA-sp IgG-secreting MBCs but also the amount of HLA-sp antibody secreted by these cells. The biological value of this new information needs to be investigated in new studies.

Main limitations of this technique are the required number of cells, the 6-day polyclonal stimulation period[55], and the necessity of a Fluorospot reader. Currently, a large pool of HLA monomers is available covering most of the HLA antigen repertoire.

A2. Detection of HLA-sp Abs in MBCs and PCs culture supernatants

The HLA antibody repertoire of MBCs can also be assessed with the analysis of the antibodies released by MBCs after polyclonal stimulation with single antigen beads on a solid-phase platform **(Figure 2d).** While a 6-day proliferation period adequately differentiates MBCs into ASCs for functional analysis, extending the culture to 10 days has shown better results as it maximizes the quantity of IgG antibodies produced in this culture[67]. Also, the antibodies present in this culture need to be concentrated with centrifugal filters before SAB assay. However, even after concentration, HLA-sp IgG levels in these cell cultures remain low and a second step of IgG isolation using protein G affinity purification results in a better detection of HLA-sp antibodies[67,68]. In a recent study, Heidt et al. showed good concordance in HLA-sp antibodies from MBCs detection between the two commercially available Luminex SAB assay (Oner Lambda and Lifecodes from Immucor), with the exception of some unspecific binding of HLA-C coated beads reported in one of the two platforms[69].

With these assays, it is important to remember that the HLA antibody concentration in the culture supernatant may not necessarily correspond to the frequency of the in vitro differentiated MBCs, as it is possible that each plasma cell secretes different amounts of HLA antibodies. In addition, such assays require ideally 10 days of culture of the MBCs for inducing in vitro differentiation, making these assays less practical when clinical results are required in a short time frame. On the other hand, potential recipients of a living-donor transplant or highly sensitized transplant candidates in the waiting lists may benefit from such detailed quantification as the outcome includes the whole HLA-sp MBC repertoire.

A3. Memory B-cell crossmatch.

Performing T and B-cell crossmatch using donor cells and MBC culture supernatant can also assess the presence of donor-specific anti-HLA MBCs[52,67,70]. While this test has not been directly compared to other methods for clinical immune-risk stratification, it may offer supplementary evidence of the ability of the antibodies produced by MBCs to effectively bind the corresponding HLA molecules expressed on donor cells surface[55].

Nevertheless, low concentrations of IgG in cell culture supernatants, particularly for those MBCs at low frequencies in circulation, is the main limitation of this assay.

A4. HLA-sp Flow cytometry assay

The assessment of circulating alloreactive MBCs using flow cytometry relies on the principle that B cells recognize antigens through their surface-bound B cell receptors (BCRs), therefore the BCR of antigen-specific B cells will bind the antigen of its specificity and, if the antigen is conjugated with a fluorophore, antigen-specific B cells could be detected using flow cytometry **(Figure 2b).** Several attempts to identify HLA-sp MBCs with flow cytometry have been done using either biotinylated HLA monomers and a fluorophore-labeled streptavidin or HLA multimers directly conjugated to a fluorochrome[71]. The low frequency of these cells described to be in the circulation (0.1% of total CD19+ cells), makes this approach difficult to extend and use in a generalized manner. Song et al[72] performed single cell culture and expansion with MS40L cell line and cytokines of a previously sorted HLA-A*02:01-specific MBCs (CD3– CD14–CD16– CD19+ CD24hi CD27+ IgM– IgD– IgG+) using an HLA A*02:01 tetramer-PE. After single-cell culture, they assessed the anti-HLA IgG specificities through SAB Luminex assay, being able to identify HLA-sp antibody-secreting clones through their BCR sequences. After functional analysis, only one of 270 clones were producing HLA-A*02:01-sp antibodies. To overcome this limitation and minimize the risk of nonspecific binding of HLA monomers or streptavidin to B cells, it is strongly recommended to employ the same HLA monomer coupled to two different fluorochromes, considering HLA-sp only those cells specifically stained with both multimers. Indeed, Kramer et al used this approach to isolate HLA-DR-specific MBCs (CD3- CD27+ IgD- tetramer-APC+ and tetramer-PE+) , finding an average of 0.008% HLA-DR-sp MBCs over total MBCs (CD3- CD27+ IgD-), although after the subsequent in vitro expansion of these MBCs, only 30% produced HLA-DR-sp antibodies[73]. After the expansion, different studies can be performed in the clones, for example, the generation of recombinant HLA-sp monoclonal antibodies (mAbs) using mRNA from the isolated B-cell clones to verify the eplets[73,74].

The limitations of this assay include its sensitivity, which is limited by the low frequencies of HLA-sp MBCs, and its inability to provide information on the capacity of MBCs to differentiate into ASCs. Additionally, defining conditions for the specific identification of

low-frequency donor-specific B cells has significant challenges and hampers its adaptation to the clinical setting. Moreover, these assays have not demonstrated the ability to reliably and specifically quantify the frequency of memory HLA-reactive B cells in humans[47,75].

2.4 Stratification of alloimmune sensitization risk in kidney transplant candidates

Biological origine of serological memory

As previously discussed, HLA sensitization can result from any exposure to HLA antigens including pregnancies, transfusions or previous transplants. Even though it has been proved that transfusions and pregnancies also lead to HLA sensitization, HLA sensitization through a previous transplant has been shown to induce stronger and longer-lasting sensitization than pregnancy or blood transfusions. In this regards, for example, Lopes D et al. reported that 75% of patients that received a transplant sensitized against mismatched HLA antigens, in comparison to 38.3% of those with pregnancies and 18.9% of those with previous transfusions[76,77]. In a similar retrospective study in 1066 cases anti-HLA antibodies were significantly higher in patients with previous transplantation (73.9%) than in previous pregnancies (57.46%) and blood transfusions (27.3%). Interestingly, while pregnancy and blood transfusion has been suggested to induce an allo-immunization rate higher for class I HLA antigens than for class II[78], no specific mechanistic explanation has been thoroughly been reported.

Physiopathology and impact of preformed DSA on kidney graft outcomes

The effect of those preformed anti-HLA antibodies in transplant outcomes has been widely studied over the years in the transplantation setting. To date, it is clear that preformed anti-HLA DSA, specially targeting A, B, DR and DQ antigens, are associated with post-transplant rejection and allograft loss, with up to 30% acute clinical or subclinical ABMR within the first year post-transplant[79]. This is due to DSAs initiating antibody-mediated injury to the graft binding to HLA antigens or other targets on the allograft endothelium. Those antibodies that are capable of complement activation have a higher pathogenic potential and are usually the ones initiating the humoral immune response against the graft[80]. If DSAs are complement activating, they activate the complement pathway through IgG binding and activation of C1q, that usually results in

a fast immune response that end with allograft loss. Also, DSAs can bind to endothelial cells and stimulate cell proliferation or induce antibody-dependent cell-mediated cytotoxicity (ADCC)[9].

While not only the presence of preformed DSAs associates with worse graft outcomes, also its quantity measured by mean fluorescence intensity (MFI) has been suggested to predict poorer transplant results. Indeed, Lefaucheur et al described that as the higher peak DSA MFI increased, also increased the relative risk for AMR and decreased the probability of graft survival[81]. In this work, patients with an MFI>6000 MFI had 100-fold higher risk for ABMR than patients with lower MFIs. On the other hand, in Ziemann et all also described that pretransplant DSAs were associated with lower overall graft survival and that even weak DSAs <3000 MFI associated also with worse graft survival specially in deceased donors[82]. This is the main reason why most allocation programs avoid kidney transplantation with the presence of preformed DSA[81,82]. Therefore, patients with prior history of sensitization events with the resultant presence of HLA-sp antibodies in serum are less likely of receiving an HLA compatible organ offer and remain for long periods of time on dialysis. Currently, patients with pretransplant cPRA rates above 80-90% are considered highly sensitized[83].

Multilayer B-cell memory favoring post-transplant ABMR

However, the status of sensitized patients is highly heterogeneous. Recent literature suggests that not all DSA are equally harmful to the graft. Additionally, advances in understanding humoral memory have shown that the absence of DSA at the time of transplantation does not necessarily rule out the presence of preformed cellular humoral memory against the graft[84]. Different groups have reported the presence of HLA-sp MBCs independently of the presence of serum DSA[68,85] and related its presence with higher risk of developing de novo DSA after transplantation[86], and higher risk of ABMR[52,54,67]. Therefore, humoral memory is both heterogeneous and multilayered, with the cellular compartment playing a significant role alongside serological memory.

For that reason, the exclusive assessment of circulating anti-HLA IgG antibodies does not fully illustrate the complete memory alloimmune response. The assessment of circulating donor (HLA)-specific MBC may significantly improve characterization of the

HLA-sp immune response of kidney transplant patients both before and after transplantation. For this reason, a novel integrative risk stratification method to stratify kidney transplant candidates was proposed by the ENGAGE group[87]. This stratification involves dividing risk into five distinct evaluation points that consider the patient's past immunological clinical background that may have created cellular memory, together with the assessment of serological alloimmune memory using CDC-crossmatch, FCM crossmatch and SAB assays. This approach aims to enhance the likelihood of a successful transplantation outcome **(Figure 3)**.

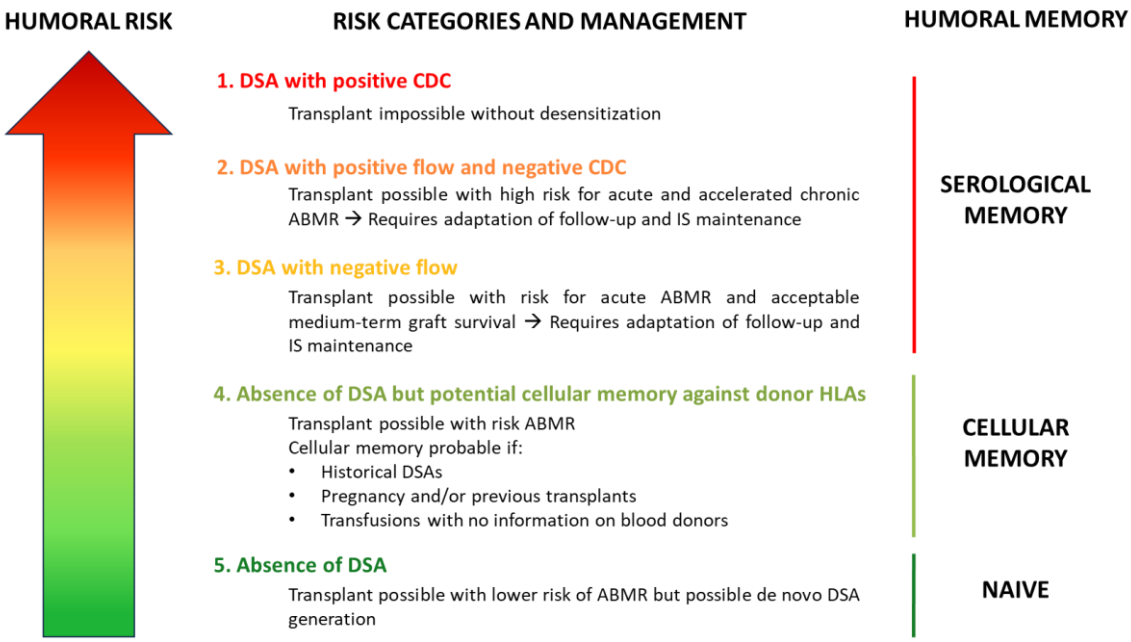

Figure 3. Humoral risk stratification of kidney transplant candidates. *Adapted from Mamode et al. 2023*[87].

2.4 Strategies to facilitate access to compatible transplantation for highly sensitized kidney transplant candidates

With the advances in alloimmune risk stratification, especially by introducing SAB assays, allocation policies have prioritized access to transplantation in absence of serum anti-HLA Ab, this is in HLA compatible transplantation thus, leaving sensitized kidney transplant candidates, and especially those with very high sensitization rates, with very low chances to receive an HLA compatible organ offer. Therefore, several strategies have been developed to enhance access to HLA compatible transplantation for highly sensitized candidates.

2.4.1 Living-donor kidney pair exchange programs

The most favorable choice is a transplant from an HLA-compatible living donor, which can be achieved by large paired-donor exchange pools when an HLA-identical sibling is not available for donation[88]. In the absence of a compatible living donor, another measure is to expand the pool of potential donors accessible to sensitized patients, which can be accomplished by revising allocation policies for deceased-donor organs to prioritize highly sensitized patients with broad antibody reactivity[88].

The kidney paired exchange program (PKE) was introduced in 1986 and began in 1999 to overcome sensitization barriers in kidney transplantation. This program allows the exchange of two or more HLA or ABO incompatible living donor's kidney pairs and recipients to achieve better HLA compatible kidneys[89]. The approaches to exchange schemes vary: for example, altruistic donation is permitted in the United Kingdom, but it is not possible in France, Poland, Greece or Switzerland. Similarly, compatible pairs are included in the United Kingdom's program, but not in France or Portugal[87,89]. Also, the European Network for Collaboration on Kidney Exchange Programs (ENCKEP), established in 2016, has contributed to aspirations for future developments, including modelling of European PKEs with the aim of future optimization[90].

Although this program increased the number of transplants for highly sensitized candidates, it suffered mainly for logistic limitations since it may not be always possible to match all the pairs and difficulties regarding the need to do simultaneous transplants[91,92].

2.4.2 Sliding-scale points prioritization programs

The use of a sliding scale priority points system for allocating deceased donor organs can increase the transplant rate for highly sensitized candidates. Several national and regional priorization programs are implemented in United States and some European countries to prioritize candidates with very high cPRA values for transplantation.

In 2014, UNOS implemented revisions to the kidney allocation system (KAS) in the US, significantly elevating allocation points for highly sensitized patients with broad antibody reactivity and granting priority at both national and regional levels to those with cPRA

scores exceeding 98%[93,94]. Additionally, these candidates are allowed to receive ABO incompatible offers (such as A2/A2B to B organs) due to their lower immunogenicity. Remarkably, kidney transplant rates among these patients dramatically increased when the scale was introduced, from 2.5% to 13.4% (80).

In spain, a similar prioritization kidney allocation system (PATHI) was also implemented in June 2015 for those patients with a cPRA>98%. PATHI's algorithm selects donors based on blood type and negative crossmatch of HLA antigens A, B, C. DRB, DQB and DQA. Although this program increased considerably the donor offers of highly sensitized candidates included, transplantation rates of those with a cPRA=100% remained very low[95].

However, while Sliding-scale points prioritization programs increased the transplantation rate among highly sensitized candidates, extremely sensitized patients with cPRA ≥99.9% still experience notably lower transplantation rates compared to their less sensitized counterparts[96].

2.4.3. Acceptable mismatch program

Another early allocation program for highly sensitized kidney transplant candidates was the Eurotransplant Acceptable Mismatch (AM) program, which prioritizes the allocation of compatible donor kidneys to highly sensitized patients (>85% cPRA), focusing on finding acceptable matches rather than to prohibit matches[97]. This system defines acceptable antigens those lacking antibody reactivity in complement-dependent cytotoxicity (CDC) assays using single HLA-expressing cell lines. The main advantage of the AM over prioritization schemes is that it entails better matching and thus may lead to better long-term outcomes[87].

The AM program increased transplantation rates for highly sensitized transplant candidates, being between more than 2,500 patients were enrolled in the program between 1989 and 2017, with 57% of them undergoing kidney transplantation. Highly sensitized patients transplanted through the AM program showed superior graft survival than highly sensitized patients transplanted only avoiding unacceptable mismatched[97–99]. Furthermore, death-censored graft survival rate is similar to the rate in non-sensitized

patients and is related to a lower chance of rejection in the highly sensitized patients included in the AM program[100]. Unfortunately, this program does not seem to increase access to transplantation for those very highly sensitized patients (>99% cPRA)[98].

Therefore, even with the implementation of national and regional allocation programs aimed at enhancing transplantation accessibility for highly sensitized candidates, a significant portion of individuals, particularly those with cPRA ≥98%, may not receive a transplant offer and frequently remain in the waiting list for long periods of time, increasing the risk of death while waiting for a compatible donor[88]. In general, highly sensitized patients account for approximately up to 30% of patients in the waiting list, and only approximately 6% of them receive a transplant each year[101]. Over the past years, the number of highly sensitized patients on the waiting list increased significantly from 2% to 5.6% from 2011 to 2019[102]. As seen previously, those patients that have more difficulties finding a compatible donor despite allocation systems are those very highly sensitized, with a cPRA>98%, being candidates with a cPRA≥99.9% the ones facing more challenges in finding a compatible donor. In Europe, very highly sensitized patients account for 5% of patients in the waiting list[8,101], among which only 9.7% are likely to receive transplant offer[103]. For that reason, desensitization therapies are then established to overcome this HLA barrier.

3. Desensitization therapies

Highly sensitized transplant candidates, especially the very highly sensitized ones that remain waitlisted for long periods of time, may need alternative strategies when these distinct allocation systems fail to facilitate access to transplantation. Therefore, different therapeutic approaches to deplete or decrease circulating anti-HLA antibodies have been developed over the last decades.

Importantly, desensitization treatments have emerged as a highly successful strategy for mitigating graft rejection by eliminating circulating antigen-specific Abs, especially against ABO antigens. This approach gained prominence in the mid-1990s and steadily grew in significance over the subsequent two decades, marking a pivotal shift in transplant practice and allowing transplantation across the ABO barriers[104]. Notably,

similar approaches have been carried out for those highly sensitized transplant candidates against HLA antigens, allowing some patients receiving a kidney allograft after achieving an acceptable crossmatch and ultimately avoid the development of hyperacute rejection and rapid graft loss. Over the last decades, a number of agents and combinations have been attempted, but very few have undergone thorough efficacy assessment through randomized clinical trials, and most of them have been reported in small, single center experiences. In fact, there is currently no approved desensitization therapy by main regulatory agencies but only the IgG endopeptidase (Ideferix®), which has been granted a conditional approval by the European Medical Agency (EMA), while awaiting the outcomes of an ongoing post-authorization study (PAES) running in Europe.

The advent of preconditioning methods with high-dose intravenous immune globulin (IVIg) or a combination of plasmapheresis and low-dose IVIg increased transplantation rates, reduced waitlist time and had promising short-term outcomes across many single center studies[104]. However, many of these data were collected before implementation of the KAS, and patients in the control group were not necessarily enrolled in kidney-pair exchange programs. While a number of desensitization strategies have been reported with different rates of success, kidney transplant outcomes of these patients have been shown to be significantly poorer than those receiving HLA compatible donors[105,106] thus, emphasizing the need of prioritizing patients to undergo these different allocations strategies to facilitate HLA compatible transplantation.

Currently, desensitization protocols have evolved significantly, benefiting from the rapid development of novel agents designed not only for treating circulating anti-HLA antibodies, but also targeting the B-cell alloimmune response from a more holistic manner, aiming at diminishing or deleting circulating anti-HLA antibodies, key cytokines or by directly targeting B cells and/or plasma cells[107].

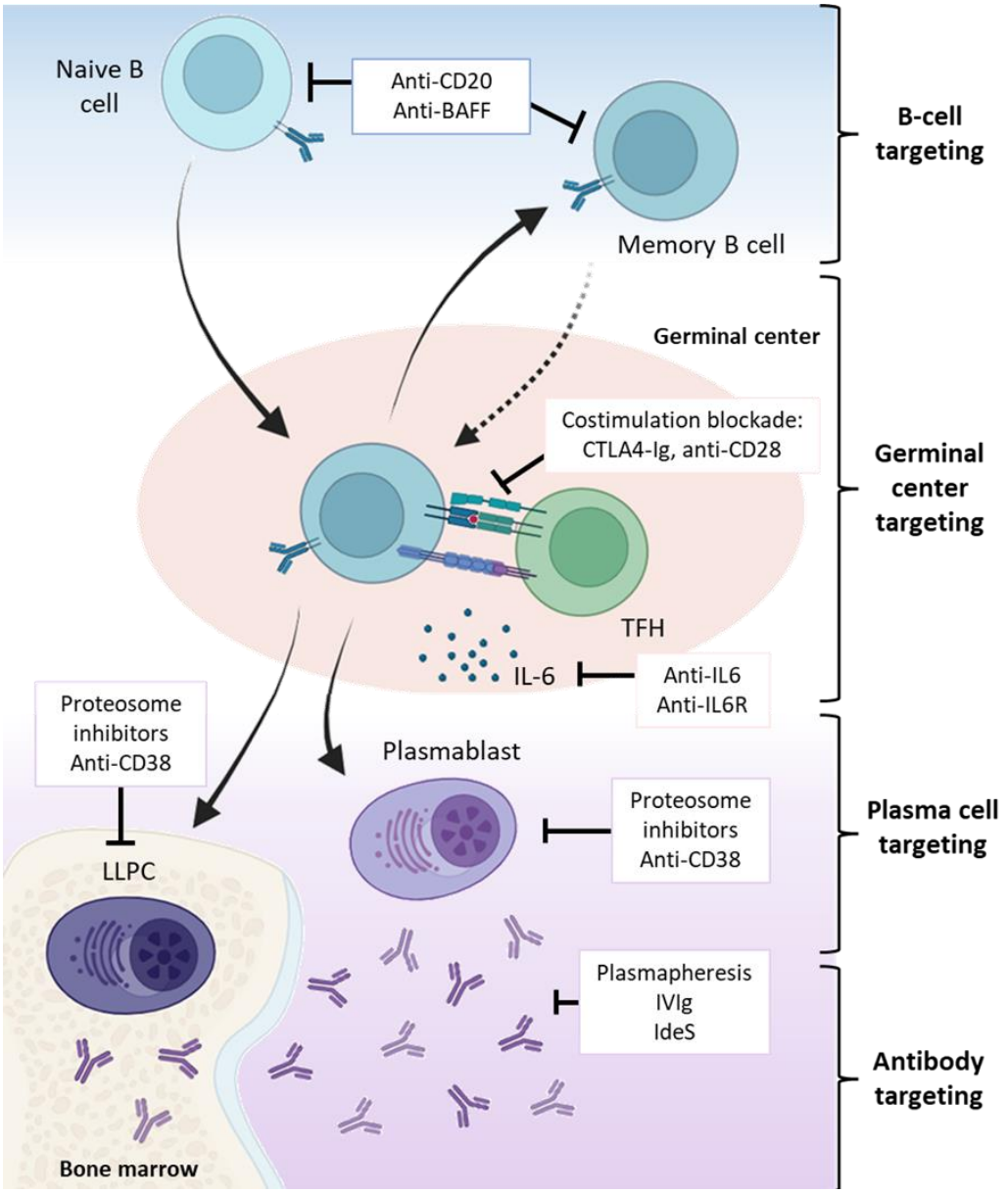

Figure 4. Desensitization treatments targeting the different B-cell compartments involved in HLA sensitization.

3.1 Classical desensitization strategies in transplantation

3.1.1 Plasmapheresis and Immunoadsorption

Plasmapheresis has served as a longstanding method for reducing circulating antibodies in various immune diseases for several decades[108]. This technique physically eliminates large molecular weight substances from the plasma, including antibodies, complement components, immune complexes, and coagulation factors[108]. The use of the double filtration plasmapheresis system involves a filtration cascade that traps larger molecules, allowing lower molecular weight components to return to the patient. When combined

with IVIg, plasmapheresis has been key in achieving successful transplantation for patients with positive crossmatches and it remains a cornerstone of desensitization protocols prior to transplantation in many medical units[109,110].

Also, in Europe and Australia, immunoadsorption (IA) utilizing a staphylococcal protein A column has been employed to eliminate antibodies from blood[111]. Together with plasmapheresis, IA stands as an effective treatment for transplantation across a positive HLA crossmatch in sensitized patients despite its main limitation being the antibody rebound following the completion of treatment sessions[112].

3.1.2 High-dose Intravenous immunoglobulin (IVIg)

IVIg is a natural modulator of inflammation, innate, and adaptive immunity, widely utilized in various inflammatory and autoimmune conditions[113]. In transplantation, it has been widely used in ABMR and desensitization treatments[114]. Despite its longstanding use in desensitization regimens, the precise mechanism of action remains elusive due to its broad spectrum of effects. Proposed mechanisms include neutralization of circulating anti-HLA antibodies with anti-idiotypic antibodies, inhibition of complement activation, and binding to Fc receptors (FcR) on immune cells inhibiting B and T-cell proliferation, as well as upregulating anti-inflammatory cytokines[115,116]. Additionally, IVIg is thought to prevent rebound of DSA post-plasmapheresis by providing a excess of circulating IgG[114].

Several studies have demonstrated the benefits of IVIg in increasing transplant rates in highly sensitized kidney transplant candidates, specially together with plasmapheresis and the anti-CD20 mAb rituximab[117].

3.1.3 Targeting B-cells with anti-CD20 monoclonal antibodies

The first anti-CD20 mAb used in transplantation is rituximab, a chimeric antibody primarily used for B cell targeting for lymphoma treatment. Besides lymphoma, its efficacy in depleting mature B cells and pre-B cells has shown benefits across various autoimmune diseases, including rheumatoid arthritis, systemic lupus, phospholipase A2 receptor (PLA2R) membranous nephropathy, and transplantation settings[118]. The mechanisms of action of rituximab include direct effects such as complement-mediated

cytotoxicity and antibody-dependent cell-mediated cytotoxicity and indirect effects such as structural changes and induction of apoptosis in CD20-expressing B cell subsets[119].

As a desensitization treatment for transplant candidates, rituximab is often combined with IVIG and plasmapheresis[120]. Several studies[120–122] of desensitization with rituximab in combination with IVIG showed a significant reduction in cPRA levels after infusion with graft survival ranging from 80-95% and ABMR occurring in 30-40% of transplanted patients. Further research by Jackson et al[123] comparing recipients desensitized with isatuximab and non-desensitized described a significantly lower rebound of anti-HLA antibodies 1 month post-transplantation in the rituximab-treated group, although repeated mismatched antigens were the hardest to deplete. Also, IVIG combined with isatuximab therapy showed significantly reduced AMR and graft loss post-transplantation compared to IVIG alone[122]. Besides rituximab, a type-2 anti-CD20 mAbs with enhanced B-cell depletion efficiency, Obinutuzumab, has also been explored for desensitization[124]. In combination with IVIG, it has showed a significant reduction in MFIs of anti-HLA antibodies although with non-substantial clinical relevance and 9/25 patients experiencing adverse infections post-transplantation.

Despite anti-CD20 mAb rituximab effectiveness, rituximab's half-life in patients with ESRD typically ranges from 9 to 14 days and sustain durable B-cell depletion for only up to six months[125]. Importantly, since plasma cells lack CD20-expression, anti-CD20 mAb do not deplete this compartment participating in the generation and maintenance of serological anti-HLA memory, thus, anti-HLA antibody production may continue despite the use of this treatment[126].

3.2 Other explored desensitization therapies

3.2.1 IgG endopeptidase

More recently, efforts have been directed towards fundamentally modifying the structure of preformed IgG antibodies. This has been achieved through the use of IgG endopeptidase or IgG-degrading enzyme of Streptococcus pyogenes (IdeS), a bacterial enzyme produced by streptococcus pyogenes that cleaves all four human IgG subclasses into F(ab) and F(c) fragments, thereby inhibiting both CDC and antibody-dependent

cytotoxicity[127]. Additionally, IdeS has further effects by cleaving the IgG present in the BCR, resulting in the downstream effect of switching off B-cell memory[128]. Notably, this therapy can only be used once, as it induces immunization that neutralizes its effect.

A trial from Jordan et all in 2017 involving 25 HS patients receiving IdeS for HLA-incompatible kidney transplantation showed a near-complete or complete abrogation of anti-HLA antibodies and DSA within 24 hours post-transplantation, facilitating transplantation in 24 out of 25 patients (96%). However, within 1-2 weeks, the levels of these antibodies rebounded. One patient experienced graft loss due to hyperacute rejection, while 10 out of 25 patients (40%) exhibited evidence of ABMR during the early post-transplant period[129]. Another study by Lonze et al. was conducted in 7 HS patients that had a positive crossmatch before transplantation that became negative after IdeS infusion and therefore could undergo transplantation. After transplant, DSA rebound occurred in 43% of patients, although almost all patients had a functional graft 8 months post-transplantation[130]. Also, an open-label phase-II multicenter trial assessing the efficacy of IdeS in converting a positive to a negative crossmatch just before transplantation showed conversion from positive to negative crossmatch in 17 out of the 19 patients included. ABMR occurred in 36.8% of patients between day 2 and 19 post-transplantation and survival rate was 88.9% at 6 months post-transplant. Interestingly, all patients who experienced AMR had a rebound in DSA post-IdeS treatment, but not all patients who had a DSA rebound developed clinical ABMR[131]. A pooled analysis of phase II trials using IdeS assessing the safety and efficacy of patients at 3 years, showed preservation of graft function and patient survival rates similar to patients having received other desensitization therapies[132].

These findings indicate that while IdeS demonstrates potent, albeit temporary, ability to reduce DSA, its utility may be enhanced in combination with strategies aimed at longer-term control of DSA rebound[131].

3.2.2 Anti-IL6 and anti-IL6R

New desensitization approaches have also focused on targeting mediators and/or survival factors necessary for the development of the humoral immune response. This is the case for those targeting soluble IL6 or its receptor. IL-6 is a versatile cytokine

produced by various cell types. Its receptor, IL6R, is found on hepatocytes and certain immune cells. However, a soluble form of IL-6R can bind IL-6, forming a complex that signals through the transmembrane cytokine receptor gp130 (trans-signaling) and is ubiquitously expressed[133]. IL-6 plays a crucial role in inflammatory pathways and is essential for the induction of TFH, needed to help naïve B cells in GCs to become MBCs and high-affinity IgG-secreting PC[134].

Tocilizumab (Actemra®), is a humanized mAb that targets both membrane-bound and soluble forms of IL-6R. It is approved to treat moderate to severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, and Castleman's disease[133]. In transplantation, Inhibition of IL-6 signaling has been investigated in experimental transplant models showing a reduction of alloantibody responses by inhibiting BM-residing PC[135].

Different studies have explored the use of tocilizumab for desensitization in highly sensitized kidney transplant candidates. Vo et al. investigated the efficacy of the combination of high-dose IVIG and tocilizumab in 10 highly sensitized patients who responded poorly to high-dose IVIG + rituximab[136]. This regimen was associated with reduced DSA number and strength, decreased waitlist time, and increased transplantation rates, without any evidence of subclinical ABMR on protocol biopsies. A recent study from Jouve et al. using tocilizumab to desensitize 13 highly sensitized kidney transplant candidates showed a significant reduction in the frequencies of circulating plasmablasts although with almost no effect on serum anti-HLA antibody levels[137]. The same group investigated the effect of tocilizumab in combination with apheresis and rituximab pre-transplantation but no impact was found in the reduction of pre-transplant MFIs[138].

3.2.3 Anti-BAFF agents

B cell activating factor (BAFF) is a cytokine member of the TNF family highly involved in B-cell development, activation and survival. It can be located on the cell surface as a transmembrane protein or released in soluble form after cleavage. BAFF is secreted by multiple cell types and binds to three separate receptors on B cells, facilitating its maturation, proliferation and differentiation[139]. Therefore, blocking BAFF has also been

assessed to abrogate anti-HLA antibody responses. Belimumab (Benlysta®), a mAb against BAFF, was the first biologic drug approved for the treatment of systemic lupus erythematosus[140]. In kidney transplantation, belimumab monotherapy was tested for desensitization in a clinical trial (NCT01025193)[141]. Unfortunately, the trial was prematurely halted due to lack of efficacy. Other anti-BAFF agents such as tabalumab, atacicept and blisibimod have not been yet evaluated as a desensitization therapy in human trials.

3.2.4 Proteosome Inhibitors: Bortezomib /Carfelzomib

Proteasome inhibitors have emerged as key agents depleting PC, notably in treating conditions characterized by abnormal PC activity like multiple myeloma (MM)[142]. In clinical practice, two main primary proteasome inhibitors have been investigated, bortezomib and carfilzomib.

Bortezomib is a first-generation reversible inhibitor of the 26S proteasomal subunit. Bortezomib has shown to effectively induce apoptosis in PC by disrupting cellular recycling mechanisms[143]. Everly et al. first described its use as effective treatment for ABMR, by means of successfully decreasing DSA levels in kidney transplant recipients[144], a result that was further reported in other small, single center trials[145,146]. However, in a first trial reported by Woodle et al., bortezomib was introduced in combination with plasmapheresis and rituximab, resulting in modest success[147], showing a mild reduction in DSAs in 86% of highly sensitized patients, with 43.2% of patients undergoing successful transplantation, with 89.5% of grafts remaining functional at a median follow-up time of 436 days. Also, Jeong et al. used a combination therapy with high-dose IVIG, rituximab, and bortezomib, resulting in a marginal reduction in the MFI value of class I PRA, although transplantation rate was higher in the treated group, with 42.1% desensitized patients compared to 23.5%[148]. Nonetheless, other reports using bortezomib monotherapy did not reproduced these data and showed very modest impact on anti-HLA antibody reduction. Moreover, significant toxicity has been associated with prolonged drug courses[149,150].

Carfilzomib is a second-generation, irreversible inhibitor targeting the 20S proteasomal subunit, which is currently indicated in patients with MM and showing superior efficacy

and improved tolerability as compared to bortezomib[151]. In a clinical trial using carfilzomib monotherapy, it demonstrated a median reduction of 72.8% in HLA antibodies and a 69.2% decrease in BM-residing PC, showing an acceptable safety and toxicity profile. However, anti-HLA antibody rebound occurred rapidly to almost all patients , with levels returning to baseline between 2-4 months later[152].

3.2.5 Costimulation signals blockade

Costimulation signals plays a crucial role in regulating B-cell and T-cell activation and thus, enables an efficient alloimmune response. In consequence, biological agents that interfere with this pathway could offer a more targeted approach to modulate the immune response, reducing non-immune adverse effects[153]. The first costimulatory molecules that moved to the clinic are the cytotoxic T-lymphocyte associated protein 4 (CTLA4)-Ig, abatacept, and its modified version, belatacept. While the former was approved in 2005 for the treatment of rheumatoid arthritis, the later was approved by the FDA in 2011 for the prevention of organ rejection in adult kidney transplant recipients.

Belatacept inhibits T-cell activation when binding its ligands CD80 and CD86 present on the surface of antigen-presenting cells, preventing its union to CD28 and the necessary co-stimulation signals for T-cell activation and proliferation[154]. While the indication of belatacept has been focused on the prevention of acute (T-cell mediated) rejection as maintenance immunosuppressive therapy sparing calcineurin-inhibitors[155,156], most interestingly, these molecules also proved to disrupt GC responses abrogating T-cell help for B-cell activation[157], successfully avoiding the development of de novo DSAs after transplantation[158]. In fact, it is now very well known, the capacity of belatacept of effectively abrogating the advent of de novo DSAs, but also to slightly reduce the strength of HLA antibodies assessed by Flow PRA, especially against class I HLA antigens in highly sensitized kidney transplant candidates[159]. In line with these data, in experimental mice transplant models, delayed administration of CTLA-4Ig treatment until day 6 after a fully mismatched heart transplantation, it effectively suppressed alloantibody production, prevented acute rejection and the generation of MBC response. These findings strongly suggested the efficacy of co-stimulation blockade at

this level impeding ongoing B-cell responses, even when donor-specific T-cell and GC B-cell responses are already established[160,161]. However, by delaying CTLA4Ig over a period of 14 days, failed to diminish donor-specific DSA levels illustrating that beyond controlling GC responses, the presence of plasmablasts and LLPCs may lead to the persistence of antibody production[161].

Altogether, these findings have led to the hypothesis that concomitant dual targeting of main compartments involved on antibody production could more effectively contribute to its control and avoid the production of anti-HLA antibodies.

3.3 Concomitant dual targeting of MBC and PC to increase desensitization efficacy

Since the anti-HLA memory B-cell response is orchestrated by different and highly specialist cell sources such as peripheral MBC and BM-residing plasma cells, all of them directly contributing to the generation of the anti-HLA antibody pool, a novel desensitization has focused on combining agents that may inhibit each compartment to ultimately reduce antibody production.

First attempts for dual targeting with proteasome inhibitors bortezomib or carfilzomib and costimulation blockade with belatacept for desensitization treatment were performed with allosensitized nonhuman primates (NHP)[162,163]. In these studies, NHP were sensitized through skin transplantation and desensitization treatment was administered during 4 weeks after primary and secondary DSA responses were stablished. After desensitization, a kidney transplant was performed with the same skin donor. They reported a significant reduction in BM PCs (CD19+ CD20+ CD38+), lymph node TFH (CD4+ ICOS+ PD1high) and proliferative GC B cells in lymph nodes (BCL-6+ CD20+) and a decrease in DSA levels after treatment. Desensitized recipients also showed significantly prolonged graft survival and reduced ABMR in comparison to the control groups. However, some recipients showed a rebound of DSAs and ABMR long-term[164].

Another study was performed in a NHP using a novel anti-CD28 mAb lulizumab and carfilzomib as desensitization treatment[165]. Sensitization was also performed through skin grafts, followed by desensitization treatment and kidney transplantation from the

previous skin donors. Desensitized subjects demonstrated a significant reduction in DSAs, TFH (CD4+PD-1+ICOS+), and proliferating B cells (CD20+Ki67+) in the lymph nodes. Interestingly, Naïve CD4 T cells (CCR7+CD45RA+) and naïve B cells (IgD+CD27-CD20+) increased in circulation. Treated recipients also showed significant prolongation in graft survival and lower ABMR compared to control animals. However, all desensitized animals eventually developed AMR and graft failure. A recent study by Manook et al also studied the efficacy of belatacept and bortezomib for desensitization of pregnancy-sensitized NHP receiving a kidney transplant with belatacept-based maintenance therapy[166]. Females undergoing desensitization exhibited only a minimal survival advantage compared to control females, but the addition of belatacept to posttransplant maintenance significantly extended graft survival while also suppresses posttransplant DSA and circulating TFH.

In humans, belatacept in combination with bortezomib has successfully reversed ABMR in 6 patients with sustained DSA disappearance[167]. Also, in four highly sensitized heart transplant candidates (cPRA>99%), Alishetti et al. reported the efficacy of this combined desensitization therapy to successfully reduce both class I and II HLA antibodies, increasing the likelihood of finding a compatible donor, achieving three negative CDC crossmatches against DSAs and its sustained suppression post-transplantation[168].

Altogether, these data strongly support the potential value of concomitant dual targeting of PC and peripheral MBC using different immunosuppressive agents to effectively downregulate the robust anti-HLA antibody responses in highly sensitized patients. Indeed, different trials are currently ongoing in highly sensitized kidney transplant candidates combining different agents targeting these two different compartments (NCT05145296; NCT04827979).

4. CD38-targeting therapies

Anti-CD38 mAb recently emerged as promising therapeutic agents for organ transplantation in view of the excellent efficacy results obtained in patients with Multiple myeloma (MM). By targeting CD38, a surface protein abundantly expressed on myeloma cells and various immune cells, these antibodies exhibit multiple mechanisms of action,

including direct cell killing, immune modulation, and interference with cell adhesion and migration[169]. Beyond their established efficacy in MM[169–171], some few studies have explored their potential utility in transplantation, particularly in the context of desensitization and rescue of ABMR to reduce anti-HLA antibodies.

4.1 Biology of CD38

CD38 is a 45-kDa single chain glycoprotein that can exist as monomeric, dimeric, or multimeric forms. It was discovered nearly four decades ago and has been found to play crucial roles in a wide range of cell types, both in normal physiological functions and pathological context[172].

CD38 can act as a type II or type III transmembrane protein depending on the orientation of the catalytic domain, whether it faces outside (type II) or inside (type III) of the membrane, exerting distinct enzymatic functions as both a receptor and an ectoenzyme[173,174]. Furthermore, a soluble form of CD38 has also been detected in biological fluids[175]. These diverse configurations suggest that CD38 can exert its functions both inside and outside of cells, influencing different cellular processes and signaling pathways[172]. The ectoenzymatic activity of CD38 operates independently of its receptor functions, catalyzing the synthesis of cADPR and NAADP from NAD and NADP, respectively, generating secondary messengers that mobilize calcium from intracellular stores and regulate calcium signaling[176].

In cells, CD38 is commonly situated on the cell surface, yet it can also be identified within various intracellular compartments including the endoplasmic reticulum, nuclear membrane, and mitochondria[173] and is also expressed, in humans, in multiple tissues such as prostatic epithelial cells, pancreatic islet astrocytes, smooth muscle cells, retinal tubes, kidney, gut, and brain. However, it exhibits its highest expression levels in hematopoietic tissues, particularly in lymph nodes and bone marrow. In immune cells, CD38 displays a dynamic pattern of expression among lymphocytes, monocytes, macrophages, dendritic cells, granulocytes, and natural killer (NK) cells, among which the expression levels on the cell surface are influenced by the cell's stage of maturation and/or activation[177]. Within the lymphocytes compartments, plasmablast and plasma

cells are the immune cells with highest CD38 expression, followed by NK cells, B cells, dendritic cells and T cells[177]. Moreover, CD38 is also expressed on cells outside of immunologic networks such as red blood cells and platelets[177].

Within the B-cell compartment, CD38 expression is also highly variable depending on B-cell maturation, state and activation[178]. Generally, CD38 is expressed in B cell precursors, GC B cells, and plasma cells. At early stages of B-cell development, human progenitor B cells in bone marrow express CD38 on surface but lose this expression after maturation. Outside the bone marrow, mature B cells start expressing CD38 in GC, being plasma cells the ones with highest CD38 expression. Moreover, mature B cells induce CD38 upon activation. Therefore, this molecule is often used as a cell activation and differentiation marker **(Figure 5)**[178].

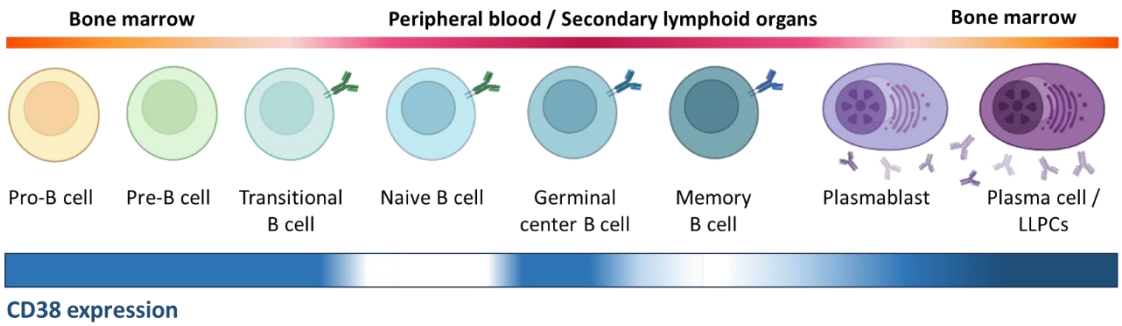

Figure 5. CD38 expression through B-cell differentiation in bone marrow and periphery. *Adapted from Crickx et al 2020*[179].

Thanks to its variable multifunctionality, human CD38 has the capability to form lateral associations with a variety of membrane proteins or complexes, modulating diverse functions within the immune system when its extensive extracellular domain engages in frontal and lateral interactions with other functional receptors [180]. These include CD16 in NK cells, the T cell receptor (TCR)/CD3 complex and CD4 in T cells, membrane immunoglobulin (Ig) and the B cell co-receptor complex (CD19/CD81) in B lymphocytes, and class II MHC in monocytes[180], potentially contributing to cell signaling in these complexes. Despite its short cytoplasmic domain lacking signaling motifs, CD38 undergoes relocalization at the immunologic synapse in T cells following TCR engagement, modulating antigen-mediated T-cell responses[181]. Similarly, crosslinking of

CD38 has been shown to reduce the threshold for B-cell activation through the BCR, evidencing its role in BCR signaling[182].

4.2 Anti-CD38 monoclonal antibodies: mechanism of action

CD38's abundant expression and its pivotal role in cellular signaling make it a highly appealing target for therapeutic antibodies, especially in conditions like MM and CD38-positive non-Hodgkin's lymphoma. Indeed, the development of various CD38 antibodies has shown their robust preclinical and clinical potential in hematologic malignancies[183]. Currently, 2 human anti-CD38 mAb are approved for clinical use: daratumumab and isatuximab. MOR202 (Morphosys) and TAK-079 (Takeda) are other candidates, also in advanced stages of development. Both isatuximab and daratumumab have direct effector mechanism and FC-independent immune mechanisms that include ADCC, CDC, and antibody-directed cellular phagocytosis (ADCP)[183].

Daratumumab was the first-in-class human anti-CD38 mAb approved for the treatment of MM[171]. Daratumumab is an Ig G1 kappa (IgG1k) human mAb that targets a specific epitope on CD38-expressing cells with high affinity. Developed through the immunization of human Ig transgenic mice with recombinant CD38 protein, daratumumab standed out among 42 human CD38-specific mAb as the sole inducer of CDC in Daudi target cells[184]. Daratumumab binding site is located in a specific discontinuous region on CD38 that includes residues located opposite to the active site of CD38 and therefore, outside the CD38 catalytic site[185]. That is the main reason why daratumumab inhibits only partially the cyclase activity of CD38[183].

In vitro studies demonstrated its ability to trigger CDC and ADCC in CD38-expressing MM cells isolated from the bone marrow of previously untreated or relapsed patients[170].Its specificity was confirmed by the absence of ADCC induction in CD38-negative cells[184]. Notably, daratumumab retained its efficacy in the bone marrow microenvironment[184].

The level of CD38 expression has been correlated with daratumumab-induced CDC and ADCC[186]. In addition to CDC and ADCC, daratumumab has been demonstrated to induce ADCP both in vitro and un vivo[187,188]. Additionally, other studies have revealed that FcR-

mediated crosslinking of daratumumab induces apoptosis of CD38-expressing tumor cells in vitro[189,190].

On the other hand, Isatuximab it's a more novel immunoglobulin G (IgG)1 mAb that also targets CD38. Isatuximab binds a specific epitope that partially encompasses, but does not block access to or alter the configuration of the CD38 ectoenzyme catalytic site, in contrast to daratumumab epitope that is located completely outside CD37 catalytic site[169]. In consequence, exposure of recombinant CD38+ cells to isatuximab produced near-complete inhibition of the CD38 cyclase activity in a dose-dependent manner. In vitro studies performed on MM cell lines demonstrated that isatuximab was more efficient than daratumumab in inhibiting the enzymatic activity of CD38[191].

Similarly to daratumumab, isatuximab binding to CD38 activates multiple mechanisms including ADCC, ADCP, CDC, and direct cytotoxicity[191].

Globally, despite its mechanistic differences, both isatuximab and daratumumab have proved to effectively deplete CD38+ cells through both Fc-Dependent and Independent mechanisms.

Immunomodulatory effects of CD38-targeting antibodies include downregulation of all those CD38+ immune subsets such as NK cells, Tregs, regulatory B cells (Bregs) and myeloid-derived suppressor cells (MDSC), the depletion of CD38high immune subsets, such as B-lymphocyte precursors, plasma cells and LLPCs[192], and the upregulation of T-cell responses (**Figure 6**)[192,193].

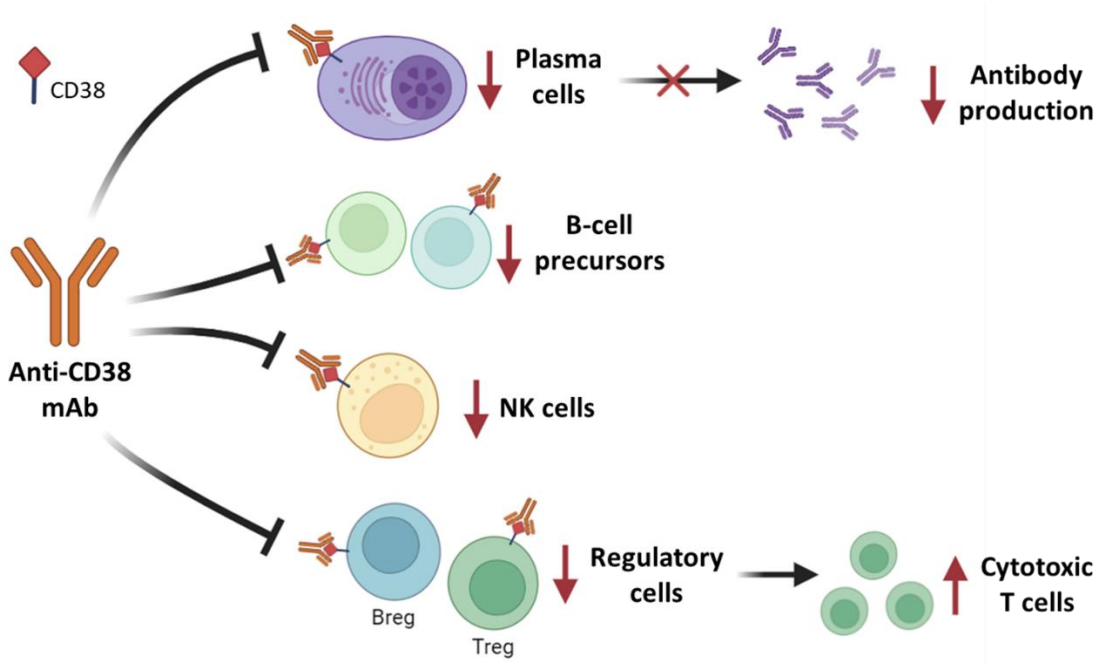

Figure 6. Immunomodulatory effects of anti-CD38 antibodies.

Daratumumab monotherapy has demonstrated an association with the expansion of CD4+ and CD8+ T cells[194]. This expansion correlates with alterations in T-cell functionality, leading to elevated antiviral and alloreactive responses and increased T-cell clonality[193,194]. These changes coincide with the depletion of immunosuppressive CD38+ cells, including Tregs, Bregs, and MDSCs. Similar to daratumumab, Isatuximab has demonstrated efficacy in reducing Tregs, blocking their trafficking and diminishing the production of inhibitory cytokines, consequently enhancing NK and T-cell responses[192]. Moreover, isatuximab has shown to overcome immune suppression mediated by the PD-1/PD-L1 pathway and inhibitory cytokines like TGF-β, suggesting potential synergy with immune checkpoint inhibitors and anti-TGF-β antibodies[195].

In B cells, daratumumab has shown to impair B-cell proliferation and survival in vitro in several studies, reducing ERK phosphorylation in B cells from peripheral blood and inhibiting IgM-BCR polarization and the colocalization of CD38 with CD19[196]. In another recent work by Verhoeven D et al, B-cell stimulation in vitro in the presence of daratumumab resulted in decreased B-cell proliferation, B-cell differentiation and IgG

production. Moreover, this effect was specially seen among sorted CD19+ IgD- CD27+ MBCs[197].

At present, two additional anti-CD38 mAb are undergoing testing in MM, autoimmunity and transplantation; felzartamab (MOR202, morphosys) and, mezagitimab (TAK-079). Both target CD38-expressing cells with mechanisms similar to those of isatuximab and daratumumab: felzartamab through ADCC and ADPC[198,199], and mezagitamab through ADCC and CDC[200,201].

4.3 Impact of anti-CD38 mAb in B-cell mediated diseases

Multiple myeloma (MM) is one of the most commons hematologic malignancies and is characterized by malignant PC accumulating in the BM[192]. Those malignant PC express high levels of CD38, and therefore anti-CD38 mAb have emerged as key therapies for this disease. At present, CD38 antibodies have been integrated into treatment regimens alongside proteasome inhibitors and immunomodulatory drugs for both newly diagnosed MM patients who are eligible or ineligible for autologous stem cell transplantation, as well as for relapsed/refractory MM patients. Both, Isatuximab and daratumumab, have demonstrated efficacy in improving clinical outcomes among MM patients[192].

In autoimmune disease, anti-CD20 or anti-CD22 mAb are frequently utilized for the treatment of systemic lupus erythematosus (SLE), vasculitis, autoimmune cytopenia, and rheumatoid arthritis, among others, to mitigate autoantibody production, antigen presentation, cytokine production, and T cell activation[202]. However, a significant challenge lies in the resistance of autoreactive LLPC to these therapies, leading to sustained autoantibody secretion[203]. Therefore, anti-CD38 mAb have emerged as promising therapies for targeting those autoreactive PC mostly residing within the BM in autoimmune diseases.

In this regard, systematic review of daratumumab effect in autoimmune diseases showed that Daratumumab therapy increased remission rate and disease improvement in 18/19 (95%) of patients for divers rheumatological diseases, including inflammatory idiopathic myopathies, SLE, lupus nephritis, ANCA-associated vasculitis patients and two

primary Sjögren's disease. Although antibody depletion was only reported in 4 cases (21%), reduction in antibodies was observed in 13 (68%) patients[204]. Moreover, daratumumab has also shown to effectively deplete PC in PBMCs isolated from systemic lupus erythematosus patients ex vivo[205]. Also, the new anti-CD38 mAb, mezagitimab (TAK-079), has also been evaluated for the treatment of MM with positive results[206,207] and for the treatment of primary immune thrombocytopenia[208,209] and systemic lupus erythematosus[210]. Furthermore, felzartamab is also being investigated in a phase 2 trial for the treatment of IgA nephropaty[211].

The expression of CD38 on plasma cells in patients with autoimmune conditions, along with the demonstrated decrease in autoantibodies following anti-CD38 mAb treatment, provides a strong basis for assessing daratumumab's efficacy in autoantibody-dependent disorders and, in extension, to treat alloimmune sensitization in the context of solid organ transplantation.

4.5 Anti-CD38 monoclonal antibodies in solid organ transplantation

Given the success of anti-CD38 mAbs in targeting MM cells and plasma cells in autoimmune diseases, the prospect of targeting CD38 appears promising in alloimmune scenarios like solid organ transplantation (SOT), appearing as a rational target to decrease HLA-sp antibodies.

4.5.1 Anti-CD38 mAb for transplant rejection rescue therapy

Some few studies have been published on the use of anti-CD38 mAb in SOT. First reports have focused on the treatment of salvage ABMR with these agents. A first experience reported a patient with refractory early active ABMR caused by anti-A isohemagglutinins after kidney transplantation from his ABO-incompatible sister[212], where daratumumab was tested as a rescue solution leading to a significant decrease of the pathogenic isohemagglutinins and resolution of tissue damage in the kidney biopsy. Kwun and colleagues also published a case report of daratumumab as a therapeutic strategy for refractory heart and kidney transplant rejection in a patient who received heart and kidney transplants due to systemic lupus[213]. Both transplant biopsies showed T cell–mediated rejection, ABMR and diffuse PC infiltration associated to the presence of

several DSA. Treatment with daratumumab lead to the resolution of both allograft function, improvement in acute kidney lesions and a decrease in PC infiltrate and the majority of DSA MFIs. Also, daratumumab successfully rescue another patient with refractory ABMR after a heart transplant[214]. Another case reported daratumumab treatment in one chronic active ABMR in a kidney allograft recipient diagnosed with MM. In this case they reported a profound reduction of BM-residing LLPC and PC and NK cells in peripheral blood, together with the abrogation of in vitro Ab production by PC from BM aspirates, leading to significant reduction in DSA levels[215]. Long term data of this case revealed no evidence of ABMR rebound after daratumumab withdrawal[216]. Most importantly, these initial experiences have been further confirmed in a recently published phase 2 clinical trial in late ABMR, showing excellent preliminary outcomes as compared to a standard of care therapy, with significant recovery and reduction of fundamental inflammatory lesions in patients receiving this new therapy[217].

4.5.2 Anti-CD38 mAb for desensitization therapy

Daratumumab as a desensitization therapy was first assessed in a preclinical fully MHC mismatched NHP model, highly sensitized trough two serial skin grafts before transplantation with a kidney from the paired skin graft donor[218]. Daratumumab and plerixafor, an anti-CXCR4 known to induce mobilization of PC from BM to peripheral blood, were given as desensitization therapy 8 to 12 weeks after sensitization and 8 weeks before kidney transplantation. Animals received for induction anti-CD4 and anti-CD8 antibodies and for maintenance immunosuppression tacrolimus, mycophenolate mofetil and a methylprednisolone taper. This desensitization therapy led to a significant reduction in preformed DSAs, exceeding a 50% reduction in comparison to baseline levels, and prolonged graft survival. Nevertheless, desensitized monkeys experienced delayed ABMR associated with DSA rebound and TCMR. Also, a decrease in regulatory B and T cells post-desensitization was observed, together with a rapid increase in activated T cells after kidney transplantation. In a clinical setting, the same authors used daratumumab in a highly sensitized heart transplant candidate remaining with high levels of anti-HLA antibodies after multiple courses of plasmapheresis, high-dose IVIG, and rituximab. A significant and persistent decrease of allosensitization was observed

after treatment, allowing a heart transplantation six months after daratumumab infusion[218].

II. HYPOTHESIS AND OBJECTIVES

1. Hypothesis

The hypothesis of this thesis is that considering the observed efficacy of anti-CD38 mAb to successfully deplete malignant cells in multiple myeloma patients, we hypothesize that anti-CD38 mAbs may target LLPCs and plasma cells also in highly sensitized transplant candidates, depleting one of the main production sources of anti-HLA antibodies leading to their elimination and increasing the probability of these patients to find an HLA compatible donor.

Additionally, the assessment of the B-cell profile of each patient prior to therapy using high dimensional analysis with high-throughput technologies such as spectral flow-cytometry, could identify specific biological features associated to a successful serological response to this therapy.

Moreover, a thorough assessment of different cell sources of HLA-sp antibodies such as bone marrow-residing plasma cells, MBCs and serum antibodies in the context of patients undergoing desensitization with CD38-targeting therapy would provide deep insight on the immune mechanisms of allosensitization.

2. Objectives

The main objectives of this thesis are the following:

- Assess the safety, pharmacokinetics and preliminary efficacy of the anti-CD38 mAb isatuximab in a phase 1/2 desensitization clinical trial (NCT04294459) in HS patients awaiting kidney transplantation.
- Assess the biological effect of CD38-targeting therapy on peripheral blood and bone marrow B-cell subsets using phenotypical and functional immune assays.
- Assessing the biological interplay of distinct HLA-sp immune memory compartments leading to serological sensitization
- Investigate whether specific biological features of peripheral B-cell components prior to anti-CD38 therapy could discriminate between patients displaying successful serological responses from those showing low or no response to this therapy.
- Validate these findings in an external, independent cohort of highly sensitized kidney transplant candidates receiving a different anti-CD38 mAb as desensitization therapy.

III. MATERIALS, METHODS AND STUDY DESIGN

III. MATERIALS, METHODS AND STUDY DESIGN

1. Patients of the study

The study consisted in two highly sensitized patient cohorts of kidney transplant candidates participating in 2 different phase 1-2 clinical trials; a first multicentric international cohort of patients receiving isatuximab as desensitization, which were investigated as derivation cohort, and a second group of French highly sensitized patients receiving daratumumab as desensitization therapy.

1.1 Derivation cohort (NCT04294459)

The derivation cohort consisted in 23 highly sensitized kidney transplant candidates participating in the anti-CD38 mAb isatuximab desensitization trial (NCT04294459) **(Appendix 2).** This was an open-label, single-arm, phase 1/2 study conducted at six centers in the United States and Spain between June 18, 2020, and May 2, 2022. The study had a screening period of up to 28 days, a treatment period of up to 12 weeks, a site-visit follow-up of up to 26 weeks after treatment had stopped, and an extended follow-up via telephone every 90 days until study cut-off date, death, or loss to follow-up. Study cutoff was planned at 26 weeks after the last patient completed the treatment period or when the last ongoing patient was lost to follow-up, whichever was earlier. The primary objective of the phase 1 study was to characterize the safety and tolerability of isatuximab in kidney transplant candidates. The primary objective of the phase 2 study was to evaluate the preliminary efficacy of isatuximab in the desensitization of patients awaiting kidney transplantation.

For the immune mechanistic study, 16/23 patients in whom PBMCs were available both prior and after therapy were investigated **(Figure 7).** These patients were investigated for the impact of isatuximab serological memory, on BM-resident cells and different peripheral blood B cells.

1.2 Isatuximab treatment

Isatuximab was administered at a starting dose of 10 mg/kg every week for 4 weeks in cycle 1 and every 2 weeks for cycles 2 and 3. Patients underwent three cycles of

treatment (a total of eight planned doses) spanning a 12-week period. Each cycle was 28 days **(Figure 7).**

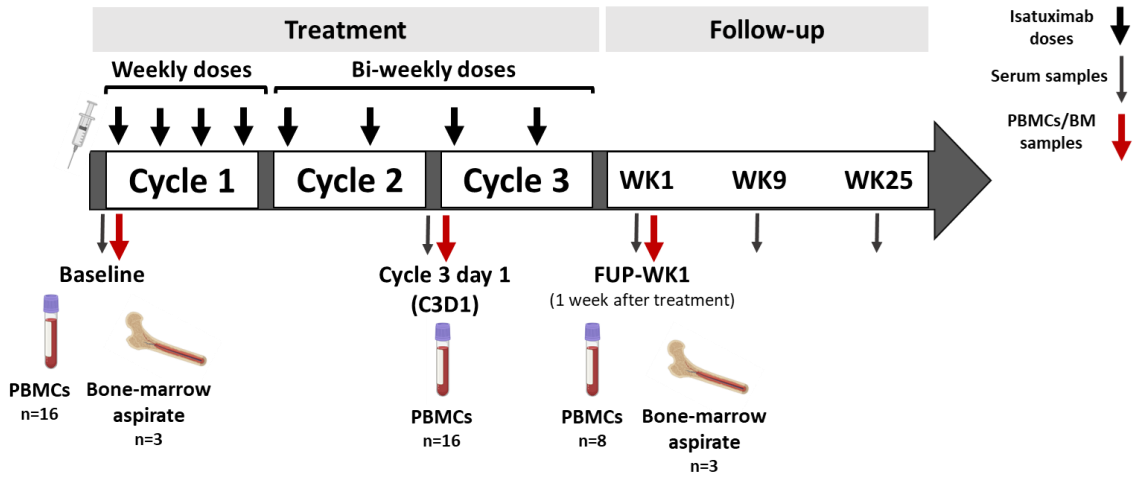

Figure 7. Derivation cohort Study design. Treatment with isatuximab in the derivation cohort was administered in 3 cycles of 28 days comprising a total of 8 doses. Serum samples were collected at baseline, day 1 of cycle 3 (C3D1) and at follow-up weeks 1, 9 and 25 (FUP-WK1, FUP-WK9 and FUP-WK25). PBMCs were obtained at baseline, C3D1 and FUP-WK1. Bone-marrow aspirates were collected at baseline and FUP-WK1.

1.3 Study endpoints

The primary end point for the phase 1 study was the proportion of patients with adverse events (AEs), serious AEs, and laboratory abnormalities. In phase 2, the primary end point was response rate (RR) which was a composite end point, as assessed by central laboratory.

RR was defined as a proportion of patients meeting at least one of the three predefined desensitization efficacy criteria: Criterion 1 was the reduction of cPRA to target levels, where target cPRA was defined as cPRA that would result in at least doubling the theoretical likelihood of finding a compatible donor[219]. Target cPRA was calculated according to the following equation[219]. Examples of target cPRA reduction are presented in **supplementary table 1**. Participants with a baseline cPRA=100% were assigned with cPRA 99.99% for computational purpose.

$$LCD = 1\ in \frac{1}{1 - cPRA}$$

Criterion 2 was the reduction of more than 2 antibody titers to reach target cPRA. Antibody titer was defined as the last dilution of serum at which positive results is obtained[220] (positive >2000 MFI). Examples of antibody titer based on MFI are illustrated in **supplementary table 2**. Finally, criterion 3 was the elimination of anti-HLA antibody as MFI reduced to <2000 for antibodies with a baseline MFI of >3000.

Secondary end points included duration of response (DoR), number of anti-HLA antibody eliminated, change in cPRA and anti-HLA antibody levels, pharmacokinetics (PK), and biomarkers. Safety assessments included adverse events (AEs) and serious AEs reported per Common Terminology Criteria for AEs v5.0, laboratory abnormalities, and incidence of anti-drug antibodies (ADAs) against isatuximab.

1.4 Obtention of biological samples

Serum samples were collected before treatment (baseline), at day one of cycle 3 (C3D1), and at 7 follow-up (FUP-WK) visits after last treatment dose, at weeks 1, 2, 9, 13, 17, 21 and 25. PBMC samples were collected before treatment (n=16), at C3D1 (n=16) and one week after the last dose (FUP-WK1) (n=8) **(Figure 7).** PBMCs were isolated through ficoll density gradient centrifugation as previously described[54]. BM aspirates from 3 patients of the study were obtained before treatment and at FUP-WK1 **(Figure 7).**

2. Pharmacokinetics Analysis

Blood samples for PK analysis were collected mainly during cycle 1 at selected time points (predose, end of infusion [EOI], EOI+1 hour or EO+4 hours, start of infusion +72 hours, and start of infusion +168 hours) and were used for isatuximab PK assessment by noncompartmental analysis. Analysis was performed with Phoenix WinNonlin version 8.2 (Pharsight). The Gyrolab Platform, a quantitative sandwich immunoassay using biotinylated anti-isatuximab antibodies bound by streptavidin beads within the Gyrolab Bioaffy CD microstructure for capture and Alexa Fluor 647-conjugated CD38 antibody for detection, was used to measure functional isatuximab (isatuximab with ≥1 site available to bind target) plasma levels, with a lower limit of quantitation of 5.0 ug/ml.

3. Anti-HLA antibodies assessment

3.1 Anti-HLA Antibody testing

Serum samples were frozen at 280°C for at least 10 minutes, then thawed at 224°C, and brought to room temperature for preparation. Aggregates were removed by centrifugation for 5 minutes at 74003g. Serum was treated with Adsorb Out Beads (ADSORB, One Lambda) according to the manufacturer's instructions, and EDTA, 0.5 M pH 8.060.1, was added to serum in a 1:20 ratio (e.g., 5 ml EDTA to 95 ml serum). Serum dilutions were performed in PBS (Beckman Coulter), and all assays were performed by one technologist. Consecutive samples from each patient were batched to minimize assay variability. Anti-HLA antibody testing was performed using LABScreen Single Antigen HLA class I (catalog LSA1A04, One Lambda) and LABScreen Single Antigen HLA class II (catalog LS2A01, One Lambda), and data were acquired on a LABScan 3D flow analyzer and analyzed in HLA Fusion 4.6 software.

3.2. cPRA per serial dilutions

At baseline, serum from each patient was tested neat and in serial doubling dilutions from 1:2 to 1:4096. Samples collected at day 1 of each treatment cycle and at site visit follow-up weeks 1, 5, 9, 13, 17, 21, and 25 were tested neat and at the relevant dilution for assessment of efficacy criterion 2. cPRA was calculated to two decimal points (e.g., 99.99%) on the basis of the cPRA calculator developed by the Organ Procurement and Transplantation Network, with unacceptable antigens defined as those with MFI ≥ 2000.

3.3 Antibody Titration Heat Maps to Compare baseline to Follow-Up

Antibody results were compared for each patient for serum samples collected at BL versus site visit follow-up weeks 9 and 25 (or the closest dated alternate follow-up samples as available). HLA class I and class II panels were analyzed separately, and beads on each panel were sorted from high to low on the basis of the patient's BL titer strength for that bead. Following the BL titer sorted values, heat maps were produced in consecutive subsequent columns of a spreadsheet. For the BL and follow-up samples, MFIs for serum tested neat, 1:16, and 1:256 were compared. Conditional formatting was used to color code MFIs in strength categories.

4. IgG analysis and conventional flow cytometry immunophenotyping

Ig and immunophenotyping assays were performed by Covance Central Laboratory Services. B-cell panels, NK and natural killer T-cell panels, and Ig assays were performed. B-cell panels were analyzed as follows—each specimen was incubated with Whole Blood Lysing Reagent and centrifuged afterward. White blood cells were then washed and prepared for immunophenotyping staining. Cells were incubated with Fc block working solution followed by incubation with CD38 FITC (Beckman Coulter), AHIgG1 FITC (Southern Biotech), CD24 PE (BioLegend), CD20 PerCPCy5.5 (BD Pharmingen), CD19 APC (BD Pharmingen), CD45 AF700 (BD Pharmingen), IgD V450 (BD Horizon), CD27 BV510 (BioLegend), and CD138 BV605 (BioLegend) in Brilliant Stain Buffer (BD Horizon). Finally, cells were fixed with 1% paraformaldehyde solution and acquired on the BD SORP FACSCanto II. For analysis of the NK and regulatory T-cell (Treg) cell panel, each specimen was washed with plain PBS without azide and the pellet was resuspended and incubated with N-hydroxysuccinimide solution. After washing, cells were incubated with Fc block working solution followed by incubation with CD38 FITC (Beckman Coulter), AHIgG1 FITC (Southern Biotech), CD25 PE (BD Pharmingen), CD127 PerCP-Cy5.5 (BioLegend), CD56 APC (BioLegend), N-hydroxysuccinimide Ester AF700 (Thermo Fisher), CD4 BV421 (BioLegend), CD8 BV510 (BD Horizon), and CD3 BV605 (BioLegend) in Brilliant Stain Buffer (BD Horizon). After washing, red blood cells were lysed with Whole Blood Lysing Reagent. Finally, cells were fixed with 1% paraformaldehyde solution and acquired on the BD FACSCanto II.

5. Functional assessment of HLA-sp memory B cells and HLA-sp bone marrow plasma cells

Functional assessment of HLA-sp MBCs was performed with both HLA-sp B cell fluorospot in 8 patients and analyzing the repertoire of anti-HLA antibodies in mBC supernatants in 10 patients. Bone marrow HLA-sp plasma cells were assessed in 3 patients through HLA-sp B-cell fluorospot assay.

5.1 In vitro expansion of mBCs for HLA-sp memory B-cell assessment

For the analysis of HLA-sp mBCs, PBMCs were stimulated polyclonally in vitro with 500 ng/ml Human CD40/TNFRSF5 Antibody (Bio-Techne R&D Systems, S.L.U), 1µg/mL imidazoquinoline resiquimod R848 (Mabtech,Sweden), 600 IU/ml human interleukin-2 (Sigma Aldrich), 100 ng/ml human interleukin-21 (Peprotech), 25 ng/ml human interleukin-10 (IL-10) (Peprotech, UK) and incubated for 6 days for HLA-sp fluorospot assay or 10 days for the assessment of anti-HLA antibodies in culture supernatant[56].

5.2 HLA-sp B-cell fluorospot assay

HLA-sp Fluorospot assay was developed in peripheral blood MBCs and bone marrow samples. Expanded mBCs or bone marrow plasma cells were seeded in a previously coated with anti-IgG mAb (Mabtech) fluorospot plate and incubated for 20 hours to release antibodies. After incubation, polyclonal IgG was detected with anti-human IgG-FITC (Merck) and HLA-sp IgGs were detected using fluorescent labeled HLA tetramers (HLA pureprotein LLC.) using an ispot fluorospot reader (Autoimmun Diagnostika GMBH, AiD)

All MBCs and BM plasma cell tested HLA specificities per patient are listed in **Supplementary Table 3**. A total of 60 HLA specificities were evaluated for HLA-sp mBCs and 17 for HLA-sp bone marrow plasma cells. All evaluated bone marrow HLAs had high MFI antibody levels in their respective sera. HLA-sp Fluorospot results are reported as HLA-sp IgG activity per 450.000 seeded cells, which summarizes the number of HLA-sp IgG-secreting spots forming units (SFU) and the quantity of HLA-sp IgG produced assessed by MFI.

5.3 Assessment of HLA-sp antibodies in memory B cell supernatants

Circulating HLA-sp IgG-producing mBCs could be assessed in 11 patients at baseline, 10 patients at C3D1, and in 5 patients at FUP-WK1. Supernatants from *in vitro* expanded mBCs were collected and IgG antibodies from each sample were purified using Magne protein G beads (Promega). All purified IgG samples were quantified by nephelometry. Samples with a total of IgG>1.2 mg/ml were considered valuable for assessment and anti-HLA antibodies were analyzed using single-antigen beads (SAB) assays on a Luminex

platform (Lifecodes, Immucor). Results from the SAB assay were then normalized by the total IgG obtained in each sample. A positive threshold was considered using the ratio MFI/IgG values for antibodies against self-typed HLA antigens means multiplied by three times the standard deviation of those values ($cut\ off = self\ HLA\ MFI\ mean * (3 * SD)$).

6. Spectral flow cytometry immunophenotyping of PBMC and BM-residing cells

A 22-antibody flow cytometry panel was developed to analyze multiple immune cell with a focus in B-cells subsets both in PBMC and BM-residing cells both prior, during and after desensitization using a 5-laser spectral flow cytometer (Cytek Aurora, Biosciences).

Antibody staining was performed in PBMCs and BM samples. Briefly, samples were incubated with 22 different fluorochrome-conjugated antibodies listed in **Supplementary Table 4** and brilliant violet buffer (BD Biosciences) for 20min at 4ºC. After incubation, stained cells were washed and stained with viability dye 7-AAD. Samples were acquired immediately after sample preparation using the spectral flow cytometer. All samples were analyzed the same day to avoid batch-to-batch effect.

Data processing and analysis was performed with the web tool OMIQ; First, quality control and data cleaning were performed with PeacoQC algorithm. Total lymphocytes and B cells were selected manually excluding dead cells and doublets and were analyzed by both manual gating and unsupervised analysis (**Supplementary Figure 1**). Dimensionality reduction of different cell subsets was performed using T-distributed stochastic neighbor embedding (t-SNE) and B-cell clustering was executed through Phenograph algorithm[221]. Cluster identification was done manually using the relative expression of all markers across clusters.

7. External validation patient cohort

An external validation cohort comprised of 10 highly sensitized kidney transplant candidates from the Groupe Hospitalo-Universitaire Chenevier Mondor (Paris, France), who were enrolled in a phase 1b/2 desensitization clinical trial with the anti-CD38 mAb daratumumab (NCT04204980) and had pre-treatment PBMC samples available were

included (n=10/13). Included patients were adults (> 18 years old) active in the waiting list for kidney transplantation and registered on the French National kidney allograft waiting list for at least three years. All patients were highly sensitized with a cPRA >95% for at least three years. Daratumumab was infused at 16 mg/kg weekly for eight weeks for a total of 8 doses of treatment (**Figure 8**). These patients were evaluated for the impact of daratumumab on anti-HLA serological responses and presence of specific B-cell subsets phenotypes prior to therapy.

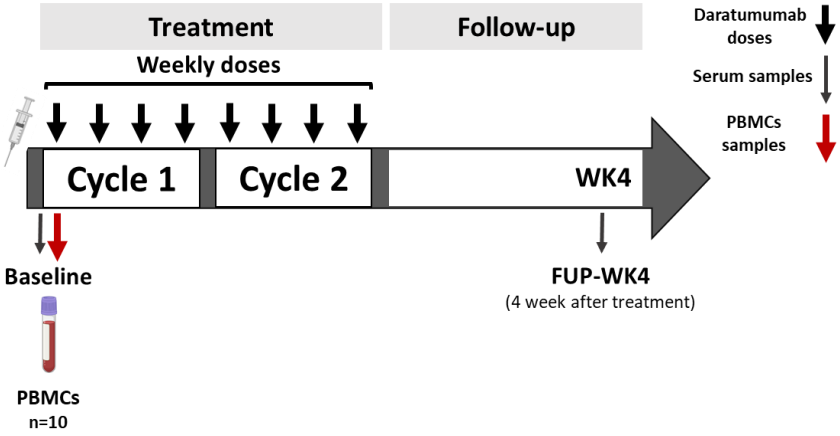

Figure 8. Validation cohort study design. Daratumumab treatment was administered weekly during 56 days for a total of 8 doses of treatment. For the validation study, PBMCs were obtained at baseline and serum samples were collected at baseline and 4 weeks after the last dose (FUP-WK4).

8. Flow-cytometry immunophenotyping in the validation cohort

Conventional flow cytometry was performed in all available baseline PBMCs samples from the validation cohort. PBMCs were washed and incubated with the following conjugated antibodies: CD27-V450 (M-T271, BD Biosciences, France), CD19-APC-Cy7 (clone HIB19, Biolegend), IgD-FITC (clone IADB6, Beckman Coulter, France), CD38-PE-Vio770 (clone REA572, Miltenyi Biotec) for 20 minutes 4ºC. Samples were acquired through a BD Canto II cytometer and analyzed using FlowJO software (FlowJo LLC, Ashland, OR). Frequencies of B-cell populations were determined following the gating strategy shown in **supplementary figure 1.**

9. Prevalence of B-cell phenotype signature of response to anti-CD38 mAb in highly sensitized transplant candidates in the waiting list.

We evaluated 39 kidney transplant candidates, 19 highly sensitized patients (cPRA > 85%) and 20 non-sensitized controls (cPRA = 0%), from the active kidney transplant waitlists of Vall d'Hebron University Hospital and Assistance Publique - Hôpitaux de Paris. B-cell phenotyping was conducted using conventional flow cytometry at each institution. B cells were defined as CD19+ lymphocytes, and MBCs were identified as CD19+ CD27+ IgD- cells.

10. Statistical analysis

Clinical trial response rate (primary composite endpoint) was calculated as proportion of patients meeting at least one of the three predefined desensitization efficacy criteria, along with corresponding two-sided 95% confidence intervals (CIs) using the Clopper–Pearson method.

Duration of response (DoR) was defined as the time from central laboratory sample collection date indicating response up to the central laboratory sample collection date when the patient was no longer meeting any response criterion (i.e., nonresponder) or up to date of death due to any cause, whichever occurred first. DoR is summarized with the Kaplan–Meier method.

For pharmacodynamic analyses, the Wilcoxon signed-rank test was used to evaluate the significance of the change posttreatment as compared with the BL. To control for multiple testing, adjusted P values have been also calculated by using the Benjamini and Hochberg method[222].

Continuous variables are presented as median with interquartile range (IQR), due to their non-parametric nature and statistical analysis was done using two-tailed Wilcoxon test and Kruskal-Wallis. P values <0.05 were considered statistically significant.

The serological desensitization effect of isatuximab and daratumumab for the mechanistic study at the individual patient level was done considering the global

changes on MFI Ab values that were detectable at baseline between baseline and after therapy and measuring the Euclidean distance between them to perform hierarchical clustering analysis; being this one of the most commonly used methods to measure distances between quantitative data[223,224].

Partial least squares discriminant analysis (PLS-DA) was performed to assess if certain patterns of B-cell population abundances were able to discriminate between responders and non-responder patients based on their serological immune response to therapy defined by the relative changes on both HLA antibody titers and MFI values. Due to the compositional nature of these data, Centered Log Ratio (CLR) transformation was previously performed[225].

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the most optimal thresholds predicting response to treatment. Data analysis was performed using SPSS Statistics and R (V 4.3.1) [226]. Figures were generated using R (V 4.3.2) and GraphPad Prism version 8.0 software (GraphPad Software).

11. Ethical Considerations

This study was conducted in accordance with the ethical standards of the Institutional Review Board (IRB) of Vall d'Hebron university hospital and Bellvitge university hospital and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Participants were provided with detailed information regarding the study's objectives, procedures, potential risks, and benefits prior to providing their consent. The study protocol was reviewed and approved by the ethics committee for drug research of Vall d'Hebron university hospital (IRB) number: IRB00002850 and Federalwide Assurance (FWA) number: FWA00003437) and Bellvitge university hospital (IRB number: IRB00005523 and FWA number: FWA00010235) **(Appendix 3).**

IV. RESULTS

STUDY I.

Clinical impact of Isatuximab Monotherapy for Desensitization in Highly Sensitized Patients Awaiting Kidney Transplant

IV. RESULTS

STUDY I. Clinical impact of Isatuximab Monotherapy for Desensitization in Highly Sensitized Patients Awaiting Kidney Transplant

1. Patients and baseline characteristics

A total of 23 patients were enrolled in this study—12 in cohort A and 11 in cohort B—of which 22 completed the study treatment period and 18 completed the extended follow-up period until study cut-off date **(Figure 9).** The median follow-up (FUP) of all treated participants was 68.0 weeks. One patient in cohort B discontinued treatment definitively on the basis of logistical reasons due to coronavirus disease 2019 positivity before the last planned dose.

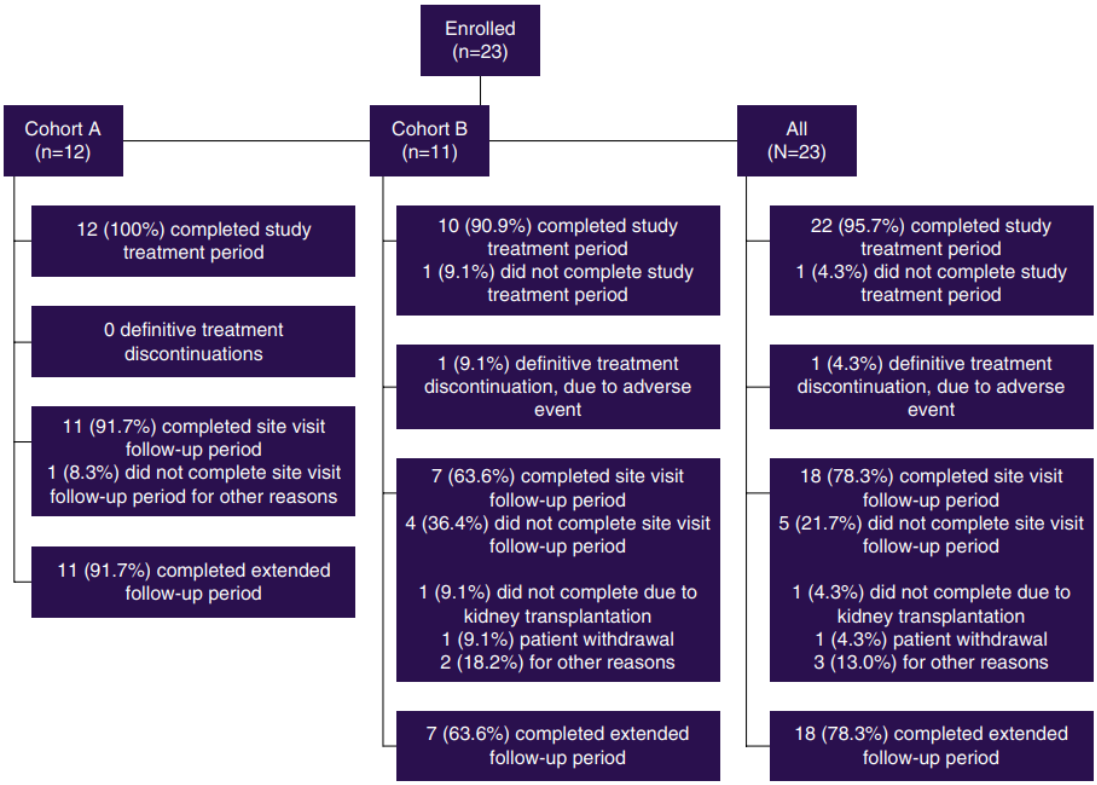

Figure 9. Patient disposition

The median age of patients was slightly higher in cohort A than cohort B (52.5 versus 48.0 years; **Table 1**). Patients in cohort A spent a median 6.0 years on the kidney transplant waitlist, while those in cohort B spent a median 3.6 years. Origins of renal insufficiency reported by investigators were also more varied in cohort B than in cohort

A, with 50.0% of patients in cohort A due to prior transplant failure, compared with 36.4% in cohort B. Origins of renal insufficiency in cohort B also included urologic disorders (18.2%), hypertension (9.1%), and autosomal dominant polycystic kidney disease (9.1%). Most patients had one prior kidney transplant (58.3% cohort A, 54.5%

Table 1. Patient disposition

Characteristic	**Cohort A (n=12)**	**Cohort B (n=11)**	**All (n=23)**
Median age, yr (min-max)	52.5 (25-68)	48 (25-69)	52 (25-69)
Sex, n (%)			
Male	6 (50)	9 (81.8)	15 (65.2)
Female	6 (50)	2 (18.2)	8 (34.8)
Race, n (%)			
White	4 (33.3)	9 (81.8)	13 (56.5)
Black or African American	3 (25)	1 (9.1)	4 (17.4)
Asian	2 (16.7)	0	2 (8.7)
American Indian or Alaska Native	0	0	0
Native Hawaiian or other Pacific Islander	0	0	0
Not reported	1 (8.3)	1 (9.1)	2 (8.7)
Unknown	2 (16.7)	0	2 (8.7)
Blood type, n (%)			
A	4/12 (33.3)	1/9 (11.1)	5 (23.8)
B	4/12 (33.3)	2/9 (22.2)	6 (28.6)
AB	0	1/9 (11.1)	1 (4.8)
O	4/12 (33.3)	5/9 (55.6)	9 (42.9)
Dialysis time, median years (min-max)	6.8 (2.9-12.9)	5.05 (0.2-24.2)	6.23 (0.2-24.2)
Waitlist time, median years (min-max)	6 (2.2-12.9)	3.6 (0.6-9.2)	5.3 (0.6-12.9)
Origin of renal insufficiency at study entry, n (%)			
Diabetes mellitus	1 (8.3)	0	1 (4.3)
Hypertension	0	1 (9.1)	1 (4.3)
Glomerular disease	2 (16.7)	2 (18.2)	4 (17.4)
ADPKD	0	1 (9.1)	1 (4.3)
Failure of previous transplants	6 (50)	4 (36.4)	10 (43.5)
Urologic disorders	0	2 (18.2)	2 (8.7)
Other	5 (41.7)	4 (36.4)	9 (39.1)
No. of prior kidney transplants, n (%)			
0	2 (16.7)	1 (9.1)	3 (13)
1	7 (58.3)	6 (54.5)	13 (56.5)
2	2 (16.7)	3 (27.3)	5 (21.7)
3	1 (8.3)	1 (9.1)	2 (8.7)
Prior sensitizing events at screening, n (%)	11 (91.7)	10 (90.9)	21 (42.9)
Pregnancy	1 (8.3)	0	1 (4.8)
Transfusion	6 (50)	3 (30)	9 (42.9)
Transplant	10 (83.3)	10 (100)	20 (95.2)
cPRA per central laboratory, median % (min-max)	99.99 (99.62-100)	99.95 (98.38-100)	99.97 (98.38-100)
cPRA per local laboratory with OPTN, median % (min-max)	99.99 (99.90-100)	99.42 (95.57-99.85)	99.90 (95.57-100)

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; cPRA, calculated panel reactive antibody; OPTN, Organ Procurement and Transplantation Network

cohort B), and over 90% of patients in both cohorts had prior sensitizing events at screening, mostly attributed to transplant and transfusion. All 23 patients were diagnosed with stage 5 CKD at the time of study entry, representing a patient population that is likely to require dialysis (22 of 23 patients were on dialysis at the time of study entry). The median cPRA per local laboratory assessment was 99.99% (99.90–100.00) and 99.42% (95.57–99.85) in cohorts A and B, respectively. When measuring cPRA by central laboratory, the median cPRA was 99.99% (99.62–100.00) and 99.95% (98.38–100.00), respectively. All patients received a median of three cycles of isatuximab, with 12 weeks of exposure. The median relative dose intensity was 98.24% in cohort A and 98.38% in cohort B.

2. Safety

Safety analysis showed any grade treatment-emergent AEs (TEAEs) occurred in 7 (30.4%) patients overall. No TEAEs were grade≥3. One death occurred in cohort A due to disease complications not related to study treatment during site visit follow-up. A safety summary of TEAEs by AE preferred term can be seen in **Table 2** and **Supplementary Table 5.** No treatment-emergent serious AEs were reported, and the only treatment-related AEs were infusion reactions. At BL, anemia as laboratory abnormality occurred in 65.2%. Post-treatment, anemia occurrence increased to 82.6% of patients. At both BL and post-treatment, most occurrences were grade 1 in severity with no occurrence of grade≥3. Lymphocytopenia as laboratory abnormality occurred in 25.0% of patients at BL, most of which were grade 1. Post-treatment, the occurrence of lymphocytopenia as laboratory abnormality was 56.3%, with most occurrences grade 1 and one grade 3 occurrence (6.3%). There were no instances of neutropenia during the trial. No patients had an on-treatment positive ADA response against isatuximab.

Table 2. Safety summary of treatment-emergent adverse events by adverse event preferred term

	All (n=23)	
No. (%)	**All Grades**	**Grade≥3**
Any event	(30.4)	0
Infusion reaction	5 (21.7)	0
Nasopharyngitis	1 (4.3)	0
Headache	1 (4.3)	0
Tachycardia	1 (4.3)	0
Nasal congestion	1 (4.3)	0
Nausea	1 (4.3)	0
Myalgia	1 (4.3)	0
Temporomandibular joint syndrome	1 (4.3)	0
Chills	1 (4.3)	0
COVID-19	1 (4.3)	0

3. Pharmacokinetics of Isatuximab in Kidney Transplant Candidates

Isatuximab was quantifiable in plasma over the whole dosing period of 1 week after the first infusion at a dose of 10 mg/kg. The overall mean isatuximab maximum plasma concentration (C_{max}) and area under the curve over 1 week ($AUC_{1\ week}$) were 290 ug/ml and 24700 ug*h/ml, respectively, with moderate variability. A PK summary can be seen in **Table 3** and **Supplemental Table 6**.

Table 3. Summary of pharmacokinetics of isatuximab after the first administration at a dose of 10 mg/kg.

Mean ± SD (CV %)	**All (n=22)**
C_{max}, ug/ml	290 ± 109 (38)
T_{max}, h	3.46 (2.00-6.03)
$AUC_{1\ week}$, ug·h/ml	24700 ± 7880 (32)

Abbreviations: AUC1 week, area under the curve over 1 week; Cmax, maximum plasma concentration; CV, coefficient of variation; tmax, time to reach maximal concentration.

4. Desensitization Activity in Highly Sensitized Kidney Transplant Candidates

The overall RR was 83.3% in cohort A and 81.8% in cohort B **(Table 4).** Median DoR was not reached in either cohort (cohort A 95% CI, 4.857 to not reached weeks; cohort B 95% CI, 4.143 to not reached weeks). Most responders had a decrease in the anti-HLA antibody level after treatment initiation which was maintained during the site visit follow-up period after stopping treatment. However, among all patients, there is minimal effect on the overall cPRA values. Only 39% of patients (4/12 and 5/11 in cohorts A and B, respectively) had reached target cPRA (*i.e.,* decrease in the cPRA level that would result in at least doubling the theoretical likelihood of finding a compatible donor[219]). Approximately 47.8% (7/12 and 4/11 in cohorts A and B, respectively) had meaningful

reduction in anti-HLA antibody titer, and 82.6% (10/12 and 9/11 in cohorts A and B, respectively) had at least one anti-HLA antibody with baseline MFI ≥3000 reduced to <2000 **(Table 4 and Supplementary table 7).** No baseline clinical characteristics or laboratory features were observed to be associated with treatment response. In particular, given the polymorphic nature of the HLA system, as well as the variability in antibody strengths and other variables, a large cohort is required to enable more detailed analysis with high confidence.

Table 4. Summary of response rate in the efficacy-evaluable population on the basis of assigned cohort using screening calculated panel reactive antibody from local laboratory assessment.

No. (%)	Cohort A (n=12)	Cohort B (n=11)	All (n=23)
No. of participants assessed	12	11	23
RR based on criterion 1	4 (33.3)	5 (45.5)	9 (39.1)
RR based on criterion 2	7 (58.3)	4 (36.4)	11 (47.8)
RR based on criterion 3	10 (83.3)	9 (81.8)	19 (82.6)
Overall RR	10 (83.3)	9 (81.8)	19 (82.6)
95% CI	51.6 to 97.9	48.2 to 97.7	61.2 to 95.0

Abbreviations: CI, confidence interval; RR, response rate.

Since cPRA alone is not sufficient to reflect a partial desensitization effect, a composite end point that also included titer reduction and assessment of anti-HLA antibody profiles was implemented to provide a better measurement. This is illustrated through the examples of partial responders, who met criterion 2 or 3 or both, but not criterion 1 (reaching target cPRA), as shown in **Figure 10.** Assessment of their titer and antibody profiles reveals more information on the desensitization effect of isatuximab. In some patients, although not considered responders based solely on cPRA, a marked and durable decrease in MFI, up to 215,000, was observed for some anti-HLA antibodies.

The example full profiles of responders, partial responders, and non-responders are presented in **Figure 11-13**. Of note, MFI values increased from baseline over time in patient 0811, a non-responder per protocol **(Figure 13).** This increase was observed from C2D1 onward, approximately 2.5 weeks after the patient received their second dose of coronavirus disease 2019 vaccine and at the time point where the highest stimulation of the immune system is expected to be observed. This potentially led to the nonspecific activation of dormant memory response to HLA.

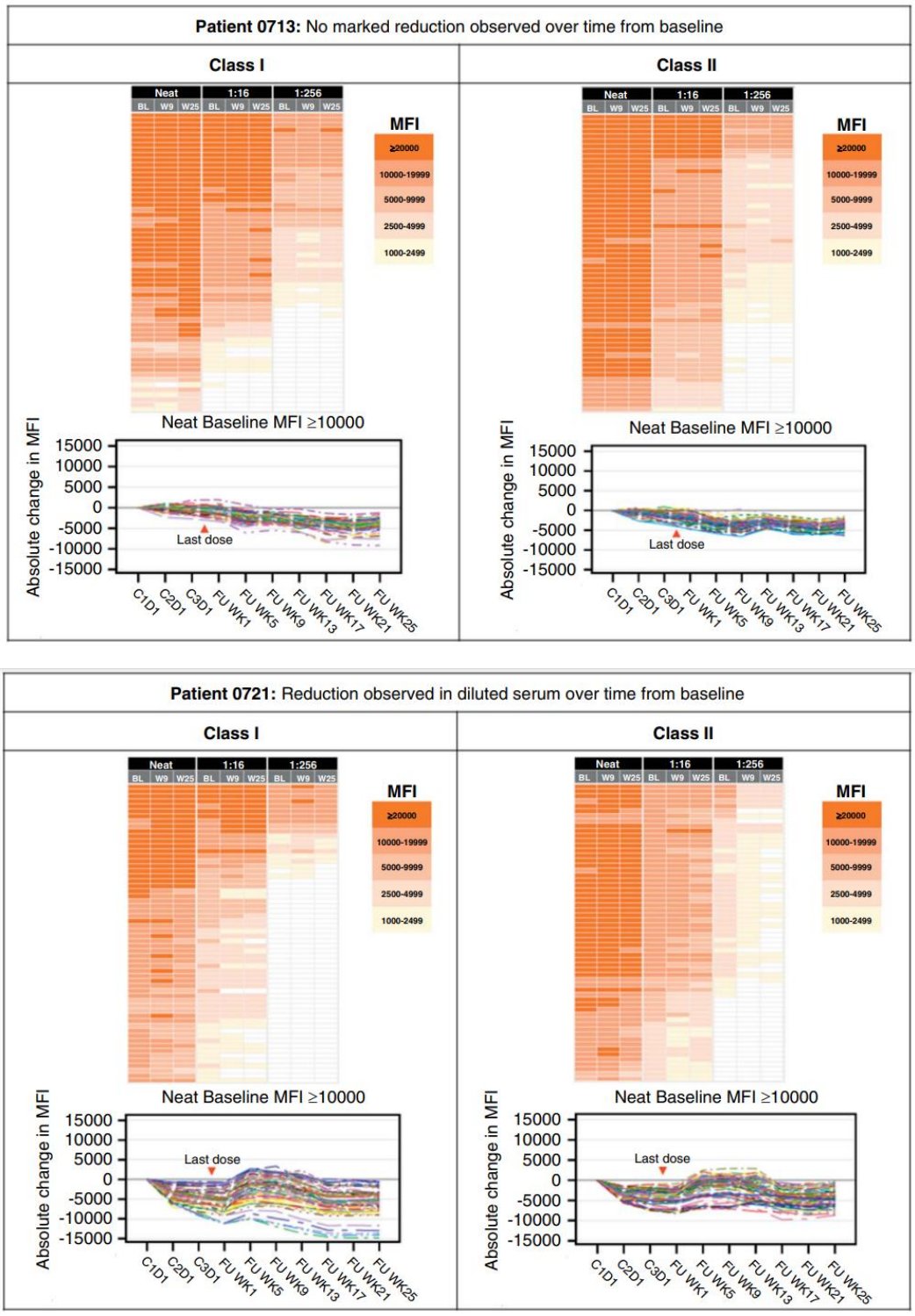

Figure 10. Examples of patients who were responders per protocol but did not meet target cPRA in criterion 1. Class I and class II anti-HLA antibody heat maps illustrate the MFI values of the top 60 HLA alleles (from the top, in descending order on the basis of their baseline titer at neat, 1:16, and 1:256 dilutions. Each dilution includes baseline, FUP-WK9 (approximately 9 weeks after the last dose), FUP-WK25 (approximately 25 weeks after the last dose). Spaghetti plots illustrate the absolute change in MFIs in neat serum of anti-HLA antibodies with BL MFI>10.000. Abbreviations: BL, baseline; C, cycle; cPRA, calculated panel reactive antibody; D, day; FUP, follow-up; MFI, mean fluorescence intensity; N, neat; WK, week.

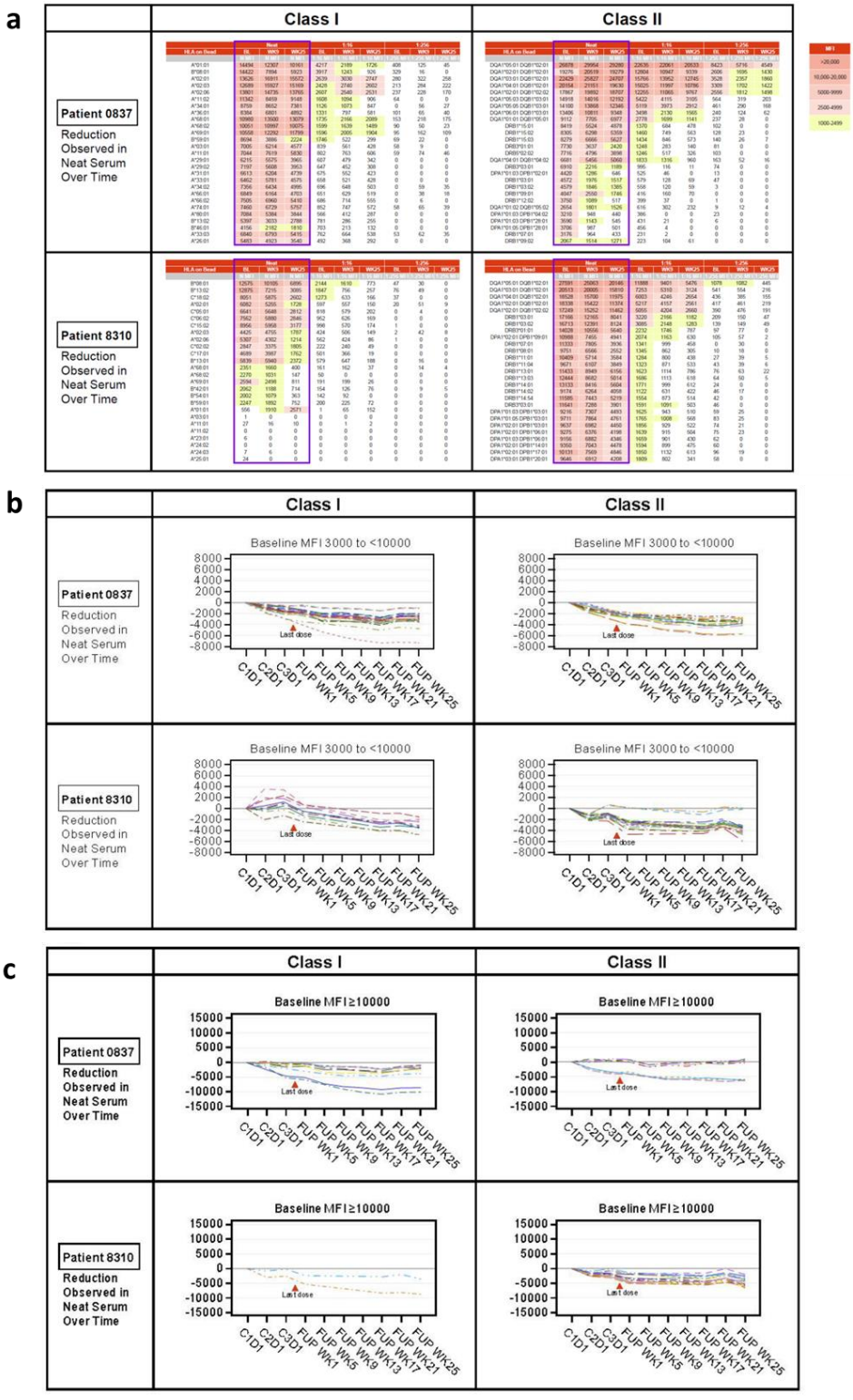

Figure 11. Examples of responder patients per protocol who met all 3 criteria. a) Antibody titration heat map. **b-c)** Plots of MFI change in anti-HLA antibody levels for antibodies with baseline MFI>3000 and <10000. Abbreviations: C, cycle; D, day; FUP, follow-up; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; N, neat; WK, week.

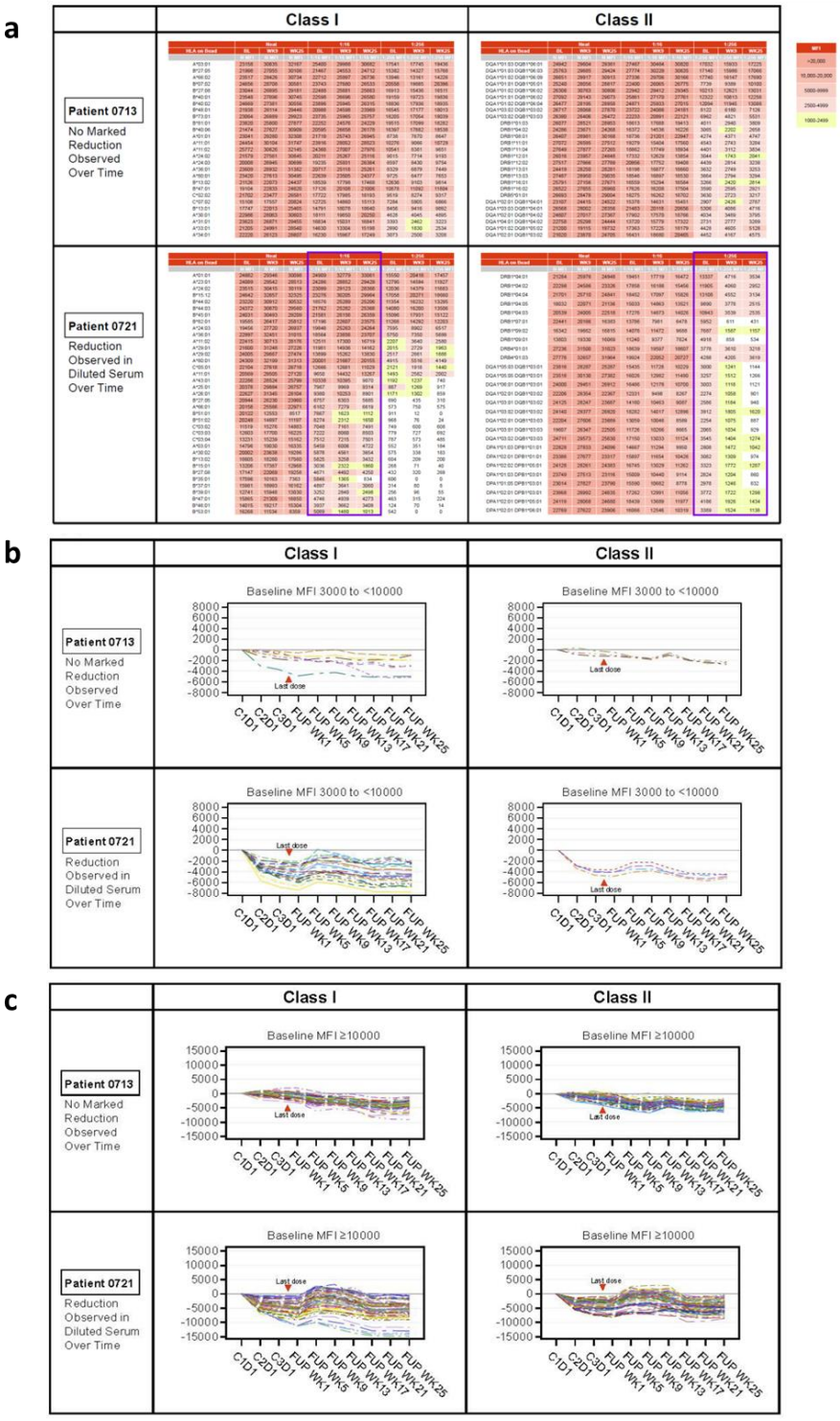

Figure 12. Examples of responder patients per protocol that did not meet target cPRA in Criterion 1. a) Antibody titration heat map. b-c) Plots of MFI change in anti-HLA antibody levels for antibodies with baseline MFI>3000 and <10000. Abbreviations: C, cycle; D, day; FUP, follow-up; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; N, neat; WK, week.

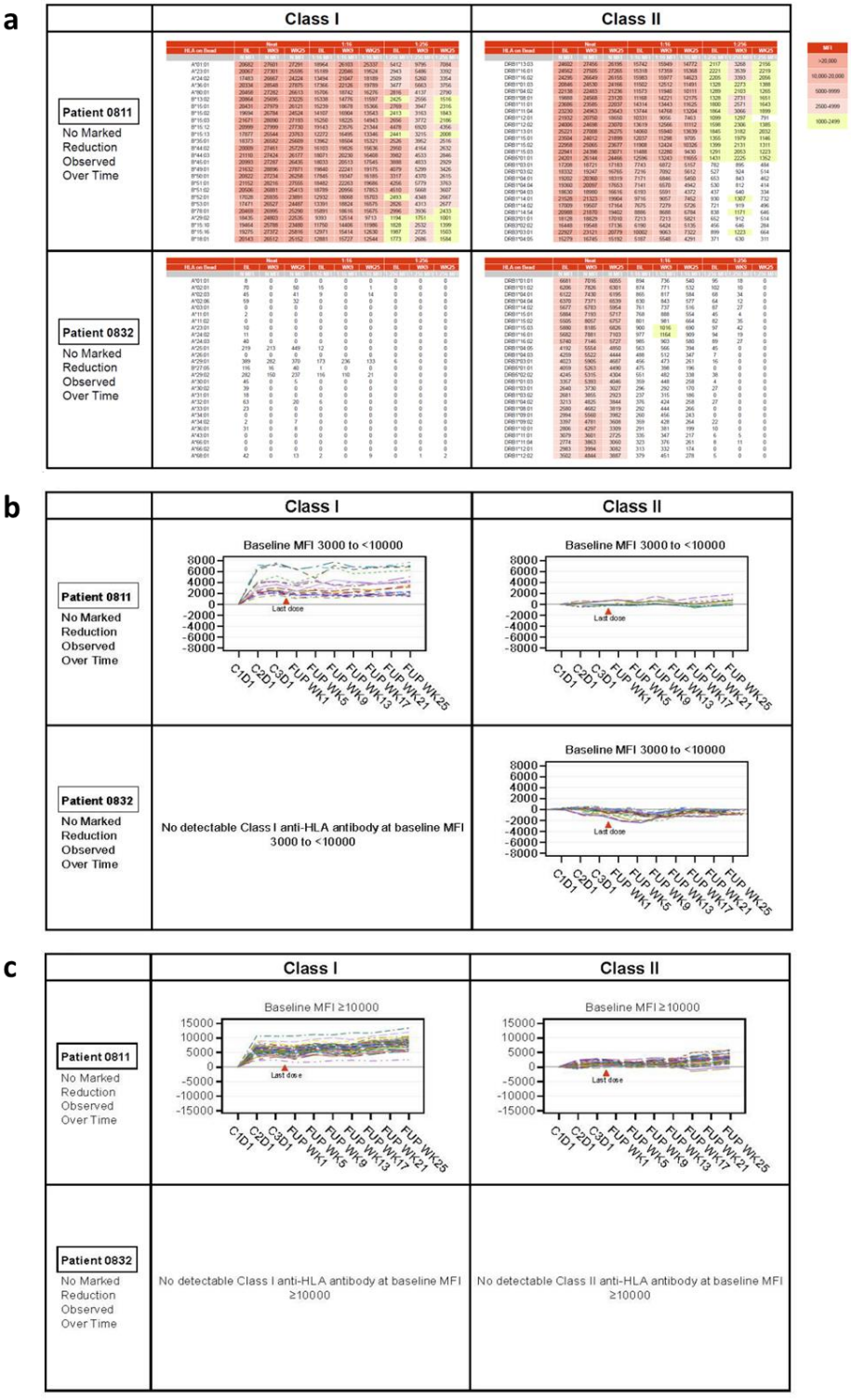

Figure 13. Examples of non-responder patients per protocol that met 0 of 3 criteria. a) Antibody titration heat map. b-c) Plots of MFI change in anti-HLA antibody levels for antibodies with baseline MFI>3000 and <10000. Abbreviations: C, cycle; D, day; FUP, follow-up; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; N, neat; WK, week.

5. Transplant Outcomes

As of study cut-off date, a total of six patients treated with isatuximab received transplant offers (three each from cohorts A and B), all of which were from deceased donors. Four transplant offers were accepted. Reasons for declining an offer were offer not suitable for transplant and poor donor quality. Among the four patients who received transplant before study cut-off date, two of four were HLA incompatible with their donors before isatuximab treatment but were negative at the time of transplant. Three grafts were functioning with no report of rejection as of study cut-off date, while one graft in cohort A was lost due to thrombosis 1 day after transplant surgery with no reported rejection. As of February 2023, 9/11 patients from the two recruitment centers in Spain received kidney transplantation, five of whom received kidneys from previously incompatible donors.

6. Pharmacodynamics and Immune Modulation of Isatuximab

Using Ig levels as a surrogate, the results from the pharmacodynamics analysis support isatuximab target engagement. A sustained and significant decrease in total Ig levels was observed up to the last analyzed time point ($p<0.001$, **Supplementary Table 8),** 17 weeks after last dose (IgG and IgM as shown in **Figure 14A-B**). Different B-cell populations were studied for biomarker analysis with conventional flow cytometry: A decrease in peripheral plasmablasts (p-value= 0.025 and adjusted P value=0.245) and plasma cells (p=0.078 and adjusted p-value=0.706) was observed at C3D1 compared with baseline, with a trend of returning to baseline at 17 weeks after the last dose (**Figure 15a-b**). A significant decrease in NK cells was observed at C3D1 compared with BL (p-value=0.001 and adjusted p-value =0.005), which is mainly driven by the depletion of $CD38^+$ NK cells as no significant change was detected in the $CD38^-$ NK cell population at all time points. No notable decreases in the total Treg population were observed, as a decrease in $CD38^+$ Tregs was compensated by an increase of the $CD38^-$ Treg compartments **(Figure 16c-e**). No notable changes in other T-cell subset data were observed during the study follow-up (data not shown).

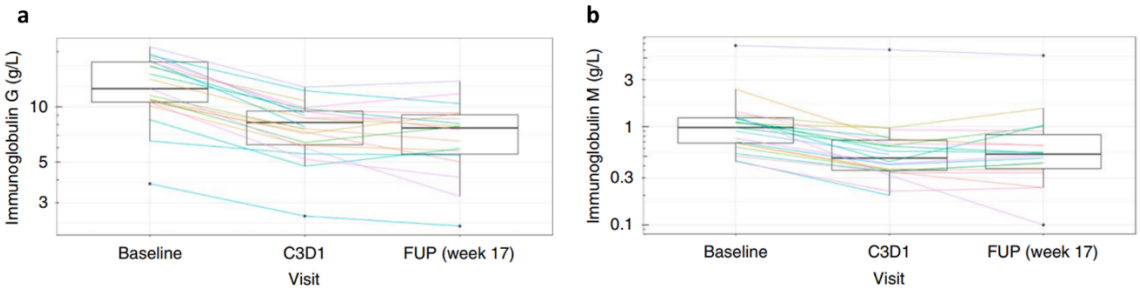

Figure 14. Sustained decrease in total IgG and IgM levels up to last analyzed timepoint. a) Evolution of total IgG levels. b) Evolution of total IgM.

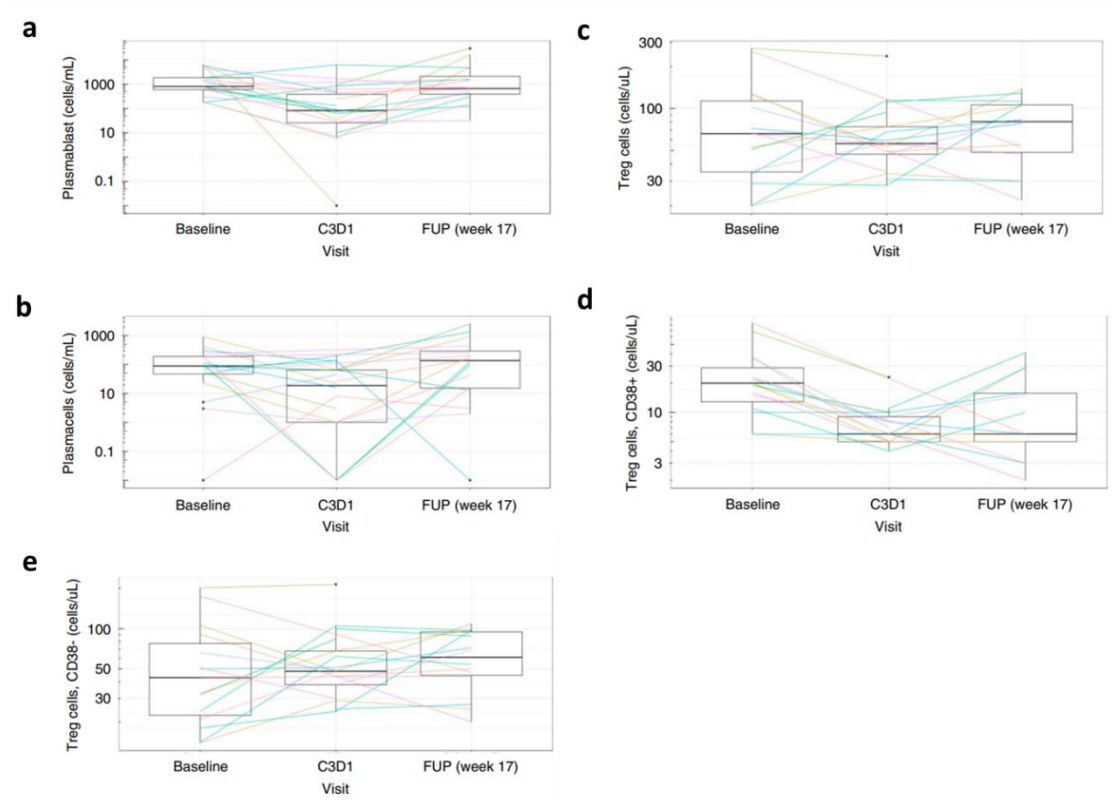

Figure 15. Evolution of lymphocyte populations up to last analyzed timepoint. a) Evolution of plasmablast. b) Evolution of plasmacells. c) Evolution of Tregs d) Evolution of CD38+ Tregs. e) Evolution of CD38- Tregs.

IV. RESULTS

STUDY II.

Anti-HLA serological response to CD38-targeting desensitization therapy is challenged by peripheral memory B-cell phenotypes in highly sensitized patients1. Patients and baseline Characteristics

1. Patients and baseline Characteristics

16 out of the 23 patients included in the isatuximab clinical trial had available PBMCs samples and were used for the mechanistical analysis as a discovery cohort. All patients were adult kidney transplant candidates, with a long time on dialysis and on the waiting lists and a high sensitization degree depicted by a mean cPRA >99%. There were no significant differences between the 23 patients of the trial and the 16 patients assessed in this study regarding main demographic, clinical or immunological characteristics **(table 5).**

Table 5. Demographics and clinical charachteristics

Characteristics	Included in clinical trial *n=23*	Included in the mechanistic study *n=16*	*P-value*
Age, yr, mean ± SD	49 ± 11.8	46.1 ± 11.8	0.457
Sex, Female, n (%)	8 (34.8)	3 (18.8)	0.234
Dialysis time, yr, mean ± SD	7 ± 5.2	6.7 ± 3.6	0.630
Waitlist time, yr, mean ± SD	5.4 ± 3	5.6 ± 3.2	0.899
Origin of renal insufficiency, n (%)			
Glomerular disease	4 (17.4)	2 (12.5)	
Urologic disorders	2 (8.7)	1 (6.3)	
Hypertension	1 (4.3)	1 (6.3)	
Diabetes Mellitus	1 (4.3)	1 (6.3)	0.974
Failure of previous transplant	6 (26.1)	4 (25)	
ADPKD	1 (4.3)	0 (0)	
Other	8 (34.8)	7 (43.8)	
Prior kidney transplants, n, mean ± SD	1.13 ± 0.8	1.37 ± 0.61	0.373
Prior sensitizing events (%)			
Transplant	12 (52.2)	11 (68.8)	0.421
Transplant/Transfusion	8 (34.4)	4 (25)	
Pregnancy/Transfusion	1 (4.3)	0 (0)	
Uknown	2 (8.7)	1 (6.3)	
Baseline cPRA, mean ± SD	99.8 ± 0.43	99.81 ± 0.41	0.767

Abbreviations: ADPKD, Autosomal dominant polycystic kidney disease; cPRA, calculated Panel Reactive Antibod; SD, standard deviation

2. Impact of isatuximab on serum anti-HLA antibodies.

Globally, anti-HLA antibody MFI levels in serum were significantly reduced at all time points as compared to baseline for all antibodies, for both class I and class II, as well as for each specific HLA allele until last follow-up (**Figure 16a supplementary table 9**). Likewise, the relative changes on MFI values for all, class I and class II, as well as for each HLA allele, antibodies over time were steadily reduced until last follow-up (FUP-WK25) **(figure 16b, supplementary table 10).**

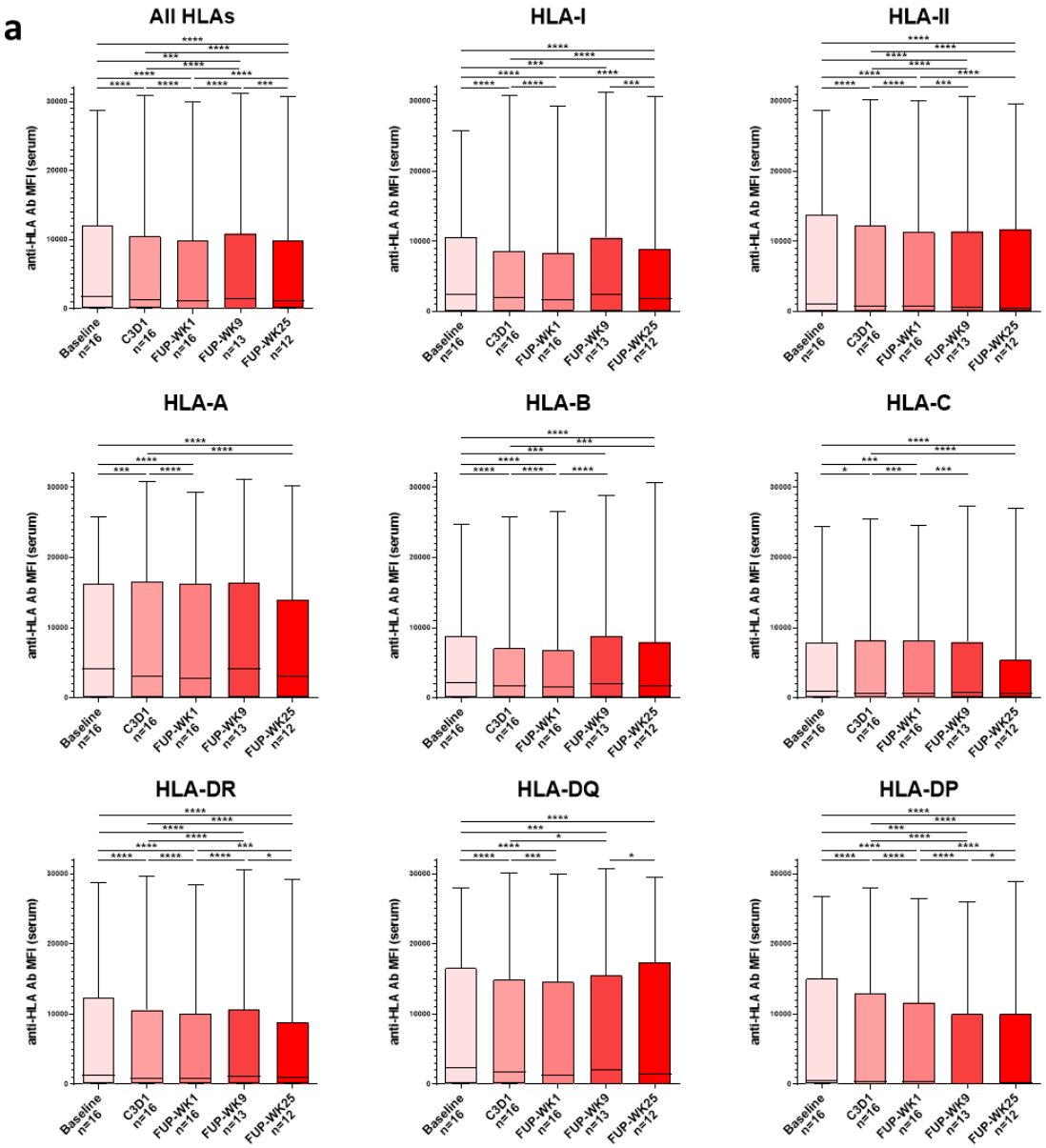

Figure 16. Effect of desensitization in global serum anti-HLA antibodies. a) Circulating anti-HLA antibodies MFI at baseline, C3D1 and FUP-WK1.

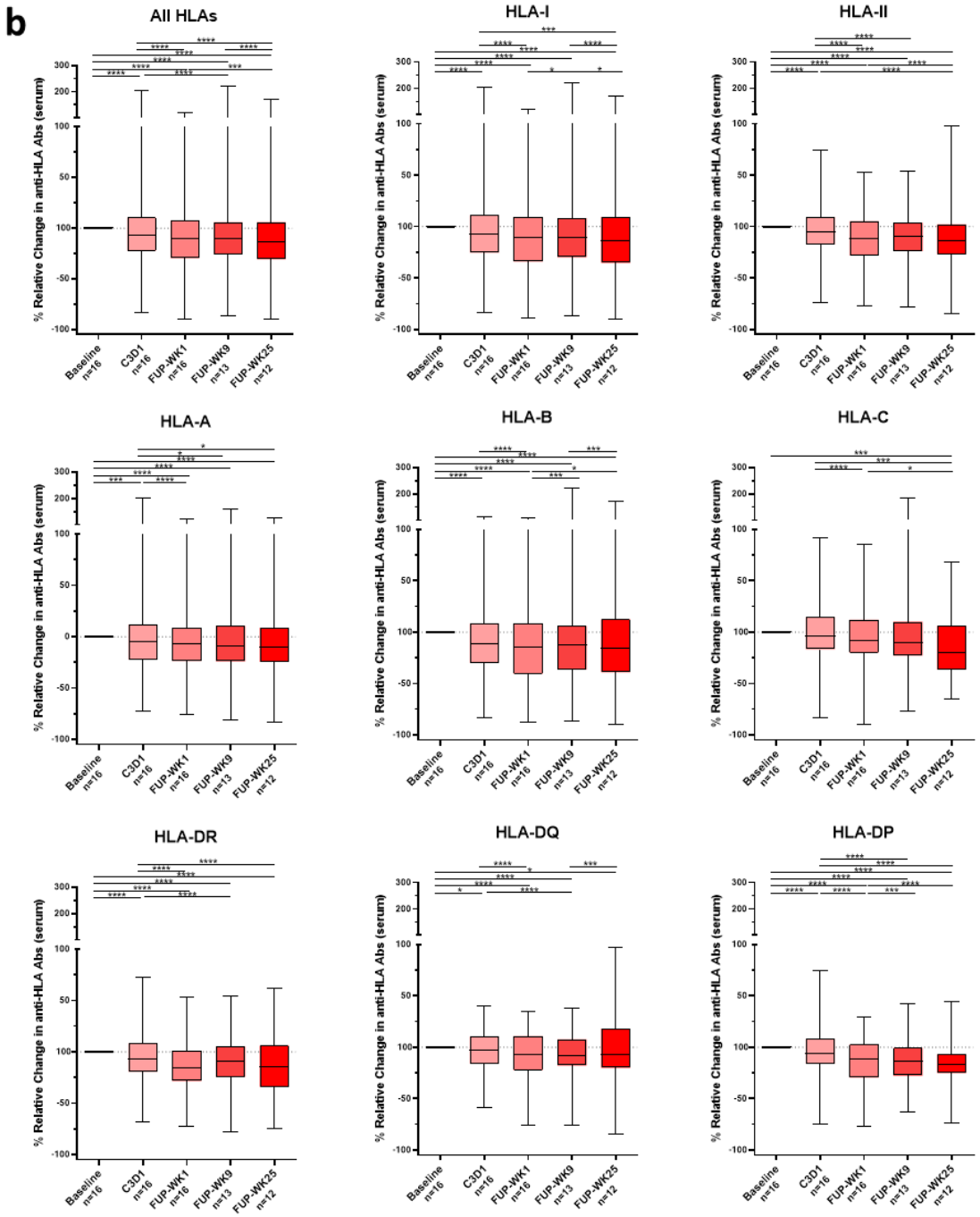

Figure 16 *(Continued)*. b) Changes (% relative change) in serum anti-HLA antibodies during desensitization until 25 weeks after end of treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3. Assessment of different BM-residing B and plasma cells

Assessment of BM-residing B-cell populations by spectral flow cytometry revealed a remarkable decrease in CD38+ expression as illustrated in the tSNE plots in **Figure 17a.** As shown in **Figure 17b**, cell subsets with a high CD38 expression, including PC (CD19+ CD20- CD27+ IgD- CD24- CD38+), LLPC (CD19+ CD20- CD27+ IgD- CD24- CD38+ CD138+) as well as B-cell precursors (CD19+ CD10+ CD38+) were effectively reduced after therapy. A decrease was also observed in CD38+ switched MBCs (CD19+ CD20+ IgD- CD27+ CD24var CD38+), but no significant changes were observed within the total switched MBC population (CD19+ CD20+ IgD- CD27+ CD24var).

Moreover, in vitro assessment of class I and class II HLA-specific IgG-producing BM-residing plasma cells through HLA-sp fluorospot assay showed a marked reduction after isatuximab (45 [18-87] vs 0 [0-14] of HLA-sp IgG activity at baseline and FUP-WK1, respectively, p=0.001, for all HLAs, 48 [8-99] vs 0 [0-27], p=0.018, for HLAs class I and 40 [22-82] vs 0 [0-8], p=0.013, for HLAs class II). **(Figure17c).**

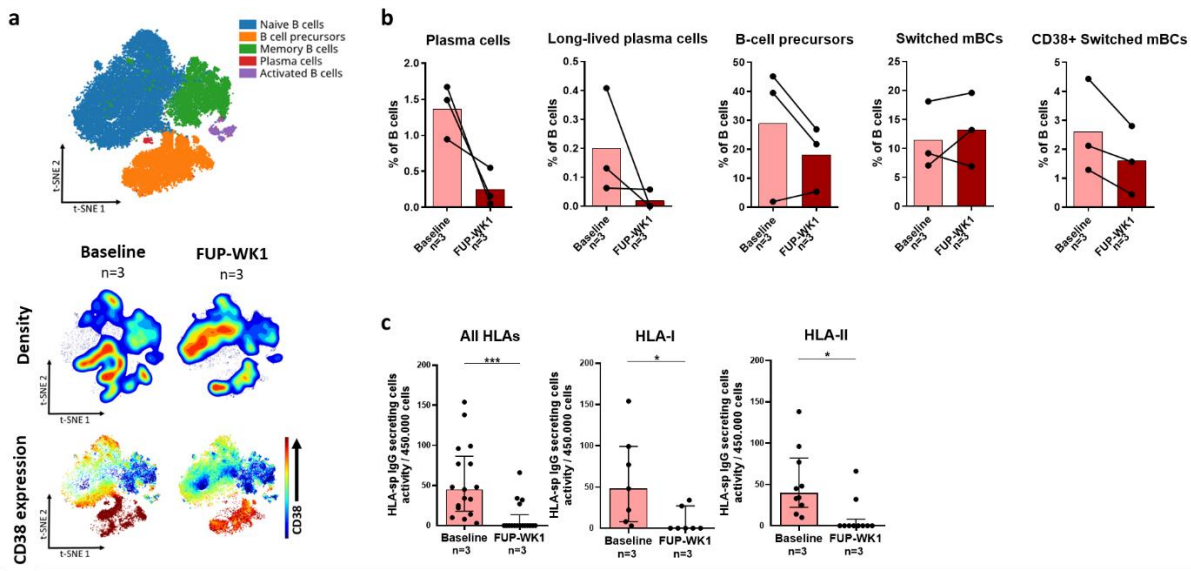

Figure 17. Effect of desensitization in the bone marrow compartment. a) T-distributed stochastic neighbor embedding (tSNE) plots displaying the major B cells subsets present in bone marrow samples. tSNE density plots and CD38 expression plots provide an overview of the changes produced by desensitization. b) Quantification of B cell populations present in bone marrow before and after isatuximab therapy. c) Activity of bone marrow IgG-secreting HLA-specific plasma cells before and after therapy. Plasma cells from 3 patients were tested against 17 HLA specificities. 7 against HLAs class I and 10 against HLAs class II through HLA-sp B-cell fluorospot. *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

4. Changes in peripheral immune cell subsets

High-dimensional spectral flow cytometry in PBMCs showed that while among main parental cell subsets, only CD16+/CD56+ NK cells were significantly decreased, a profound depletion of all those CD38+ expressing cell subsets, and especially within the CD4+ T, CD56+/CD16+ NK and CD19+ B-cell compartments was already observed after two cycles of therapy (C3D1) (**Figure 18a-b)**. Unsupervised phenograph clustering analysis on CD19+ B cells identified 15 B-cell clusters with distinct differentiation and activation phenotypes (**Figure 18c**). Out of these 15 clusters, those with high CD38 expression were markedly reduced (clusters 1, 9, 10 and 13), whereas clusters not expressing CD38 persisted unchanged after treatment (cluster 4,8,14 and 15), being clusters 1, 4 and 9 those showing significant statistical changes (**Figure 18d**). As illustrated in **Figure 18e,** while the percentage of total switched MBCs (CD19+ CD20+ CD27+ IgD- CD24var) over total B cells remained unchanged, the percentages of plasmablast (CD19+ CD20- CD24- CD27+ IgD- CD38+), transitional B cells (CD19+ CD10+) and CD38+ switched MBCs (CD19+ CD20+ CD27+ IgD- CD24var CD38+) were significantly reduced.

5. Impact of isatuximab on circulating HLA-specific memory B cell responses

HLA-sp IgG-secreting MBC responses were evaluated both at the single cell level with an HLA-sp B-cell fluorospot and evaluating the whole HLA antigen repertoire in expanded MBCs culture supernatants. Both approaches showed a strong positive correlation both at baseline ($r=0.8$, $p<0.0001$) and after treatment ($r=0.88$, $p<0.0001$) **(Figure 19).**

Globally, there was a significant decrease in both class I and class II HLA-specific IgG-producing MBC after isatuximab therapy, both when assessed at the single cell level **(Figure 20a)** and evaluated with SAB assay. (**Figure 20b, supplemental Table 11**). Concretely, 30% of HLA-sp MBCs producing anti-HLA antibodies at baseline were undetectable after treatment (**Figure 20c**).

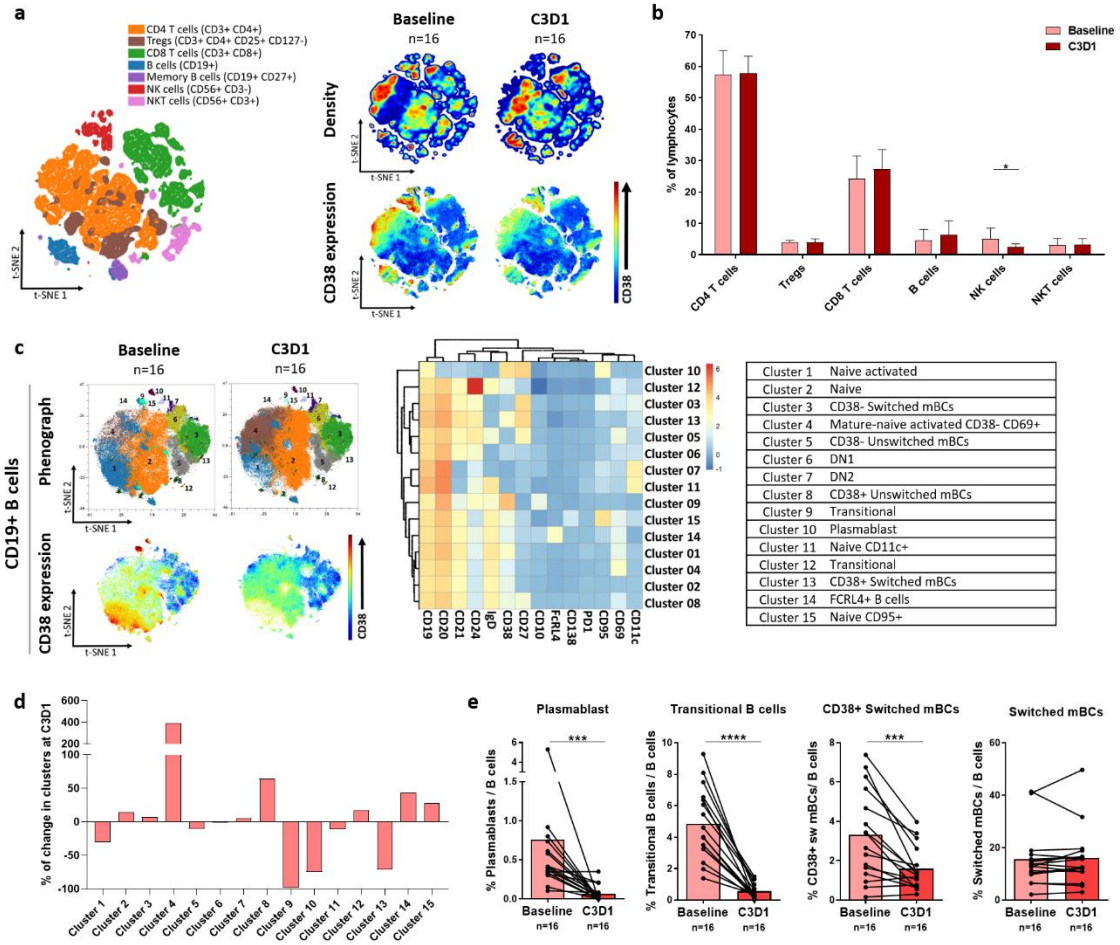

Figure 18. Effect of desensitization in peripheral immune cell populations. a) Individual t-distributed stochastic neighbor embedding (tSNE) plots displaying the major immune cell subsets present in PBMCs. tSNE density plots and CD38 expression plots provide an overview of the changes produced by desensitization and its effect on the different immune cell populations. b) Quantification of major PBMCs subsets before and after treatment; CD4 T cells (CD4+ CD3+). Tregs (CD3+ CD4+ CD25+ CD127-). CD8 T cells (CD3+ CD8+). B cells (CD19+/CD20+). NK cells (CD3- CD16+/CD56+). NKT cells (CD3+. CD16+/CD56+). *p<0.05 c) Unsupervised cluster analysis of CD19+ B cells using self-organizing maps (PhenoGraph) and consensus hierarchical clustering identified 15 different B cell clusters. CD38 expression and phenograph scatterplots displayed in tSNEs show the changes in clusters abundance at baseline and at C3D1. Clusters were identified as different B cell populations based on its expression of surface markers shown in the heatmap. d) Changes (% change) in cluster abundance at C3D1 in comparison to baseline. Abundance of cluster 1, 4 and 9 changed significantly after desensitization (p=0.0015, p=0.003 and p=0.003, respectively). e) Quantification of specific B cell populations before and after treatment. (13.3 [10-17] vs 14 [10.9-18.3] of switched MBCs at baseline and C3D1, p=0.365, 0.41 [0.32-0.7] vs 0.02 [0-0.08] of plasmablast at baseline and C3D1, respectively, p=0.001, 4.3 [3.2-6.5] vs 0.38 [0.2-0.9] of transitional B cells, p<0.0001 and 24.4 [15-34] vs 4.3 [3-7] of transitional B cells. *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

HLA-sp IgG-producing MBC responses showed a positive correlation with the respective HLA-specific antibody MFI in the serum for all HLA alleles at all time points (**figure 21a-c**). At baseline, HLA-specific MBCs were detected in all evaluated patients against different class I and class II HLA specificities (18.4% ± 13.5%) ranging from 3.4% to 39.4% in some patients. Out of all detectable class I and class II anti-HLA antigen repertoire at baseline, 64.6% specificities were only detected by antibodies in the serum, 31.6% were observed in both antibodies and MBC, whereas 3.8% were only detected in MBC (**Figure 22**). These percentages were similar for most HLA alleles, but almost half of anti-DQ responses were detected both in serum antibodies and MBC (47.6%) whereas 12% were observed only in MBC. When focusing on previously recognized mismatch HLA antigens in previous transplants, a significantly higher contribution of the MBC compartment as compared to non-repeated antigens was observed (p<0.0001 for all HLAs and HLA-I and p=0.001 for HLA-II) (**Figure 23a**). Moreover, both responses at baseline against repeated antigens were also significantly stronger than those against non-repeated HLA antigens and were not reduced after isatuximab therapy (**Figure 23b, supplemental table 12**).

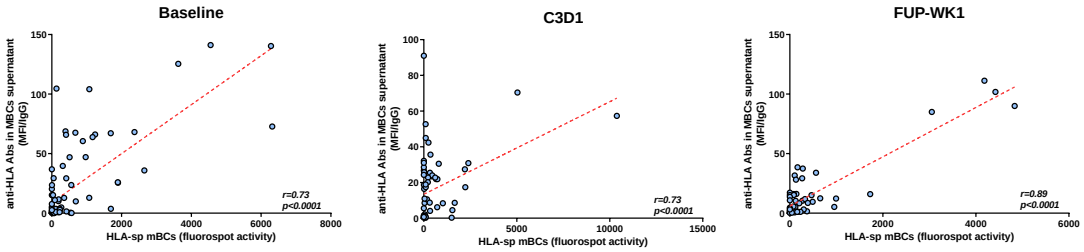

Figure 19. Correlation of HLA-sp MBCs detection techniques. Correlation between the assessment of anti-HLA antibodies in MBCs supernatants (MFI/IgG) and the evaluation of HLA-sp MBCs using HLA-sp B-cell fluorospot (fluorospot well activity)

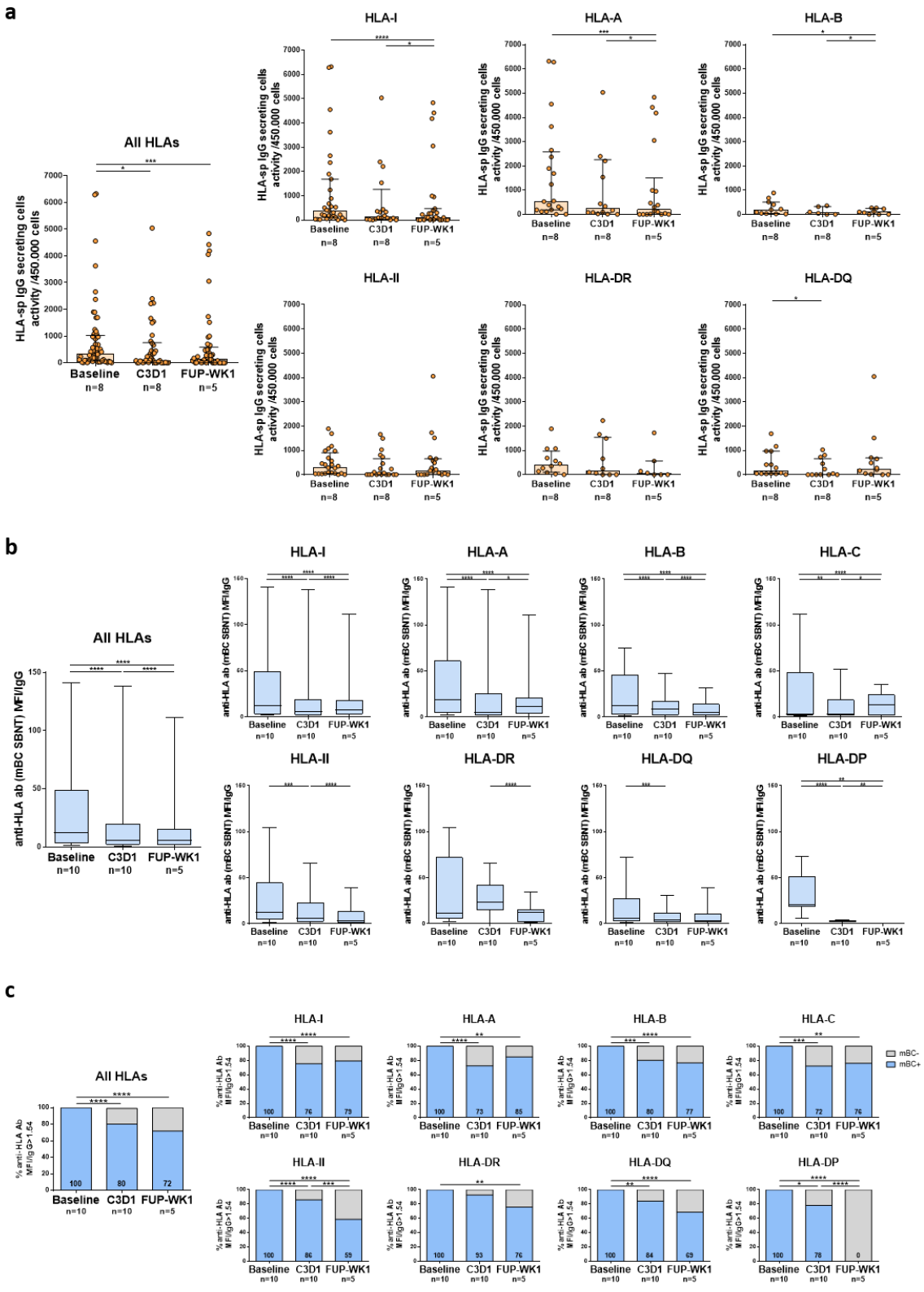

Figure 20. Effect of isatuximab on HLA-sp MBC responses. a) Evolution of HLA-sp MBCs evaluated with HLA-sp B-cell fluorospot assay (fluorospot activity). b) Evolution of anti-class I and class II anti-HLA antibodies (MFI/IgG) from MBCs over time after isatuximab therapy. c) Percentages of HLA-sp antibodies detected in MBCs supernatants before and after therapy. *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

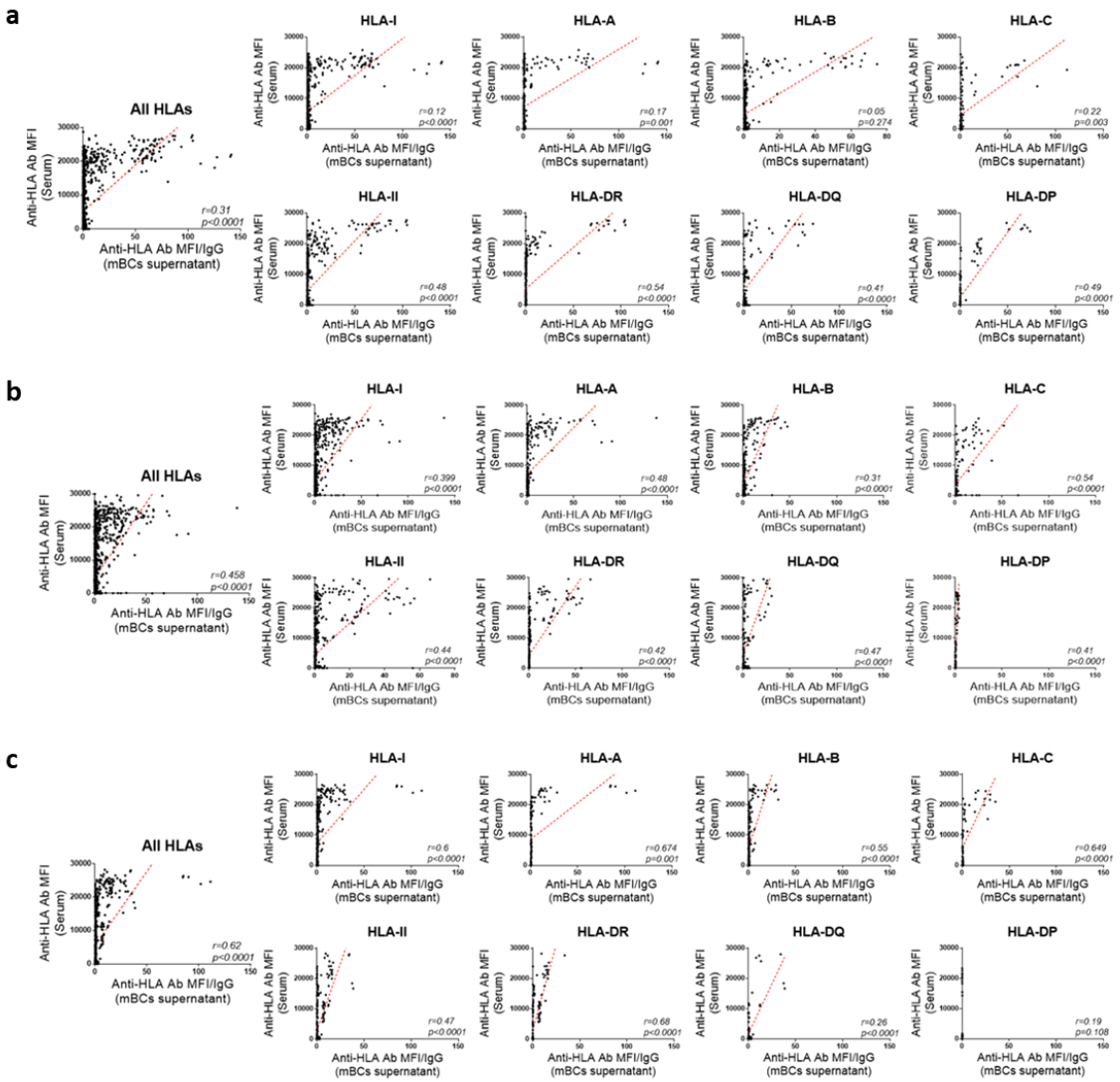

Figure 21. Correlation of serum anti-HLA antibodies and HLA-sp MBCs. Correlation of anti-HLA Abs in serum (MFI) versus antibodies produced by HLA-sp mBCs (MFI/ total IgG) in a) baseline b) C3D1 and c) FUP-WK1.

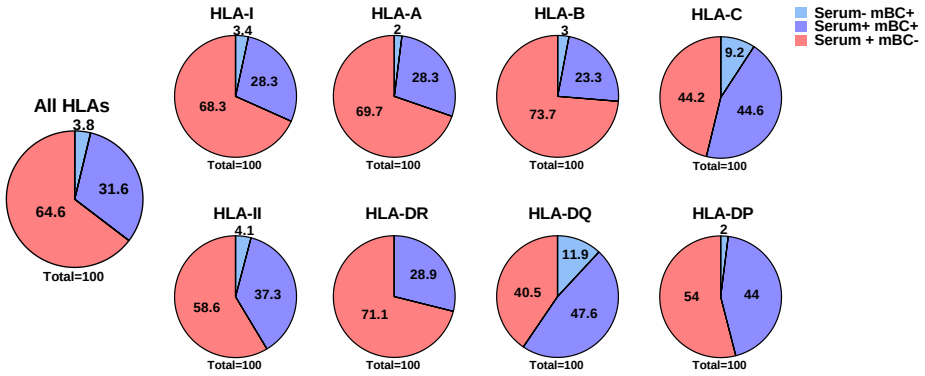

Figure 22. Contribution of the anti-HLA antigen repertoire of serum antibodies and mBC against distinct HLA antigens. Percentage of HLA specificities found both in serum and MBCs (purple), only in serum (red) or only in mBCs (blue).

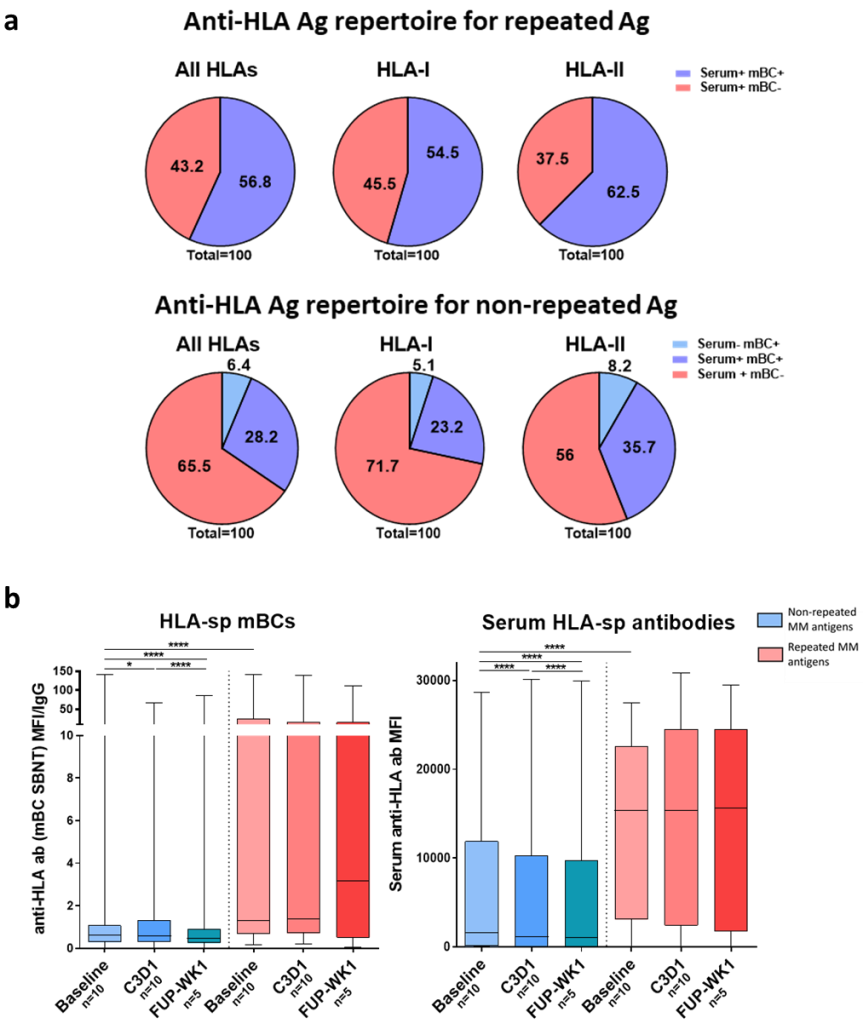

Figure 23. Responses against repeated and non-repeated HLA antigens. a) Contribution of the anti-HLA antigen repertoire of serum antibodies and MBC against repeated or non-repeated HLA mismatch antigens. b) Serum anti-HLA antibody and MBC responses against repeated HLA antigens were significantly stronger than against non-repeated HLA antigens. Moreover, anti-HLA antibody and MBC responses against non-repeated HLA antigens and were not reduced after isatuximab therapy whereas those against non-repeated mismatch antigens were significantly decreased. *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

6. Distinct patient's serological responses to isatuximab therapy

Hierarchical clustering analysis to identify different serological antibody responses to isatuximab therapy revealed three distinct patterns of response: high responders (HR, n=4), showing a significant reduction of MFI antibody levels after treatment, ranging between 34-65% of their baseline levels; low responders (LR, n=8), patients with a modest reduction of MFI Ab levels, ranging between 74-95% of their baseline values, and non-responders (NR, n=4), in whom MFI values did not change or even increased between 114-123% from baseline (**Figure 24**). As illustrated in **Figure 24c**, these

differences between the 3 groups remained unchanged at all time-points of follow-up. These three different clusters were also similarly observed when the reduction of antibody titers through serial dilutions was analyzed (**figure 25a-b**). Moreover, 3 out of 4 HR patients also showed a numerically higher reduction in their cPRA after treatment and until last follow-up, whereas none of NR and only 2 LR patients showed any reduction on their cPRA (f**igures 25c**). No major clinical, demographic or immunological differences were observed between the three groups, but HR patients had previously been transplanted more times than NR (**Table 6).**

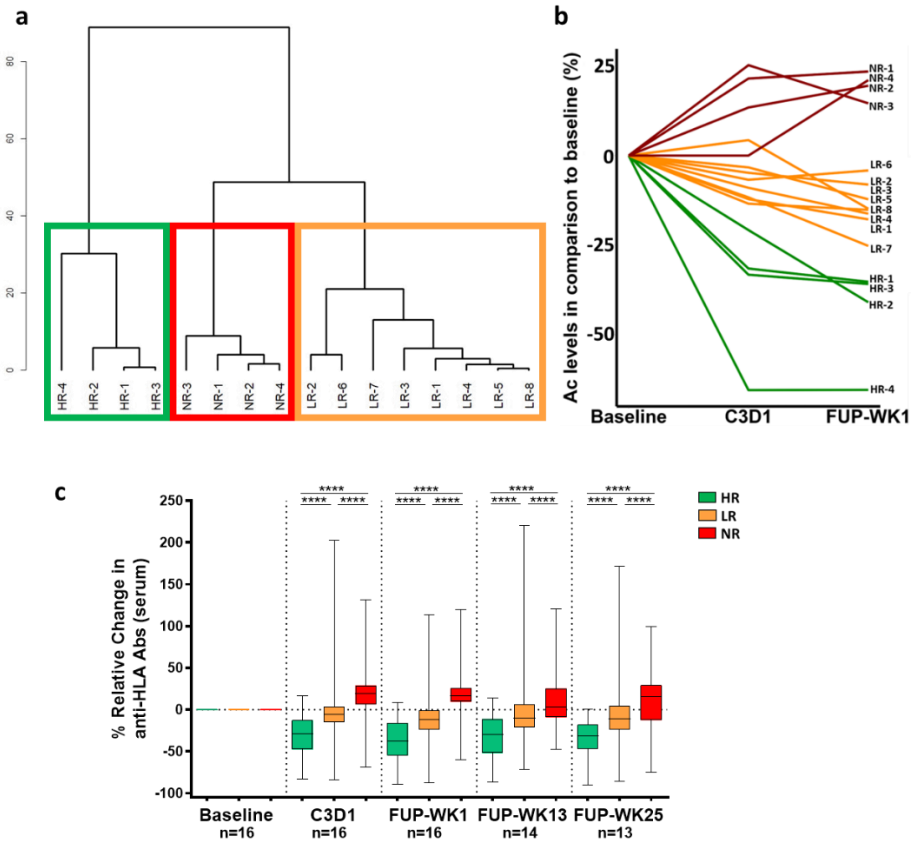

Figure 24. Patterns of response to desensitization treatment according to reduction of HLA-sp Ab. a) Dendrogram representing clustering of patients in three response groups according to the Euclidean distance between them based on its change in levels (MFI) of anti-HLA antibodies one week after treatment. b) Mean of anti-HLA Ab relative change (%) per patient at C3D1 and FUP-WK1. c) Evolution of HLA-sp Abs (% relative change) at C3D1 and at weeks 1, 13 and 25 of follow-up (65 [44-84], 57 [38-81], 64 [43-82] and 65 [48-81] % of reduction in MFI from baseline at C3D1, at FUP-WK1, at FUP-WK13 and at FUP-WK25, respectively, for HR patients, 93 [81-104], 87 [71-99], 94 [81-119] and 88 [72-104] for LR patients and 118 [104-129], 118 [108-127], 108 [76-125] and 113 [83-130] in NR individuals, p<0.0001 between all groups.) *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

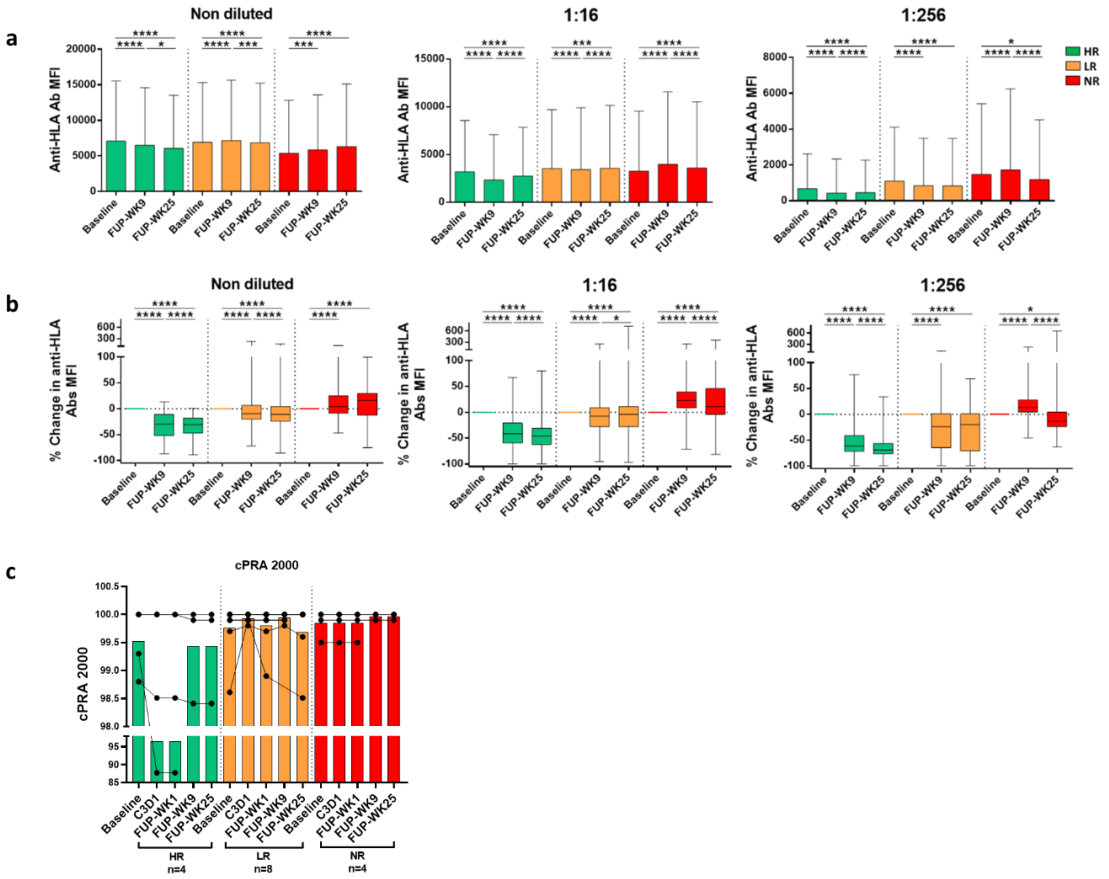

Figure 25. Evolution of serum anti-HLA abs after treatment. a) Levels (MFI) of anti-HLA abs in serum in non-diluted, diluted 1:16 and diluted 1:256 serum samples at baseline, FUP-WK9 and FUP-WK25. b) Percentage of relative change in positive serum anti-HLA abs (MFI>2000) in non-diluted, diluted 1:16 and diluted 1:256 serum samples at baseline, FUP-WK9 and FUP-25. c) cPRA considering only positive (MFI>2000) abs at baseline, C3D1 and 1, 9 and 25 weeks after the last dose. *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

Table 6. Demographics and clinical characteristics of high, low and non-responders

	High responders (HR)	Low responders (LR)	Non responders (NR)	
Highly sensitized patients (n=16)	***n=4***	***n=8***	***n=4***	*P-value*
Age, yr, mean ± SD	41.5 ± 12	47.8 ± 14.1	47.3 ± 7.3	0.701
Sex, Female, n (%)	1 (25)	2 (25)	0 (0)	0.54
Dialysis time, yr, mean ± SD	5.7 ± 1.8	6.8 ± 4.4	7.6 ± 4	0.784
Waitlist time, yr, mean ± SD	3.8 ± 2.2	5.61 ± 3	7.35 ± 4	0.299
Origin of renal insufficiency, n (%)				
Glomerular disease	0 (0)	1 (12.5)	1 (25)	
Urologic disorders	1 (25)	0 (0)	0 (0)	
Hypertension	1 (25)	0 (0)	0 (0)	0.336
Diabetes Mellitus	0 (0)	0 (0)	1 (25)	
Failure of previous transplant	1 (25)	2 (25)	1 (25)	
Other	1 (25)	5 (62.5)	1 (25)	
Prior kidney transplants, n, mean ± SD	2 ± 0.8[a]	1.25 ± 0.5	1[a]	0.06[a]
Prior sensitizing events (%)				
Transplant	3 (75)	6 (75)	3 (75)	0.597
Transplant/Transfusion	1 (25)	1 (12.5)	1 (25)	
Uknown		1 (12.5)		
Baseline cPRA, mean ± SD	99.86 ± 0.17	99.78 ± 0.57	99.81 ± 0.24	0.373

Abbreviations: cPRA, calculated Panel Reactive Antibody; SD, standard deviation

[a]Significant differences between HR and NR

7. Cellular and functional immune patterns predicting serological responses to therapy

Aiming at interrogating whether the three distinct patient groups of serological responses (HR, LR and NR) could be discriminated prior to desensitization therapy, we performed PLS-DA analysis using the baseline abundances of B-cell clusters assessed by spectral flow cytometry. A clear stratification between HR and both LR and NR was observed (**figure 26a**). As shown in **Figure 26b**, 7 specific B-cell clusters (12, 13, 8, 2, 14, 15) correlated with HR patients whereas 8 other different B-cell subsets (1, 10, 9, 11, 15, 7, 6, 5, 3) correlated with LR and/or NR patients**.** Notably, while clusters 5 ($CD38^{neg}$ unswitched mBc) and 6 (double-negative B cells) tended to be lower among HR than in LR and NR (1.8 [0.85-6.4] vs 6.2 [3.4-9.4] vs 7.3 [5.5-8.2] in HR, LR and NR patients, respectively, p=0.11 for HR vs LR and p=0.2 for HR vs NR, for cluster 5; and 3 [2.1-7] vs 6.3 [5.1-7.8] vs 8.7 [5.2-12.1] in HR, LR and NR patients, respectively, p=0.15 for HR vs LR and p=0.11 for HR vs NR, for cluster 6), cluster 3 ($CD38^{neg}$ switched mBc) was statistically significantly lower in HR patients as compared to the other 2 suboptimal responder groups (SoR: NR and LR) (5.9 [2.4-9.7] vs 14 [12.3-16.9] vs 16.6 [11.8-30.4], in HR, LR and NR, respectively, p=0.008 for HR vs LR and p=0.03 for HR vs NR) (**figure 26c**).

Immune-functional analysis of circulating HLA-specific IgG-producing mBc revealed that SoR patients displayed significantly higher frequencies of HLA-specific IgG-producing mBCs than HR patients (3 [2.6-4.3] vs [10.3 [2.9-44] vs 16.9 [4-56.6] in HR, LR and NR patients, respectively, p=0.0001 for HR vs LR, p<0.0001 for HR vs NR and p=0.02 for LR vs NR patients) (**Figure 26d**). Furthermore, HR displayed significantly less proportion of positive HLA-specific mBc than SoR (4% vs 21% vs 26% in HR, LR and NR, respectively, p<0.0001 for HR vs LR and HR vs NR and p=0.103 for LR vs NR) (**Figure 26e**). A ROC curve analysis found that 15.6% of circulating switched mBc over total CD19+ B cells discriminated HR from SoR with high accuracy (AUC 0.958, 0.860-1.000, p=0.009) (**Figure 27a)**,

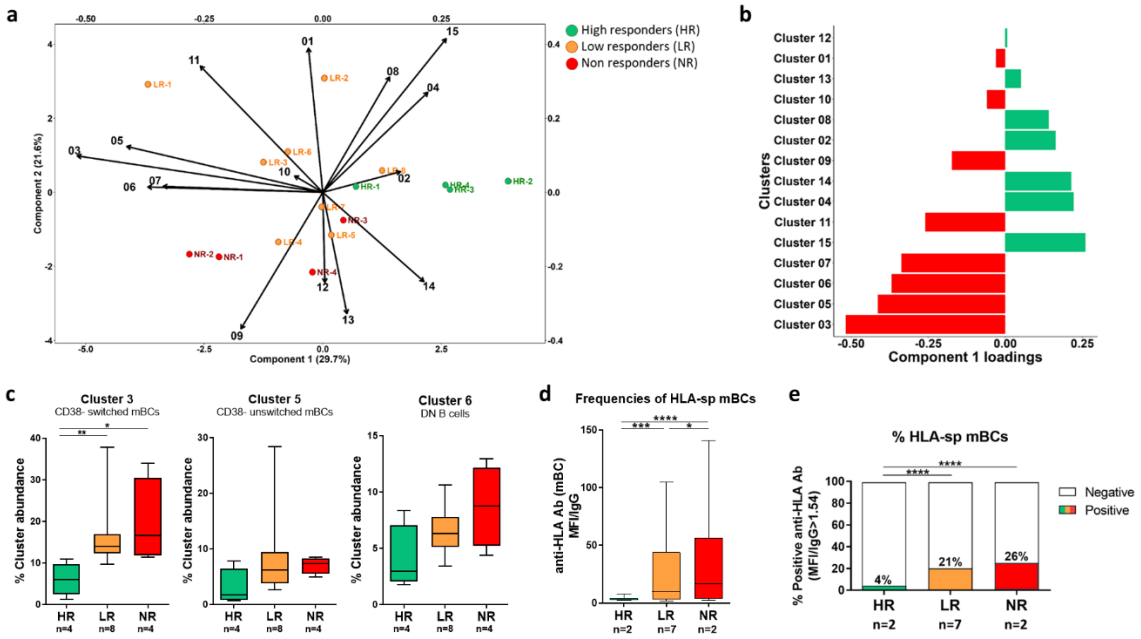

Figure 26. Phenotypical and functional characterization of response to treatment groups at baseline. a) Multiway partial least squares discriminant analysis (N-PLS-DA) plot. N-PLS-DA was used to identify features that could discriminate between HR (green), LR (orange) and NR (red) patients. The PLS-DA scores plot shows the clear separation between groups using baseline abundances of the 15 B cell clusters. As shown, component 1 is able to discriminate HR from LR and NR patients. b) Chord diagram representing the weight of each cluster in component 1 loading. c) Boxplot of clusters 3, 5 and 6 frequencies at baseline in HR, LR and NR patients. d) Frequencies of HLA-specific IgG-producing mBCs at baseline in the 3 groups. e) Percentage of HLA-specific IgG-producing mBCs at baseline in the 3 different groups. *p<0.05,**p<0.01, ***p<0.001, ****p<0.0001.

8. Validation of phenotypic and functional immune patterns of response to therapy in an independent external cohort of highly sensitized patients treated with the anti-CD38 mAb daratumumab.

We next sought to confirm our findings in an independent, external cohort of 10 HS patients enrolled in the desensitization trial with the anti-CD38 mAb daratumumab (NCT04204980). As shown in **table 7**, there were no major statistical differences regarding main clinical, demographic and immunological characteristics between the derivation and validation cohorts, but there were more women in the validation cohort. Based on the cellular discrimination threshold found in derivation cohort discriminating responder patients from SoR (**Figure 27a**), 3/10 patients showed low levels of $CD38^{neg}$ switched MBC and were classified as HR, whereas 7/10 displayed high cell percentage and were classified as SoR (**Figure 27b)**. When changes in anti-HLA antibody MFI values

after daratumumab therapy were analyzed according to these two groups, responder patients showed a significantly higher reduction of anti-HLA antibody MFI levels than SoR patients (-65.6 [-77.9—22] % reduction in responders vs -34.6 [-57.6—10.3] % reduction in SoR, p<0.0001) (**Figure 27c**). The reduction of cPRA levels after therapy was more pronounced in responders than in SoR patients (**figure 27d**). Notably, baseline frequencies of HLA-specific IgG-producing MBCs were also significantly lower in responder patients than in the SoR group (11.4 [2.6-13.7] vs 19.7 [16-689.5], p<0.0001) (**Figure 27e**).

Table 7. Demographics and clinical characteristics of the derivation and validation cohort.

Characteristics	Discovery *n=16*	Validation *n=10*	*P-value*
Age, yr, mean ± SD	46,1 ± 11,8	48,7 ± 12,3	p=0.844
Sex, Female, n (%)	3 (18,8)	6 (60)	p=0.042
Dialysis time, yr, mean ± SD	6,7 ± 3,6	6,5 ± 2,7	p=0.614
Waitlist time, yr, mean ± SD	5,6 ± 3,2	6, ± 2,7	p=0.881
Origin of renal insufficiency, n (%)			
Glomerular disease	2 (12,5)	0 (0)	p=0.126
Urologic disorders	1 (6,3)	0 (0)	
Hypertension	1 (6,3)	0 (0)	
Diabetes Mellitus	1 (6,3)	0 (0)	
Failure of previous transplant	4 (25)	0 (0)	
Other	7 (43,8)	10 (100)	
Prior kidney transplants, n, mean ± SD	1,37 ± 0,6	1,1 ± 0,3	p=0.363
Prior sensitizing events (%)			
Transplant	11 (68,8)	3 (30)	p=0.066
Transplant/Transfusion	4 (25)	3 (30)	
Pregnancy	0 (0)	0 (0)	
Transplant/Pregnancy	0 (0)	3 (30)	
Transplant/Pregnancy/Transfusion	0 (0)	1	
Uknown	1 (6,3)	0 (0)	
cPRA 2000, mean ± SD	99,7 ± 0,45	95,6 ± 8	p=0.150

Abbreviations: cPRA, calculated Panel Reactive Antibody; SD, standard deviation

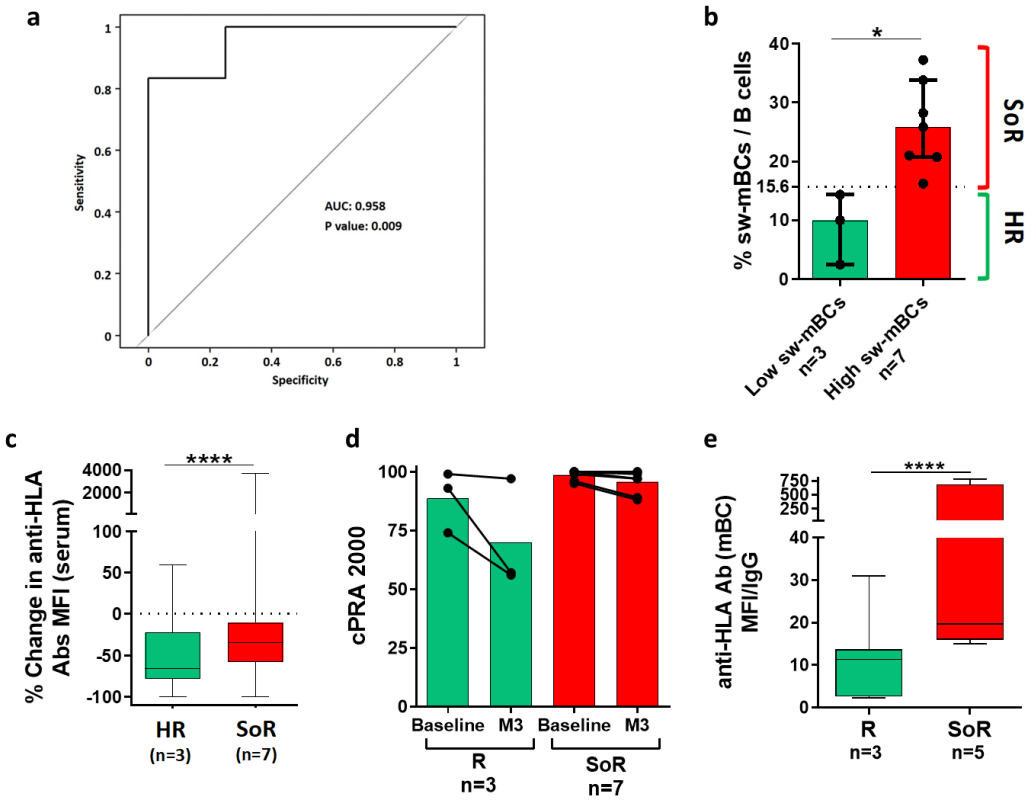

Figure 27. Validation of switched MBCs frequencies predicting response to treatment. a) ROC curve analysis of the baseline switched MBC frequencies predicting response to treatment in the discovery cohort (n=16). A frequency of 15.6% of switched MBCs over total B cells was shown as the most accurate cut-off value discriminating HR patients at baseline (AUC=0.958; CI 0.860-1.000, p=0.009). b) Flow cytometry assessment of % of sw-MBCs (CD19+ CD20+ CD27+ IgD-) and determination of responder (R) and non-responder (NR) groups. c) Relative change in serum anti-HLA Abs 3 months after the first dose of treatment (-65.6 [-77.9—22] % reduction in R group vs-34.6 [-57.6—10.3] % reduction in NR group, p<0.0001). d) Levels of cPRA 2000 in R and NR patients at baseline and after treatment. e) Functional analysis of HLA-sp MBC frequencies. Anti-HLA Abs in MBC supernatants were evaluated through SAB assay and MFI values were normalized by total IgG of each sample.

9. Proportion of highly sensitized kidney transplant candidates displaying the biological signature of successful response to anti-CD38 mAb

To identify potential responders to anti-CD38 mAb desensitization therapy among kidney transplant candidates on the waiting list, we performed flow cytometry phenotyping on 29 patients from Vall d'Hebron University Hospital and Assistance Publique - Hôpitaux de Paris. We evaluated 19 highly sensitized patients (cPRA > 85%) and 20 non-sensitized controls (cPRA = 0%). No significant differences were observed in the abundances of B-cells (CD19+) or switched MBCs (CD19+ CD27+ IgD-) between highly sensitized and non-

sensitized transplant candidates (**Figure 28a**). Among the 19 highly sensitized patients analyzed, 10 (53%) exhibited frequencies of MBCs below the defined threshold, categorizing them as potential responders to anti-CD38 therapy, while 9 (47%) had high frequencies of these cells in circulation, indicating they may be suboptimal responders (**Figure 28b**).

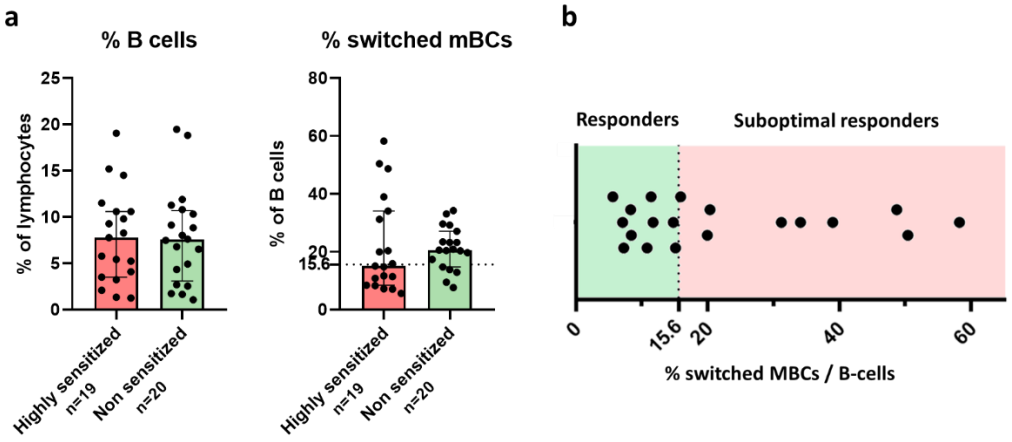

Figure 28. Phenotyping of transplant candidates on the waiting list for kidney transplantation. a) B cells (CD19+) and switched MBCs (CD19+ IgD- CD27+) frequencies of highly sensitized and non sensitized kidney transplant candidates assessed by flow cytometry. b)) distribution of highly sensitized kidney transplant candidate's switched MBCs frequencies to determine their possible response to anti-CD38 treatment.

V. DISCUSSION

1. Discussion

Preformed humoral memory against HLAs is a significant barrier for successful kidney transplantation. Sensitization events, such as previous transplants, transfusions, or pregnancies, can activate an immune response, predominantly driven by alloreactive MBCs and plasma cells producing anti-HLA antibodies, ultimately leading to allograft rejection and accelerated graft loss.

Highly sensitized patients, typically defined as those with a calculated panel reactive antibody (cPRA) of ≥80.00%, constitute an increasing proportion of kidney transplant candidates within the waiting lists worldwide. Despite the implementation of different organ allocation policies, such as national prioritization programs, the acceptable mismatch program and living-donor kidney-pair exchange programs, access to transplantation remains challenging for some (very) highly sensitized patients, particularly those with the highest sensitization rates (cPRA>99.90%) or uncommon HLA types. Indeed, only 6.5% of highly sensitized patients with cPRA ≥80.00% receive a compatible kidney transplant each year, and almost none of these patients have a cPRA ≥99.9%[87]. Consequently, a large proportion of those transplant candidates spend long periods of time on dialysis with poorer quality of life and reduced life expectancy compared to transplant recipients.

Currently, there is no standardized desensitization regimen for kidney transplant candidates and most treatments are based on off-label therapies based on either removing circulating antibodies or targeting those immune cells directly involved in antibody production such B cells and plasma cells. Unfortunately, many of these approaches show limited efficacy in consistently reducing anti-HLA antibodies and frequently result in antibody rebound, primarily due to their incomplete depletion of antibody-producing plasma cells. Therefore, there is an urgent need for novel desensitization strategies to address this clinical challenge.

Anti-CD38 mAbs have shown high efficacy in depleting myeloma plasma cells and achieving high remission rates in patients with multiple myeloma. Consequently, they represent a unique opportunity for eliminating HLA antibodies as desensitization

therapy in kidney transplant candidates. While some few studies and clinical case reports are available on the use of CD38 antibodies for desensitization, they have demonstrated various levels of reduction in DSAs, indicating their potential in this context.

The work comprehending this doctoral thesis has fundamentally focused in various goals related to the clinical and biological impact of novel CD38-targeting mAb anti-HLA B-cell responses in highly sensitized kidney transplant candidates in the waiting list; more specifically, we have thoroughly assessed i) the safety, pharmacokinetics and clinical desensitization effect of the novel anti-CD38 mAb isatuximab facilitating access to transplantation of highly sensitized transplant candidates participating in a clinical trial, ii) its impact reducing the quantity, quality and types of serum anti-HLA alloantibodies, iii) the biological effect on peripheral blood and BM-residing B-cell subsets and specifically on functionally active HLA-specific MBC, and v) assess the biological basis of different treatment responses to anti-CD28 mab differentiating patients with significant reduction of anti-HLA antibodies from those with very weak or no response at all to help personalize the use of these new therapies to successfully desensitize highly sensitized transplant candidates and ultimately facilitate their access to HLA compatible kidney transplantation.

In the first study, we designed a clinical trial to investigate whether the anti-CD38 therapy, isatuximab, had the potential to be a new effective and safe desensitization therapy, addressing a therapeutic gap left by currently available regimens.

The trial consisted of two patient cohorts, representing patients who could benefit from desensitization. Patients in cohort A represented the largest proportion of patients under the US *kidney allocation system* (KAS) and the Spanish *Programa de Acceso al trasplante para pacientes hiperimmunizados* (PATHI) within the 100% cPRA category, with a significantly lower transplant rate and who are unlikely to undergo transplantation within a reasonable timeframe. Cohort B patients were those with cPRA 80.0%–99.89% who also receive prioritization allocation points in both allocation systems. Despite this, the median cPRA in cohort B was similar to cohort A. Baseline characteristics showed that patients were on the waiting list for a kidney transplant for years (median 5.3 years,

range 0.6–12.9) despite the recent advances in the field, demonstrating that there is still significant unmet clinical need under the current kidney allocation system.

We first evaluated the safety and tolerability of isatuximab monotherapy in highly sensitized transplant recipients, together with the Pharmacokinetic evaluation by measuring the elimination of nonspecific IgG and IgM.

Isatuximab monotherapy was well tolerated and showed a good safety profile in kidney transplant candidates, with a grade 1–2 infusion reaction rate of approximately 21%. No treatment-related infections were reported throughout the study, although the risk of hypogammaglobulinemia has been raised as a concern with anti-CD38 therapy due to CD38 being expressed in normal PC, and as seen with the nonspecific elimination of IgG and IgM[227,228]. In this regard, a comprehensive population PK analysis in patients with relapsed/refractory multiple myeloma did not identify any effect of renal impairment on isatuximab PK[229]. In this study, where renal function was even worse due to majority of patients being on dialysis, isatuximab PK exposure was comparable with those from other studies[230–232]. Overall, our results complement the previous analyses mentioned above, showing no effect of renal impairment on isatuximab PK exposure. However, these data were expected as isatuximab is a mAb, and thus a large molecule, and is eliminated by catabolism.

Three specific desensitization efficacy criteria were stablished to evaluate a clinically meaningful response to treatment, considering cPRA reduction, likelihood to receive an organ offer and also, the elimination of anti-HLA antibodies with an MFI higher than 3000MFI. Notably, isatuximab demonstrated a durable decrease in anti-HLA antibodies, which appeared to be persistent during site visit follow-up period after stopping treatment (approximately 26 weeks after the last dose). Isatuximab also demonstrated partial desensitization activity by eliminating or lowering the titer of some antibodies, with minimal effect on the overall cPRA values. Nevertheless, Schinstock et al. show that a mild reduction in cPRA to 99.50%–99.89% may drastically increase the probability of transplant on the based on the current KAS[103], and the same data was recently published related to the PATHI Spanish prioritization program[233]. The minimal decrease in cPRA values is unsurprising as most broadly sensitized patients often have high titers of HLA

antibody. As demonstrated in this study, desensitization activity cannot be reflected through cPRA values alone. Examining antibody titer reduction across the entire anti-HLA antibody profile of each patient provides a better assessment of desensitization efficacy. Therefore, a composite end point that included antibody elimination, titer reduction, and cPRA reduction proposed in this study may be more suitable for assessing desensitization therapies. The proposed criteria account for and minimize potential assay variability by incorporating multiple measures of antibody response, allowing a more informed assessment of treatment efficacy. For example, in criterion 3, only a reduction of antibodies to MFI <2000 from a baseline of >3000 would be considered as antibody elimination on the basis of a potential 25% assay variability[44]. Most importantly, by study cutoff (median follow-up of 68 weeks), six patients received transplant offers, of which four were accepted.

We next explored the impact of isatuximab on main peripheral cellular subsets using conventional flow cytometry to assess with a first high-level overview the immune modulation effect of isatuximab at baseline, day 1 of cycle 3 and until 17 weeks after treatment. As described, only a mild decrease on CD3-CD56+ NK cells was observed, but no changes in other major cell compartments such as total CD19+ B cells, CD4+ and CD8+ T cells or CD4+CD25+CD127- regulatory T cells (Tregs). Prior publications investigating daratumumab hypothesized anti-CD38 treatment decreases Treg cells, increasing the risk for TCMR. In rhesus macaques with two sequential mismatched skin allografts desensitized with daratumumab and plerixafor before transplant, DSA levels were significantly reduced but this reduction was not maintained as all recipients showed a rapid rebound of antibodies, experienced T-cell–mediated rejection (TCMR), and developed rejection within 30 days of transplantation[218]. Jordan et al. reported a case of a patient treated with daratumumab for standard-of-care resistant ABMR resolved with minimal AEs, with significant reductions in circulating HLA class I and reductions in HLA class II, but the patient developed TCMR[215]. In our study, no notable changes implicated in transplant rejection were observed among T cells. Sufficient data are lacking to suggest whether the risk of TCMR was increased after treatment with isatuximab. However, no TCMR was observed in the transplanted patients treated with isatuximab as of study cut-off date. One patient transplanted approximately 15 months after the last

dose of isatuximab (after study cut-off date) experienced acute rejection that was successfully treated with plasmapheresis and intravenous Ig, where the pathologic diagnosis includes mixed acute ABMR and TCMR due to the presence of interstitial infiltrate and tubulitis in addition to severe peritubular capillaritis with diffuse C4d staining.

The pharmacodynamic effect of isatuximab was also investigated in BM-resident B-cell subsets by phenotypical and functional anti-HLA antibody production. Using spectral flow cytometry, we found an intense depletion of plasmablasts and LLPCs, along with other B-cell subsets with high CD38 expression such as B-cell precursors and CD38+ class-switched MBCs. This results paralleled the functional abrogation of class I and class II HLA-sp IgG-producing plasma cell frequencies, similarly to what has been reported in multiple myeloma patients[195,234]. A more in-depth evaluation of B-cell populations was also performed in peripheral blood using spectral flow cytometry and unsupervised clustering. Among the 15 different B-cell clusters detected by phenograph, those with CD38-expressing B cells subsets, including plasmablast, PC, transitional B cells as well as CD38+ MBC were reduced after treatment, while global class-switched MBC remained unchanged. The reduction of CD38+ MBC may account for the decrease in IgG-secreting HLA-sp B cells assessed with functional assays, highlighting the role of CD38-expressing MBCs in anti-HLA antibody production. In fact, it is well-known that besides BM-residing HLA-sp plasma cells, other cellular compartments such as peripheral MBCs directly contribute to the global burden of HLA sensitization[52,54].

In this regard, we comprehensively analyzed the complete HLA-antigen repertoire of IgG-producing MBC in a functional manner and found that while most serum anti-HLA antibodies are produced by BM-residing long-LLPC, 30% are concomitantly released by circulating MBC and up to 5-10% are exclusively produced by the MBC compartment, in line with previous studies that showed the detection of HLA-sp MBCs without the presence of the correspondent circulating anti-HLA antibody[54]. Interestingly, both antibodies and MBC specific against repeated HLA mismatch antigens showed the highest baseline levels and were more resistant to therapy. These data underscore that sensitization due to previous transplants leads to a strong and long-lasting sensitization,

mostly generated after a persistent exposure to donor HLA antigens, leading to prolonged germinal center reactions producing high levels of HLA-sp MBC and PCs[15,235]. While CD38 expression in germinal-center MBC is generally low[31,33], and the efficacy of anti-CD38 mAb seems to rely on CD38 receptor surface density[234], it has also been reported the capacity of these therapies to directly decrease B-cell proliferation, differentiation, and IgG production in vitro, especially among class-switched MBC[197].

However, the effect of isatuximab on the reduction or elimination of antibody levels showed high interpatient variability. Indeed, hierarchical clustering analysis based on the changes on MFI anti-HLA antibody reduction levels between last isatuximab dose and baseline showed 3 clearly different patient groups: high-responders (HR), showing a high decrease in anti-HLA antibodies (ranging between 34-65% of their baseline levels), low-responders (LR), with a week serological response (ranging between 74-95% of their baseline values), or non-responders (NR), in whom MFI values did not change or even increased between 14-23% from baseline. Notably, these different groups remained unchanged until the last follow-up period, up to 25 weeks post-final dose. Furthermore, these three different clusters were also similarly observed when analyzing changes of antibody titers through serial dilutions and reductions in their cPRA values.

Interestingly, no main clinical, immunological or demographic characteristics could differentiate between these 3 groups prior to therapy, although high responders had received previous kidney transplant more often than NR patients. While this association may seem counter-intuitive, it could be argued that HLA antigens from previous grafts only account for approximately 5% of anti-HLA Abs among highly sensitized patients thus, isatuximab does reduce the vast majority of serum anti-HLA antibodies.

Considering this different effect of isatuximab between highly sensitized patients together with the important role of the peripheral MBC compartment contributing to anti-HLA antibody production, we hypothesized that differences between responder and non-responder patients could rely on the presence and abundance of specific peripheral B-cell immune phenotypes related to antibody production. As shown, a PLS-DA analysis evaluating baseline B-cell cluster abundances and changes on anti-HLA antibody responses could successfully discriminated HR patients from suboptimal responders

(SoR), this is, both LR and NR patients. Notably, three main B-cell clusters that most distinguished between the two groups were those with very low CD38 expression, which were significantly more abundant among SoR as compared to HR patients. Notwithstanding, the cluster with the highest weight in the PLS-DA analysis distribution corresponded to CD38- MBC, especially CD38- class-switched MBCs. Furthermore, when we evaluated functional responses of IgG-secreting HLA-sp MBC at baseline among these 3 groups, SoR (LR and NR) patients displayed significantly higher frequencies than HR. These data strongly suggest that while a subset of MBC display high CD38 expression and are consequently targeted by isatuximab, there are some other MBC subsets that do not express or display very low CD38 levels and retain the ability to secrete HLA-specific antibodies despite this therapy.

In order to obtain a cellular frequency cut-off enabling discriminating highly sensitized patients susceptible to respond to anti-CD38 mAb, we performed a ROC curve analysis. Interestingly, we found a precise threshold of the percentage of class-switched MBC over total CD19+ B cells (15.6%) differentiating between HR and SoR patients with high sensitivity and specificity (AUC 0.958, p=0.009). Subsequently, we aimed to validate these findings in an external, independent cohort of highly sensitized French kidney transplant candidates participating in desensitization clinical trial with the anti-CD38 mAb daratumumab (NCT04204980). Here, 3/10 patients were classified as HR based on the low percentage of circulating class-switched MBC, and showed significantly higher serological responses by means of reduction of both anti-HLA antibody MFI levels and cPRA values than those classified as SoR. In addition, HR patients did also show significantly lower HLA-specific IgG-producing MBC than non-responders.

Finally, in view of these data we were also interested in analyzing the prevalence of this cellular-based signature of successful response to anti-CD38 mAb in highly sensitized patients (cPRA>99%) from two different waiting lists for kidney transplantation at 2 different transplant centers, at Vall d'Hebron University Hospital (Barcelona, Spain) and at the Groupe Hospitalo-Universitaire Chenevier Mondor (Paris, France), and we found that half (53%) of the evaluated patients were classified as responders to this treatment

and could eventually benefit from successful desensitization therapy with anti-CD38 mAbs.

2. Limitations of this work

The studies that comprise this thesis have some limitations that are worthwhile to mention.

First, we analyzed a relatively low number of patients in these two studies. However, all patients evaluated perfectly illustrated the profile of the highly sensitized participating in two distinct clinical trials, from geographically different areas (US, EU) and found comparable results. Furthermore, while we evaluated patients receiving two different anti-CD38 mAbs with distinct epitopes and mechanisms of action, the comparable findings observed in both groups, strongly suggests similar pharmacodynamics, ultimately leading to antibody reduction.

We also acknowledge that patients in the two clinical trials received a limited number of doses of anti-CD38 mAb and were subsequently followed up for 26 weeks on anti-HLA antibody level and immune cell profiling. The short treatment period and long follow-up duration design intended to explore the temporal mechanisms of antibody rebound if it occurred. A desired desensitization treatment regimen with isatuximab may then be adjusted based on the observed data. Indeed, patients could be screened for the presence of the cellular signature of response to therapy and complete a first round of desensitization treatment with these agents and then be prepared for activating the novel cPRA to local or national histocompatibility labs to eventually receive HLA-compatible organ offers. Moreover, due to its well-tolerated safety profile, we cannot exclude the possibility that additional serial doses of anti-CD38 mAb could further reduced the anti-HLA antibody burden and provide an even longer durable responses or that some of the so-called non-responders could even become high-responder after a longer course of this therapy.

Finally, this study is a biomarker-based mechanistic study investigating whether anti-CD38 mAb monotherapy can durably decrease titers of anti-HLA antibody. Although

there were patients transplanted during the study, these cannot be clearly attributed to isatuximab treatment, which needs to be tested in a randomized controlled study. However, whereas transplantability may seem to be a clinically meaningful measure of direct clinical benefit, there are intrinsic biases associated with patient selection, donor availability, different organ allocation policies across countries and regions, skill and aggressiveness of a transplant center, and difference in crossmatch positivity cut-off across transplant centers. It would also be challenging to test this hypothesis in a randomized controlled study in patients waiting for a deceased donor using transplant rate as the primary end point due to confounding factors, such as donor availability, varying criteria on donor crossmatch, and inconsistent use of desensitization regimens across transplant centers.

3. Next steps and future directions on HLA desensitization

Our study has allowed to describe the safety, pharmacokinetics, pharmacodynamics as well as finding a predictive cell-based biological marker of successful desensitization response to two anti-CD38 mAb, isatuximab and daratumumab in highly sensitized kidney transplant candidates in the waiting list. Most importantly, based on the strategies followed in these two trials using specific doses of anti-CD38 mAb, patients could now be selected to receive this therapy in a personalized manner and benefit of anti-HLA antibody reduction to ultimately facilitate access to HLA-compatible transplantation, while avoid its use in patients that most likely not respond to this treatment.

However, these therapies could be further investigated as an option for successful desensitization following distinct strategies; i) it could be used as adjunct therapy to existing desensitization therapy for patients on the kidney transplant waiting list to provide better durable responses due to its well-tolerated safety profile, moreover ii) as anyi-CD38 mAb target PC and does not deplete serum anti-HLA antibodies, time is needed for the desensitization effect to be observed, considering the half-life of Immunoglobulins thus, combining it with an initial session of plasmapheresis or imlifidase could be an effective strategy to achieve fast and durable desensitization, and iii) since it seems that anti-CD38 mAb efficacy in desensitization is challenged by high

frequencies of HLA-sp class-switched MBC, in patients with high levels of these cells, anti-CD38 mAb could be considered in combination with other immunosuppressive agents that more selectively target MBC or GC B-cell compartments such as anti-CD20 and anti-IL6 mAb, or alternatively with costimulation blockers such as CTLA4Ig/belatacept and anti-CD40 or anti-CD40L mAb to prevent the further differentiation of MBCs to antibody-secreting PC. In fact, combining anti-CD38 mAb with belatacept are currently being investigated in two desensitization clinical trials (NCT05145296; NCT04827979).

Finally, since CD38 is a highly attractive target for many antibody-producing PC-driven diseases other than anti-HLA desensitization, the findings of this work could be explored to evaluate the role of circulating antigen-specific MBC in different clinical settings such as in transplant patients developing acute or chronic ABMR, as well as in patients developing autoreactive B-cell mediated immune diseases.

VI. CONCLUSIONS

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- The anti-CD38 mAb isatuximab used as monotherapy for anti-HLA desensitization in highly sensitized kidney transplant candidates was well tolerated and showed an optimal safety profile, with manageable infusion reactions and no significant treatment-related infections.

- Isatuximab reduced anti-HLA antibody MFI and titers and showed a partial desensitization activity, opening the door to some patients to receive a transplant offer and in some of undergoing kidney transplantation. Noteworthy, an important interpatient variability of serological response to therapy was observed.

- Isatuximab effectively depleted functionally active BM-residing CD38-expressing B-cell subsets, including plasmablasts and LLPCs, which are main producers of serum anti-HLA antibodies.

- Isatuximab did not impact on global circulating CD4+/CD8+ T cells, Tregs and B-cell numbers, but significantly depleted from peripheral blood total NK cells and specific CD38-expressing B-cell subsets such as transitional B cells, plasmablast, PC and class-switched-MBCs.

- While isatuximab treatment did not impact on total circulating class-switched MBC numbers, it reduced the global burden of HLA-specific IgG-producing MBCs frequencies.

- The proportion of HLA-specific MBC frequencies are significantly higher against HLA antigens harbored in previous kidney allografts than against other non-repeated HLA antigens, and seem to be less sensitive to depletion with isatuximab therapy

  Specific circulating memory B-cell phenotypes, particularly CD38- class-switched MBC subset prior to isatuximab desensitization monotherapy, successfully distinguished between highly sensitized patients displaying a relevant reduction of anti-HLA antibodies (responders) from others that barely or not responded at

all (sub-optimal responder). These findings were confirmed in an external, independent cohort of highly sensitized patients receiving anti-CD38 desensitization monotherapy with daratumumab.

- Assessing HLA-specific class-switched memory B-cell frequencies in highly sensitized kidney transplant candidates could help identifying patients susceptible to successfully respond to anti-CD38 desensitization therapies, thereby potentially improving treatment outcomes by implementing personalized desensitization therapy decision-making.

- Continuous investigation is warranted to further optimize dosing regimens and explore different combination therapies, preferably through randomized controlled trials, eventually embedded with biomarker guided strategies, to ultimately confirm these encouraging results.

VII. REFERENCES

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1. Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med*. 1999;341(23):1725-1730. doi:10.1056/NEJM199912023412303

2. Fergie R, Maxwell AP, Cunningham EL. Latest advances in frailty in kidney transplantation: A narrative review. *Transplant Rev Orlando Fla*. 2024;38(2):100833. doi:10.1016/j.trre.2024.100833

3. Venkataraman S, Kendrick J. Barriers to kidney transplantation in ESKD. *Semin Dial*. 2020;33(6):523-532. doi:10.1111/sdi.12921

4. Wadström J, Ericzon BG, Halloran PF, et al. Advancing Transplantation: New Questions, New Possibilities in Kidney and Liver Transplantation. *Transplantation*. 2017;101(2):S1. doi:10.1097/TP.0000000000001563

5. Coemans M, Süsal C, Döhler B, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int*. 2018;94(5):964-973. doi:10.1016/j.kint.2018.05.018

6. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2018 Annual Data Report: Kidney. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2020;20 Suppl s1:20-130. doi:10.1111/ajt.15672

7. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2012;12(5):1157-1167. doi:10.1111/j.1600-6143.2012.04013.x

8. Heidt S, Claas FHJ. Transplantation in highly sensitized patients: challenges and recommendations. *Expert Rev Clin Immunol*. 2018;14(8):673-679. doi:10.1080/1744666X.2018.1498335

9. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol*. 2012;8(6):348-357. doi:10.1038/nrneph.2012.81

10. M M Kenneth, Casey W. *Janeway's Immunobiology: Ninth International Student Edition*. W.W. Norton & Company; 2016.

11. HLA Nomenclature @ hla.alleles.org. https://hla.alleles.org/alleles/index.html

12. Ting JPY, Baldwin AS. Regulation of MHC gene expression. *Curr Opin Immunol*. 1993;5(1):8-16. doi:10.1016/0952-7915(93)90074-3

13. Hasnain SZ, Lourie R, Das I, Chen AC, McGuckin MA. The interplay between endoplasmic reticulum stress and inflammation. *Immunol Cell Biol*. 2012;90(3):260-270. doi:10.1038/icb.2011.112

14. Callemeyn J, Lamarthée B, Koenig A, Koshy P, Thaunat O, Naesens M. Allorecognition and the spectrum of kidney transplant rejection. *Kidney Int*. 2022;101(4):692-710. doi:10.1016/j.kint.2021.11.029

15. Abbes S, Metjian A, Gray A, et al. HLA sensitization in solid organ transplantation: a primer on terminology, testing, and clinical significance for the apheresis practitioner. *Ther Apher Dial Off Peer-Rev J Int Soc Apher Jpn Soc Apher Jpn Soc Dial Ther*. 2017;21(5):441-450. doi:10.1111/1744-9987.12570

16. Torija A, Favà A, Meneghini M, Crespo E, Bestard O. Novel insights into the pathobiology of humoral alloimmune memory in kidney transplantation. *Curr Opin Organ Transplant*. 2020;25(1):15. doi:10.1097/MOT.0000000000000717

17. Benichou G, Gonzalez B, Marino J, Ayasoufi K, Valujskikh A. Role of Memory T Cells in Allograft Rejection and Tolerance. *Front Immunol*. 2017;8. doi:10.3389/fimmu.2017.00170

18. Bestard O, Crespo E, Stein M, et al. Cross-validation of IFN-γ Elispot assay for measuring alloreactive memory/effector T cell responses in renal transplant recipients. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2013;13(7):1880-1890. doi:10.1111/ajt.12285

19. Espinosa JR, Samy KP, Kirk AD. Memory T cells in organ transplantation: progress and challenges. *Nat Rev Nephrol*. 2016;12(6):339-347. doi:10.1038/nrneph.2016.9

20. Kwak K, Akkaya M, Pierce SK. B cell signaling in context. *Nat Immunol*. 2019;20(8):963-969. doi:10.1038/s41590-019-0427-9

21. Foster WS, Lee JL, Thakur N, et al. Tfh cells and the germinal center are required for memory B cell formation & humoral immunity after ChAdOx1 nCoV-19 vaccination. *Cell Rep Med*. 2022;3(12):100845. doi:10.1016/j.xcrm.2022.100845

22. Corcoran LM, Tarlinton DM. Regulation of germinal center responses, memory B cells and plasma cell formation—an update. *Curr Opin Immunol*. 2016;39:59-67. doi:10.1016/j.coi.2015.12.008

23. Akkaya M, Kwak K, Pierce SK. B cell memory: building two walls of protection against pathogens. *Nat Rev Immunol*. 2020;20(4):229-238. doi:10.1038/s41577-019-0244-2

24. Moran I, Nguyen A, Khoo WH, et al. Memory B cells are reactivated in subcapsular proliferative foci of lymph nodes. *Nat Commun*. 2018;9(1):3372. doi:10.1038/s41467-018-05772-7

25. Tangye SG, Tarlinton DM. Memory B cells: effectors of long-lived immune responses. *Eur J Immunol*. 2009;39(8):2065-2075. doi:10.1002/eji.200939531

26. Palm AKE, Henry C. Remembrance of Things Past: Long-Term B Cell Memory After Infection and Vaccination. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.01787

27. Chu VT, Berek C. The establishment of the plasma cell survival niche in the bone marrow. *Immunol Rev*. 2013;251(1):177-188. doi:10.1111/imr.12011

28. Niiro H, Clark EA. Regulation of B-cell fate by antigen-receptor signals. *Nat Rev Immunol*. 2002;2(12):945-956. doi:10.1038/nri955

29. Noelle RJ, Erickson LD. Determinations of B cell fate in immunity and autoimmunity. *Curr Dir Autoimmun*. 2005;8:1-24. doi:10.1159/000082084

30. Zuccarino-Catania GV, Sadanand S, Weisel FJ, et al. CD80 and PD-L2 define functionally distinct memory B cell subsets that are independent of antibody isotype. *Nat Immunol*. 2014;15(7):631-637. doi:10.1038/ni.2914

31. Sanz I, Wei C, Jenks SA, et al. Challenges and Opportunities for Consistent Classification of Human B Cell and Plasma Cell Populations. *Front Immunol*. 2019;10:2458. doi:10.3389/fimmu.2019.02458

32. Kaminski DA, Wei C, Qian Y, Rosenberg AF, Sanz I. Advances in human B cell phenotypic profiling. *Front Immunol*. 2012;3:302. doi:10.3389/fimmu.2012.00302

33. Ridderstad A, Tarlinton DM. Kinetics of establishing the memory B cell population as revealed by CD38 expression. *J Immunol Baltim Md 1950*. 1998;160(10):4688-4695.

34. Fuggle SV, Martin S. Tools for human leukocyte antigen antibody detection and their application to transplanting sensitized patients. *Transplantation*. 2008;86(3):384-390. doi:10.1097/TP.0b013e31817c90f5

35. Monteiro F, Rodrigues H, Kalil J, et al. Pre- and Posttransplant Monitoring of Alloantibodies by Complement-Dependent Cytotoxicity and Luminex Methodologies in Liver Transplantation. *Transplant Proc*. 2012;44(8):2411-2412. doi:10.1016/j.transproceed.2012.07.005

36. Tait BD, Süsal C, Gebel HM, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation*. 2013;95(1):19-47. doi:10.1097/TP.0b013e31827a19cc

37. Hönger G, Krähenbühl N, Dimeloe S, Stern M, Schaub S, Hess C. Inter-individual differences in HLA expression can impact the CDC crossmatch. *Tissue Antigens*. 2015;85(4):260-266. doi:10.1111/tan.12537

38. Tait BD, Hudson F, Cantwell L, et al. Review article: Luminex technology for HLA antibody detection in organ transplantation. *Nephrol Carlton Vic*. 2009;14(2):247-254. doi:10.1111/j.1440-1797.2008.01074.x

39. Zachary AA, Vega RM, Lucas DP, Leffell MS. HLA antibody detection and characterization by solid phase immunoassays: methods and pitfalls. *Methods Mol Biol Clifton NJ*. 2012;882:289-308. doi:10.1007/978-1-61779-842-9_17

40. Tang MY, Wang QH, Wang J, Gao X, Wu L, Tan JM. Strength of donor-specific antibodies with the use of Luminex single-antigen beads is a reliable predictor of acute rejection in living-relative kidney recipients. *Transplant Proc*. 2015;47(2):309-312. doi:10.1016/j.transproceed.2014.11.039

41. Pei R, Lee J how, Chen T, Rojo S, Terasaki PI. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. *Hum Immunol*. 1999;60(12):1293-1302. doi:10.1016/S0198-8859(99)00121-4

42. Cecka JM. Calculated PRA (CPRA): The New Measure of Sensitization for Transplant Candidates. *Am J Transplant*. 2010;10(1):26-29. doi:10.1111/j.1600-6143.2009.02927.x

43. Leffell MS, Cherikh WS, Land G, Zachary AA. Improved definition of human leukocyte antigen frequencies among minorities and applicability to estimates of transplant compatibility. *Transplantation*. 2007;83(7):964-972. doi:10.1097/01.tp.0000258588.09356.d7

44. Reed EF, Rao P, Zhang Z, et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2013;13(7):1859-1870. doi:10.1111/ajt.12287

45. Koefoed-Nielsen P, Møller BK. Donor-specific anti-HLA antibodies by solid phase immunoassays: advantages and technical concerns. *Int Rev Immunol*. 2019;38(3):95-105. doi:10.1080/08830185.2018.1525367

46. Tambur AR, Campbell P, Claas FH, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2018;18(7):1604-1614. doi:10.1111/ajt.14752

47. Tambur AR, Bestard O, Campbell P, et al. Sensitization in transplantation: Assessment of Risk 2022 Working Group Meeting Report. *Am J Transplant*. 2023;23(1):133-149. doi:10.1016/j.ajt.2022.11.009

48. Ouranos K, Panteli M, Petasis G, et al. Complement and Non-Complement Binding Anti-HLA Antibodies Are Differentially Detected with Different Antigen Bead Assays in Renal Transplant Recipients. *J Clin Med*. 2023;12(24):7733. doi:10.3390/jcm12247733

49. Llorente S, Boix F, Eguia J, et al. C1q-Fixing Human Leukocyte Antigen Assay in Immunized Renal Patients: Correlation Between Luminex SAB-C1q and SAB-IgG. *Transplant Proc*. 2012;44(9):2535-2537. doi:10.1016/j.transproceed.2012.09.084

50. Wong ZE, Downing J, De Santis D, et al. C3d-binding assay for the detection of complement activating HLA antibodies: A useful tool for allocation to highly sensitised recipients in the post-CDC era? *HLA*. 2023;102(1):13-27. doi:10.1111/tan.15010

51. Lan JH, Gjertson D, Zheng Y, et al. Clinical utility of complement-dependent C3d assay in kidney recipients presenting with late allograft dysfunction. *Am J Transplant*. 2018;18(12):2934-2944. doi:10.1111/ajt.14871

52. Lúcia M, Luque S, Crespo E, et al. Preformed circulating HLA-specific memory B cells predict high risk of humoral rejection in kidney transplantation. *Kidney Int*. 2015;88(4):874-887. doi:10.1038/ki.2015.205

53. Crotty S, Ahmed R. Immunological memory in humans. *Semin Immunol*. 2004;16(3):197-203. doi:10.1016/j.smim.2004.02.008

54. Luque S, Lúcia M, Melilli E, et al. Value of monitoring circulating donor-reactive memory B cells to characterize antibody-mediated rejection after kidney transplantation. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2019;19(2):368-380. doi:10.1111/ajt.15055

55. Kervella D, Torija A, Zúñiga JM, Bestard O. How to measure human leukocyte antigen-specific B cells. *Curr Opin Organ Transplant*. 2023;28(5):345-354. doi:10.1097/MOT.0000000000001097

56. Luque S, Lúcia M, Crespo E, Jarque M, Grinyó JM, Bestard O. A multicolour HLA-specific B-cell FluoroSpot assay to functionally track circulating HLA-specific memory B cells. *J Immunol Methods*. 2018;462:23-33. doi:10.1016/j.jim.2018.07.011

57. Saletti G, Çuburu N, Yang JS, Dey A, Czerkinsky C. Enzyme-linked immunospot assays for direct ex vivo measurement of vaccine-induced human humoral immune responses in blood. *Nat Protoc*. 2013;8(6):1073-1087. doi:10.1038/nprot.2013.058

58. Czerkinsky CC, Nilsson LA, Nygren H, Ouchterlony O, Tarkowski A. A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. *J Immunol Methods*. 1983;65(1-2):109-121. doi:10.1016/0022-1759(83)90308-3

59. Walsh PN, Friedrich DP, Williams JA, et al. Optimization and qualification of a memory B-cell ELISpot for the detection of vaccine-induced memory responses in HIV vaccine trials. *J Immunol Methods*. 2013;394(1):84-93. doi:10.1016/j.jim.2013.05.007

60. Pannus P, Depickère S, Kemlin D, et al. Third dose of COVID-19 mRNA vaccine closes the gap in immune response between naïve nursing home residents and healthy adults. *Vaccine*. 2023;41(17):2829-2836. doi:10.1016/j.vaccine.2023.03.047

61. Jourdan M, Caraux A, De Vos J, et al. An in vitro model of differentiation of memory B cells into plasmablasts and plasma cells including detailed phenotypic and molecular characterization. *Blood*. 2009;114(25):5173-5181. doi:10.1182/blood-2009-07-235960

62. Buisman AM, de Rond CGH, Oztürk K, Ten Hulscher HI, van Binnendijk RS. Long-term presence of memory B-cells specific for different vaccine components. *Vaccine*. 2009;28(1):179-186. doi:10.1016/j.vaccine.2009.09.102

63. Fan X, Ang A, Pollock-Barziv SM, et al. Donor-specific B-cell tolerance after ABO-incompatible infant heart transplantation. *Nat Med*. 2004;10(11):1227-1233. doi:10.1038/nm1126

64. Perry DK, Pollinger HS, Burns JM, et al. Two novel assays of alloantibody-secreting cells demonstrating resistance to desensitization with IVIG and rATG. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2008;8(1):133-143. doi:10.1111/j.1600-6143.2007.02039.x

65. Heidt S, Roelen DL, de Vaal YJH, et al. A NOVel ELISPOT assay to quantify HLA-specific B cells in HLA-immunized individuals. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2012;12(6):1469-1478. doi:10.1111/j.1600-6143.2011.03982.x

66. Karahan GE, de Vaal YJH, Roelen DL, Buchli R, Claas FHJ, Heidt S. Quantification of HLA class II-specific memory B cells in HLA-sensitized individuals. *Hum Immunol*. 2015;76(2-3):129-136. doi:10.1016/j.humimm.2015.01.014

67. Karahan GE, Krop J, Wehmeier C, et al. An Easy and Sensitive Method to Profile the Antibody Specificities of HLA-specific Memory B Cells. *Transplantation*. 2019;103(4):716-723. doi:10.1097/TP.0000000000002516

68. Snanoudj R, Claas FHJ, Heidt S, Legendre C, Chatenoud L, Candon S. Restricted specificity of peripheral alloreactive memory B cells in HLA-sensitized patients awaiting a kidney transplant. *Kidney Int*. 2015;87(6):1230-1240. doi:10.1038/ki.2014.390

69. Karahan GE, de Vaal Y, Bakker K, Roelen D, Claas FHJ, Heidt S. Comparison of different luminex single antigen bead kits for memory B cell-derived HLA antibody detection. *HLA*. 2021;98(3):200-206. doi:10.1111/tan.14356

70. Akalan H, Şirin DY, Yılmaz I, et al. Alloreactive memory B cell detection by flow cytometric cross match using polyclonally activated memory B cell culture supernatants. *Transpl Immunol*. 2022;73:101642. doi:10.1016/j.trim.2022.101642

71. Burton H, McLaughlin L, Shiu KY, et al. The phenotype of HLA-binding B cells from sensitized kidney transplant recipients correlates with clinically prognostic patterns of interferon-γ production against purified HLA proteins. *Kidney Int*. 2022;102(2):355-369. doi:10.1016/j.kint.2022.02.041

72. Song S, Kwun J, Jackson AM, Knechtle SJ, Kelsoe G. Allo-Specific Humoral Responses: New Methods for Screening Donor-Specific Antibody and Characterization of HLA-Specific Memory B Cells. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.705140

73. Kramer CSM, Franke-van Dijk MEI, Bakker KH, et al. Generation and reactivity analysis of human recombinant monoclonal antibodies directed against epitopes on HLA-DR. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2020;20(12):3341-3353. doi:10.1111/ajt.15950

74. Bezstarosti S, Kramer CSM, Franke-van Dijk MEI, et al. HLA-DQ-Specific Recombinant Human Monoclonal Antibodies Allow for In-Depth Analysis of HLA-DQ Epitopes. *Front Immunol*. 2021;12:761893. doi:10.3389/fimmu.2021.761893

75. Tambur AR, Campbell P, Chong AS, et al. Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report. *Am J Transplant*. 2020;20(10):2652-2668. doi:10.1111/ajt.15937

76. Lopes D, Barra T, Malheiro J, et al. Effect of Different Sensitization Events on HLA Alloimmunization in Kidney Transplantation Candidates. *Transplant Proc*. 2015;47(4):894-897. doi:10.1016/j.transproceed.2015.03.014

77. Montgomery RA, Tatapudi VS, Leffell MS, Zachary AA. HLA in transplantation. *Nat Rev Nephrol*. 2018;14(9):558-570. doi:10.1038/s41581-018-0039-x

78. Pandey P, Pande A, Mandal S, et al. Effects of different sensitization events on HLA alloimmunization in renal transplant cases; a retrospective observation in 1066 cases. *Transpl Immunol*. 2022;75:101680. doi:10.1016/j.trim.2022.101680

79. Ixtlapale-Carmona X, Arvizu A, De-Santiago A, et al. Graft immunologic events in deceased donor kidney transplant recipients with preformed HLA-donor specific antibodies. *Transpl Immunol*. 2018;46:8-13. doi:10.1016/j.trim.2017.09.006

80. Lefaucheur C, Viglietti D, Hidalgo LG, et al. Complement-Activating Anti-HLA Antibodies in Kidney Transplantation: Allograft Gene Expression Profiling and Response to Treatment. *J Am Soc Nephrol*. 2018;29(2):620-635. doi:10.1681/ASN.2017050589

81. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting Donor-Specific HLA Antibodies Predict Outcome in Kidney Transplantation. *J Am Soc Nephrol*. 2010;21(8):1398. doi:10.1681/ASN.2009101065

82. Ziemann M, Altermann W, Angert K, et al. Preformed Donor-Specific HLA Antibodies in Living and Deceased Donor Transplantation: A Multicenter Study. *Clin J Am Soc Nephrol*. 2019;14(7):1056. doi:10.2215/CJN.13401118

83. Kasiske BL, Skeans MA, Leighton TR, Ghimire V, Leppke SN, Israni AK. OPTN/SRTR 2011 Annual Data Report: international data. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2013;13 Suppl 1:199-225. doi:10.1111/ajt.12026

84. Bestard O, Couzi L, Crespo M, Kessaris N, Thaunat O. Stratifying the humoral risk of candidates to a solid organ transplantation: a proposal of the ENGAGE working group. *Transpl Int*. 2021;34(6):1005-1018. doi:10.1111/tri.13874

85. Han M, Rogers JA, Lavingia B, Stastny P. Peripheral blood B cells producing donor-specific HLA antibodies in vitro. *Hum Immunol*. 2009;70(1):29-34. doi:10.1016/j.humimm.2008.10.013

86. Zachary AA, Kopchaliiska D, Montgomery RA, Leffell MS. HLA-Specific B Cells: I. A Method for Their Detection, Quantification, and Isolation Using HLA Tetramers. *Transplantation*. 2007;83(7):982. doi:10.1097/01.tp.0000259017.32857.99

87. Mamode N, Bestard O, Claas F, et al. European Guideline for the Management of Kidney Transplant Patients With HLA Antibodies: By the European Society for Organ Transplantation Working Group. *Transpl Int Off J Eur Soc Organ Transplant*. 2022;35:10511. doi:10.3389/ti.2022.10511

88. Bestard O, Thaunat O, Bellini MI, et al. Alloimmune Risk Stratification for Kidney Transplant Rejection. *Transpl Int*. 2022;35:10138. doi:10.3389/ti.2022.10138

89. Biró P, Haase-Kromwijk B, Andersson T, et al. Building Kidney Exchange Programmes in Europe-An Overview of Exchange Practice and Activities. *Transplantation*. 2019;103(7):1514-1522. doi:10.1097/TP.0000000000002432

90. Biró P, van de Klundert J, Manlove D, et al. Modelling and optimisation in European Kidney Exchange Programmes. *Eur J Oper Res*. 2021;291(2):447-456. doi:10.1016/j.ejor.2019.09.006

91. Kher V, Jha PK. Paired kidney exchange transplantation - pushing the boundaries. *Transpl Int Off J Eur Soc Organ Transplant*. 2020;33(9):975-984. doi:10.1111/tri.13693

92. Chandra Shrestha P, Bhandari TR, Adhikari R, Baral H, Verma RK, Shrestha KK. Living donor kidney paired exchange: An observational study. *Ann Med Surg*. 2022;78:103761. doi:10.1016/j.amsu.2022.103761

93. Wilk AR. The Kidney Allocation System (KAS) - The First Two Years.

94. Cron DC, Husain SA, Adler JT. The New Distance-Based Kidney Allocation System: Implications for Patients, Transplant Centers, and Organ Procurement Organizations. *Curr Transplant Rep*. 2022;9(4):302-307. doi:10.1007/s40472-022-00384-z

95. Valentin M, Vega RR, Martin CC, Dominguez-Gil BB. The Spanish Prioritization System for Highly Sensitized Patients: A Successful Tool. *Transplantation*. 2018;102:S219. doi:10.1097/01.tp.0000542879.12460.06

96. Jackson KR, Covarrubias K, Holscher CM, et al. The national landscape of deceased donor kidney transplantation for the highly sensitized: Transplant rates, waitlist mortality, and posttransplant survival under KAS. *Am J Transplant*. 2019;19(4):1129-1138. doi:10.1111/ajt.15149

97. Heidt S, Haasnoot GW, van Rood JJ, Witvliet MD, Claas FHJ. Kidney allocation based on proven acceptable antigens results in superior graft survival in highly sensitized patients. *Kidney Int*. 2018;93(2):491-500. doi:10.1016/j.kint.2017.07.018

98. Heidt S, Witvliet MD, Haasnoot GW, Claas FHJ. The 25th anniversary of the Eurotransplant Acceptable Mismatch program for highly sensitized patients. *Transpl Immunol*. 2015;33(2):51-57. doi:10.1016/j.trim.2015.08.006

99. Heidt S, Haasnoot GW, Claas FHJ. How the definition of acceptable antigens and epitope analysis can facilitate transplantation of highly sensitized patients with excellent long-term graft survival. *Curr Opin Organ Transplant*. 2018;23(4):493-499. doi:10.1097/MOT.0000000000000545

100. Heidt S, Haasnoot GW, Witvliet MD, et al. Allocation to highly sensitized patients based on acceptable mismatches results in low rejection rates comparable to nonsensitized patients. *Am J Transplant*. 2019;19(10):2926-2933. doi:10.1111/ajt.15486

101. Jordan SC, Choi J, Vo A. Kidney transplantation in highly sensitized patients. *Br Med Bull*. 2015;114(1):113-125. doi:10.1093/bmb/ldv013

102. Heidt S, Haasnoot GW, van der Linden-van Oevelen MJH, Claas FHJ. Highly Sensitized Patients Are Well Served by Receiving a Compatible Organ Offer Based on Acceptable Mismatches. *Front Immunol*. 2021;12:687254. doi:10.3389/fimmu.2021.687254

103. Schinstock CA, Smith BH, Montgomery RA, et al. Managing highly sensitized renal transplant candidates in the era of kidney paired donation and the new kidney allocation system: Is there still a role for desensitization? *Clin Transplant*. 2019;33(12):e13751. doi:10.1111/ctr.13751

104. Kumar V, Locke JE. New Perspectives on Desensitization in the Current Era - An Overview. *Front Immunol*. 2021;12:696467. doi:10.3389/fimmu.2021.696467

105. Montgomery Robert A., Lonze Bonnie E., King Karen E., et al. Desensitization in HLA-Incompatible Kidney Recipients and Survival. *N Engl J Med*. 2011;365(4):318-326. doi:10.1056/NEJMoa1012376

106. Manook M, Koeser L, Ahmed Z, et al. Post-listing survival for highly sensitised patients on the UK kidney transplant waiting list: a matched cohort analysis. *Lancet Lond Engl*. 2017;389(10070):727-734. doi:10.1016/S0140-6736(16)31595-1

107. Vo A, Ammerman N, Jordan SC. Advances in desensitization for human leukocyte antigen incompatible kidney transplantation. *Curr Opin Organ Transplant*. 2024;29(2):104. doi:10.1097/MOT.0000000000001131

108. Pusey CD, Levy JB. Plasmapheresis in immunologic renal disease. *Blood Purif*. 2012;33(1-3):190-198. doi:10.1159/000334155

109. Thielke J, DeChristopher PJ, Sankary H, Oberholzer J, Testa G, Benedetti E. Highly successful living donor kidney transplantation after conversion to negative of a previously positive flow-cytometry cross-match by pretransplant plasmapheresis. *Transplant Proc*. 2005;37(2):643-644. doi:10.1016/j.transproceed.2004.12.063

110. Warren DS, Simpkins CE, Cooper M, Montgomery RA. Modulating alloimmune responses with plasmapheresis and IVIG. *Curr Drug Targets Cardiovasc Haematol Disord*. 2005;5(3):215-222. doi:10.2174/1568006054064735

111. Lorenz M, Regele H, Schillinger M, et al. Peritransplant immunoadsorption: a strategy enabling transplantation in highly sensitized crossmatch-positive cadaveric kidney allograft recipients. *Transplantation*. 2005;79(6):696-701. doi:10.1097/01.tp.0000148732.26761.fa

112. Choi AY, Manook M, Olaso D, et al. Emerging New Approaches in Desensitization: Targeted Therapies for HLA Sensitization. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.694763

113. Sibéril S, Elluru S, Negi VS, et al. Intravenous immunoglobulin in autoimmune and inflammatory diseases: More than mere transfer of antibodies. *Transfus Apher Sci*. 2007;37(1):103-107. doi:10.1016/j.transci.2007.01.012

114. Jordan SC, Vo A, Tyan D, Toyota M. Desensitization Therapy with Intravenous Gammaglobulin (IVIG): Applications in Solid Organ Transplantation. *Trans Am Clin Climatol Assoc*. 2006;117:199-211.

115. Fernández-Cruz E, Alecsandru D, Sánchez Ramón S. Mechanisms of action of immune globulin. *Clin Exp Immunol*. 2009;157 Suppl 1(Suppl 1):1-2. doi:10.1111/j.1365-2249.2009.03955.x

116. Stapleton NM, Einarsdóttir HK, Stemerding AM, Vidarsson G. The multiple facets of FcRn in immunity. *Immunol Rev*. 2015;268(1):253-268. doi:10.1111/imr.12331

117. Palsson R, Riella LV. Desensitization in transplantation: is intravenous immunoglobulin the holy grail? *J Bras Nefrol*. 2022;44(4):470. doi:10.1590/2175-8239-JBN-2022-E010en

118. Rastetter W, Molina A, White CA. Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Annu Rev Med*. 2004;55:477-503. doi:10.1146/annurev.med.55.091902.104249

119. Weiner GJ. Rituximab: mechanism of action. *Semin Hematol*. 2010;47(2):115-123. doi:10.1053/j.seminhematol.2010.01.011

120. Vo AA, Lukovsky M, Toyoda M, et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med*. 2008;359(3):242-251. doi:10.1056/NEJMoa0707894

121. Vo AA, Peng A, Toyoda M, et al. Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. *Transplantation*. 2010;89(9):1095-1102. doi:10.1097/TP.0b013e3181d21e7f

122. Vo AA, Choi J, Cisneros K, et al. Benefits of rituximab combined with intravenous immunoglobulin for desensitization in kidney transplant recipients. *Transplantation*. 2014;98(3):312-319. doi:10.1097/TP.0000000000000064

123. Jackson AM, Kraus ES, Orandi BJ, Segev DL, Montgomery RA, Zachary AA. A closer look at rituximab induction on HLA antibody rebound following HLA-incompatible kidney transplantation. *Kidney Int*. 2015;87(2):409-416. doi:10.1038/ki.2014.261

124. Redfield RR, Jordan SC, Busque S, et al. Safety, pharmacokinetics, and pharmacodynamic activity of obinutuzumab, a type 2 anti-CD20 monoclonal antibody for the desensitization of candidates for renal transplant. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2019;19(11):3035-3045. doi:10.1111/ajt.15514

125. Vieira CA, Agarwal A, Book BK, et al. Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: 1. Safety, pharmacodynamics, and pharmacokinetics. *Transplantation*. 2004;77(4):542-548. doi:10.1097/01.tp.0000112934.12622.2b

126. Genberg H, Hansson A, Wernerson A, Wennberg L, Tydén G. Pharmacodynamics of rituximab in kidney allotransplantation. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2006;6(10):2418-2428. doi:10.1111/j.1600-6143.2006.01497.x

127. Johansson BP, Shannon O, Björck L. IdeS: A Bacterial Proteolytic Enzyme with Therapeutic Potential. *PLOS ONE*. 2008;3(2):e1692. doi:10.1371/journal.pone.0001692

128. Järnum S, Bockermann R, Runström A, Winstedt L, Kjellman C. The Bacterial Enzyme IdeS Cleaves the IgG-Type of B Cell Receptor (BCR), Abolishes BCR-Mediated Cell Signaling, and Inhibits Memory B Cell Activation. *J Immunol*. 2015;195(12):5592-5601. doi:10.4049/jimmunol.1501929

129. Jordan SC, Lorant T, Choi J, et al. IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation. *N Engl J Med*. 2017;377(5):442-453. doi:10.1056/NEJMoa1612567

130. Lonze BE, Tatapudi VS, Weldon EP, et al. IdeS (Imlifidase): A Novel Agent That Cleaves Human IgG and Permits Successful Kidney Transplantation Across High-strength Donor-specific Antibody. *Ann Surg*. 2018;268(3):488. doi:10.1097/SLA.0000000000002924

131. Jordan SC, Legendre C, Desai NM, et al. Imlifidase Desensitization in Crossmatch-positive, Highly Sensitized Kidney Transplant Recipients: Results of an International Phase 2 Trial (Highdes). *Transplantation*. 2021;105(8):1808-1817. doi:10.1097/TP.0000000000003496

132. Kjellman C, Maldonado AQ, Sjöholm K, et al. Outcomes at 3 years posttransplant in imlifidase-desensitized kidney transplant patients. *Am J Transplant*. 2021;21(12):3907-3918. doi:10.1111/ajt.16754

133. Rossi JF, Lu ZY, Jourdan M, Klein B. Interleukin-6 as a therapeutic target. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2015;21(6):1248-1257. doi:10.1158/1078-0432.CCR-14-2291

134. Chavele KM, Merry E, Ehrenstein MR. Cutting edge: circulating plasmablasts induce the differentiation of human T follicular helper cells via IL-6 production. *J Immunol Baltim Md 1950*. 2015;194(6):2482-2485. doi:10.4049/jimmunol.1401190

135. Kim I, Wu G, Chai N ning, Klein AS, Jordan S. Anti-interleukin 6 receptor antibodies attenuate antibody recall responses in a mouse model of allosensitization. *Transplantation*. 2014;98(12):1262-1270. doi:10.1097/TP.0000000000000437

136. Vo AA, Choi J, Kim I, et al. A Phase I/II Trial of the Interleukin-6 Receptor-Specific Humanized Monoclonal (Tocilizumab) + Intravenous Immunoglobulin in Difficult to Desensitize Patients. *Transplantation*. 2015;99(11):2356-2363. doi:10.1097/TP.0000000000000741

137. Jouve T, Laheurte C, Noble J, et al. Immune responses following tocilizumab therapy to desensitize HLA-sensitized kidney transplant candidates. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2022;22(1):71-84. doi:10.1111/ajt.16709

138. Jouve T, Daligault M, Noble J, et al. Tocilizumab Evaluation in HLA-Desensitization before Kidney Transplantation as an Add-On Therapy to Apheresis: The TETRA Study. *J Clin Med*. 2023;12(2):424. doi:10.3390/jcm12020424

139. Mackay F, Browning JL. BAFF: A fundamental survival factor for B cells. *Nat Rev Immunol*. 2002;2(7):465-475. doi:10.1038/nri844

140. Dubey AK, Handu SS, Dubey S, Sharma P, Sharma KK, Ahmed QM. Belimumab: First targeted biological treatment for systemic lupus erythematosus. *J Pharmacol Pharmacother*. 2011;2(4):317-319. doi:10.4103/0976-500X.85930

141. Agarwal D, Luning Prak ET, Bharani T, et al. BLyS neutralization results in selective anti-HLA alloantibody depletion without successful desensitization. *Transpl Immunol*. 2021;69:101465. doi:10.1016/j.trim.2021.101465

142. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *N Engl J Med*. 2008;359(9):906-917. doi:10.1056/NEJMoa0801479

143. Kouroukis TC, Baldassarre FG, Haynes AE, Imrie K, Reece DE, Cheung MC. Bortezomib in multiple myeloma: systematic review and clinical considerations. *Curr Oncol Tor Ont*. 2014;21(4):e573-603. doi:10.3747/co.21.1798

144. Everly MJ, Everly JJ, Susskind B, et al. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation*. 2008;86(12):1754-1761. doi:10.1097/TP.0b013e318190af83

145. De Sousa-Amorim E, Revuelta I, Diekmann F, et al. Bortezomib for refractory acute antibody-mediated rejection in kidney transplant recipients: A single-centre case series. *Nephrol Carlton Vic*. 2016;21(8):700-704. doi:10.1111/nep.12659

146. Tzvetanov I, Spaggiari M, Joseph J, et al. The use of bortezomib as a rescue treatment for acute antibody-mediated rejection: report of three cases and review of literature. *Transplant Proc*. 2012;44(10):2971-2975. doi:10.1016/j.transproceed.2012.02.037

147. Woodle ES, Shields AR, Ejaz NS, et al. Prospective iterative trial of proteasome inhibitor-based desensitization. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2015;15(1):101-118. doi:10.1111/ajt.13050

148. Jeong JC, Jambaldorj E, Kwon HY, et al. Desensitization Using Bortezomib and High-dose Immunoglobulin Increases Rate of Deceased Donor Kidney Transplantation. *Medicine (Baltimore)*. 2016;95(5):e2635. doi:10.1097/MD.0000000000002635

149. Moreno Gonzales MA, Gandhi MJ, Schinstock CA, et al. 32 Doses of Bortezomib for Desensitization Is Not Well Tolerated and Is Associated With Only Modest Reductions in Anti-HLA Antibody. *Transplantation*. 2017;101(6):1222-1227. doi:10.1097/TP.0000000000001330

150. Sberro-Soussan R, Zuber J, Suberbielle-Boissel C, et al. Bortezomib as the Sole Post-Renal Transplantation Desensitization Agent Does Not Decrease Donor-Specific Anti-HLA Antibodies. *Am J Transplant*. 2010;10(3):681-686. doi:10.1111/j.1600-6143.2009.02968.x

151. Siegel DS, Martin T, Wang M, et al. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. *Blood*. 2012;120(14):2817-2825. doi:10.1182/blood-2012-05-425934

152. Tremblay S, Driscoll JJ, Rike-Shields A, et al. A prospective, iterative, adaptive trial of carfilzomib-based desensitization. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2020;20(2):411-421. doi:10.1111/ajt.15613

153. van der Zwan M, Hesselink DA, van den Hoogen MWF, Baan CC. Costimulation Blockade in Kidney Transplant Recipients. *Drugs*. 2020;80(1):33-46. doi:10.1007/s40265-019-01226-6

154. Ippoliti G, D'Armini AM, Lucioni M, Marjieh M, Viganò M. Introduction to the use of belatacept: a fusion protein for the prevention of posttransplant kidney rejection. *Biol Targets Ther*. 2012;6:355-362. doi:10.2147/BTT.S27565

155. Vincenti F, Larsen CP, Alberu J, et al. Three-year outcomes from BENEFIT, a randomized, active-controlled, parallel-group study in adult kidney transplant recipients. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2012;12(1):210-217. doi:10.1111/j.1600-6143.2011.03785.x

156. Vincenti F, Charpentier B, Vanrenterghem Y, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2010;10(3):535-546. doi:10.1111/j.1600-6143.2009.03005.x

157. Chen J, Yin H, Xu J, et al. Reversing endogenous alloreactive B cell GC responses with anti-CD154 or CTLA-4Ig. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2013;13(9):2280-2292. doi:10.1111/ajt.12350

158. Bray RA, Gebel HM, Townsend R, et al. De novo donor-specific antibodies in belatacept-treated vs cyclosporine-treated kidney-transplant recipients: Post hoc analyses of the randomized phase III BENEFIT and BENEFIT-EXT studies. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2018;18(7):1783-1789. doi:10.1111/ajt.14721

159. Vincenti Flavio, Rostaing Lionel, Grinyo Joseph, et al. Belatacept and Long-Term Outcomes in Kidney Transplantation. *N Engl J Med*. 2016;374(4):333-343. doi:10.1056/NEJMoa1506027

160. Young JS, Chen J, Miller ML, et al. Delayed Cytotoxic T Lymphocyte-Associated Protein 4-Immunoglobulin Treatment Reverses Ongoing Alloantibody Responses and Rescues Allografts From Acute Rejection. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2016;16(8):2312-2323. doi:10.1111/ajt.13761

161. Yang J, Chen J, Young JS, et al. Tracing Donor-MHC Class II Reactive B cells in Mouse Cardiac Transplantation: Delayed CTLA4-Ig Treatment Prevents Memory Alloreactive B-Cell Generation. *Transplantation*. 2016;100(8):1683-1691. doi:10.1097/TP.0000000000001253

162. Kwun J, Burghuber C, Manook M, et al. Successful desensitization with proteasome inhibition and costimulation blockade in sensitized nonhuman primates. *Blood Adv*. 2017;1(24):2115-2119. doi:10.1182/bloodadvances.2017010991

163. Burghuber CK, Manook M, Ezekian B, et al. Dual targeting: Combining costimulation blockade and bortezomib to permit kidney transplantation in sensitized recipients. *Am J Transplant*. 2019;19(3):724-736. doi:10.1111/ajt.15067

164. Ezekian B, Schroder PM, Mulvihill MS, et al. Pretransplant Desensitization with Costimulation Blockade and Proteasome Inhibitor Reduces DSA and Delays Antibody-Mediated Rejection in Highly Sensitized Nonhuman Primate Kidney Transplant Recipients. *J Am Soc Nephrol JASN*. 2019;30(12):2399-2411. doi:10.1681/ASN.2019030304

165. Schroder PM, Schmitz R, Fitch ZW, et al. Preoperative carfilzomib and lulizumab based desensitization prolongs graft survival in a sensitized non-human primate model. *Kidney Int*. 2021;99(1):161-172. doi:10.1016/j.kint.2020.08.020

166. Manook M, Olaso D, Anwar IJ, et al. Desensitization and belatacept-based maintenance therapy in pregnancy-sensitized monkeys receiving a kidney transplant. *Sci Adv*. 2023;9(20):eadg1448. doi:10.1126/sciadv.adg1448

167. Jain D, Rajab A, Young JS, et al. Reversing donor-specific antibody responses and antibody-mediated rejection with bortezomib and belatacept in mice and kidney transplant recipients. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2020;20(10):2675-2685. doi:10.1111/ajt.15881

168. Alishetti S, Farr M, Jennings D, et al. Desensitizing highly sensitized heart transplant candidates with the combination of belatacept and proteasome inhibition. *Am J Transplant*. 2020;20(12):3620-3630. doi:10.1111/ajt.16113

169. Leleu X, Martin T, Weisel K, et al. Anti-CD38 antibody therapy for patients with relapsed/refractory multiple myeloma: differential mechanisms of action and recent clinical trial outcomes. *Ann Hematol*. 2022;101(10):2123-2137. doi:10.1007/s00277-022-04917-5

170. Touzeau C, Moreau P. Daratumumab for the treatment of multiple myeloma. *Expert Opin Biol Ther*. 2017;17(7):887-893. doi:10.1080/14712598.2017.1322578

171. Giri S, Grimshaw A, Bal S, et al. Evaluation of Daratumumab for the Treatment of Multiple Myeloma in Patients With High-risk Cytogenetic Factors: A Systematic Review and Meta-analysis. *JAMA Oncol*. 2020;6(11):1759-1765. doi:10.1001/jamaoncol.2020.4338

172. Glaría E, Valledor AF. Roles of CD38 in the Immune Response to Infection. *Cells*. 2020;9(1):228. doi:10.3390/cells9010228

173. Deaglio S, Mehta K, Malavasi F. Human CD38: a (r)evolutionary story of enzymes and receptors. *Leuk Res*. 2001;25(1):1-12. doi:10.1016/s0145-2126(00)00093-x

174. Bruzzone S, Guida L, Franco L, Zocchi E, Corte G, De Flora A. Dimeric and tetrameric forms of catalytically active transmembrane CD38 in transfected HeLa cells. *FEBS Lett*. 1998;433(3):275-278. doi:10.1016/s0014-5793(98)00929-6

175. Funaro A, Horenstein AL, Calosso L, et al. Identification and characterization of an active soluble form of human CD38 in normal and pathological fluids. *Int Immunol*. 1996;8(11):1643-1650. doi:10.1093/intimm/8.11.1643

176. Malavasi F, Deaglio S, Funaro A, et al. Evolution and Function of the ADP Ribosyl Cyclase/CD38 Gene Family in Physiology and Pathology. *Physiol Rev*. 2008;88:841-886. doi:10.1152/physrev.00035.2007

177. Piedra-Quintero ZL, Wilson Z, Nava P, Guerau-de-Arellano M. CD38: An Immunomodulatory Molecule in Inflammation and Autoimmunity. *Front Immunol*. 2020;11. doi:10.3389/fimmu.2020.597959

178. Funaro A, Morra M, Calosso L, Zini MG, Ausiello CM, Malavasi F. Role of the human CD38 molecule in B cell activation and proliferation. *Tissue Antigens*. 1997;49(1):7-15. doi:10.1111/j.1399-0039.1997.tb02703.x

179. Crickx E, Weill JC, Reynaud CA, Mahévas M. Anti-CD20-mediated B-cell depletion in autoimmune diseases: successes, failures and future perspectives. *Kidney Int*. 2020;97(5):885-893. doi:10.1016/j.kint.2019.12.025

180. Funaro A, De Monte LB, Dianzani U, Forni M, Malavasi F. Human CD38 is associated to distinct molecules which mediate transmembrane signaling in different lineages. *Eur J Immunol*. 1993;23(10):2407-2411. doi:10.1002/eji.1830231005

181. Muñoz P, Mittelbrunn M, de la Fuente H, et al. Antigen-induced clustering of surface CD38 and recruitment of intracellular CD38 to the immunologic synapse. *Blood*. 2008;111(7):3653-3664. doi:10.1182/blood-2007-07-101600

182. Lund FE, Yu N, Kim KM, Reth M, Howard MC. Signaling through CD38 augments B cell antigen receptor (BCR) responses and is dependent on BCR expression. *J Immunol Baltim Md 1950*. 1996;157(4):1455-1467.

183. van de Donk NWCJ, Janmaat ML, Mutis T, et al. Monoclonal antibodies targeting CD38 in hematological malignancies and beyond. *Immunol Rev*. 2016;270(1):95-112. doi:10.1111/imr.12389

184. de Weers M, Tai YT, van der Veer MS, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. *J Immunol Baltim Md 1950*. 2011;186(3):1840-1848. doi:10.4049/jimmunol.1003032

185. Lee HT, Kim Y, Park UB, Jeong TJ, Lee SH, Heo YS. Crystal structure of CD38 in complex with daratumumab, a first-in-class anti-CD38 antibody drug for treating multiple

myeloma. *Biochem Biophys Res Commun*. 2021;536:26-31. doi:10.1016/j.bbrc.2020.12.048

186. Nijhof IS, Groen RWJ, Lokhorst HM, et al. Upregulation of CD38 expression on multiple myeloma cells by all-trans retinoic acid improves the efficacy of daratumumab. *Leukemia*. 2015;29(10):2039-2049. doi:10.1038/leu.2015.123

187. Overdijk MB, Verploegen S, Bögels M, et al. Antibody-mediated phagocytosis contributes to the anti-tumor activity of the therapeutic antibody daratumumab in lymphoma and multiple myeloma. *mAbs*. 2015;7(2):311-321. doi:10.1080/19420862.2015.1007813

188. Zhukova OV, Kovaleva TF, Arkhipova EV, Ryabov SA, Mukhina IV. Tumor-associated macrophages: Role in the pathological process of tumorigenesis and prospective therapeutic use (Review). *Biomed Rep*. 2020;13(5):47. doi:10.3892/br.2020.1354

189. Overdijk MB, Jansen JHM, Nederend M, et al. The Therapeutic CD38 Monoclonal Antibody Daratumumab Induces Programmed Cell Death via Fcγ Receptor-Mediated Cross-Linking. *J Immunol Baltim Md 1950*. 2016;197(3):807-813. doi:10.4049/jimmunol.1501351

190. Jansen JHM, Boross P, Overdijk MB, van Bueren JJL, Parren PWHI, Leusen JHW. Daratumumab, a Human CD38 Antibody Induces Apoptosis of Myeloma Tumor Cells Via Fc Receptor-Mediated Crosslinking. *Blood*. 2012;120(21):2974. doi:10.1182/blood.V120.21.2974.2974

191. Martin TG, Corzo K, Chiron M, et al. Therapeutic Opportunities with Pharmacological Inhibition of CD38 with Isatuximab. *Cells*. 2019;8(12):1522. doi:10.3390/cells8121522

192. Bisht K, Fukao T, Chiron M, et al. Immunomodulatory properties of CD38 antibodies and their effect on anticancer efficacy in multiple myeloma. *Cancer Med*. 2023;12(20):20332-20352. doi:10.1002/cam4.6619

193. Joher N, Matignon M, Grimbert P. HLA Desensitization in Solid Organ Transplantation: Anti-CD38 to Across the Immunological Barriers. *Front Immunol*. 2021;12:688301. doi:10.3389/fimmu.2021.688301

194. Krejcik J, Casneuf T, Nijhof IS, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. *Blood*. 2016;128(3):384-394. doi:10.1182/blood-2015-12-687749

195. Zhu C, Song Z, Wang A, et al. Isatuximab Acts Through Fc-Dependent, Independent, and Direct Pathways to Kill Multiple Myeloma Cells. *Front Immunol*. 2020;11:1771. doi:10.3389/fimmu.2020.01771

196. Camponeschi A, Kläsener K, Sundell T, et al. Human CD38 regulates B cell antigen receptor dynamic organization in normal and malignant B cells. *J Exp Med*. 2022;219(9):e20220201. doi:10.1084/jem.20220201

197. Verhoeven D, Grinwis L, Marsman C, et al. B-cell targeting with anti-CD38 daratumumab: implications for differentiation and memory responses. *Life Sci Alliance*. 2023;6(9):e202302214. doi:10.26508/lsa.202302214

198. Maixnerova D, El Mehdi D, Rizk DV, Zhang H, Tesar V. New Treatment Strategies for IgA Nephropathy: Targeting Plasma Cells as the Main Source of Pathogenic Antibodies. *J Clin Med*. 2022;11(10):2810. doi:10.3390/jcm11102810

199. NCATS Inxight Drugs — FELZARTAMAB. https://drugs.ncats.io/substance/3O9FA4XC02

200. Korver W, Carsillo M, Yuan J, et al. A Reduction in B, T, and Natural Killer Cells Expressing CD38 by TAK-079 Inhibits the Induction and Progression of Collagen-Induced Arthritis in Cynomolgus Monkeys. *J Pharmacol Exp Ther*. 2019;370(2):182-196. doi:10.1124/jpet.119.256602

201. NCATS Inxight Drugs — MEZAGITAMAB. https://drugs.ncats.io/substance/0XMR8L9LOL

202. Dörner T, Radbruch A, Burmester GR. B-cell-directed therapies for autoimmune disease. *Nat Rev Rheumatol*. 2009;5(8):433-441. doi:10.1038/nrrheum.2009.141

203. Hiepe F, Dörner T, Hauser AE, Hoyer BF, Mei H, Radbruch A. Long-lived autoreactive plasma cells drive persistent autoimmune inflammation. *Nat Rev Rheumatol*. 2011;7(3):170-178. doi:10.1038/nrrheum.2011.1

204. Holzer MT, Ruffer N, Huber TB, Kötter I, Ostendorf L, Krusche M. Daratumumab for autoimmune diseases: a systematic review. *RMD Open*. 2023;9(4):e003604. doi:10.1136/rmdopen-2023-003604

205. Cole S, Walsh A, Yin X, et al. Integrative analysis reveals CD38 as a therapeutic target for plasma cell-rich pre-disease and established rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Res Ther*. 2018;20(1):85. doi:10.1186/s13075-018-1578-z

206. Abadier M, Estevam J, Berg D, Fedyk ER. Mezagitamab Induces Immunomodulatory Effect in Patients with Relapsed/Refractory Multiple Myeloma (RRMM). *Blood*. 2020;136(Supplement 1):9. doi:10.1182/blood-2020-140527

207. Study Details | A Study of Mezagitamab in Adults With Primary Immunoglobulin A Nephropathy Receiving Stable Background Therapy | ClinicalTrials.gov. https://clinicaltrials.gov/study/NCT05174221

208. Al-Samkari H, Neufeld EJ. Novel therapeutics and future directions for refractory immune thrombocytopenia. *Br J Haematol*. 2023;203(1):65-78. doi:10.1111/bjh.19078

209. Study Details | A Study of TAK-079 in Adults With Persistent/Chronic Primary Immune Thrombocytopenia | ClinicalTrials.gov. https://clinicaltrials.gov/study/NCT04278924

210. McDonnell SRP, Nguyen VA, Walton NM, et al. Mezagitamab in systemic lupus erythematosus: clinical and mechanistic findings of CD38 inhibition in an autoimmune disease. *Lupus Sci Med*. 2024;11(1):e001112. doi:10.1136/lupus-2023-001112

211. Clinical Trial to Assess Efficacy and Safety of the Human Anti-CD38 Antibody Felzartamab (MOR202) in IgA Nephropathy - Full Text View - ClinicalTrials.gov. https://classic.clinicaltrials.gov/ct2/show/NCT05065970

212. Spica D, Junker T, Dickenmann M, et al. Daratumumab for Treatment of Antibody-Mediated Rejection after ABO-Incompatible Kidney Transplantation. *Case Rep Nephrol Dial*. 2019;9(3):149-157. doi:10.1159/000503951

213. Zaninoni A, Giannotta JA, Gallì A, et al. The Immunomodulatory Effect and Clinical Efficacy of Daratumumab in a Patient With Cold Agglutinin Disease. *Front Immunol*. 2021;12:649441. doi:10.3389/fimmu.2021.649441

214. Aguilera Agudo C, Gómez Bueno M, Krsnik Castello I. Daratumumab for Antibody-mediated Rejection in Heart Transplant-A Novel Therapy: Successful Treatment of Antibody-mediated Rejection. *Transplantation*. 2021;105(3):e30-e31. doi:10.1097/TP.0000000000003505

215. Doberer K, Kläger J, Gualdoni GA, et al. CD38 Antibody Daratumumab for the Treatment of Chronic Active Antibody-mediated Kidney Allograft Rejection. *Transplantation*. 2021;105(2):451-457. doi:10.1097/TP.0000000000003247

216. Mayer KA, Doberer K, Eskandary F, Halloran PF, Böhmig GA. New concepts in chronic antibody-mediated kidney allograft rejection: prevention and treatment. *Curr Opin Organ Transplant*. 2021;26(1):97-105. doi:10.1097/MOT.0000000000000832

217. Mayer Katharina A., Schrezenmeier Eva, Diebold Matthias, et al. A Randomized Phase 2 Trial of Felzartamab in Antibody-Mediated Rejection. *N Engl J Med*. 0(0). doi:10.1056/NEJMoa2400763

218. Kwun J, Matignon M, Manook M, et al. Daratumumab in Sensitized Kidney Transplantation: Potentials and Limitations of Experimental and Clinical Use. *J Am Soc Nephrol*. 2019;30(7):1206-1219. doi:10.1681/ASN.2018121254

219. Kransdorf EP, Pando MJ. Calculated panel reactive antibody with decimals: A refined metric of access to transplantation for highly sensitized candidates. *Hum Immunol*. 2017;78(3):252-256. doi:10.1016/j.humimm.2016.12.009

220. Tambur AR, Glotz D, Herrera ND, et al. Can solid phase assays be better utilized to measure efficacy of antibody removal therapies? *Hum Immunol*. 2016;77(8):624-630. doi:10.1016/j.humimm.2016.05.025

221. Levine JH, Simonds EF, Bendall SC, et al. Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. *Cell*. 2015;162(1):184-197. doi:10.1016/j.cell.2015.05.047

222. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J R Stat Soc Ser B Methodol*. 1995;57(1):289-300. doi:10.1111/j.2517-6161.1995.tb02031.x

223. Kim MJ, Ahn EY, Hwang W, et al. Association between fever pattern and clinical manifestations of adult-onset Still's disease: unbiased analysis using hierarchical clustering. *Clin Exp Rheumatol*. 2018;36(6 Suppl 115):74-79.

224. Hasan MN, Malek MB, Begum AA, Rahman M, Mollah MNH. Assessment of Drugs Toxicity and Associated Biomarker Genes Using Hierarchical Clustering. *Med Kaunas Lith*. 2019;55(8):451. doi:10.3390/medicina55080451

225. Aitchison J. The Statistical Analysis of Compositional Data. *J R Stat Soc Ser B Methodol*. 1982;44(2):139-160. doi:10.1111/j.2517-6161.1982.tb01195.x

226. R Core team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Published online 2023. https://www.R-project.org/

227. Lancman G, Sastow D, Aslanova M, et al. Effect of Intravenous Immunoglobulin on Infections in Multiple Myeloma (MM) Patients Receiving Daratumumab. *Blood*. 2020;136(Supplement 1):6-7. doi:10.1182/blood-2020-140056

228. Korst CLBM, van de Donk NWCJ. Evidence-Based Minireview: Should all newly diagnosed MM patients receive CD38 antibody–based treatment? *Hematology*. 2020;2020(1):259-263. doi:10.1182/hematology.2020000161

229. Fau JB, El-Cheikh R, Brillac C, et al. Drug-Disease Interaction and Time-Dependent Population Pharmacokinetics of Isatuximab in Relapsed/Refractory Multiple Myeloma Patients. *CPT Pharmacomet Syst Pharmacol*. 2020;9(11):649-658. doi:10.1002/psp4.12561

230. Carlo-Stella C, Zinzani PL, Sureda A, et al. A phase 1/2, open-label, multicenter study of isatuximab in combination with cemiplimab in patients with lymphoma. *Hematol Oncol*. 2023;41(1):108-119. doi:10.1002/hon.3089

231. Simonelli M, Garralda E, Eskens F, et al. Isatuximab plus atezolizumab in patients with advanced solid tumors: results from a phase I/II, open-label, multicenter study. *ESMO Open*. 2022;7(5). doi:10.1016/j.esmoop.2022.100562

232. Zucali PA, Lin CC, Carthon BC, et al. Targeting CD38 and PD-1 with isatuximab plus cemiplimab in patients with advanced solid malignancies: results from a phase I/II open-label, multicenter study. *J Immunother Cancer*. 2022;10(1):e003697. doi:10.1136/jitc-2021-003697

233. Valentin MO, Crespo M, Fernandez C, et al. Improving the Access of Highly Sensitized Patients to Kidney Transplantation From Deceased Donors: The Spanish PATHI Program With Allocation Based on the Virtual Crossmatch. *Transplantation*. 2024;108(3):787-801. doi:10.1097/TP.0000000000004824

234. Moreno L, Perez C, Zabaleta A, et al. The Mechanism of Action of the Anti-CD38 Monoclonal Antibody Isatuximab in Multiple Myeloma. *Clin Cancer Res*. 2019;25(10):3176-3187. doi:10.1158/1078-0432.CCR-18-1597

235. Amanna IJ, Slifka MK. Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. *Immunol Rev*. 2010;236(1):125-138. doi:10.1111/j.1600-065X.2010.00912.x

APPENDIX I

Supplementary table 1. Examples of target cPRA reduction

Baseline		Target	
cPRA	**LCD**	**cPRA**	**LCD**
99.99%	1:10000	99.98%	1:5000
99.90%	1:1000	99.80%	1:500
99.80%	1:500	99.60%	1:250
99.60%	1:250	99.20%	1:125
99.50%	1:200	99.00%	1:100
99.00%	1:100	98.00%	1:50
97.50%	1:40	95.00%	1:20
95.00%	1:20	90.00%	1:10
90.00%	1:10	80.00%	1:5
80.00%	1:5	60.00%	1:2.5

Abbreviations: cPRA, calculated panel reactive antibodies; LCD, likelihood of compatible donor.

Supplementary table 2. Examples of antibody titer.

Abbreviations: MFI, mean fluorescence intensity.

	Neat serum	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**Titer**
Antigen 1 MFI	13430	12492	6250	3123	1550	790	8
Antigen 2 MFI	18320	20820	10501	5206	2604	1302	16
Antigen 3 MFI	11020	5493	2750	1384	688	360	4

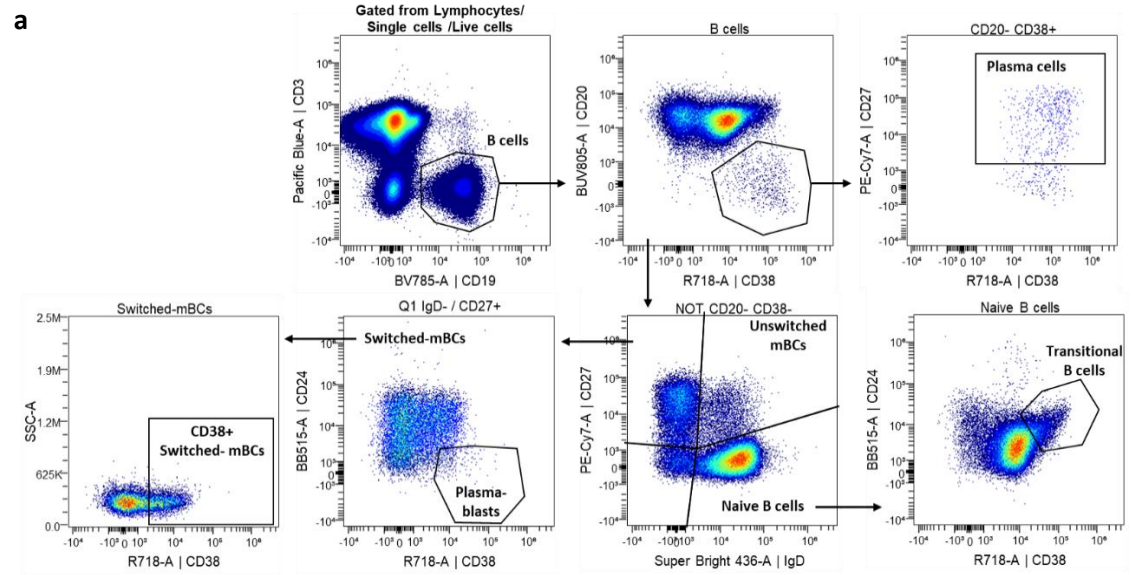
Supplementay Table 3. HLA specificities assessed in circulating memory B cells and bone marrow plasma cells with the HLA-specific B-cell Fluorospot in each patient

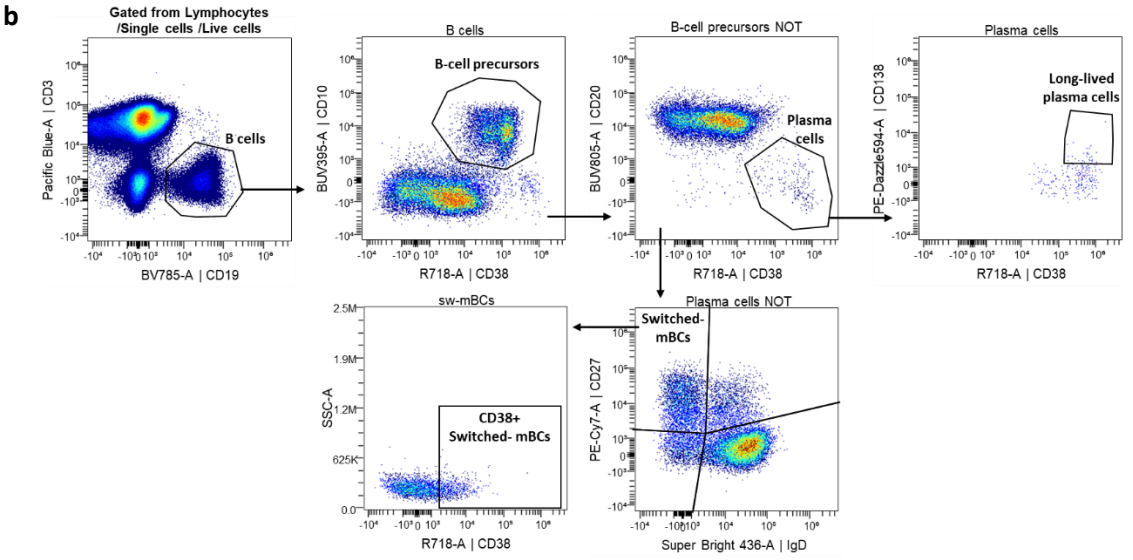
Patient ID	HLA specificities	Patient ID	HLA specificities
Circulating HLA-specific memory B cells		Bone marrow-residing HLA-specific Plasma cells	
712	A*23:01	712	A*24:02
712	A*24:02	712	A*24:03
713	A*03:01	712	A*34:02
713	A*11:01	712	DRB1*14:01
713	B*07:02	712	DRB3*02:02
713	DRB1*01:03	712	DRB3*03:01
713	DRB1*11:04	714	A*68:02
714	A*02:01	714	B*37:01
714	B*57:01	714	B*67:01
714	DRB1*01:01	714	DQB1*03:02 DQA1*02:01
714	DRB1*04:02	714	DRB1*01:02
714	DRB1*07:01	714	DRB1*04:01
714	DRB1*10:01	714	DRB1*07:01
714	DRB1*11:01	714	DRB1*10:01
716	A*36:01	714	DRB1*15:01
717	A*01:01	714	DRB4*01:03
717	A*03:01	714	DRB5*02:02
717	A*24:02	724	B*27:05
717	A*29:02		
717	A*80:01		
717	B*07:02		
717	B*15:03		
717	B*15:12		
717	B*44:02		
717	B*45:01		
717	B*55:01		
717	DQB1*05:02/DQA1*01:02		
717	DQB1*06:03/DQA1*01:03		
717	DQB1*02:01/DQA1*02:01		
717	DQB1*02:02/DQA1*02:01		
717	DQB1*04:01/DQA1*02:01		
717	DQB1*02:01/DQA1*03:01		
717	DQB1*04:02/DQA1*04:01		
718	A*01:01		
718	A*23:01		
718	A*24:02		
718	A*36:01		
718	B*57:01		
718	DRB1*10:01		
718	DRB1*15:03		
719	A*01:01		
719	A*36:01		
719	DQB1*03:02/DQA1*02:01		
719	DQB1*04:01/DQA1*02:01		
719	DQB1*03:01/DQA1*06:01		
722	A*23:01		
722	B*07:02		
722	B*51:01		

Supplemental Table 4. Antibodies used for spectral flow cytometry analysis

Specificity	Clone	Fluorochrome	Source	Ref Num
CD19	HIB19	BV785	Biolegend	302239
CD20	2H7	BUV805	BD Biosciences	612906
CD27	O323	PECy7	Biolegend	302837
IgD	IA6-2	SuperBirght 436	ThermoFisher	62-9868-42
CD24	ML5	BB515	BD	564522
CD38	HB7	R718	BD	567987
CD138	MI15	PE-Dazzle594	Biolegend	356529
FcRL4	A1	A647	BD Biosciences	566587
CD21	HB5	PerCP-eF710	ThermoFisher	46-0219-42
CD95	DX2	BV605	Biolegend	305627
CD11c	B-ly6	BUV661	BD Biosciences	612968
CD10	HI10a	BUV395	BD Biosciences	563871
CD69	FN50	BV711	BD Biosciences	563836
PD1	EH12.1	BV421	BD Biosciences	565935
CD3	SK7	PB	Biolegend	344823
CD4	SK3	AF660	Biolegend	344675
CD127	HIL-7R-M21	BUV737	BD Biosciences	612794
CD25	M-A251	PE	BD Biosciences	555432
CD8	HIT8a	APC-H7	BD Biosciences	566856
CD16	3G8	BV510	Biolegend	302047
CD56	NCAM16.2	BB700	BD Biosciences	566574

Supplementary Figure 1. Gating strategy for flow cytometry assay. a) Gating strategy for PBMCs samples b) Gating strategy for bone marrow samples.





Supplementary table 5. Summary of safety by cohort

n (%)	Cohort A (n=12)		Cohort B (n=11)	
	All grades	Grade ≥3	All grades	Grade ≥3
Any event	3 (25.0)	0	4 (36.4)	0
Infusion-related reaction	2 (16.7)	0	3 (27.3)	0
Nasopharyngitis	1 (8.3)	0	0	0
Headache	1 (8.3)	0	0	0
Tachycardia	1 (8.3)	0	0	0
Nasal congestion	1 (8.3)	0	0	0
Nausea	1 (8.3)	0	0	0
Myalgia	1 (8.3)	0	0	0
Temporomandibular Joint Syndrome	1 (8.3)	0	0	0
Chills	1 (8.3)	0	0	0
COVID-19	0	0	1 (9.1)	0

COVID-19, coronavirus disease.

Supplementary Table 6. Summary of pharmacokinetics of isatuximab by cohort after the first administration at a dose of 10 mg/kg

Mean ± SD [CV%]	Cohort A (n=11)	Cohort B (n=11)
C_{max} (µg/mL)	295 ± 128 [43]	285 ± 94 [33]
t_{max}* (h)	3.67 (2.00–6.03)	3.40 (2.25–4.63)
$AUC_{1\ week}$ (µg•h/mL)	29400 ± 7400 [25]†	20000 ± 5240 [26]†

*Median (min–max), t_{max} was generally at end of infusion

†n=10

$AUC_{1\ week}$, area under the curve over 1 week; C_{max}, maximum plasma concentration; CV, coefficient of variation; SD, standard deviation; t_{max}, time to reach maximal concentration

Supplementary Table 7. Summary of number of anti-HLA-antibody with baseline MFI ≥3000 reduced to

	Cohort A (n=12)	Cohort B (n=11)	All (N=23)
Maximum number of anti-HLA-antibody reduced [n (%)]			
Number of participants assessed	12	11	23
None	2 (16.7)	2 (18.2)	4 (17.4)
1-5	4 (33.3)	4 (36.4)	8 (34.8)
>5-10	4 (33.3)	4 (36.4)	8 (34.8)
>10-15	1 (8.3)	0	1 (4.3)
>15	1 (8.3)	1 (9.1)	2 (8.7)
Baseline MFI 3000<6000			
None	2 (16.7)	2 (18.2)	4 (17.4)
1-5	5 (41.7)	5 (45.5)	10 (43.5)
>5-10	3 (25.0)	3 (27.3)	6 (26.1)
>10-15	1 (8.3)	0	1 (4.3)
>15	1 (8.3)	1 (9.1)	2 (8.7)
Baseline MFI 6000<10000			
None	9 (75.0)	9 (81.8)	18 (78.3)
1-5	3 (25.0)	1 (9.1)	4 (17.4)
>5-10	0	1 (9.1)	1 (4.3)
>10-15	0	0	0
>15	0	0	0
Baseline MFI ≥10000			
None	12 (100)	11 (100)	23 (100)

Supplementary Table 8. Summary of statistical testing in pharmacodynamic changes from baseline to post-treatment

Biomarker	C3D1	C3D1 (p-value)	C3D1 (adjusted p-value)	FUP (Week 17)	FUP Week 17 (p-value)	FUP (Week 17) adjusted p-value
Treg cells (cells/µL)	13	0.889	1.000	10	0.557	1.000
Treg cells, CD38+ (cells/µL)	13	0.004	0.046	10	0.221	1.000
Treg cells, CD38- (cells/µL)	13	0.376	1.000	10	0.275	1.000
Plasma cells (cells/mL)	14	0.078	0.706	12	0.380	1.000
Plasmablasts (cells/mL)	14	0.025	0.245	12	0.791	1.000
Immunoglobulin G (g/L	20	<0.001	<0.001	16	<0.001	<0.001
Immunoglobulin M (g/L)	20	<0.001	0.001	16	0.002	0.022
NK cells, CD38- (cells/µL)	13	0.085	1.000	10	0.200	1.000
NK cells, CD38+ (cells/µL)	13	<0.001	0.005	10	0.232	1.000
NK cells (cells/µL)	13	<0.001	0.005	10	0.275	1.000
Memory B-cell CD38+ (cells/mL)	16	0.083	0.706	N/A	N/A	N/A
Memory B-cell CD38- (cells/mL)	16	0.006	0.069	N/A	N/A	N/A

Supplementary table 9. Statistics of HLA-sp antibodies in serum (MFI)

MFI	Baseline	C3D1	FUP-WK1	FUP-WK9	FUP-WK25
All HLAs	1750 [49-12600]	1272 [22-11222]	1118 [21-10458]	2162 [21-12522]	1907 [29-12022]
HLA-I	2176 [50-10613]	1672 [30-8980]	1490 [32-8602]	2253 [34-10126]	2281 [43-9911]
HLA-II	1182 [46-14703]	922 [17-12989]	864 [16-11940]	1981 [13-14453]	1412 [20-14092]
HLA-A	4081 [59-17296]	3098 [44-17494]	2843[35-16779]	4270 [34-17441]	3831 [51-17157]
HLA-B	1677 [55-8573]	1306 [30-6850]	1147 [33-6381]	1885 [29-7424]	1764 [25-7139]
HLA-C	942 [32-9639]	640 [9-9136]	626 [5-8841]	806 [55-10468]	1598 [118-8920]
HLA-DR	2114 [59-13169]	1333 [26-11316]	1303 [24-10561]	3199 [26-1286]	1366 [19-10545]
HLA-DQ	2388 [98-17066]	1913 [41-15891]	1288 [24-15325]	3736 [24-16798]	1867 [20-17749]
HLA-DP	444 [25-15878]	328 [7-13770]	350 [6-12788]	476 [0-14594]	1003 [20-15685]

p-values	Baseline vs C3D1	Baseline vs FUP-WK1	Baseline vs FUP-WK9	Baseline vs FUP-WK25	C3D1 vs FUP-WK1	C3D1 vs FUP-WK9	C3D1 vs FUP-WK25	FUP-WK1 vs FUP-WK9	FUP-WK1 vs FUP-WK25	FUP-WK9 vs FUP-WK25	total
All HLAs	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.171	p<0.0001	p=0.06	p<0.0001
HLA-I	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.028	p<0.0001	p=0.44	p=0.197	p=0.043	p<0.0001
HLA-II	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.013	p<0.0001	p=0.619	p<0.0001
HLA-A	p<0.0001	p<0.0001	p=0.001	p<0.0001	p<0.0001	p=0.218	p=0.001	p=0.821	p=0.271	p=0.644	p<0.0001
HLA-B	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.084	p=0.017	p=0.194	p=0.729	p=0.018	p<0.0001
HLA-C	p=0.014	p=0.001	p=0.002	p<0.0001	p<0.0001	p=0.381	p=0.011	p=0.630	p=0.085	p=0.565	p<0.0001
HLA-DR	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.856	p=0.175	p=0.648	p<0.0001
HLA-DQ	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.092	p=0.085	p=0.666	p=0.005	p<0.0001
HLA-DP	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.008	p<0.0001	p=0.128	p<0.0001

Supplementary Table 10. Statistics of relative change in serum HLA-sp antibodies MFI

% Change in MFI	Baseline	C3D1	FUP-WK1	FUP-WK9	FUP-WK25
All HLAs	0	-6,6 [-22,6-10,3]	-10,8 [-29,9-7,4]	-10,3 [-26,1-5,7]	-14 [-30,8-5,7]
HLA-I	0	-7,5 [-25,6-10,9]	-10,6 [-34,1-9,4]	-10,6 [-30-8,27]	-14 [-35,2-9,2]
HLA-II	0	-5,4 [-17,3-9,2]	-11,3 [-28,1-5,4]	-10 [-24-3,8]	-14,2 [-27-2,1]
HLA-A	0	-5,4 [-22,5-11,8]	-7,4 [-23,8-8,7]	-8,8 [-24,1-10,4]	-10,6 [-24,8-8,7]
HLA-B	0	-11,8 [-29,8-8,3]	-15,1 [-40,9-8,6]	-12,3 [-36,6-6]	-15,6 [-39,1-12,3]
HLA-C	0	-4 [-16,8-14,6]	-8,4 [-20,2-12]	-10,4 [-22,8-10,1]	-19,6 [-36,6-6,3]
HLA-DR	0	-7,4 [-19,7-8,3]	-15,3 [-28,6-0,9]	-9,6 [-24,5-5,1]	-15 [-34,2-5,9]
HLA-DQ	0	-2,9 [-16-10,5]	-7,1 [-22,7-10,9]	-7,9 [-18-7,6]	-7,5 [-19,9-18,3]
HLA-DP	0	-6 [-16,3-8,2]	-11,3 [-30-3]	-13,8 [-28,6--0.6]	-16,6 [-25,1--6,7]

p-values	Baseline vs C3D1	Baseline vs FUP-WK1	Baseline vs FUP-WK9	Baseline vs FUP-WK25	C3D1 vs FUP-WK1	C3D1 vs FUP-WK9	C3D1 vs FUP-WK25	FUP-WK1 vs FUP-WK9	FUP-WK1 vs FUP-WK25	FUP-WK9 vs FUP-WK25	total
All HLAs	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.658	p=0.001	p=0.051	p<0.0001
HLA-I	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.112	p=0.005	p=0.019	p=0.863	p=0.038	p<0.0001
HLA-II	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.053	p<0.0001	p=0.369	p<0.0001
HLA-A	p=0.001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.043	p=0.018	p=0.882	p=0.273	p=0.309	p<0.0001
HLA-B	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.810	p=0.659	p=0.003	p=0.023	p=0.008	p<0.0001
HLA-C	p=0.501	p=0.062	p=0.064	p=0.001	p<0.0001	p=0.727	p=0.001	p=0.660	p=0.034	p=0.253	p<0.0001
HLA-DR	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.404	p=0.103	p=0.710	p<0.0001
HLA-DQ	p=0.014	p<0.0001	p<0.0001	p=0.026	p<0.0001	p<0.0001	p=0.343	p=0.144	p=0.378	p=0.001	p<0.0001
HLA-DP	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.002	p<0.0001	p=0.103	p<0.0001

Supplemental Table 11. Statistics of HLA-sp Abs in mBCs supernatants (MFI/IgG)

Parameter	Baseline n=10	C3D1 n=10	FUP-WK1 n=5	Baseline vs C3D1	Baseline vs FUP1	C3D1 vs FUP1
All HLAs	12.3 [2.9-49.7]	5.7 [1.8-20.2]	5.5 [1.3-15.6]	*p<0.0001*	*p<0.0001*	*p<0.0001*
HLA-I	12 [2.6-49.5]	5.8 [1.6-19]	7.3 [2.6-18]	*p<0.0001*	*p<0.0001*	*p<0.0001*
HLA-II	12.5 [4.4-44.7]	5.5 [1.9-22.9]	3.1 [0.3-13.3]	*p=0.001*	*p=0.193*	*p<0.0001*
HLA-A	19 [4-61.3]	4.7 [1.4-25.4]	11.1 [3.1-21.3]	*p<0.0001*	*p<0.0001*	*p=0.044*
HLA-B	12.6 [2.6-46.2]	8.5 [2-17.6]	5.2 [1.7-14.2]	*p<0.0001*	*p<0.0001*	*p<0.0001*
HLA-C	3 [2.1-48.1]	3 [1.4-19]	13.6 [1.6-24.3]	*p=0.004*	*p<0.0001*	*p=0.033*
HLA-DR	11 [5-72.1]	23.44 [14-42.4]	12.1 [1.65-15.75]	*p=0.627*	*p=0.563*	*p<0.0001*
HLA-DQ	5.9 [2.7-27.2]	4.4 [1.6-11.9]	3.1 [1.4-10.7]	*p=0.001*	*p=0.352*	*p=0.959*
HLA-DP	20.7 [18-51.2]	2.3 [1.6-3.27]	0.16 [0.13-0.22]	*p<0.0001*	*p=0.005*	*p=0.005*

Supplemental Table 12. Statistics of repeated and non-rerpeated HLA-sp Abs in serum (MFI) and mBc supernatants (MFI/IgG)

Serum HLA-sp Abs MFI	Baseline	C3D1	FUP-WK1	Baseline vs C3D1	Baseline vs FUP-WK1	Baseline vs FUP-WK9	total
Non-repeated MM antigens	1306 [44-1118]	921 [23-9826]	842 [13-8749]	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Repeated MM antigens	15360 [3077-22571]	15360 [2388-24507]	15645 [1743-24492]	p=0.230	p=0.521	p=0.101	p=0.359

mBCs HLA-sp Abs MFI/IgG	Baseline	C3D1	FUP-WK1	Baseline vs C3D1	Baseline vs FUP-WK1	Baseline vs FUP-WK9	total
Non-repeated MM antigens	0.58 [0.27-1.06]	0.55 [0.32-1.36	0.46 [0.24-0.91]	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Repeated MM antigens	1.28 [0.66-25.61]	1.37 [0.69-16.41]	3.15 [0.5-15.62]	p=0.108	p=0.574	p=0.172	p=0.131

APPENDIX II

OPEN

Isatuximab Monotherapy for Desensitization in Highly Sensitized Patients Awaiting Kidney Transplant

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ABSTRACT

Background Patients with calculated panel reactive antibody (cPRA) ≥80.00%, particularly those with cPRA ≥99.90%, are considered highly sensitized and underserved by the Kidney Allocation System. Desensitization removes circulating reactive antibodies and/or suppresses antibody production to increase the chances of a negative crossmatch. CD38 is expressed highly on plasma cells, thus is a potential target for desensitization.

Methods This was an open-label single-arm phase 1/2 study investigating the safety, pharmacokinetics, and preliminary efficacy of isatuximab in patients awaiting kidney transplantation. There were two cohorts, cohorts A and B, which enrolled cPRA ≥99.90% and 80.00% to <99.90%, respectively.

Results Twenty-three patients (12 cohort A, 11 cohort B) received isatuximab 10 mg/kg weekly for 4 weeks then every 2 weeks for 8 weeks. Isatuximab was well tolerated with pharmacokinetic and pharmacodynamic profiles that indicated similar exposure to multiple myeloma trials. It resulted in decreases in $CD38^+$ plasmablasts, plasma cells, and NK cells and significant reductions in HLA-specific IgG-producing memory B cells. Overall response rate, on the basis of a predefined composite desensitization end point, was 83.3% and 81.8% in cohorts A and B. Most responders had decreases in anti-HLA antibodies that were maintained for 26 weeks after the last dose. Overall, cPRA values were minimally affected, however, with only 9/23 patients (39%) having cPRA decreases to target levels. By study cutoff (median follow-up of 68 weeks), six patients received transplant offers, of which four were accepted.

Conclusions In this open-label trial, isatuximab was well tolerated and resulted in a durable decrease in anti-HLA antibodies with partial desensitization activity.

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INTRODUCTION

Patients may become sensitized to HLAs through pregnancy, after a blood product transfusion, or after solid organ transplantation.[1] Although there is no standardized definition, highly sensitized patients can be regarded as patients with a calculated panel reactive antibody (cPRA) ≥80.00%.[2,3]

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Only 6.5% of highly sensitized patients with cPRA ≥80.00% receive a compatible kidney transplant each year, and almost none of these patients have a cPRA ≥99.90%.[4]

The implementation of the kidney allocation system (KAS) in 2014 dramatically increased organ equity for highly sensitized patients and increased the likelihood of finding a compatible donor for transplantation. However, post-KAS implementation, candidates with cPRA ≥99.90% continue to have very low rates of kidney transplantation. These patients on average receive less than one organ offer per decade and have the greatest need for desensitization.[5]

The aim of desensitization is to remove circulating HLA-reactive antibodies and/or reduce or eliminate antibody production, thus increasing the chances of a negative crossmatch and of matching with a compatible donor, reducing time on dialysis, and improving clinical outcome.[6] However, there is no standard desensitization regimen, and most regimens involve off-label plasmapheresis, intravenous Ig, and anti-CD20 therapies, such as rituximab.[4] Other trials have also investigated IL-6 targeting therapies, such as tocilizumab and clazakizumab, or proteasome inhibitors, such as bortezomib and carfilzomib.[1] Imlifidase, a cysteine protease that cleaves all IgG subclasses, was recently approved by the European Medicines Agency for desensitization treatment of highly sensitized adult kidney transplant recipients with positive crossmatch against an available deceased donor.[7,8] However, imlifidase lacks durability of effect, requires repeated dosing that would not be feasible due to antidrug antibody (ADA) response, and leaves patients still susceptible to antibody-mediated rejection (ABMR) due to antibody rebound.[7]

CD38 is a commonly found ectoenzyme on plasma cells and multiple myeloma cells.[9] Alloantibody-producing plasma cells express CD38 at a higher level than other $CD38^+$ hematopoietic cells.[9] There is thus a rationale for depleting plasma cells producing alloantibodies or donor-specific antibodies (DSAs) for desensitization in kidney transplant patients with CD38-targeting antibodies. Few studies are ongoing, and few clinical case reports are available regarding the use of anti-CD38 antibodies, which have demonstrated various levels of DSA reduction, for desensitization in patients awaiting transplantation.[10–14]

Isatuximab is an anti-CD38 monoclonal antibody approved in combination with pomalidomide and dexamethasone, and in combination with carfilzomib and dexamethasone, for the treatment of patients with relapsed/refractory multiple myeloma.[15] Isatuximab binding to CD38 triggers a number of Fc-dependent mechanisms—antibody-dependent cellular cytotoxicity, complement dependent cytotoxicity, and antibody-directed cellular phagocytosis—and direct apoptosis.[16–18] Isatuximab has demonstrated the induction of apoptosis in primary cells from bone marrow aspirates of patients with multiple myeloma.[16] It is hypothesized that isatuximab can target long-lived plasma cells, depleting the production source of alloantibodies and DSAs, leading to their sustained removal.

Significance Statement

There is no standardized desensitization regimen for kidney transplant candidates. CD38, expressed by plasma cells, could be targeted for desensitization to deplete plasma cells producing alloantibodies and donor-specific antibodies. Few studies and case reports are available regarding the use of CD38 antibodies for desensitization in patients awaiting kidney transplant. This study shows that isatuximab, a CD38-targeting therapy, was well tolerated in kidney transplant candidates, with a durable decrease in anti-HLA antibodies and partial desensitization activity. The short treatment period and long follow-up of this study allowed for the understanding of the mechanism and timing for any antibody rebound. Isatuximab could be further investigated as an option for adjunct therapy to existing desensitization for patients on the kidney transplant waitlist.

In this phase 1/2 study report (NCT04294459), we describe the safety, pharmacokinetics (PK), and preliminary efficacy of isatuximab in patients awaiting kidney transplantation. The study included two cohorts on the basis of patient's baseline (BL) cPRA. Cohorts A and B enrolled patients with cPRA ≥99.90% and cPRA 80.00% to <99.90%, respectively, where the former represents a sample of a population particularly poorly served by the current KAS.

METHODS

This was an open-label, single-arm, phase 1/2 study conducted at six centers in the United States and Spain between June 18, 2020, and May 2, 2022. The study had a screening period of up to 28 days, a treatment period of up to 12 weeks, a site-visit follow-up of up to 26 weeks after treatment had stopped, and an extended follow-up *via* telephone every 90 days until study cut-off date, death, or loss to follow-up. Study cutoff was planned at 26 weeks after the last patient completed the treatment period or when the last ongoing patient was lost to follow-up, whichever was earlier. The primary objective of the phase 1 study was to characterize the safety and tolerability of isatuximab in kidney transplant candidates. The primary objective of the phase 2 study was to evaluate the preliminary efficacy of isatuximab in the desensitization of patients awaiting kidney transplantation.

Isatuximab Treatment

Isatuximab was administered at a starting dose of 10 mg/kg every week for 4 weeks in cycle 1 and every 2 weeks for cycles 2 and 3. Patients underwent three cycles of treatment (a total of eight planned doses) spanning a 12-week period. Each cycle was 28 days.

Study End Points

The primary end point for the phase 1 study was the proportion of patients with adverse events (AEs), serious AEs, and laboratory abnormalities. In phase 2, the primary end point was response rate (RR) which was a composite

end point, as assessed by central laboratory. RR was defined as a proportion of patients meeting at least one of the three predefined desensitization efficacy criteria (see Supplemental Appendix 1 for details). Criterion 1 was the reduction of cPRA to target levels, where target cPRA was defined as cPRA that would result in at least doubling the theoretical likelihood of finding a compatible donor.[19] Criterion 2 was the reduction of >2 antibody titers to reach target cPRA, and criterion 3 was the elimination of anti-HLA antibody as mean fluorescence intensity (MFI) reduced to <2000 for antibodies with BL MFI of ≥3000. Secondary end points included duration of response (DoR), number of anti-HLA antibody eliminated, change in cPRA and anti-HLA antibody levels, PK, and biomarkers. Safety assessments included AEs and serious AEs reported per Common Terminology Criteria for AEs v5.0, laboratory abnormalities, and incidence of ADAs against isatuximab.

PK Analysis

Blood samples were collected mainly during cycle 1 at selected time points (predose, end of infusion [EOI], EOI+1 hour or EOI+4 hours, start of infusion+72 hours, and start of infusion+168 hours) and were used for isatuximab PK assessment by noncompartmental analysis. Analysis was performed with Phoenix WinNonlin version 8.2 (Pharsight). The Gyrolab Platform, a quantitative sandwich immunoassay using biotinylated anti-isatuximab antibodies bound by streptavidin beads within the Gyrolab Bioaffy CD microstructure for capture and Alexa Fluor 647-conjugated CD38 antibody for detection, was used to measure functional isatuximab (isatuximab with ≥1 site available to bind target) plasma levels, with a lower limit of quantitation of 5.0 μg/ml.

Anti-HLA Antibody Testing

Serum samples were frozen at −80°C for at least 10 minutes, then thawed at 2−4°C, and brought to room temperature for preparation. Aggregates were removed by centrifugation for 5 minutes at 7400×*g*. Serum was treated with Adsorb Out Beads (ADSORB, One Lambda) according to the manufacturer's instructions, and EDTA, 0.5 M pH 8.0±0.1, was added to serum in a 1:20 ratio (*e.g.*, 5 μl EDTA to 95 μl serum). Serum dilutions were performed in PBS (Beckman Coulter), and all assays were performed by one technologist. Consecutive samples from each patient were batched to minimize assay variability. Anti-HLA antibody testing was performed using LABScreen Single Antigen HLA class I (catalog LSA1A04, One Lambda) and LABScreen Single Antigen HLA class II (catalog LS2A01, One Lambda), and data were acquired on a LABScan 3D flow analyzer and analyzed in HLA Fusion 4.6 software.

cPRA per Serial Dilutions

At BL, serum from each patient was tested neat and in serial doubling dilutions from 1:2 to 1:4096. Samples collected at day 1 of each treatment cycle and at site visit follow-up weeks 1, 5, 9, 13, 17, 21, and 25 were tested neat and at the relevant dilution for assessment of efficacy criterion 2. cPRA was calculated to two decimal points (*e.g.*, 99.99%) on the basis of the cPRA calculator developed by the Organ Procurement and Transplantation Network, with unacceptable antigens defined as those with MFI ≥2000.

Antibody Titration Heat Maps to Compare BL to Follow-Up

Antibody results were compared for each patient for serum samples collected at BL versus site visit follow-up weeks 9 and 25 (or the closest dated alternate follow-up samples as available). HLA class I and class II panels were analyzed separately, and beads on each panel were sorted from high to low on the basis of the patient's BL titer strength for that bead. Following the BL titer sorted values, heat maps were produced in consecutive subsequent columns of a spreadsheet. For the BL and follow-up samples, MFIs for serum tested neat, 1:16, and 1:256 were compared. Conditional formatting was used to color code MFIs in strength categories.

Biomarkers Analysis

Ig and immunophenotyping assays were performed by Covance Central Laboratory Services. B-cell panels, natural killer (NK) and natural killer T-cell panels, and Ig assays were performed.

B-cell panels were analyzed as follows—each specimen was incubated with Whole Blood Lysing Reagent and centrifuged afterward. White blood cells were then washed and prepared for immunophenotyping staining. Cells were incubated with Fc block working solution followed by incubation with CD38 FITC (Beckman Coulter), AHIgG1 FITC (Southern Biotech), CD24 PE (BioLegend), CD20 PerCP-Cy5.5 (BD Pharmingen), CD19 APC (BD Pharmingen), CD45 AF700 (BD Pharmingen), IgD V450 (BD Horizon), CD27 BV510 (BioLegend), and CD138 BV605 (BioLegend) in Brilliant Stain Buffer (BD Horizon). Finally, cells were fixed with 1% paraformaldehyde solution and acquired on the BD SORP FACSCanto II.

Memory B cells (mBC) were assessed both phenotypically and functionally. mBC phenotypes were assessed using peripheral blood mononuclear cells that were characterized by flow cytometry (Cytek Aurora CS) with the following markers: CD19 BV785 (BioLegend), CD20 BUV805 (Beckton Dickinson), CD27 PE-Cy7 (BioLegend), IgD SuperBright-436 (Thermo Fisher), CD24 BB515 (Beckton Dickinson), and CD38 R718 (Beckton Dickinson). Switched mBCs were defined as $CD19^+$ $CD20^+$ $CD27^+$ IgD and analyzed according to the number of B cells/ml. For the evaluation of HLA-specific mBC function, mBCs were polyclonally stimulated as previously described in Luque *et al.*,[20] seeded in an anti-IgG precoated FluoroSpot plate, and incubated overnight to release antibodies. HLA-sp mBC detection was performed

with a HLA-sp B-cell FluoroSpot assay using different class I and class II fluorophore-conjugated HLA tetramers (Pure MHC; LLC, Oklahoma). All tested HLA specificities per patient and time point are listed in Supplemental Table 1. The results of the assay are reported as the number of IgG-secreting anti-HLA mBCs per 450,000 seeded cells as median and interquartile range.

For analysis of the NK and regulatory T-cell (Treg) cell panel, each specimen was washed with plain PBS without azide and the pellet was resuspended and incubated with *N*-hydroxysuccinimide solution. After washing, cells were incubated with Fc block working solution followed by incubation with CD38 FITC (Beckman Coulter), AHIgG1 FITC (Southern Biotech), CD25 PE (BD Pharmingen), CD127 PerCP-Cy5.5 (BioLegend), CD56 APC (BioLegend), *N*-hydroxysuccinimide Ester AF700 (Thermo Fisher), CD4 BV421 (BioLegend), CD8 BV510 (BD Horizon), and CD3 BV605 (BioLegend) in Brilliant Stain Buffer (BD Horizon). After washing, red blood cells were lysed with Whole Blood Lysing Reagent. Finally, cells were fixed with 1% paraformaldehyde solution and acquired on the BD FACSCanto II.

The bone marrow–residing HLA-specific plasma cell response analyses were performed as follows. Bone marrow aspirates were performed before first treatment and at the end of cycle 3 in three patients of the study. Bone marrow cells were isolated from bone marrow aspirates, seeded to an anti-IgG precoated FluoroSpot plate, and incubated for antibody release. HLA-sp IgG-secreting bone marrow plasma cells were detected and reported, as described previously for HLA-sp mBCs. HLA antigen specificities tested were randomly selected because of their antibody presence in the sera. See Supplemental Table 1 where all specificities tested in each patient are described.

Statistical Analysis

Patients treated at the phase 2 dose during phase 1 were included in the efficacy analyses together with the phase 2 patients. RR (primary composite endpoint) was calculated as proportion of patients meeting at least one of the three predefined desensitization efficacy criteria, along with corresponding two-sided 95% confidence intervals (CIs) using the Clopper–Pearson method.

DoR was defined as the time from central laboratory sample collection date indicating response up to the central laboratory sample collection date when the patient was no longer meeting any response criterion (*i.e.*, nonresponder) or up to date of death due to any cause, whichever occurred first. DoR is summarized with the Kaplan–Meier method.

For pharmacodynamic analyses, the Wilcoxon signed-rank test was used to evaluate the significance of the change post-treatment as compared with the BL. To control for multiple testing, adjusted *P* values have been also calculated by using the Benjamini and Hochberg method.[21]

RESULTS

Patients and BL Characteristics

A total of 23 patients were enrolled in this study—12 in cohort A and 11 in cohort B—of which 22 completed the study treatment period and 18 completed the extended follow-up period until study cut-off date (Figure 1). The median follow-up of all treated participants was 68.0 weeks. One patient in cohort B discontinued treatment definitively on the basis of logistical reasons due to coronavirus disease 2019 positivity before the last planned dose.

The median age of patients was slightly higher in cohort A than cohort B (52.5 versus 48.0 years; Table 1). Patients in cohort A spent a median 6.0 years on the kidney transplant waitlist, while those in cohort B spent a median 3.6 years. Origins of renal insufficiency reported by investigators were also more varied in cohort B than in cohort A, with 50.0% of patients in cohort A due to prior transplant failure, compared with 36.4% in cohort B. Origins of renal insufficiency in cohort B also included urologic disorders (18.2%), hypertension (9.1%), and autosomal dominant polycystic kidney disease (9.1%). Most patients had one prior kidney transplant (58.3% cohort A, 54.5% cohort B), and over 90% of patients in both cohorts had prior sensitizing events at screening, mostly attributed to transplant and transfusion. All 23 patients were diagnosed with stage 5 CKD at the time of study entry, representing a patient population that is likely to require dialysis (22 of 23 patients were on dialysis at the time of study entry).

The median cPRA per local laboratory assessment was 99.99% (99.90–100.00) and 99.42% (95.57–99.85) in cohorts A and B, respectively. When measuring cPRA by central laboratory, the median cPRA was 99.99% (99.62–100.00) and 99.95% (98.38–100.00), respectively.

All patients received a median of three cycles of isatuximab, with 12 weeks of exposure. The median relative dose intensity was 98.24% in cohort A and 98.38% in cohort B.

Safety

Safety analysis showed any grade treatment-emergent AEs (TEAEs) occurred in 7 (30.4%) patients overall. No TEAEs were grade ≥3. One death occurred in cohort A due to disease complications not related to study treatment during site visit follow-up. A safety summary of TEAEs by AE preferred term can be seen in Table 2 and Supplemental Table 2. No treatment-emergent serious AEs were reported, and the only treatment-related AEs were infusion reactions.

At BL, anemia as laboratory abnormality occurred in 65.2%. Post-treatment, anemia occurrence increased to 82.6% of patients. At both BL and post-treatment, most occurrences were grade 1 in severity with no occurrence of grade ≥3. Lymphocytopenia as laboratory abnormality occurred in 25.0% of patients at BL, most of which were grade 1. Post-treatment, the occurrence of lymphocytopenia as laboratory abnormality was 56.3%, with most occurrences grade 1 and one grade 3

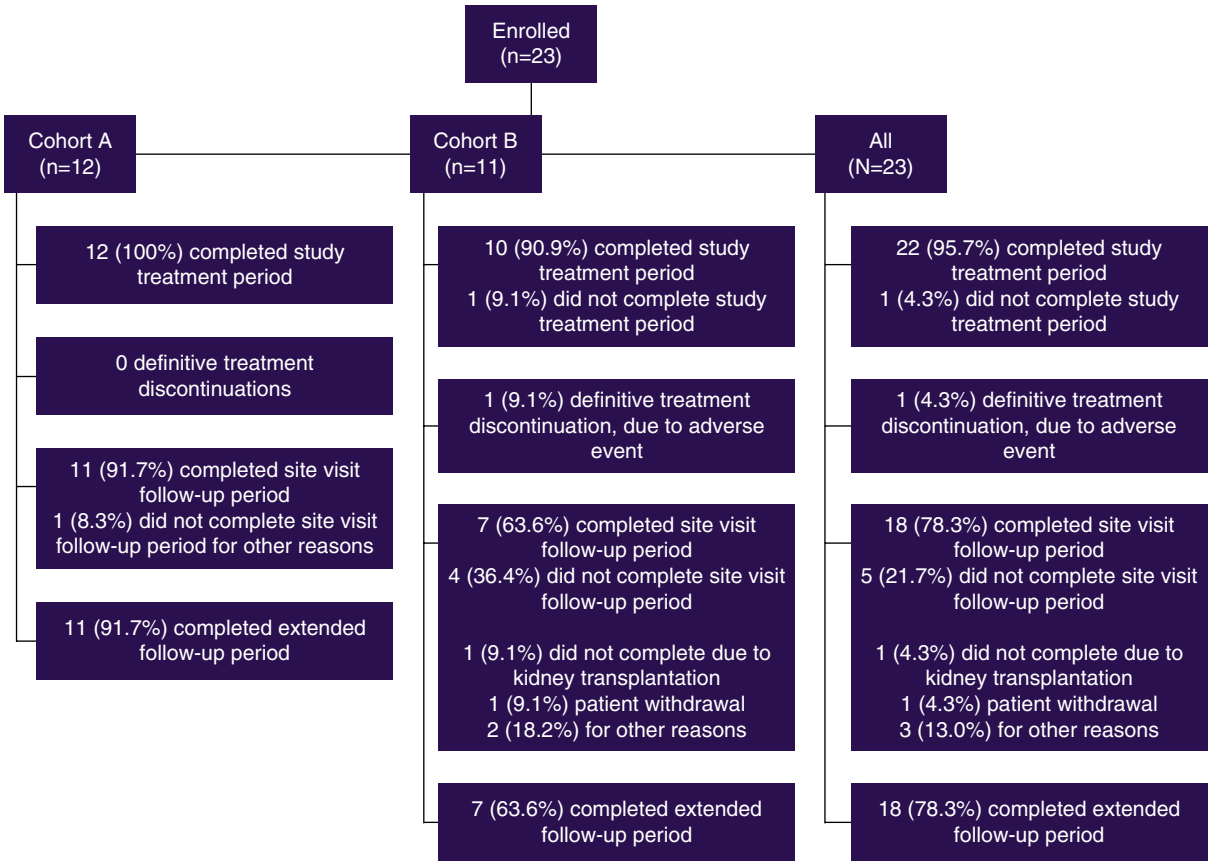

Figure 1. Patient disposition.

occurrence (6.3%). There were no instances of neutropenia during the trial. No patients had an on-treatment positive ADA response against isatuximab.

PK of Isatuximab in Kidney Transplant Candidates

Isatuximab was quantifiable in plasma over the whole dosing period of 1 week after the first infusion at a dose of 10 mg/kg. The overall mean isatuximab maximum plasma concentration (C_{max}) and area under the curve over 1 week ($AUC_{1\ week}$) were 290 μg/ml and 24,700 μg·h/ml, respectively, with moderate variability. A PK summary can be seen in Table 3 and Supplemental Table 3.

Desensitization Activity in Highly Sensitized Kidney Transplant Candidates

The overall RR was 83.3% in cohort A and 81.8% in cohort B (Table 4). Median DoR was not reached in either cohort (cohort A 95% CI, 4.857 to not reached weeks; cohort B 95% CI, 4.143 to not reached weeks). Most responders had a decrease in the anti-HLA antibody level after treatment initiation which was maintained during the site visit follow-up period after stopping treatment. However, among all patients, there is minimal effect on the overall cPRA values. Only 39% of patients (4/12 and 5/11 in cohorts A and B, respectively) had reached target cPRA (*i.e.*, decrease in the cPRA level that would result in at least doubling the theoretical likelihood of finding a compatible donor).[19] Approximately 47.8% (7/12 and 4/11 in cohorts A and B, respectively) had meaningful reduction in anti-HLA antibody titer, and 82.6% (10/12 and 9/11 in cohorts A and B, respectively) had at least one anti-HLA antibody with BL MFI ≥3000 reduced to <2000 (Table 4 and Supplemental Table 4). No BL clinical characteristics or laboratory features were observed to be associated with treatment response. Indeed, the small cohort sample size precludes any meaningful predictive biomarker analysis. In particular, given the polymorphic nature of the HLA system, as well as the variability in antibody strengths and other variables, a large cohort is required to enable more detailed analysis with high confidence.

cPRA alone is not sufficient to reflect a partial desensitization effect, and a composite end point that also included titer reduction and assessment of anti-HLA antibody profiles was therefore implemented to provide a better measurement. This is illustrated through the examples of partial responders, who met criterion 2 or 3 or both, but not criterion 1 (reaching target cPRA), as shown in Figure 2. Assessment of their titer and antibody profiles reveals more information on the desensitization effect of isatuximab. In some patients, although

Table 1. Baseline characteristics

Characteristic	Cohort A (*n*=12)	Cohort B (*n*=11)	All (*N*=23)
Median age, yr (min–max)	52.5 (25–68)	48.0 (25–69)	52.0 (25–69)
Sex, *n* (%)			
Male	6 (50.0)	9 (81.8)	15 (65.2)
Female	6 (50.0)	2 (18.2)	8 (34.8)
Race, *n* (%)			
White	4 (33.3)	9 (81.8)	13 (56.5)
Black or African American	3 (25.0)	1 (9.1)	4 (17.4)
Asian	2 (16.7)	0	2 (8.7)
American Indian or Alaska Native	0	0	0
Native Hawaiian or other Pacific Islander	0	0	0
Not reported	1 (8.3)	1 (9.1)	2 (8.7)
Unknown	2 (16.7)	0	2 (8.7)
Blood type, *n* (%)			
A	4/12 (33.3)	1/9 (11.1)	5 (23.8)
B	4/12 (33.3)	2/9 (22.2)	6 (28.6)
AB	0	1/9 (11.1)	1 (4.8)
O	4/12 (33.3)	5/9 (55.6)	9 (42.9)
Dialysis time, median years (min–max)	6.80 (2.9–12.9)	5.05 (0.2–24.2)	6.23 (0.2–24.2)
Waitlist time, median years (min–max)	6.0 (2.2–12.9)	3.6 (0.6–9.2)	5.3 (0.6–12.9)
Origin of renal insufficiency at study entry, *n* (%)			
Diabetes mellitus	1 (8.3)	0	1 (4.3)
Hypertension	0	1 (9.1)	1 (4.3)
Glomerular disease	2 (16.7)	2 (18.2)	4 (17.4)
ADPKD	0	1 (9.1)	1 (4.3)
Failure of previous transplant	6 (50.0)	4 (36.4)	10 (43.5)
Urologic disorders	0	2 (18.2)	2 (8.7)
Other	5 (41.7)	4 (36.4)	9 (39.1)
No. of prior kidney transplants, *n* (%)			
0	2 (16.7)	1 (9.1)	3 (13.0)
1	7 (58.3)	6 (54.5)	13 (56.5)
2	2 (16.7)	3 (27.3)	5 (21.7)
3	1 (8.3)	1 (9.1)	2 (8.7)
Prior sensitizing events at screening, *n* (%)	11 (91.7)	10 (90.9)	21 (91.3)
Pregnancy	1 (9.1)	0	1 (4.8)
Transfusion	6 (54.5)	3 (30.0)	9 (42.9)
Transplant	10 (90.9)	10 (100)	20 (95.2)
cPRA per central laboratory, median % (min–max)	99.99 (99.62–100.00)	99.95 (98.38–100.00)	99.97 (98.38–100.00)
cPRA per local laboratory with OPTN, median % (min–max)	99.99 (99.90–100.00)	99.42 (95.57–99.85)	99.90 (95.57–100.00)

ADPKD, autosomal dominant polycystic kidney disease; cPRA, calculated panel reactive antibody; OPTN, Organ Procurement and Transplantation Network.

not considered responders based solely on cPRA, a marked and durable decrease in MFI, up to −15,000, was observed for some anti-HLA antibodies.

Table 2. Safety summary of treatment-emergent adverse events by adverse event preferred term

No. (%)	All (*N*=23)	
	All Grades	Grade ≥3
Any event	7 (30.4)	0
Infusion reaction[a]	5 (21.7)	0
Nasopharyngitis	1 (4.3)	0
Headache	1 (4.3)	0
Tachycardia	1 (4.3)	0
Nasal congestion	1 (4.3)	0
Nausea	1 (4.3)	0
Myalgia	1 (4.3)	0
Temporomandibular joint syndrome	1 (4.3)	0
Chills	1 (4.3)	0
COVID-19	1 (4.3)	0

COVID-19, coronavirus disease 2019.
[a]Treatment-related.

The example full profiles of responders, partial responders, and nonresponders are presented in Supplemental Figures 1–3. Of note, MFI values increased from BL over time in patient 0811, a nonresponder per protocol (Supplemental Figure 3C). This increase was observed from C2D1 onward, approximately 2.5 weeks after the patient received their second dose of coronavirus disease 2019 vaccine and at the time point

Table 3. Summary of pharmacokinetics of isatuximab after the first administration at a dose of 10 mg/kg

Mean±SD (CV %)	All (*N*=22)
C_{max}, μg/ml	290±109 (38)
t_{max}[a], h	3.46 (2.00−6.03)
$AUC_{1\ week}$, μg·h/ml	24,700±7880 (32)[b]

$AUC_{1\ week}$, area under the curve over 1 week; C_{max}, maximum plasma concentration; CV, coefficient of variation; t_{max}, time to reach maximal concentration.
[a]Median (min−max), t_{max} was generally at end of infusion.
[b]*n*=20.

Table 4. Summary of response rate in the efficacy-evaluable population on the basis of assigned cohort using screening calculated panel reactive antibody from local laboratory assessment

No. (%)	Cohort A (*n*=12)	Cohort B (*n*=11)	All (*N*=23)
No. of participants assessed	12	11	23
RR based on criterion 1	4 (33.3)	5 (45.5)	9 (39.1)
RR based on criterion 2	7 (58.3)	4 (36.4)	11 (47.8)
RR based on criterion 3	10 (83.3)	9 (81.8)	19 (82.6)
Overall RR	10 (83.3)	9 (81.8)	19 (82.6)
95% CI	51.6 to 97.9	48.2 to 97.7	61.2 to 95.0

CI, confidence interval; RR, response rate.

where the highest stimulation of the immune system is expected to be observed. This potentially led to the non-specific activation of dormant memory response to HLA.

Transplant Outcomes

As of study cut-off date, a total of six patients treated with isatuximab received transplant offers (three each from cohorts A and B), all of which were from deceased donors. Four transplant offers were accepted. Reasons for declining an offer were offer not suitable for transplant and poor donor quality. Among the four patients who received transplant before study cut-off date, two of four were HLA incompatible with their donors before isatuximab treatment but were negative at the time of transplant. Three grafts were functioning with no report of rejection as of study cut-off date, while one graft in cohort A was lost due to thrombosis 1 day after transplant surgery with no reported rejection. As of February 2023, 9/11 patients from the two recruitment centers in Spain received kidney transplantation, five of whom received kidneys from previously incompatible donors.

Pharmacodynamics and Immune Modulation of Isatuximab

Using Ig levels as a surrogate, the results from the pharmacodynamics analysis support isatuximab target engagement. A sustained and significant decrease in total Ig levels was observed up to the last analyzed time point ($P < 0.05$, Supplemental Table 5), 17 weeks after last dose (IgG and IgM as shown in Figure 3, A and B). A decrease in peripheral plasmablasts ($P = 0.025$ and adjusted P value $= 0.245$) and plasma cells ($P = 0.078$ and adjusted P value $= 0.706$) was observed at C3D1 (week 9) compared with BL, with a trend of returning to BL at 17 weeks after the last dose (Figure 4, A and B). Although no notable change was observed in the total mBC population (data not shown), there was a decreasing trend in $CD38^+$ switched mBCs (Figure 4C, $P = 0.069$) and an increase in $CD38^-$ mBCs (Figure 4D, $P = 0.006$ and adjusted P value $= 0.069$). A significant decrease in NK cells was observed at C3D1 compared with BL ($P < 0.001$ and adjusted P value $= 0.005$), which is mainly driven by the depletion of $CD38^+$ NK cells as no significant change was detected in the $CD38^-$ NK cell population at all time points. No notable decreases in the total Treg population were observed, as a decrease in $CD38^+$ Tregs was compensated by an increase of the $CD38^-$ Treg compartments (Figure 4, E–G). No notable changes in other T-cell subset data were observed during the study follow-up (data not shown).

The functional analyses of circulating HLA-specific mBCs revealed that treatment with isatuximab significantly reduced the frequencies of both class I and class II HLA-specific IgG-secreting mBCs, which was mostly achieved after receiving the total eight doses (Figure 5). Furthermore, a drastic reduction of HLA-specific IgG-producing bone marrow–residing plasma cells specific against classes I and II HLA antigens was also observed after three cycles of isatuximab therapy (Figure 6, A and B). Representative images of mBCs HLA-sp FluoroSpot before and after treatment are illustrated in Supplemental Figure 4. The analysis of the relationship between reduction of anti-HLA antibodies MFI from the sera and the respective HLA-sp mBC revealed that while 24.5% (12/49) of HLA antibody specificities were reduced in both compartments and 12% (6/49) were still concomitantly present both in the sera and by mBCs, up to 51% (25/49) of the Abs that were reduced in serum were unchanged in the functional in vitro assay by circulating mBC, harboring the same HLA specificity.

DISCUSSION

This study was designed to investigate whether the anti-CD38 therapy, isatuximab, has the potential to be an effective desensitization therapy, addressing a therapeutic gap left by currently available regimens. The study consisted of two cohorts, representing patients who may potentially benefit from desensitization.

Patients in cohort A represent the largest proportion of patients under the US KAS within the 100% cPRA category, with a significantly lower transplant rate and who are unlikely to undergo transplantation within a reasonable timeframe. Cohort B patients were those with cPRA 80.0%–99.89% who also receive prioritization allocation points. Despite this, the median cPRA in cohort B was similar to cohort A. BL characteristics also showed patients were on the waiting list for a kidney transplant for years (median 5.3 years, range 0.6–12.9) despite the recent advances in the field, demonstrating that there is still significant unmet clinical need under the current KAS.

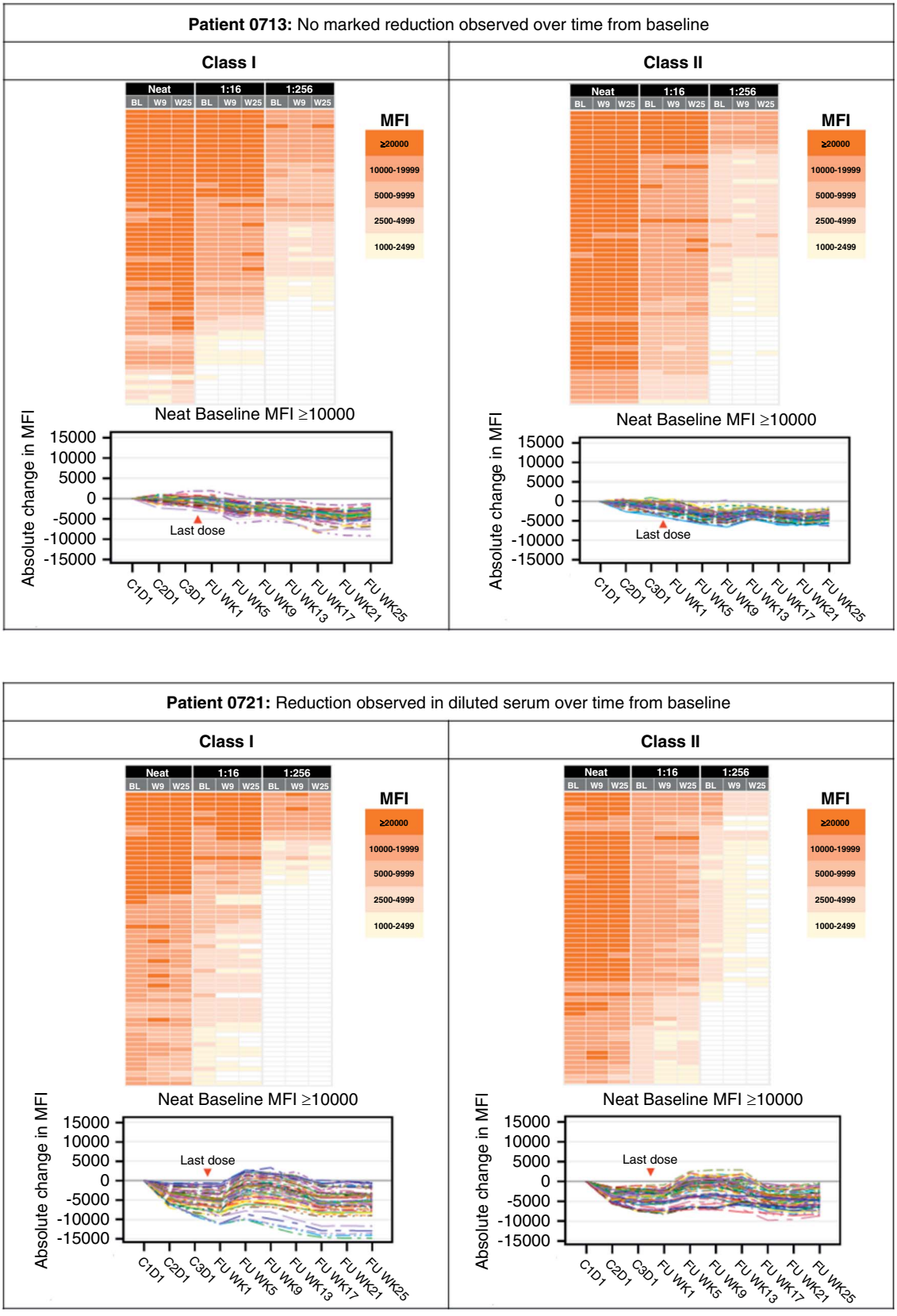

Figure 2. Examples of patients who were responders per protocol but did not meet target cPRA in criterion 1. Class I and class II anti-HLA antibody heat maps illustrate the MFI values of the top 60 HLA alleles (from the top, in descending order on the basis of their

Figure 2. *(Continued)* BL titer) at neat, 1:16, and 1:256 dilutions. Each dilution includes BL, W9 (approximately 9 weeks after the last dose), W25 (approximately 25 weeks after the last dose). Spaghetti plots illustrate the absolute change in MFIs in neat serum of anti-HLA antibodies with BL MFI ≥10,000. BL, baseline; C, cycle; cPRA, calculated panel reactive antibody; D, day; FUP, follow-up; MFI, mean fluorescence intensity; N, neat; WK, week.

Isatuximab monotherapy was well tolerated with a good safety profile in kidney transplant candidates, with a grade 1–2 infusion reaction rate of approximately 21%. No treatment-related infections were reported throughout the study, although the risk of hypogammaglobulinemia has been raised as a concern with anti-CD38 therapy due to CD38 being expressed in normal plasma cells, and as seen with the nonspecific elimination of IgG and IgM (Figure 2).[22,23]

A comprehensive population PK analysis in patients with relapsed/refractory multiple myeloma did not identify any effect of renal impairment on isatuximab PK.[24] In this study, where renal function was even worse due to majority of patients being on dialysis, isatuximab PK exposure was comparable with those from other studies.[25–27] Overall, these results complement the previous analyses mentioned above, showing no effect of renal impairment on isatuximab PK exposure. These results were expected as isatuximab is a monoclonal antibody, and thus a large molecule, and is eliminated by catabolism.

The data presented here support isatuximab's mechanism of action and achievement of target engagement *via* a sustained decrease in Ig levels. A decrease in peripheral $CD38^+$ plasmablasts and plasma cells was observed, supported by a robust and sustained decrease of Ig levels after treatment was stopped for >17 weeks. In addition, HLA-specific IgG antibodies produced by circulating mBCs were also partially reduced, a finding that may account for the depleting effect of isatuximab to the mature class-switched mBC subset compartment expressing CD38. However, the peripheral cell population was small, and data should be interpreted cautiously. Notably, a significant reduction in HLA-specific IgG antibody production of bone marrow–residing plasma cells was observed in patients with evaluable bone marrow aspirates, highlighting the efficacy of isatuximab in targeting this central lymphoid compartment.

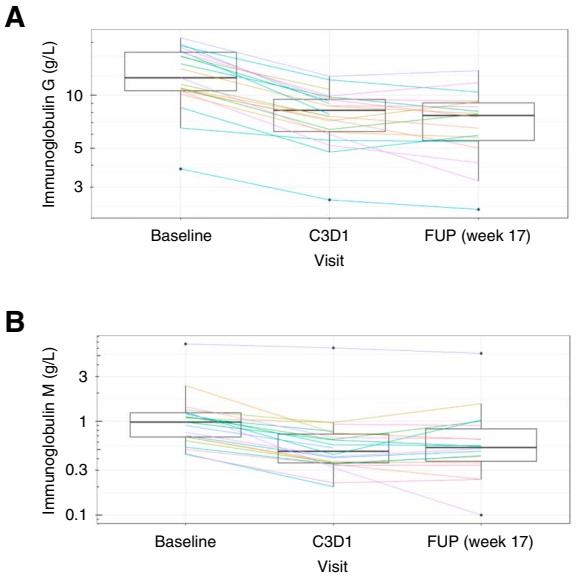

Figure 3. Sustained decrease in total IgG and IgM levels up to last analyzed timepoint. Evolution of (A) total IgG levels and (B) total IgM levels.

Prior publications investigating daratumumab hypothesized anti-CD38 treatment decreases Treg cells. In rhesus macaques with two sequential mismatched skin allografts desensitized with daratumumab and plerixafor before transplant, DSA levels were significantly reduced but this reduction was not maintained as all recipients showed a rapid rebound of antibodies, experienced T-cell–mediated rejection (TCMR), and developed rejection within 30 days of transplantation.[10] Jordan *et al.* reported a case of a patient treated with daratumumab for standard-of-care resistant ABMR—ABMR resolved with minimal AEs, with significant reductions in circulating HLA class I and reductions in HLA class II, but the patient developed TCMR.[28]

In this study, no notable changes implicated in transplant rejection were observed in NK cells and T cells. Sufficient data are lacking to suggest whether the risk of TCMR was increased after treatment with isatuximab. However, no TCMR was observed in the transplanted patients treated with isatuximab as of study cut-off date. One patient transplanted approximately 15 months after the last dose of isatuximab (after study cut-off date) experienced acute rejection that was successfully treated with plasmapheresis and intravenous Ig, where the pathologic diagnosis includes mixed acute ABMR and TCMR due to the presence of interstitial infiltrate and tubulitis in addition to severe peritubular capillaritis with diffuse C4d staining.

Isatuximab demonstrated a durable decrease in anti-HLA antibodies, which appeared to be persistent during site visit follow-up period after stopping treatment (approximately 26 weeks after the last dose). Isatuximab also demonstrated partial desensitization activity by eliminating or lowering the titer of some antibodies, with minimal effect on the overall cPRA values. Nevertheless, Schinstock *et al.* show that a mild reduction in cPRA to 99.50%–99.89% may drastically increase the probability of transplant on the based on the current KAS.[29] The minimal decrease in cPRA values is unsurprising as most broadly sensitized patients often have

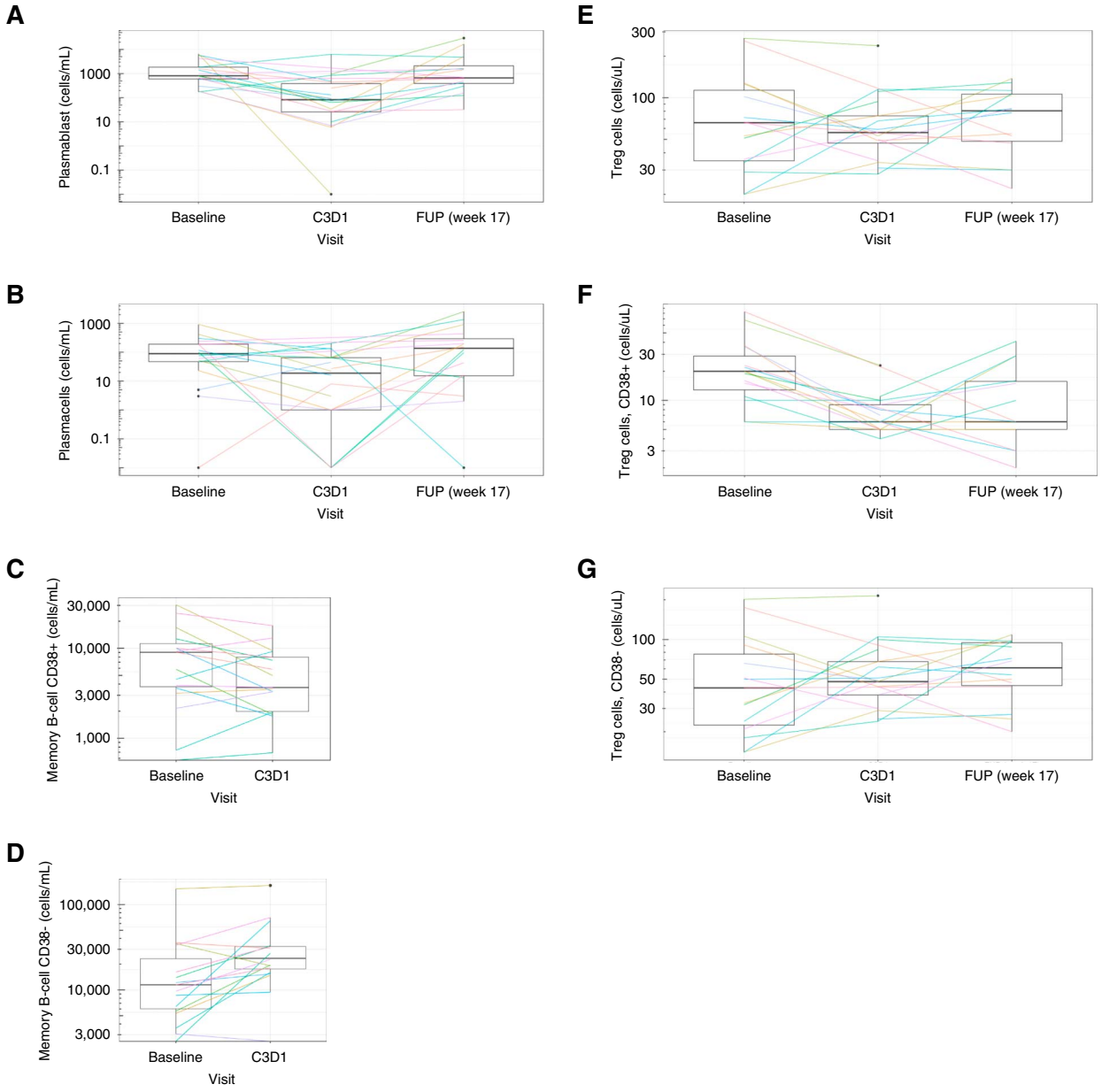

Figure 4. No notable changes were observed in the total mBC or total Treg population, although decreases in plasmablasts and plasma cells were observed at C3D1 that returned to baseline by week 17 after last dose. (A) Plasmablasts, (B) plasma cells, (C) $CD38^{+}$ mBCs, (D) $CD38^{-}$ mBCs, (E) overall Treg cells, (F) $CD38^{+}$ Treg cells, and (G) $CD38^{-}$ Treg cells over isatuximab treatment from BL to follow-up at week 17* *Plotted with logarithmic scale for ease of visualization. "0" values were replaced with "0.01." mBC, memory B cell.

high titers of HLA antibody. As demonstrated in this study, desensitization activity cannot be reflected through cPRA values alone. Examining antibody titer reduction across the entire anti-HLA antibody profile of each patient provides a better assessment of desensitization efficacy. Therefore, a composite end point that included antibody elimination, titer reduction, and cPRA reduction proposed in this study may be more suitable for assessing desensitization therapies. The proposed criteria account for and minimize potential assay variability. For example, in criterion 3, only a reduction of antibodies to MFI <2000 from a BL of ≥ 3000 would be considered as antibody elimination on the basis of a potential 25% assay variability.[30]

Patients in this study received a limited number of doses of isatuximab and were subsequently followed up for 26 weeks on anti-HLA antibody level and immune cell profiling. The short treatment period and long follow-up duration design intended to explore the temporal mechanisms of antibody rebound if it occurred. A desired desensitization treatment regimen with isatuximab may then be adjusted based on the observed data. It is possible to administer isatuximab while the patient is on the waiting

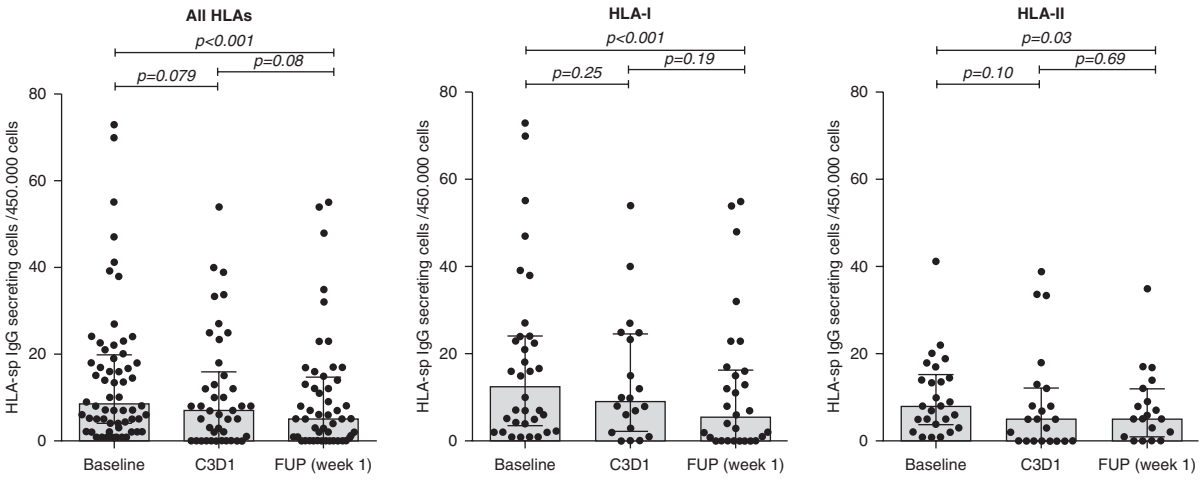

Figure 5. Boxplots of mBC function. Median IgG-secreting HLA-sp mBCs against all HLA antigens at distinct time points were 8.5 (4–19.75) at BL, 7 (0–15.7) at C3D1 and 5 (0.5–14.5) at FUP week 1, against class I HLA antigens were 12.5 (3.5–24) at BL, 9 (2.25–24.5) at C3D1, and 5.5 (0–16.3) at FUP week 1 and against class II HLA antigens were 8 (3.8–15.2) at BL, 5 (0–12.3) at C3D1, and 5 (1–12) at FUP week 1. ns, nonsignificant.

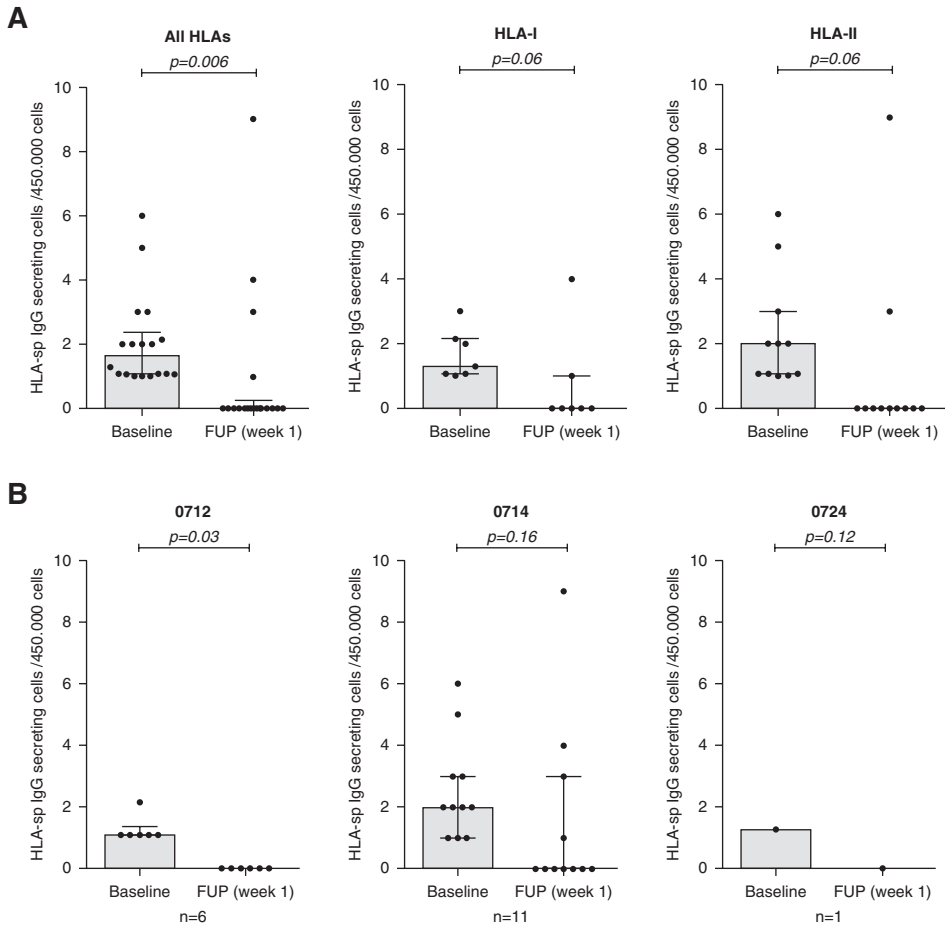

Figure 6. Boxplots of HLA-specific IgG-producing bone marrow–residing plasma cells. (A) Median IgG-secreting plasma cell frequencies at two distinct time points against all HLA antigens in the three tested patients were 1.6 (1.1–2.4) at BL and 0 (0–0.25) at FUP week 1, against HLA-I antigens were 1.3 (1.1–2.1) at BL and 0 (0–1) at FUP week 1, and against HLA-II antigens were 2 (1.1–3) at BL and 0 (0–0) at FUP week 1. (B) The median IgG-secreting plasma cell frequencies at BL and FUP week 1 in patient 0712 was 1.1 (1.1–1.3) versus 0 (0–0), in patient 0714 was 2 (1–3) versus 0 (0–3), and in patient 0724 was 1.28 versus 0.

list for a deceased donor and start retreatment as needed to maintain their desensitized status. This may be as frequent as every 6 months on the basis of the available biomarker data indicating when plasma cells and plasmablasts start to return to BL levels. However, further study will be required to optimize the retreatment frequency.

Isatuximab may be further investigated as an option for adjunct therapy to existing desensitization therapy for patients on the kidney transplant waiting list to provide better durable responses due to its well-tolerated safety profile. For instance, isatuximab could be combined with a therapy that targets the mBC or germinal center B-cell compartments that do not express CD38, such as anti-CD20 antibody or belatacept, a fusion receptor protein that inhibits T-cell activation.[31] As isatuximab targets plasma cells and does not target circulating anti-HLA antibodies, time is needed for the desensitization effect to be observed, considering the half-life of Ig. Combining isatuximab with an initial session of plasmapheresis or imlifidase may be an effective strategy to achieve fast and durable desensitization. However, these combinations need further clinical investigation, particularly to ascertain if there are adverse interactions with isatuximab. There is also potential for further investigation of the prevention or treatment of ABMR as an adjunct therapy on the basis of the durable plasma cell depletion and DSA suppression observed in this study.

This study is a biomarker-based mechanistic study investigating whether isatuximab monotherapy can durably decrease titers of anti-HLA antibody. Although there were patients transplanted during the study, these cannot be clearly attributed to isatuximab treatment, which needs to be tested in a randomized controlled study. However, whereas transplantability may seem to be a clinically meaningful measure of direct clinical benefit, there are intrinsic biases associated with patient selection, donor availability, different organ allocation policies across countries and regions, skill and aggressiveness of a transplant center, and difference in crossmatch positivity cutoff across transplant centers. It would also be challenging to test this hypothesis in a randomized controlled study in patients waiting for a deceased donor using transplant rate as the primary end point due to confounding factors, such as donor availability, varying criteria on donor crossmatch, and inconsistent use of desensitization regimens across transplant centers.

In summary, isatuximab was well tolerated in kidney transplant candidates, and monotherapy demonstrated a durable decrease in anti-HLA antibodies, with partial desensitization activity. The durable decrease in the anti-HLA antibody level observed was accompanied by reduction in alloantibody production sources (including long-lived plasma cells and in mBC function) after isatuximab treatment. The ultimate benefit of isatuximab as a monotherapy or as potential adjunct therapy in facilitating transplantation from previous incompatible donors will require corroboration from future controlled trials.

DISCLOSURES

G. Abbadessa also reports Patents or Royalties: Sanofi; and Advisory or Leadership Role: Biond Biologics and HiFi Bio. G. Abbadessa, M. Dudek, H. Lee, L. Lépine, J.-A. Paul, D. Semiond, and N. Ternes are Sanofi employees and may hold stock/stock options in the company. N. Ali reports institutional funding for the present study; and Research Funding: Regeneron and Sanofi—clinical trials, PI. O. Bestard reports a grant from Sanofi for the memory B-cell experiments; and Patents or Royalties: Oxford Immunotec. J.M. Cruzado reports consulting fees: Diaverum; payment or honoraria: Chiesi and Vifor; participation on a Data Safety Monitoring Board or Advisory Board: Astra Zeneca, Chiesi, MSD, Sanofi, and Vifor; he serves as an Associate Editor for the *American Journal of Transplantation* and *Clinical Kidney Journal*. A.O. Gaber reports support for the present manuscript by Sanofi; Grants or contracts: Hansa Pharma, Novartis, and Veloxis; he serves on the American Society of Transplant Surgeons Council to which Sanofi provides meeting and educational support. A.O. Gaber also reports Ownership Interest: adaptive immune therapy, Eli Lilly, Novonordisk, oil companies and financials, and Technology; Research Funding: Amplyx Therapeutics, Angion, CareDx, Hansa Biopharma, Medior Therapeutics, Novartis, Sanofi, and Veloxis; Honoraria: Europhins, Optum Health, Sanofi, and Veloxis; Patents or Royalties: Research patent pending, developed in my lab; and Other Interests or Relationships: Chairman of BOD for Nora's Gift Foundation. R.A. Montgomery reports Consultancy: CareDx, Genzyme, Hansa Medical, Indyairone, Sanofi-Aventis, and Veloxis Pharmaceuticals; Research Funding: Hansa Biopharma and United Therapeutics; Honoraria: CareDx, Genzyme, Hansa Medical, Indyairone, Sanofi-Aventis, and Veloxis Pharmaceuticals; and Advisory or Leadership Role: CareDx, Hansa Biopharma, Genzyme, and Sanofi-Aventis US. J.-A. Paul also reports Ownership Interest: Amazon, Apple, Pfizer, and Tesla. D. Semiond reports Employer: Sanofi; Ownership Interest: Sanofi; and Patents or Royalties: Sanofi. D. Seron reports Sanofi support for this clinical trial, including medical writing support. M. Stegall reports consulting fees from Sanofi. M. Stegall reports Consultancy: Aiosyn, eGenesis, Hansa, and Novartis; Research Funding: Janssen, Talaris, and Veloxis; and Advisory or Leadership Role: Aisosyn and eGenesis. A.R. Tambur reports consulting fees from Sanofi; Honoraria: Thermo Fisher/One Lambda; Advisory or Leadership Role: Sanofi, and was also the central laboratory for this clinical trial; and Speakers Bureau: Thermo-Fisher. A. Torija reports a grant from Sanofi for the memory B-cell experiments. F. Vincenti reports grants or contracts from Sanofi. F. Vincenti also reports Research Funding: Angion, Astellas, CSL Behring, Merck, Novartis, Pfizer, and Viela Bio; and Honoraria: Sanofi and Veloxis. All remaining authors have nothing to disclose.

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Writing – review & editing: Giovanni Abbadessa, Nicole Ali, Oriol Bestard, Amarpali Brar, Josep M. Cruzado, Markus Dudek, A. Osama Gaber, Lucie Lépine, Helen Lee, Robert A. Montgomery, Jo-Anne Paul, Dorothée Semiond, Daniel Seron, Ruby J. Siegel, Mark Stegall, Anat R. Tambur, Nils Ternes, Alba Torija, Flavio Vincenti.

DATA SHARING STATEMENT

All data is included in the manuscript and/or supporting information.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://links.lww.com/JSN/E568.

Supplemental Appendix 1. Desensitization response criteria.

Supplemental Table 1. HLA specificities assessed in circulating memory B cells and bone marrow plasma cells with the HLA-specific B-cell FluoroSpot in each patient.

Supplemental Table 2. Summary of safety by cohort.

Supplemental Table 3. Summary of pharmacokinetics of isatuximab by cohort after the first administration at a dose of 10 mg/kg.

Supplemental Table 4. Summary of number of anti-HLA antibody with baseline MFI ≥3000 reduced to <2000 as measured in a SAB assay (using central laboratory assessment).

Supplemental Table 5. Summary of pharmacodynamic changes from baseline to post-treatment.

Supplemental Figure 1. Examples of responder patients per protocol who met all three criteria.

Supplemental Figure 2. Examples of responder patients per protocol that did not meet target cPRA in criterion 1.

Supplemental Figure 3. Examples of nonresponder patients per protocol that met zero of three criteria.

Supplemental Figure 4. Representative images of mBCs and bone marrow plasma HLA-sp FluoroSpot before and after treatment.

REFERENCES

1. Noble J, Jouve T, Malvezzi P, Rostaing L. Desensitization in crossmatch-positive kidney transplant candidates. *Transplantation*. 2023;107(2):351–360. doi:10.1097/TP.0000000000004279
2. Gebel HM, Kasiske BL, Gustafson SK, et al. Allocating deceased donor kidneys to candidates with high panel-reactive antibodies. *Clin J Am Soc Nephrol*. 2016;11(3):505–511. doi:10.2215/CJN.07720715
3. Sypek MP, Kausman JY, Watson N, et al. The introduction of cPRA and its impact on access to deceased donor kidney transplantation for highly sensitized patients in Australia. *Transplantation*. 2021;105(6):1317–1325. doi:10.1097/TP.0000000000003410
4. Jordan SC, Choi J, Vo A. Kidney transplantation in highly sensitized patients. *Br Med Bull*. 2015;114(1):113–125. doi:10.1093/bmb/ldv013
5. Kumar V, Locke J. New perspectives on desensitization in the current era - an overview. *Front Immunol*. 2021;12:696467. doi:10.3389/fimmu.2021.696467
6. Heidt S, Haasnoot GW, van der Linden-van Oevelen MJH, Claas FHJ. Highly sensitized patients are well served by receiving a compatible organ offer based on acceptable mismatches. *Front Immunol*. 2021;12:687254. doi:10.3389/fimmu.2021.687254
7. Jordan SC, Legendre C, Desai NM, et al. Imlifidase desensitization in crossmatch-positive, highly sensitized kidney transplant recipients: results of an international phase 2 trial (Highdes). *Transplantation*. 2021;105(8):1808–1817. doi:10.1097/TP.0000000000003496
8. European Medicines Agency. *Idefirix*; 2023. Accessed March 24, 2023. https://www.ema.europa.eu/en/medicines/human/EPAR/idefirix
9. D'Agostino M, Innorcia S, Boccadoro M, Bringhen S. Monoclonal antibodies to treat multiple myeloma: a dream come true. *Int J Mol Sci*. 2020;21(21):8192. doi:10.3390/ijms21218192
10. Kwun J, Matignon M, Manook M, et al. Daratumumab in sensitized kidney transplantation: potentials and limitations of experimental and clinical use. *J Am Soc Nephrol*. 2019;30(7):1206–1219. doi:10.1681/ASN.2018121254
11. US National Library of Medicine. *Desensitization in Kidney Allograft Using Daratumumab (DARDAR)*. 2023. Accessed March 24, 2023. https://clinicaltrials.gov/ct2/show/NCT04204980
12. US National Library of Medicine. *Study of Daratumumab for Decreasing Circulating Antibodies in Sensitized Patients Awaiting Heart Transplantation*. 2020. Accessed March 24, 2023. https://clinicaltrials.gov/ct2/show/NCT04088903
13. Curtis A, Guha A, Bhimaraj A, et al. Use of daratumumab for desensitization prior to cardiac transplantation: a case report. *J Heart Lung Transplant*. 2021;40(4):S493. doi:10.1016/j.healun.2021.01.2015
14. Ibrahim U, Keyzner A. Daratumumab for donor-specific anti-HLA antibody desensitization in a case of HLA-mismatched allogeneic stem cell transplantation. *Hematol Transfus Cell Ther*. 2023;45(4):510–512. doi:10.1016/j.htct.2021.11.019
15. Sanofi Sarclisa. *Prescribing Information*. 2020. Accessed March 20, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761113s000lbl.pdf
16. Deckert J, Wetzel MC, Bartle LM, et al. SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent antitumor activity in models of multiple myeloma and other CD38+ hematologic malignancies. *Clin Cancer Res*. 2014;20(17):4574–4583. doi:10.1158/1078-0432.CCR-14-0695
17. Jiang H, Acharya C, An G, et al. SAR650984 directly induces multiple myeloma cell death via lysosomal-associated and apoptotic pathways, which is further enhanced by pomalidomide. *Leukemia*. 2016;30(2):399–408. doi:10.1038/leu.2015.240
18. Moreno L, Perez C, Zabaleta A, et al. The mechanism of action of the anti-CD38 monoclonal antibody isatuximab in multiple myeloma. *Clin Cancer Res*. 2019;25(10):3176–3187. doi:10.1158/1078-0432.CCR-18-1597
19. Kransdorf EP, Pando MJ. Calculated panel reactive antibody with decimals: a refined metric of access to transplantation for highly sensitized candidates. *Hum Immunol*. 2017;78(3):252–256. doi:10.1016/j.humimm.2016.12.009
20. Luque S, Lucia M, Crespo E, Jarque M, Grinyó JM, Bestard O. A multicolour HLA-specific B-cell FluoroSpot assay to functionally track circulating HLA-specific memory B cells. *J Immunol Methods*. 2018;462:23–33. doi:10.1016/j.jim.2018.07.011
21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series*

B Stat Methodol. 2018;57(1):289–300. doi:10.1111/j.2517-6161.1995.tb02031.x
22. Korst C, van de Donk N. Should all newly diagnosed MM patients receive CD38 antibody-based treatment? *Hematol Am Soc Hematol Educ Program*. 2020;2020(1):259–263. doi:10.1182/hematology.2020000161
23. Lancman G, Sastow D, Aslanova M, et al. Effect of intravenous immunoglobulin on infections in multiple myeloma (MM) patients receiving daratumumab. *Blood*. 2020;136(suppl 1):6–7. doi:10.1182/blood-2020-140056
24. Fau JB, El-Cheikh R, Brillac C, et al. Drug-disease interaction and time-dependent population pharmacokinetics of isatuximab in relapsed/refractory multiple myeloma patients. *CPT Pharmacometrics Syst Pharmacol*. 2020;9(11):649–658. doi:10.1002/psp4.12561
25. Carlo-Stella C, Zinzani PL, Sureda A, et al. A phase 1/2, open-label, multicenter study of isatuximab in combination with cemiplimab in patients with lymphoma. *Hematol Oncol*. 2023;41(1):108–119. doi:10.1002/hon.3089
26. Simonelli M, Garralda E, Eskens F, et al. Isatuximab plus atezolizumab in patients with advanced solid tumors: results from a phase I/II, open-label, multicenter study. *ESMO Open*. 2022;7(5):100562. doi:10.1016/j.esmoop.2022.100562
27. Zucali PA, Lin CC, Carthon BC, et al. Targeting CD38 and PD-1 with isatuximab plus cemiplimab in patients with advanced solid malignancies: results from a phase I/II open-label, multicenter study. *J Immunother Cancer*. 2022;10(1):e003697. doi:10.1136/jitc-2021-003697
28. Jordan S, Vescio R, Toyoda M, et al. *Daratumumab for Treatment of Antibody-Mediated Rejection in a Kidney Transplant Recipient*. American Transplant Congress; 2019.
29. Schinstock CA, Smith BH, Montgomery RA, et al. Managing highly sensitized renal transplant candidates in the era of kidney paired donation and the new kidney allocation system: is there still a role for desensitization? *Clin Transplant*. 2019;33(12):e13751. doi:10.1111/ctr.13751
30. Reed EF, Rao P, Zhang Z, et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. *Am J Transplant*. 2013;13(7):1859–1870. doi:10.1111/ajt.12287
31. Klasener K, Jellusova J, Andrieux G, et al. CD20 as a gatekeeper of the resting state of human B cells. *Proc Natl Acad Sci U S A*. 2021;118(7):e2021342118. doi:10.1073/pnas.2021342118

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APPENDIX III

DICTAMEN DEL COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CON MEDICAMENTOS (CEIm) PARA SOLICITUD INICIAL

El Dr. Enric Sospedra Martínez, Secretario del Comité de Ética de la Investigación con Medicamentos del Hospital Universitari de Bellvitge.

CERTIFICA

Que el El Comité de Ética de la Investigación con medicamentos del Hospital Universitari de Bellvitge, en su reunión de fecha 12/03/2020 (Acta 05/20), ha evaluado la siguiente documentación presentada por el Promotor Sanofi-Aventis Recherche & Developpement, para el ensayo clínico con nuestra ref. **AC050/19**, titulado:

ESTUDIO DE FASE 1B/2 PARA EVALUAR LA SEGURIDAD, LA FARMACOCINÉTICA Y LA EFICACIA PRELIMINAR DE ISATUXIMAB (SAR650984) EN PACIENTES EN ESPERA DE TRASPLANTE RENAL, código **TED16414**, Núm. EudraCT **2019-004154-28**

Documentos con versiones:

Protocolo	Versión 1, 07-Nov-2019
Hoja de Información al Paciente y Consentimiento Informado	[Versión inglesa nº 1]. Versión 1.1 (castellano), de fecha 13/02/2020
Hoja de Información al Paciente y Consentimiento Informado	SEGUIMIENTO DEL EMBARAZO DE LA PAREJA DE UN PACIENTE VARÓN [Versión inglesa nº 1]. Versión 1.1 (castellano), de fecha 13/02/2020
Memoria económica_CEIm	Versión 2 del 13/02/2020
Manual del Investigador	Edition: 10 Amendment 1, Date 14-Oct-2019

Y considera que:

- El procedimiento para obtener el consentimiento informado (incluyendo las hojas de información al sujeto de ensayo y consentimientos informados mencionados en el encabezamiento), y el plan de reclutamiento de sujetos previsto son adecuados y cumplen con los requisitos para la obtención del consentimiento informado previstos en el capítulo II del Real Decreto 1090/2015.
- Las compensaciones previstas a los participantes son adecuadas, así como las previsiones de indemnización por daños y perjuicios que pueda sufrir el participante.
- El procedimiento previsto para el manejo de datos personales es adecuado.
- El uso futuro de las muestras biológicas obtenidas durante el ensayo se adecua a lo previsto en el Real Decreto 1716/2011.

- Para la realización del ensayo se consideran adecuados los centros e investigadores previstos en el anexo II a este dictamen, teniendo en cuenta las declaraciones de idoneidad emitidas por el promotor y por los responsables de las instituciones correspondientes.

Tras estudiar toda la documentación presentada, este CEIm ha acordado otorgar

DICTAMEN FAVORABLE

Que en dicha reunión se cumplieron los requisitos establecidos en la legislación vigente –Real Decreto 1090/2015 – para que la decisión del citado CEIm sea válida.

Que el CEIm del Hospital Universitari de Bellvitge, tanto en su composición como en sus procedimientos, cumple con las normas de BPC (CPMP/ICH/135/95) y con la legislación vigente que regula su funcionamiento, y que la composición del CEIm del Hospital Universitari de Bellvitge, es la indicada en el anexo I.

Que en dicha reunión del El Comité de Ética de la Investigación con medicamentos se cumplió el quórum preceptivo legalmente.

Que no se ha declarado ningún conflicto de interés por parte de ninguno de los miembros del comité que impida su participación en la evaluación y dictamen de la solicitud de autorización de al ensayo clínico.

Lo que firmo en L'Hospitalet de Llobregat a 12 de marzo de 2020

SOSPEDRA MARTINEZ ENRIQUE - 36986426B

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Dr. Enric Sospedra Martínez
Secretario
CEIm Hospital Universitari de Bellvitge

ANEXO I

COMPOSICION DEL CEIm

Presidente	Dr. Francesc Esteve Urbano	Médico - Medicina Intensiva
Vicepresidenta	Dra. Pilar Hereu Boher	Médico - Farmacología Clínica
Secretario	Dr. Enric Sospedra Martínez	Farmacéutico - Farmacia Hospitalaria
Vocales:	Dr. Jordi Adamuz Tomás	Enfermero - Enfermería
	Dra. Concepción Cañete Ramos	Médico - Neumología
	Dr. Enric Condom Mundo	Médico - Anatomía Patológica
	Sra. Consol Felip Farrás	Miembro Laico - Docencia
	Dr. José Luis Ferreiro Gutiérrez	Médico - Cardiología
	Dra. Ana María Ferrer Artola	Farmacéutica - miembro sanitario
	Dr. Xavier Fulladosa Oliveras	Médico - Nefrología
	Dra. Margarita García Martín	Médico - Oncología Médica
	Dr. Carles Lladó i Carbonell	Médico - Urología
	Dr. Josep Manel Llop Talaveron	Farmacéutico – Farmacia Hospitalaria
	Sra. Sonia López Ortega	Graduado Social - Atención a la Ciudadanía
	Dr. Sergio Morchón Ramos	Médico - Medicina Preventiva
	Dr. Joan Josep Queralt Jiménez	Jurista
	Dra. Gemma Rodríguez Palomar	Farmacéutica – Atención Primaria
	Dra. Nuria Sala Serra	Bióloga - miembro no sanitario
	Dr. Petru Cristian Simon	Médico - Farmacología Clínica
	Dra. Laura Villagrasa Álvarez	Derecho – DPD

ANEXO II

CENTROS E INVESTIGADORES PRINCIPALES PARTICIPANTES EN ESPAÑA

AC050/19 ESTUDIO DE FASE 1B/2 PARA EVALUAR LA SEGURIDAD, LA FARMACOCINÉTICA Y LA EFICACIA PRELIMINAR DE ISATUXIMAB (SAR650984) EN PACIENTES EN ESPERA DE TRASPLANTE RENAL, código **TED16414**, Núm. EudraCT **2019-004154-28**

Investigador principal	**Centro de realización del estudio**
Dr. Daniel Serón Micas	Hospital Universitari Vall d'Hebron
Dr. Oriol Bestard Matamoros	Hospital Universitari de Bellvitge

Fecha actualización Anexo II: 12/03/2020

INFORME DEL COMITÉ DE ÉTICA DE INVESTIGACIÓN CON MEDICAMENTOS Y COMISIÓN DE PROYECTOS DE INVESTIGACIÓN DEL HOSPITAL UNIVERSITARI VALL D'HEBRON

Sra. Mireia Navarro Sebastián, Secretaria del COMITÉ DE ÉTICA DE INVESTIGACIÓN CON MEDICAMENTOS del Hospital Universitari Vall d'Hebron,

CERTIFICA

Que el Comité de Ética de Investigación con Medicamentos del Hospital Universitario Vall d'Hebron, en el cual la Comisión de proyectos de investigación está integrada, se reunió en sesión ordinaria nº 501 el pasado 17/09/2021 y evaluó el proyecto de investigación **PR(AG)485/2021** cuyo promotor Vall d'Hebron Institut de Recerca (VHIR), titulado *"Sub-estudi projecte FIS (PI19/01710): "Avaluació funcional de la resposta de memòria cel.lular i humoral donant-específica mitjançant tècniques de diagnòstic cel.lular""* que tiene como investigador principal al Dr. Oriol Bestard Matamoros del Servicio de Nefrología de nuestro Centro.

Versión de documentos

Memoria de Proyecto	versión 1.0 del 11/08/2021
HIP/CI	versión 2.0 del 13/07/2021
Resumen en español de Protocolo	versión con fecha 04/08/2021

El resultado de la evaluación fue el siguiente:

Aprobado

El Comité tanto en su composición como en los PNT cumple con las normas de BPC (CPMP/ICH/135/95) y con el Real Decreto 1090/2015, y su composición actual es la siguiente:

Presidente:
- SOLEDAD GALLEGO MELCÓN - Médico

Vicepresidente:
- JOAN SEGARRA SARRIES - Abogado

Secretario:
- MIREIA NAVARRO SEBASTIAN - Química

Vocales:
- LLUIS ARMADANS GIL - Médico
- FERNANDO AZPIROZ VIDAUR - Médico

- VALENTINA BALASSO - Médico
- INES M DE TORRES RAMÍREZ - Médico
- ELADIO FERNÁNDEZ LIZ - Farmacéutico Atención Primaria
- INMACULADA FUENTES CAMPS - Médico Farmacólogo
- JAUME GUARDIA MASSÓ - Médico
- JUAN CARLOS HORTAL IBARRA - Profesor de Universidad
- MARIA LUJAN IAVECCHIA - Médico Farmacólogo
- ALEXIS RODRIGUEZ GALLEGO - Médico Farmacólogo
- JUDITH SANCHEZ RAYA - Médico
- MARTA SOLÉ ORSOLA - Personal de Enfermería
- PILAR SUÑÉ MARTÍN - Farmacéutica Hospital
- VÍCTOR VARGAS BLASCO - Médico
- ESTHER CUCURULL FOLGUERA - Médico Farmacólogo
- GLORIA GÁLVEZ HERNANDO - Personal de Enfermería
- ORIOL ROCA GAS - Médico
- ESPERANZA ZURIGUEL PEREZ - Personal de Enfermería
- ANA BELÉN ESTÉVEZ RODRÍGUEZ - Abogada experta en protección de datos

En dicha reunión del Comité de Ética de Investigación con Medicamentos se cumplió el quórum preceptivo legalmente.

En el caso de que se evalúe algún proyecto del que un miembro sea investigador/colaborador, éste se ausentará de la reunión durante la discusión del proyecto.

MIREIA NAVARRO SEBASTIAN

Firmado digitalmente por MIREIA NAVARRO SEBASTIAN
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Sra. Mireia Navarro

Secretaria técnica CEIM HUVH