# Novel tools for transplant outcome prediction

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Kidney transplantation is the optimal treatment option for end-stage renal failure as it is cost-effective and improves patients' survival and quality of life. Personalizing immunosuppression in solid organ transplantation has been a long-standing challenge since the introduction of this treatment option. Currently, immunosuppressive protocols are tailored according to functional and histological assessment, as well as signs of drug toxicity and/or infections. There is much discussion on non-invasive assessment of the alloimmune response. Blood biomarkers, such as HLA donor-specific antibodies (DSA) and non-HLA donor-specific antibodies, are routinely used to assess the risk for humoral rejection and transplant loss. Also, donor-derived cell-free DNA (dd-cfDNA) is currently being tested as a surrogate marker of allograft injury. This non-invasive test provides a more frequent, safer and quantitative assessment of antibody-mediated rejection (ABMR) and is recommended for periodical screening in patients at risk for ABMR and negative biopsy findings. Another method for individualized immunotherapy in transplantation is gene expression profiling (GEP). It assesses the expression of immune response-related genes, which can indicate the presence of acute rejection and is more related to cellular rejection. However, GEP results should be interpreted with caution as they often do not correlate with biopsy results in terms of ABMR and/or T cell-mediated rejection (TCMR). Nevertheless, according to Park et al, simultaneous use of GEP and dd-cfDNA assays could improve rejection detection and provide safer and less invasive monitoring for subclinical rejection.

| Commercial Assay | Study | Methodology | Targets | Number | Threshold | %AR | Sen/Spec | PPV/NPV | AUC |
|---|---|---|---|---|---|---|---|---|---|
| Allosure | Bloom et al,[62] 2017 DART | NGS | 266 SNPs | 102P/107B | 1% | 24% | 59/85 81/83 | 61/84 44/96 | 0.74 0.87 |
| | Jordan et al,[63] 2018 DART | | 266 SNPs | 87P 33 DSA+ | 1% | 16% AMR | 81/82 for AMR in DSA+ | 81/83 for AMR in DSA+ | 0.86 |
| | Huang et al,[64] 2019 | | 266 SNPs | 63P | 1% (0.74%)* | 54% | 68/72 (79/72)* | 74/65 (77/75)* | 0.71 |
| Prospera | Sigdel et al,[65] 2019 | NGS | 13,392 SNPs | 217B | 1% | 18% | 88.7/72.6 | 52/95 (if 25% prevalence) | 0.87 |
| Noncommercial | Gielis et al,[66] 2019 | NGS | 1027 SNPs | 107P | 0.88% | 24 | 38/85 | 9/98 | 0.64 |
| | Lee et al,[67] 2017 | ddPCR | 36 SNPs | 34P | NA | NA | NA | NA | NA |
| | Whitlam et al,[59] 2019 | ddPCR | 38 CNV | 55P/61B | 0.75% 21 cp/mL | 21.3% | 80/71† 90/88‡ | 35/95† 60/98‡ | 0.85† 0.92‡ |
| | Oellerich et al,[58] 2019 | ddPCR | 41 SNPs | 189P | 0.43% 52 cp/mL | 8% | 73/69 73/73‡ | NA | 0.73 0.83‡ |
| | Zhang et al,[68] 2020 | NGS | 56,049 SNPs | 37P | 1% | 49% | 89/74 | 76/87 | 0.9 |
| | Dauber et al,[69] 2020 | qPCR | 34 INDELs | 29P | 2.7% | 27.6% | 88/81 | 64/94 | 0.84 |

Figure 1. Studies assessing ddcfDNA in Plasma for Diagnosis of Acute Rejection

**Recent and ongoing projects on monitoring immunosuppression in renal transplant patients**

The European BIO-DrIM (*BIOmarker-Driven personalized IMmmunosuppression*) consortium initiated a project focusing on the implementation of biomarker-driven strategies for personalizing immunosuppression. One of the five multi-centre clinical trials in this project, the CELLIMIN trial (*Prospective donor-specific Cellular alloresponse assessment for Immunosuppression Minimization in de novo renal transplantation*), was designed to evaluate the usefulness of assessing pretransplant donor-reactive T cell memory using an interferon-γ (IFN-γ) ELISpot marker to stratify kidney transplant recipients into "low" and "standard-of-care" immunosuppressive regimen. The results strongly

underline the benefit of refining current immune-risk stratification by monitoring preformed T cell memory and primary alloimmune activation with the IFN-γ ELISPOT assay and HLA eplet mismatching.

Another BIO-DrIM study, the Rimini trial (Tacrolimus After Rabbit Antithymocyte Globulin And Infliximab Induction immunosuppression In Kidney Transplantation), aimed to assess the clinical efficacy and safety of an induction regimen with rabbit anti-thymocyte globulin (rATG) (Thymoglobuline) and infliximab. This combination is expected to reduce freshly activated naïve T-cells and expand the pool of patients who are suitable for a low dose of immunosuppression (tacrolimus).

The recently initiated TWO study (Transplantation Without Overimmunosuppression), a phase 2b randomised control trial of regulatory T cell therapy in living donor kidney transplant recipients, is expected to confirm the safety and explore the efficacy of this novel treatment strategy. It will randomize 60 patients to Treg therapy or standard clinical care (control) and analyze rates of biopsy-proven acute rejection over the following 18 months. Secondary endpoints include immunosuppression burden, chronic graft dysfunction and drug-related complications.

Another multicentre prospective phase 2 clinical trial is comparing rejection and infection rates between Torque teno virus (TTV) guided immunosuppression and standard of care. TTV is a highly prevalent and non-pathogenic microorganism which is associated with the grade of host immunosuppression. Non-interventional studies suggest that TTV-guided immunosuppression is superior to standard strategies. This approach could also be a proof of principle for TTV-based immune assessment, not just for transplants, but also for all other patients receiving immunosuppression. The issue of personalized immunosuppression is tackled by other approaches as well, such as software solutions and applications.

An ongoing prospective randomized controlled multi-centre trial is assessing the use of Predigraft software based on iBOX to predict allograft survival in the follow-up of kidney transplanted patients. Patients in the interventional arm will use Predigraft to receive therapeutic education content (videos, facts sheets, short messages, questionnaires), exchange documents with their doctors and interact via messaging with them, and physicians will be able to calculate a score to predict allograft survival at 3, 5 and 7 years. This trial paves the way for the larger adoption of artificial intelligence-based medical device software in transplantology.



Predictive software
- Dynamic allograft survival probabilities for clinical follow-up (up to 10 years predictivity)
- Advanced risk stratification
- Response to therapy
- Surrogate endpoint for clinical research

| Mandatory data input | Additional data input | Output |
| --- | --- | --- |
| • Time from transplant<br><br>• eGFR (mL/Min/1.73m2)<br><br>• Proteinuria (g/g) | • Banff lesions grading:<br>  g,i,t,ptc,cg,IFTA<br><br>• Histology diagnoses<br><br>• Anti HLA DSA (MFI) | • Individual patient prediction of long-term allograft survival for 3, 5 and 7 years from evaluation time |

Figure 2. Predigraft: decision-making tool based on iBox predicting patient individual risk of graft failure

**Graft-related interventions to improve transplant outcome**

In the past years, there has been a resurgence of interest in normothermic machine perfusion (NMP), to expand the donor pool. There is evidence that this form of graft preservation is superior to static cold storage, especially in the context of higher-risk and extended-criteria donor organs. A study led by Annemarie Weissenbacher found that NMP of human kidneys is feasible even for 24 hours and that urine recirculation facilitates the maintenance of perfusate volume and homeostasis. The Cambridge group led by John R. Ferdinand compared the effect of NMP with that of cold storage on the global kidney transcriptome. They found that cold storage led to a global reduction in gene expression, including inflammatory pathway genes and those required for energy generation processes, such as oxidative phosphorylation (OXPHOS). In contrast, during NMP, there was marked upregulation of OXPHOS genes, but also of several immune and inflammatory pathway genes. Using biopsies from grafts subjected to NMP that were subsequently transplanted, the group found that higher inflammatory gene expression occurred in organs with prolonged delayed graft function (DGF). Therefore, they used a hemoadsorber to remove pro-inflammatory cytokines from the perfusate. This attenuated inflammatory gene expression, increased OXPHOS pathway genes, and had potentially clinically important effects on reducing the expression of a DGF-associated gene signature. Together, their data suggest that the adsorption of pro-inflammatory mediators from the perfusate may improve graft viability.
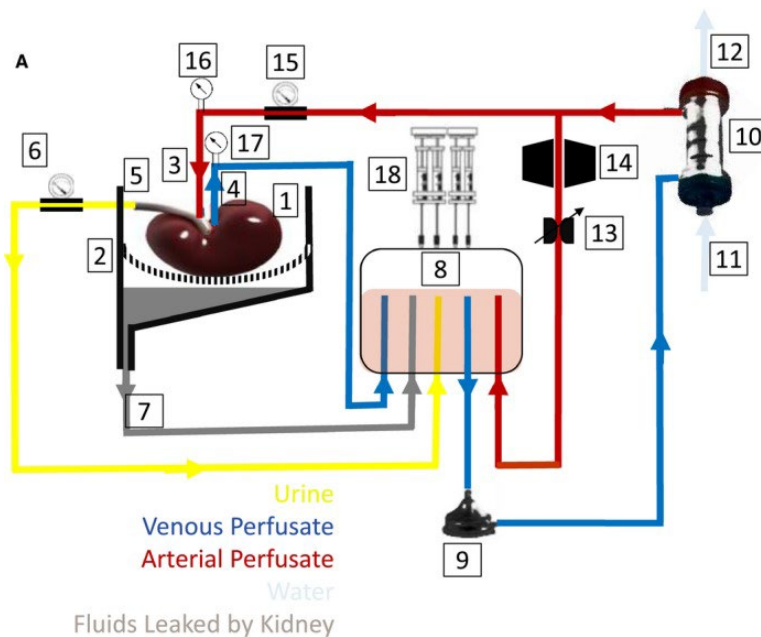


Figure 3. Diagram of the prototype device for long-term normothermic kidney perfusion

Human kidney NMP beyond 24 hours could create the most optimal environment for a kidney in the *ex-situ* setting, provide for better viability assessment, optimize the preconditioning concept for clinical applicability and provide a better treatment onset for the extended criteria organs pre-transplant. A trial led by Annemarie Weissenbacher proved that 48-hour normothermic graft preservation with urine recirculation is possible with stable perfusion hemodynamics and perfusate homeostasis. Besides possible treatment of the organ, preserving kidneys beyond 24 hours offers potential pre-treatment of immunologically complex recipients.

Finally, the advances in lung transplantation may be useful in kidney transplantation. The group led by Aizhou Wang used *ex vivo* enzymatic treatment to convert blood type A donors' lungs into universal blood type lungs. This research paved the way for the group from the University of Cambridge led by Prof. M Nicholson to enzymatically remove the B blood type antigen from a normothermically perfused kidney.

**Key points**

1. There has been considerable progress made in monitoring transplant patients and detecting immunological imbalances.
2. Prospective trials evaluating the implementation of biomarker-driven strategies for personalizing immunosuppression and software-based allograft survival prediction are currently ongoing.
3. A clear pathway for clinical immunosuppression and its tailoring is needed to achieve patient improvement and personalised medicine.
4. Ex-vivo kidney perfusion, ex-vivo treatment and pre-conditioning can serve as helpful tools to desensitize the organs and lower the immunological burden for the recipients.

**Further reading**

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