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ENGAGE II

International Workshop on Immunomodulation and Desensitisation

27-28 NOVEMBER 2024

Barcelona, Spain

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ENGAGE CONSENSUS

A PROJECT BY THE EUROPEAN SOCIETY FOR ORGAN TRANSPLANTATION (ESOT)

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ENGAGE II International Workshop on Immunomodulation and Desensitisation

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Scientific Programme – 27 November 2024

Session 1 Dissemination of ENGAGE II (14:30 17:45)

Moderator: Lucrezia Furian

14:30	Welcome Address	Lucrezia Furian
14:40	**Engage II** Conclusions	Lucrezia Furian
15:10	**Interactive Case Study:** *How to transplant patients with a CDC positive X match?*	Alice Koenig
15:40	**Interactive Case Study:** *HLAi kidney transplantation with flow cytometry crossmatch pretransplant positivity: IKEM cases*	Ondřej Viklický
16:40	**Interactive Case Study:** *Day-zero DSA of undetermined significance: how to manage?*	Kevin Louis
17:10	**Interactive Case Study:** *Beyond DSA, looking for hidden memory*	Delphine Kervella

Scientific Programme – 28 November 2024

Session 2 Paving the way for ENGAGE III (09:00 12:00)

Moderator: Georg Böhmig, Olivier Thaunat

09:00	Introduction	Olivier Thaunat
09:05	*Pathophysiology of AMR*	Olivier Thaunat
09:35	*Present treatment options*	Georg Böhmig
10:35	*Paving the way for ENGAGE III*	Panel discussion
11:35	Closing	Georg Böhmig Olivier Thaunat

1. Executive Summary

The ENGAGE II International Workshop on Immunomodulation and Desensitisation was held in Barcelona, Spain, on 27–28 November 2024.

The event brought together 60 nephrologists, HLA specialists, transplant surgeons, and experts in immunomodulation and desensitisation from 21 European countries (Albania, Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Greece, Hungary, Italy, Lithuania, Norway, Portugal, Slovakia, Spain, The Netherlands, Turkey, and the United Kingdom).

Over the course of the two-day workshop, participants reviewed the outcomes of the EuropeaN Guidelines for the mAnagement of Graft rEcipients (ENGAGE) and ENGAGE II initiatives and discussed recent advancements in desensitisation and immunomodulation strategies in kidney transplantation.

Case studies were presented to illustrate clinical decision-making based on the ENGAGE-defined humoral risk stratification system, in alignment with the ENGAGE II recommendations for tailored desensitisation and immunomodulation approaches according to each risk category.

The discussion then shifted toward the development of the next phase of the program, ENGAGE III. Despite the numerous open questions in such a complex field with many different interconnected and overlapping aspects, there was broad agreement on the need to work toward a consensus, to provide recommendations on the management of antibody-mediated rejection (AMR) post-transplantation.

This consensus is urgently needed given the current heterogeneity in treatment practices across transplant centres, the low level of supporting evidence, and the limited number of adequately powered studies, many of which have yielded inconclusive or negative results.

2. Tackling sensitisation in kidney transplant recipients: outcomes and future of the ENGAGE initiative

For patients on a transplantation waiting list, the presence of circulating antibodies directed against human leukocyte antigen (HLA) molecules —defining them as 'sensitised patients'— significantly affects access to transplantation, leading to prolonged waiting times and higher mortality rates, and graft failure when the anti-HLA antibodies are specific to the selected donor. Sensitisation occurs through different modalities, namely prior transplantation, blood transfusions, and pregnancies. Sensitised individuals are at a higher risk of developing post-transplant antibody-mediated rejection (AMR). Among them, those with calculated panel-reactive antibody (cPRA) >85% are categorised as highly sensitised patients who have developed antibodies against a large variety of HLA antigens. High levels of donor-specific HLA antibodies (DSA) are strongly associated with a higher incidence of AMR and are therefore regarded as a contraindication for transplantation. However, the status of sensitised patients is highly heterogeneous. Emerging evidence indicates that the pathogenic potential of DSA varies considerably: not all DSA exert the same degree of impact on graft outcomes, and the absence of detectable DSA at the time of transplantation does not necessarily exclude the presence of preformed cellular humoral memory against the graft (DSA-negative sensitised recipients).

Over the past decade, the development of a wide array of very sensitive assays for assessing alloimmune humoral memory has contributed to an increasing number of transplant candidates being classified as highly sensitised worldwide. Currently, country-specific reports show a percentage of highly sensitised candidates varying from 20% to 30% depending on the assay utilised.

Desensitisation strategies are employed before transplantation to prevent rejection in patients with high levels of preformed DSA, increasing transplant candidate's access to transplantation. After transplantation, induction and maintenance immunosuppression can be modulated from day zero onward based on the recipient's immunological risk profile, aiming at personalised treatments and reduced likelihood of subsequent AMR and graft loss.

The primary goal of desensitisation and immunomodulation in high immunological risk patients is to enable transplantation and improve post-transplant outcomes. However, due to the current lack of robust evidence guiding the optimal management of these patients, a considerable number of them remain on chronic dialysis. This not only compromises their quality of life but also imposes a substantial financial burden on healthcare systems.

To address this critical gap, the European Society for Organ Transplantation (ESOT) launched the EuropeaN Guidelines for the mAnagement of Graft rEcipients (ENGAGE) program in 2021. The ENGAGE initiative is aimed at providing a global view of the current management of sensitised kidney recipients and to provide the community with an evidence-based consensus on how to combine desensitisation and immunomodulation strategies according to a patient's risk of humoral rejection.

In 2021, the ENGAGE working group suggested to stratify the risk of AMR and graft loss into five categories based on patient's "immunological risk" combined with data on the presence or absence of DSA and results of complement-dependent cytotoxicity (CDC) and flow cytometry (FCXM) crossmatch assays. According to this stratification, the risk of AMR decreases from Category 1 (high risk: patients with day zero DSA and positive CDC crossmatch) to 5 (low risk: patients with no DSA and no cellular memory) (Figure 1).

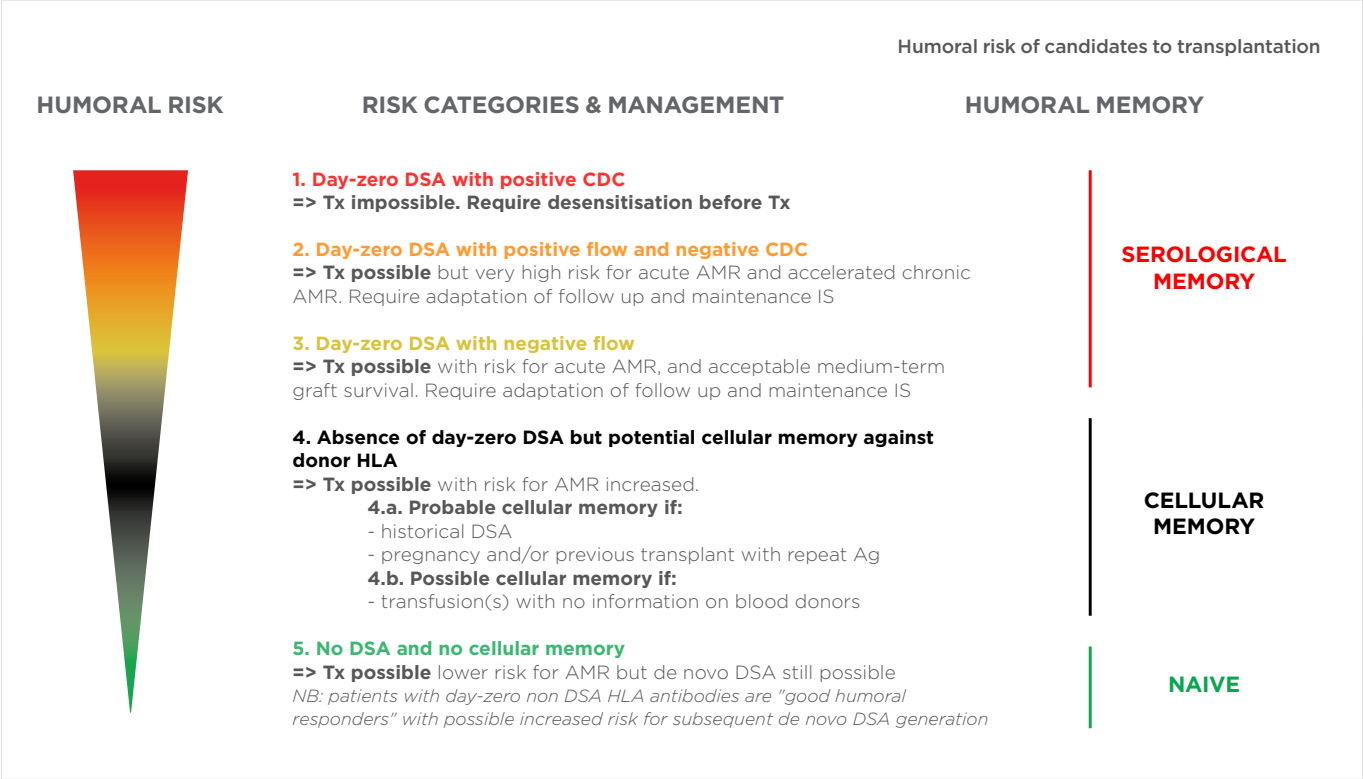

Figure 1. Categorisation of humoral risk for solid organ transplant candidates (from Bestard et al., 2021; *Transplant International;* DOI: 10.1111/tri.13874).

The establishment of well-defined strata for humoral risk in transplantation marked a crucial first step toward implementing targeted interventions based on the graded risk of AMR and toward deciphering the complex landscape of humoral alloimmune memory. This stratification accounts not only for serological memory —reflected by the presence of preformed anti-HLA antibodies (Categories 1–3)— but also for the influence of potential cellular memory (Category 4).

Following the publication of the ENGAGE strata classification in 2021, the European guidelines for the management of kidney transplant patients with HLA antibodies published by ESOT in 2022 recommended using this stratification to define humoral risk in kidney transplantation.

In addition, the ENGAGE II working group was convened to evaluate how patient management strategies could be optimally tailored to each of the five ENGAGE immunological risk categories.

Through a process involving systematic literature searching, statement development, and Delphi-based consensus, a series of recommendations for desensitisation and immunomodulation strategies, aligned with the risk categories, were formulated and published in April 2024 (see Table 1A-E).

The aim of this international workshop on immunomodulation and desensitisation was to bring together nephrologists, HLA specialists, transplant surgeons, and experts in immunomodulation and desensitisation to deepen their understanding of desensitisation and immunomodulation strategies based on patient risk, and to lay the groundwork for the next phase of consensus on the management of AMR, considering that treatment strategies are still based on low level of evidence, with relatively few studies available and many yielding negative outcomes—highlighting a significant unmet need for effective, approved therapeutical strategies.

A) Category 1 patients (DSA present with positive CDC crossmatch at day zero)

TRANSPLANTATION:

Kidney transplantation should be avoided unless no other options are available (**agreement rate 98%**). If transplantation is considered, CDC negative crossmatch must be achieved through desensitisation before transplantation, with strategies to prevent and treat antibody rebound carefully planned (**agreement rate 96%**).

MONITORING:

Clinical surveillance, DSA screening and surveillance biopsy (**agreement rate 96%**).

DESENSITISATION STRATEGIES:

Plasma exchange (PEX) and intravenous immunoglobulin (IVIg) should be part of the first-line desensitisation strategy (**agreement rate 75%**). Imlifidase may be considered for deceased donor transplants in selected cases (**agreement rate 92%**).

INDUCTION THERAPY:

T-lymphocyte depleting agents should be used rather than IL-2RA (**agreement rate 94%**). T-cell depleting therapy such as alemtuzumab or antithymocyte globulins (ATG) can be used (**agreement rate 94%**). The B-cell depleting agent rituximab might be considered as an adjunct to prevent antibody rebound (**agreement rate 89%**).

MAINTENANCE IMMUNOSUPPRESSION:

Tacrolimus, mycophenolate, and steroids are recommended (**agreement rate 91%**), with mTOR inhibitors in combination with tacrolimus, as an alternative to mycophenolate in cases of intolerance or infectious complications (**agreement rate 81%**).

IMMUNOSUPPRESSION MANAGEMENT:

Planned minimisation or withdrawal of immunosuppression should be avoided (**agreement rate 92%**).

B) Category 2 patients (DSA present with positive flow and negative CDC crossmatch at day zero)

TRANSPLANTATION:

Kidney transplantation should be avoided unless no other options are available (**agreement rate 83%**). If transplantation is considered, FC negative crossmatch must be achieved through desensitisation before transplantation, with strategies to prevent and treat antibody rebound carefully planned (**agreement rate 86%**).

MONITORING:

Clinical surveillance, DSA screening, and surveillance biopsy (**agreement rate 96%**).

DESENSITISATION STRATEGIES:

Plasma exchange (PEX) and intravenous immunoglobulin (IVIg) should be part of the first-line desensitisation strategy (**agreement rate 77%**). Imlifidase may be considered for deceased donor transplants in selected cases (**agreement rate 91%**).

INDUCTION THERAPY:

T-lymphocyte-depleting agents should be used, rather than interleukin 2 receptor antagonists (IL-2RAs; **agreement rate 93%**). Alemtuzumab or antithymocyte globulins (ATG) can be used (**agreement rate 91%**). The B-cell depleting agent rituximab might be considered as an adjunct to antibody mediated injury (**agreement rate 91%**).

MAINTENANCE IMMUNOSUPPRESSION:

Tacrolimus, mycophenolate, and steroids are recommended (**agreement rate 93%**), with mTOR inhibitors in combination with tacrolimus, as an alternative to mycophenolate in cases of intolerance or infectious complications (**agreement rate 83%**).

IMMUNOSUPPRESSION MANAGEMENT:

Planned minimisation or withdrawal of immunosuppression should be avoided (**agreement rate 91%**).

C) Category 3 patients (DSA present and negative flow and CDC crossmatch at day zero)

TRANSPLANTATION:

Other options for transplantation i.e. compatible living donor transplants or kidney paired donation should be considered due to higher immunological risk compared to Categories 4 and 5 (**agreement rate 83%**). Risk/benefit analysis, and strategies to prevent and treat antibody rebound need to be carefully planned (**agreement rate 96%**).

MONITORING:

Clinical surveillance, DSA screening and surveillance biopsy (**agreement rate 94%**).

DESENSITISATION STRATEGIES:

Plasma exchange (PEX) and intravenous immunoglobulin (IVIg) might be considered (**agreement rate 77%**), with rituximab as an adjunct to prevent antibody-mediated injury (**agreement rate 79%**).

INDUCTION THERAPY:

T-lymphocyte-depleting agents should be used rather than IL-2RAs (**agreement rate 85%**). Alemtuzumab or ATG can be used (**agreement rate 89%**).

MAINTENANCE IMMUNOSUPPRESSION:

Tacrolimus, mycophenolate, and steroids are recommended (**agreement rate 93%**), with mTOR inhibitors in combination with tacrolimus, as an alternative to mycophenolate in cases of intolerance or infectious complications (**agreement rate 89%**).

IMMUNOSUPPRESSION MANAGEMENT:

Planned minimisation or withdrawal of immunosuppression should be avoided (**agreement rate 81%**).

D) Category 4 patients (without DSA on day zero but with potential cellular memory against donor HLA)

CATEGORY 4a: with "probable" cellular memory, in case of positive history of DSA, pregnancy and/or previous transplant with repeated antigens.

TRANSPLANTATION:

Candidates for kidney transplantation in this category are at increased risk of AMR compared to patients in category 4b and 5. Post-transplant monitoring and strategies to control antibody-mediated injury need to be considered (**agreement rate 89%**).

MONITORING:

Clinical surveillance, DSA screening and surveillance biopsy (**agreement rate 87%**).

INDUCTION THERAPY:

Lymphocyte-depleting agents should be used, rather than IL-2RAs (**agreement rate 76%**). Alemtuzumab or ATG can be used (**agreement rate 81%**).

MAINTENANCE IMMUNOSUPPRESSION:

Tacrolimus, mycophenolate, and steroids are recommended (**agreement rate 87%**), with mTOR inhibitors in combination with tacrolimus, as an alternative to mycophenolate in cases of intolerance or infectious complications (**agreement rate 94%**).

IMMUNOSUPPRESSION MANAGEMENT:

Planned minimisation or withdrawal of immuno-suppression should be avoided (**agreement rate 81%**).

CATEGORY 4b: "possible" cellular memory if history of transfusions and/or pregnancies with no information on the HLA type patient was exposed to.

Given the current lack of routinely accessible tests to evaluate the humoral cellular memory of kidney transplant candidates, patients in Category 4b do not necessitate additional treatment beyond the standard of care (**agreement rate 81%**).

E) Category 5 patients (with no DSA and no cellular memory)

Based on existing data, patients in Category 5 do not necessitate additional treatment beyond the standard of care (**agreement rate 93%**).

Table 1. Summary of ENGAGE II consensus recommendations (adapted from Furian et al. 2024; *Transplant International;* DOI: 10.3389/ti.2024.12475).

3. Workshop highlights

On Day 1 of the 2-day workshop, speakers presented data on how patients requiring kidney transplantation are managed in their respective hospitals, based on the classification of humoral risk.

On Day 2, discussions focused on the pathophysiology of AMR, current treatment options, and the objectives of ENGAGE III.

Below is a summary of the presentations.

HLA-incompatible kidney transplantation in patients with pretransplant positive crossmatch

Among patients with baseline DSA, those with a positive complement-dependent cytotoxicity crossmatch (CDCXM) —classified as Category 1 in the ENGAGE stratification— are at the highest risk for AMR and poor long-term allograft survival, representing the highest level of humoral risk among solid organ transplant candidates, posing a significant immunologic barrier to successful transplantation.

Higher risk of AMR and inferior allograft outcomes are also observed in patients with baseline DSA and a positive flow cytometry crossmatch (FCXM), even in the absence of a positive CDCXM. These patients fall under Category 2 of the ENGAGE risk stratification.

In these highly sensitised candidates for kidney transplantation, the ideal approach is to prioritise HLA-compatible transplant through available kidney allocation systems. In the absence of any other option, HLA-incompatible transplantation may be considered; however, in such cases, prior desensitisation is essential to reduce DSA levels below the threshold capable of triggering acute allograft injury and to maintain these levels at a minimum during the immediate post-transplant period. Recent data underscore the lifesaving benefits of desensitisation followed by transplantation, when compared to remaining on dialysis. A variety of pre-transplant desensitisation protocols have been employed, some with controversial efficacy, often resulting in incomplete removal of DSA.

According to the ENGAGE II consensus statements, plasma exchange (PEX) and intravenous immunoglobulin (IVIg) should be part of the first line desensitisation strategy to provide a negative crossmatch prior to transplantation. Moreover, imlifidase might be considered as a desensitisation strategy for deceased kidney transplantation in very selected patients for whom there are no other treatment options.

During the workshop, the use of imlifidase as a desensitisation strategy was reported for patients in categories 1 and 2 undergoing deceased donor transplantation by Alice Koenig and Ondřej Viklický, respectively.

The results demonstrated that all positive XM (CDCXM and FCXM) quickly converted to negative within 2-6 hours following treatment with imlifidase prior to transplantation and HLA-antibodies disappeared, corroborating findings from previous studies. Despite the occurrence of DSA rebound and AMR in some cases, no graft loss or patient death was observed during follow-up (ranging from 3 months to up to 2.5 years).

These data further demonstrate that in highly sensitised patients, where living donation is not an option, imlifidase effectively removes HLA antibodies within a time frame compatible with performing transplantation across an initially positive crossmatch.

To note that in some of the cases presented by the speakers, there was no correlation between antibody rebound, occurrence of rejection, and kidney function. Predicting which antibodies are likely to rebound —and whether they will trigger AMR— would have significant implications for organ selection, but further research is needed to define unacceptable antigens and guide post-transplant management. Longer follow-ups are also necessary to identify patients at risk of rebound and AMR.

> *"Highly immunised patients with activating complement anti-HLA antibodies are increasingly common. A step-by-step strategy should be proposed to these patients: always favour an HLA compatible transplant. In the absence of any other alternative, consider active desensitisation."* A. Koenig
>
> *"Patients' selection criteria to access desensitisation treatment are paramount. Sometimes patients are too old and with too many comorbidities to be suitable candidates."* A. Koenig
>
> *"The advantage of imlifidase is that it allows transplantation in those patients at high risk who would never be transplanted."* O. Viklický

Day-zero DSA of undetermined significance

Cases involving Category 3 patients —those with day zero DSA in the absence of a positive crossmatch (XM)— were presented by Kevin Louis. These cases illustrated that the presence of DSA, even with a negative XM, can lead to variable graft outcomes. Importantly, not all DSA carry the same risk for AMR and graft injury, and correlating the immunogenicity of specific antibodies with clinical outcomes remains challenging.

The discussion emphasised the need to keep assessing risks/benefits of treatments and plan close clinical, immunological, and histological monitoring to prevent and treat antibody rebound in these patients. Useful tools beyond clinical surveillance are 1) DSA monitoring, with regular screening, i.e. at 1-, 3- and 12-months post-transplant, considering DSA class/specificity (A, B, DR, *vs*. Cw, DP) to improve risk evaluation, and assessing DSA dynamics (resolved ***vs.*** persistent) and 2) surveillance biopsy to assess injury in patients with graft dysfunction and also in those without graft dysfunction, performing protocol biopsy at 3 and 12 months.

The use of adjunctive tools now available to clinicians —though still requiring further studies to establish their clinical utility — may be considered alongside DSA monitoring and biopsies, in cases where standard assessments yield inconclusive results.

Molecular profiling of graft biopsy may assist in resolving inconclusive diagnoses; however, lack of standardisation of thresholds for clinical diagnosis and inter-centre agreement on interpretation prevent broader clinical implementation.

An alternative assay when graft biopsy is not possible/contraindicated could be the detection of donor-derived cell-free DNA (ddcfDNA), i.e. fragments of DNA released into the blood by cells damaged by apoptosis or necrosis, a non-invasive biomarker for allograft injury after kidney transplantation, with high sensitivity, and high negative predicting value, potentially an early marker, preceding DSA elevation. The half-life of dd-cfDNA in the blood is very short— approximately 2.5 hours— making this test an indicator of the actual clinical status.

> *"My key message is the importance of carefully monitoring DSA [...]. After induction therapy and initiation of immunosuppressive regimen, some DSAs may resolve spontaneously — likely these will not be harmful. However, for those that persist with high MFI levels, increased monitoring is warranted. The ENGAGE II consensus showed 94% agreement on DSA monitoring for category 3 patients, but we now need to define more precisely how to implement it to guide clinical decisions."* K. Louis

> *"We should biopsy patients when there is graft dysfunction. In the absence of graft dysfunction, for the centres that have access to biopsy, it remains the best way to assess if there is pathogenicity of DSA."* K. Louis

Beyond DSA: looking for hidden memory

Cellular alloreactivity may occur without humoral activation and plays a critical role in the initiation and mediation of allograft rejection; however, current immunological risk assessment for transplantation is exclusively based on the detection of preformed circulating DSA.

Assays have been developed to study allogeneic T-cell memory, HLA-specific B-cell memory — both pre- and post-transplant— and their combined impact. Although these tools are not yet integrated into clinical practice, their clinical relevance is increasingly recognised.

Refining baseline immune-risk stratification through such biomarkers could help guide immunosuppression strategies, which is particularly relevant in category 4 patients where cellular memory seems likely despite absence of serum antibodies.

Seemingly "non-immunised" re-transplant candidates or husband-to-wife or child-to-mother transplantations, historically considered at higher immunological risk due to the potential presence of memory B cells, represent key patient groups for targeted screening.

In this regard, Delphine Kervella discussed a representative case study for ENGAGE category 4a (including patients with historical DSA or a strong immunising event like pregnancy or previous transplant) concerning a female recipient with a history of two pregnancies, no transfusions, and no detectable DSA prior to transplantation (cPRA: 0%). She received standard-of-care immunosuppression for patients with low immunological risk. On post-transplant day 12, she developed acute AMR, C4d-negative, with high levels of DSA targeting HLA-B35:01. To investigate the rapid emergence of DSA post-transplant, HLA typing of the patient's husband was performed. HLA-B35:01, shared by both the donor and the husband, was identified as a likely sensitising antigen from previous pregnancies, potentially explaining the early alloimmune response.

Analysis of the patient's memory B cells both pre-transplant and on post-transplant day 12 revealed the presence of memory B cells specific for HLA-B35:01: despite the absence of DSA prior to transplantation, humoral memory existed in this patient prior to transplantation.

The acute rejection episode was likely driven by a rapid recall immune response triggered by re-exposure to the antigen, highlighting the clinical relevance of assessing cellular alloimmune memory.

Several assays have been designed to assess the peripheral alloreactive memory B cell pool, with the most commonly used method involving the *in vitro* differentiation of memory B cells into antibody-secreting cells. This approach enables quantification of HLA-specific IgG-producing B cells; it typically uses the interferon-γ Enzyme-Linked ImmunoSpot (IFN-γ ELISPOT) assay in conjunction with specialised HLA detection systems.

Pre-formed memory T cells as well is associated with bad outcomes after transplantation, i.e., biopsy-proven acute rejection (BPAR) and T cell–mediated rejection (TCMR). Using the sensitive IFN-γ ELISPOT assay combined with HLA eplet mismatching may represent a valid approach for detection of preformed T cell memory and evaluation of the risk of primary alloimmune activation.

To broaden clinical experience and ultimately enable their successful translation into routine clinical practice, these approaches should be applied prospectively to larger, well-defined cohorts.

> *"The difficulty in category 4a patients is that we are talking about possible cellular memory, so we need to validate more biomarkers to better identify which patients have cellular memory and adapt immunosuppressive therapy."* D. Kervella

Current treatment options for AMR

During the second day of the workshop, Olivier Thaunat presented an in-depth overview of the pathophysiology of AMR while Georg Böhmig discussed current treatment options.

AMR remains a leading cause of kidney graft loss, and its impact is anticipated to increase with the growing number of sensitised recipients undergoing transplantation. A thorough understanding of AMR pathophysiology is an essential prerequisite for the development of innovative, effective, and personalised therapeutic strategies.

Despite research efforts, treatment strategies are based on low level of evidence, with relatively few studies available and many yielding negative outcomes — highlighting a significant unmet need for effective, approved therapies.

To address this gap, in 2019 the Transplantation Society convened an international panel of experts to establish a consensus on appropriate treatment strategies for active and chronic active AMR. The aim was to reach a consensus for standard of care treatment against which new therapies could be evaluated. While it was agreed that the aims of treatment are to preserve renal function, reduce histological injury, and reduce the titre of donor-specific antibody, there was insufficient conclusive evidence to support any specific therapy. Consequently, the resulting treatment recommendations were predominantly grounded in observational studies, low-level evidence, and expert opinion. Despite the clear lack of evidence, defining a standard of care for AMR was considered essential as a benchmark for future research and prospective clinical trials.

The current landscape of clinical trials in AMR reveals the development of different therapeutic strategies, each targeting specific steps of antibody-mediated injury, namely B cell activation and differentiation, DSA depletion, or interference with deleterious effector mechanisms, such as complement activation or NK cells.

Some of the completed studies yielded unclear or negative results, some were prematurely terminated due to financial constraints or difficulties in patient recruitment.

Innovative therapies are currently under investigation, including felzartamab, a monoclonal antibody targeting CD38, which is highly expressed in NK cells and antibody-producing plasma cells. In a phase 2 trial, this compound has demonstrated potential in resolving molecular and morphologic rejection activity and injury, predominantly by targeting NK cell effector function.

Daratumumab is another anti-CD38 monoclonal antibody approved for the treatment of multiple myeloma. Several case reports suggest its efficacy in the treatment of AMR.

These promising findings give hope for the development of effective treatments but underscore the urgent need for new trial designs and cooperation within large transnational consortia.

> *"Understanding the pathophysiology of AMR is a mandatory prerequisite to design innovative/efficient/personalised therapies."* O. Thaunat
>
> *"My feeling is that targeting CDC38 is a promising concept and we should focus on that."* G. Böhmig

4. Tackling remaining challenges

The heterogeneity within the kidney transplant population and the complexity of the alloimmune response are mirrored by the diverse landscape of clinical practices and treatment options, fuelled by the rapid emergence of prognostic and predictive biomarkers to assess individual risk of graft failure and response to AMR treatment as well as by the availability of new agents for desensitisation, immunomodulation and AMR treatment.

In such an evolving context, the central challenge lies in delivering the right treatment to each patient based on their alloimmune risk. Ultimately, a personalised approach minimises drug-related toxicity while preserving therapeutic efficacy.

During this workshop, some of the current treatment's options and practices have been discussed and are summarised below.

In highly sensitised patients for whom living donation is not an option, **imlifidase appears as a promising desensitisation agent**, eliminating DSA within about 4 hours from administration thus enabling kidney transplantation across a positive crossmatch (CDCXM or FCXM, corresponding to ENGAGE categories 1 and 2, respectively), as shown in clinical trials and confirmed by results presented by different groups during the workshop. Currently, the European Medicines Agency (EMA) has conditionally approved imlifidase only for deceased donor kidney transplantation in patients who are highly unlikely to receive a compatible kidney offer.

Imlifidase has the potential to transform the field of HLA-incompatible kidney —and other solid organ— transplantation representing an immediate, efficient desensitisation modality and enabling higher immunological risk procedures. However, due to the significant rebound observed following imlifidase treatment, future approaches will likely require combining imlifidase with other drugs chosen for their time-dependent role in preventing immunological damage. Tailored studies are needed to determine optimal drug combinations that can effectively mitigate DSA rebound following treatment with this protease.

Day-0 DSA in the absence of positive crossmatch (XM) demonstrates highly variable graft outcomes. Importantly, **not all DSAs carry the same risk for AMR or graft injury**; moreover, the immunogenicity of different antibodies does not always correlate clearly with clinical outcomes, which represents a significant challenge in risk stratification.

The heterogenous phenotypes frequently observed in this category of patients highlights the importance of monitoring DSA dynamic and specificity, as well as performing regular surveillance biopsies. The **use of adjunctive tools**, now available to clinicians, may support monitoring strategies, though still requiring further studies to establish their clinical utility, particularly when standard assessments are inconclusive or contraindicated. A potential novel approach showing promising results include incorporating **donor derived cell-free DNA** levels assessment.

It is now evident that the alloimmune response should not be separated into cellular and antibody-mediated but rather considered as a continuous process in which different components predominate at various time points after transplantation. This underlines the importance of

evaluating donor-specific T- and B-cell memory to improve risk assessment. While refining pre-transplant risk stratification to prevent allograft rejection should remain the primary objective, memory T and B-cell detection may also prove valuable in guiding post-transplant management of kidney allograft recipients. Despite the development of several *in vitro* assays, there is an unmet need for further clinical validation and standardisation in clinical trials.

AMR is the leading cause of kidney allograft failure. There exists a relatively large number of studies, although most were underpowered and gave inconclusive results. Because of that, there are currently no approved treatments for AMR, resulting in significant heterogeneity in practice across transplant centres.

The current landscape of clinical trials in AMR reveals different therapeutic strategies, targeting specific steps of antibody-mediated injury, namely the process of B cell activation and differentiation, DSA depletion, or interfering with deleterious effector mechanisms, such as complement activation or NK cells.

Among the promising therapies under investigation, **felzartamab** is a monoclonal antibody targeting the surface molecule CD38, which is highly expressed in NK cells and antibody-producing plasma cells. In a phase 2 trial, this compound has demonstrated potential in resolving molecular and morphologic rejection activity and injury, predominantly by targeting NK cell effector function.

Daratumumab is another anti-CD38 monoclonal antibody approved for the treatment of multiple myeloma. Several case reports have been published suggesting its efficacy in the treatment of AMR.

Perspectives

The results presented at this workshop fuelled the discussion on follow-up initiatives that would help reduce graft failure in highly sensitised patients.

There is a consensus that to cope with the heterogeneity of patients' responses to treatment, ENGAGE III should focus on a well-defined cohort of patients characterised using standardised criteria and agreed-upon methods.

Participants discussed the possibility of designing a study protocol for a future clinical trial to be conducted outside of the ENGAGE III. Many emphasised that, before moving forward, an in-depth discussion is essential to establish clear criteria for patient enrolment, diagnosis, and outcome endpoints. For instance, a precise and shared definition of 'standard of care' is crucial, given the significant variation across countries and institutions. Reaching a consensus on this definition early in the project is critical, and it may even lead to the exclusion of certain treatments due to insufficient evidence.

In addition, the feasibility of implementing novel therapies and diagnostic tools must be carefully considered and validated, as not all countries have access to, or regulatory approval for, some treatments.

The assembly agreed that pathologists should be involved in the study design, given the critical importance of biopsy histology in monitoring graft health and assessing AMR.

Finally, there is a desire to involve experts with experience in designing innovative clinical trials and, potentially, to engage with the European Medicines Agency (EMA) at an early stage of the process.

5. Setting the stage for ENGAGE III

What's next for the ENGAGE initiative? How can we build on the foundations laid by ENGAGE II?

The complexity of the alloimmune response, along with the diversity of the immune players involved, contributes to heterogeneous clinical phenotypes, limiting the ability to predict accurately the risk of AMR, characterise its various presentations, and develop targeted therapeutic strategies.

In the context of a heterogeneous and multi-layered kidney transplant population and based on the outcomes of ENGAGE II, workshop participants agreed that the next phase of the ENGAGE initiative should focus on post-transplant management. A key objective would be **to reach a consensus on standardising the diagnosis and treatment of AMR**, based on well-defined and homogeneous patient groups.

A first step would be the characterisation of the different forms of AMR based on pathophysiological mechanisms, histological features, and clinical and genetic phenotypes. AMR can present with overt allograft dysfunction occurring early post-transplant, or have an insidious or subclinical onset, manifesting later in the post-transplant course. Anti-HLA antibodies can be present prior to transplantation (preexisting DSA) or emerge afterward (*de novo* DSA). In some instances, histological lesions consistent with AMR are observed despite the absence of detectable anti-HLA antibodies. Therefore, integrating histological findings with immunological data (DSA monitoring) could enable more precise stratification of patients according to AMR subtype. Such stratification could serve as a framework to guide personalised therapeutic approaches based on currently available treatment options, as well as represent a benchmark for future studies and clinical trials.

Regarding the methodological process, participants agreed that once the key clinical questions are defined, the same approach adopted for ENGAGE II —comprising systematic literature review, structured statement development, and Delphi-based expert consensus— should be employed to develop a new set of evidence-based recommendations.

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