DESENSITIZATION IN HIGH IMMUNOLOGIC RISK RECEPIENT

Bahareh Marghoob.MD

IUMS

WE REWIEW HERE

- when desensitization in kidney transplantation should be considered
- The outcomes of HLA incompatible transplantation
- Conventional desensitization
- Novel therapeutics
- Desensitization endpoints
- Strategies for future study

WHAT IS SENSITIZATION AND HOW IS IT MEASURED?

- Terms such as **highly sensitized** are routinely used in the field of transplantation without a universal meaning.
- Historically even patients with a cPRA of low as 30 percent may have been considered highly sensitized prior to the widespread use of kidney paired donation (KPD) programs
- current era, it is best to avoid terms such as highly sensitized and instead report the cPRA and mode of sensitization.

WHO DERIVES THE MOST BENEFIT FROM DESENSITIZATION IN THE CURRENT ERA?

- Desensitization protocols are generally used for the following two reasons:
- 1) **To increase transplant candidates**' access to transplantation by decreasing HLA antibody and the number of unacceptable antigens for listing (e.g. reduction in cPRA),
- 2) **To decrease known DSA** prior to a planned positive crossmatch transplant to reduce the risk of immediate graft loss from catastrophic hyperacute rejection

HLA INCOMPATIBLE

- HLA incompatible transplants are associated with **reduced allograft survival**, **increased expense** , and **increased hospital readmission rates**
- In a large retrospective series among a French cohort, the incidence of **early active ABMR** was 36.4% with a baseline DSA MFI of 3001-6000 and 51.3% with a baseline DSA MFI of > 6000 .
- A key message is that transplantation in the context of **low level DSA** results in acceptable outcomes when other options are not available.

HLA INCOMPATIBLE

- A multicenter observational study of **living donor transplants** performed at 22 centers in the United States:
- The 1 and 5 year unadjusted **all-cause graft loss** was 3.9% and 16.6% among patients without DSA at transplant,
- 3.8% and 20.2% when SAB were **positive for DSA** but the flow crossmatch was negative,
- 6.9% and 28.8% when the flow cytometric crossmatch was positive,
- 19.4% and 39.9% when the cytotoxic crossmatch was positive

SENSITIZED PATIENTS

- More than **one third** of patients awaiting a renal transplant in the US are sensitized to HLA antigens
- Sensitization developed during the course of **pregnancies** , **blood transfusions** or **previous failed transplant**.
- Preformed Ab has the risk of hyperacute rejection or accelerated acute rejection
- This results in direct sensitization against the partner, potentially making the partner and/or her child an unsuitable donor

SENSITIZATION AND DSA IDENTIFICATION

- **screening antibodies**:
- Complement-dependent cytotoxicity assay
- The enzyme-linked immunoabsorption
- Multiplexed particle-based flow cytometry (Luminex).
- Single antigen beads are used to characterize the preformed DSAs before transplant as well as any *de novo* development of DSAs after transplant

SENSITIZATION AND DSA IDENTIFICATION

- **Luminex assay** can characterize the preformed HLA antibodies in sensitized patients awaiting transplant
- The recurrent antibodies or highly expressed antibodies are considered clinically significant.
- A patient will not be offered a kidney from the deceased donor who expresses an unacceptable HLA antigen (positive virtual crossmatch)

DSA PATHOGENESIS

- DSA is a well established biomarker predicting poor transplant outcomes, including high incidence of **antibody-mediated rejection**, **graft dysfunction**, and **inferior graft survival**.
- The development of *de novo* DSAs after kidney transplant was reported in 13%–30% of previously nonsensitized patients

DSA PATHOGENESIS

- The risk factors for *de novo* DSA include the following:
- **High HLA mismatches** (especially DQ mismatches)
- **Inadequate immunosuppression**
- **Nonadherence**
- **Graft inflammation**, such as viral infection, cellular rejection, or ischemia injury, which can increase graft immunogenicity

COMPARISON OF OF CLASSES 1 AND 2 DONOR-SPECIFIC ANTIBODIES

	Class 1 Donor-Specific Antibodies	Class 2 Donor-Specific Antibodies
HLA		
Antigens	A, B, and C	DR, DQ, and DP
Epitopes location	α-chain	α- and β-chains
Expression	All nucleated cells	Antigen-presenting cells

DSA in kidney transplant recepient -clin j Am Soc Nephrol 2018

Preformed DSA → Class 1 → Class 2

Important	very	less
Positive crossmatch	T cells	B cells
Transplant decision	No transplant	permissible

De novo DSA → Class 1 → Class 2

Detection	Sooner	Later
IgG subclasses	IgG1, IgG3	IgG2, IgG4
Complement binding	Strong	Weak/no
Frequency	Fewer	Common, especially DQ

AMR	Class 1	class2
Phenotypes	Acute	Chronic, subclinical
Presentation	Early	Later
Graft dysfunction	Rapidly	Slowly
C4d deposit	Positive	Negative
Treatment	More responsive	Less responsive
Graft loss	Early	Later

DSA STRENGTH

- The DSA strength (or titer) is usually expressed as the **mean fluorescence intensity** by Luminex solid-phase assay.
- High titer of DSA has been correlated with complement binding capability and more severe tissue injuries
- The thresholds reported for clinically significant mean fluorescence intensity vary widely between studies from 1000 to 10,000 depending on the antigen specificities
- **False positive** or **false negative** may be seen

ACUTE PHENOTYPES OF ANTIBODY-MEDIATED REJECTION

- **Hyper acute rejection**
- Irreversible vascular rejection, intravascular thrombosis, and graft necrosis, and graft nephrectomy is usually indicated.
- Du to **preformed Ab**

DSA in kidney transplant recepient -clin j Am Soc Nephrol 2018

ACUTE PHENOTYPES OF ANTIBODY-MEDIATED REJECTION

- **Accelerated acute rejection** (or delayed hyper acute rejection)
- Can occur within 24 hours to several days after transplant.
- Response by **memory B** and **plasma cells** from prior sensitization.
- Even a negative crossmatch before transplant may not prevent it, because of low titer of preformed DSA

ACUTE PHENOTYPES OF ANTIBODY-MEDIATED REJECTION

- **Acute antibody-mediated rejection**
- More common than hyperacute rejection and accelerated acute rejection, and it may be further divided into **early** and **late** subtypes.
- **Early acute antibody-mediated** rejection occurs sooner after transplant from rising titer of preformed DSA.
- **Late acute antibody-mediated rejection** develops due to the emergence of *de novo* DSA after kidney transplant

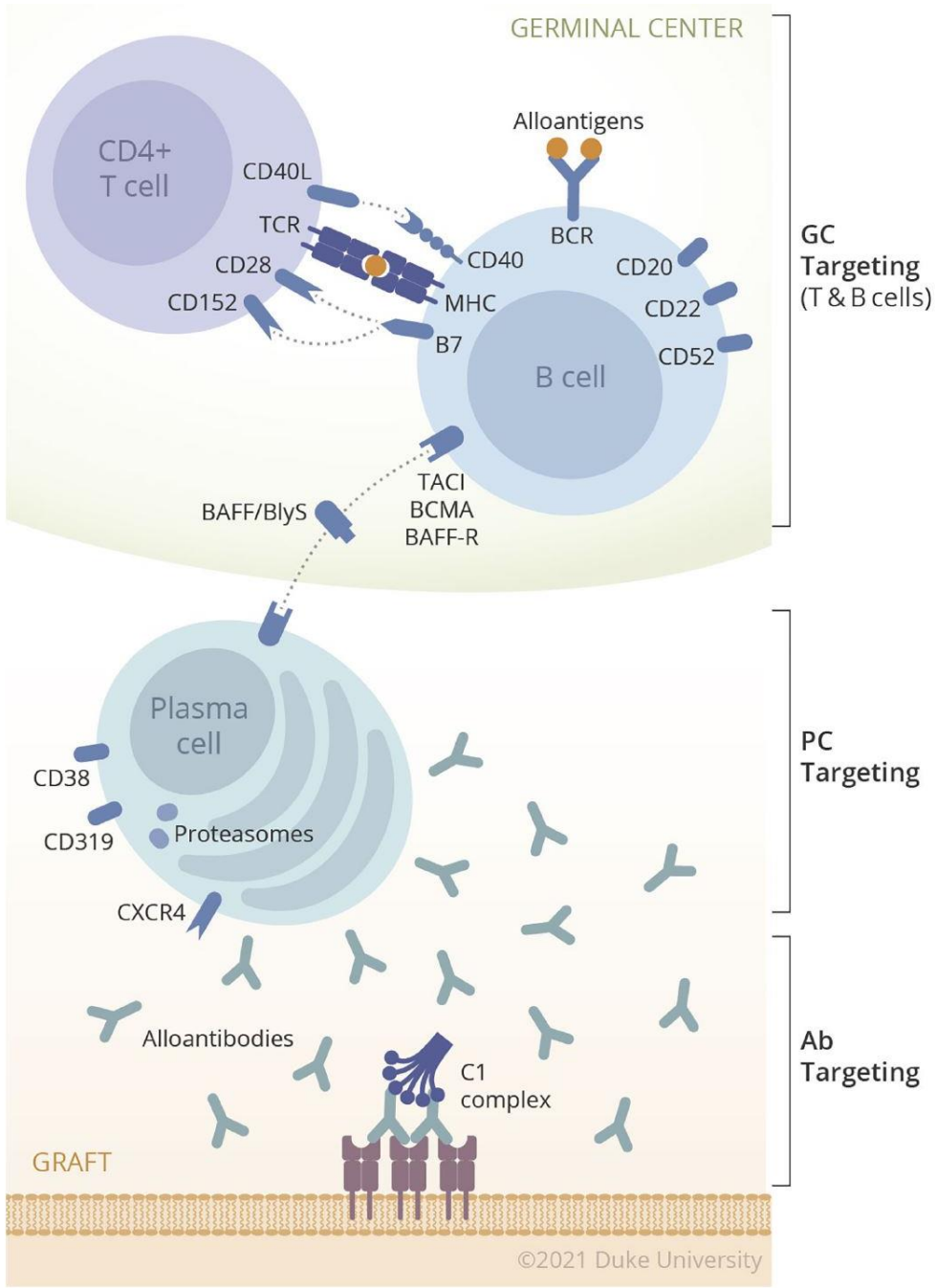
ACUTE PHENOTYPES OF ANTIBODY-MEDIATED REJECTION

- Antibody-mediated rejection can also develop in a graft already suffering from **delayed graft function**.
- This can be difficult to be recognized if the patient remains anuric or oliguric .
- Therefore, any new kidney transplant with delayed graft function should have **serial DSA monitoring** and **protocol biopsies** to detect covert rejection and be treated properly

EMERGING NEW APPROACHES IN DESENSITIZATION

- **Circulating antibody**, the final product of the humoral immune response, has been the **primary target of desensitization** and AMR treatment
- Compared to patients with absent or low cPRA, the highly sensitized candidates could expect to wait twice as long for a compatible transplant in both the USA & UK

Multiple components of humoral immunity in organ transplantation



POSSIBLE THERAPEUTICS FOR DESENSITIZATION REGIMENS

- B Cells
- Plasma Cells
- T Follicular Cells
- Ubiquitin-Proteasome Inhibitors
- Circulating Abs

OVERVIEW OF DESENSITIZATION

- Immunomodulation of recipient immune system
- Depletion of B cell population
- Removal of Anti-HLA Abs

PLASMAPHERESIS AND PLASMA EXCHANGE

- **Plasma exchange** is widely used in desensitization protocols along with rituximab for patients with an incompatible crossmatch to reduce the effect of ABMR
- The number of plasmaphereses is dependent on the **antibody levels** and the degree of mismatch
- There is evidence of the positive effect of plasmapheresis on the **survival** of highly sensitized patients

PLASMAPHERESIS OR IMMUNOADSORPTION

- The main mechanisms is **lowering circulating antibody** .
- Removes large molecular weight substances from the plasma, including antibodies, complement components immune complexes an coagulation factors
- In Europe and Australia immunoadsorption (IA) using staphylococcal protein A column has been applied in eliminating antibodies
- PP or IA has a limitation of **antibody rebound** after the completion of treatment sessions so are beneficial in the setting of planned transplantation

INTRAVENOUS IMMUNOGLOBULIN

- **The main mechanisms** are neutralization of circulating anti-HLA antibodies, the inhibition of complement activation , and binding to Fc receptors on immune cells.
- **IVIG** following plasmapheresis **prevents rebound of DSA**, by providing an abundant quantity of circulating IgG.
- IVIG has been used in various doses according to protocol from 100mg/kg to 2.0g/kg in desensitization prior to living donor KT or for deceased donor KT of patients with high PRA

HIGH-DOSE IVIG ALONE

- There are no randomized trials comparing these two protocols.
- The NIH IGO2 study, a controlled clinical, multi-center, double blinded trial of IVIG (2g/kg, monthly 4 times) versus placebo in sensitized patients, HLA antibody levels were reduced further, and the transplantation rate was higher in the IVIG group than in the placebo group.

HIGH-DOSE IVIG PLUS RITUXIMAB

- The addition of rituximab to IVIG is superior to IVIG alone
- Jordan et al.reported successful transplantation outcomes with two doses of 2 g/kg IVIG on day 0 and day 30 plus rituximab 1 gr on days 7 and 22 in 20 patients.
- In this study, 16 patients among 20 could receive KT within 6 months.
- In their subsequent series, they used high-dose IVIG 2g/kg, 3 times on day 1, day 30 and at the time of transplantation with rituximab.
- Mean PRA levels decreased from 77% to 44%

LOW DOSE IVIG PLUS PP

- Alternate day plasmaphresis plus 100 mg/kg IVIG after each session
- Protocols are differents
- Rituximab 375 mg/m² after last session of pp
- ATG for induction

PP WITH LOW-DOSE IVIG VS. HIGH-DOSE IVIG ALONE

- Desensitization protocols using high-dose IVIG or low-dose IVIG + PP with rituximab have relative advantages and disadvantages.
- Regardless of whether high-dose IVIG or low-dose IVIG with PP were used, **acute AMR** rate as well as **acute cellular rejection** rates were higher in desensitized patients than in **non-sensitized patients**

PP WITH LOW-DOSE IVIG VS. HIGH-DOSE IVIG ALONE

- In a study that included surveillance biopsy of desensitized KT recipients, the **subclinical AMR** rate was 31% at 3 months post-transplantation, and patients with subclinical AMR at 3months post-transplantation had **higher C4d**, ptc and arteriosclerosis scores post-transplantation at 1 year than the patients without subclinical AMR at 3 months post transplantation
- **Transplant glomerulopathy** was reported at a rate of 44% at a mean of 18 months post-transplantation.
- **After desensitization, long-term outcomes of KT seems to be worse than for unsensitized patients**

ANTI-CD20 ANTIBODY (RITUXIMAB)

- **Rituximab** is an anti-CD20 monoclonal antibody that binds to CD20 expressed on immature and mature B-lymphocytes, inducing apoptosis via antibody-dependent cytotoxicity, complement-dependent cytotoxicity or direct apoptosis process lead to depletion of short lived plasmacell in blood in short time and depletion of CD 27+ cell in long term

- In transplantation, rituximab was introduced to deplete B cells with the goal of **reducing donor-specific antibody (DSA) production**.

ANTI-CD20 ANTIBODY (RITUXIMAB)

- Rituximab has been used as an additional therapy as part of desensitization treatments, in conjunction with plasmapheresis & IvIg.
- The half-life of rituximab in patients with end-stage renal disease is known to be 9-14 days.
- Rituximab administration can maintain durable B-cell depletion for at least six months, but rituximab does not bind to plasma cells as they do not express CD20

ANTI-CD 20 MONOCLONAL ANTIBODIES

- even after treatment, nearly 50% of patients had ABMR within 30 days post-transplant
- **Obinutuzumab** has been studied in desensitization. This **3rd generation anti CD 20 monoclonal antibody** has been associated with a more profound depletion of B cells and is used outside of transplant as a second line agent for hematologic malignancies **refractory to rituximab**
- Obinutuzumab is associated with depletion of peripheral and lymph node B cells, but its effect on MFI, number of unacceptable antigens, and cPRA has been shown to be limited and does not appear to be clinically meaningful

TARGETING PLASMA CELLS

- Alloantibody secreting cells predominantly exist as **long-lived plasma cells** (LLPC) in the bone marrow compartment
- **Bortezomib**, a proteasome inhibitor (PI) which depletes non-malignant plasma cells, was proposed to reduce anti-donor HLA antibody.

TARGETING PLASMA CELLS

- **Long-lived plasma cells** (LLPC) can produce antibodies whole life; therefore, they play an important role in antibody-mediated rejection (ABMR).
- Since LLPCs are **independent of B-cell precursors**, proteasome inhibitors can be used to address the issue of ABMR
- It has apoptotic abilities, which inhibit the function of proteasomes, which is crucial against allogeneic HLA

TARGETING PLASMA CELLS

- Woodle et al. in the first trial with **bortezomib** variably combined with **plasmapheresis** and **rituximab** showed modest success with a reduction in the immunodominant DSA of 38/44 (86%) highly sensitized patients, successful transplantation of 19/44 (43.2%), and 17/19 (89.5%) of grafts functional at a median follow-up of 436 days

TARGETING PLASMA CELLS

- Jeong et al. used a combination of **high dose IVIG**, **rituximab**, and **bortezomib** and demonstrated a small reduction in the MFI value of class I PRA, and an increased rate of deceased donor kidney transplantation (8/19 or 42.1% of desensitized patients vs. 4/17 or 23.5% of controls, p = 0.004) with no graft loss in the desensitized group at a median follow-up of 23 months.
- Studies using **bortezomib as monotherapy** for desensitization have shown less promising results with poor reduction of anti-HLA antibodies and significant toxicity with longer courses of the drug

PROTEASOME INHIBITORS

- The irreversible proteasome inhibitor **carfilzomib** has been shown to deplete plasma cells and decrease HLA antibody, but its effects were transient and antibody levels returned to baseline in less than 6 months

NEW PHARMACOLOGIC STRATEGIES FO DESENSITIZATION

- **Targeting Antibodies**
- **IgG Endopeptidases**
- Alter the structure of preformed antibody, using IgG endopeptidase (IdeS) which is a bacterial enzyme produced by S. pyogenes that cleaves all four human IgG subclasses into F(ab) & F (c) fragments, thus inhibiting both complement-dependent cytotoxicity and antibody-dependent cytotoxicity
- IdeS has additional effects by cleaving the IgG present in the B-cell receptor complex (BCR), thus switching off B-cell memory as a downstream effect.

IGG ENDOPEPTIDASES

- Jordan et al. recently completed a trial of IdeS in 25 highly sensitized patients prior to HLA-incompatible kidney transplantation.
- All patients had near-complete or complete reductions of anti-HLA antibodies and donor-specific antibodies at 24 hours post-transplant, which allowed successful transplantation in 24/25 (96%).

IGG ENDOPEPTIDASES

- However, in 1-2 weeks the levels of these antibodies rebounded.
- Ultimately, one patient had graft loss from hyperacute rejection, while 10/25 (40%) had evidence of **antibody-mediated rejection** in the early post-transplant period.
- These findings suggest that IdeS has strong, albeit **transient**, ability to reduce DSA that may make this therapy useful in combination with strategies that allow for longer-term control of DSA rebound.

ENDOPEPTIDASE

- **Imlifidase**
- This endopeptidase rapidly cleaves all IgG into F(ab') and Fc fragments to impair the effector function from all circulating IgG
- In both phase 1 and 2 desensitization trials, this agent led to a precipitous drop in DSA within hours, and therefore is a valuable tool for deceased donor positive crossmatch transplantation to avoid hyperacute rejection
- **In the future, it may be used instead of pretransplant plasmapheresis to rapidly reduce circulating DSA**.

NEW PHARMACOLOGIC STRATEGIES FOR DESENSITIZATION

- **Anti-FcRn Approach** :
- A neonatal IgG receptor that is closely related to the MHC Class I receptor and increased half-life of IgG and albumin in human serum.
- Strategies that block the IgG-FcRn interaction are hypothesized to promote IgG degradation and decrease pathogenic autoantibodies and alloantibodies.
- Treatment with aFcRn prior to transplantation significantly reduced the levels of total and donor-specific alloantibody.
- The anti-FcRn approach demonstrated promising applications in lowering alloantibody levels in transplantation

ANTI-CD38 MONOCLONAL ANTIBODIES

- **Daratumumab**, an anti CD38 monoclonal antibody, has been studied for desensitization in a nonhuman primate model. This treatment was associated with reduced DSA and prolonged renal survival but was also followed by a **rebound in DSA** and a severe combined antibody and T cell mediated rejection
- A similar but more potent anti CD38 monoclonal antibody, **isatuximab**, is currently being studied in a phase 1b/2 trial to evaluate the safety, pharmacokinetics, and efficacy for desensitization in kidney transplant candidates

COMPLEMENT INHIBITORS

- **Eculizumab** is a terminal complement inhibitor that does not decrease antibody but has been added to desensitization regimens to minimize the effect of a high level of DSA on the allograft
- It has been shown to decrease the incidence of early active ABMR in a small single center cohort from nearly 41.2% in the historical control group compared to 7.7% in the treatment arm
- **eculizumab has not been shown to improve long term allograft survival when added to desensitization**

TARGETING MEDIATORS/SURVIVAL FACTOR

- **Interleukin-6 Receptor Inhibition**
- IL-6 is critical for many **inflammatory pathways** and has a key role in the induction of follicular helper T cells,which direct naïve B cells in the germinal center to differentiate to memory B cells and high-affinity, IgG-secreting plasma cells
- Dysregulated production of IL-6 has been associated with chronic diseases such as diabetes, systemic lupus erythematosus, rheumatoid arthritis, cancer, end-stage renal disease, crescentic glomerulonephritis, and graft versus host disease

INTERLEUKIN-6 RECEPTOR INHIBITION

- IL-6 has also been associated with deviation of T cells towards a Th17 phenotype, reduction of the proportion of Treg cells, and potentiation of allograft rejection in kidney transplantation.
- **Tocilizumab** (Actemra) is a humanized monoclonal antibody with activity against both the membrane and soluble forms of IL-6R approved to treat moderate to severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, and Castleman's disease
- **This therapy reduces alloantibody responses by inhibition of bone marrow plasma cells and induction of Treg cells**

INTERLEUKIN-6 RECEPTOR INHIBITION

- Vo et al. recently examined the efficacy of **high dose IVIG** + **tocilizumab** in 10 highly sensitized patients who were poorly responsive to high dose IVIG + rituximab .
- This regimen was associated with reduced donor specific antibody number and strength, decreased wait list time, and increased rate of transplantation.
- No transplanted patients had evidence of antibody-mediated rejection on protocol biopsies.

ANTI-BAFF AGENTS

- **B cell activating factor** (BAFF) is a homotrimer and member of the tumor necrosis factor (TNF) family that is found on the cell surface
- BAFF is secreted by multiple cell types and is critical for the maturation of B cells and B cell proliferation and differentiation
- Therefore, blocking this molecule may be essential when targeting allo-B cell response.

ANTI-BAFF AGENTS

- Monoclonal antibody against BAFF, **Belimumab** (Benlysta), was the first targeted biologic approved for the treatment of systemic lupus erythematosus
- Belimumab monotherapy was tested for desensitization in kidney transplantation (NCT01025193), but this trial was closed early due to a reported lack of efficacy
- Other studies are needed

A MULTI-MODAL APPROACH TO DESENSITIZATION

- The concept of desensitization has been expanded from only targeting alloantibody (IVIG/IA/plasmapheresis) to instead targeting the upstream sources of antibody such as B cells (Rituximab) and PC (proteasome inhibitor).
- The conventional desensitization concept, removal of preformed antibody, may prevent hyperacute rejection or acute AMR but without longlasting impact on humoral alloimmunity

Living-Donor Kidney Transplant With Preformed Donor-Specific Antibodies

Yoshihiro Itabashi,[1] Atsushi Aikawa,[1] Masaki Muramatsu,[1] Youji Hyoudou,[1] Kazunobu Shinoda,[1] Yusuke Takahashi,[1,2] Kei Sakurabayashi,[1] Toshihide Mizutani,[1] Hideyo Oguchi,[1] Taichi Arai,[1] Takeshi Kawamura,[1] Yuko Hamasaki,[1,2] Ken Sakai,[1] Seiichiro Shishido[1,2]

LIVING-DONOR KIDNEY TRANSPLANT WITH PREFORMED DSA

- Patients with high titers of DSAs (mean fluorescence intensity [MFI] > 1000) should be desensitized pretransplant to avoid acute AMR posttransplant.
- Desensitization was initiated using calcineurin inhibitors, methylprednisolone, and mycophenolate mofetil 30 days pretransplant, with rituximab administered 1 and 10 days pretransplant. Patients underwent plasmapheresis 1, 3, and 5 days pre-transplant.
- Antithymocyte globulin was administered for 5 days posttransplant as induction therapy

RESULTS

- T-cell complement-dependent cytotoxicity crossmatch was negative in all 15 recipients, but T-cell and B-cell flow cytometry was positive in 8 and 14 recipients, respectively.
- Anti-HLA class I antibodies became negative, except in 1 recipient 3 months posttransplant.
- Class II antibodies remained positive in 8 recipients 3 months posttransplant.

RESULTS

- No clinical or subclinical T-cell-mediated rejection occurred, but 1 recipient experienced clinical acute antibody-mediated rejection.
- At 3 and 12 months posttransplant, 8 and 5 recipients had subclinical acute antibody-mediated rejection.
- Cytomegalovirus test showed positivity in 14 recipients, but none developed cytomegalovirus disease.
- BK viremia was detected in 2 recipients, with 1 developing BK virus nephropathy, which was reversed by reducing immunosuppression

CONCLUSIONS

- Transplant patients with preformed donor-specific antibodies showed good outcomes in terms of desensitization and immunosuppression.
- However, most anti-HLA class II donor-specific anti -bodies remained, and microvascular inflammation score could indicate long-term risk of renal allograft dysfunction.

AT THE END

- When living donors are available, paired exchange should be attempted to avoid the cost and risk associated
- Proceeding with desensitization for those highly sensitized patients without living donors, where paired exchange is not possible and expected wait time is considered unacceptable, may be a reasonable consideration
- Although we know about complications of desensitization the long term risk of death is lower in transplant compared with dialysis.

- Thank you