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Individualised immunosuppression with intravenously administered donor-derived modified immune cells compared with standard of care in living donor kidney transplantation (TOL-2 Study): protocol for a multicentre, open-label, phase II, randomised controlled trial

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ABSTRACT

Introduction Donor-derived modified immune cells (MIC) induced long-term specific immunosuppression against the allogeneic donor in preclinical models of transplantation. In a phase I clinical trial (TOL-1 Study), MIC treatment resulted in a cellular phenotype that was directly and indirectly suppressive to the recipient's immune system allowing for reduction of conventional immunosuppressive therapy. Here, we describe a protocol for a randomised controlled, multicentre phase-IIb clinical trial of individualised immunosuppression with intravenously administered donor MIC compared with standard-of-care (SoC) in living donor kidney transplantation (TOL-2 Study).

Methods and analysis Sixty-three living donor kidney transplant recipients from six German transplant centres are randomised 2:1 to treatment with MIC (MIC group, N=42) or no treatment with MIC (control arm, N=21). MIC are manufactured from donor peripheral blood mononuclear cells under Good Manufacturing Practice conditions. The primary objective of this trial is to determine the efficacy of MIC treatment together with reduced conventional immunosuppressive therapy in terms of achieving an operational tolerance-like phenotype compared with SoC 12 months after MIC administration. Key secondary endpoints are the number of patient-relevant infections as well as a composite of biopsy-proven acute rejection, graft loss, graft dysfunction or death. Immunosuppressive therapy of MIC-treated patients is reduced during follow-up under an extended immunological monitoring including human leucocyte antigen-antibody testing, and determination of lymphocyte subsets, for example, regulatory B lymphocytes (Breg) and antidonor T cell response. A Data Safety Monitoring Board has been established to allow an independent assessment of safety and efficacy.

Ethics and dissemination Ethical approval has been provided by the Ethics Committee of the Medical Faculty of the University of Heidelberg, Heidelberg, Germany (AFmu-580/2021, 17 March 2022) and from the Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich-Institute, Langen, Germany (Vorlage-Nr. 4586/02, 21 March 2022). Written informed consent will be obtained from all patients and respective donors prior to enrolment in the study. The results from the TOL-2 Study will be published in peer-reviewed medical journals and will be presented at symposia and scientific meetings.

Trial registration number NCT05365672.

INTRODUCTION

Kidney transplantation is the best treatment modality for patients with stage 5 chronic

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STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Randomised controlled trial comparing a novel cell therapy together with reduced conventional immunosuppression head-to-head to standard-of-care in kidney transplantation.
⇒ Adequately powered study to reach the innovative primary study endpoint, confirmatory hypothesis testing of two key secondary endpoints.
⇒ Comprehensive clinical and immunological monitoring of patients with a biomarker-guided reduction of conventional immunosuppressive therapy.
⇒ The open-label trial design is a limitation.
⇒ Restricted to immunological low-risk living donor kidney transplant recipients.

kidney disease, but long-term graft and patient survival are still limited for two reasons: first, conventional immunosuppressive therapy is still inadequate to prevent the development of de novo donor-specific human leucocyte antigen (HLA) antibodies and chronic rejection, which is responsible for more than half of long-term graft losses, and second, the therapy has serious side effects such as an unfavourable cardiovascular risk profile, infectious complications and an increased frequency of malignancies.[1 2] Thus, there is a great need for an antirejection therapy with fewer side effects and higher efficacy. The ideal therapy would involve donor-specific immunosuppression in the absence of systemic downregulation of the immune response.

We have recently shown that administration of modified immune cells (MIC) prior to kidney transplantation led to specific immunosuppression against the allogeneic donor, which may allow reduction of conventional immunosuppressive therapy.[3 4] MIC are donor-derived peripheral blood mononuclear cells (PBMC) that are treated ex vivo by an alkylating agent. This results in a cellular phenotype that is directly and indirectly suppressive to the immune system.[5–10] Transferred in the living kidney transplantation model, PBMC are taken from the kidney donor by unstimulated leukapheresis 7 days before surgery. PBMC are treated with an alkylating agent under Good Manufacturing Practice (GMP) conditions and administered to the kidney recipient after washout of the alkylating agent. As a consequence, the recipient's immune system no longer recognises the characteristics of the donor tissue as non-self. If a kidney is subsequently transplanted from the same donor, the organ is not rejected.

In preclinical models, MIC induced long-term specific immunosuppression against the allogeneic donor. In the rat heart transplantation model, a single treatment with MIC without further immunosuppressive therapy resulted in a clinically and statistically significant prolongation of graft survival of 65±17 days compared with 9±0.3 days in untreated controls.[9] Similar results were shown in a hindlimb transplantation model in the rat and a kidney transplantation model in the pig.[7 9 10] In these animal models, MIC therapy did not lead to broad immunosuppression, but specifically abolished the immune response against the transplant: if a tolerant recipient treated with MIC of strain A was transplanted with an organ of strain B, this organ of strain B was rejected, whereas an organ of strain A was accepted. The animals that tolerated the donor organ had increased numbers of regulatory T lymphocytes (Treg) in the blood, lymphoid organs and graft, that mediated this immunosuppressive effect.[9] No safety-related problems occurred in the preclinical models. In the toxicity studies, there were no abnormalities in the blood counts or chemistry or in the histological analyses of various organs 7 and 30 days after administration of MIC (unpublished results). In particular, there was no accelerated transplant rejection after treatment with different doses of MIC as a possible indication of sensitisation related to the administration of donor cells.[7 9 10]

In phase I TOL-1 clinical trial, 10 adult patients with chronic kidney disease (CKD) stages 4 or 5 received a pretransplant MIC infusion in addition to post-transplant immunosuppression with cyclosporine A, enteric-coated mycophenolate sodium (EC-MPS) and corticosteroids.[3] Transplant recipients received intravenously either 1.5×10^6 MIC per kg of body weight (b.w.) (N=3, group A) or 1.5×10^8 MIC per kg b.w. (N=3, group B) 2 days before surgery, or 1.5×10^8 MIC per kg b.w. (N=4, group C) 7 days before surgery. Data collected during the first 30 days after transplantation showed that MIC infusions were well tolerated. A total of 69 adverse events (AE) including 3 severe AE occurred in the 10 treated patients that were unlikely to be related (N=1) or not related (N=68) to MIC infusion. No positive crossmatch results, de novo donor-specific antibodies or rejection episodes were recorded, and all patients had stable kidney graft function. Group C patients with low immunosuppression (reduced doses of cyclosporine A and EC-MPS and stopping corticosteroids) during follow-up showed no in vitro reactivity against stimulatory donor blood cells 360 days after transplantation, whereas reactivity against third-party cells was still preserved. Frequencies of $CD19^{+}CD24^{hi}CD38^{hi}$ transitional B lymphocytes (Breg) increased from a median of 6% before MIC infusion to 20% on day 180, which was 19-fold and 68-fold higher, respectively, than in two independent cohorts of transplanted controls. The majority of Breg produced the immunosuppressive cytokine interleukin (IL) 10.[3] Clinical and immunological findings in the 10 patients were stable with now more than 5 years of follow-up[4] (unpublished results). In summary, MIC therapy was well tolerated in the phase I clinical trial and did not lead to humoral sensitisation or rejections.

The in vitro findings of an absence of specific cell stimulatory reactivity against donor cells after transplantation, paralleled by a rise in Breg numbers and IL-10 levels, led us to hypothesise that MIC conditioning may constitute a promising method for inducing donor-specific immunosuppression in renal transplantation, which should be followed up in phase II clinical trials.

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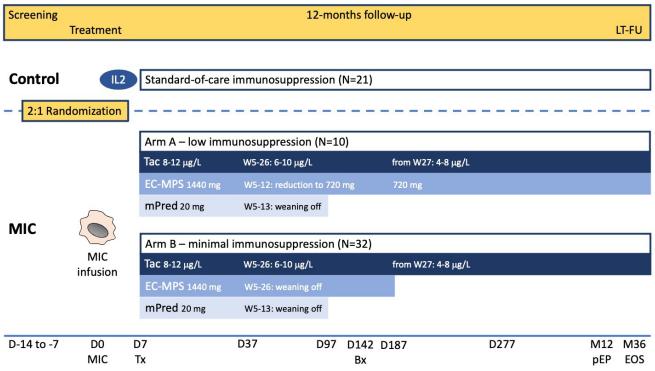


Figure 1 Flowchart of the TOL-2 Study. Bx, biopsy; D, day; EC-MPS, enteric-coated mycophenolate sodium; EOS, end of study; LT-FU, long-term follow-up; M, month; IL2, interleukin 2-receptor antibody induction therapy; MIC, modified immune cells; mPred, methylprednisolone; pEP, primary endpoint; Tac, tacrolimus; Tx, kidney transplantation, W, week.

METHODS AND ANALYSIS

Summary

An open-label, randomised, controlled, multicentre, phase II clinical trial of individualised immunosuppression with intravenously administered donor MIC compared with standard-of-care (SoC) in living donor kidney transplantation.

Trial design

This is an open, randomised, controlled, multicentre, phase IIb clinical trial in patients with stage 5 CKD who are due to undergo living donor kidney transplantation. The primary objective of this trial is to determine the efficacy of MIC treatment together with reduced conventional immunosuppressive therapy in terms of achieving an operational tolerance-like phenotype compared with SoC therapy. In total, 63 transplant couples, consisting of donor and transplant recipient, are planned to be enrolled (figure 1). The first transplant couple was enrolled on 4 May 2022, and as of 10 October 2022, six transplant couples have been screened for inclusion into the trial. Planned completion of the trial is the second quarter 2026. The 63 transplant recipients are randomised 2:1 to treatment with MIC (MIC group, N=42) or no treatment with MIC (control arm, N=21). Patients to be treated with MIC will be assigned to low immunosuppression (MIC arm A, N=10) and, after successful completion of MIC arm A, to minimal immunosuppression (MIC arm B, N=32). All patients of the control arm receive immunosuppression according to SoC.[11] To achieve balance across the key baseline factors, patient inclusion will be stratified by the patient's risk for transplant rejection, that is, lowest risk (HLA-identical), low risk (no sensitising event, haplotype identical living-related transplantation) and normal risk (all other patients). Kidney donors for patients randomised to MIC treatment are assigned to leukapheresis and PBMC donation for MIC manufacture. Patients who leave the trial before MIC administration or kidney transplantation (MIC arms and control arm) are replaced. The clinical trial includes a screening period, a treatment period (MIC treatment of patients 7±1 days prior to the scheduled living donor kidney transplantation) and a follow-up period of 12 months after MIC administration including living donor kidney transplantation and immunosuppressive therapy, followed by a long-term follow-up period of another 24 months. The trial is being conducted at multiple centres in Germany. The University Hospital Heidelberg is the main centre where leukapheresis, MIC manufacturing (GMP Core Facility) and MIC administration to patients is performed. Screening and follow-up assessments are performed in the patients' centres. An independent Data Safety Monitoring Board (DSMB) is established to allow an independent assessment of safety and efficacy data to assure that trial participants are not exposed to unnecessary or unreasonable risks (especially in MIC arm B), and to ensure scientific integrity of the trial.

Trial objectives and endpoints

Primary, key secondary, secondary and other endpoints are given in table 1.

The primary endpoint was chosen based on other studies of tolerance induction and included: (1) the primary efficacy endpoint requested by the European Medicines Agency, for example, no biopsy-proven acute rejection (>Banff borderline), graft loss, graft dysfunction with an estimated glomerular filtration rate (eGFR)<30 mL/min, or death at year 1, together with the absence of harmful donor-specific HLA antibodies, (2) the achievement of the desired immunosuppression reduction and (3) a phenotype that is specifically found in operational tolerance, that is, the induction of graft-protective transitional $CD24^{hi}CD38^{hi}$ B lymphocytes (ie, Breg).[12–19] The aim is to develop a new immunosuppressant to improve efficacy and safety outcomes of well-established immunosuppressive regimens, and to introduce a new treatment approach such as tolerance induction and exclusion of maintenance therapy to replace well-established regimens. Achieving tolerance has already been defined as the primary endpoint for competitive products in clinical investigations. Key secondary endpoints are defined to assess safety and efficacy of MIC treatment versus SoC therapy based on the number of patient-relevant infections as well as a composite of biopsy-proven acute rejection, graft loss, graft dysfunction or death. Further secondary endpoints include safety endpoints, efficacy endpoints, patient-reported outcomes such as quality of life, immunological endpoints and endpoints that are relevant to payers such as health insurances and to hospitals.

Risk-benefit assessment

A risk-benefit assessment with regard to the donor is difficult. The risks of unstimulated leukapheresis as a well proven standard procedure are very low, but there is no (medical) benefit for the donor. The donors are healthy

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Table 1 Trial objectives and endpoints

	Objectives	Endpoints
Primary	Determine efficacy of MIC treatment in terms of achieving an operational tolerance-like phenotype compared with the standard-of-care (SoC) therapy	Proportion of patients who achieve an operational tolerance-like phenotype defined on visit day 367 as fulfilling all of the following criteria: ► No biopsy-proven acute rejection (>Banff borderline), graft loss, graft dysfunction (estimated glomerular filtration rate (eGFR) <30 mL/min) or death on visit day 367. ► No development of de novo donor-specific human leucocyte antigen (HLA) antibodies (DSA ≥1000 mean fluorescence intensity (MFI)) until visit day 367, as measured by Luminex single antigen test. ► Induction of Breg ≥3% measured on visit day 367 (patient has to be infection-free at time-point of measurement). ► Patient on tacrolimus therapy with ≤720 mg EC-MPS and no corticosteroids (as well as no other immunosuppressive drug) on visit day 277 and remaining on this therapy until visit day 367.
Key secondary	Determine safety of MIC treatment vs SoC therapy based on number of patient-relevant infections as well as efficacy in terms of biopsy proven acute rejection, graft loss, graft dysfunction or death	► Number of patient-relevant infections during the first year after transplantation. ► Proportion of patients with biopsy-proven acute rejection (>Banff borderline), graft loss, graft dysfunction (eGFR<30 mL/min) or death on visit day 367.
Secondary	Determine safety and efficacy of MIC treatment based on further parameters	► AEs including serious AEs and AEs of special interest. ► Frequency of local or systemic reactions as result of MIC application. ► Patient, graft and death-censored graft survival. ► Incidence of biopsy-proven acute rejections and time to first rejection (>Banff borderline) according to Banff 2018 criteria and confirmed by a blinded central pathologist. ► Molecular scores in molecular microscope diagnostic system (MMDx) reading on visit day 367. ► Percentage of patients who achieved tacrolimus and EC-MPS dual therapy (MIC arm A, control arm) or tacrolimus monotherapy (MIC arm B) on visit day 367. ► Development of donor-specific HLA-antibodies (≥1000 MFI) until visit days 5, 187 and 367, as measured by Luminex single antigen test. ► Occurrence of delayed function of the kidney graft after transplantation, defined as dialysis within the first week after transplantation, except for one dialysis for hyperkalaemia. ► eGFR (according to chronic kidney disease epidemiology collaboration (CKD-EPI)). ► Incidence of CMV reactivation (CMV-DNA≥1000 copies/mL). ► Incidence of BK virus replication ≥10 000 copies/mL. ► Incidence of BK virus associated nephropathy. ► Incidence of hospital readmissions after transplant surgery. ► Days in hospital, on intensive care (ICU/IMC) and hours on mechanical ventilation on readmission. ► Change of quality of life (SF-36) compared with baseline. ► Incidence of new-onset diabetes mellitus after transplantation (fasting plasma glucose ≥7.0 mmol/L/126 mg/dL with no calorie intake for at least 8 hours). ► TIS and blood pressure on visit day 367 compared with baseline. ► Breg percentage. ► Antidonor T cell response to the donor. ► Cumulative steroid dose until visit day 367.
Other	Determine safety and efficacy of MIC treatment based on other parameters	► Infection: time, type of infection, duration, type and amount of antibiotic/antifungal/antiviral treatment, hospitalisation. ► Protein excretion (protein/creatinine ratio). ► Incidence of post-transplant lymphoproliferative disorder/malignancy. ► Hypercholesterolaemia ≥250 mg/dL. ► Days off work and reason. ► Donor-derived cell free (dd-cf)DNA during eIM. ► Torque Teno virus (TTV) level during eIM.

An adjudication committee will be established to confirm the definition of patient-relevant infections as adverse events of special interest (AESIs).
AE, adverse events; AESIs, adverse events of special interest; CMV, cytomegalovirus; EC-MPS, enteric-coated mycophenolate sodium; eIM, extended immunological monitoring; MIC, modified immune cells; SF-36, Short Form 36 Health Survey.

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and therefore do not require any treatment per se. Leukapheresis for recovery of PBMC, just like kidney donation, serves only to improve the health of the recipient.

MIC is a new 'Advanced Therapy Medicinal Product' (ATMP). The experience with this ATMP is limited, so possible side effects could occur in the recipients, which are not anticipated at this time. For this reason, patients are closely monitored during the infusion of the product and the postadministration period. In a previous clinical phase I trial (TOL-1), no AE/reactions nor severe AE/reactions related to the MIC therapy have been recorded. There were also no adverse side effects in preclinical animal trials. Potential side effects that cannot be excluded based on the limited experience are adequately addressed during the TOL-2 Study and comprise an acute anaphylactic reaction, physical complications (ie, embolisms with cell aggregates), immunological complications (ie, sensitisation of the recipient to the donor before transplantation with the need to postpone the transplantation, or after transplantation a rejection reaction of the kidney transplant), infections caused by the cell preparation due to contamination during manufacturing of the preparation (MIC will be produced under GMP conditions to minimise the risk), infection due to an undetected infection of the donor (the donor will be retested in advance to minimise the risk), side effects of traces of the alkylating agent in MIC, side effects of the buffer, haematological side effects, in particular blood count changes, neoplasia caused by malignant cell transformation, and overimmunosuppression by combination of cell therapy and drug immunosuppression.

Inclusion and exclusion criteria

Inclusion and exclusion criteria for donors and transplant recipients are given in boxes 1 and 2, respectively.

For the donor, the usual inclusion and exclusion criteria apply as they apply to living kidney donors in general. In addition, communicable diseases or previous interventions that may have resulted in a communicable disease are considered exclusion criteria.

For the kidney transplant recipient, again the usual inclusion and exclusion criteria apply as they apply to living kidney transplant recipients in general. Only immunological low-risk recipients are considered, that is, they are ABO-blood group identical or compatible with the donor, receive a first kidney transplant with a negative complement dependent cytotoxicity (CDC) crossmatch result with the donor, have CDC-panel reactive antibodies <20%, and no detection of a donor-specific HLA-antibody (DSA) in the Luminex-Assay (cut-off: mean fluorescence intensity (MFI) ≤1000). Female patients who have a child with the donor or were pregnant from the donor are excluded from the study due to possible sensitisation. In addition, kidney transplant recipients are excluded from the study if they have a condition or treatment that could interfere with MIC treatment, such as immunosuppressive therapy, collagenosis and vasculitis, or splenectomy.

Box 1 Inclusion and exclusion criteria for donors

Inclusion criteria

1. Age ≥18 years and able to consent.
2. Ability to understand the nature and scope of the clinical trial.
3. Written consent form given prior to any trial-related procedures (including PBMC donation).

Exclusion criteria

1. Pregnant or breast feeding.
2. Participation in an interventional clinical trial within 30 days prior to screening or in observation period of a competing study.
3. Severe psychiatric disease.
4. Severe cardiovascular diseases (ie, heart insufficiency of grade NYHA III or IV).
5. Severe neurological diseases.
6. Severe liver and kidney diseases.
7. Any acute or chronic disease that may put the donor at risk in case of cell donation by leukapheresis.
8. Malignant neoplasms, except in situ carcinoma after complete removal.
9. Known infections or exposures to HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), West Nile virus (WNV; testing only required during WNV season (1 June to 30 November of a year)), gonorrhoea or syphilis, with the risk of transmission of infection.
10. Active bacterial, mycotic or viral infection.
11. Known malaria infection; known infection of tuberculosis, Q fever, Salmonella typhi and paratyphi, or osteomyelitis (if not medically documented to have been cured for 2 years); known toxoplasmosis (except if symptom free for 6 months); after completion of treatment for rheumatic fever (except if treatment was completed for 2 years).
12. Known transmissible spongiform encephalopathies.
13. Known protozoonosis (babesiosis, trypanosomiasis (eg, chagas), leishmaniosis), known chronic bacterial infections as brucellosis, rickettsiosis, leprosy, relapsing fever, melioidosis, tularemia (except after assured healing according to documented medical assessment).
14. Autoimmune diseases requiring systemic immunosuppressive therapy.
15. Allergies requiring systemic immunosuppressive therapy.
16. Immunosuppressive therapy within 6 months prior screening.
17. Known or suspected abuse of alcohol, drugs or medicinal products.
18. Unexplained night sweats, unexplained fever, unexplained weight loss, prolonged unexplained cough or diarrhoea, unexplained skin lesions, lymph gland swelling or thrush.
19. Dura mater and/or cornea grafts, allogeneic organ transplants, xenotransplants, pituitary hormones of human origin received.
20. Stay of longer than 6 months in the UK between 1980 and 1996 and/or an operation and/or blood transfusion in the UK after 1 January 1980.
21. Operations or other invasive interventions (eg, endoscopies, biopsies, catheter applications, acupunctures (except acupuncture with sterile and/or disposable needles)) within 4 months prior to screening.
22. Any invasive exposure to blood (ie, allogeneic blood components or plasma derivatives) or blood-contaminated injection needles or instruments, tattoos or piercings within 4 months prior to screening.

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Box 1 Continued

23. Positive PCR test result for SARS-CoV-2 at screening.
24. Haemoglobin <80 g/L, thrombocytes <80 000/µL and/or leucocytes <3000/µL.
25. Known history of hypersensitivity to components used in the leukapheresis setting (ie, components of the anticoagulant acid citrate dextrose solution).
26. Any finding or medical condition prohibiting the inclusion in the trial according to the judgement of the responsible leukapheresis physician.

    For donors who meet the following exclusion criteria, the inclusion in the clinical trial will be temporarily postponed:
27. Travel history: differentiated time-limited postponement of enrolment depending on areas with temporarily increased risk of transmission of infectious disease.
28. Vaccinations/sera of animal origin: temporary postponement of enrolment by 1 week to 12 months (4 weeks for SARS-CoV-2 vaccination).
29. Infectious diseases/diarrhoeal diseases of unknown cause: temporary postponement of enrolment for 4 weeks after symptoms subside.
30. Uncomplicated infection/tooth extraction: temporary postponement of enrolment for 1 week after symptoms subside.

PBMC, peripheral blood mononuclear cells.

Treatments

PBMC from the kidney donor of patients randomised to the MIC arm are taken by unstimulated leukapheresis 7 (±1) days before the scheduled transplantation (visit 2) (figure 1). The PBMC are subsequently modified through treatment with an alkylating agent under GMP conditions. After washing out of the alkylating agent from the supernatant of the MIC preparation, the resulting ATMP is administered as a single infusion of 1.5×10^8 MIC per kg of body weight (b.w.) to the prospective transplant recipient within 24 hours. Dose and time of administration were chosen based on results of the TOL-1 Study, in which a good safety and efficacy profile was seen. One day before transplantation (visit 3), all enrolled patients treated with MIC will undergo a crossmatch test including DSA. Patients who develop an immune reaction to MIC, shown by positive results in crossmatch and Luminex single antigen test (a highly sensitive method for determining HLA-antibodies/DSA) at visit 3, will be withdrawn from the trial and the planned transplantation will be temporarily stopped. If there are no safety concerns, the kidney transplantation will be performed, and the immunosuppression therapy will be started 7 (±1) days after MIC treatment.

Patients treated with MIC receive immunosuppression consisting of tacrolimus, mycophenolic acid derivative and corticosteroids without IL-2 receptor antibody induction therapy. A structured weaning of immunosuppression will begin after 28 days, aiming for low immunosuppression (MIC arm A) or minimal immunosuppression (MIC arm B). In both MIC arms, the tacrolimus dose will be gradually reduced to achieve a target blood concentration

Box 2 Inclusion and exclusion criteria for kidney transplant recipients (patients)

Inclusion criteria

1. Patient with CKD in stage 5 (eg, eGFR<15 mL/min AND/OR on renal replacement therapy), who are in preparation for kidney transplantation from a live donor.
2. Age ≥18 years, <70 years.
3. ABO-blood group identical OR compatible with donor.
4. First kidney transplantation.
5. Complement dependent cytotoxicity (CDC)-panel reactive antibodies <20%.
6. No detection of a donor-specific HLA-antibody in the Luminex-Assay (cut-off: MFI≤1000).
7. Negative CDC crossmatch with the donor.
8. Negative PCR test result for SARS-CoV-2 at screening.
9. Patient's living donor gave written consent for trial participation.
10. Ability to understand the nature and scope of the clinical trial.
11. Written informed consent given prior to any trial-related procedures.
12. Female patients of childbearing potential must:
    a. Have a negative pregnancy test (blood) at screening.
    b. Either commit to true abstinence from heterosexual contact or agree to use, and be able to comply with, two highly effective measures of contraception control (failure rate less than 1% per year when used consistently and correctly) without interruption, during the trial participation. Patients who discontinue mycophenolic acid derivate during the trial participation can switch to one highly effective contraceptive method 6 weeks after the end of mycophenolic acid derivative treatment. Reliable methods for this trial are: combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, sexual abstinence or vasectomised sexual partner. Abstinence from heterosexual contact is only accepted as true abstinence: when this is in line with the preferred and usual lifestyle of the patient. (Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods and withdrawal) is not an acceptable method of contraception.) Postmenopausal (no menses for at least 1 year without alternative medical cause) or surgically sterile female patients (tubal ligation, hysterectomy or bilateral oophorectomy) may be enrolled.
    c. Agree to abstain from breast feeding during the trial participation.
13. Male patients must practice true abstinence or agree to use a condom during sexual contact with a pregnant woman or a woman of childbearing potential during the trial participation and for at least 90 days after the end of mycophenolic acid derivative treatment, even if he has undergone a successful vasectomy.

Exclusion criteria

1. Pre-existing severe psychiatric disorder.
2. Heart insufficiency of grade NYHA III or IV.
3. Severe liver disease (aspartate aminotransferase or alanine aminotransferase or gamma glutamyl transpeptidase ≥3-fold of upper norm).
4. Active infection of HIV, HBV, HCV or syphilis.
5. Active bacterial, mycotic or viral infection.
6. Negative serological test result for antibodies specific for Epstein-Barr virus (EBV) antigens (note: EBV negative patients can be included if the donor is confirmed EBV negative).

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Box 2 Continued

7. Malignant disease within 2 years prior to screening, except basal cell carcinomas of the skin and in situ carcinomas.
8. Immunosuppressive therapy (eg, for the treatment of an autoimmune disease) within 6 months prior screening.
9. Pre-existing vasculitis or collagenosis.
10. Known presence of irregular antibodies in Coombs test.
11. Vaccination within 4 weeks prior to screening.
12. Spleen removed.
13. Known or suspected abuse of alcohol, drugs or medicinal products.
14. Pregnant or breast feeding.
15. Female patients who have a child with the donor or were pregnant from the donor due to possible sensitisation.
16. Known history of hypersensitivity to the cellular components or to any other constituent/excipient in the pharmaceutical formulation of MIC (eg, components of the SSP+ buffer as electrolytes (sodium chloride, potassium chloride, magnesium), citrate and phosphate, traces of mitomycin C, human albumin or EDTA).
17. Any finding or medical condition prohibiting the inclusion in the trial according to the judgement of the investigator.
18. Participation in an interventional clinical trial within 30 days prior to screening or in observation period of a competing study.
19. Employees of the sponsor, or employees or relatives of the investigator.

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HCV, hepatitis C virus; HLA, human leucocyte antigen; MFI, mean fluorescence intensity.

of 4–8 µg/L at day 183 (week 27) and the corticosteroid treatment (eg, methylprednisolone) will be stopped at day 92 (week 14) after gradual dose reduction. In MIC arm B, the mycophenolic acid derivative for example, EC-MPS will be stopped between days 141 and 182 (weeks 21 to 26) after gradual dose reduction and only tacrolimus monotherapy is given permanently. The first 10 MIC treatment patients will be treated according to MIC arm A. The scheduled complete weaning of mycophenolic acid derivative in MIC arm B will occur only after successful weaning of immunosuppression in MIC arm A, as assessed by the DSMB.

From the time of living donor kidney transplantation, patients of the control arm receive SoC immunosuppression according to the Efficacy Limiting Toxicity Elimination symphony scheme, that is, IL-2 receptor antibody induction therapy, tacrolimus, mycophenolic acid derivative and corticosteroids. In immunological lowest risk (HLA-identical) and low-risk recipients (no sensitising event, haplotype identical living-related transplantation), corticosteroids may be withdrawn from week 14. A placebo arm will not be included as it will not significantly improve the understanding of safety and would unnecessarily expose the donor to the risks of leukapheresis.

The weaning of immunosuppressive therapy of the patients in the MIC arms and the control arm has to be stopped permanently and immunosuppression has to be done according to SoC if at least one of the following criteria will occur: rejection classified higher than Banff borderline (according to Banff 2018 criteria), and/or detection of a DSA in the Luminex-Assay (cut-off: ≥1000 MFI; confirmed by repeated measurement). The weaning of immunosuppression therapy with EC-MPS below a dose of 720 mg per day in patients of the MIC arms and control arm has to be stopped intermittently if one of the following criteria will occur: Breg <3% and/or antidonor T cell response >5%. If the patients again do not fulfil the criteria during further follow-up, weaning may be continued at the investigator's discretion.

Extended immunological monitoring and biopsy assessment

Blood sampling will be done for an extended immunological monitoring at time points indicated in the schedule of assessment for patients (table 2).

The following measurements will be performed: DSA using Luminex single antigen test, lymphocyte subsets for example, T, B (including Treg and Breg) and NK cells using flow cytometry, cytokines using Luminex assay, and antidonor T cell response using mixed lymphocyte reaction assay as previously described.[3]

In addition, Torque Teno virus level, donor-derived cell-free DNA using PCR and sequencing, and genomic and proteomic analyses using PCR and array technology will be performed retrospectively. These latter parameters will not determine the changes of immunosuppression during (primary) follow-up.

Tissue samples from the transplanted kidney will be obtained by fine needle biopsy during the transplantation (if possible) and at time points indicated in the schedule of assessment for patients (table 2). Histological biopsy analysis for rejection assessment according to Banff 2018 criteria will be done locally. In addition, secondary reporting by a blinded central pathologist and molecular biopsy analysis using the molecular microscope diagnostic system (MMDx) will be done. MMDx is a central diagnostic system that uses microarrays to measure transcript levels in biopsies. An algorithm is then applied to compare the biopsy to a reference set of samples and assign quantitative scores to stratify rejection and injury risk in kidney transplant patients.[20]

Safety assessments

Safety oversight will be conducted by an independent DSMB. The DSMB will advise regarding the scheduled discontinuation of the mycophenolic acid derivative for minimal immunosuppression in MIC arm B and the continuation of the clinical trial. In addition, the DSMB will review safety data ad hoc in the following cases: detection of de novo donor-specific HLA-antibodies in highly sensitive Luminex testing (cut-off ≥1000 MFI) and/or positive CDC-crossmatch results for B/T cells or unseparated (U) cells at visit 3 in MIC treated patients, graft loss and death. After such safety reviews, the DSMB will make recommendations whether to continue, modify or stop the trial. Further ad hoc DSMB meetings may be scheduled on request, for example, for newly identified safety issues.

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Table 2 Patient schedule of assessments (simplified version)

Procedures for patient	Screening	Treatment		12-month follow-up including transplantation and immunosuppression								LT-FU
	V1	V2		V3	Tx	V4	V5	V6	V7	V8	V9	V10 V11
Day	−14 to −7	0		6	7	37	97	142	187	277	367	M24 M36
Arm		Control	MIC	MIC								
Written informed consent	x											
Inclusion and exclusion criteria	x		x									
Demographic data	x		x									
Medical history	x		x									
Previous medication	x											
Risk of transplant rejection	x											
Pregnancy test	x		x									
SARS-CoV-2 PCR	x		x									
Serology/PCR tests	x		x									
CMV/BKV DNA	x		x	x		x	x	x	x	x	x	x
Routine lab	x	x	x	x		x	x	x	x	x	x	x
Blood group	x		x									
Physical examination	x	x	x	x		x	x	x	x	x	x	x
ECG	x											
Vital signs	x	x	x	x		x	x	x	x	x	x	x
Randomisation	x											
MIC administration			x									
Crossmatch and DSA				x								
Urine analysis						x	x	x	x	x	x	x
Biopsy							x*	x*			x	
eIM†		x	x	x		x	x	x	x	x	x	x
Status assessment				x		x	x	x	x	x	x	x
Days off work and reason	x					x	x	x	x	x	x	x
Quality of life (SF-36)	x										x	x
Concomitant medication		x	x	x		x	x	x	x	x	x	x
AEs, AESIs	x	x	x	x		x	x	x	x	x	x	x

*May be performed at visit day 97 or 142.
†Extended immunological monitoring comprising HLA antibody screening, lymphocyte subsets, cytokines (only patients at main centre), antidonor T-cell response and blood sampling for donor-derived cell free DNA, Torque Teno virus level, genomic and proteomic analysis.
AE, adverse event; AESIs, adverse events of special interest; BKV, BK virus; CMV, cytomegalovirus; DSA, donor-specific HLA antibody; eIM, extended immunological monitoring; LT-FU, long-term follow-up; M, month(s); MIC, modified immune cells; SF-36, Short Form 36 Health Survey; V, visit.

Sample size calculation

The sample size calculation is based on the primary endpoint assessed on visit day 367 (360 days after transplantation). It can be assumed that in the MIC arm the operational tolerance-like phenotype within 12 months can be up to 100%. In the control arm, operational tolerance-like phenotype can be assumed to be not higher than 20%. With rates of 80% versus 20%, a sample size of 24 patients (MIC arm: 16, control arm: 8) is necessary to achieve 90% power using the χ^2 test with a significance

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level of 5% and an allocation ratio of 2:1 (MIC:Control). With more conservative assumptions of 70% versus 30%, a sample size of 63 patients (MIC arm: 42, control arm: 21) would be necessary. Besides the primary endpoint, the aim of this trial is to decide whether to continue with a large confirmatory phase III trial and to gain information on candidate outcomes as primary endpoint for the phase III trial. Therefore, the sample size was chosen not only based on the primary endpoint of this trial but also in view of secondary endpoints.

Data analysis plan

The primary analysis for all safety and efficacy endpoints will be performed after the end of the 12-month follow-up period and will be based on the full analysis set (all randomised patients who underwent kidney transplantation). The primary endpoint 'proportion of patients achieving the operational tolerance-like phenotype on visit day 367' as well as the key secondary endpoint 'proportion of patients with biopsy-proven acute rejection, graft loss, graft dysfunction or death on visit day 367' will be compared in the MIC and the control arms using Cochran-Mantel-Haenszel test, adjusted for the stratification factor patient's risk for transplant rejection. The key secondary endpoint 'number of patient-relevant infections during the first year after transplantation' in the MIC arm compared with the control arm will be analysed by a generalised linear negative binomial model with patient's risk for transplant rejection as a covariate and logarithm of observation period length (years) as offset variable. For the analysis MIC arms A and B will be combined into one MIC arm which will be compared with the control arm. The primary and the key secondary endpoints will be analysed with a hierarchical testing procedure using a global significance level of 0.05 (two-sided). Confirmatory hypothesis testing in the predefined order will stop once the first non-significant test result is obtained and the analysis of remaining endpoints will be performed in an exploratory manner. All other endpoints will be analysed exploratorily. In addition to the primary analysis two further exploratory analyses will be performed 24 and 36 months after visit day 0.

Patient and public involvement

None.

ETHICS AND DISSEMINATION

This manuscript is based on the TOL-2 Study clinical trial protocol version 4.0 from 1 March 2022. The TOL-2 Study received approval from the Ethics Committee of the Medical Faculty of University of Heidelberg, Heidelberg, Germany (AFmu-580/2021, 17 March 2022), and from the Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich-Institut, Langen, Germany (Vorlage-Nr. 4586/02, 21 March 2022). The MIC cell product is manufactured under GMP conditions under the manufacturing authorisation DE_BW-01_MIA_2020_0118/DE_BW_01_Uniklinik HD_Med Klinik V GMP-Facility of the Regierungspräsidium Tübingen, Tübingen, Germany. The trial will be performed in accordance with the ICH guideline for good clinical practice (GCP; CPMP/ICH/135/95/ICH GCP E6 (R2)), the appropriate national regulations ('Arzneimittelgesetz' and 'GCP-Verordnung') and the Declaration of Helsinki in its current version. The TOL-2 Study is registered with ClinicalTrials.gov (first registered 3 May 2022).

Written informed consent will be obtained from patients and respective donors prior to enrolment in the study (patient consent forms are available as online supplemental files 1 and 2).

Primary sponsor and contact point for further information on the trial is TolerogenixX GmbH (CEO: Professor Dr M Schaier), Im Neuenheimer Feld 162, 69 120 Heidelberg, Germany. Coordinating investigator and contact for scientific queries is Professor Dr C Morath, christian.morath@med.uni-heidelberg.de, Department of Nephrology, Heidelberg University Hospital, Im Neuenheimer Feld 162, 69 120 Heidelberg, Germany, Phone: +49-6221-9112 207.

The results from the TOL-2 Study will be published in peer-reviewed medical journals and will be presented at symposia and scientific meetings.

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ASchmitt, MSchmitt, LW, CK, SS, M-LS, AK, AH-K, BN. Immunological monitoring: LW, THT, SS, GAB, VD. Patient recruitment and treatment: CM, ASchmitt, VS, SK, MF, MStangl, IAH, CSommerer, CN, FK, CSpeer, LB, CB, AM, MZ.

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Competing interests CM, ASchmitt, MS, CK, GO, PT, MZ and MSchaier together with the University of Heidelberg, are cofounders of TolerogenixX GmbH, Heidelberg, Germany, a biotechnology company that holds licenses for MIC treatment. CK, GO, and PT hold a patent for MIC treatment. CM, ASchmitt, MSchmitt, CK, GO, CSüsal, PT, MZ, VD and MSchaier together with the University of Heidelberg and TolerogenixX GmbH filed a patent application for MIC treatment. JR is cofounder and shareholder of Trisaq, a biopharmaceutical company that develops novel therapy for kidney diseases.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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