Supplemental Data – Table of contents

Supplemental Methods

Control group for lymphocyte proliferation assay

Controls for Figure 2C: Six stable unsensitized ABO compatible patients a median of 328 days (range, 222-616 days) after living donor kidney transplantation served as controls. The patients were selected because there was sufficient biomaterial available from both donor and recipient. Two patients were on cyclosporine A (CyA) therapy with trough levels of 111 and 179 µg/L, 4 patients were on tacrolimus therapy with a median trough level of 6.7 µg/L (range, 6-8.4 µg/L). Median enteric-coated mycophenolate sodium (EC-MPS) dose was 720 mg (range, 720-1440 mg) and all patients had 4 mg of methylprednisolone. Patients were recruited at Heidelberg University Hospital from October 23, 2018 to November 27, 2018.

Control groups for the measurement of regulatory B lymphocytes

Controls for Figure 4C consisted of 40 measurements in 31 patients at different time points after living or deceased donor kidney transplantation. Patients were selected because they had comparable immunosuppression to group C patients with CyA, with or without EC-MPS and with or without corticosteroids (Supplemental Figure 3). Patients were recruited at Heidelberg University Hospital from August 28, 2018 to November 07, 2018.

Controls for Figure 4D and Supplemental Figure 6 consisted of 15 healthy controls, 20 dialysis patients, 81 patients on different immunosuppressive regimens after living or deceased donor kidney transplantation (median 112 days; range, 4-13,579 days), and 1 operationally tolerant patient off immunosuppressive therapy for more than 13 years without signs of allograft dysfunction. Patients were recruited at Heidelberg University Hospital from September 25, 2018 to October 21, 2019.

Modified immune cell product manufacturing

The modified immune cell (MIC) product was based on donor peripheral blood mononuclear cells (PBMCs) that were harvested by unstimulated donor leukapheresis with a Spectra Optia® apheresis device (Terumo BCT, Eschborn, Germany). The leukapheresis product had a median volume of 138 mL (range, 101-234 mL) with $1.61x10^{10}$ nucleated cells (range, 0.65-$3.16x10^{10}$) thereof 93% $CD45^+$ leukocytes (range, 81%-96%), 18% monocytes (range, 11%-23%), 2% granulocytes (range, 1%-9%), and 8 mL red blood cells (range, 4-20 mL). The leukapheresis products were processed under Good Manufacturing Practice (GMP) conditions according to the manufacturing license obtained from the regional (DE_BW_01_MIA_2015_0032/DE_BW_01_Uniklinik

HD_Med Klinik V GMP-Facility, Regierungspräsidium, Tübingen, Germany) and national (Vorlagen-Nr. 2252/01, Paul-Ehrlich Institute, Langen, Germany) regulatory authorities. Cells were incubated with mitomycin C followed by a 3 times washing procedure. The sterile final product (MIC) consisted of human cells of the peripheral blood resuspended in a buffer solution to a maximum total volume of 100 mL.

Quality control

Quality controls of the final products were performed for cell count, cell viability, and for percentage of monocytes by multiparametric flow cytometry. Further quality controls were operated at the external lab BioChem (BioChem GmbH, Karlsruhe, Germany) for sterility by direct inoculation method, for endotoxin by Limulus amebocyte lysate (LAL) test, and for mitomycin C concentration by high-performance liquid chromatography (HPLC) according to European Pharmacopoeia (Ph. Eur.). The concentration of mitomycin C had to be ≤0.5 μg/mL and endotoxin concentrations <5 IU/kg bw/h (Supplemental Table 10).

Potency Assay

In a first step, a potency assay was developed in which MICs were co-cultured with immature dendritic cells (DCs) (1). The expression of surface markers on resulting DCs, and the immunosuppressive capacity of both, MICs and resulting DCs, on the proliferation and cytokine release of antigen-specific $CD8^+$ T cells, were assessed for the potency assay (Supplemental Table 11).

Coculture

To avoid cellular variations among different clinical recipients and to establish a standardized potency assay, PBMCs from a third party donor who is both human leukocyte antigen (HLA)-A2 and cytomegalovirus (CMV) positive were used in the potency assay to mimic the clinical recipient. Third party immature DCs (iDCs) were prepared from monocytes using DC differentiation medium, e.g. RPMI medium (Gibco, Grandisland, NY, US) supplemented with 1% human serum (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), 500 IU/mL IL-4 (Miltenyi Biotec, Bergisch Gladbach, Germany), and 800 IU/mL GM-CSF (Peprotech, Hamburg, Germany), 3 days before Good Manufacturing Practice (GMP) production of MICs for the TOL-1 study. Each GMP-batch of MICs was introduced to $2x10^5$ iDCs at a ratio of 0:1, 1:1, 10:1, 20:1 for 2 hours then the DC maturation cocktail, e.g. RPMI medium (Gibco) supplemented with 1% human serum (Sigma-Aldrich), 500 IU/mL IL-4 (Miltenyi Biotec), 800 IU/mL GM-CSF (Peprotech), 10 ng/mL LPS (Sigma-Aldrich) and 100 IU/mL IFN-γ (Peprotech) was added for overnight co-culture. Resulting DCs were purified by magnetic negative separation using Miltenyi

Pan-DC Enrichment Kit (Miltenyi Biotec) in order to get rid of the influence of MICs. The morphology of resulting DCs was analyzed under a light microscope (Helmut Hund, Wetzlar, Germany, magnification: x40).

Flow cytometry

Cells were stained with different combinations of antibodies and reagents: HLA-DR FITC (eBioscience, San Diego, CA, US), CD8 APC (BioLegend, San Diego, CA, US), CD14 PerCP (BioLegend), CD19 PerCP (BioLegend), CD80 APC (BioLegend), CD83 PE (eBioscience), CD86 BV421 (BD Biosciences, New Jersey, US), CD103 PE-Cy7 antibodies (eBioscience), 7AAD (BD Biosciences) and cytomegalovirus(CMV) phosphoprotein65 (495-503) (CMV-A2)/HLA-A*0201 tetramer (synthetic peptides were provided by the DKFZ, Heidelberg, Germany, monomers to construct tetramer by the NIH, Bethesda, MD, US). All measurements were performed on an LSRII device (BD Biosciences) and data were analyzed using BD FACSDiva software (BD Biosciences). Briefly, after 15 minutes blocking with blocking buffer (50% FACS buffer), e.g. PBS (Gibco), 1% bovine serum albumin (Miltenyi Biotec) and 2 mM EDTA (Sigma-Aldrich) together with 50% human albumin (Carl Roth, Karlsruhe, Germany) at 4°C, cells were stained with antibodies for 30 minutes at room temperature in the dark or stained with CMV tetramer for 45 minutes at room temperature in the dark, followed by staining with the other antibodies.

Mixed lymphocyte peptide culture (MLPC)

The proliferation of CMV-specific T cells was evaluated by tetramer staining followed by one-week MLPC. Briefly, $2x10^5$ third party PBMCs were cocultured with either resulting DCs or mature DCs (mDCs) or MICs at 4:1 ratio in the presence of 1 μg/mL CMVpp65 peptide. After 7 days of culture, T cells were harvested and the proliferation of CMV-specific $CD8^+$ T cells was evaluated by CMV tetramer staining.

Enzyme-Linked ImmunoSpot (ELISpot) assay

The secretion of interferon-γ by CMV-specific T cells was measured by ELISpot assay. Briefly, $1x10^5$ third party PBMCs were mixed with either resulting DCs or mDCs or MICs at a ratio of 2:1 and plated in triplicate. CMVpp65 (495-503) (NLVPMVATV) peptide was added directly to the experimental well at a concentration of 1 μg/mL. Image analysis of ELISpot plates was performed with an ImmunoSpotTM Analyzer (CTL, Bonn, Germany).

This potency assay necessitates at least 6 days. When using MICs for the treatment of patients, the whole procedure from leukapheresis until injection takes only 12-15 hours. The ideal would be an assay whose result would already be available before the start of the MIC infusion. Therefore, in a second step, gene expression of donor PBMCs after compared to before mitomycin C treatment (MIC modification) was analyzed. Candidate genes for a new potency assay are shown in Supplemental Table 12. This assay needs further validation.

Transcriptional profiling

The total RNA prepared from individual samples was analysed on Illumina Whole Genome Expression HumanHT-12 beadarray (Illumina, San Diego, CA, US). To synthesize first and second strand cDNA and for amplifying biotinylated cRNA, the Illumina Totalprep RNA Amplification kit was used. Hybridisation to the BeadChip was performed according to the manufacturer's instructions. Subsequently, the arrays were scanned with a BeadArray Reader (Illumina).

Data analysis

Raw data were exported from the Illumina Beadstudio software (Illumina) and processed by R/Bioconductor scripts (open source software). The data was quantile normalised and log2 transformed (2). Significant differentially expressed transcript features were detected using the LIMMA package of R/Bioconductor by pairwise comparisons of the groups (3). Resulting p-values were adjusted for multiple testing using Benjamini-Hochberg's false discovery rate (FDR) method; features with a FDR<0.05 were considered significant.

Pathway analysis of differentially expressed genes

For a functional enrichment analysis, the Ingenuity Pathways Analysis (IPA) software (version 6.3, Ingenuity Systems, Redwood City, CA, US) was applied. Differentially expressed transcripts were mapped onto a molecular network developed from information contained in the Ingenuity knowledge base (Ingenuity Systems). Ingenuity Pathway Analysis ranks the resulting biofunctions by calculating a significance score corresponding to the negative log of p-value. Furthermore, pathway core analysis identified the pre-specified canonical pathways that were most over-represented in the data set.

HLA antibody detection and crossmatch techniques

HLA antibodies were detected using the complement-dependent cytotoxicity-panel-reactive antibody (CDC-PRA), enzyme-linked immunosorbent assay (ELISA) and Luminex Single Antigen methodologies.

Crossmatches were performed using the CDC and ELISA techniques. PRA against total lymphocyte- (mainly T cells) or B lymphocyte panels were determined using the CDC method in the absence or presence of dithiothreitol (DTT). The presence of HLA class I and II antibodies of the IgG isotype were determined using the AbScreen ELISA kits of BioRad (Munich, Germany), which utilize pooled HLA molecules on 96-well microtiter plates or using the LABScreen Luminex kits of One Lambda (Canoga Park, CA, US), which utilize single HLA-coated beads and enable the identification of IgG alloantibody specificities in heat-inactivated sera against HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1 antigens. For the evaluation of pre- and post-transplant HLA antibodies, the positivity cut-off was set at 1000 MFI. ELISA crossmatches were performed using the AbCross test kit of BioRad, in which solubilized donor HLA molecules are used to detect donor-specific HLA antibodies (DSAs). Results of optical density greater than or equal to double of the negative control were considered positive. CDC crossmatches were performed using the patient's serum and isolated T, B, and unseparated lymphocytes of the donor. Following standard procedure, the patient's serum with and without DTT was incubated with lymphocytes, complement was added and the trays were read using a fluorescent microscope (Leica, Wetzlar, Germany). PRA of >5% was considered positive.

Quantitative chimerism analysis in peripheral blood

Polymerase chain reaction (PCR) of donor and recipient peripheral blood samples at day -2 / -7 (before administration of the MIC product) and from recipient at day -1/-6 (after administration of the MIC product but before kidney transplantation) and on day 7 and day 30 after kidney transplantation was performed using the AmpFISTR Profiler PCR amplification kit, AmpFlSTR Profiler Plus PCR Amplification kit in combination with AmpFlSTR Cofiler PCR Amplification kit and AmpFlSTR Identifiler Plus PCR Amplification Kit (Applied Biosystems, Weiterstadt, Germany) as recommended by the manufacturer. Separation and detection of the amplified PCR products were performed on an ABI 310 automated sequencer and genetic analyzer 3500 (Applied Biosystems). The analysis of the results was performed using the Genescan 2.1 software (Applied Biosystems). The minimum detectable proportion of recipient in chimeric samples was 1%-5% (4).

Lymphocyte proliferation assay

Lymphocytes were separated from heparinized whole blood using lymphocyte gradient centrifugation (Lymphodex, Innotrain, Kronberg, Germany). Lymphocytes were washed and adjusted to a cell number of 10^6/mL. 100 µl of cell suspension (10^5 lymphocytes) were given in each well of a 96-well cell culture plate. Cells were stimulated polyclonally for 3 days with 100 µL of the mitogens PWM (Pokeweed mitogen, Sigma-

Aldrich), PHA (phytohemagglutinin, Remel, Thermo Scientific, Dartford, UK), ConA (Concanavalin A, Sigma-Aldrich) or CD3 monoclonal antibody (BD Biosciences). In addition, recipient cells were stimulated for 5 days in a mixed lymphocyte culture (MLC) antigen-specifically with 10^5 cells of the original transplant donor per well or unspecifically with 10^5 pooled third party cells per well. The pool consisted of lymphocytes from 4 MHC class II-incompatible cell donors. Stimulator cells were irradiated to prevent their proliferation. At the beginning of the cell culture, all cells were stained with carboxyfluorescein succinimidyl ester (CFSE, Cell Trace, Invitrogen, Fisher Scientific, Schwerte, Germany). After 3 and 5 days of cell culture, respectively, green fluorescence intensity of cells was analyzed in the blast region of a CFSE/ Fw-Sc dot plot using a FACSCalibur flow cytometer (BD Biosciences). Proportion of blasts with low CFSE fluorescence (CFSElow blasts) was determined and background proliferation was subtracted determined in a cell culture without stimulus run in parallel. All assays were carried out in triplicate.

Determination of lymphocyte subsets in peripheral blood

Relative and absolute numbers of lymphocyte subsets were determined in heparinized whole blood using trucount tubes, monoclonal antibodies and a FACSCalibur flow cytometer (all from BD Biosciences). Briefly, CD45 PerCP, CD3 FITC, CD4 APC, CD8 PE, CD19 APC, CD56+CD16 PE, CD20 APC, CD25 PE, and HLA-DR PE monoclonal antibodies were incubated with heparinized whole blood for 15 minutes at room temperature in the dark. Red cells were lysed for 15 minutes using NH_4Cl lysis (Lysing solution, BD Biosciences). Thereafter, cells were ready for flow cytometric analysis.

When regulatory T lymphocytes (Tregs) were determined, CD4 PerCP, CD25 APC, and CD127 FITC monoclonal antibodies were incubated with heparinized whole blood for 30 minutes at room temperature in the dark. Red cells were lysed for 10 minutes using NH_4Cl lysis (BD Biosiences). Then, cells were washed with PBS (Gibco), permeabilized (Permeabilizing Solution 2, BD Biosciences) for 10 minutes at room temperature in the dark and washed again. Foxp3 PE monoclonal antibody was added for 30 minutes. Cells were washed, incubated in PBS for 30 minutes and washed again. Absolute and relative numbers of Tregs were analyzed using a FACSCalibur flow cytometer (BD Biosciences).

When regulatory B lymphocytes (Bregs) were determined, CD19 APC, CD24 PE, CD38 PerCP, and CD27 FITC monoclonal antibodies were incubated with freshly obtained heparinized whole blood for 30 minutes at room temperature in the dark. Red cells were lysed using NH_4Cl lysis (BD Biosciences) and washed in PBS (Gibco). Additionally, Bregs were determined from frozen samples of the patients. After separation by density gradient centrifugation, cells were frozen at 10^7 cells/mL with the freezing media containing 90% heat-inactivated fetal

bovine serum (FBS, Gibco) and 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich) in the liquid nitrogen tank until start of analysis. After thawing, the cells were incubated with CD19 APC, CD24 PE, CD38 PerCP, and CD27 FITC monoclonal antibodies for 30 minutes at room temperature in the dark, washed and analyzed using flow cytometry. In a separate analysis, cells were incubated with CD19 APC, CD24 PE, and CD38 PerCP monoclonal antibodies for 30 minutes, washed with PBS, permeabilized, washed, incubated with IL-10 FITC monoclonal antibody and washed again. Absolute and relative numbers of Bregs were determined using a FACSCalibur flow cytometer (BD Biosciences).

Determination of cytokine and chemokine levels in plasma

Plasma levels of cytokines and chemokines were determined according to the instructions of the manufacturer using the Luminex Performance Assays: Human High Sensitivity Cytokine Base Kit A, Human Cytokine Base Kit A and Magnetic Luminex Performance Assay Base Kit, TGF-β (R&D systems, Wiesbaden, Germany). Briefly, 350 µL plasma were incubated with antibody-coated beads for 3 hours, followed by biotin-labeled antibody for 1 hour and fluorochrome-conjugated streptavidin for 30 minutes. Assays were analyzed using the Luminex LX100/200 system (Luminex B.V., MV 's-Hertogenbosch, The Netherlands).

Proteomic analysis of patient serum samples

Antibody microarray production and analysis were performed as described in detail before (5, 6). In brief, a total of 1,439 antibodies from various manufacturers were used on the microarray. The full list of the antibodies is disclosed elsewhere (6). Each antibody was taken up at a concentration of 1 µg/µL in spotting buffer composed of 50 mM carbonate buffer, pH 8.5, 1.0 mM MgCl 2.5% trehalose, 0.005% Tween-20. The antibodies were spotted in quadruplicates on epoxysilane-coated glass slides (Nexterion-E, Schott, Jena, Germany) using a MicroGrid-2 robot (BioRobotics, Cambridge, UK) at controlled humidity (55-65%) and temperature (4-8°C). After printing, the slides equilibrated at a humidity of 40%-45% overnight and were stored in dry and dark conditions at 4°C until use.

Patient serum samples were collected at various time points and stored at -80°C. For analysis, they were thawed at room temperature and diluted to a protein concentration of 1.0 mg/mL in 50 mM saline-bicine buffer, pH 8.5. The protein was labeled with the fluorescent dye DY-649 (Dyomics, Jena, Germany) at a molar dye/protein ratio of 7.5 in the dark, at 4°C for two hours. Any un-reacted dye was quenched by adding 0.1 volume 10% (w/v) glycine in 50 mM bicine buffer, pH 8.5. The microarrays were blocked at room temperature in 137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM disodium hydrogen phosphate, 1.76 mM sodium dihydrogen

phosphate, pH 7.4, 0.05% (w/v) Tween-20 (PBST) and 10% (w/v) non-fat dry milk (Biorad, Munich, Germany). A pooled protein mixture extracted from 23 cell lines and labeled with a different fluorescent dye (DY-549, Dyomics) was used as reference for microarray normalization (7). After blocking, each array was incubated with 50 µg of the individual labeled sample as well as a similar amount of the labeled pool reference. The incubation of sample was performed in PBST containing 1% milk at 4°C overnight. The following day, arrays were washed with PBST four times, rinsed shortly with deionized water, and dried in a ventilating chamber at room temperature. The microarrays were scanned under constant laser power using a Tecan Power Scanner (Tecan, Grödig, Austria). The analysis of the resulting images was done with the software package GenePix Pro version 6.0 (Molecular Devices, Sunnyvale, CA, US).

The signal intensities obtained from the microarray analysis were loaded into the software package Chipster and normalized as described elsewhere (8, 9). The median of the signal intensities obtained at the four spots of each antibody in the red (DY-649/sample) and green (DY-549/pool reference) channels was used to calculated ratios. They were normalized using the Loess method with background correction offset [0, 50] of the normexp process (9). The paired student t-test was used to investigate significance. A p-value of less than 0.05 was considered significant. Functional annotation of any differentially abundant protein was elucidated with the IPA software (Ingenuity Systems). Prediction of variations in biological functions was performed using z-score values of +2 or -2, respectively, as threshold for significance.

Gene expression analysis from peripheral blood

The original Immune Tolerance Network (ITN) signature consisted of 3 genes, namely IGKV1D-13, IGLL1, and IGKV4-1 that were expressed at comparable levels in healthy controls and tolerant renal transplant recipients, but were suppressed in renal transplant recipients receiving standard immunosuppression (10). In line with previous reports, we found that the amplification of IGLL1 was unreliable (11, 12). Therefore, we used the remaining 2 gene signature (IGKV1D-13 and IGKV4-1) which has proven sufficient to distinguish between tolerant and non-tolerant patients (11, 12).

Peripheral EDTA-blood samples were collected and stabilized using the RNA-Stabilization Reagent for Blood and Bone Marrow of Roche Molecular Biochemicals (Mannheim, Germany). RNA was isolated with the Roche High Pure RNA Kit, including DNAase I and DNAase Incubation Buffer, according to manufacturer's instructions with the following two changes: a) as the RNA Stabilization Reagent inactivates potentially degrading enzymes parallel to cell lysis, EDTA-blood was directly pipetted onto the filter, and b) Wash Buffer II was used twice to obtain high quality RNA. Synthesis of cDNA was conducted using the Taqman Reverse

Transcription Kit of Applied Biosystems Inc. (ABI, Foster City, CA, US) using random hexamers. RNA was stored at -80 °C, cDNA at -20°C. Real-Time PCR reactions were performed using the ABI PRISM 7500 Sequence Detection System with PrimaQuant 2x qPCR Mastermix plus (high) Rox (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany), containing the passive reference dye ROX. For the determination of threshold cycle (CT) during the exponential phase of amplification, we used real-time monitoring of fluorescent emission after cleavage of sequence-specific probes by nuclease activity of Taq polymerase. Smaller quantities of transcripts generate threshold-exceeding fluorescence during later cycles resulting in higher CT values. Threshold level is set individually and lies within the exponential amplification stage of each gene. Threshold for IGKV4-1 and IGKV1D-13 was 0.06 and RPLPO 0.05. To normalize target quantities, RPLPO gene transcripts were used as endogenous control. Target Pre-Developed Assay Reagents for IGKV4-1 gene expression were obtained from Life Technologies (Darmstadt, Germany; Sequence ID: AIN1E62). IGKV1D-13 gene expression was measured using the custom-made 5´GGG CTT CTG CTG CTC TGG3´ forward and 5´TGG AGA CTG GGT CAA CTG GAT3´reverse primers and the 6FAM-CCA GGT GCC AGA TGT G-NFQ probe (Life Technologies). Cycling parameters were: 2 min at 50°C, 10 min at 95°C, 43 cycles of 15 sec at 95°C and 1 min at 60°C. 4 ng/µL/well of cDNA was used (7.5 µL cDNA/well = 30 ng cDNA/well).

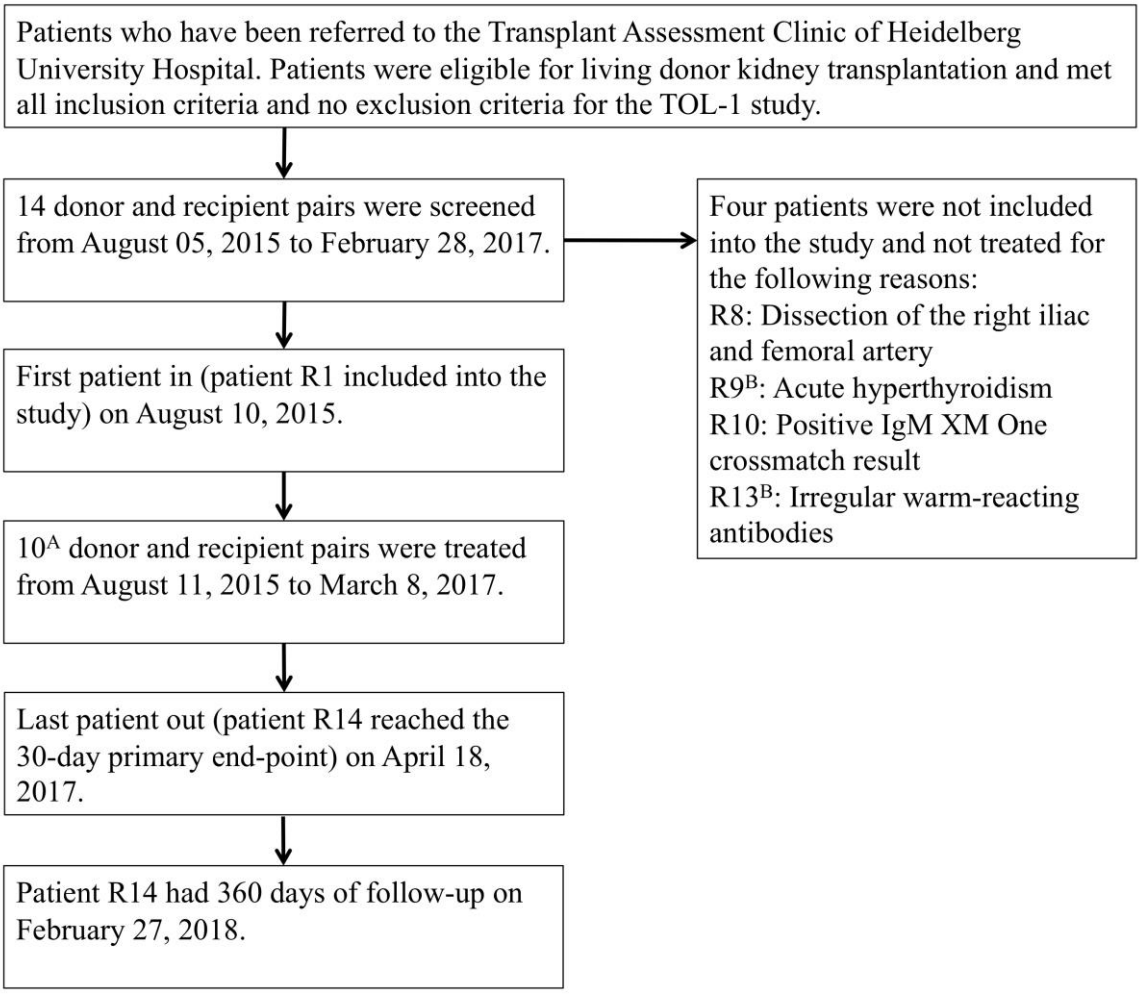
In order to correct for differences in RNA amount of different samples, endogenous control CT values were used to normalize target gene expression for each sample, referred to as Target Gene "Delta C_T" (ΔC_T) value:

ΔC_T (target gene) = C_T (target gene) – C_T (endogenous control).

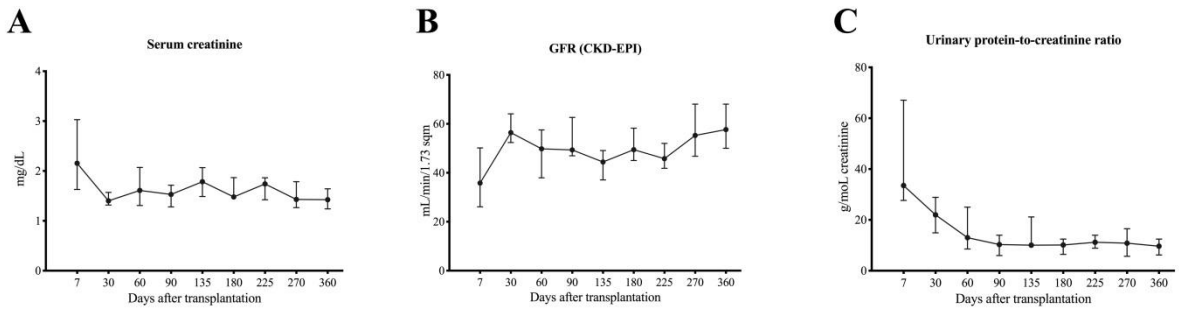
Relative expression was calculated according to the formula:

Relative gene expression (amount of target) = $2^{-\Delta CT}$.

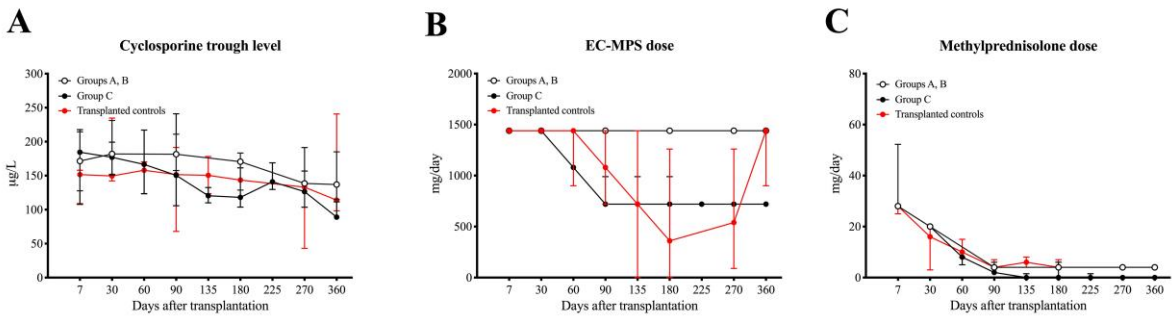
Supplemental Figures and Figure Legends



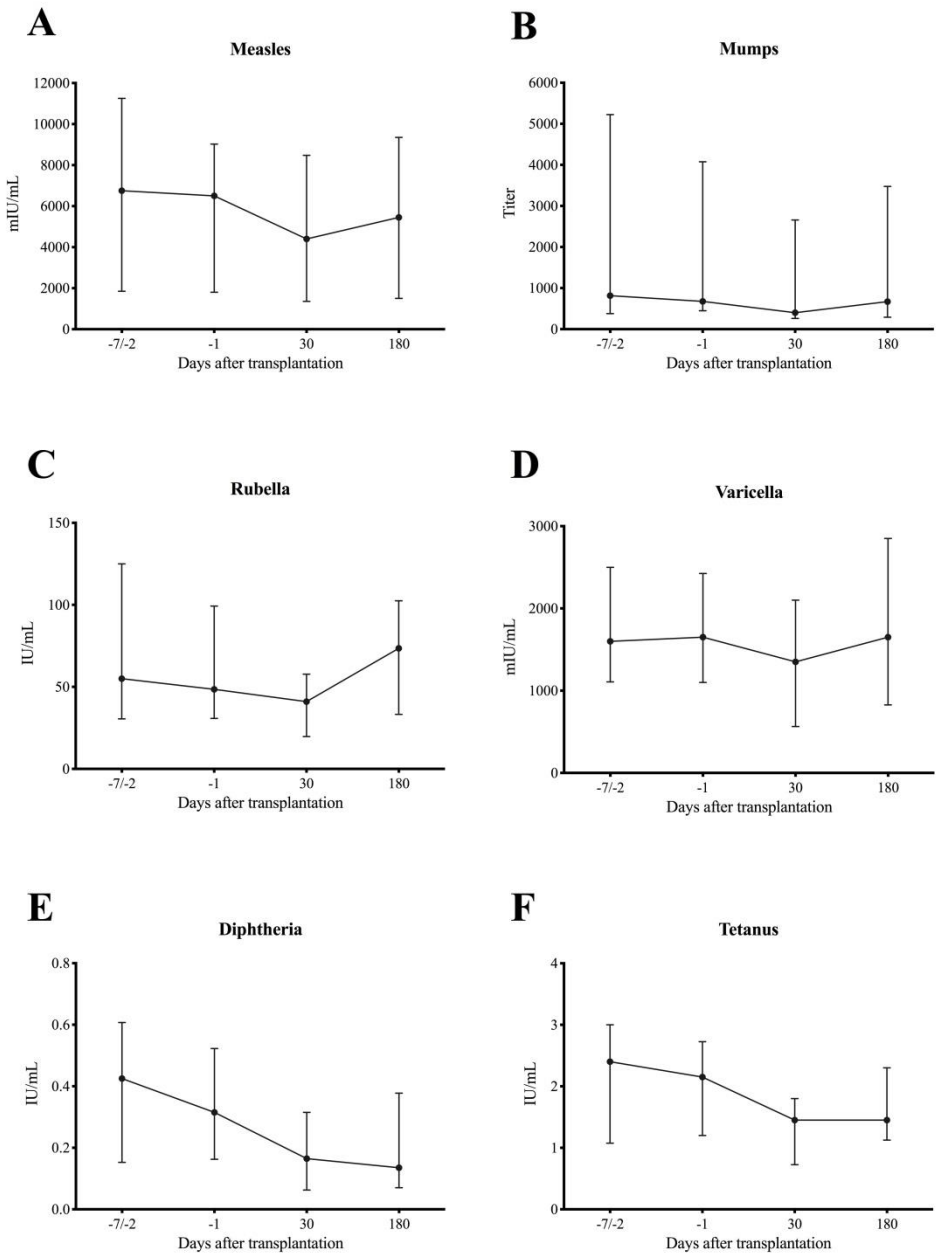
Supplemental Figure 1: Flow chart for study inclusion
[A]R1-R7, R11, R12, R14. [B]Leukapheresis was performed in donor D9 and D13 but cells were not further processed and were discarded.



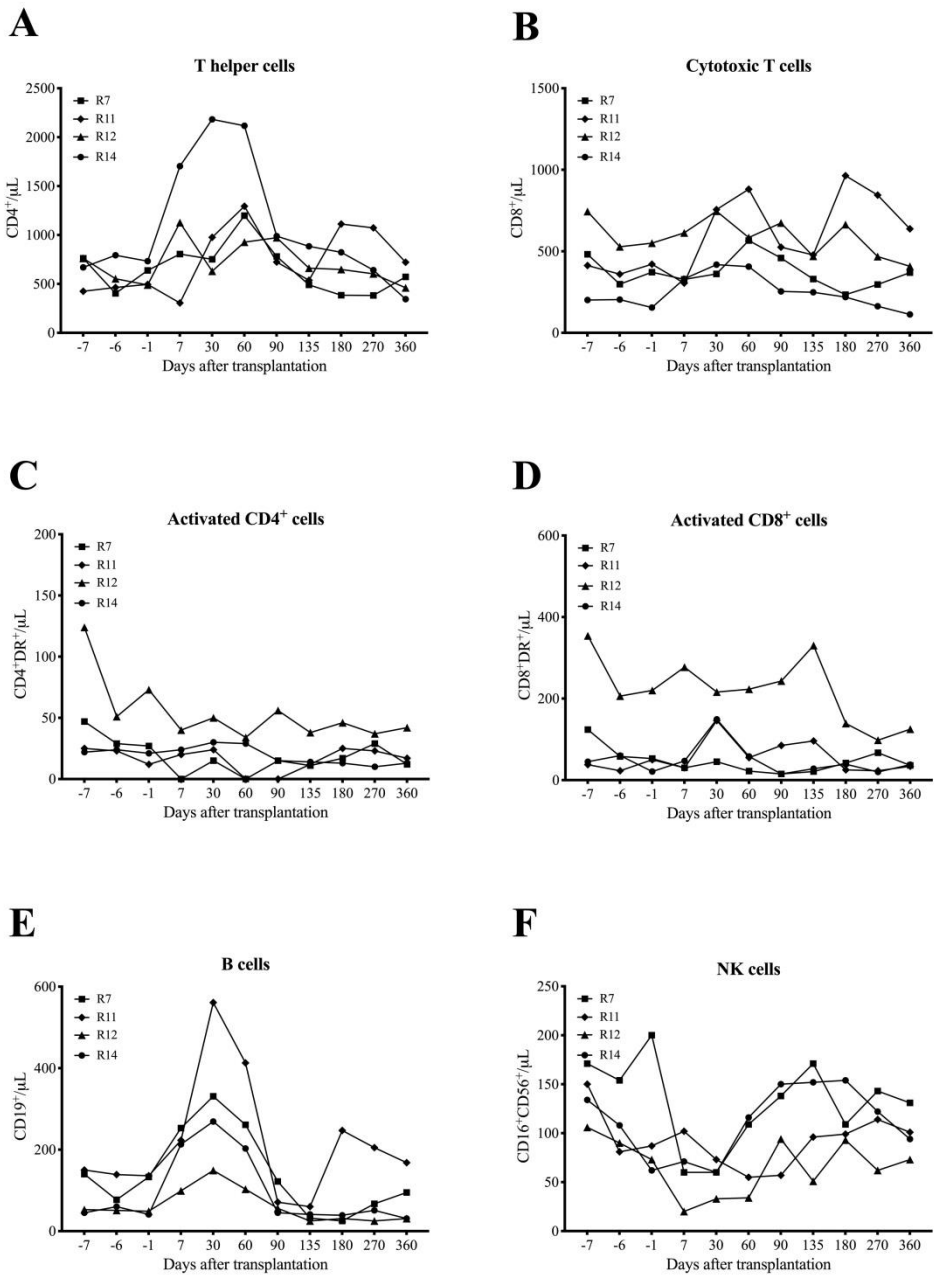
Supplemental Figure 2: Kidney function and integrity
Serum creatinine (A), eGFR according to CKD-EPI formular (B) and urinary protein excretion (C) in 10 patients (TOL-1 study phase and follow-up to day 360, median and interquartile range). All patients showed stable kidney graft function with no proteinuria to day 360 after surgery.



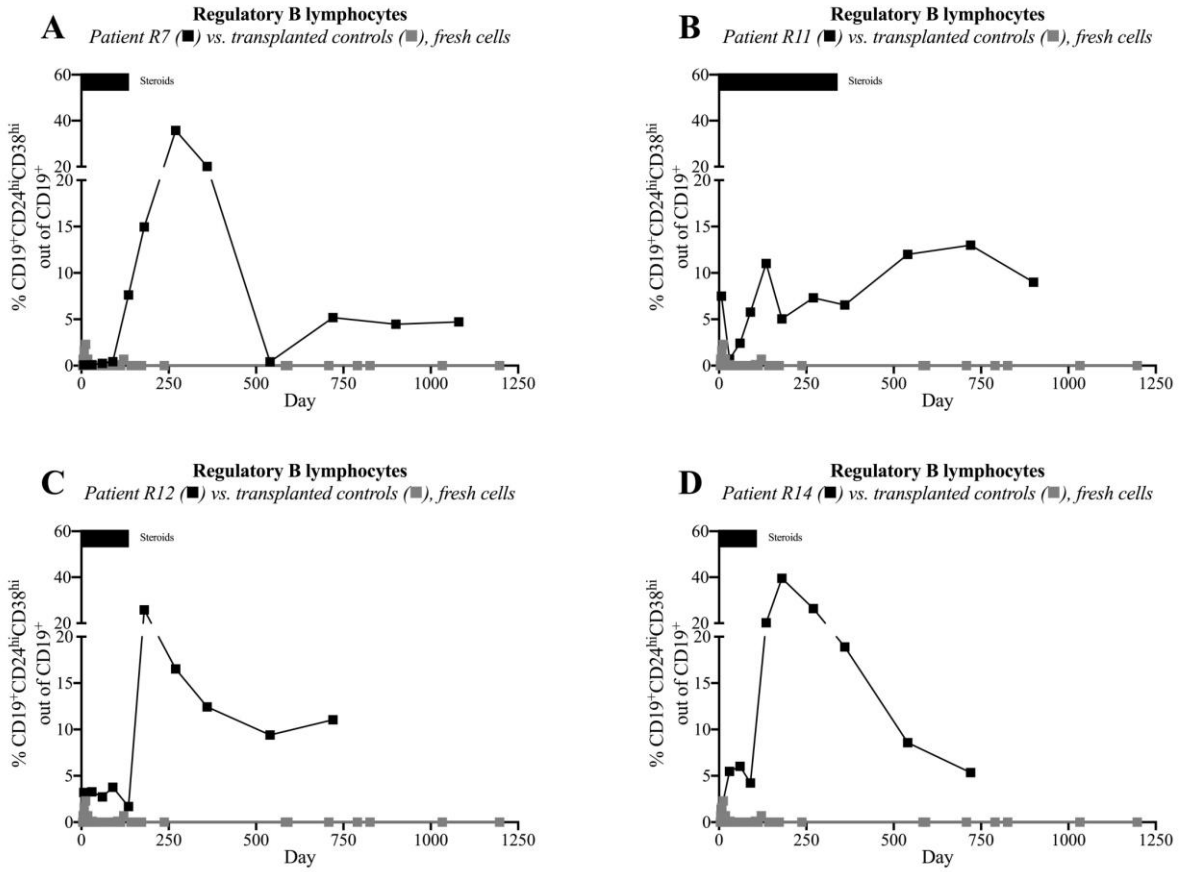
Supplemental Figure 3: Immunosuppressive therapy
Cyclosporine A trough level (A), total daily enteric-coated mycophenolate sodium dose (EC-MPS) (B) and daily methylprednisolone dose (C) in patients of groups A and B (○), group C (●) and transplanted controls for the measurement of regulatory B lymphocytes (●) for TOL-1 study phase and follow-up to day 360 (median and interquartile range).



Supplemental Figure 4: Antibody titers of bacterial and viral immunizations
Antibody titers against measles (A), mumps (B), rubella (C), varicella (D), diphtheria (E), tetanus (F) in 10 patients (TOL-1 study phase and follow-up to day 180, median and interquartile range). Titer for all tested bacteria and viruses remained constant during follow-up.

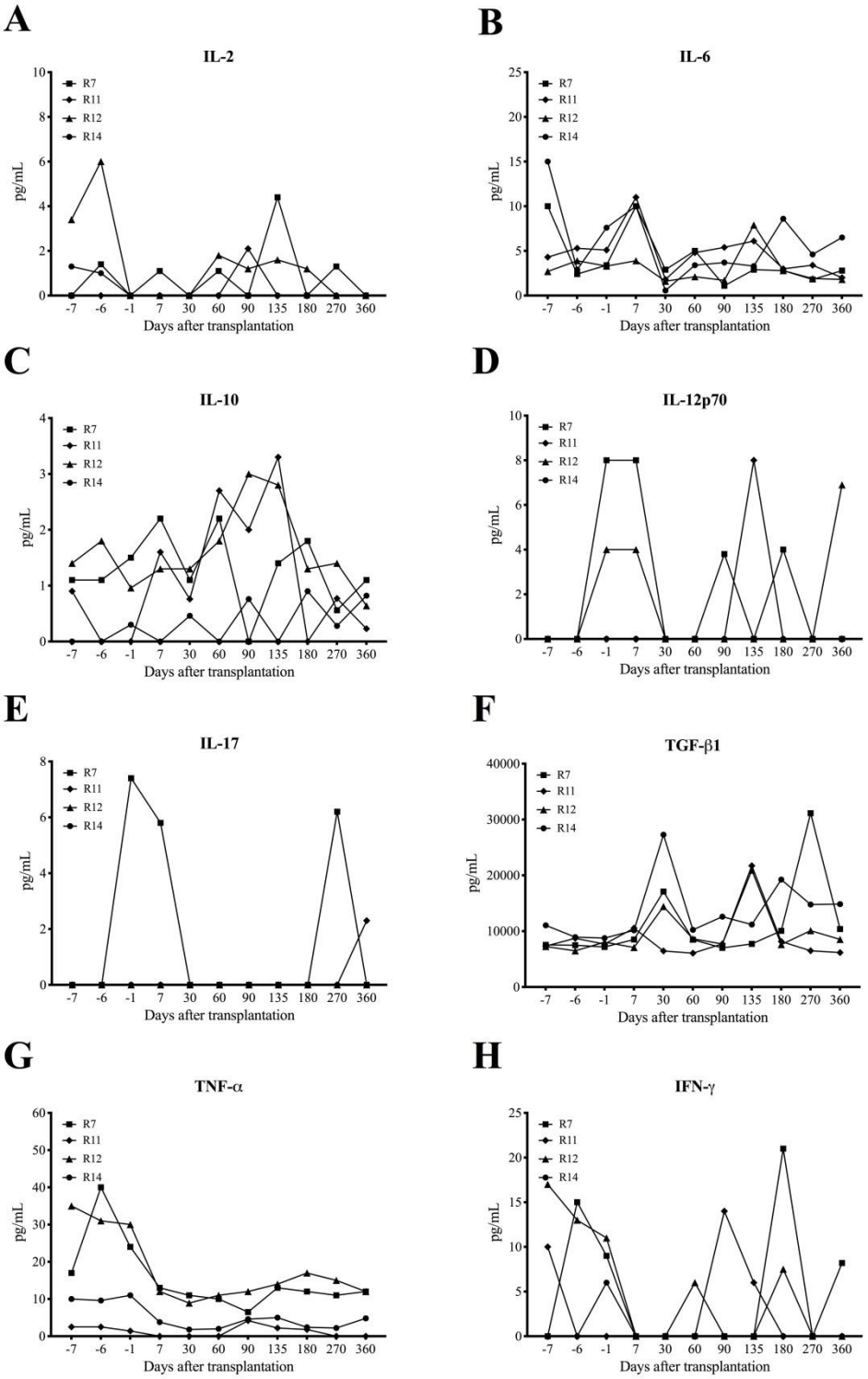


Supplemental Figure 5: Lymphocyte subpopulations
Individual measurements for $CD4^+$ T lymphocytes (A), $CD8^+$ T lymphocytes (B), activated $CD4^+$ T lymphocytes (C), activated $CD8^+$ T lymphocytes (D), $CD19^+$ B lymphocytes (E), $CD16^+CD56^+$ NK cells (F) in patients R7 (■), R11 (♦), R12 (▲), R14 (●) for TOL-1 study phase and follow-up to day 360. Evolution of $CD4^+/CD8^+$ T lymphocytes and activated $CD4^+/CD8^+$ T lymphocytes remained constant after kidney transplantation while $CD19^+$ B lymphocytes showed a maximum 30 days after surgery but decreased to day 180. $CD16^+CD56^+$ NK cells behaved inversely.

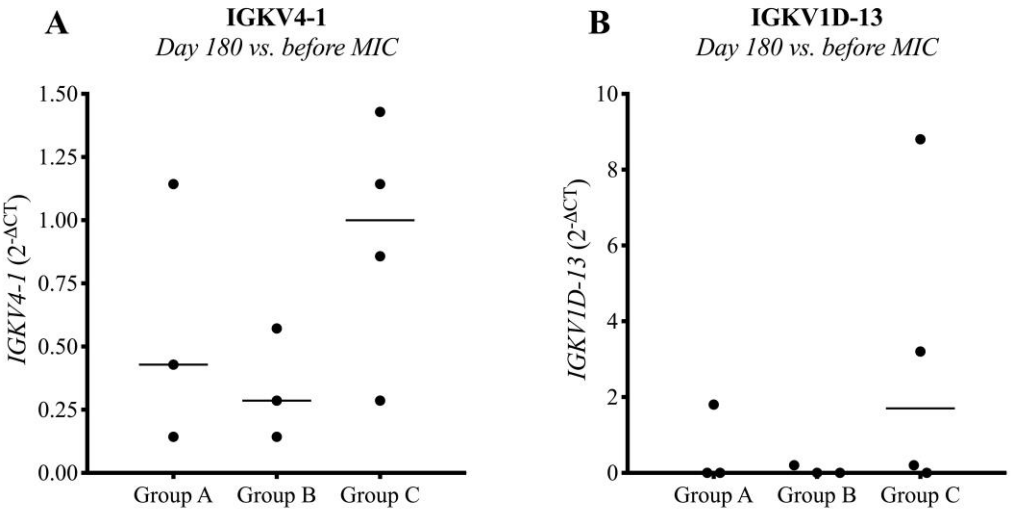


Supplemental Figure 6: Regulatory B lymphocytes in MIC-treated patients

(A-D) Individual measurements for percentage of $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs in patients R7, R11, R12, R14 (■) from day 0 to day 1080 are shown as compared to measurements in transplanted controls (■)**.** On day 135 and before steroids were stopped in 3 out of 4 patients, Bregs were a median of 9% (range, 2%-20%). At last follow-up (median 810 days; range, 720-1080 days) after transplantation and more than 1.5 years after steroid withdrawal in all MIC-treated patients, Bregs with a median of 7% (range, 5%-11%) were 24 times higher than Breg percentages in transplanted controls without steroid treatment.



Supplemental Figure 7: Cytokines
Individual measurements for IL-2 (A), IL-6 (B), IL-10 (C), IL-12p70 (D), IL-17 (E), TGF-β1 (F), TNF-α (G), IFN-γ (H) in patients R7 (■), R11 (♦), R12 (▲), R14 (●) for TOL-1 study phase and follow-up to day 360. Immunosuppressive cytokines IL-10 and TGF-β1 are found constantly high in patients after kidney transplantation and with immunosuppressive therapy. Only patient R11 who needed high-dose methylprednisolone therapy had an undetectable IL-10 level on day 180.



Supplemental Figure 8: Immune Tolerance Network (ITN) operational tolerance signature
Gene expression analysis of IGKV4-1 and IGKV1D-13 in patients of group A-C. Individual measurements and median are shown. On day 180, IGKV4-1 expression was higher in group C with a median of 1.00 (range, 0.29-1.43) compared to group A with a median of 0.43 (range, 0.14-1.14) and group B with a median of 0.29 (range, 0.14-0.57) patients. The same numbers for IGKV1D-13 were 1.70 (range, 0-3.2) in group C, compared to 0 (range, 0-1.8) in group A and 0 (range, 0-0.2) in group B patients.

Supplemental Table 1: HLA-A, -B, -DR, -DQ mismatches for transplanted patients

Group	TOL pat. no.	HLA-A	HLA-B	HLA-DRB1	HLA-DQB1
A	R1	1	1	1	0
A	R2	1	2	2	1
A	R3	0	0	0	0
B	R4	1	1	1	1
B	R5	1	1	1	1
B	R6	1	2	2	1
C	R7	1	1	1	0
C	R11	0	1	1	1
C	R12	0	0	1	1
C	R14	0	0	0	0

HLA = human leukocyte antigen, pat. = patient

Supplemental Table 2: Detailed overview of the reported AEs[A] and SAEs in 14 screened patients (Primary outcome measure, TOL-1 study phase to day 30).

AE no.	Group	TOL pat. no.	Grade (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death)	Relationship to study intervention (1=definitely, 2=probably, 3=possibly, 4=unlikely, 5=not related, 6=not assessable)	Outcome (1=resolved, 2=condition improving, 3=ongoing, 4=recovered with major sequelae, 5=death, 6=unknown)	Action taken (1=none, 2=medical intervention, 3=other)	SAE (no=0, yes=1)	Metabolism and nutrition disorders	Injury, poisoning and procedural complications	Gastrointestinal disorders	Investigations	Infections and infestations	Vascular disorders	General disorders and administration site conditions	Renal and urinary disorders	Endocrine disorders	Immune system disorders	Cardiac disorders	Nervous system disorders	
1	A	R1	1	5	1	3	0	1												
2	A	R1	1	5	1	2	0	1												
3	A	R1	1	5	1	2	0	1												
4	A	R2	1	5	1	3	0			1										
5	A	R2	1	4	1	3	0					1								
6	A	R2	1	5	1	2	0			1										
7	A	R2	1	5	1	1	0		1											
8	A	R2	1	5	2	1	0				1									
9	A	R2	1	5	1	1	0				1									
10	A	R3	1	5	2	2	0	1												
11	A	R3	1	5	1	2	0	1												
12	A	R3	1	5	3	1	0	1												
13	A	R3	1	5	2	1	0		1											
14	A	R3	1	5	1	1	0		1											
15	A	R3	1	5	1	1	0				1									

AE no.	Group	TOL pat. no.	Grade (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death)	Relationship to study intervention (1=definitely, 2=probably, 3=possibly, 4=unlikely, 5=not related, 6=not assessable)	Outcome (1=resolved, 2=condition improving, 3=ongoing, 4=recovered with major sequelae, 5=death, 6=unknown)	Action taken (1=none, 2=medical intervention, 3=other)	SAE (no=0, yes=1)	Metabolism and nutrition disorders	Injury, poisoning and procedural complications	Gastrointestinal disorders	Investigations	Infections and infestations	Vascular disorders	General disorders and administration site conditions	Renal and urinary disorders	Endocrine disorders	Immune system disorders	Cardiac disorders	Nervous system disorders	
16	A	R3	1	5	1	2	0						1							
17	B	R4	1	5	1	2	0			1										
18	B	R4	1	5	1	1	0		1											
19	B	R4	1	5	1	1	0							1						
20	B	R4	1	5	1	1	0			1										
21	B	R4	1	5	1	2	0			1										
22	B	R4	1	5	1	1	0											1		
23	B	R4	1	5	1	2	0								1					
24	B	R5	1	5	1	2	0			1										
25	B	R5	1	5	1	1	0												1	
26	B	R5	1	5	1	2	0			1										
27	B	R5	1	5	1	1	0						1							
28	B	R5	1	5	1	1	0											1		
29	B	R5	1	5	1	2	0	1												
30	B	R5	1	5	1	1	0							1						
31	B	R5	1	5	2	1	0		1											
32	B	R5	1	5	2	2	1		1											

AE no.	Group	TOL pat. no.	Grade (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death)	Relationship to study intervention (1=definitely, 2=probably, 3=possibly, 4=unlikely, 5=not related, 6=not assessable)	Outcome (1=resolved, 2=condition improving, 3=ongoing, 4=recovered with major sequelae, 5=death, 6=unknown)	Action taken (1=none, 2=medical intervention, 3=other)	SAE (no=0, yes=1)	Metabolism and nutrition disorders	Injury, poisoning and procedural complications	Gastrointestinal disorders	Investigations	Infections and infestations	Vascular disorders	General disorders and administration site conditions	Renal and urinary disorders	Endocrine disorders	Immune system disorders	Cardiac disorders	Nervous system disorders	
33	B	R5	1	5	1	2	0	1												
34	B	R6	1	5	2	2	0						1							
35	B	R6	1	5	1	2	0			1										
36	B	R6	1	5	1	2	0			1										
37	B	R6	1	5	1	2	0			1										
38	B	R6	1	5	1	1	0							1						
39	B	R6	2	5	1	3	0		1											
40	B	R6	1	5	1	2	0	1												
41	C	R7	2	5	2	2	0	1												
42	C	R7	2	5	1	2	0						1							
43	C	R7	1	5	1	1	0											1		
44	C	R7	2	5	1	3	0		1											
45	C	R7	1	5	1	2	0	1												
46	C	R7	1	5	1	2	0			1										
47	C	R7	1	5	1	1	0			1										
48	C	R7	1	5	1	1	0							1						
49	C	R7	1	5	1	2	0			1										

AE no.	Group	TOL pat. no.	Grade (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death)	Relationship to study intervention (1=definitely, 2=probably, 3=possibly, 4=unlikely, 5=not related, 6=not assessable)	Outcome (1=resolved, 2=condition improving, 3=ongoing, 4=recovered with major sequelae, 5=death, 6=unknown)	Action taken (1=none, 2=medical intervention, 3=other)	SAE (no=0, yes=1)	Metabolism and nutrition disorders	Injury, poisoning and procedural complications	Gastrointestinal disorders	Investigations	Infections and infestations	Vascular disorders	General disorders and administration site conditions	Renal and urinary disorders	Endocrine disorders	Immune system disorders	Cardiac disorders	Nervous system disorders	
50	C	R7	2	5	1	3	1								1					
51	-	R9	2	5	3	2	0									1				
52	C	R11	1	5	1	2	0						1							
53	C	R11	1	5	1	2	0			1										
54	C	R11	2	5	1	3	1			1										
55	C	R11	1	5	1	1	0			1										
56	C	R11	2	5	1	3	0		1											
57	C	R11	1	5	1	1	0					1								
58	C	R11	1	5	1	1	0			1										
59	C	R11	1	5	1	0	0			1										
60	C	R11	1	5	1	2	0	1												
61	C	R11	1	5	2	2	0	1												
62	C	R12	1	5	1	1	0			1										
63	C	R12	1	5	3	1	0		1											
64	C	R12	1	5	2	1	0						1							
65	-	R13	2	5	1	1	0										1			
66	-	R13	1	5	2	3	0	1												

AE no.	Group	TOL pat. no.	Grade (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death)	Relationship to study intervention (1=definitely, 2=probably, 3=possibly, 4=unlikely, 5=not related, 6=not assessable)	Outcome (1=resolved, 2=condition improving, 3=ongoing, 4=recovered with major sequelae, 5=death, 6=unknown)	Action taken (1=none, 2=medical intervention, 3=other)	SAE (no=0, yes=1)	Metabolism and nutrition disorders	Injury, poisoning and procedural complications	Gastrointestinal disorders	Investigations	Infections and infestations	Vascular disorders	General disorders and administration site conditions	Renal and urinary disorders	Endocrine disorders	Immune system disorders	Cardiac disorders	Nervous system disorders	
67	C	R14	1	5	2	1	0						1							
68	C	R14	1	5	2	2	0	1												
69	C	R14	1	5	1	2	0	1												
70	C	R14	1	5	1	1	0								1					
71	C	R14	1	5	1	1	0								1					
72	C	R14	1	5	1	2	0	1												
																				Total
							3	17	10	19	3	2	7	4	4	1	1	3	1	72

[A]according to Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. AE = adverse event, pat. = patient, SAE = serious AE

Supplemental Table 3: Detailed overview of the reported AEs[A] and SAEs in 14 screened donors (TOL-1 study phase to day -1/-6).

AE no.	Group	TOL pat. no.	Grade (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death)	Relationship to study intervention (1=definitely, 2=probably, 3=possibly, 4=unlikely, 5=not related, 6=not assessable)	Outcome (1=resolved, 2=condition improving, 3=ongoing, 4=recovered with major sequelae, 5=death, 6=unknown)	Action taken (1=none, 2=medical intervention, 3=other)	SAE (no=0, yes=1)	Metabolism and nutrition disorders	injury, poisoning and procedural complications	Gastrointestinal disorders	Investigations	Infections and infestations	Vascular disorders	General disorders and administration site conditions	Renal and urinary disorders	Endocrine disorders	Immune system disorders	Cardiac disorders	Nervous system disorders	
1	C	D7	1	5	1	1	0												1	
																				Total
							0	0	0	0	0	0	0	0	0	0	0	0	1	1

[A]according to Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. AE = adverse event, pat. = patient, SAE = serious AE

Supplemental Table 4: Biopsy results in 10 patients (TOL-1 study phase and follow-up to day 360)

Group	TOL pat. no.	Type of biopsy	Day after tx	Result
A	R1	Protocol	7	mm0, g0, cg0, t0, ct0, i0, ci0, v0, cv0, ah0, ptc0, C4d0
A	R1[C]	Indication	77	mm0, g0, cg0, t1, ct1, i1, ci1, v0, cv0, ah0, ptc0, C4d0
A	R2	Protocol	10	mm0, g0, cg0, t1, ct0, i2, ci0, v0, cv0, ah0, ptc1, C4d0
A	R2[C]	Indication	46	mm0, g0, cg1, t1, ct0, i0, ci0, v0, cv0, ah0, ptc0, C4d0
A	R3	Protocol	10	mm0, g0, cg0, t0, ct1, i0, ci0, v0, cv0, ah0, ptc0, C4d0
B	R4	Protocol	8	mm0, g0, cg0, t1, ct0, i1, ci0, v0, cv0, ah0, ptc0, C4d0
B	R5[A]	Protocol	-	No protocol biopsy procedure due to a complicated post-operative course
B	R6[A]	Protocol	-	No protocol biopsy procedure due to a complicated post-operative course
C	R7	Protocol	22	mm0, g0, cg0, t1, ct0, i1, ci0, v0, cv0, ah2, ptc0, C4d0
C	R11[A]	Protocol		No protocol biopsy procedure due to a complicated post-operative course
C	R11[C]	Indication	159	mm0, g0, cg0, t1, ct1, i3, ci1, v0, cv0, ah0, ptc1, C4d0
C	R12	Protocol	8	Insufficient biopsy specimen
C	R14[B]	Protocol		Patient refused protocol biopsy procedure
C	R14[C]	Indication	119	mm0, g0, cg0, to, ct0, i0, ci0, v0, cv0, ah1, ptc0, C4d0

[A]Due to a complicated post-operative course, 3 patients (R5, R6 and R11) did not undergo the protocol biopsy procedure. [B]Patient R14 refused the investigation. [C]Indication biopsies due to a transient rise in serum creatinine were performed in patients R2 and R14 but showed no abnormalities. In both patients, serum creatinine returned to baseline without further measures. Patient R1 from group A, who received only 1% of the MIC cell number administered to groups B and C, was found to have borderline changes in a biopsy on day 77. In this patient, the biopsy was performed after a rise in serum creatinine of 0.2 mg/dL from baseline. Patient R11 was found to have *E. coli* urinary tract infection on day 128 after surgery requiring a quinolone antibiotic. Serum creatinine rose from 1.31 mg/dL before antibiotic treatment to a maximum of 1.81 mg/dL on day 157. A biopsy procedure revealed severe interstitial inflammation (i3) with nearly absent tubulitis (t1) indicative for allergic interstitial nephritis. High-dose methylprednisolone was given and serum creatinine returned to baseline in both patients. ah = arteriolar hyalinosis, C4d = complement split product 4d, cg = glomerular double contours, ci = interstitial fibrosis, ct = tubular atrophy, cv = vascular fibrous intimal thickening, g = glomerulitis, i = inflammation, mm = mesangial matrix, ptc = peritubular capillaritis, t = tubulitis, tx = transplantation, v = intimal arteritis

Supplemental Table 5: Chimerism analysis in 10 patients (TOL-1 study phase to day 30)

Patient		**Quantitative chimerism analysis**					
Group	**TOL pat. no.**	**Day -7**	**Day -6**	**Day -2**	**Day -1**	**Day 7**	**Day 30**
A	R1	---	---	p/p	p/p	p/p	p/p
A	R2	---	---	p/p	p/p	p/p	p/p
A	R3	---	---	p/p	p/p	p/p	p/p
B	R4	---	---	p/p	p/p	p/p	p/p
B	R5	---	---	p/p	p/p	p/p	p/p
B	R6	---	---	p/p	p/p	p/p	p/p
C	R7	p/p	p/p	---	p/p	p/p	p/p
C	R11	p/p	p/p	---	p/p	p/p	p/p
C	R12	p/p	p/p	---	p/p	p/p	p/p
C	R14	p/p	p/p	---	p/p	p/p	p/p

The lower detection limit was at 1%, b.w. = body weight, MICs = modified immune cells, pat./p = patient

Supplemental Tables 6-9: Proteome analysis in sera of MIC-treated patients. The table gives the list of 20 proteins with the largest decreases or increases in expression after (day -1) compared to before (day -2/-7) MIC administration (Supplemental Tables 6 and 7) and the list of 20 proteins with the largest decreases or increases in expression on day 180 after transplantation compared to before (day -2/-7) MIC administration (Supplemental Tables 8 and 9).

Supplemental Table 6: Pre-transplant (largest decrease after MIC therapy)

Symbol	Name	UniProt ID	Log FC	P value
CD74	HLA class II histocompatibility antigen gamma chain (HLA-DR)	P04233	-1.428	0.019
CHKA	Choline kinase alpha	P35790	-0.791	0.046
CSTA	Cystatin-A	P01040	-0.711	0.043
PTPRC	Receptor-type tyrosine-protein phosphatase C	P08575	-0.625	0.022
FAP	Prolyl endopeptidase FAP	Q12884	-0.586	0.023
C2CD4B	C2 calcium-dependent domain-containing protein 4B	A6NLJ0	-0.575	0.022
TRIM22	E3 ubiquitin-protein ligase TRIM22	Q8IYM9	-0.548	0.047
MUC17	Mucin-17	Q685J3	-0.474	0.049
KRT8	Keratin, type II cytoskeletal 8	P05787	-0.462	0.033
TNFRSF8	Tumor necrosis factor receptor superfamily member 8 (CD30)	P28908	-0.438	0.028
KLHL12	Kelch-like protein 12	Q53G59	-0.431	0.049
IGFBP4	Insulin-like growth factor-binding protein 4	P22692	-0.402	0.025
MTA2	Metastasis-associated protein MTA2	O94776	-0.385	0.047
RPS6KA2	Ribosomal protein S6 kinase alpha-2	Q15349	-0.361	0.045
CD33	Myeloid cell surface antigen CD33	P20138	-0.351	0.023
MMP1	Interstitial collagenase	P03956	-0.322	0.048
MMP3	Stromelysin-1	P08254	-0.303	0.004
NME2	Nucleoside diphosphate kinase B	P22392	-0.23	0.030
VDR	Vitamin D3 receptor	P11473	-0.169	0.048
DDIT3	DNA damage-inducible transcript 3 protein	P35638	-0.538	0.500[A]

[A]not significant

Supplemental Table 7: Pre-transplant (largest increase after MIC therapy)

Symbol	Name	UniProt ID	Log FC	P value
SMAD3	Mothers against decapentaplegic homolog 3	P84022	0.755	0.011
TSPAN12	Tetraspanin-12	O95859	0.589	0.025
ADAM9	Disintegrin and metalloproteinase domain-containing protein 9	Q13443	0.537	0.021
SERPINE2	Glia-derived nexin	P07093	0.526	0.024
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2, isoform CRA_a	A0A024RDD4	0.497	0.006
MCM5	DNA replication licensing factor MCM5	P33992	0.489	0.022
GJB1	Gap junction beta-1 protein	P08034	0.456	0.031
SDC1	Syndecan-1	P18827	0.455	0.004
F13B	Coagulation factor XIII B chain	P05160	0.448	0.011
IL10	Interleukin-10	P22301	0.448	0.042
SNCG	Gamma-synuclein	O76070	0.44	0.007
MAGEA8	Melanoma-associated antigen 8	P43361	0.437	0.030
HMGB1	High mobility group protein B1	P09429	0.429	0.037
LGALS4	Galectin-4	P56470	0.421	0.011
THBS2	Thrombospondin-2	P35442	0.42	0.008
TNF	Tumor necrosis factor	P01375	0.417	0.025
PLEC	Plectin	Q15149	0.413	0.006
NUPR1	Nuclear protein 1	O60356	0.41	0.009
MAPK1	Mitogen-activated protein kinase 1	P28482	0.38	0.011
KDM5A	Lysine-specific demethylase 5A	P29375	0.368	0.024

Position 34 (not shown in the Table). Symbol: TGFB1, Name: Transforming growth factor beta-1 proprotein,

UniProt ID: P01137, Log FC: 0.301, P value: 0.035

Supplemental Table 8: Post-transplant (largest decrease after MIC therapy)

Symbol	Name	UniProt ID	Log FC	P value
IGFBP4	Insulin-like growth factor-binding protein 4	P22692	-1.182	0.000
CHKA	Choline kinase alpha	P35790	-0.949	0.042
PSPHP1	Putative phosphoserine phosphatase-like protein	O15172	-0.686	0.018
IL32	Interleukin-32	P24001	-0.679	0.002
AQP6	Aquaporin-6	Q13520	-0.659	0.014
CD53	Leukocyte surface antigen CD53	P19397	-0.604	0.003
CCN3	CCN family member 3	P48745	-0.498	0.010
DKK1	Dickkopf-related protein 1	O94907	-0.482	0.032
CD44	CD44 antigen	P16070	-0.48	0.017
SOD1	Superoxide dismutase [Cu-Zn]	P00441	-0.475	0.027
CLDN10	Claudin-10	P78369	-0.46	0.001
INPP5D	Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1	Q92835	-0.45	0.031
	hypothetical protein DKFZp761E1347.1	-	-0.447	0.045
LAMP2	Lysosome-associated membrane glycoprotein 2	P13473	-0.444	0.007
ZNF345	Zinc finger protein 345	Q14585	-0.431	0.029
MME	Neprilysin	P08473	-0.429	0.003
HSBP1	Heat shock factor-binding protein 1	O75506	-0.417	0.022
FYN	Tyrosine-protein kinase Fyn	P06241	-0.417	0.044
CAV2	Caveolin-2	P51636	-0.409	0.004
VEGFB	Vascular endothelial growth factor B	P49765	-0.402	0.014

Supplemental Table 9: Post-transplant (largest increase after MIC therapy)

Symbol	Name	UniProt ID	Log FC	P value
KRT4	Keratin, type II cytoskeletal 4	P19013	1.605	0.042
MSLN	Mesothelin	Q13421	1.575	0.037
CDKN2A	Cyclin-dependent kinase inhibitor 2A	P42771	1.383	0.024
CD4	T cell surface glycoprotein CD4	P01730	1.254	0.025
CD47	Leukocyte surface antigen CD47	Q08722	0.982	0.023
CD27	CD27 antigen	P26842	0.955	0.023
TFRC	Transferrin receptor protein 1 (CD71)	P02786	0.74	0.023
IL10	Interleukin-10	P22301	0.707	0.009
CTNNB1	Catenin beta-1	P35222	0.689	0.018
SELL	L-selectin	P14151	0.647	0.029
N4BP2L2	NEDD4-binding protein 2-like 2	Q92802	0.643	0.005
CTNNB1	Catenin beta-1	P35222	0.622	0.030
HLA-DMB	HLA class II histocompatibility antigen, DM beta chain	P28068	0.606	0.033
CCL5	C-C motif chemokine 5	P13501	0.538	0.015
CSF2	Granulocyte-macrophage colony-stimulating factor	P04141	0.503	0.042
TGFB1	Transforming growth factor beta-1 proprotein	P01137	0.493	0.005
IL3RA	Interleukin-3 receptor subunit alpha	P26951	0.49	0.042
CTNNBL1	Beta-catenin-like protein 1	Q8WYA6	0.466	0.008
CTGF	Connective tissue growth factor	P29279	0.454	0.017
CD34	Hematopoietic progenitor cell antigen CD34	P28906	0.446	0.032

Supplemental Table 10: Specification of the final product (MIC)

Production			**Clinical parameter**			**GMP**			**Quality control**		
Group	**Lot no.**	**TOL pat. no.**	**Blood group donor**	**Blood group recipient**	**Achieved cell dose per kg b.w.**	**MIC product volume (mL)**	**Absolut nuclear cell amount x10^8 (Neubauer)**	**CD14$^+$ cells (%)**	**Sterility (Ph.Eur.)**	**Endotoxin (EU/mL, Ph.Eur.)**	**Mitomycin C (µg/mL, Ph.Eur.)**
A	MMC-01	D1	A Rh. pos.	A Rh. pos.	1.5x10^6	85.1	1.3	11.7	sterile	0.79	<LOD
A	MMC-02	D2	A Rh. pos.	A Rh. pos.	1.5x10^6	80.7	1.2	10.4	sterile	1.38	0.1
A	MMC-03	D3	O Rh. pos.	O Rh. pos.	1.5x10^6	79.1	1.2	19.2	sterile	1.56	0.0
B	MMC-04	D4	A Rh. pos.	A Rh. pos.	1.5x10^8	93.8	105.0	23.2	sterile	0.72	0.0
B	MMC-05	D5	O Rh. pos.	O Rh. pos.	1.5x10^8	85.0	87.0	17.2	sterile	1.72	0.0
B	MMC-06	D6	O Rh. pos.	O Rh. neg.	0.4x10^{8A}	91.6	43.0	16.2	sterile	1.31	0.0
C	MMC-07	D7	O Rh. neg.	O Rh. neg.	1.5x10^8	64.3	134.0	16.4	sterile	1.08	0.0
No infusion	MMC-08	D9	O Rh. pos.	O Rh. pos.	1.5x10^8	n.a.	n.a.	21.1	n.a.	n.a.	n.a.
C	MMC-09	D11	O Rh. neg.	A Rh. neg.	1.5x10^8	91.8	96.0	15.9	sterile	1.97	0.5
C	MMC-10	D12	O Rh. pos.	O Rh. pos.	1.3x10^{8B}	96.7	126.0	17.3	sterile	1.39	0.3
No infusion	MMC-11	D13	O Rh. pos.	A Rh. pos.	1.5x10^8	n.a.	n.a.	11.9	n.a.	n.a.	n.a.
C	MMC-12	D14	O Rh. pos.	A Rh. pos.	1.5x10^8	95.7	124.5	9.9	sterile	1.23	0.2

[A]In donor D6, only 60% of the targeted blood volume was processed during leukapheresis due to venous access problems and therefore patient R6 (male, 101 kg b.w.) received a reduced dose of only 0.4x10^8 MICs per kg b.w.. [B]In donor D12, leukapheresis was performed per protocol but the cells obtained were only sufficient for a cell dose of 1.3x10^8 MICs per kg b.w. in patient R12 (male, 105 kg b.w.). b.w. = body weight, CD = cluster of differentiation, EU = endotoxin units, GMP = Good Manufacturing Practice, LOD = limit of detection, MICs = modified immune cells, n.a. = not applicable, pat. = patient, Ph.Eur. = Pharmacopoea Europaea

Supplemental Table 11: Results of the potency assay

Marker	Method	Sample N	Result
Characterization of resulting DCs after co-culture with MICs			
CD80	Flow cytometry	9/12	reduced expression
CD86	Flow cytometry	6/12	reduced expression
CD83	Flow cytometry	6/12	reduced expression
HLA-DR	Flow cytometry	6/12	reduced expression
CD103	Flow cytometry	7/7	increased expression
Ag-specific $CD8^+$ T cell proliferation	MLPC	4/5	inhibited
Interferon-γ secretion of Ag-specific $CD8^+$ T cell	ELISpot assay	6/7	inhibited
Characterization of MICs			
Ag-specific $CD8^+$ T cell proliferation	MLPC	7/9	inhibited
Interferon-γ secretion of Ag-specific $CD8^+$ T cell	ELISpot assay	4/4	inhibited

Ag = antigen, CD = cluster of differentiation, DCs = dendritic cells, ELISpot assay = Enzyme-Linked ImmunoSpot assay, HLA = human leukocyte antigen, MICs = modified immune cells, MLPC = Mixed lymphocyte peptide culture

Supplemental Table 12: Top canonical pathways, diseases, and biofunctions related to genes up- or down-regulated after mitomycin C treatment of PBMCs (MIC modification)

Name	P value or P value range	Overlap or number of molecules
Canonical pathways		
Sirtuin signaling pathway	$1.3x10^{-9}$	8.6% (25/292)
Systemic lupus erythematosus signaling	$4.5x10^{-8}$	8.7% (20/230)
mTOR signaling	$5.2x10^{-8}$	9.0% (19/210)
Regulation of eIF4 and p70S6K signaling	$1.2x10^{-7}$	10.2% (16/157)
CD28 signaling in T helper cells	$1.3x10^{-7}$	11.7% (14/120)
Diseases and disorders		
Immunological disease	$4.1x10^{-6}$-$9.5x10^{-22}$	182
Infectious diseases	$7.4x10^{-7}$-$1.0x10^{-20}$	127
Connective tissue disorders	$1.8x10^{-7}$-$1.2x10^{-20}$	106
Inflammatory disease	$1.2x10^{-6}$-$1.2x10^{-20}$	160
Organismal injury and abnormalities	$4.5x10^{-6}$-$1.2x10^{-20}$	430
Molecular and cellular functions		
Cell death and survival	$4.7x10^{-6}$-$1.0x10^{-22}$	208
Cell cycle	$4.6x10^{-6}$-$6.4x10^{-19}$	128
Cellular compromise	$2.0x10^{-6}$-$1.0x10^{-18}$	80
Cellular movement	$3.7x10^{-6}$-$2.3x10^{-16}$	137
Cellular growth and proliferation	$4.9x10^{-6}$-$6.4x10^{-16}$	166
Physiological system development and function		
Hematological system development and function	$5.0x10^{-6}$-$1.7x10^{-17}$	145
Tissue morphology	$4.1x10^{-6}$-$1.7x10^{-17}$	138
Lymphoid tissue structure and development	$3.2x10^{-6}$-$6.4x10^{-16}$	103
Immune cell trafficking	$3.6x10^{-6}$-$1.3x10^{-15}$	105
Organismal survival	$4.1x10^{-8}$-$7.9x10^{-13}$	151

A number of 554 genes were significantly up- or down-regulated in all 4 MIC samples. Of special relevance was down-regulation of CD86, HLA-DQ1, IL-6 and up-regulation of ADM. These genes may serve as candidates for a future potency assay. MICs = modified immune cells, PBMCs = peripheral blood mononuclear cells

References

1 Mevorach D, et al. Single infusion of donor mononuclear early apoptotic cells as prophylaxis for graft-versus-host disease in myeloablative HLA-matched allogeneic bone marrow transplantation: a phase I/IIa clinical trial. *Biol Blood Marrow Transplant.* 2014;20(1):58-65.

2 Ritchie ME, Dunning MJ, Smith ML, Shi W, Lynch AG. BeadArray expression analysis using bioconductor. *PLoS Comput Biol.* 2011;7(12):e1002276.

3 Ritchie ME, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 2015;43(7):e47.

4 Odriozola A, Riancho JA, Colorado M, Zarrabeitia MT. Evaluation of the sensitivity of two recently developed STR multiplexes for the analysis of chimerism after haematopoietic stem cell transplantation. *Int J Immunogenet.* 2013;40(2):88-92.

5 Alhamdani MS, Schröder C, Hoheisel JD. Analysis conditions for proteomic profiling of mammalian tissue and cell extracts with antibody microarrays. *Proteomics.* 2010;10(17):3203-3207.

6 Mustafa S, et al. Comparison of the tumor cell secretome and patient sera for an accurate serum-based diagnosis of pancreatic ductal adenocarcinoma. *Oncotarget*. 2017;8(7):11963-11976.

7 Alhamdani MS, et al. Immunoassay-based proteome profiling of 24 pancreatic cancer cell lines. J *Proteomics.* 2012;75(12):3747-3759.

8 Kallio MA, et al. Chipster: user-friendly analysis software for microarray and other high-throughput data. *BMC Genomics.* 2011;12:507.

9 Ritchie ME, et al. A comparison of background correction methods for two-colour microarrays. *Bioinformatics*. 2007;23(20):2700-2707.

10 Newell KA, et al. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest.* 2010;120(6):1836-1847.

11 Bottomley MJ, et al. Application of Operational Tolerance Signatures Are Limited by Variability and Type of Immunosuppression in Renal Transplant Recipients: A Cross-Sectional Study. *Transplant Direct.* 2016;3(1):e125.

12 Moreso F, et al. Gene expression signature of tolerance and lymphocyte subsets in stable renal transplants: results of a cross-sectional study. *Transpl Immunol*. 2014;31(1):11-16.

Morath et al., Donor-derived modified immune cell (MIC) infusion as a novel therapy in kidney transplantation: a phase I clinical trial

TREND Statement Checklist 133595-JCI-CMED-1

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	✓	3
		• Structured abstract recommended	✓	3
		• Information on target population or study sample	✓	3
Introduction				
Background	2	• Scientific background and explanation of rationale	✓	5,6
		• Theories used in designing behavioral interventions	n.a.	
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	✓	18
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	✓	SD 11
		• Recruitment setting	✓	SD 11
		• Settings and locations where the data were collected	✓	SD 11
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:	✓	19,20
		○ Content: what was given?	✓	19,20
		○ Delivery method: how was the content given?	✓	19,20
		○ Unit of delivery: how were the subjects grouped during delivery?	✓	19,20
		○ Deliverer: who delivered the intervention?	✓	19,20
		○ Setting: where was the intervention delivered?	✓	19,20
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	✓	19,20
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	✓	19,20
		○ Activities to increase compliance or adherence (e.g., incentives)	n.a.	
Objectives	5	• Specific objectives and hypotheses	✓	18
Outcomes	6	• Clearly defined primary and secondary outcome measures	✓	18
		• Methods used to collect data and any methods used to enhance the quality of measurements	✓	18
		• Information on validated instruments such as psychometric and biometric properties	n.a.	
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	n.a.	
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	✓	SD 11
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	✓	SD 11, 18, 19
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	n.a.	

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	n.a.	
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	n.a.	
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	n.a.	
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	n.a.	
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	n.a.	
		• Methods for imputing missing data, if used	n.a.	
		• Statistical software or programs used	n.a.	
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	✓	SD11
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	✓	SD11
		○ Assignment: the numbers of participants assigned to a study condition	✓	SD11
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	✓	SD11
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	✓	SD11
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	✓	SD11
		• Description of protocol deviations from study as planned, along with reasons	✓	SD11, 19
Recruitment	13	• Dates defining the periods of recruitment and follow-up	✓	SD11
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	✓	34,35
		• Baseline characteristics for each study condition relevant to specific disease prevention research	n.a.	
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	n.a.	
		• Comparison between study population at baseline and target population of interest	n.a.	
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	n.a.	

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	✓	SD 11
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	n.a.	
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	✓	36, 37
		• Inclusion of null and negative findings	✓	
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	n.a.	
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	✓	18
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	✓	36, 37 SD 20-25
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	✓	14-17
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	✓	14-17
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	✓	14-17
		• Discussion of research, programmatic, or policy implications	✓	14-17
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	✓	14-17
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	✓	14-17

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/