Final report of the workshop ‘Management of transplant patients with HLA antibodies’

14 April 2023, Vienna, Austria

Programme

11:00 – 11:20	Welcome coffee break and introduction	
11:20 – 11:50	Current landscape – definition of HLA sensitisation – consensus between units	Christian Morath | Heidelberg, Germany Stefan Schaub | Basel, Switzerland Gottfried Fischer | Vienna, Austria
11:50 – 12:10	Procedere and Impact of the B-list in ET’s-AM program	Michael Fischereder | Munich, Germany Farsad Eskandary | Vienna, Austria
12:10 – 12:30	Improving risk stratification	Fabian Halleck | Berlin, Germany Klemens Budde | Berlin, Germany Roman Reindl-Schwaighofer | Vienna, Austria
12:30 – 12:50	Paired exchange programs in small countries	Ondrej Viklicky | Prague, Czech Republic Georg Böhmig | Vienna, Austria Annemarie Weissenbacher | Innsbruck, Austria Karine Hadaya | Geneve, Switzerland
12:50 – 13:15	Defining the patient population who would benefit from imlifidase	Discussion moderator: Rainer Oberbauer | Vienna, Austria All
13:15 – 14:00	***Lunch break***	

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14:00 – 14:30	Discussion ● Safe, effective and equitable implementation of an Imlifidase enabled kidney transplant pathway ● Protocol standardisation and data collection - A transnational view	Discussion moderator: Rainer Oberbauer | Vienna, Austria All
14:30 – 14:50	Future potential of T regulatory cell therapies	Thomas Wekerle | Vienna, Austria
14:50 – 15:10	Management options for treating ABMR post-Imlifidase	Klemens Budde | Berlin, Germany Georg Böhmig | Vienna, Austria
15:10 – 15:30	Discussion and final wrap up	All

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Attendees

Bernhard Banas | Regensburg, Germany
Georg Böhmig | Vienna, Austria
Klemens Budde| Berlin, Germany
Farsad Eskandary | Vienna, Austria
Gottfried Fischer | Vienna, Austria
Michael Fischereder | Munich, Germany
Edward Geissler | Regensburg, Germany
Karine Hadaya | Geneve, Switzerland
Fabian Halleck | Berlin, Germany
Johannes Kläger | Vienna, Austria
Nicolas Kozakowski | Vienna, Austria
Andreas Kronbichler | Innsbruck, Austria
Christian Morath | Heidelberg, Germany
Thomas Müller | Zurich, Switzerland
Rainer Oberbauer | Vienna, Austria
Heinz Regele | Vienna, Austria
Roman Reindl-Schwaighofer | Vienna, Austria
Stefan Schaub | Basel, Switzerland
Ondrej Viklicky | Prague, Czech Republic
Annemarie Weissenbacher | Innsbruck, Austria
Thomas Wekerle | Vienna, Austria

Workshop rationale

Discussion of the management of highly sensitised kidney transplant patients, based on the framework of recent European guidelines authored by an ESOT working group and published in *Transplant International*, and assessment of how these apply to kidney transplant programmes across the DACHCZ region.

Welcome and introduction

Rainer Oberbauer

Current landscape: Definition of HLA sensitisation – Consensus between units

Christian Morath

Presentation

- The main method of detecting human leukocyte antigen (HLA) antibodies is Luminex single antigen bead (SAB) using One Lambda
    - Frequency: annually, after sensitising events, graft removal, implausible screening results (quarterly), within 2 months before a living donor transplantation
- Plausibility testing: immunological history is recorded annually, and complement-dependent cytotoxicity (CDC) T and B cell testing and Luminex LABScreen Mixed are performed quarterly
- Positive results (SAB) are defined as:
    - Mean fluorescence intensity (MFI) ≥1,000 (pretransplant)
    - Exceptions: antibodies against denatured antigens, natural antibodies (Gombos. *AJT* 2013), autoantibodies
    - Positive specificity does not always equal an unacceptable antigen for patients on the waiting list
- Main methods of donor-specific antibody (DSA) assignment:
    - Donor: HLA typing for allocation by EuroTransplant (ET)
        - Intermediate resolution typing – reverse transcription polymerase chain reaction (RT-PCR) FluoGene (inno-train) for 11 HLA loci
        - Subsequent sequencing in the process is by Sanger or next-generation sequencing (NGS)
    - Patient: defining unacceptable antigens (Süsal. *Tissue Antigens*. 2015)
        - Luminex: MFI ≥5,000 in patients with no history of sensitising events
        - Luminex: MFI ≥3,000 in patients with allo-immunisation and those who are Luminex LABScreen Mixed positive for HLA Class I and II antibodies
        - CDC: all specificities that are detected by CDC T or B cell assays. To date, CDC-crossmatch (XM) for T and B lymphocytes has been performed prospectively using unseparated lymphocytes ± dithiothreitol; from April onwards, there is a planned change to CDC-XM before or in parallel with transplantation for selected patients
    - DSAs are then assigned risk categories which affect whether patients receive a transplantation and if so, what immunosuppression they receiveIn ET, virtual XM (V-XM) negative patients' risk is judged:
        - Low if no DSAs and CDC-XM negative
        - Intermediate-low if no DSAs and CDC-XM negative but virtual panel-reactive antibody (vPRA) positive
        - Intermediate-high if CDC-XM negative but with a DSA ≥1,000
    - Independently of MFI, patients are judged high risk if:
        - In the acceptable mismatch (AM) programme

        - Double positive in Luminex LABScreen Mixed
        - Retransplant and Class I positive in Luminex LABScreen Mixed
    - No transplant is given to (ET) V-XM negative, CDC-XM positive patients
    - No organ is offered to (ET) V-XM positive patients

Stefan Schaub

- Main detection of HLA-antibodies is by Luminex SAB (all labs use the same product from the same vendor for consistency), performed annually and after sensitising events. CDC testing has not been used for the past 6 years
- Hierarchy of sensitisation is: transplant > pregnancy > transfusion > heterologous immunity. HLA typing is also performed on spouse and children
- Plausibility: HLA antibody pattern is checked to identify problematic beads that commonly give false-positive results such as identifying distinctive eplets
    - Serum from non-sensitised males can be used as a negative control
- Definition of a positive result is based on >500 MFI in Basel and >1,000 MFI in some other Swiss centres
    - Antibodies that appear not to be plausible are excluded
    - If a clear reactivity pattern is seen below 500 MFI they can be assigned as positive (false negatives)
- Main methods of DSA assignment
    - Donor: HLA-typed by LinkSeq or Q-Type (11 loci)
        - Retrospective high-resolution HLA typing by NGS
        - High-resolution HLA typing by NGS of all living donors
        - High-resolution HLA typing by Oxford Nanopore sequencing in evaluation
        - Prospective CDC-XM is only performed in the deceased donor setting if the V-XM is positive
    - Patient: high-resolution HLA typing by NGS of all recipients
- DSA risk categories:
    - Standard risk if V-XM negative
    - Risk for transplantation if low-level DSAs present: V-XM positive, CDC-XM negative (or not plausible). Anti-thymocyte globulin (ATG) induction is given
    - Contraindication for transplantation with high levels of DSAs present: VXM positive, CDC-XM positive

Gottfried Fischer

- Main detection of HLA antibodies is by Luminex and CDC-XM
    - Luminex screening is performed quarterly
    - SAB is performed routinely annually and after sensitising events, significant rise in MFI or changes in reaction pattern
    - CDC-XM is performed quarterly in sensitised patients or annually in unsensitzed patients

- Definition of positive result: reactivity against self-antigen is used as a baseline and to determine the threshold. Also check for problem beads, cross checking with flow-XM or ImmunoCore
    - Unacceptable antigens >1,000 MFI with repeated mismatches, or >10,000 with CDC specificities
    - >2,000 MFI Luminex only – represents a heightened risk
- Main methods of DSA assignment
    - V-XM
    - Retrospective flow-XM
- Four DSA risk categories:
    - I MFI 1,000–2,000 (considered as 'noise' or grey zone)
    - II MFI 2,000–5,000
    - III MFI 5,000–10,000
    - IV MFI >10,000
    - Flow-XM (living donation)

Summary

	Heidelberg	**Basel**	**Vienna**
HLA antibody detection Main methods	Luminex SAB	Luminex SAB	Luminex Screening & SAB, CDC
HLA antibody detection Plausibility testing	Immunological history CDC T/B Luminex LABScreen Mixed Known antibodies against denatured antigens	Sensitisation history Bead behaviour Reactivity pattern	Sensitisation history Test artefacts
HLA antibody detection Definition of a positive result	Luminex SAB MFI ≥1,000 Unacceptables: MFI ≥3,000/5,000, CDC	Luminex SAB MFI>500	MFI >1,000 (plausible antibodies) MFI >2,000 (means risk) MFI >10,000 (means UAG)
DSA assignment Main methods	V-XM based on 'unacceptables' CDC-XM	V-XM CDC-XM	V- XM Flow XM
DSA assignment Risk categories	V-XM negative as prerequisite, then vPRA negative or positive, if vPRA positive, then non-DSA or DSA (≥1,000 MFI) + CDC-XM negative HD algorithm criteria	V-XM negative VXM positive/CDC negative VXM positive/CDC positive	MFI 1,000–2,000 MFI 2,000–5,000 MFI 5,000–10,000 MFI >10,000

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Procedure and impact of the B list in ET's AM programme

Farsad Eskandary
Michael Fischereder

The current situation and the need for solutions for sensitised patients was outlined by MF who chairs the EuroTransplant Kidney Advisory Committee (ETKAC) and is also member of the Imlifidase Working Group.

- Although priority programmes have helped some sensitised people to have access to donor organs (~80%), the very highly sensitised with ≥99.99% PRA remain unlikely to receive a kidney transplantation or even an offer of a kidney
- In Europe, the current solutions include:
    - The AM programme within ET countries
    - EUROSTAM, a Europe-wide AM programme within which only 27% of patients are are receiving a transplant
- Currently, there remains a need for some sort of desensitisation and these include:
    - Plasmapheresis/immunoadsorption (IAS) plus rituximab, etc.
    - Imlifidase
- At present, ET forbids transplantation in the presence of unacceptable HLA antigens and will not assign kidneys to these patients, which would preclude the use of imlifidase – this leads to a need for the 'B' list
    - "In ET, patients unlikely to be transplanted under the available kidney allocation system for highly sensitised patients (i.e. the AM programme) need to be identified and offered an organ after revision of unacceptable HLA antigens (i.e. B list) that are present in the HLA phenotype of the donor"
- FE continued the presentation by outlining the background to the development of the B list, before looking at the criteria for both inclusion on the list and, by extension, the need to define the patients for whom imlifidase represents the most appropriate treatment pathway
- Proposed imlifidase pilot scheme within ET (potentially benefiting around 154 patients who have been on the AM waiting list for >3 years):
    - Patients remain active within the AM programme with the originally defined acceptable antigens to allow for a compatible offer
    - For the imlifidase programme, additional acceptable antigens are defined, based on immunization history and MFI
    - Within the 'B-Lisr' imlifidase acceptable antigens are used for allocation
    -
    - Imlifidase acceptable antigens are not restricted to the AM programme inclusion criteria
        - A resulting vPRA of <85% is allowed
        - Unacceptable antigens used for AM inclusion can be listed as imlifidase-acceptable
    - An extra allocation tier will be added between the AM programme and the EuroTransplant Kidney Allocation System (ETKAS) for donors aged <65 years:
        - AM programme

        - Imlifidase programme
        - ETKAS 000 mismatch
        - ETKAS on point score
- Representative patient examples were shown from HLA lab, Vienna, where delisting antigens led to an increase in potential donor frequency
    - Patient 1 zero offers over more than 3 years to potential frequency of 0.85%
    - Patient 2 zero offers over 6 years to a potential frequency of 1.14%
- Note was taken of the limited long-term results after transplantations following desensitisation with imlifidase, especially with regard to the proportion of patients on the 06 study experiencing antibody-mediated rejection (AMR; 38.9%) (Jordan *Transplantation* 2021)
- It was also noted that in the longer-term follow-up analysis (Kjellman *AJT* 2021), 10.5% (2/18) of patients in the 06 study were positive for a T cell CDC-XM test
- Because of concerns over AMR rates and potential for graft loss, the ETKAC working group has limited which antigens are acceptable to delist for transplantation using imlifidase to:
    - CDC negative
    - Luminex positive, flow-XM positive
- The group also advise, “Formulation of imlifidase acceptable antigens should be performed on an individual patient level, depending on strength and origin of antibodies, and change in donor frequency vs the extra immunological risk”
- In the Hansa post-approval efficacy study (PAES) protocol, the statement reads, “delisting of antigens, no longer considered a medical contraindication to transplantation using imlifidase, may be performed on removal of the least risky antigens for that specific patient”
- An example patient case from Sebastian Heidt was run through:
    - vPRA 99.32% with 0.01% chance of receiving a donor organ
    - By delisting antigens that avoid repeated mismatches and shared epitopes with the sensitising events, the chances increase to 0.3% (i.e. 30 times more likely)
    - Using a less conservative approach – accepting all DSAs below 10,000 MFI, the chances increase to 0.69%, or 69 times more likely
    - The question was posed in the presentation as to whether these increases via delisting mean that imlifidase may not then be necessary
- The current status of progress towards implementing the new system includes:
    - Resolving logistical issues
        - Distribution and coordination of patients by country
        - Identification of which patients to include first
    - Review of outcomes and processes after 20 transplantations
    - If successful, the programme may be extended to patients outside the AM programme
- Sebastian Heidt was thanked for the use of some slides

Discussion and comment

- RO commented on the observations that acceptable mismatch programmes were not able to cover all patients and that although delisting can help to a degree, the transplantation rate in very highly sensitised patients is still low. This is affected by risk determination, which was to be explored in the next topic, especially with respect to which patients may benefit from desensitisation

Improving risk stratification

Fabian Halleck
Klemens Budde
Roman Reindl-Schwaighofer

Case study 1 was presented by FH to illustrate strategies for evaluating individual risk.

- He noted that when treating within the ET B list, it may be better not to start with the most dangerous or highest risk patients, so careful evaluation was important
- The patient was a referral, female, 35 years old, blood group A with comorbidities: systemic lupus erythematosus (SLE), secondary (triple positive) antiphospholipid syndrome
    - Vascular access was becoming difficult with thrombophilia
    - Highly sensitised (calculated panel-reactive antibody [cPRA] 99% for HLA Class I and II) by pregnancy, SLR, previous transplantation in 2012 with subsequent graft loss in 2017 due to thrombosis
    - Predicted donor frequency 0.03% and was deemed to have no chance to receive a transplant
- She had several living donor offers, so desensitisation while on the waiting list was attempted using rituximab and several rounds of IAS, with very little effect (cPRA remained at 99%)
- Because the patient entered high urgency status, they decided to perform a living kidney donation using the patient's mother (best match)
    - Immunological status ABO incompatible (ABOi), T and B cell CDC-XM positive,
      four DSAs with high MFI:
        - HLA-B*07:02 (B7) – MFI 26,000 – complement binding
        - HLA-C*07:02 (Cw7) – MFI 16,000
        - HLA-DRB1*11:04 (DR11) – MFI 17,000 – complement binding
        - HLA-DRB3*02:02 (DR52) – MFI 12,000 – complement binding
- The patient received standard immunosuppression (tacrolimus/Mycophenolate Mofetil [MMF]/prednisolone) from 5 days pre-surgery, daratumumab (anti-CD38 monoclonal antibody) 2 days pre-surgery, horse ATG (hATG) starting 1 day pre-surgery for 5 days and imlifidase on the day of surgery. Post-surgery, rituximab was given at day 6, intravenous immunoglobulin (IVIG) days 7–9 and the patient was re-dosed with daratumumab on day 10
- All DSAs were eliminated by imlifidase, with 2/4 (both HLA-DR) showing sharp rebound around day 6–7, falling to below pre-imlifidase levels by around day 17 and

inexplicably remaining low until past day 60. Most other antibodies returned to near pre-imlifidase levels, although other HLA-DR/DQ showed a similar pattern to the two DR DSAs

- The patient had a complicated clinical course with bleeding issues and no graft function for the first month, during which time she underwent dialysis. At around day 30 the creatinine levels spontaneously stabilised and remained low (1.4 mg/dL) from day 40 onwards
    - Biopsies were performed at days 7 and 14, which showed no signs of AMR/rejection
    - Levels of selected non-DSA immunoglobulin (Ig) G antibodies against dsDNA, β2 glycoprotein IgG, cardiolipin IgG and phosphatidylserine IgG were sensitive to imlifidase treatment and have remained low (near day 80 so far)
    - Analysis of immune cell levels showed a rapid return of T cells after ATG treatments, indicating a lack of efficacy with hATG and daratumumab treatments affected mainly peripheral natural killer (NK) cells
        - There was a large expansion in peripheral plasmablasts after day 10, despite anti-CD20 and anti-CD30 treatment, and there were no measurable peripheral B cells
    - The patient contracted SARS-CoV2 on day 35, which was treated with tixagevimab/cilgavimab, but was already mounting a humoral response by herself as measured by the presence of nucleocapsid IgG. She had mild symptoms and was discharged on day 47
- In summary: the individual patient risk evaluation included donor/patient characteristics and the immunological characteristics led the team to decide that her young age and non-frailty, high-urgency situation, low chance of obtaining a donor organ the usual way even after delisting, coupled with the availability of a living donor, meant that the risk was acceptable. She had a good response to the desensitisation strategy and is living well with no further complications

RR-S then discussed a generalised approach to the immunological factors that contribute to risk of development of DSAs in kidney transplant patients.

- HLA mismatch is a predominant risk factor for reduced long-term graft survival; however, immunosuppression is not stratified to account for the level of HLA mismatching in most transplant centre protocols
    - Graft survival declines over time and the loss curve is steeper for each extra mismatch (2005–2014 data shown from Collaborative Transplant Study Registry)
- Available data are retrospective but may be able to contribute to improving prospective studies in the future, and to show that HLA is a risk factor for developing DSAs, which represent the most important factor leading to graft loss
- It has long been known that antibodies develop against polymorphic structures – most importantly HLA molecules, but a relatively new breakthrough understanding is viewing the HLA molecule as a compound of different self and non-self epitopes, and various structures are prone to recognition by the host's immune system. This is only recently entering into the thinking of kidney practice

- Antibodies usually have a polymorphic epitope, surrounded by structural epitopes that are similar to the host epitopes, and the degree of risk is dependent on the extent of the differences between these structures
- Current concepts to reduce allo-sensitisation include stratification of immune suppression in patients with a high risk of developing antibodies or reduction of mismatches and optimisation of matching
- For example, a single antigen/molecule mismatch can have a very different magnitude of effect depending on its location in the HLA molecule (Wiebe. *AJT*. 2018) ranging from very few, right up to 30 different mismatch epitopes
    - This means antigen-based risk assessment of DSA development and graft failure is therefore incomplete as a tool, with a need for more granular assessment based on epitope assessment
    - Other methods include single molecule eplet mismatch and DSAs, epitope mismatch and adherence, all of which show a correlation in that they represent genetic polymorphism, with cumulative effect on the risk of developing DSAs
- Prospective studies are needed to drive the knowledge, and to see if this approach will result in lower sensitisation in transplant patients
- There has been one more recent Canadian study that suggests that centres would need a waiting list size of 250 patients to enable transplantation of 80–90% of patients with an epitope mismatch of ≥10 (suggested cut-off for increased risk of sensitisation). This would represent a cohort with a low risk of iso-sensitisation
- In summary, there are two current approaches to limit sensitisation:
    - Utilise epitope or molecular mismatch data, but this would be dependent on having a large waiting list
    - Perhaps more feasible, and currently in clinical trials, is to reduce immunosuppression depending on individual risk

Discussion and comment

- It was especially noted that, in the case study, despite the delayed graft function, no severe immune activity was seen in the biopsies, and graft function stabilised and remains so
- The longer-term outcomes will be informative
- A consensus has not yet been reached on the different types of incompatibility

Paired exchange programmes in small countries

Ondrej Viklicky
Georg Böhmig
Annemarie Weissenbacher
Karine Hadaya

This session aimed to outline the options and initiatives that are being used or developed for enabling paired exchange programmes in countries that have small populations, and

hence a small pool of donors and recipients, thereby reducing the likelihood of matching up suitable donor-recipient pairs.

- OV first outlined the kidney paired donation (KPD) systems that are currently operation in Europe, illustrating the range from large, advanced programmes such as those seen in the UK and Spain, through new, smaller programmes (including France, Italy, Czech Republic, Austria, Portugal), programmes in preparation or with *ad hoc* exchanges (Sweden, Switzerland, Slovenia and Greece) to countries with no KPD activity (Iceland, Republic of Ireland, Finland)
- The first international KPD was a simple swap between two incompatible couples to achieve full compatibility in 2011
    - The patients are still doing well at present
- Since then, the exchanges mostly remained within the individual countries, until 2016 when international exchanges recommenced (n=10) (Viklicky *Transpl Int.* 2020)
- There were differences in approaches between the two centres, with Prague referrals having a large proportion (71.3%) of ABOi patients, only 21% HLA-incompatible and 7.7% both. By contrast, Vienna referrals had roughly equal proportions (37%, 29.2% and 31.5%, respectively)
- For KPD, this meant that most ABOi transplants were performed in the home countries, but by changing practice they have increased the numbers of international transplantations
- Israel has now been added to the mix, which unlike the Czech–Austrian exchanges is non-simultaneous, so this generally increases the cold ischaemia time (CIT) by around 2–7 hours. Although not without challenges, the exchanges have worked well
- With current learnings, they now use the Transplant Matching Programme from Institut Klinické a Experimentální Medicíny (IKEM) in Prague, which has also been used in Vienna and Belgium
    - This allows the definition of unacceptable/acceptable antigens, including ABO
- They are now using non-simultaneous extended altruistic donor (NEAD) chains because of the small donor pool that is available to such small countries
- In one chain, one altruistic donor in 2018 led to a chain of 19 kidney transplantations with some short periods where donors needed to wait until a suitable recipient could be identified
- Shorter NEAD chains can be used in problematic cases, although he was uncertain if this would be applicable to imlifidase patients. For example, a patient who had previously been a living donor for a relative with IgA nephropathy, who later developed IgA nephropathy himself and entered kidney failure. Two countries aided in sourcing a match and closed the chain from Austria to help him get a match
- In small countries, KPD is only for a very limited set of patients because of the small donor pool, but this may be modified in the future

GB then reported on a collaboration between the KPD programmes in Vienna and Australia (Paola Ferrari and Samantha Fidler.

- The Australian algorithm involves:
    - Luminex based (SAB)
    - High-resolution HLA typing: HLA A, B, C, DR, DQ, DP
    - Crossmatching: CDC-XM and flow-XM
    - Calculation of HLA-compatible pairs, ABO-compatible or -incompatible
    - Two- and three-way loops
- The Austrian team had performed two crossover transplantations before engaging with the Australian team's algorithm to try to build a systemic programme
- Validation study together with GF and the Australian team examined 16 HLA-incompatible pairs, with or without ABOi (Böhmig. *Human Immunol.* 2013) to demonstrate a good correlation between matching using V-XM and actual flow and cytotoxic crossmatches
    - Negative crossmatch cut-off of 2,000 MFI in Luminex – below this value a negative CDC-XM is predicted
- The first kidney paired exchange (KPE) using this algorithm was a simple two-pair exchange and was successful with no rejection in either case
- The next case involved three pairs; this was challenging because the surgeries were performed in the same centre on the same day to avoid any risk of a donor reconsidering
    - This policy has now been amended to include more than one centre to ease the burden on any one centre and a collaboration established with Prague as described by OV in the previous talk
    - The first cross-border KPE was successful although needing IAS desensitisation in one of the involved recipients to reduce residual HLA antibody incompatibility
- Since the first exchange, Prague has included more ABOi pairs than Vienna which meant they could do more exchanges, although these proportions have since changed to similar numbers to Vienna
- Since 2019, Innsbruck has also participated as an independent KPD partner.This was followed by a kidney exchange with Prague. Until now, no exchange between Vienna and Innsbruck was performed.
- Israel has many incompatible pairs and is now in partnership with Prague and Vienna and has successfully made exchanges with both countries. This has doubled the size of the potential donor pool
- Current status for Vienna is 19 KPD transplantations, eight of which are international: seven from Prague and one from Israel, with a further imminent exchange with Prague
    - Three active countries
    - 32 potential pairs
- Hopes that Germany may also join; however, there are barriers to this regarding anonymity between the KPD pairs, the rules for which are different in Germany. If this changes, the number of potential exchanges will significantly increase.

Discussion and comment

Comment from RO: noted that in paired exchanges, non-sequential donors rarely refuse, even after some time. It can be a concern in theory but seems to be a lesser factor in practice.

AW next spoke about the Innsbruck experience with KPD, as well as the experience of the effect of machine perfusion for organ preservation on delayed graft function in sensitised patients.

- The Innsbruck team has so far performed two paired exchanges via the international platform:
    - Two-pair exchange with Prague in 2020 – second kidney of their female patient, who remains well (27 March 2023: serum creatinine 0.87 mg/dL, urea 30.5 mg/dL, estimated glomerular filtration rate [eGFR] >60 mL/min/1.73 m^2)
    - Two-pair exchange within Austria – two female recipients, two male donors. Both patients were doing well at last follow-up with good kidney function
    - Three more exchanges were planned, but two patients received kidneys via other routes and one needed a blood transfusion leading to additional sensitisation
    - Two more exchanges are planned

- LifePort hypothermic machine perfusion (HMP) has been part of clinical practice since 2014; since 2019 an institutional standard operating procedure for the application of HMP has been in place
- Since then, the proportion of deceased donor kidney transplantations using HMP increased from 26.5% in the period 2016–2018, to 46.8% in the period 2019–2022
- Delayed graft function (DGF) has dropped from 39.3% of 328 kidneys to 27.6% of 380 kidneys, although overall CIT has increased from 13.9 (3.6–26.7) hours to 14.1 (4.3–40.4) hours
- This has been especially important as they are accepting more extended-criteria deceased donors, 1.2% increased to 7.9% in the same time periods. They cannot decrease the CIT but the DGF has still fallen by 2%
- HMP is used either to perform IAS pre-transplantation or await crossmatch results
- HMP was compared with static cold storage (SCS) in sensitised patients (15 and 10 kidneys, respectively): 11/15 HMP recipients were previously transplanted vs 9/10 SCS patients
    - The CIT was significantly longer in the SCS group than it had been before HMP was introduced
    - There was no significant difference in CIT overall between groups
    - There was no significant difference in DGF between groups, although rates were higher in the HMP group: 8/15 (53.3%) in HMP vs 3/10 (30%) in SCS; p=0.26
    - No significant difference was seen at 3 months post transplantation between groups in mean serum creatinine or eGFR (although non-significantly higher in the HMP group) despite the longer preservation period and longer DGF

    - This suggests that with increased CIT the use of machine perfusion is not deteriorating the kidney, allowing desensitisation to be performed
- The team hopes to use HMP when using imlifidase to mitigate any CIT increase necessitated by the mandate in Austria to have a confirmed negative crossmatch before initiating the transplant

KH from Geneva, the final speaker in this section, spoke about the Swiss Kidney Paired Donation System (KiPaDoS).

- The first exchange in the Western world between a Swiss and German pair was performed at Basel hospital in 1999. However, legal complications precluded any further KPE until 2011
- Legal changes enabled cross-over exchanges and two further two-pair exchanges and a single three-pair loop then took place
    - The donors travelled to recipient centres where all parties chose to meet
    - All surgeries occurred on the same day; data were shared and showed good outcomes
- These successes led to the first Swiss Meeting in September 2012 between six transplant centres that between them had 38 incompatible pairs unable to donate to native recipients in a country of 6 million inhabitants
    - It was decided that a national programme similar to the Netherlands (59 pairs in 16 million inhabitants) was worthwhile
    - No legal details on KPD in Swiss transplant law; altruistic donor allocation is to the best-matched patient on the waiting list
- In consultation with the Dutch Transplant Foundation in Leiden they established a National Coordinator and developed software based on current allocation protocols for deceased donors
    - Luminex-based DSA with MFI cut-off values decided by the centre
    - CDC-XM and flow-XM
    - *De novo* ABOi patients were accepted, as long as their native antibodies were <1/64 to be on time for the procedures
    - Two- or three-way loops
    - Aligned donor acceptance criteria
        - Bylaw was passed in 2018: no chain initiation by a non-directed (altruistic) donor, presurgical anonymity, but post-surgery meet-ups were permitted if all parties agree
- Pre-bylaw changes (2011–2018), 45 KPD procedures were performed ‘hand to hand’; since then (2019–2022), 15 procedures using the software have been done, and there is a desire to work out how to increase numbers
- Factors include:
    - Post-run decline of offers for immunological reasons
    - Inclusion of ABOi pairs leads to a decrease in the proportion of HLA-sensitised patients
    - Need to sometimes persuade patients to wait a little longer for a more compatible donor so they need less immunosuppression. A comment was made, however, that it may be up to 9 months before the next match run is

performed, so some patients may wish to go ahead anyway to avoid initiating dialysis during this intermission
    - New legal changes in 2025 mean that inclusion of altruistic donors into KPE chains may be permitted, which will increase opportunity for chain initiation
    - The restricted donor pool from being a small country means they are actively seeking international European collaboration opportunities, otherwise they are restricted to an average of 20 pairs

Defining the patient population who would benefit from imlifidase: Discussion

Moderator Rainer Oberbauer

RO posed questions on how to define HLA incompatibility, when to use historic methods for desensitisation and when to use products such as imlifidase.

- For imlifidase, there is a need for a general protocol. Which DSAs are acceptable when delisting, other medicinal products, induction, AMR management, etc. Currently, there are only the Summary of Product Characteristics and the PAES protocol
- Should delisting occur before the ET rules have changed?
- After discussions in Vienna on who should be delisted, there were four or five patients with 100% cPRA – does it make sense to delist them all (DSAs) because after 7 days the sensitised state returns?
- GB thought there was a need to share experiences and data, especially in cases where severe rejection has occurred. These data will help to optimise immunosuppression and which risks can be taken
- KB explained that there is a desire within ET to have a registry or ability to collect the data. There are no B-list data yet within ET. However, in an earlier meeting, PAES Hansa protocol seemed to show a lot of rejections, not unexpectedly. This shows the need to collect all the data, and perhaps draw further conclusions. It may need to be done in connection with Hansa, but ET as a body cannot make a recommendation for immunosuppression
- RO agreed that colleagues are sharing data as episodic reports, without certainty that the DSA profile in each case would have required imlifidase:
    - Three cases from Lucrezia Furian in Italy
    - One case from John Boletis in Greece, in which they used everything available to treat the patient
- He feels, therefore, that it is very important generally, and for the B list, to standardise protocols and uniformly measure some outcomes to see whether or not imlifidase is useable
- KB commented that in Germany, they have the highest demand for organs and do not want to ‘waste’ any:
    - There may be a dialysis patient next to a sensitised patient who may have much better outcomes with the kidney, so why give it to the sensitised patient?

    - They may already have had two or three previous transplantations, perhaps lost because of non-adherence to immunosuppression
    - There is an ethical dimension that is related to successful transplantation and the definition of success
- KB also spoke from the ET perspective – they want to focus on outcomes after the first 20 B-list patients and if there is for example high rejection rates then they need to stop or do something different. As a research programme, it is acceptable to spare 20 of 2,000 kidneys to further the research, but ultimately, they are also responsible to non-sensitised dialysis patients who might otherwise have received the organ
- RO acknowledged this position and noted that the conditional authorisation granted to imlifidase by the European Medicines Agency (EMA) recognised the need for more information, and pointing out that graft failure, rather than rejection is the main parameter
- MF noted that the AM programme has not been a waste of organs and has worked well. He also noted that by delisting the right antigens, some patients at 99% cPRA may not need to be that high. He suggested that delisting the right antigens will be the art to gaining similar results in the B-list as in the AM programme. Delisting too many antigens could lead to bad outcomes as immunologically there will only be a week-long safe period
- The group generally agreed that this will be important and reiterated that sharing experience on delisting, how to choose antigen, how to measure risk, learn techniques from each other, especially in cases that were problematic. For example, in the Greek case that needed a great deal of treatment, many antigens had been ignored and delisted
- There was a point made on the indication and the difficult patient population – that imlifidase might be better suited to, for example, heart transplantation where there is no alternative to transplantation. The single lung transplant case for which imlifidase has been used was noted. For him, a highly sensitised child who would not get a transplant otherwise, or a kidney patient with an incompatible living donor are relevant recipients
- The discussion also considered the definition of an HLA-incompatible transplantation. Historically, CDC-XM defined incompatibility, but more modern assays have changed this
    - RO suggested that the current literature seems to have a general consensus that a positive flow-XM or CDC-XM would suggest HLA incompatibility
    - KB observed that ESOT has recommended that the immunological risk stratification should be based on Luminex assays. Determining sensitisation is difficult, and epitopes may be important as well. This means using Luminex, plus T and B cell crossmatch. Early papers on imlifidase are very mixed, so it is difficult to determine a risk stratification. Where people have ignored CDC positive crossmatch, there were poorer outcomes. ESOT has worked at improving the risk stratification
    - KB felt that plausibility is an important issue, and detailed information shared on all aspects is very important

- GB questioned how best they can defend imlifidase use – perhaps to a decision board or committee overseen by Sebastian Heidt. Can very different immunological profiles be compared? Doing this locally has proved to be difficult
- KB felt an imlifidase board to oversee use in Germany would be appropriate, to generate consensus on unacceptable antigens then work out what to delist and ascertain the risk factors
    - Together with Sebastian Heidt, ET, HLA specialists and clinicians, a risk stratification can be performed
- KB offered the calculation that if ET has 2,000 kidneys each year and a donor frequency of 0.5, theoretically this should result in the allocation of 10 kidneys per year
- RO noted that, when selecting patients for the initial 20 who would be transplanted under the ET imlifidase B list, it was important to ensure that allocation happened according to the number of patients waiting from each country
- CM felt that you need to get the donor frequency to 0.3-0.4 to have a chance of getting an organ offer but not higher. team has 10 highly sensitised patients who have spent >10 years on their waiting list, and they hope they will now be able to transplant them
- FE suggested a stepwise approach, beginning with conservative delisting (e.g. shift frequency from 0.1 to 0.3), wait for a year and if there is no offer then delist more antigens bringing the donor frequency to around 0.6%
- AW noted that increasing the donor pool was also an important factor in increasing frequency. ET will be HLA-typing older donors who would otherwise be included in the EuroTransplant Senior Programme, as a wider age gap may be acceptable if there is a good match. This is opening up more resource to the whole population, not just sensitised patients

Safe, effective and equitable implementation of an imlifidase-enabled kidney transplant pathway

Protocol standardisation and data collection – A transnational view

Moderator Rainer Oberbauer

After the break, RO moved the discussion on to the place for imlifidase in the treatment pathway for highly sensitised patients. Is it necessary to remove the biologically plausible DSAs in the mid-strength category?

- There was varied opinion, with some saying they would use plasmapheresis or IAS instead if there were only a few, or medium-/low-titre DSAs
- SS suggested that imlifidase can be used for patients with a CDC-XM positive or based on high MFI, which shows a correlation with a positive CDC-XM and seems to indicate a higher risk of AMR. There should be some standardisation on thresholds, but it is difficult to define patterns of epitopes and eplets in highly sensitised patients because almost all are likely to be positive. It is therefore difficult

to determine which specificities are not harmful and can be delisted. Achieving a standardisation would be useful. He felt that imlifidase is not needed when the MFIs are low

- KB agreed that data on the use of imlifidase in CDC-XM positive patients were very scarce. CDC-XM negative patients can be transplanted within ET using IAS. He felt IAS may be more appropriate than imlifidase in low-risk patients and suggested that a head-to-head trial between imlifidase and IAS would be valuable in determining this
- GF felt that the low-titre or low-affinity antibodies that they deal with in their living donor, can probably be delisted for the ET B list. Those antibodies could be dealt with on rebound using IAS and are therefore imlifidase-acceptable antigens. However, repeated mismatches or many different DSAs are likely to be unacceptable
- GB noted in relation to MFI levels and AMR risk correlation that as a rule, they estimate a 50% risk (their risk limit) of AMR if the MFI levels are above 10,000. Although long-term outcomes are ultimately similar, patients who experience AMR will need more immunosuppression. Step-by-step delisting according to strength may be needed
- Overall, there was an agreement that a range of around 2,000–5,000 MFI was a more manageable risk level, but that the presence of other factors, such as repeat mismatches and positive CDC-XM should be avoided when using imlifidase
- There was some thought that centres who see very low volumes of highly sensitised patients will not necessarily acquire the expertise they need

- Discussions on the potential use of IAS in conjunction with imlifidase noted that it would need to be performed before imlifidase administration; however, there was little appetite for this kind of pre-desensitisation. It was also noted that IAS can also remove other classes of antibodies as well as IgG

- AW requested clarification on the ET rule requiring a negative crossmatch before administering imlifidase and the effect on CIT. In particular, would there be a potential second recipient in place in the event of a negative crossmatch not being achieved after the first dose, or would the kidney remain at the initial centre?
    - The general feeling was that it is very rare for there to be a negative crossmatch after 4 hours. The advice was to take a pre-imlifidase sample, give imlifidase, then take another sample 4 hours later, then process both samples together. This means only one set of crossmatch processing time (3–4 hours)
    - AK informed participants that, from another perspective, he has seen in the imlifidase Anti-Glomerular Basement Membrane Disease study that antibodies are cleaved in a few minutes and return after 5–10 days. At that point, they need to be ready with PLEX or IAS. Imlifidase is buying some time which is very important in anti-glomerular basement membrane disease
    - It was also noted that in animal studies kidney-bound IgG antibodies are also rapidly cleaved – not just those in circulation

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- KB asked if there were any immunological effects of imlifidase beyond elimination of IgG antibodies.
    - FE replied that plasma cells have compensatory mechanisms for detecting/balancing levels of IgG in the blood and may become activated in response to the decrease in IgG levels. This may be a reason for the strong rebound seen in some patients
    - The relative level of cleavage for different isotypes of IgG was mentioned, noting that IgG3 is cleaved to a slightly lesser extent than the rest. It was also noted that the B cell receptor is also cleaved by imlifidase
    - TM noted that one-time removal of antibodies is performed quite often, and this is not a dangerous thing to do *per se*. The mode of action of imlifidase is well defined and dependable, but cumulative immunosuppression may be one of the key factors for consideration when using imlifidase

- TM discussed their application for imlifidase in Switzerland to the Diagnosis Related Group (DRG) for inclusion on the speciality list. There is a desire by the DRG to keep the application simple, but it has been difficult to define a particular patient group as the potentially eligible population is very heterogeneous. He would like to have a set of criteria, such as age, time on waiting list, cPRA limit (suggested ≥99%), and the number of acceptable/unacceptable DSAs
    - CM observed that this was similar to the criteria that are being considered in Germany. He was concerned that imlifidase is a one-shot strategy, requiring thought being put into several options for managing rebound and AMR

- CM also suggested that a technique his team has used in the past, of desensitising and observing rebound while patients are on the waiting list to identify unacceptable antigens, may also be a useful tool to inform delisting when using imlifidase
    - RO noted that some patients appear to be DSA negative on transplantation but have early severe disease (AMR?), suggesting that they have a memory response along with plasma cells that would not have been detected before transplantation. Should extra tests or biopsies be considered in the few patients for whom imlifidase is being considered, or is flow-XM sufficient?
    - GF noted that there were differences in transplanting kidney, lung and heart and that the right conditioning of the patient, as well as immunosuppression was important

- There was a question on whether there should be a study to compare outcome in patients desensitised using imlifidase vs standard of care. RO felt obtaining academic financing for such a study would be challenging at this point, although it may be interesting to ETKAS as a subsequent study
- KB and RO discussed the possibility that the current inclusion of higher risk patients in current imlifidase case studies may show higher rates of AMR than reported in the initial studies, led to a potential call for the EMA and ESOT to lend support for requesting a controlled comparative study before users will be comfortable in using imlifidase. There was a feeling that the current PAES should have a control arm

- GB felt that although imlifidase itself is not dangerous, it is impeding the transplantation-associated treatment. Because of its mode of action, rabbit ATG (rATG) cannot be administered until 4 days post transplantation. There was a comment noting that not all countries have access to hATG, so the PAES protocol was developed to allow the use of rATG instead. RO felt that the delay in initiating ATG therapy is somewhat counter-productive and could lead to harm

- TM initiated a discussion on patient risk, noting that their oncology colleagues are always very happy to have a new treatment and will use it much more readily than the nephrologists and with less aversion to the cost; however, KB pointed out that there is a risk of wasting organs, not just money. RO felt the sensitivity for potential side effects is different in the two fields. TM countered that they have a powerful drug, they can do the transplantations
    - KB suggested that there is no real immunological effect from imlifidase, it is more like very effective plasmapheresis, but they need a solution to prevent rebound
    - TM suggested that this was the case for all high-content biologics; they do not give a long-term perfect effect. For imlifidase, they now have data to show the grafts have lasted 3 years so far, although KB thought that imlifidase was not necessary in at least half of the patients
- RO did not feel they would currently be able to resolve the different approaches to risk and concluded the discussion on desensitisation and imlifidase

Future potential of T regulatory cell therapies

Thomas Wekerle

TW spoke next on perspectives for the future – can cell therapy with regulatory cells modulate the allo-response in sensitised patients? In particular, could T regulatory (T_{reg}) cells remove the need for immunosuppression by regulating the T effector (T_{eff}) cells?

- The approach appears to have had success in liver transplantation, with a small study in Japan showing 7/10 liver transplant patients could cease immunosuppression within 18 months of initiating therapy (Todo *Hepatology* 2016)
- No success has been seen in kidney transplantation with the same cell therapy (Koyama Transplantation 2020; 104:2415). In 7/16 HLA-mismatched living donor transplantations, recipients developed acute rejection and none of the 16 was able to cease immunosuppression after T_{reg} therapy
- However, the ONE study has brought preliminary evidence for efficacy in immunologically low-risk patients with PRA <40%
- The results show that protolerogenic protocols applicable to one organ may not translate to another
- Genetically modified chimeric antigen receptor (CAR) T_{reg} cells have the antibody-binding portion of an antibody spliced onto a modified signalling portion of a T cell receptor, which also comprises all the relevant domains to ensure correct configuration and stabilisation, and to provide first and second signals to activate the T cell. Fourth-generation CAR T cells (TRUCKS T cells redirected for antigen-unrestricted cytokine-initiated killing) use a transgene to combine the direct action of

the CAR T cell with the immune modulating capacities of the delivered cytokine, anticoagulants, etc. (Kaljanac, Abken. *Transplantation.* 2023) CAR T_{reg} cells are of great interest in organ transplantation, as they are more potent than polyclonal T_{reg} cells
    - *In vitro* data show that CAR T_{reg} cells directed towards the donor HLA antigen are more potent compared with polyclonal T_{reg} cells (Lombardi. *AJT*. 2017)
    - *In vivo* mouse models of heart transplantation show a similar pattern, but eventually the suppression fails, even at a dose of 4×10^6 CAR T_{reg} cells. With an HLA-A2 mismatch and several major histocompatibility complex types, CAR T_{reg} cells alone were not capable of inducing permanent graft acceptance; however, there was some delay in rejection when used in combination with rapamycin (Tang. *AJT*. 2022)
    - These data suggest that CAR T_{reg} cells are potent but insufficient to induce tolerance without additional treatment
- In mouse skin transplantation models with sensitised vs non-sensitised recipients, CAR T_{reg} cells failed to control the allo response in sensitised recipients, although there was a significant delay seen in naïve recipients (Levings. *AJT*. 2020)
- There are ongoing CAR T_{reg} trials in human organ transplantation on non-sensitised adults: STEADFAST (Sangamo) in kidney transplantation and LIBERATE (Quell) after liver transplantation. Both studies have yet to report results, which if positive would boost the field (Scheeb. *Kid Int Rep*. 2022)
- TW is interested in the concept of chimerism; transplanting stem cells from the organ donor along with the kidney. All protocols to date include myelosuppressive preconditioning of some kind, and his group want to avoid this (Mahr. *Front Immunol*. 2017)
- In mouse models, the combination of a donor bone marrow graft with either skin or heart, plus recipient-strain T_{reg} cells, and a combination of CTLA4Ig + anti-CD40L monoclonal antibody + rapamycin, promotes chimerism-induced tolerance (Pilat. *AJT*. 2010; Pilat. *Transplantation.* 2011; Pilat. *J Heart Lung Transplant*. 2014; Pilat. *J Immunol Res*. 2015; Hock. *Eur J Immunol*. 2015; Pilat. *JCI Insight*. 2016; Granofszky. *Front Immunol*. 2017; Mahr. *AJT*. 2017; Mahr. *AJT*. 2019; Mahr. *AJT*. 2021)
- TW's group has now progressed to a pilot clinical study in collaboration with RO and others, in which recipients are given a living donor kidney plus donor bone marrow, then, within the first 3 days, they receive an infusion of autologous *in vitro*-expanded polyclonal T_{reg} cells plus an anti-IL-6 monoclonal antibody (Oberbauer *Front Med*. 2021). The recipients do not receive any myelosuppression, and the study aim is feasibility, safety and induction of transient chimerism
    - The trial is still ongoing, preliminary data showed that Treg therapy together with donor bone marrow infusion is tolerated without any immediate adverse events.

Discussion and comment

- EG commented that this approach is unusual in that rather than trying to block something, as past approaches have done, something is being added. Blocking always eventually fails. In oncology, certain approaches such as checkpoint inhibitors are also trying to boost anti-tumour immune function. He acknowledged that the data and development of T_{reg} cells are still ongoing, but the preliminary results were encouraging
    - EG also noted that the inclusion of controls in the study added to the validity of the study
- TW felt this approach of basically copying the self-tolerance mechanism was promising, suggesting that it could also be attempted by other means
- FE suggested that adding more immunosuppression at the beginning may prolong the longevity of the T_{reg} cells
- TW outlined two possibilities:
    - Apart from mTOR inhibitors, other known immunosuppressive drugs could negatively affect T_{reg} cells; they had discussed belatacept and calcineurin inhibitors and both can have a negative effect on T_{reg} cells
    - Second, persistence of T_{reg} cells is still an unknown factor in many models and is one of the goals in CAR design

Management options for treating AMR post-imlifidase

Klemens Budde
Georg Böhmig

The final pair of presentations discussed the options for treating AMR when it occurs; they were split into early active and late chronic, as the treatments for each situation differ.

- KB began by suggesting that the success of desensitisation depends on the frequency of rejection and the success of rejection treatments. After desensitisatioon most centers have a rejection rate of ~40% however the diagnosis of rejection may differ between centers and according to the evolution of the Banff categories
- Outlining the treatment suggestions in the KDIGO guidelines, which were published 13 years ago, KB noted that he felt they needed updating in light of more recent developments:
    - "…suggest treating antibody-mediated acute rejection with one or more of the following alternatives, with or without corticosteroids (2C):
        - Plasma exchange
        - Intravenous immunoglobulin
        - Anti CD20 antibody
        - Lymphocyte-depleting antibody"
    - He commented that there are issues related to interpretation of study results, which always comprise a variable mix of interventions, early vs late, pre-formed vs *de novo* DSA and changes in Banff classification. These factors make it difficult to draw sound conclusions
- By examining the pathways of humoral rejection, many potential therapeutic concepts have been developed, including imlifidase. Targets include B cells, IL-6,

plasma cells, NK cells and antibodies. Analyses of publication frequency and use in main centres can be a tool to discover trends in therapeutic approaches (Roberts. *Transplantation*. 2012; Burton. *Clin Transplant*. 2015; Sood. *Clin Transplant*. 2022)
    - A survey in 26 US centres in 2014, showed plasmapheresis, IVIG, corticosteroids and rituximab as the most frequently used at that time
    - Trends vary by the type of AMR, with bortezomib and eculizumab featuring more strongly in recurrent AMR
    - However, a 2018 meta-analysis showed low evidence for any treatment other than antibody removal by plasmapheresis or IAS (Wan. *Transplantation*. 2018)
    - A randomised controlled trial (RCT) in 10 patients using IAS with protein A vs standard of care without IAS, showed a statistically significant benefit in graft survival with IAS (Böhmig. *AJT.* 2007) which KB suggested was indicative of the removal of antibodies being the key to successful treatment of AMR
    - Combination therapy (plasmapheresis/IVIG/rituximab) vs IVIG alone showed IVIG is insufficient as a single agent, indicating that it needs to be used with plasmapheresis and steroids, and perhaps rituximab, although the latter leads to a greater side effect burden and overall costs (Lefaucheur. *AJT.* 2009)
    - Rituximab vs placebo was studied in the double-blind prospective RITUX-ERAH trial which showed no benefit of rituximab either short or long term (Sautenet. *Transplantation.* 2016)
    - In a study over 7 years from Bob Montgomery's group in severe AMR after desensitisation, 14 patients were treated with splenectomy, five with eculizumab and five with both. While the combination was slightly better, 2/3 had graft loss or transplant glomerulopathy (TG) by the end of the first year.
- KB was part of a consensus meeting in 2019, which concluded that histology alone was insufficient to make a sufficient analysis of AMR for treatment decisions (Schinstock. *Transplantation*. 2020). Important factors include distinguishing between preformed or *de novo* DSAs, and the time after transplantation. This allows classification into phenotypes of early active AMR and late chronic AMR
- Early active AMR – memory B cell response on days 7–14, pre-existing DSA, CD4d positive with frequent thrombotic microangiopathy leading to graft loss within days if left untreated. Even after treatment there is frequent TG
    - For this phenotype, the consensus for standard of care treatment was one of:
        - Plasmapheresis (frequency base in DSA titre)
        - IVIG either fractions after each plasmapheresis treatment or one larger dose once plasmapheresis ends
        - Corticosteroids
    - Adjunctive treatments were complement inhibitors, rituximab and splenectomy
    - There was no conclusive evidence for any specific therapy
- Overall, more clinical studies are needed to define therapies or combinations of therapies. In the meantime, a combination of steroid, plasmapheresis and IVIG as the mainstay to prevent early graft loss, with splenectomy, eculizumab or rituximab

as adjunctive therapy. New therapies are under investigation, including imlifidase, but more evidence is needed

GB continued the section, speaking on late chronic AMR, which can happen even many years after transplantation.

- In a previous trial evaluating bortezomib in late ABMR (BORTEJECT), around 50% of patients experiencing late chronic AMR had pre-formed DSAs and had received desensitization treatment at the time of transplantation.
- There are no recommendations from the consensus paper for treating chronic AMR beyond optimising immunosuppression (Schinstock. *Transplantation*. 2020)
    - The OutSMART study has shown that this approach is ineffective at delaying failure of graft loss (Stringer. *eClinMed*. 2023), but several limitations of the study impeded a valid interpretation of results.
- There are many ongoing or recent studies with varying targets (Mayer. *Expert Opin Emerg Drug*. 2022). Of those that have reported results (some were terminated early, mostly due to slow patient recruitment or financial issues), the outcomes are mainly negative. GB then summarised the published study results:
    - Results from TRITON, a Spanish study in 25 patients with TG, who were DSA positive with an active AMR phenotype. Patients were randomised to either IVIG and rituximab or no treatment. There was no effect on either eGFR or immunodominant DSA MFI (Moreso. *AJT*. 2018;18:927)
    - A Phase 2 RCT, BORTEJECT, was performed in Vienna, on 44 patients with DSAs and AMR morphology ≥6 months post transplantation and with eGFR ≥20 mL/min/1.73 m^2 with patients randomised to receive two cycles of either bortezomib or placebo. The primary endpoint of eGFR slope was the same in both groups, as were the secondary endpoints of measured GFR, DSA MFI, 24-month biopsy and survival (Eskandary. *J Am Soc Nephrol*. 2018;29:591)
    - An observational study of the IL-6 inhibitor tocilizumab in 36 patients with chronic AMR, showed stable eGFR on tocilizumab, and relatively good graft survival, but the lack of a control arm made the results difficult to interpret
    - Other small observational studies are ongoing, but GB felt the lack of controls and low patient numbers mean they are controversial
    - In collaboration with KB, the centre in Vienna undertook an RCT of the anti-IL-6 antibody clazakizumab, in 20 patients with late AMR who were randomised 1:1 to receive clazakizumab or placebo for the 12 weeks of the RCT. At 12 weeks, all patients received clazakizumab until week 32 in an open-label, uncontrolled phase. In retrospect, they feel that the 12-week RCT phase was too short as there were no differences seen between the active and control arms during this period.
      At 51 weeks they saw slightly higher eGFR with clazakizumab than placebo, a lower molecular AMR score (significant between week 11 and 51) and disappearance of C4d staining over weeks 11 to 51 in some individuals, despite the study not being powered for clinical significance (Doberer. *J Am Soc Nephrol*. 2021)
    - The results were promising enough for the company to proceed to a Phase 2 RCT, IMAGINE, in 350 planned patients with chronic active AMR at >80 sites

throughout North America, Europe and Australia. Patients are DSA positive, eGFR 25–65 mL/min/1.73 m^2, >6 months post kidney transplantation. Patients will be randomised to receive clazakizumab or placebo for 260 weeks and followed up for 5 months after the last dose (Nickerson. *Trials*. 2022). Currently, around 150 patients have been included in the study.

- GB felt that CD38 is an interesting target for research as it works on more than one cell type: plasma cells and NK cells, which appear to play a role in AMR. In a case study of a patient with smouldering multiple myeloma and a chronic active AMR phenotype, treatment with daratumumab (along with steroids) led to some stabilisation of the eGFR and a reversal of rejection by morphologic and molecular microscope results. He felt they were intriguing results, but as it is only one patient, acknowledged that the results may be chance
    - Now they have initiated a study together with the Berlin group, in 22 patients who will be randomised 1:1 to receive the CD38 antibody felzartamab or placebo for 24 weeks, with two follow-up , one after 6 and one after 12 months
        - Primary endpoint: safety and tolerability
        - Secondary endpoints: DSA levels and characteristics, follow-up biopsy results, leukocyte subpopulations, serum urine markers of inflammation and endothelial activation injury, Torque teno virus load, eGFR slope, proteinuria, iBOX
        - Results are due in 2024
- Overall, late chronic AMR is associated with adverse graft survival and there are no currently proven effective treatments. Promising concepts are under evaluation, but so far the evidence is low and anecdotal. New concepts should be tested in well-designed RCTs, such as the IMAGINE study, and surrogate endpoints to accurately predict graft failure need to be defined. There has been some discussion on the iBOX for this purpose, but it not yet accepted as an endpoint

Discussion and comment

- RO thanked both speakers for their talks commenting that both types of AMR treatment are important to cover, each with different treatment modalities, outcomes and risks
- RO noted the relevance to imlifidase use regarding the treatment of potential rebound and AMR after 5 days or so, and asked the participants what they felt should or could be done to mitigate these.
- AW questioned whether regularly monitoring any rebound antibodies after IAS could help define which ones could be delisted for imlifidase and which should not. CM replied that they have been doing this as a routine but still see some completely unexpected behaviour in antibodies after desensitisation
- BB asked whether prevention of AMR may be achieved by keeping the basal level of immunosuppression as high as possible, including after imlifidase treatment. KB replied that patients receiving transplantations after desensitisation with imlifidase will have received steroids, high-dose tacrolimus and MMF, but they still get rejection from day 7. He cited a study of prophylactic treatment with eculizumab

where patients had less AMR after desensitisation, but still had 40–50% transplant glomerulopathy after 1 year.. His opinion is that if the intention is to prevent AMR, then eculizumab should be given. RO commented that this is a rare situation, but they still need to make decisions

- GB noted that in the UK OutSMART study, there was a link between DSAs and AMR, but no difference in graft survival between the optimised and non-optimised immunosuppression arms. However, he felt that although the trial was negative, there were some confounding factors and several limitations impeding interpretation of results. Therefore it makes sense to maximise MMF and retain steroid immunosuppression in high-risk patients
- MF noted that in high-risk patients it would be unlikely that anyone would risk reducing the immunosuppression. Both presentations showed that the only recommended intervention early on is plasmapheresis. He noted the ET recommendation that centres giving imlifidase should have 24/7 capacity to treat with IAS. In the imlifidase studies, he noted that AMR was treated with plasmapheresis and that is as good as they can do
- RO posed the question: if we have a transplantation, and on day 7 there is an AMR, what do we do? Results from a show of hands were:

Eculizumab	2	Rituximab	0
Daratumumab	0	IVIG	~3
Splenectomy	0	ATG	
IAS/plasmapheresis	~5		

- There was a comment about the possibility of treating with hATG before day 4, or rATG depending on time post imlifidase
    - FH commented that, in the case study he presented earlier, they had used daratumumab 3 days before imlifidase, then rituximab to counter a slight increase in B cells, then daratumumab on day 10. They did not use eculizumab and have had no sign of rejection so far
- RO asked if there was a place for any other complement-depleting or blocking antibodies, but no-one had any suggestions
- TM wanted to know the group's thoughts on any benefit of IAS vs plasmapheresis. GB thought that the volume of plasma can affect the decision and that plasmapheresis is non-selective, removing any type of IgG. By contrast, with IAS and membrane filtration, complement can be targeted with good results in case studies
- FE described patients who were under full immunosuppressive therapy but still developing AMR, in which they performed Luminex assays and found IgM antibodies were present. These are more difficult to assign as their response is 'messier'. Discussion showed there was very little information on different immunoglobulins, and a lack of knowledge on the pathophysiology and responses
- KB considered that cyclophosphamide might be a candidate for investigation as well, although it would be working through a different B cell response, it may well be worth revisiting in a different disease area. There was a comment that it has been used with rituximab with good results, allowing reduction or removal of steroids

- RO asked what the group thought of CD25 as a new drug and its suitability for use with imlifidase if available for acute AMR
- There was some general discussion on complement inhibitors and risk of glomerulonephritis, with no obvious conclusion as to efficacy or suitability
- TM felt that more studies are needed, including the use of the molecular microscope on more biopsies to better define the antibodies present, as the presence of IgM may indicate that some reactions are not IgG AMR
- There was general agreement that more studies are needed

Close and next steps

RO closed the meeting by thanking everyone for attending and informed participants that they would like to draw up a work plan to make suggestions. Eventually, if the B list becomes more available or there are new data, they can repeat a similar meeting next year. ESOT was thanked for arranging the meeting.

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