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Novel insights in the clinical management of hyperimmune patients before and after transplantation

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ABSTRACT

Despite improvements in anti-Human Leucocyte Antigens antibody detection, identification, and characterization offer a better in peri-operative management techniques, antibodies remain a serious cause of morbidity and mortality for patients both before and after organ transplantation. Hyperimmune patients are disadvantaged by having to wait longer to receive an organ from a suitably matched donor. They could benefit from desensitization protocols in both pre- and post-transplantation period. Clinical studies are underway to highlight which best desensitization strategies could be assure the best outcome in both heart and kidney transplantation. Although most clinical evidence about desensitization strategies by using anti-CD20 monoclonal antibodies, proteasome inhibitors, anti-CD38 monoclonal antibodies, interleukin-6 blockade, cysteine protease and complement inhibitors, comes from kidney transplantation studies, many of the debated novel concepts can be easily applied to desensitization also in heart transplantation.

Here, we discuss the candidates and recipients' management by using most common standard of care and novel therapeutics, desensitization endpoints, and strategies for future studies.

1. Management of hyperimmune subject in the waiting list for organ transplantation

1.1. Definition of hyperimmune patients

Hyperimmune subjects have exceptionally high antibody levels against human leucocyte antigens (HLA) that react to foreign tissue. High anti-HLA antibody levels are harder to match for donor organs. Sensitization to allogeneic HLA occurs following a previous exposure to foreign tissue, as a prior transplant, blood transfusion or pregnancy (Heidt and Claas, 2018). Thus, each year only a low percentage of highly sensitized patients receive a transplant (Lonze, 2017). They must wait three to four times longer than unsensitized patients for a compatible deceased donor (Lonze, 2017). Indeed, the extensive immunization status precludes transplantation through regular assignment schemes for deceased donors, which are based on the exclusion of donor HLA-antigens to which the antibodies are directed (unacceptable antigens) (Ziemann et al., 2022). The immunological barrier, which is linked both to an increased risk of antibody-mediated rejection and poor graft survival, remains a major deterrent to transplant (Benincasa et al., 2022; Cozzi et al., 2017).

Even when highly sensitized patients eventually are transplanted, they are at increased risk of antibody-mediated allograft rejection, also when the crossmatch is negative at the time of transplantation (Novotný et al., 2021). This phenomenon is due to a higher alloreactivity and/or to donor-specific antibodies (DSAs) that were overlooked in the crossmatch tests or antibody screenings (Novotný et al., 2021). For this reason, it is mandatory an accurate identification and monitoring of anti-HLA antibodies for all patients awaiting transplantation (Kransdorf et al., 2017).

1.2. Anti-HLA antibodies detection

Panel-reactive antibody (PRA) testing has been widely adopted since

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the 1960s in the evaluation both of sensitization and presence of circulating antibodies (Picascia et al., 2014). PRA measures anti-human antibodies in the blood. The PRA score is expressed as a percentage, which can range from 0 to 99 percent representing the likelihood of your blood having an antibody against a particular donor (Kransdorf et al., 2017). A PRA of 20% means you have antibodies against approximately to 20% of the population. Having antibodies against foreign tissues makes it difficult to find a compatible organ donor.

In 2007, the United Network of Organ Sharing (UNOS) approved a proposal by the Histocompatibility Committee to use calculated PRA (cPRA) (Kransdorf et al., 2017). The cPRA is based on antibody specificity (not positive reactions on a panel) and it is calculated from HLA frequencies among donors (Kransdorf et al., 2017). Thus, a transplantation candidate with a cPRA of 80% would be provided to be incompatible with 80% of available donors (Kransdorf et al., 2017).

The detection and characterization of HLA antibodies have significantly improved over the past 15 years (Tait, 2016). Although today it is very easy to detect antibodies in solid-organ transplantation, the clinical significance of these antibodies is not well understood (Rosser and Sage, 2021).

The goal of this testing is to predict the presence of DSAs which can cause a positive HLA T- or B-cell crossmatch between donor and recipient (Matsumoto and Rosen-Bronson, 2021). Solid-phase immunoassays (SPIs) by using flow cytometer and/or Luminex technology are used and result in much greater sensitivity in the detection of specific HLA antibodies, especially those that are donor specific (Picascia et al., 2014, 2016; Koefoed-Nielsen and Møller, 2019).

According both to the mean fluorescence intensity (MFI) for the Luminex-based assays and the mean/median channel shift with the flow cytometer, HLA antibodies can be semi-quantified into low or high (Sullivan et al., 2017). It must be borne in mind that the antibody titer remains the true quantitative measure of the HLA antibody levels, and it is not always true that high titer corresponds to high MFI values (Sullivan et al., 2017). The limitations of SPIs are based mostly on the interpretation of the semiquantitative numeric results which requires individualized immunological risk assessment (Koefoed-Nielsen and Møller, 2019). Indeed, establishing standardized MFI cutoff values for positivity due to varying correlations between MFI, soluble dye equivalent unit molecules, antibody titers, crossmatch results and clinical outcomes still remains under debate. Even today the MFI limit values for the determination of unacceptable HLA will vary between transplant centers. In general, IgG MFI levels >1000 or 5000 are used for reporting positive results; levels >8000 or 10,000 may be corresponding to cytotoxic antibody and clinically significant.

Modifications of the SPIs by using C4d, C3d, and C1q assays are made to distinguish between complement-fixing and non–complement-fixing antibodies (Lan and Tinckam, 2018). Furthermore, SPIs false-negative results due to the presence of high concentrations of complement components and/or high IgM levels in the serum interfere with reporter binding can be minimized by using ethylene diamine tetra acetic acid (EDTA) or dithiothreitol (DDT) (Visentin et al., 2016). Other techniques to minimize the interfering effect of complement or prozone include heat inactivation or dilution (Jain et al., 2018).

1.3. Defining unacceptable antigens and predicting the Virtual Crossmatch

The use of single-antigen assays by SPIs has facilitated the prospective crossmatch obtaining a suitable donor for sensitized patients (Wade et al., 2022). For this purpose, Virtual Crossmatch (VXM) compares recipient HLA antibody detected by Luminex single antigen beads (SABs) with donor HLA. The threshold to identify the significance both of an antibody and of the corresponding unacceptable antigen can be determined from MFI and is center specific (Wade et al., 2022).

Overall, VXM requires significant HLA laboratory expertise, good knowledge of the patient HLA antibody history, and close communication between the referring HLA laboratory and the transplant center (Wade et al., 2022). It is performed electronically in a central location as a "first-pass" crossmatch to facilitate organ acquisition (Wade et al., 2022). VXM is a useful tool performed before kidney allocation since prevent the need for reallocation due to an unexpected positive prospective crossmatch related to a previously undetected HLA antibody or a false-positive reaction (Bhaskaran et al., 2022).

Therefore, it is very reliable but not foolproof in heart allocation since cold ischemia time is so short and physical crossmatches can be performed retrospectively immediately after transplant (Hsiao and Khush, 2022).

1.4. Desensitization approaches before organ transplantation

Identification of anti-HLA antibodies and desensitization strategies for reduce the antibodies titer remains a paramount goal of patient care before transplantation to prevent organ rejection and allowing a successful transplantation (Abu Jawdeh et al., 2014). Desensitization approaches are generally used to increase access to transplantation by reducing HLA antibody and the number of unacceptable antigens for listing (e.g., reduction in cPRA), or to decrease known DSAs prior to a planned positive crossmatch transplant to reduce the risk of immediate graft loss from catastrophic hyperacute rejection. They may be based on a combined strategy to reduce/remove circulating antibodies with plasmapheresis (PP) and agents that decrease the production of antibodies or block their actions (Kuppachi and Axelrod, 2020), described in detail below. Indeed, many transplant centers adopt strategies to temporarily remove circulating antibodies and/or antibody production by desensitization treatment, creating a window of opportunity for deceased or living donor organ transplantation in the presence of a negative crossmatch (Cooper, 2019). This is not always a successful solution since a rebound of antibodies often occurs and relatively high rates of antibody-mediated acute rejection and chronic rejection are observed (Keith and Vranic, 2016). However, the gold option for highly sensitized patients remains transplantation with a negative crossmatch donor without any additional therapeutic intervention.

2. Graft surveillance of the hyperimmune transplanted patients

The first few weeks following transplantation are identified as a critical clinical phase in the alloimmune response. Thus, it is required a careful monitoring to distinguish pre-transplant antibodies from *de novo* formation following transplantation (Morath et al., 2014; Kar and Bhattacharya, 2019).

According to a 2013 Transplantation Society Consensus Guideline addressing antibody management after solid organ transplantation, frequency of monitoring should be based to their pretransplant risk for antibody-mediated rejection (AMR) (Fig. 1) (Morath et al., 2014; Gilbert and Chang, 2017; Kar and Bhattacharya, 2019). Risk stratification for monitoring and treatment of patient after transplantation is often common for both kidney and heart transplanted patients (Morath et al., 2014; Gilbert and Chang, 2017; Kar and Bhattacharya, 2019).

Patients who were negative to HLA antibody before transplantation and received their first allograft are considered "low risk" patients. Thus, the screening must be carried out at least in the period from 3 to 12 months after transplantation (Morath et al., 2014; Kar and Bhattacharya, 2019).

The "intermediate risk" patients are those not sensitized at the time of transplantation but had DSAs in previous screening, thus, this group should be monitored already during the first month. Specifically, for cardiac transplantation it has been suggested to include in "intermediate risk group" also patients treated with mechanical circulatory support such as left ventricular assist device (LVAD), homografts for congenital heart defects and numerous prior childbirths (Kar and Bhattacharya, 2019; Montisci et al., 2021).

Until the first year no further testing is recommended for "low risk"

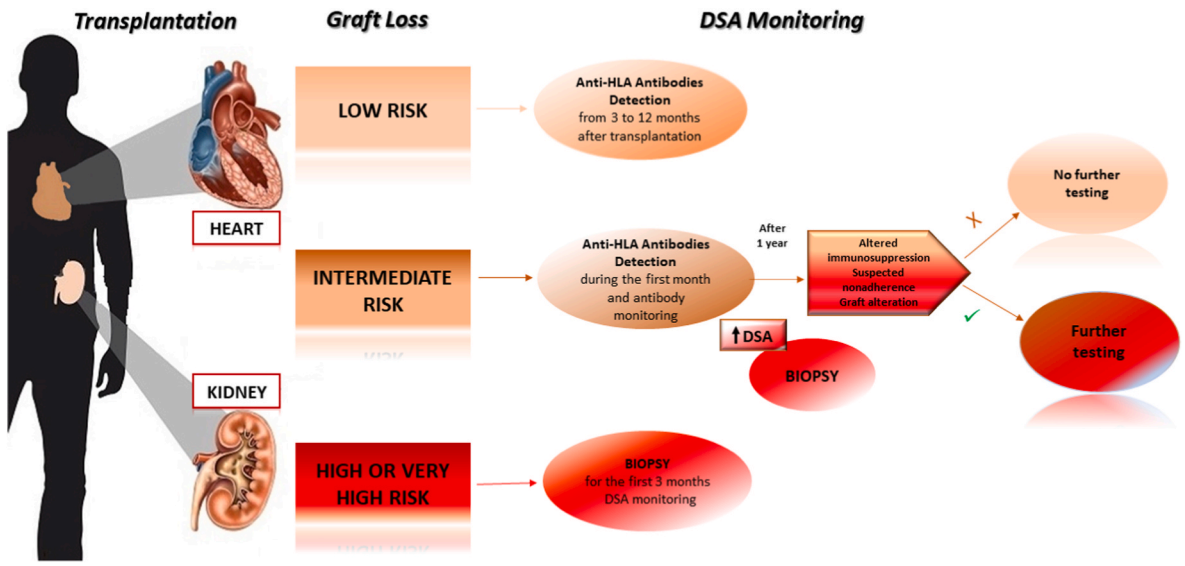

Fig. 1. Risk stratification for monitoring and treatment of patient after heart and/or kidney transplantation, Post-transplantation Group: (a) In low-risk patients DSA screening has been carried out at least once 3–12 months after transplantation. (b) Intermediate-risk patients should be screened for DSAs already during the first month. If DSA is evidenced, a biopsy should be performed. (c) In high and very high risk patients the measurement of DSAs and a biopsy is recommended for all patients for the first 3 months after transplantation. In all these groups, the recommendations for subsequent treatment are based on the biopsy results.

and "intermediate risk" groups, unless immunosuppression is altered, nonadherence is suspected, graft alteration occurs, or patient transfer to a remote outside center. A biopsy should be required if DSA is detected at any time, and if its result is positive treatment of AMR should be recommended (Morath et al., 2014; Gilbert and Chang, 2017; Kar and Bhattacharya, 2019; Napoli and Maiello, 2020; Palmieri et al., 2021).

Otherwise, in DSA-positive "high risk" patients and "very high risk" patients (desensitized crossmatch-positive), a biopsy is recommended for all patients for the first 3 months after transplantation, plus DSA screening (Morath et al., 2014; Kar and Bhattacharya, 2019). For these two groups, a rapidly DSA increasement, or a subclinical rejection evidenced by biopsy is necessary to initiate AMR treatment, especially in diabetic patients (Marfella et al., 2020). If AMR have not been evidenced, DSA should be monitored, and immunosuppression maintained at higher levels.

Although, no routine DSA monitoring is recommended for the four risk groups beyond year one, a minority of members within the guidelines group supported HLA antibody monitoring at least once a year in all patients to exclude antibody-mediated allograft injury at its earliest stage (Morath et al., 2014; Gilbert and Chang, 2017; Kar and Bhattacharya, 2019; Palmieri et al., 2021).

If DSA are observed beyond year one, patients should be treated and monitored essentially as described above during the first year following transplantation (Morath et al., 2014; Kar and Bhattacharya, 2019).

DSAs play an essential role in the development of the histological lesions defining AMR and their presence is associated with a higher risk of allograft failure (Loupy and Lefaucheur, 2018). Moreover, a significant percentage of patients who develop histological rejection do not have circulating DSAs (Senev et al., 2019).

Despite several evidence have investigated the association between antibodies against different non-HLA targets and graft failure (Delville et al., 2019; Dragun et al., 2016), the obtained results were often not reproducible and conflicting (Reindl-Schwaighofer et al., 2020). Recent evidence indicates the involvement of direct natural killer (NK) cell activation in the occurrence of microvascular inflammation in the absence of DSAs (Callemeyn et al., 2022). Moreover, it has been shown that donor-recipient HLA mismatch is at least partially involved in developing the histological presentation of AMR and its defining lesions in an HLA antibody–independent process, regardless of the way of calculating the donor-recipient HLA disparity (Senev et al., 2019, 2022; Palmieri et al., 2021). Thus, the lesions suggestive of antibody activity are not specific for antibody involvement but probably due to primary T cell activation as an initiating process (Senev et al., 2019).

Furthermore, several studies have shown that *de novo* DSAs after kidney transplantation are associated with AMR, which leads to allograft loss (Mohan et al., 2012; Jung et al., 2018). It was reported that DSAs against class II HLA are associated with a poor prognosis (Mohan et al., 2012; Wiebe et al., 2012) and that MFI values (Yamamoto et al., 2016) and complement fixing activity of DSA is correlated with the risk of AMR and allograft loss (Sicard et al., 2015).

Additionally, an increasing interest in detecting and understanding the clinical importance of non-HLA antibodies it has also been described (Kardol-Hoefnagel et al., 2021) as well as the role of angiotensin II type-1 receptor antibodies and endothelin-1 type-A receptor antibodies on AMR and graft function in kidney transplanted patients are still under investigation (Liu et al., 2022).

About heart transplantation, there was a strong association between the presence of de-novo HLA antibodies, particularly class I antibodies, and cellular rejection, although no association was found with AMR (Kobashigawa et al., 2018). Class II antibodies were highly prevalent among patients with cardiac allograft vasculopathy (CAV) and were associated with CAV at 3 years; 55% of patients with CAV had Class II antibodies compared with 14% in those who did not have CAV. There was also a strong correlation between transplant-related death and the presence of Class II antibodies (Kobashigawa et al., 2018).

However, the exact characteristics of *de novo* DSAs in terms of pathogenic capacity that are directly linked to AMR and allograft loss still remain to be established at the bedside.

3. Desensitization strategies after organ transplantation

The goal of desensitization is to reduce circulating antibodies, to increase the size of the donor pool, to prevent hyperacute rejection and to allow a successful transplantation. Although it has been evident that desensitization confers a benefit to the patients, the principal debates regard when to desensitize.

There are strategies that aim to obtain a prospective negative crossmatch and those who instead proceed with the first available organ

and subsequently mitigate the possible negative impact of post-operative DSAs (Kobashigawa et al., 2018).

Indeed, living cross-over kidney transplantation has emerged as a valuable tool to minimize immunological risks and facilitate successful transplantation (Leeser et al., 2020; Viklicky et al., 2020).

After transplantation desensitization strategies have found a wide field of applications, mainly when complement binding donor-specific antibodies lead to graft loss, compromising the transplantation outcome (Sicard et al., 2015; Choi et al., 2021).

Furthermore, these protocols sometimes fail to achieve efficient removal of all DSAs and long-term outcomes of patients with persistent DSAs are far worse when compared to non-sensitized patients (Choi et al., 2021).

3.1. Desensitization approaches in heart and kidney transplanted patients

Most common standard of care in both heart and kidney transplanted patients include intravenous immunoglobulin (IVIg), plasmapheresis (PP), semi-selective immunoadsorption (IA), and rituximab (Resse et al., 2013; Choi et al., 2021).

The extracorporeal photopheresis is a type of apheresis which is indicated in the treatment of lung, heart, and liver rejection after transplantation (Marques and Adamski, 2014; Padmanabhan et al., 2019). Its use is still debated in renal transplantation due to the lack of studies on the safety and effectiveness of the technique (Kusztal et al., 2011). Recent evidence has indicated photopheresis as a suitable treatment in reduction of antibody titer and renal failure progression in patients with chronic renal AMR, modulating the immune cellular and humoral responses, without complications (Gregorini et al., 2021; Xipell et al., 2022).

Although PP effectively removes harmful antibodies from the circulation, this technique is not specific for removing alloantibodies resulting in the reduction of all plasma proteins among them clotting factors (Rodrigo et al., 2020).

Moreover, this removal is only short-lived with antibodies rebounding to pretreatment levels following re-equilibration between intravascular and interstitial compartments (Xipell et al., 2022). Among the desensitization treatments, it is a poor treatment choice as sole therapy because the plasmapheresis does not affect ongoing antibody production by plasma cells and cause a lot of side effects as coagulopathy, hypocalcemia, thrombocytopenia, hypotension and catheter-related infection and sepsis (Rodrigo et al., 2020).

Conversely, recent study has evidenced the efficacy of anti-HLA antibody removal after PP and semi-selective IA protocols before and after organ transplantation (Sipahi et al., 2019; Jambon et al., 2021). Furthermore, also a reduction of initial MFI value in relationship with the number of PP and IA has been observed (Yamada et al.,2015; Maillard et al., 2015; Pinelli et al., 2019). Although IA, with a high number of sessions, has shown effectiveness, its high-cost limits its widespread use.

IVIg is a blood product derived from the gamma globulin fraction of plasma from pooled donors has been shown to regulate cellular immunity, including innate and adaptive components (Kobashigawa et al., 2018). IVIg-based desensitization can be divided into two general approaches: combined with alternate day PP at a low dose (100 mg/kg); or used at a high dose (1–2 g/kg). Overall, IVIg (2 g/kg) appeared more effective than PP with a better safety profile (PP required longer treatment and was associated with more infections). IVIg at a high dose (3 g/kg) in patients resistant to 2 g/kg is effective in reducing sensitization but is associated with a reversible renal insufficiency (Kobashigawa et al., 2018).

Cyclophosphamide has traditionally been used in desensitization regimens in combination with IVIg and PP, although its use has declined in recent years (Takeuchi et al., 2018).

To date, for all desensitization strategies, infectious complications and adverse side effects may limit their utility. Therefore, clinical efforts in therapeutic strategies to reduce anti-HLA antibodies PRA and to prevent AMR after organ transplantation are aimed to allow not only an efficient desensitization drug but also a better safety profile. To this purpose, standard of care and novel drugs result under investigation before and after both heart and kidney transplantation (Tables 1 and 2).

Emerging therapeutics for desensitization include anti-CD20 monoclonal antibodies aimed to deplete B cells and minimize the memory response (Macklin et al., 2014).

Rituximab has been shown to reduce the PRA increasing the rate of transplantation and has been used successfully as combination therapy with IVIg (with or without PP) both in cardiac (Jordan et al.2010; Kobashigawa et al., 2018; Starling et al.,2019) (NCT01278745) and in kidney desensitization protocols (Vo et al., 2008; Vo AA et al.,2014) (NCT01178216, NCT00642655).

3.2. Novel application in kidney transplantation

Recently, Obinutuzumab, a 3rd generation anti-CD20 monoclonal antibody, has been evaluated in desensitization protocols in kidney transplantation (NCT02586051). Until now, in kidney did not appear to be clinically meaningful (Redfield et al., 2019). Indeed, the effect of Obinutuzumab on both anti-HLA alloantibodies and cPRA appear to be limited and inconsistent (Redfield et al., 2019). In addition, patients who were considered refractory to Rituximab and IVIG were studied in a small phase I/II nonrandomized study by using the IL-6 inhibitor Tocilizumab as desensitization agent (Vo et al., 2015a,b) (NCT01594424). Tocilizumab was able to reduce immunodominant DSA score based on MFI (Vo et al., 2015a,b). A randomized, placebo-controlled multicenter clinical trial (IMAGE) of Clazakizumab, a soluble IL-6 inhibitor, for chronic active antibody mediated rejection (cAMR) is underway (NCT03744910). A pilot study by using Clazakizumab treatment on a small cohort of kidney transplanted patients with cAMR has shown a trend toward stabilization of estimated glomerular filtration rate (eGFR) and reductions in DSA and graft inflammation (Jordan et al., 2022).

Proteasome inhibitors such as Bortezomib and Carfilzomib have been showed to induce apoptosis of plasma-cells (Tremblay et al., 2020; Choi et al., 2021). In clinical studies for kidney transplantation (NCT01502267, NCT02442648), these drugs led to modest reductions in alloantibody, and were not well tolerated (Jeong et al., 2016; Woodle et al., 2015; Choi et al., 2021). In addition, their effects were transient and antibody levels returned to baseline in less than 6 months (Tremblay et al., 2020). There are several clinical studies to test the safety and efficacy of Eculizumab to prevent AMR in kidney transplantation (Table 1). Preliminary findings suggest a potential benefit for eculizumab compared with standard of care in preventing acute AMR in recipients sensitized to their donor kidney transplants (Marks et al., 2019) (NCT01399593).

A novel agent that has shown promise in desensitization is Imlifidase, a cysteine protease. In both phase 1 and 2 desensitization trials (NCT02426684, NCT04935177, NCT05369975), this drug led to a precipitous decrease in DSA within hours, and therefore is a valuable tool for deceased donor positive crossmatch transplantation to avoid hyperacute rejection (Jordan et al., 2021). The main limitation for this agent is that antibody levels begin to have a brisk rebound within 3–7 days (Jordan et al., 2021). Thanks to its characteristics, Imlifidase could be a promising agent instead of pre-transplant plasmapheresis to rapidly reduce circulating DSA (Jordan et al., 2017) (NCT02426684, NCT05369975).

Ongoing evidence is aimed to unravel the therapeutical efficacy of Belatacept also in kidney transplantation (Leibler et al., 2014, 2018; Vincenti et al.,2016; Bray et al.,2018; Jain et al., 2020) (NCT05145296).

3.3. Desensitization attempts in heart transplantation

Although plasmapheresis and Bortezomib appeared to decrease cPRA in patients awaiting heart transplantation, refractory to

Table 1
Clinical studies on desensitization therapies in kidney transplantation.

NIH registration number	Study Type	Study Phase	Drug	N patients	Conditions	References	Results
NCT01178216	N/A	Phase 1Phase 2	Rituxan	41	End Stage Renal Disease	Not Provided	–
NCT00642655	Non-Randomized	Phase 1 Phase 2	IVIG and Rituximab	20	Kidney Transplant	Vo et al. (2008)	IVIG and Rituximzab combination may prove effective as a desensitization regimen for patients awaiting a transplant.
NCT02586051	Non-Randomized	Phase 1	Obinutuzumab Intravenous Immunoglobulin	24	Kidney Failure, Chronic	Redfield et al. (2019)	Profound peripheral blood B cell depletion and reduction of B cells substantially in lymph nodes.
NCT01594424	N/A	Phase 1 Phase 2	Tocilizumab Intravenous Immunoglobulin	10	End Stage Renal Disease (ESRD)	Vo et al. (2015)	Targeting the IL-6/IL-6R pathway could offer a novel alternative for difficult to desensitize patients.
NCT03744910	Randomized	Phase 3	Clazakizumab Physiologic saline solution	350	Antibody-mediated Rejection	Not provided	–
NCT01502267	N/A	Phase 4	Bortezomib	2	Patients Awaiting a Living Kidney Donation	Jeong et al. (2016)	The posttransplant outcomes, after this well tolerated desensitization regimen, were acceptable.
NCT02442648	Non-Randomized	Phase 1	Carfilzomib Rituximab	32	Transplants and Implants	Not Provided	–
NCT04294459	Non-Randomized	Phase 1 Phase 2	Isatuximab	21	Immune System Disorder	Not Provided	–
NCT01919346	Randomized	Phase 2	Eculizumab	21	Delayed Graft Function Kidney Transplantation Complement Activity	Not Provided	-
NCT01327573	Randomized	Phase 1	Eculizumab	16	Kidney Complications Allograft	Not Provided	–
NCT00670774	N/A	Phase 1	Eculizumab	31	Kidney Transplant	Stegall et al. (2011)	The incidence of AMR in highly sensitized renal allograft recipients was significantly decreased.
		Phase 2				Bentall et al. (2014)	
NCT01403389	Randomized	Phase 2	Eculizumab	8	Delayed Function of Renal Transplant	Not Provided	–
NCT01895127	Randomized	Phase 2	Eculizumab	11	Antibody-mediated Rejection Humoral Rejection	Not Provided	–
NCT01399593	Randomized	Phase 2	Eculizumab	102	Antibody Mediated Rejection	Marks et al. (2019)	The incidence of acute AMR in highly sensitized kidney recipients was lower than the control group.
NCT01567085	N/A	Phase 2	Eculizumab	80	Stage V Chronic Kidney Disease	Not Provided	–
NCT02145182	Randomized	Phase 2 Phase 3	Eculizumab	288	Delayed Graft Function	Not Provided	–
NCT01756508	Randomized	Phase 2	Eculizumab	57	End-Stage Renal Disease	de Vries et al. (2013)	Is associated with better early graft function and improved graft morphology.
					Kidney Failure Graft Reperfusion Injury	Kaabak et al. (2018)	
NCT05145296	N/A	Not Applicable	Combination Product: Each patient will undergo in the first step of the study belatacept treatment and in the second apheresis and daratumumab	12	Sensitization	Leibler et al. (2014, 2018)	This desensitization regimen lead to a reduction of preexixting DSA levels in posttransplantation period.
					Highly Sensitized Dialysis Patients	Vincenti et al. (2016), Bray et al. (2018), Kwun et al. (2019) Jain et al. (2020)	
NCT01025193	N/A	Phase 2	Belimumab	8	Desensitization	Not Provided	–
NCT03380962	N/A	Phase 1	Clazakizumab	20	Kidney Failure, Chronic	Not Provided	–
		Phase 2			End-Stage Renal Disease		

(continued on next page)

Table 1 (*continued*)

NIH registration number	Study Type	Study Phase	Drug	N patients	Conditions	References	Results
					Transplant Glomerulopathy Transplant; Failure, Kidney Kidney Transplant Failure and Rejection Antibody-mediated Rejection Kidney Transplant; Complications		
NCT05369975	Non-Randomized	Phase 3	Imlifidase	225	Kidney Transplantation in Highly Sensitized Patients	Not Provided	–
NCT02790437	N/A	Phase 2	IdeS	19	Kidney Failure, Chronic	Kjellman et al. (2021) Jordan et al. (2021)	Converted positive crossmatches to negative, and enabled patients with a median calculated PRA of 99.83% to undergo kidney transplantation.
NCT04935177	Interventional	Phase 3	Imlifidase	64	Kidney Transplantation in Highly Sensitized Patients	Not Provided	–
NCT01134510	Randomized	Phase 1 Phase 2	C1 Esterase Inhibitor	20	Kidney Transplantation	Vo et al. (2008) Jordan et al. (2004) Shapiro (2008) Vo (2015)	Resulted in significant elevations of C1–INH levels, C3, C4, and reduced C1q + HLA antibodies.
NCT02426684	N/A	Phase 1 Phase 2	IdeS® (Imlifidase)	17	Renal Disease	Jordan et al. (2017)	Reduced or eliminated donor-specific antibodies and permitted HLA-incompatible transplantation.
NCT00476515	Non-Randomized	Phase 1	Rituximab	0	Kidney Insufficiency	Not Provided	–
NCT04827979	Non-Randomized	Phase 1 Phase 2	Daratumumab	15	Highly Sensitized Prospective Kidney Transplant Recipients	Not Provided	–
NCT01842074	N/A	Phase 4	Bortezomib	10	Patients Awaiting a Living Kidney Donation	Not Provided	–
NCT01911546	N/A	Phase 2	Everolimus + low-dose tacrolimus	20	Highly-sensitized Kidney Transplant Recipients	Not Provided	–
NCT01147302	Randomized	Phase 2	C1 Esterase Inhibitor	18	Graft Rejection	Montgomery et al. (2016)	No discontinuations, graft losses, deaths, or study drug-related serious adverse events occurred.
NCT05345717	N/A	Phase 1 Phase 2	Belatacept Injection	5	Kidney Transplantation End Stage Kidney Disease (ESRD)	Not Provided	–
NCT05092347	Non-Randomized	Phase 1 Phase 2	REGN5459 REGN5458	60	Chronic Kidney Disease (CKD)	Not Provided	–

desensitization with IVIg/rituximab, the desensitization protocol by using Bortezomib seems to be associated with an increased risk of infection (Patel et al.,2011). Thus, further studies are needed to determine whether the benefits of desensitization using this strategy outweigh the risks in heart transplantation (NCT01556347, NCT01769443).

Recent preliminary study has shown efficacy of intensive proteosome inhibitor-based desensitization protocols added Belatacept in a cohort of highly sensitized heart transplant candidates (Alishetti et al., 2020). Specifically, it has been evidenced a reduction of class I and II HLA antibodies with a negative CDC crossmatch against multiple previously high-level, complement binding antibodies (Alishetti et al., 2020).

Daratumumab, an anti CD38 monoclonal antibody, studied in kidney recipients (Kwun et al.,2019) where plasma cell and NK cell depletion may be an effective strategy to counteract AMR (Doberer et al., 2021) has already been evaluated for desensitization in heart transplantation (NCT04610320, NCT05300451). The preliminary findings in heart transplantation indicate a decrease of HLA antibodies and an improved AMR (Jordan et al., 2021). However, concerns for antibody rebound, B-reg depletion and cellular mediated rejection prompting may limit efficacy. NK cell depletion by daratumumab is likely responsible for improvements in AMR where no impact on DSA was seen (Jordan et al., 2021).

Currently, a phase 1 b/2 clinical trial is evaluating the safety, pharmacokinetics, and efficacy of Isatuximab for the desensitization in kidney transplant candidates (NCT04294459) and no evidence, at best of our knowledge, have been found about heart transplantation.

Complement inhibitors including Eculizumab have been evaluated in

Table 2
Clinical studies on desensitization therapies in heart transplantation.

NIH registration number	Study Type	Study Phase	Drug	N patients	Conditions	Ref.	Results
CT01278745	Randomized	Phase 2	Rituximab	362	Cardiac Allograft Vasculopathy Heart Transplant Recipients	Starling et al. (2019)	The rate of treated rejection was 24.7% rituximab versus 32.4% placebo.
NCT01556347	N/A	Phase 2	Bortezomib,	2	Heart Transplantation	Not Provided	–
NCT01769443	Randomized	Phase 2	Bortezomib	2	Primary Heart Transplant Heart Transplantation Heart Transplant	Not Provided	The therapeutic and safety profile of IVIg seem to appear to be superior to plasmapheresis.
NCT04610320	N/A	Phase 1	Daratumumab-SC	12	Allosensitization Heart Transplant Failure and Rejection	Not Provided	-
NCT05300451	Non-Randomized	Phase 2	Daratumumab and hyaluronidase-fihj	6	Antibody-mediated Rejection Cardiac Transplant	Not Provided	–
NCT02013037	N/A	Phase 3	Eculizumab	36	Antibody-mediated Rejection Hyperacute Rejection of Cardiac Transplant Left Ventricular Dysfunction Cardiac Allograft Vasculopathy Heart Graft Dysfunction	Stegall et al. (2011) Patel et al. (2021)	Eculizumab treatment was associated with a dramatic decrease in the incidence of biopsy-proven AMR.
NCT04180085	Randomized	Phase 2	Belatacept Injection	25	Heart Transplant Failure	Not Provided	–

desensitization regimens to minimize the effect of a high level of DSA on the cardiac allograft (Stegall et al.,2011; Patel et al.,2021) (NCT02013037). Eculizumab has not been shown to improve long term allograft survival when added to desensitization (Schinstock et al., 2019).

The use of novel drugs is under definition according to clinical trials both for heart and kidney transplantation (Tables 1 and 2).

4. Conclusions

Organ allocation to hyperimmune recipients remains one of the major clinical challenges in transplantation.

The Eurotransplant Acceptable Mismatch (AM) program has allowed an efficient way to prioritize the transplantation of highly sensitized patients by using an extended HLA phenotype for allocation and giving priority whenever a compatible donor organ becomes available. This approach resulted to increase not only the transplantation rate with excellent results but also maximization of the transplant longevity.

Moreover, technological breakthroughs in antibody characterization, coupled with the advent of emerging therapeutic modalities and antibody elimination protocols, have had a profound impact on the treatment options for highly sensitized patients.

Current therapeutic desensitization protocols, particularly in hyperimmune subjects, show variable results, often resulting in transient or incomplete reduction in HLA antibodies attended by frequent increases in antibody levels. Thus, it often becomes difficult to find an acceptable donor and to prevent post-transplant allosensitization responses.

Novel approaches to desensitization can rapidly remove antibodies but, at the same time, they do not to prevent their recurrence in the long-term follow-up. This is because there is not yet an ideal agent effective in removing antibodies while suppressing resurgence and the risk of AMR.

Furthermore, for all desensitization strategies, infectious complications and adverse side effects may limit their utility.

Although the new agents appear to show promise in overcoming the immune barrier effectively and safely, they are still widely used chronic immunosuppressive therapies for the solid organ transplantation patients included combinations of prednisone, azathioprine, cyclosporine, mycophenolate, and tacrolimus, which varied by institution and care provider. To date, immunotherapy with chimeric anti-CD20 monoclonal antibody (Rituximab) has demonstrated some efficacy by offering a potentially safe and effective treatment (Green et al., 2019). The same is true for removal of antibodies through regular plasmapheresis, immunoadsorption (Fuchs et al., 2022) or possibly proteasome inhibition (Alishetti et al., 2020) that could be methods to reduce the risk of allograft failure.

However, these novel therapeutic approaches remain to be investigated in large clinical trials.

CRediT authorship contribution statement

Vincenzo Grimaldi: Conceptualization, Writing – original draft, Writing – review & editing. **Martina Pagano:** Data curation, Writing – original draft. **Giusi Moccia:** Data curation, Writing – original draft. **Ciro Maiello:** Visualization. **Paride De Rosa:** Visualization. **Claudio Napoli:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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